

Role of Dual Inoculation of *Rhizobium* and Arbuscular Mycorrhizal (AM) Fungi on Pulse Crops Production

**Erneste HAVUGIMANA^{*}, Balkrishna Sopan BHOPLE,
Emmanuel BYIRINGIRO and Jean Pierre MUGABO**

Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara, India

(*Corresponding author's e-mail: haverinestus@gmail.com)

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Abstract

Legume crops are useful as human and animal feed, wood energy, and as soil-improving components of agricultural and agro forestry systems through its association with bio-fertilizers. The later have a potential environment friendly inputs that are supplemented for proper plant growth. Bio-fertilizers are preparations containing living cells of microorganisms that help crop plants in the uptake of nutrients by their interactions in the rhizosphere. Arbuscular mycorrhizal (AM) fungi are beneficial symbionts for plant growth. They are associated with higher plants by a symbiotic association and benefit plants in the uptake of phosphorus nutrients, production of growth hormones, increase of proteins, lipids and sugars levels, helps in heavy metal binding, salinity tolerance and disease resistance. In nature symbiotic association of *Rhizobium* and leguminous plants fixes atmospheric nitrogen. Indeed, research has proved that the association of mycorrhizae fungi and *Rhizobium*, with pulse crops, increased the beneficial aspects comparatively more than their single associations with the host plants. This review focuses on the role of dual inoculation of AM fungi and *Rhizobium* on different pulse crops.

Keywords: Dual inoculation, *Rhizobium*, AM fungi, pulse crops

Introduction

Legume crops are important not only for human and animal consumption but also for the environment as they can be grown in water deficient and low nutrient environmental soil due to their ability to form symbiosis with both nitrogen fixing *Rhizobium* and arbuscular mycorrhizal (AM) fungi. Symbiosis is a biological phenomenon involving dynamic changes in the genome metabolism and signaling network [1]. These 2 symbionts namely mycorrhizal fungi and *Rhizobium* with leguminous plants establish a tripartite association capable of supplying Nitrogen and Phosphorous content to plants [2]. The mycorrhizal fungi and *Rhizobium* both have a high ability to convert nutritional elements N and P from unavailable forms to available forms through biological processes and act as bio-fertilizer [3,4]. The legume crops have high nutritional value as sources of protein, phosphorus, carbohydrate, minerals and different vitamins but are commonly used as fodder and green manure [5]. The scarcity of food containing high levels of protein, micronutrients and various vitamins sources is an increasing problem affecting most people in developing countries. New technologies are needed to meet the challenge of food scarcity and malnutrition in poor families across the world [6]. To improve the quality and yield of economically important legumes, farmers apply large quantities of chemical fertilizers, which have detrimental effects on the soil, such as the accumulation of toxic salt while application of bio-fertilizers should be the best solution as it is environmental friendly and highly cost effective [7].

Much research in the past few years has been carried out on various aspects of root symbionts and has shown that the dual interaction of AM fungi and *Rhizobium* improve not only the growth, nodulation

and yield of pulse crops, but also nutrient status in the soil [8,9]. Most farmers are not familiar with bio-fertilizer application especially the dual inoculation of *Rhizobium* and AM fungi on pulse crops. Objective of this review article is to address the effectiveness of the results revealed by different research on dual inoculation of AM fungi and *Rhizobium* on pulse crops.

Bio-fertilizers and their major potential

Bio-fertilizer is defined as a substance which contains a living microorganism and when applied to seeds, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant [10]. Bio-fertilizers are not fertilizers, because fertilizers directly increase soil fertility by adding nutrients to the soil while bio-fertilizers add nutrients through the natural processes of fixing atmospheric nitrogen, solubilising Phosphorus, and stimulating plant growth through the synthesis of growth promoting substances [11].

Bio-fertilizers enhance the nutrient availability to crop plants by processes like fixing atmosphere N or dissolving P present in the soil, and also impart better health to plants and soil thereby enhancing crop yields in a moderate way [10]. It is a natural method without any problems like salinity, alkalinity and soil erosion. In areas of low input agriculture and oil seeds production, these products will be of much use to give sustainability to production and improve organic farming and reduce the effects of chemical fertilizers on the ecosystem [7].

