Assessment of *Rhizobium* strains and their impact on Urdbean productivity

Abstract

The study sought to assess the impact of inoculation by several Rhizobium strains on field-grown urdbean (Blackgram). Rhizobia, which promotes the growth of legume plants. The bacteria that live in the soil and generate the root nodules where symbiotic biological nitrogen fixing occurs are called rhizobia. Four AICRP centers *viz*., Kalaburagi (GUR5 & GUR8), Akola (WUR 12-1), Vamban (VUC), and Pantnagar (A-3, P-5 & PUR 34), provided a total of seven *Rhizobium* isolates, which were employed in the experiment together with the best *Rhizobium* BMBS 47. Urdbean seeds were treated with the *Rhizobium* isolates and sown. Observations were recorded at 45th DAS, to assess their effect on plant growth parameters *viz.*, number of **nodules/plants**, root length, shoot length, nodule **dry weight,** and yield parameters were recorded during harvest. Among the ***Rhizobium* isolates,** VUC (Vamban) recorded a greater number of nodules/plant (30 / plant) and nodule dry weight (0.038 g/plant), which is significantly higher than other isolates. The same isolate recorded the highest root length (13.50 cm). The **treatment T_9** recorded the maximum shoot length of 27.26 cm. Crop was harvested at 65 DAS and yield parameters *viz.*, number of pods per plant, plant dry weight and yield were recorded and tabulated (Table 2). Among the different *Rhizobium* isolates, VUC (Vamban) performed better and recorded the higher values for yield parameters. The highest yield of 1162 kg/ha was recorded by T_5 which received VUC through seed treatment followed by T_6 treatment A-3 (Pantnagar) which recorded 1077.6 kg/ha of seed yield.

Keywords: Yield, Inoculation, Symbiotic, *Rhizobium*

Introduction

"Nitrogen (N) is the **macronutrient** that is required in the largest amount for plant growth, development, and yield" (Good, 2018). "Its deficiency in the soils adversely impacts the overall agricultural productivity. A quick method to supply N to plants is *via* synthetic industrial production of reactive N-fertilizers to maintain higher crop yields in highly agricultural intensification systems" (Allen, 2008; Good, 2018). "However, in low-input production systems, such as in many smallholder farms in Africa, food production is hampered by the use of little or no N" (Masso et al., 2017; Bindraban et al., 2020). "Other impediments to high crop yields in sub-Saharan Africa (SSA) are the inherent low N status of soils, a high nutrient depletion rate (which is exacerbated by crop removals), and non-replenishment" (Manyong et al., 2001; Jemo et al., 2015; Bado and Bationo, 2018). "Increasing events of drought associated with uncertainties in climate change are also observed in many areas of West African regions, which will also likely aggravate the yield gaps, specifically for legume crops that are often grown toward the end of the rainy season" (Cernay et al., 2016; van Loon et al., 2018; Defrance et al., 2020; Paliwal et al., 2020). Therefore, exploration of alternatives to synthetic N sources and fertilization, such as the natural biological N_2 fixation (BNF), is paramount to increasing crop yields in smallholder farms, particularly in Ghana.

Unlike most non-legumes, legume crops establish a beneficial root association with bacteria in the α- and ß-proteobacteria groups, where rhizobia convert abundant N_2 present in the atmosphere into usable N *via* specialized plant structures called “nodules” (Udvardi and Poole, 2013; Basu and Kumar, 2020). “Through this process, legumes satisfy their N demand and that of other nonlegume plants when grown in rotation or association. This process contributes to the agricultural sustainability of production systems for the benefit of millions of smallholder farmers” (Giller, 2001; Desbrosses and Stougaard, 2011; Franke et al., 2018; Ulzen et al., 2018; Adjei-Nsiah et al., 2019). However, various abiotic and biotic stress factors, such as drought (Jemo et al., 2017), low phosphorus (P) availability (Sulieman and Tran, 2015), non-competitive *Rhizobium* strains, and diseases (Jiménez-Guerrero et al., 2021), may affect the BNF process and limit legume productivity (van Loon et al., 2018).

