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Research Application Summary

# Optimisation of rhizobium and arbuscular mycorrhizal fungi benefits for grain legume production in acid soils

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**Abstract**

This study was conducted to evaluate the symbiotic effectiveness of *Rhizobium* isolates using soybean and *Phaseolus* bean as host plants grown in acid soil (pH 4.5 and 5) from two southern districts of Rwanda. For this purpose, 10 and 33 isolates for soybean and *Phaseolus* bean respectively were studied. The two isolates with highest nitrogen fixing capacity were combined with mycorrhizal species for enhancing legume nodulation and production in 10 treatments (control with/ without 882mg of TSP/ arbuscular mycorrhizal fungi (AMF), *Rhizobium* isolates with/without 882mg of TSP and arbuscular mycorrhizal fungi in 4 kg). These results indicated that effective *Rhizobium* isolates had potential for use as inoculants on soybean and bean.

Key words: Arbuscular mycorrhizal fungi, nitrogen fixation, *Phaseolus* beans, phosphorus, *Rhizobium* species, Rwanda soils, soybean

**Résumé**

Cette étude a été menée pour évaluer l'efficacité symbiotique des isolats de *Rhizobium* utilisant le soja les haricots *Phaseolus* comme des plantes hôtes cultivées dans un sol acide (pH 4,5 et 5) de deux districts du sud du Rwanda. À cette fin, 10 et 33 isolats de soja et de haricot *Phaseolus* respectivement ont été étudiés. Les deux isolats avec la plus grande capacité de fixation de l'azote ont été combinés avec les espèces mycorhiziennes pour améliorer la nodulation et la production des légumineuses dans 10 traitements (contrôle avec ou sans 882mg de TSP / champignons mycorhiziens arbusculaires (AMF), des isolats *Rhizobium* avec ou sans 882mg de TSP et champignons mycorhiziens arbusculaires à 4 kg). Ces résultats indiquent que les isolats de *Rhizobium* efficaces ont un potentiel d'utilisation comme inoculants sur le soja et le haricot.

Mots clés: Champignons mycorhiziens à arbuscules, fixation de l'azote, haricots *Phaseolus*, phosphore, espèces de *Rhizobium*, sols du Rwanda, soja

## Background

Soil acidity is a problem constraining agricultural production in many parts of the world. The productivity of legume systems which support nitrogen fixation has been particularly affected (Graham and Vance, 2000). One way to enhance the performance of the biological system is the possible exploitation of synergy between *Rhizobium* and mycorrhizale. The two microorganisms enter cortex of roots to obtain carbon from their host plants, while assisting the plants to get nitrogen from atmosphere, and with the uptake of phosphorus and the other mineral nutrients from soil (Auge, 2001). This association is beneficial to plants because, nitrogen and phosphorus are a major essential element for growth and development.

The present study was designed to evaluate improvement in performance of grain legumes grown on an acidic soil as affected by *Rhizobium* and Arbuscular Mycorrhizal fungal associations.

## Literature Summary

The symbiotic interrelationship amongst *Rhizobium*, mycorrhizal fungi and plants demonstrates the importance of soil organisms for plant health. Mycorrhizas are able to form symbioses with about 80% of all terrestrial plants and *Rhizobium* contribute $35x10^{12}$ tonnes equivalent to 47% of the total $N_2$ fixed annually to the global nitrogen budget or 24-584 kg N $ha^{-1}$ $yr^{-1}$ (Elkan, 1992). These fungi have well known effects on plant nutrition and seem to have played a major role when plants colonised land four hundred million years ago (Smith and Read, 1997).

A primary reason for the enhanced microbial activity in the rhizosphere is the supply of nutrients from a host plant root exudates containing various stimulatory chemical molecules, although most soil microorganisms scavenge the rhizosphere for nutrients for their growth and reproduction. This activity does not appear to be common for all organisms. There are a few groups of microorganisms which respond to chemical signals released by plant roots and actively colonise the rhizosphere and endorhizosphere. For example, the arbuscular mycorrhizal fungi (AMF) colonise the roots of most known land plants and influence plant growth. Similarly, members of the Rhizobiaceae colonise most leguminous hosts and typically enhance plant growth by fixing atmospheric nitrogen (Brockwell *et al*., 1995).

