**The impact of *Bradyrhizobium japonicum* inoculation and Nitrogen fertilisation on leguminous crop: A review**

Tope Daniel Bitire1, 2 Michael Abberton2, Olubukola Oluranti Babalola1\* and Olaniyi Oyatomi2

1 Food Security and Safety Niche Area, Faculty of Natural and Agricultural Sciences, North-West University, Private bag X2046 Mmabatho, 2735, South Africa.

2 Genetic Resources Center, International Institute of Tropical Agriculture (IITA), PMB 5320, Oyo Road, Ibadan, Nigeria.

\*Corresponding author: Olubukola.babalola@nwu.ac.za

**Abstract**

The use of microbial inoculant to improve the growth and development of legumes as been embrace by small holder farmers to reduce the excessive cost spent on inorganic N fertilizer to increase production of legumes as most farmers usually apply below manufactural recommended rate due to cost. A review was carried out to determine the effect of *Bradyrhizobium japonicum* over the N fertilizer application (urea 46%) on leguminous crops. Several studies reveal that the inoculation of *Bradyrhizobium japonicum* significantly enhanced the growth, nitrogen fixation, yield and yield components of legumes than the inorganic N fertilizer application. While other studies reveals that combine inoculation of *Bradyrhizobium japonicum* and inorganic N fertilizer application also significantly enhanced the growth and yield parameters of leguminous crop than when the treatments are singly applied on legumes. The high cost of procuring the inorganic fertilizers and adverse effect on soil after use also limit the use and recommendation than the *Bradyrhizobium japonicum* as it has no residual effect on the soil after use and also affordable for the farmers compared to the N fertilizer application.

**Keywords**-Bacterial inoculant, inorganic N fertilizer, inoculation, legumes

**Introduction**

In the last decades, rapid increase in human population has increase and it is expected to increase to 2 billion in the nearest future and agricultural sustainability is essential to meet the demand of the growing population (Meena et al. 2017). To counter the food shortage crisis, farmers are reported to increase pressure on agricultural systems to provide adequate food for the high population (Ochieng et al. 2016). Symbiotic interaction between legume and nitrogen fixing bacteria generally called rhizobia has been the focus of research over 12 decades due to the importance in sustainable agriculture, minimizing the cost of the agriculturalist, improve the soil fertility and improving plant tolerance to drought (Igiehon and Babalola 2018).The use of inorganic fertilizer, chemical nutrients and plant protection chemicals in agriculture result in negative effect on environment, soil and water (Nath et al. 2017). Similarly, improper use of chemical nutrients and pesticides recorded detrimental impacts on soil properties like reduction of soil fertility, physical properties, organic matter, water holding capacity, quality reduction of food and water due to agrochemicals as well as negative impact on biodiversity. Unfortunately, these inorganic inputs contribute to environmental degradation (Abou-Shanab et al. 2017). Also, the increasing cost acquiring chemical fertilizers renders it unaffordable to most resource-poor smallholder farmers (Argaw 2013). Organic farming, usually do not involve the use of any synthetic pesticide or synthetic fertilizer, rather relies on crop rotation, use of plant residues, animal wastes, off-farm organic wastes, mineral grade rock additives and other biological systems of nutrient mobilization, solubilization and plant protection (Reganold and Wachter 2016). The inoculation of beneficial soil microorganisms in organic farming is an intensive smart-technology that could be used to reduce the use of inorganic fertilizers (N’cho et al. 2015) and with ability to promote soil fertility and maximize nutrient cycles. *Bradyrhizobia* species are major components of soil microbiota that are essential in soil fertility, plant growth, nutrition and play an important role in organic agriculture by compensating for the reduced application of inorganic fertilizers and other agrochemicals. *Bradyrhizobia* colonize the roots of leguminous plants and form symbiotic association that leads to enhanced water and nutrient uptake to support growth and development of legume (Mahanty et al. 2017; Srivastava et al. 2017). *Bradyrhizobia* are able to biologically fix atmospheric nitrogen (N) and help accessing other nutrients such as nitrogen and phosphorous from soil stocks and organic fertilizers (Ballesteros-Almanza et al. 2010). Thus, inoculation of legumes with *bradyrhizobia* constitutes one of the major agronomic practices targeting improvement of symbiosis in sustainable agriculture. The aim of this review is to determine the interplay between *Bradyrhizobium japonicum* and Nitrogen fertilisation on legume and the effect of the combination of both treatment on legumes.