“Seed inoculation with effective *Rhizobium* at sowing is a recommended agronomic practice for pulse production technology. The soil contains many types of microorganisms such as bacteria, actinomycetes, fungi, and algae. These soil microorganisms are important because they affect the soil's physical, chemical, and biological properties through different processes” (Datta et al., 2015). “Among the soil bacteria, there is a unique group called rhizobia that has a beneficial effect on the growth of legumes. Rhizobia are soil-inhabiting bacteria that form the root nodules where symbiotic biological nitrogen fixation takes place. In root nodules, the nitrogen-fixing rhizobia exist as irregular cells called **bacteroids,** which are often club and Y-shaped whereas regular rod-shaped when they are living freely in the soil” (Datta et al., 2015; Bahati 2015). Different groups of “Rhizobia genera include *Rhizobium, Bradyrhizobium, Sinorhizobium, Mesorhizobium, Allorhizobium, and Azorhizobium,* which can form symbiotic associations with different legumes” (Sessitsch et al., 2002). The formation of root nodules (Nyaguthii, 2017) in symbiotic legumes involves complex molecular signaling between the legume host and the rhizobial microsymbiont (Jaiswal et al., 2021). “Sinorhizobium consists of all fast-growing acid-producing rhizobia while Bradyrhizobium contains the slow-growing, alkali-producing rhizobia” (Nyaguthii, 2017). “Nowadays, global agricultural production increased considerably. But due to the global human population increase, the demand for higher crop production has also increased substantially. Because of this, to achieve **higher agricultural yields**, farmers have adopted the extensive application of chemical fertilizers and pesticides, which causes soil degradation and a decrease in soil fertility” (Díaz Valle A. et al., 2019). “Inoculants are products composed of living microorganisms capable of benefiting the development of different plant species. Rhizobia are the first microorganisms used as inoculants. Currently, the use of inoculants is widespread and indicated in agriculture, mainly for legumes such as soybeans, common beans, faba beans, and cowpea, but the production of inoculants for other legumes and **nonlegumes** has to be increased to obtain the greater yield" (Emanoel et al., 2020). "*Rhizobium* inoculants are selected strains of beneficial soil microorganisms cultured in a laboratory and packed in with or without a carrier. They are host-specific, low-cost, and an environmentally friendly source of nitrogen. Rhizobia inoculants coated on legume seeds before planting **enhance** the growth, yield of legume **crops** and provide nitrogen and organic carbon for subsequent or associated crops. Seeds coated with rhizobia inoculants are not exposed to chemical nitrogen fertilizer. The coated seeds must be planted in moist soil as soon as possible" (Daniel, 2019; 37. Buernor AB et al., 2022). The present paper highlights the *Rhizobium* sp. effect on nodulation and productivity in urdbean under field conditions.

Materials and methods

To assess the performance of *Rhizobium* strains at various locations, a trial was laid out with *Rhizobium* strains received from various AICRP centres. The experimental details are mentioned below.

The field experiments were conducted during kharif 2022 in red sandy loam soil of pH 7.3 and low in organic C, and available N and medium in available N and P. The ten treatments consisted of inoculation with different *Rhizobium* strains (**T1-BMBS 47** (Coimbatore), T2-GUR 5 (Kalaburagi), T3-GUR8 (Kalaburagi), T4-WUR 12-1 (Akola), T5-VUC **(Vamban),** T6-A-3 (Pantnagar) T7-P-5 (Pantnagar), T8-PUR 34 (Pantnagar Ch. strain), T9- N as per RDF, T10-uninoculated control were tested following Randomized Block Design in three replicates in plot of 4.0 x 3.0 m size. Urdbean seeds (VBN8) were treated with the required inoculants (200g/10 kg seeds) at the time of sowing and crop was raised as per the recommended agronomic practices.

Seven *Rhizobium* isolates were obtained from four AICRP centres *viz.*, Kalaburagi (GUR5 & GUR8), Akola (WUR 12-1), Vamban (VUC), and Pantnagar (A-3, P-5 & PUR 34), along with the local best *Rhizobium* BMBS47 were used for the experiment. Urdbean seeds were treated with the *Rhizobium* isolates and sown. Observations were recorded at 45th DAS, to assess their effect on plant growth parameters *viz.*, number of **nodules/plants,** root length, shoot length, nodule dry weight and yield parameters were recorded during harvest.