Advantageous characteristics of *Rhizobium* and AM fungi

Rhizobium

Rhizobium belongs to a group of bacteria, in the family of *Rhizobiaceae* and is a symbiotic nitrogen fixer in association with legume plants only. They are the most efficient bio-fertilizers in terms of per quantity of nitrogen fixed. The bacteria infect the legume root and form root nodules within which they reduce molecular nitrogen to ammonia which is utilized by the plant to produce valuable proteins, vitamins and other nitrogen containing compounds [12]. It is useful for pulse legumes like chickpea, red-gram, pea, lentil, black gram, and oil-seed legumes like soybean and groundnut and forage legumes like *berseem* and *Lucerne*. Successful nodulation of leguminous crops by *Rhizobium* largely depends on the availability of a compatible strain for a particular legume [13]. *Rhizobium* has the ability to fix atmospheric nitrogen and its population in the soil depends on the presence of legume crops in the field; in absence of legumes, the population decreases. It colonizes the roots of specific legumes to form tumor like growths called root nodules, which act as factories of ammonia production [14]. Artificial seed inoculation is often needed to restore the population of effective strains of the *Rhizobium* near the rhizosphere to hasten N-fixation. Each legume requires a specific species of *Rhizobium* to form effective nodules [10].

AM fungi

Phosphate solubilizing microorganisms of different bacterial species have the ability to solubilize insoluble inorganic phosphate compounds, such as tri-calcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate. The species of *Pseudomonas*, *Bacillus* and *Aspergillus* secrete organic acids and lower the pH in their vicinity to bring about dissolution of bound phosphates in the soil [13]. AM fungi is a type of mycorrhizae in which the fungus penetrates the cortical cells of the roots of a vascular plant. The term mycorrhizal denotes “fungus roots”. It is a symbiotic association between host plants and certain groups of fungi at the root system, in which the fungus obtains its carbon requirements from the photosynthates of the host and the host in turn benefits by obtaining much needed nutrients with the help of the fine absorbing hyphae of the fungus especially phosphorus, calcium, copper, zinc etc., which are otherwise inaccessible to it [15].

Symbiotic association of AM fungi and *Rhizobium* on some important pulses

The scarcity of available phosphorus and the imbalance of trace elements in the ecosystem actually limit legume plant establishment and nitrogen fixation. But, when associated with mycorrhizal, it has

been shown that there is an increase in plant establishment. Arbuscular mycorrhizae are by far the most widespread in nature and the most common natural association makers with the nodulated nitrogen fixing legumes [16]. After forming symbiotic associations with legume plant roots, AM fungi develop an extra radical mycelium that links the roots and the soil environment and helps the plants to more efficiently use soil nutrients, particularly those that diffuse slowly towards the root surface, such as phosphate and trace elements [17]. In addition, the symbiosis enhances the ability of the plant to become established and cope with stress situations (nutrient deficiency, drought, trace element imbalance, soil disturbance), which are typical in desert situations. Nitrogen content increased in plants inoculated with the *Glomus* species than in those inoculated only with *Rhizobium* [16]. The largest nitrogen content corresponded to the treatment with both *Glomus* and *Rhizobium*, in *G. fasciculatum* and *Rhizobium* inoculated *P. juliflora*, treated with tannery effluent showed a higher dry weight, increased rate of photosynthesis, higher protein content, increased sugars, lipid and amino acid levels, increased activity of enzymes catalyze, peroxides, phosphates, nitrate reductase, increased level of growth hormones IAA, gibberellins and cytokines, higher N, P, K and Ca, Fe, Co, Mo levels in roots and shoots, than their single inoculations. Also, the translocation of heavy metals such as Cd, Cr and Zn are highly restricted in the extra radical hyphae of AM fungi, in dual inoculated treatments, when compared with single inoculations. Thus, the symbiotic association of AM fungi with *Rhizobium* benefits the plants more than single symbiotic or no-inoculated plants [18]. **Table 1** summarizes clearly the positive synergistic effects of dual inoculation of AM fungi and *Rhizobium* on different pulses crops.