**Response of *Bradyrhizobium japonicum* and N fertilizer application on legumes**

During an investigation to observe the effects of inoculation and N fertilization on seed yield and protein concentration of soybean under three irrigation systems (severe drought, moderate drought and control, no drought stress), significant differences was recorded in seed yield associated with inoculation and irrigation and there were no significant differences in the seed yield associated with N fertilization. The study concluded that adding N fertilizer is not always recommended, especially when seeds are inoculated before being sown while enhanced protein concentration can be enhanced by applying N fertilizer whether seed were pre inoculated or not (Basal and Szabó 2018) . In a recent study conducted to determine the effect of inoculation of *Bradyrhizobium japonicum* and N Fertilizer application on seed of soybean, result reveals that both cultivars of soybean used in the study responded with an increase in the seed yield after seed inoculation with *Bradyrhizobium japonicum* strains (HiStick and Nitragina) and N fertilizer application (30 kg N-1 and 60 kg N-1), HiStick with application of 30 kg N-1 and 60 kg N-1, as well as with Nitragina with 60 kg N-1 than singly application of fertilizer and uninoculated control (Prusiński et al. 2020). The process of inoculating legumes with high superior inoculant strains usually result in high increased in nodulation, N2 fixation and optimum grain yield (Kyei-Boahen et al. 2017). Also in another recent research conducted to study the co-inoculation of *rhizobium* and *Bradyrhizobium* strains on Common Bean, result shows that the nodule weight was similar in both co-inoculation treatments and supersede when the treatment are inoculated singly and the uninoculated control, also no significant difference was observed in the N content in the shoot in the co-inoculation of the *rhizobium* and *Bradyrhizobium* strains and when inoculated singly (de Carvalho et al. 2020). In another study that shows the response of cowpea and groundnut to fiverhizobia inoculant strains and N fertilizer in Ghana, result reveals that three rhizobia strains recorded highest shoot biomass in peanut and groundnut also no significant difference was recorded among therhizobia inoculation and N treatments in the nodule number compare to uninoculated control but significant difference was observed in nodule dry weight in both cowpea and groundnut (Mintah et al. 2020). Rhizobia inoculation significantly influenced the number of pod and weight of cowpea and groundnut. In another experiment set up to study the effect of N, P fertilizer rate and *Bradyrhizobium* inoculation on common bean in Ethiopia result disclose that N fertilizer had the highest nodule number and nodule dry weight, the result also revealed that nitrogen promote nodulation when introduce in small amount as a starter fertilizer during inoculation (Yoseph et al. 2017). In another research conducted to determine the effect of co-inoculation of *Bradyrhizobium* to stimulate efficiency of *rhizobium* on common bean, the result reveals that the inoculation of *Bradyrhizobium* increased biomass accumulation in shoot, root and nodules and the number of nodules. Co-inoculation of common bean to inoculation in relation to growth, the shoot dry weight was positively correlated with inoculation dose (da Conceição Jesus et al. 2018). Recent studies shows that inoculation of *rhizobium* with N fertilizer using soybean as a test crop, reveals that the highest plant height was recorded at the 50kg N ha-1. The height of the plant measured shows decrease as fertilizer is increasing and the lowest height was recorded in the 200kg ha-1 (Ntambo et al. 2017b). Moreso the inoculation of soybean to rhizobium shows greater plant height compare to the uninoculated plant (Alam et al. 2015; Janagard and Ebadi-Segherloo 2016). The control in the study and 50kg N/ha treatments responded to the inoculation having the highest nodule number and nodule dry weight per plant, which was significantly different from 100 and 200 kg N/ha, which reflect that increasing nitrogen levels decreased nodule number and nodule dry weight per plant in the inoculated plants (Ntambo et al. 2017a). Another research conducted also reveal that the inoculation of *rhizobium* and urea fertilizer (80kg ha-1) to velvet beans (*Mucuna pruriens*) showed almost similar biomass accumulation, nodule number, nodule dry weight compared to the uninoculated control, the effectiveness of the inoculation and urea fertilizer application show similar results (Paudyal and Gupta 2018). A research conducted on soybean to determine the effect of inoculation of *Bradyrhizobium japonicum* and Nitrogen fertilisation (0, 30, 60 kg·ha−1) on the fatty acid profile of soybean seeds reveals that N fertilizer application did not produce the expected changes, suggesting the use of only a “starter” rate of 30 kg ha−1 N. while the inoculation of *Bradyrhizobium japonicum* (control, HiStick®, Soy, Nitragina) was recommended as it will cause a decrease in soybean fatty acid and acid level (Szpunar-Krok et al. 2021). Also in a study to determine the effect of the inoculation of *Bradyrhizobium japonicum* on the seed of soybean in a study conducted in Poland, it was discovered that the inoculation significantly enhanced the nodule number, dry weight and number of pod per plant than the uninoculated control. Also protein and fat yield was higher after seed inoculation by 318kg ha-1 and 101kg ha-1 compare to uninoculated control (Jarecki 2020). In another investigation using soybean as test crop, soybean yield was most favorably affected by the combined used of inoculation and nitrogen fertilization with increased in seed yield by 42%, protein yield by about 28% Księżak and Bojarszczuk (2022).