Fig 1: Experimental field

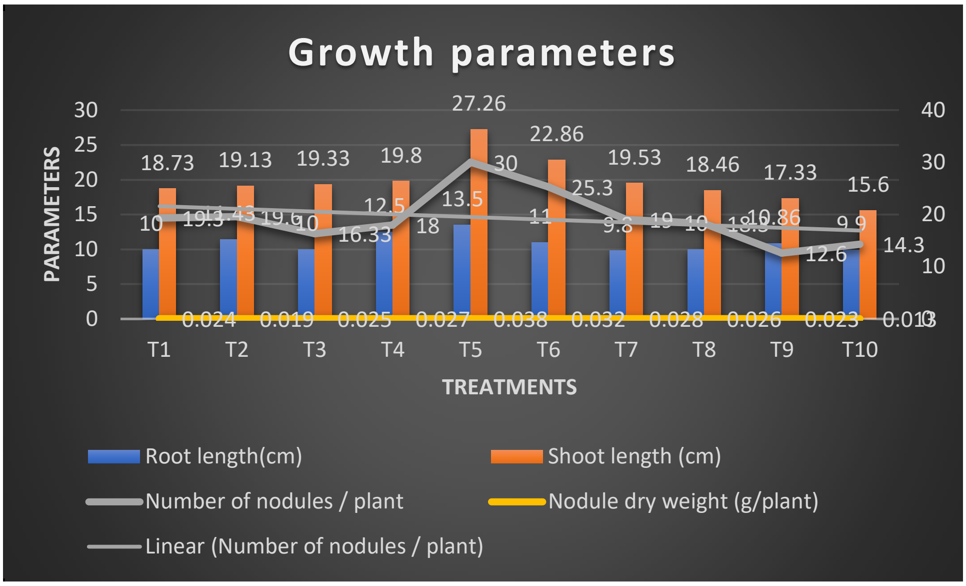
Results and discussion

The plant growth parameters viz., root length, shoot length, number of nodules / plant and nodules dry weight (g/plant) were observed after vegetative growth and the results were tabulated in Table1& Fig.2.

Table 1. Multilocation testing of *Rhizobium* strains for Urd bean – Plant growth parameters

Treatments	Root length(cm)	Shoot length (cm)	Number of nodules / plant	Nodule dry weight (g/plant)
T1	10.00	18.73	19.30	0.024
T2	11.43	19.13	19.60	0.019
T3	10.00	19.33	16.33	0.025
T4	12.50	19.80	18.00	0.027
T5	13.50	27.26	30.00	0.038
T6	11.00	22.86	25.30	0.032
T7	9.80	19.53	19.00	0.028
T8	10.00	18.46	18.30	0.026
T9	10.86	17.33	12.60	0.023
T10	9.90	15.60	14.30	0.013
SE (D)	0.64	0.90	2.23	0.002
CD (0.05%)	1.35	1.88	4.68	0.003

