Research Article
Volume 6:4,2022

Journal of Microbiology and Pathology

ISSN:2952-8119 Open Access

The Effect of Rhizobacteria on the Physical Properties of Cowpea *Vigna unguiculata* (L.Walp.)

Ajayi Olaoluwa O[1,2*], Dianda Mahamadi[2,3], Fagade Obasola E[1], Nwadike Blessing[1]

[1]*Department of Microbiology, University of Ibadan, Ibadan, Nigeria*

[2]*Department of Tropical Agriculture, University of Ibadan, Ibadan, Nigeria*

[3]*Laboratoire de Microbiologie Forestière (INERA/DEF)BP 7047 Ouagadougou 03, Burkina Faso, West Africa*

Abstract

Seventeen isolates including nine rhizobial and eight Plant Growth Promoting Rhizobacteria (PGPR) were obtained from root nodules of cowpea recovered from three local governments in Nassarawa State Nigeria. *Rhizobial spp* were co-innoculated with these Plant growth promoting rhizobacteria and applied as treatments to experimental cowpea (Tvx 3236) plants. Treatments were replicated four times using block design. The height of the experimental plants were monitored every two weeks to observe if the co-inoculation of rhizobial spps with NRMs at two different applications of nutrient solution was able to enhance physical properties of the plant such as height, flowering and podding. It was observed that plant height, flowering and podding were enhanced by co-inoculation of *Rhizobial spp*. with a consortium of *Agrobacterium spp., Pseudomonas sp. Paenibacillus sp*. while a consortium of *Azotobacter spp*. improved flowering and podding but showed no ability to improve plant height. It is therefore shown that plant growth promoting rhizobacteria have positive effects on the maturation of plant with respect to flowering and podding of legumes and also on their height.

Keywords: Maturation • Height • Rhizobia • Plant growth promoting rhizobacteria

Introduction

Nitrogen fixing bacteria especially members of the family Rhizobiacea have been known to be able to fix nitrogen when they are found to be in friendly symbiotic relationships with leguminous plants. This phenomenon is described as nitrogen fixation and is known as nature's nitrogen factory [1]. Although much work has been done on legume-rhizobium symbiosis, it has been found that other microorganisms are also important and useful in fixation of nitrogen. These other endophytic bacteria which can be found in legume nodules are able to penetrate the nodules alongside rhizobia but they have been ignored for a long time [2,3] thus the diversity of bacteria co-exsisting in nodules with rhizobial bacteria and attention has not been paid to their own salient roles in nitrogen fixation, development and growth of leguminous plant nodules are the site where nitrogen fixation occurs nodules which contain efficient rhizobia are usually large, and pink in colour and contain leaghaemoblobin while nodules formed by inefficient rhizobia are small and white [4].

Agrobacterium spp were isolated from root nodules of five different leguminous plant. It was observed that when *agrobacterium* was inoculated alone into the plant host *spp,* there was formation of proliferate and multibranched secondary roots in tested plants, it was also observed that there were variations in protein, lipid and glycogen content of the plant indicating that agrobacterium had effect on plant growth [5]. It has also been suggested that some other nitrogen fixing bacteria, *Panebacillus spabuli, P. amyliticus* and *Methylobacterium mesophillium* have been shown to reside inside the plant formed by sillus tomenous on Pivum contortavar latifolia roots [6].

Other Plant growth promoting rhizobacteria (PGPR) such as Psedomonass, *Micromonospora spp, Phyllobacterium spp, Ochrobacterium, Devosia spp, Bosae spp, Kribbella sp, Cohnella, Paracoccus, Herbaspirillum sp, Microvigra spp, Shinella sp, Burkholderia spp, Cupriavidus spp, Paenibacillus spp* were found in association with the nodules of legumes but their specific roles are yet to be determined.

Cow pea (*Vigna unguiculata*) is a food and animal feed crop grown in Africa, Asia, Europe, [7-9]. It is an annual herbecious plant [10,11]. Nigeria accounts for about 5.2 million tonnes of the total 7.56 million tonnes of cowpea produced annually [12,13]. All the parts are rich in nutrient and fibre [14-16]. In Africa, humans consume the

***Address for Correspondence:** Dr. Ajayi Olaoluwa O, Department of Microbiology, University of Ibadan, Ibadan, Nigeria, Tel:+2348182928935; E-mail: Oluwafuntoajayi@yahoo.com*

Date of Submission: 22 April, 2020, Manuscript No. JMBP-20-001-PreQc-20; **Editor assigned:** 27 April, 2020, PreQC No. JMBP-20-001-PreQc-20; **Reviewed:** 15 May, 2020, QC No. JMBP-20-001-PreQc-20; **Revised:** 22 Jul, 2022, Manuscript No. JMBP-20-001-PreQc-20; **Published:** 29 July, 2022; DOI: 10.37421/2952-8119.6.159

young leaves, immature seed and the matured dried seed [17]. They are usually cooked together with vegetables [18] and alongside cereals [19].