The amount of $N_2$ fixed by legume depends on plant species and cultivars, on rhizobial strains and on the environmental conditions (Giller, 2001). Soybean and *Phaseolus* bean have the potential to establish symbiosis with arbuscular mycorrhizal fungi (AMF) that improves the uptake of low mobile nutrients such as phosphorus, from the soil (Jakobsen, 2002). Both rhizobial and mycorrhizal symbioses can act synergistically on promoting plant growth and fitness. This often result in increased yield (Jia, 2004).

## Study Description

Laboratory and screen house experiments were carried out at Makerere University in Uganda, between August 2009 and August 2010. Soil samples were obtained from two southern districts of Rwanda, namely Huye and Nyamagabe, at the depth of 0-20 cm. Both districts occur at 1500 meters above sea level but differ in soil acidity; Huye has pH 4.5 and Nyamagabe pH 5. Five hundred grammes of soil were taken for nutrients characterisation, legume nitrogen bacteria (LNB) identification and isolation using soybean and *Phaseolus* bean as trapping crops.

To select effective rhizobial isolates, a Completely Randomised Design (CRD) experiment consisting of 13 and 33 *Rhizobium* isolates for soybean and *Phaseolus* bean, respectively to two strains like *TAL*102, CIAT899 and uninoculated crop. Pure *Rhizobium* isolates were used to inoculate the host plants. At early flowering, soybean and *Phaseolus* bean were harvested for analysis of nitrogen content, nodule count and effectiveness (Vincent, 1970).

To evaluate the improvement of performance of the two grain legumes on an acidic soil through joint exploitation of biological nitrogen fixation (BNF) of the abovementioned isolates and Arbuscular Mycorrhizal associations, a completely randomised design (CRD) experiment was established in a screenhouse consisting ten treatments as follows: (1) control – uninoculated seedlings free of nitrogen, (2) control + nitrogen, (3) TSP, (4) AMF (5) *Rhizobium* isolate 1 inoculation, (6) *Rhizobium* isolate 2, (7) *Rhizobium* isolate1+TSP, (8) *Rhizobium* isolate 2+TSP, (9) *Rhizobium* isolate 1+AMF and (10) *Rhizobium* isolate 2+AMF. There were three replicates.

To study the diversity of the rhizobial isolates, DNA was extracted using phenol/chloroform technique and run on RAPD-PCR using 30 primers. PCR products were run on 1.2% gel electrophoresis (the study is still in preliminary stages).

According to leghemoglobin formation inside nodules, seven rhizobial isolates (S1B, S1A, S2E, S1D, S2D, S2G and S2F) were effective in forming nodules in soybean, as indicated by the red colour of nodules. Three more effective than standard (*TAL*102) and with/without N application in the remaining three (S1F, S2A and S1E) were considered effective (Fig.1).

In case of *Phaseolus* bean, 30 rhizobial isolates promoted high nodulation, also as shown by red colour of nodules. Two of them were especially more effective than the standard (CIAT899) with/without N application. B1TT and B2O were considered ineffective (Fig. 2).