Fig 2: Growth Parameters of various treatments of plant



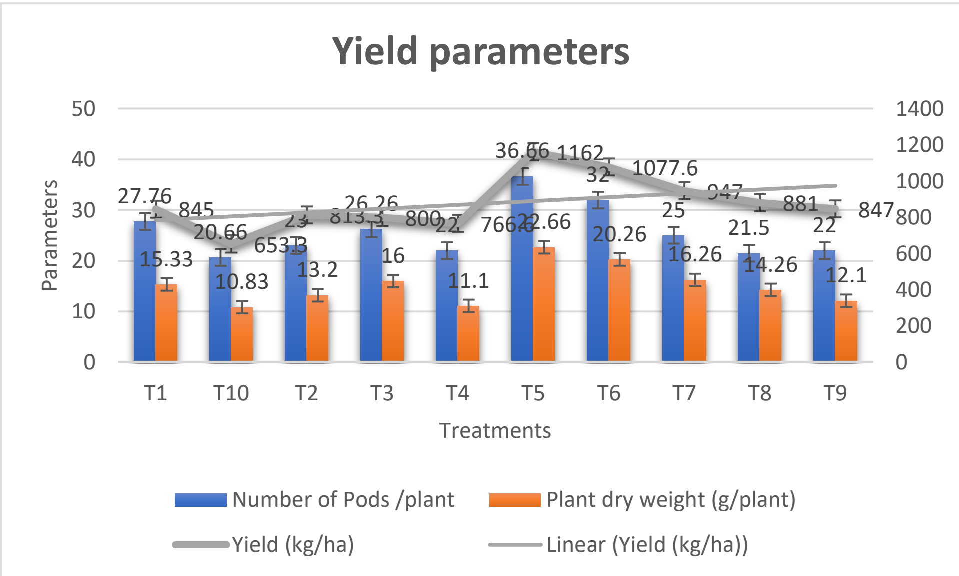
The above table concluded the findings in which among the *Rhizobium* isolates, VUC (Vamban) recorded a greater number of nodules/plant (30/plant), and nodule dry weight (0.038 g/plant), which is significantly higher than other isolates. The same isolate recorded the highest root length (13.50 cm). The treatment T_9 recorded the maximum shoot length of 27.26 cm. The findings corroborated with the findings of Jinwen et al., 2016 indicated that the seeds treating with *Rhizobium* **significantly increase root length**, nodule number on a plant root, and plant biomass. Native rhizobial inoculation significantly affects the shoot dry weight of cowpea, this improvement is because rhizobia increase plant growth and improve the plant biomass by providing nitrogen through symbiotic fixation. Inoculated common beans have a higher shoot dry weight compared to the control indicating that inoculation with native isolates improve the growth of plants (Erana et al., 2020). Also compared with the findings of Mfilinge et al., 2014 and concluded that *Rhizobium* inoculation significantly affects the growth and yield components like number of pod-bearing branches per plant, number of pods per plant, number of seeds per pod, and 1000-seed weight. The study on the effects of *Rhizobium* inoculation in *Vigna mungo* and *Vigna radiata* shows that inoculated plants possess improved height, fresh weight, number of roots, nodules, number of leaves, shoots, pods, length of pods, seed weight, over the uninoculated control. Tairo and Ndakidemi (2013) on soybean found that plant height for field experiment increases with *Rhizobium* inoculation for the entire interval of the soybean growth.

Crop was harvested at 65 DAS, and yield parameters *viz.*, number of pods per plant, plant dry weight, and yield were recorded and tabulated (Table 2 and Fig.3). Among the different *Rhizobium* isolates, VUC (Vamban) performed better and recorded the higher values for yield parameters. The highest yield of 1162 kg/ha was recorded by $T_{5,}$ which received VUC through seed treatment followed by T_6 treatment A-3 (Pantnagar) which recorded 1077.6 kg/ha of seed yield. The above results agreed with the findings of Asante et al., 2020, indicated that the Table 2

Table 2. Multilocation testing of Rhizobium strains for Urd bean – Yield parameters

Treatments	Number of pods /plant	Plant dry weight (g/plant)	Yield (kg/ha)
T1	27.76	15.33	845.00
T2	23.00	13.20	813.30
T3	26.26	16.00	800.00
T4	22.00	11.10	766.60
T5	36.66	22.66	1162.00
T6	32.00	20.26	1077.60
T7	25.00	16.26	947.00
T8	21.50	14.26	881.00
T9	22.00	12.10	847.00
T10	20.66	10.83	653.30
SE (D)	3.74	2.01	54.12
CD (0.05%)	7.86	4.23	113.70

Fig 3: Yield Parameters of Various treatments of plant



inoculation of legumes with rhizobia triggers plant growth, development, and yield and it is used as a substitute for mineral nitrogen fertilizer which is often costly. However, the *Rhizobium* is host-specific, as certain species can only infect specific legumes. Inoculation of soybean seed with a mixture of bradyrhizobia strains increases nodulation and total plant nitrogen. **Zennouhi O et al., 2020 reported that the infectivity and efficiency are commonly used to evaluate the ecological and evolutionary relationships between *Rhizobium* strains and their hosts. Although all the retained strains induce nodule formation on the roots of the host plant as well as the degree of infectivity is clearly differentiate between treatments**. Several researchers have found that inoculation of soybean seeds before planting results in increases in nodulation, percentage N, plant growth, seed yield, and yield components compared to un-inoculated control (Miriko 2015). Growth and yield of leguminous crops can be triggered by rhizobial inoculation directly by producing various metabolites/substances such as plant hormones, ACC deaminase enzymes, LCOs, siderophores, lumichrome, riboflavin, etc., and or by fixing as well as solubilizing and increasing the uptake of mineral nutrients (Naveed et al., 2015). Inoculated soybean plants show improvement in nodulation, growth, and yield (Zerihun and Lijalem, 2020).