Materials and Methods

Sample site and collection

Samples were collected from three sites in Nassarawa State which are Shamage Local government (Nassarawa State) N 080 37| 47.7| E 0070 46| 48.4| Elevation 244 m, Ogba/ Ubbe Egon local government ((Nassarawa State) N 080 51| 55.4| E 080 25| 34.5| Elevation 399 m), Mandara Kokona local government (Nassarawa State) N 08050| 29.8| E 008 12| 37.1|Elevation 364 m using a hand trowel to a depth of about 20 cm and 15 cm wide around the plant after which soil particles were carefully removed from the root material mechanically. Secondary roots were carefully removed from the plant to avoided loss of nodules [20,21]. Nodules were trapped using a 0.5 mm size and mesh sieve while washing the root sample in a gentle stream of water. Nodule samples were wrapped in Aluminum foil paper and transported to the laboratory.

Isolation of microorganisms from root nodules: Microorganisms were isolated from nodules on Congo red agar [22,23] using spread plate method. Five undamaged nodules samples were picked from each site. They were place in sterile water for about 15 to 20 mins to rehydrate them after which they were surface sterilized using 3% sodium hypochlorite for 3 minutes. They were then rinsed with sterile water after which they were further sterilized with 95% ethanol and then rinsed with six changes of sterile water. A loop full of crushed nodule was streaked on congo red agar and then incubated at 28˚C for 5-7 days. All isolates present were picked and the ones that are suspected to be rhizobial were selected based on their cultural appearances i.e their ability to absorb congo red dye thus appearing white on the congo red media [23]. Purified isolates were stored on yeast mannitol agar slants and identified using biochemical methods and the bergy's manual [24,25].

Experimental design: The experimental design used in carrying out this research was the complete randomized block design. 20 treatments (18 treatments were used in addition to two controls (N+ treatment i.e to which nitrogen was applied and normal soil) with four replicates were used. Sterlized seeds were planted in pots containing sterile sand in a screen house for germination before inoculation. Pure cultures of the rhizobia isolates were multiplied using yeast-mannitol broth in duplicates. The pure cultures of the Non rhizobial microorganism. Which were introduced into one of the duplicates rhizobial broths The inoculated broth solutions were incubated at room temperature (25-30°C) on a rotary shaker for 5-7 days. 7 days after planting (1WAP), viable plants were inoculated with 1 ml of the inoculums (containing about 1*109 cells ml^{-1}).

Plants were allowed to grow for 8 week during which two replicates were given 20 ml of nutrient solution while two were given 40 ml of nutrient solution weekly. Watering was done with sterile distilled water. The nutrient solution consisted of micro and macro nutrient [26]. The plant height were also recorded for the 2nd, 4th, 6th, and 8th week using a meter rule to take the height from the base of the stem to the tip of the plant following the method [27]. Flowering and podding were monitored in plants from the 6th to the 8th week and recorded in percentage.

Results

Isolates obtained

Rhizobia isolates were obtained from the three sites, three from Shamage local government, two from Mandara Kokona and four from Ogba/Ubbe Egon local government The other microorganisms isolated include, *Azotobacter spp, Azotobacter indicus, Panibacillus wynnii, Agrobacterium spp,* and *Psuedomonas sp* (Table 1).

Effect of co-inoculation of rhizobia with PGPR on plant height: The treatments with PGPR were taller than their counterpart except for R3, R4, and R6 which were all shorter both at 20 ml and 40 ml nutrient per week than their counterpart treatment containing only rhizobia. At 20 ml nutrient per week, 22% were shorter than the N+ treatment while at 40 ml nutrient per week, 33% were shorter. The heights of plants with 40 ml nutrient per week were higher than those with 20 ml nutrient per week and all the treatments were taller than the plant from potted non sterile soil (NST) (Figures 1 and 2).