Common bean (*P. vulgaris*)

The study carried out by Tajini *et al.* [19] on a comparison of the response of common bean (*P. vulgaris*) to AM fungi and *Rhizobium* strain inoculation, 2 genotypes of common beans coco T and flamingo varying their effectiveness for N₂ fixation were inoculated with *Glomus intraradices* and the *Rhizobium* strain of the common bean. The combined inoculation of AM fungi and *Rhizobium* showed a significant increase in nitrogen and phosphorus in shoots and high P use efficiency. The P use efficiency has been observed in both varieties with dual inoculation which illustrates the direct link between the P use efficiency and symbiotic nitrogen fixation [20], and showed the relationship between colonization by AM fungi and the mechanism which affects the P use efficiency and its absorption and utilization by plants [6]. Inoculation with AM fungi improves plant growth parameters and nutrient uptake, expressing that the colonization of AM fungi also significantly reduced the negative effect of P deficiency. Also, a significant correlation between growth and nodulation proves a synergistic effect between *Rhizobium* and AM fungi on common bean growth [21]. It is also thought that the plant-*Rhizobium* system benefits from the presence of AM fungi because the mycorrhizae ameliorate not only P deficiency but also any other nutrient deficiencies that might be limiting factor to *Rhizobium* and plant mutualism [11]. Increased mineral nutrient levels in the plants would not only benefit *Rhizobium* directly, but also would lead to the increase of photosynthesis, making a greater proportion of photosynthates available to the *Rhizobium* nodules with rhizobia and AM fungi significantly enhanced the number of nodules and the dry weight per plant [12]. Thus, the results reported, show that AM symbiosis improved nodulation, N₂ fixation and phosphorus use efficiency.

Green gram (*Vigna radiata*)

Research has been conducted to investigate the effect of *Rhizobium* and arbuscular mycorrhizal fungi and inoculation individually and both in combination on growth parameters and chlorophyll content of *Vagina unguolata* (L.), the seed inoculated with *Rhizobium*; AM fungi and a combination of both have been grown differently in sterilized circular earthen pots. Plants inoculated with *Rhizobium* or AM fungi significantly increase all vegetative parameters taken (shoot length, root length, number of nodules, dry weight of nodules) compared to the control [22]. But the maximum value was shown in dual inoculation plants on all tested parameters when compared to plant inoculated individually and the control which is similar to the preview research reported that dual inoculation showed more nodulation compared to the non mycorrhizal plant [23]. Plants inoculated with AM fungi and dual inoculation showed a positive root colonization by AM fungi while the plants individually inoculated by *Rhizobium* and control plants not

have colonization due to the indirect response of the host plant that affect the lower part of the root [24]. Plants inoculated with AM fungi showed a significance increase in chlorophyll a, b and total chlorophyll content due to an increase in stomata conductance, photosynthesis rate, transpiration activity, and plant growth enhancement [25], and the presence of numerous and large bundle sheath chloroplasts in the inoculated leaves crops [26].

Pigeon pea (*Cajanus cajan*)