**Response of *Bradyrhizobium japonicum* and N fertilizer application on nitrogen fixing potential of Legumes**

Leguminous crops are not usually fertilised with mineral N. In a research conducted in Italy to examine grain yield, nodulation capacity and biological nitrogen fixation in four common grain legume, Chickpea (Cicer arietinum L.), field bean (Vicia faba L.), pea (Pisum sativum L.) and white lupin (Lupinus albus L.) were grown in growth boxes contain soil for two cropping seasons with five nitrogen fertilisation rates 0, 40, 80, 120 and 160 kg ha−1. It was observed that the high supply of N fertilizer decreased the level of N2 fixed. The four grain legumes used in the study responded differently to N fertilisation, the amount of nitrogen derived from N2 fixation decreased rapidly with increasing N supply as a result of a reduction in nodulation and N2 fixed per unit mass of nodules in white lupin and chickpea, also in field bean and pea, the decrease in N2 fixation was only due to a reduction in nodule biomass since nodule fixation activity increased with N supply (Pampana et al. 2018). In another study conducted to determine the effectiveness of rhizobium inoculation on nodulation and nitrogen fixation of faba bean (Vicia faba L.) in two locations, *Rhizobium* strains significantly enhanced the nodulation, nitrogen fixation, nutrient uptake and soil nitrogen balance. The inoculation with NSFBR-12 and NSFBR-15 strains resulted in the highest nitrogen fixed, nutrient uptake and soil nitrogen balance. Result revealed that inoculation with competitive and effective *rhizobium* strains can improve soil nitrogen balance and nutrient uptake in faba bean (Allito et al. 2020). Futhermore in another study set up to determine the interplay between nitrogen fertilizer and biological nitrogen fixation in soybean, fertilizer reduced the peak of biological nitrogen fixation up to 16% in applications at the full flowering stage. Seed yield declined to 13kg ha-1 when compare to control (Tamagno et al. 2018).

**Conclusion**

The conclusion derived from the review reflect the importance of inoculation of *Bradyrhizobium japonicum* over the N fertilizer application. The inoculation of *Bradyrhizobium japonicum* to legume is usually affordable by small holder farmers than the procurement of the inorganic N fertilizer that most farmers cannot afford and those that can afford applied below manufacturer recommendation. The combine inoculation of the nitrogen fixing bacteria (*Bradyrhizobium japonicum*) and nitrogen fertilisation often result into greater production as recorded in some studies than when both are singly apply as a treatments to legume. The use of inorganic N fertilizer often result to adverse effect on the soil and its environment, pollution and soil degradation. It is of utmost importance for small holder farmers to embrace the use of bacteria inoculant to increase legume production has it gives no residual effect on the soil after use, easily affordable, improve nitrogen fixation and give better yield than the inorganic N fertilizer.