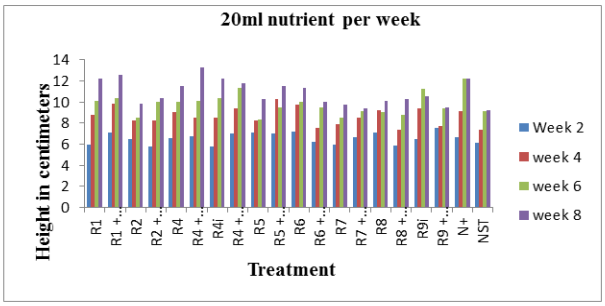

Figure 1. Height of plant with 20 ml nutrient per week.

Note: (■) Week 2, (■) Week 4, (■) Week 6, (■) Week 8.

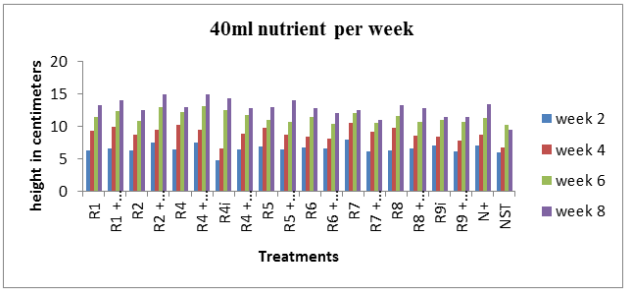

Figure 2. Height of plant with 40 ml nutrient per week.

Note: (■)Week 2, (■) Week 4, (■) Week 6, (■) Week 8.

Effect of co-inoculation of rhizobia with PGPR on flowering and podding: The first signs of flowering were observed by the end of the sixth week and plants to which treatments were added all started flowering before those with N+ treatments and those from potted non sterile soil, indicating that they reached maturity faster than the plants in N+ treatments and in non-sterile potted soil. By the end of the seventh week, all treatments including the N+ had started flowering but those plants in the potted non sterile soil had not yet shown any sign of flowering, and by the eighth week before they were to be harvested, those in potted non sterile soil still had not shown signs of flowering. About 82% of the plants with treatments had produced visible cowpea pods before they were harvested by theeighth week but both controls i.e N+ and plants in non-sterile potted soil were yet to show any sign of pod formation (Table 1).

Table 1. Percentage flowering and podding in experimental cowpea plant.

Treatment	6 WAP		7 WAP		8 WAP	
	Flowering	Podding	Flowering	Podding	Flowering	Podding
R1	-	-	100%	-	100%	100%
R1+ consortium A	-	-	100%	-	100%	75%
R2	-	-	100%	-	100%	100%
R2+ consortium A	50%	-	100%	50%	100%	100%
R3	-	-	75%	-	100%	100%
R3+ consortium m A	-	-	75%	-	100%	100%
R4	-	-	100%	-	100%	100%
Rh4+ consortium B	-	-	100%	-	100%	75%
R5	-	-	75%	-	75%	75%
R5+ consortium B	25%	-	75%	-	100%	75%
R6	-	-	100%	25%	100%	100%
R6+ consortium C	-	-	100%	-	100%	100%
R7	25%	-	100%	25%	100%	50%
R7+ consortium C	25%	-	100%	25%	100%	75%
R8	-	-	100%	-	100%	75%
R8+ consortium C	-	-	100%	-	100%	75%
R9	-	-	100%	-	100%	50%
R9+ consortium C	-	-	100%	-	100%	75%
N+	-	-	100%	-	100%	-
NST	-	-	-	-	-	-

Note: NST: Non Sterile Soil; N+: Nitrogen Treatment; WAP: Week After Planting; Consortium A: *Agrobacterium spp, Azotobacter agilis, Panibacillus wynii;* Consortium B: *Panibacillus wynii, Pseudomonas aeroginos;* Consortium C: *Azotobacter sp, Azotobacter indicus*

Discussion

The heights of plants with 40ml nutrient per week were higher than those with 20 ml nutrient per week and all the treatments were taller than the plant from potted non sterile soil this similar to the result [24]. At 20 ml nutrient per week, 22% were shorter than the N+ treatment while at 40 ml nutrient per week, 33% were shorter. The treatments with PGPR were taller than their counterpart except for R3, R4, and R6 which were all shorter both at 20 ml and 40 ml nutrient per week than their counterpart treatment containing only rhizobia this agreed with the work [28] and [29] where co-innoculation of *Bradhyrhizobium* with PGPRs increased soybean plant vigour. It was observed that plant height, flowering and podding were enhanced by co-inoculation of *Rhizobial spp.* with a consortium of *Agrobacterium spp., Pseudomonas sp. Paenibacillus sp.* while a consortium of *Azotobacter spp.* showed no ability to improve plant height. This work also showed that the height of plants was also increased with increased nutrient concentration.