Several studies had been carried out on pigeon pea as an important pulse crop able to fix atmospheric nitrogen and these studies support dual inoculation of *Rhizobium* and AM fungi which positively influence the yield and quality of pigeon pea (*Cajanus Cajan* L.). The pot experiment carried out by Bhattacharjee and Sharma, (2012) investigated the effect of AM (*Glomus fasciculatum*) and *Rhizobium* on the chlorophyll, nitrogen and phosphorus content of pigeon pea (*Cajanus Cajan* L.). The result of this research showed that the chlorophyll content was higher in treated plants than in the control, although *Glomus fasciculatum* improved the chlorophyll content [27,28] the maximum was found in dual inoculated plants, this showed evidence that the efficacy of arbuscular mycorrhizal activities was boosted by co-inoculation with *Rhizobium* and vice versa. This is also attributed to the increase of photosynthesis and finally increases plant nutrient content and its growth [26]. Dual inoculation of AM fungi and *Rhizobium* significantly increased chlorophyll contents in *Vigna unguiculata* (L.) [29]. Inoculated plants with *Glomus fasciculatum* or *Rhizobium* also increased nitrogen and phosphorus contents in pigeon peas. However, the maximum nitrogen and phosphorus contents were recorded in the plants dually inoculated with *Glomus fasciculatum* and *Rhizobium* [27]. Inoculation of legumes with *Rhizobium* increased the nodulation of legumes causing more nitrogen fixation and making it available for the plants and therefore, it is used as an alternative for urea to minimize the cost of production [30].

AM fungi help in the nitrogen fixation process by providing to root plants the phosphorus and other immobile nutrients which are essential for nitrogen fixation [22]. Effectiveness of AM fungi can enhance the performance of Rhizobial infection and vice versa.

Chick pea (*Cicer arietinum*)

The combined inoculation of *Glomus fasciculatum* and *Rhizobium* has a synergistic effect resulting in the improvement of nitrogen and phosphorus availability to plants [31]. As in other different studies worked out on the effect of dual inoculation or the synergetic effect of *Rhizobium* and AM fungi on several legumes crops, most pea group crops in which chickpea is included showed high performance due to dual inoculation of AM fungi and *Rhizobium*. In 2013, Gosavi determined the influence of AM fungi (arbuscular mycorrhizae) on *Rhizobium*-legume interactions, after the experiment showed that dual inoculation of *Rhizobium* with AM fungi enhanced seed yield of chickpea and golden green gram [32].

The result showed the synergetic effect of co-inoculation of *Rhizobium* with *Glomus fasciculatum* in improving growth parameters and biomass when compared to single inoculation of one of them as a response to the ability of *Rhizobium* to fix atmospheric nitrogen and make it available to the plant as needed and the effect of VAM fungal association on P uptake [33]. According to different investigations the use of bio-fertilizers are recommended as they are eco-friendly, cost effective alternatives to chemical fertilizers.

Table 1 Effect of dual inoculation of *Rhizobium* and AM fungi on grain yield of different pulse crops.

Pulses' name		Grain yield (kg/ha)				Reference
English name	Botanical name	C	RH	AMF	RH + AMF	
Field pea	<i>Pisum sativum</i>	85.37	94.81	93.33	102.78	[34]
Green gram	<i>Vigna radiata</i>	616	749	703	875	[35]
Common beans	<i>Phaseolus vulgaris</i>	1729.9	1903.9	2037.8	2664.3	[36]
Pigeon pea	<i>Cajanus cajan</i>	1324	2361.4	1551	2581.4	[37]
Soy bean	<i>Glycine max</i>	576.9	804.1	760.2	956.6	[38]
Chick pea	<i>Cicer arietinum</i>	1253.13	1568.75	1671.88	2012.5	[39]
Cow pea	<i>Vigna unguiculata</i>	726.8	792.4	838.7	905.9	[40]
Lentil	<i>Lens culinaris</i>	270	372	328	445	[41]

C: control, RH: *Rhizobium*, AMF: Arbuscular mycorrhizal fungi

Conclusions

The benefit of combined association of AM fungi and *Rhizobium* is comparatively more than single inoculation, especially in legume member hosts. The current studies clearly reveal that the dual inoculation with AM fungi and *Rhizobium* bio-fertilizers is more effective in increasing growth, nutrition, chlorophyll content and biomass production of legumes. It is also essential to study the possibility of inoculating pulses crops with selected strains of AM fungi and *Rhizobium* for higher production purposes, especially dual inoculation on legume crops to improve the soil's organic carbon to sustain soil quality and sustainable agricultural productivity [10]. Bio-fertilizers have an important role to play in improving nutrient supplies and their crop availability in the years to come. These are environmental friendly, non-bulky and low cost agricultural inputs.

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