**Acknowledgements**

The authors are grateful to the Genetic Resources Center of the International Institute of Tropical Agriculture, Ibadan, Nigeria for germplasm, facilities and financial support.

**Declaration of competing interest**

The authors declare that they have no conflict of interest.

**Author Contributions**

TDB conceptualization, methodology, writing original draft preparation, OOB and OO supervision and editing, MA project administration and fund acquisition. All authors discussed the result and contributed to the final manuscripts.

**References**

Abou-Shanab RA, Wongphatcharachai M, Sheaffer CC, Orf JC, Sadowsky MJ (2017) Competition between introduced Bradyrhizobium japonicum strains and indigenous bradyrhizobia in Minnesota organic farming systems. Symbiosis 73(3):155-163

Alam F, Bhuiyan M, Alam SS, Waghmode TR, Kim PJ, Lee YBJ (2015) Effect of Rhizobium sp. BARIRGm901 inoculation on nodulation, nitrogen fixation and yield of soybean (Glycine max) genotypes in gray terrace soil. 79(10):1660-1668

Allito BB, Ewusi-Mensah N, Logah V (2020) Legume-rhizobium strain specificity enhances nutrition and nitrogen fixation in faba bean (Vicia faba L.). Agronomy 10(6):826

Argaw A (2013) Evaluation of symbiotic effectiveness and size of resident Rhizobium leguminosarum var. viciae nodulating lentil (Lens culinaris medic) in some Ethiopian soils. Archives of Agronomy and Soil Science 59(7):929-945

Ballesteros-Almanza L, Altamirano-Hernandez J, Pena-Cabriales J, Santoyo G, Sanchez-Yanez J, Valencia-Cantero E, Macias-Rodriguez L, Lopez-Bucio J, Cardenas-Navarro R, Farias-Rodriguez R (2010) Effect of co-inoculation with mycorrhiza and rhizobia on the nodule trehalose content of different bean genotypes. The open microbiology journal 4:83

Basal O, Szabó A (2018) The effects of inoculation and N fertilization on soybean [Glycine max (L.) Merrill] seed yield and protein concentration under drought stress. Agricultural Science & Technology (1313-8820) 10(3)

da Conceição Jesus E, de Almeida Leite R, do Amaral Bastos R, da Silva Aragão OO, Araújo AP (2018) Co-inoculation of Bradyrhizobium stimulates the symbiosis efficiency of Rhizobium with common bean. J Plant Soil 425(1):201-215

de Carvalho RH, da Conceição Jesus E, Favero VO, Straliotto R, Araújo APJ (2020) The co-inoculation of Rhizobium and Bradyrhizobium increases the early nodulation and development of common beans. Journal of Soil Science Plant Nutrition 1-5

Igiehon NO, Babalola OO (2018) Rhizosphere microbiome modulators: contributions of nitrogen fixing bacteria towards sustainable agriculture. International journal of environmental research and public health 15(4):574

Janagard MS, Ebadi-Segherloo AJ (2016) Inoculated soybean response to starter nitrogen in conventional cropping system in Moghan. Journal of Agronomy 15(1):26

Jarecki W (2020) Reaction of soybean [Glycine max (L.) Merr.] to seed inoculation with Bradyrhizobium japonicum bacteria. Plant, Soil and Environment 66(5):242-247

Księżak J, Bojarszczuk J (2022) The Effect of Mineral N Fertilization and Bradyrhizobium japonicum Seed Inoculation on Productivity of Soybean (Glycine max (L.) Merrill). Agriculture 12(1):110