The first signs of flowering were observed by the end of the sixth week and plants to which treatments were added all started flowering before those with N+ treatments and those from potted non sterile soil, indicating that they reached maturity faster than the plants in N+ treatments and in non-sterile potted soil. By the end of the seventh week, all treatments including the N+ had started flowering but those plants in the potted non sterile soil had not yet shown any sign of flowering, and by the eighth week before they were to be harvested, they still had not shown signs of flowering [30]. About 82% of the plants with treatments had produced visible cowpea pods before they were harvested by the eighth week but both controls i.e N+ and plants in non-sterile potted soil were yet to show any pod formation. Flowering and podding were enhanced by co-inoculation of *Rhizobial spp.* with a consortium of *Agrobacterium spp., Pseudomonas sp. Paenibacillus sp.* and a consortium of *Azotobacter*. This agreed with the work [31] where co-innoculation of *Bradhyrizobium* with PGPRs increased seed yield in soybean plants, but differs from the result [32], where rhizobial inoculation induced late flowering and pod formation in soybean.

Conclusion

Plant height was affected positively both by the presence of Plant Growth Promoting Rhizobacteria (PGPR) and also by increase in nutrient application. Flowering and podding were also improved in plants in which Rhizobial spp. were co-inoculated with NRMs. Plant height, flowering and podding were enhanced by co-inoculation of *Rhizobial spp.* with a consortium of *Agrobacterium spp., Pseudomonas sp. Paenibacillus sp.* while a consortium of *Azotobacter spp.* improved flowering and podding but showed no ability to improve plant height.

Acknowledgement

This study was supported by the International Institute of Tropical Agriculture IITA. (Presented at the Isteam Conference Ghana, 2018).

References

1. Peoples, Mark B, and Eric T. Craswell. "Biological Nitrogen Fixation: Investments, Expectations and Actual C ontributions to Agriculture." *Plant and soil* 141(1992): 13-39.
2. Pandya, Maharshi, Gattupalli Naresh Kumar, and Shalini Rajkumar. "Invasion of Rhizobial Infection Thread by Non-rhizobia for Colonization of Vigna Radiata Root Nodules." *FEMS microbiology letters* 348(2013): 58-65.