Conclusion

Rhizobia inoculants are used in biological nitrogen fixation, which is a superior substitute for other N sources. It is a more economical and non-polluting method of increasing soil fertility. There are significant ecological, economic, and environmental advantages to using rhizobial inoculants in the production of legumes. Reducing the usage of chemical fertilizers and their negative effects on the environment can be achieved by expanding the use of biofertilizers like *Rhizobium*. Therefore, because *Rhizobium* inoculants are economical and environmentally friendly, using them to increase nitrogen fixation and grain legume productivity is highly recommended. Of the *Rhizobium* isolates, VUC (Vamban) had the longest root (13.50 cm), the most nodules per plant (30 per plant), and the highest nodule dry weight (0.038 g/plant). At 65 days after sowing, the crop was harvested, and the yield parameters *viz*., number of pods per plant, plant dry weight, and yield were noted (Table 2). VUC (Vamban) outperformed the other *Rhizobium* isolates and reported the highest yield of 1162 kg/ha.

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Author's contribution

RU collected the literature, compilation of the content, wrote the article and writing the review.JP suggesting his ideas to shape the review and TS helped in revising the article. All the authors read and approved the manuscript.

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References

1. Adjei-Nsiah, S., Kumah, J. F., Owusu-Bennoah, E., and Kanampiu, F. (2019). Influence of P sources and rhizobium inoculation on growth and yield of soybean genotypes on Ferric Lixisols of Northern Guinea savanna zone of Ghana. Commun. Soil Sci. Plant Anal. 50, 853–868. doi: 10.1080/00103624.2019.1589489
2. Allen, R. C. (2008). The nitrogen hypothesis and the English agricultural revolution: a biological analysis. J. Econ. Hist. 68, 182–210. doi: 10.1017/S0022050708000065
3. Asante M., Ahiabor B. D. K., and Atakora W. K. (2020). Growth, nodulation, and yield responses of groundnut (Arachis hypogaea L.) as influenced by combined application of rhizobium inoculant and phosphorus in the Guinea Savanna Zone of Ghana. International Journal of Agronomy. Volume 2020, Article ID 8691757, 7 pages.
4. Bado, V. B., and Bationo, A. (2018). "Integrated management of soil fertility and land resources in Sub-Saharan Africa," in Advances in Agronomy, Vol. 150, ed D. L. Sparks (Cambridge: Academic Press), 1–33.
5. Bahati L. S. (2015). Effect of rhizobia inoculant on soybean nodulation and arbuscular mycorrhiza fungi colonization under greenhouse and field conditions. A thesis submitted in partial fulfillment of the requirements for the award of Master of Science Degree in Integrated Soil Fertility Management, Department of Agricultural Resource Management, Kenyatta University, Kenya
6. Basu, S., and Kumar, G. (2020). "Nitrogen fixation in a legume-rhizobium symbiosis: the roots of a success story," in Plant Microbe Symbiosis, eds A. Varma, S. Tripthi, and R. Prasad (Cham: Springer International Publishing), 35–53. Bindraban, P. S., Dimkpa, C. O., and Pandey, R. (2020). Exploring phosphorus fertilizers and fertilization strategies for improved human and environmental health. Biol. Fertil. Soils 56, 299–317. doi: 10.1007/s00374-019-01430-2
7. Cernay, C., Pelzer, E., and Makowski, D. (2016). A global experimental dataset for assessing grain legume production. Sci. Data 3, 160084. doi: 10.1038/sdata.2016.84
8. Daniel Keskse Bahru (2019). Review on: Response of Legume Crops to Rhizobium Inoculation and Inorganic Fertilizers (N, P, K and S) Application in Ethiopia. Intern. J. Res. and Innov. in Earth Sci. 6(4): 2394-1375 ISSN (Online)
9. Dashti, N., Zhang, F., Hynes, R. and Smith, D.L. Plant growth promoting rhizobacteria accelerate nodulation and increase nitrogen fixation activity by field grown soybean (Glycine max (L.) Merr.) under short season conditions. Plant and Soil. 1998; 200: 205-213.
10. Datta A., Singh R. K., Kumar S., Kumar S. (2015). An effective and beneficial plant growth promoting soil bacterium "Rhizobium": A Rev. Annals of Plant Sciences, 2015, 4 (01), 933-942.
11. Defrance, D., Sultan, B., Castets, M., Famien, A. M., and Baron, C (2020). Impact of climate change in West Africa on cereal production per capita in 2050. Sustainability 12, 7585. doi: 10.3390/su12187585 Desbrosses, G. J., and Stougaard, J. (2011). Root Nodulation: a paradigm for how plant-microbe symbiosis influences host developmental pathways. Cell Host Microbe 10, 348–358. doi: 10.1016/j.chom.2011.09.005
12. Díaz-Valle A, López-Calleja, A.C. and Alvarez-Venegas R. (2019). Enhancement of pathogen resistance in common bean plants by inoculation with rhizobium etli. Front. Plant Sci. 10:1317. doi: 10.3389/fpls.2019.01317
13. Dube, S. K. Co-inoculation of phosphate solubilizing bacteria with Bradyrhizobium japonicum to increase phosphate availability of rained soybean in Vertisol. Journal of the Indian Society of Soil Science. 1997; 45: 506-509.
14. Emanoel G. Moura, Cristina S. Carvalho, Cassia P. C. Bucher, Juliana L. B. Souza, Alana C. F. Aguiar, Altamiro S. L. Ferraz Junior, Carlos A. Bucher and Katia P. Coelho. (2020). Diversity of rhizobia and importance of their interactions with legume trees for feasibility and sustainability of the tropical agrosystems. Review. Diversity, 12:206
15. Erana K., Berhanu A., Anteneh A. & Solomon T. (2020). Symbiotic effectiveness of cowpea (Vignaunguiculata (L.) Walp.) nodulating rhizobia isolated from soils of major