Kyei-Boahen S, Savala CE, Chikoye D, Abaidoo R (2017) Growth and yield responses of cowpea to inoculation and phosphorus fertilization in different environments. J Frontiers in plant science 8:646

Mahanty T, Bhattacharjee S, Goswami M, Bhattacharyya P, Das B, Ghosh A, Tribedi P (2017) Biofertilizers: a potential approach for sustainable agriculture development. Environmental Science and Pollution Research 24(4):3315-3335

Meena VS, Meena SK, Verma JP, Kumar A, Aeron A, Mishra PK, Bisht JK, Pattanayak A, Naveed M, Dotaniya M (2017) Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: a review. Ecological Engineering 107:8-32

Mintah F, Mohammed Y, Lamptey S, Ahiabor B (2020) Growth and Yield Responses of Cowpea and Groundnut to Five Rhizobial Inoculant Strains in the Guinea Savanna Zone of Ghana. J Advances in Agriculture 2020

N’cho CO, Lesueur D, Yusuf AA (2015) Combined microbial inoculation as a promising approach to enhance promiscuous soybean nodulation and nitrogen content in Sudan Savanna. Int J Sustain Agric Res 2:86-97

Nath D, Maurya BR, Meena VS (2017) Documentation of five potassium-and phosphorus-solubilizing bacteria for their K and P-solubilization ability from various minerals. Biocatalysis and agricultural biotechnology 10:174-181

Ntambo M, Chilinda IS, Taruvinga A, Hafeez S, Anwar T, Sharif R, Chambi C, Kies LJ (2017a) The effect of rhizobium inoculation with nitrogen fertilizer on growth and yield of soybeans (Glycine max L.). Int J Biosci 10(3):163-172

Ntambo M, Chilinda IS, Taruvinga A, Hafeez S, Anwar T, Sharif R, Chambi C, Kies LJI (2017b) The effect of rhizobium inoculation with nitrogen fertilizer on growth and yield of soybeans (Glycine max L.). Int J Biosci 10(3):163-172

Ochieng J, Kirimi L, Mathenge M (2016) Effects of climate variability and change on agricultural production: The case of small scale farmers in Kenya. NJAS-Wageningen Journal of Life Sciences 77:71-78

Pampana S, Masoni A, Mariotti M, Ercoli L, Arduini I (2018) Nitrogen fixation of grain legumes differs in response to nitrogen fertilisation. Experimental Agriculture 54(1):66-82

Paudyal SP, Gupta V (2018) Substitution of chemical fertilizer nitrogen through rhizobium inoculation technology. Our Nature 16(1):43-47

Prusiński J, Baturo-Cieśniewska A, Borowska M (2020) Response of Soybean (Glycine max (L.) Merrill) to Mineral Nitrogen Fertilization and Bradyrhizobium japonicum Seed Inoculation. Agronomy 10(9):1300

Reganold JP, Wachter JM (2016) Organic agriculture in the twenty-first century. Nature plants 2(2):1-8

Srivastava P, Saxena B, Giri B (2017) Arbuscular mycorrhizal fungi: Green approach/technology for sustainable agriculture and environment Mycorrhiza-Nutrient Uptake, Biocontrol, Ecorestoration. Springer, pp 355-386

Szpunar-Krok E, Wondołowska-Grabowska A, Bobrecka-Jamro D, Jańczak-Pieniążek M, Kotecki A, Kozak M (2021) Effect of Nitrogen Fertilisation and Inoculation with Bradyrhizobium japonicum on the Fatty Acid Profile of Soybean (Glycine max (L.) Merrill) Seeds. Agronomy 11(5):941

Tamagno S, Sadras VO, Haegele JW, Armstrong PR, Ciampitti IA (2018) Interplay between nitrogen fertilizer and biological nitrogen fixation in soybean: implications on seed yield and biomass allocation. Scientific reports 8(1):1-11

Yoseph T, Baraso B, Ayalew T (2017) Influence of Bradyrhizobia inoculation on growth, nodulation and yield performance of cowpea varieties. J African Journal of Agricultural Research 12(22):1906-1913