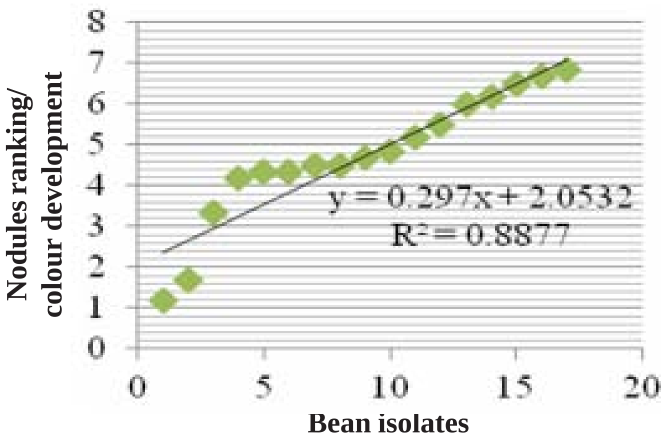


**Figure 1. *Rhizobium* isolates ranking through pink/red color development of inside nodules.**

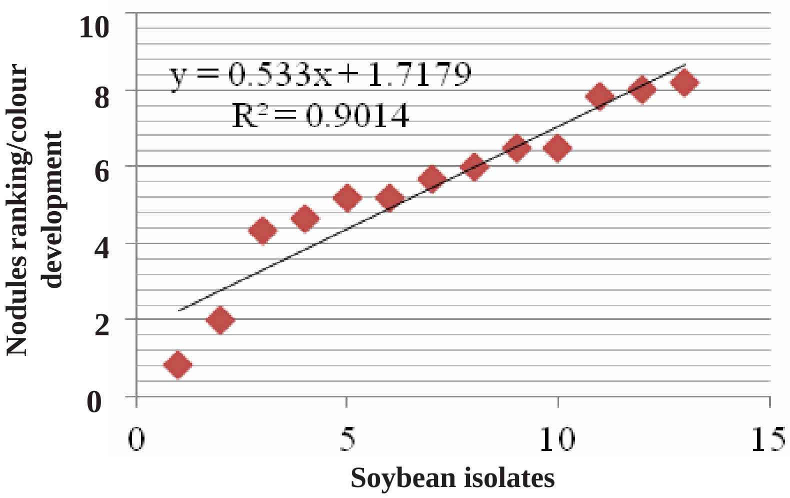


**Figure 2. *Rhizobium* isolates ranking through pink/red color development of inside nodules.**

## Research Application

This study examined the population of soybean and bean nodulating rhizobia recovered from acid soils of Rwanda. As was shown elsewhere (Anyango, 1995) the sensitivity of

CIAT899 to acidity was observed at pH 5.0. A group of rhizobia isolated in Huye and Nyamagabe district were able to nodulate soybean and *Phaseolus* bean at pH 4.5 and 5.0.

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## References

Anyango, B. 1995. Diversity of rhizobia nodulating *Phaseolus vulgaris* L. in two Kenyan soils with contrasting pHs. *Applied and Environmental Microbiology* 61:4016-4021.

Auge, R.M. 2001. Water relations, drought and VA mycorrhizal symbiosis. *Mycorrhiza* 11:3-42.

Brockwell, J., Bottomley, P.J. and Thies, J.E. 1995. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: A critical assessment. *Plant and Soil* 174:143-180.

Elkan, G. H. 1992. Biological nitrogen fixation systems in tropical ecosystems: An overview. In: Biological nitrogen fixation and sustainability of tropical agriculture. K. Mulongoy, M. Gueye and D. S. C. Spencer (Eds.), John Wiley and Sons, Chichester, UK. pp. 40.

Giller, K.E. 2001. Nitrogen fixation in tropical cropping systems. Wallingford, London, UK.

Graham, P.H. and Vance, C.P. 2000. Nitrogen fixation in respective: an overview of research and extension needs. *Field Crops Research* 65:93-106.

Jakobsen, I., Smith, S.E. and Smith F.A. 2002. Function and diversity of arbuscular mycorrhizae in carbon and mineral nutrition. Mycorrhizal Ecology. pp. 92.

Jia, Y., Gray, V.M. and Straker, C.J. 2004. The influence of *Rhizobium* and arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by *Vicia faba*. *Annals of Botany* 94:251-258.

Smith, S.E. and Reag, D.J. 1997. Mycorrhizal Symbiosis. Academic, San Diego, 2nd Ed.

Vincent, J.M. 1970. A manual for the practical study of root-nodule bacteria. International Biological Programme. Handbook No. 15, Blackwell, Oxford, U.K. ISBN 0-63206410-2. Rhizobial strains from *Gliricidia sepium.* pp. 709.