cowpea producing areas in Ethiopia, Cogent Food & Agriculture, 6:1, 1763648, DOI: 10.1080/23311932.2020.1763648

16. Franke, A. C., van den Brand, G. J., Vanlauwe, B., and Giller, K. E. (2018). Sustainable intensification through rotations with grain legumes in Sub-Saharan Africa: a review. Agric. Ecosyst. Environ. 261, 172–185. doi: 10.1016/j.agee.2017.09.029
17. Giller, K. E. (2001). Nitrogen Fixation in Tropical Cropping Systems. Wallingford: CABInternational.
18. Good, A. (2018). Toward nitrogen-fixing plants. Science 359, 869–870. doi: 10.1126/science.aas8737
19. Jemo, M., Nwoke, C., Pypers, P., and Vanlauwe, B. (2015). Response of maize (Zea mays) to the application of foliar fertilizers in the Sudan and Guinea savanna zone of Nigeria. J. Plant Nutr. Soil Sci. 178, 374–383. doi: 10.1002/jpln.201400524
20. Jemo, M., Sulieman, S., Bekkaoui, F., Olomide, O. A. K., Hashem, A., Abd_Allah, E. F., et al. (2017). Comparative analysis of the combined effects of different water and phosphate levels on growth and biological nitrogen fixation of nine cowpea varieties. Front. Plant Sci. 8, 2111. doi: 10.3389/fpls.2017.02111
21. Jiménez-Guerrero, I., Moreno-De Castro, N., and Pérez-Montaño, F. (2021). One door closes, another opens: when nodulation impairment with natural hosts extends rhizobial host-range. Environ. Microbiol. 23, 1837–1841. doi: 10.1111/1462-2920.15353
22. Jinwen H., Afshar R. K., and Chen C. (2016). "Lentil response to nitrogen application and rhizobia inoculation." Communications in Soil Science and Plant Analysis 47(21): 2458-2464. DOI: 10.1080/00103624.2016.1254786.
23. Khurana, A.L., Namdeo, S. L., Patil, B.J., and Dudeja, S.S. On farm experiments on rhizobial inoculants: Problems and possible solutions. In: Extending Nitrogen Fixation Research to Farmers field. (Rupela, O.P., Johansen.C. and Herridge, D.F. Eds). ICRISAT, Patancheru, India. 1997; pp 217-266.
24. Manyong, V. M., Makinde, K. O., Sanginga, N., Vanlauwe, B., and Diels, J. (2001). Fertilizer use and definition of farmer domains for impact-oriented research in the northern Guinea savanna of Nigeria. Nutr. Cycl. Agroecosyst. 59, 129–141. doi: 10.1023/A:101752202266
25. Masso, C., Baijukya, F., Ebanyat, P., Bouaziz, S., Wendt, J., Bekunda, M., et al. (2017). Dilemma of nitrogen management for future food security in sub Saharan Africa- a review. Soil Res. 55, 425. doi: 10.1071/SR16332
26. Mfilinge A., Mtei K. and Ndakidemi P. A. (2014). Effects of rhizobium inoculation and supplementation with P and K, on growth, leaf chlorophyll content and nitrogen fixation of bush bean varieties. American J.Res. Commun. 2(10): 49- 87. www.usajournals.com, ISSN: 2325-4076.