3. Peix, Alvaro, Martha H Ramírez-Bahena, Encarna Velázquez and Eulogio J. Bedmar. "Bacterial Associations with Legumes." *Crit Rev Plant Sci* 34(2015): 17-42.
4. Valverde, Angel, Jose M Igual, Alvaro Peix and Emilio Cervantes, et al. "*Rhizobium Lusitanum sp*. nov. a Bacterium that Nodulates Phaseolus vulgaris." *Int J Syst Evol Microbiol* 56(2006): 2631-2637.
5. Murugesan, S, C Manoharan, R Vijayakumar, and A Panneerselvam. "Isolation and Characterization of Agrobacterium Rhizogenes from the Root Nodules of Some Leguminous Plants." *Intl J Microbiol Res* 1(2010): 92-96.
6. Paul, L. R. "Nitrogen Fixation Associated with Tuberculate ectomycorrhiza on lodgepole pine (Pinus contorta)." PhD thesis (2002).
7. Bressani, R. Nutritive value of cowpea. In Cowpea Research, Production, and Utilization, Edited by S.R Singh and K.O. Rachie. John Wiley and Sons, Chichester, UK. 353-360. (1985).
8. Singh, BBOL, O Chambliss, and B Sharma. "Recent advances in Cowpea Breeding." (1997).
9. Singh, BB, DR Mohan Raj, KE Dashiell, and LEN Jackai. "Advances in Cowpea Research, co Publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), IITA, Ibadan, Nigeria." Ibadan Nigeria. IITA. JIRCAS (1997).
10. Awonaike, KO, KS Kumarasinghe, and SKA Danso. "Nitrogen Fixation and Yield of Cowpea (Vigna unguiculata) as Influenced by Cultivar and Bradyrhizobium Strain." *Field Crops Res* 24(1990): 163-171.
11. Peksen, AYŞUN, E. Peksen, and H. Bozoglu. "Relationships Among Some Seed Traits, Laboratory Germination and Field Emergence in Cowpea (Vigna Unguiculata (L.) Walp.) Genotypes." *Pak J Bot* 36(2004): 311-320.
12. IITA. Cowpea. IITA annual report. http://www.iita.org/cms/details/cowpea_ project details.aspx?Zoneid =63&articleid=269. (2007).
13. IITA. Crops and farming systems, http://www.iita.org/cowpea.html. (200.)
14. Ayodele, JT and IR Yalwa. "Amino Acid Composition of Vigna Dekindtiana." *Biological and Environmental Sciences Journal for the tropics*. 1(2004): 120-126.
15. Ayodele JT, Yalwa IR. Nutritional and Trace Element Composition of Vigna dekindtiana. *Biological and Environmental Sciences Journal for the Tropics*. 2(2005): 62-66.
16. Onwugbuta-Enyi, J. "Water Balance and Proximate Composition in Cowpea (Vigna Unguiculata (L). Walps) Seedlings Exposed to Drought and Flooding Stress." (2004).
17. Guzma-Maldonado SH and Paredes-Loez O. Functional Products of Plants Indigenous of Latin America: Amaranth, Quinoa, Common Beans and Botanicals. In: Functional Foods. Biochemical and Processing Aspects; Mazza G (Eds). Thechnomic. (1998).
18. Valenzuela H and Smith J. Cowpea: Cooperative Extension Service, College of Tropical Agriculture and Human Resources, University of Hawai at Manoa. (2002).
19. Fuller, Dorian Q. "Contrasting Patterns in Crop Domestication and Domestication Rates: Recent Archaeobotanical Insights from the Old World." *Ann Bot* 100(2007): 903-924.
20. Somasegaram P and Hoben HJ. Methods in Legume-rhizobium Technology NifTAL Project and MIRCEN. University of Hawaii Maui. (1985).
21. Woomer, Paul L, Nancy Karanja, Stanley M. Kisamuli and Mazvita Murwira, et al. "A Revised Manual for Rhizobium Methods and Standard Protocols Available on the Project Website." A Revised Manual for Rhizobium Methods and Standard Protocols Available on the Project Website (2011).
22. Dye, M. "Function and Maintenance of a Rhizobium collectin." Recent Advances in Biologicaa Nitrogen Fixation (1979): 435-471.
23. Vincent, James Matthew. "A Manual for the Practical Study of the Root-Nodule Bacteria." A Manual for the Practical Study of the Root-nodule Bacteria. (1970).
24. Olutiola, PO, Famurewa O, Sonntag HG. An Introduction to General Microbiology (A Practical approach), Reprinted. Nigeria: Bolabay Press Ltd. (2000).
25. Harrigan, WF and McCance, ME. Laboratory Methods in Microbiology. Department of Bacteriology, the West of Scotland. Agricultural College Auchicurive. (1966).
26. Somers, Ellen, J Vanderleyden, and M. Srinivasan. "Rhizosphere Bacterial Signalling: A Love Parade Beneath Our Feet." *Crit Rev Microbiol.* 30(2004): 205-240.
27. Eutropia VT and Patrick AN. Bradhyrhizobium Japonicum Inoculation and Phosphorous Supplementation on Growth and Chlorophyll Accumulation in Soybean (Glycine max L.). *Am J Plant Sci* 4(2013): 2281-2289.
28. Verma, DPS, MG Fortin, J Stanley and VP Mauro, et al. "Nodulins and Nodulin Genes of Glycine Max." *Plant Mol Biol* 7(1986): 51-61.
29. Li, De-Ming, and Martin Alexander. "Co-inoculation with Antibiotic-Producing Bacteria to Increase Colonization and Nodulation by Rhizobia." *Plant and Soil* 108(1988): 211-219.
30. Yahalom, Eli, Yaacov Okon, and Amos Dovrat. "Azospirillum Effects on Susceptibility to Rhizobium Nodulation and on Nitrogen Fixation of Several Forage Legumes." *Can J Microbiol* 33(1987): 510-514.
31. Iruthayathas, EE, S Gunasekaran, and K Vlassak. "Effect of Combined Inoculation of Azospirillum and Rhizobium on Nodulation and N2-fixation of Winged Bean and Soybean." *Sci Hortic* 20(1983): 231-240.
32. Alagawadi, AR, and AC Gaur. "Associative Effect of Rhizobium and Phosphate-solubilizing Bacteria on the Yield and Nutrient Uptake of Chickpea." *Plant and Soil* 105(1988): 241-246.

How to cite this article: Olaoluwa O, Ajayi, Dianda Mahamadi, Fagade Obasola E and Nwadike Blessing. "The Effect of Rhizobacteria on the Physical Properties of Cowpea *Vigna unguiculata* (L.Walp.)". *J Microbiol Pathol* 6 (2022): 159.