27. Miriko M.C. (2015). (BSc. Horticulture) (2015). Effect of phosphorus and rhizobia inoculation on growth and yield of desmodium and lucerne in Kiambu Country, Kenya. MSc Thesis. Kenyatta University, Kenya.
28. Naveed M., Mehboob I., Hussain M. B., and Zahir A. Z. (2015). Perspectives of rhizobial inoculation for sustainable crop production. N.K. Arora (ed.), Plant Microbes Symbiosis: Applied Facets, 209 DOI 10.1007/978-81-322-2068-8_11, © Springer India 2015. Chapter 11.
29. Nyaguthii M. C. (2017). Soybean (Glycine max) response to rhizobia inoculation as influenced by soil nitrogen levels. MSc Thesis. Kenyatta University, Kenya.
30. Paliwal, R., Abberton, M., Faloye, B., and Olaniyi, O. (2020). Developing the role of legumes in West Africa under climate change. Curr. Opin. Plant Biol. 56, 242–258. doi: 10.1016/j.pbi.2020.05.002
31. Sessitsch, J.G. Howieson, X. Perret, H. Antoun, and E. Martínez-Romero. (2002). Advances in rhizobium research. Critical Reviews in Plant Sciences, 21(4):323–378
32. Tairo E. V., Ndakidemi P.A. (2013). Possible benefits of rhizobial inoculation and phosphorus supplementation on nutrition, growth and economic sustainability in grain

legumes. American J. Res. Commun., 1(12):532-556 www.usajournals.com, ISSN: 2325-4076

33. Udvardi, M., and Poole, P. S. (2013). Transport and Metabolism in legume-Rhizobia symbioses. Annu. Rev. Plant Biol. 64, 781–805. doi: 10.1146/annurev-arplant-050312-120235
34. Ulzen, J., Abaidoo, R. C., Ewusi-Mensah, N., and Masso, C. (2018). On-farm evaluation and determination of sources of variability of soybean response to Bradyrhizobium inoculation and phosphorus fertilizer in northern Ghana. Agric. Ecosystems Environ. 267: 23–32. doi: 10.1016/j.agee.2018.08.007
35. van Loon, M. P., Deng, N., Grassini, P., Edreira, J. I. R., Wolde-meskel, E., Baijukya, F., et al. (2018). Prospect for increasing grain legume crop production in East Africa. Eur. J. Agron. 101, 140–148. doi: 10.1016/j.eja.2018. 09.004
36. Zennouhi O, Rfaki A, Mderssa ME, Ibijbijen J, Nassiri L.(2020). Evaluation of the Symbiotic Efficiency of Rhizobium Strains Applied to Bituminaria bituminosa (L.) Stirton. Microbiol. Res. J. Int.30(9):45-5. Available from: https://journalmrji.com/index.php/MRJI/article/view/1128
37. Buernor AB, Kabiru MR, Bechtaoui N, Jibrin JM, Asante M, Bouraqqadi A, Dahhani S, Ouhdouch Y, Hafidi M, Jemo M. Grain legume yield responses to rhizobia inoculants and phosphorus supplementation under Ghana soils: a meta-synthesis. Frontiers in Plant Science. 2022 Jun 23;13:877433.