



Plant Growth Promoting Rhizobacteria Enhance Physical Properties of Cowpea *Vigna Ungiculata* [(L.) Walp.].

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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-inoculated with these Plant growth promoting rhizobacteria and applied as treatments to experimental cowpea (Tx 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 spp 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.

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1. INTRODUCTION

Nitrogen fixing bacteria especially members of the family Rhizobiaceae 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 (3) 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-existing 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 leghaemoglobin 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, *paenibacillus* spabuli, *p* amylicus and *methylobacterium* mesophilium have been shown to reside inside the plant formed by *sillus tomenous* on *pivum contortav* *latifolia* roots (6). Other Plant growth promoting rhizobacteria (PGPR) such as *Pseudomonas*, *Micromonospora* spp, *Phyllobacterium* spp, *Ochrobacterium*, *Devosia* spp, *Bosae* spp, *Kribbella* sp, *Cohnella*, *Paracoccus*, *Herbaspirillum* sp, *Microvigna* 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 (4)



Cow pea (*Vigna unguiculata*) is a food and animal feed crop grown in Africa, Asia, Europe, (10, 11, 12). It is an annual herbecious plant (13, 14). Nigeria accounts for about 5.2 million tonnes of the total 7.56 million tonnes of cowpea produced annually (15, 16). All the parts are rich in nutrient and fibre (17, 18, 19). In Africa, humans consume the young leaves, immature seed and the matured dried seed (20). They are usually cooked together with vegetables (21) and alongside cereals (22).

2. MATERIAL AND METHODS

2.1 Sample site and collection

Samples were collected from three sites in Nassarawa State which are Shamage Local government [(Nassarawa State) N 080 37¹ 47.7¹ E 007⁰ 46¹ 48.4¹ Elevation 244 m], Ogba/ Ubbe Egon local government [(Nassarawa State) N 080 51¹ 55.4¹ E 080 25¹ 34.5¹ Elevation 399 m], Mandara Kokona local government [(Nassarawa State) N 08⁰50¹29.8¹ E 008 12¹37.1¹ 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 avoid loss of nodules (25, 28). 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.

2.2 Isolation of microorganisms from root nodules

Microorganisms were isolated from nodules on Congo red agar (23, 24) using spread plate method. Five undamaged nodule samples were picked from each site. They were placed 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 for 5 - 7 days (25). 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 (25). Purified isolates were stored on yeast mannitol agar slants (26, 27)

2.3 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. Sterilized 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 duplicate rhizobial broths table 3.1. 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 inoculum (containing about 1×10^9 cells ml⁻¹). Plants were allowed to grow for 8 weeks 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 (28, 29). The plant height was 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 of Eutropia *et al.*, (2013) (30). Flowering and podding were monitored in plants from the 6th to the 8th week and recorded in percentage.

3. RESULTS

3.1 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 *Pseudomonas* sp See table 1

3.2 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). See fig 1 and fig 2

3.3 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 the eighth 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.

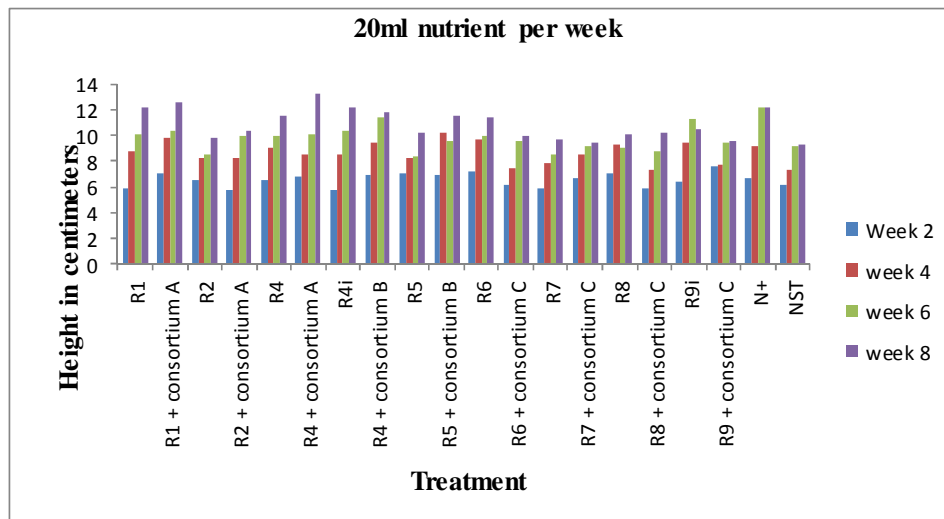


Fig 3.1: 20 ml Nutrient per Week treatment vs height

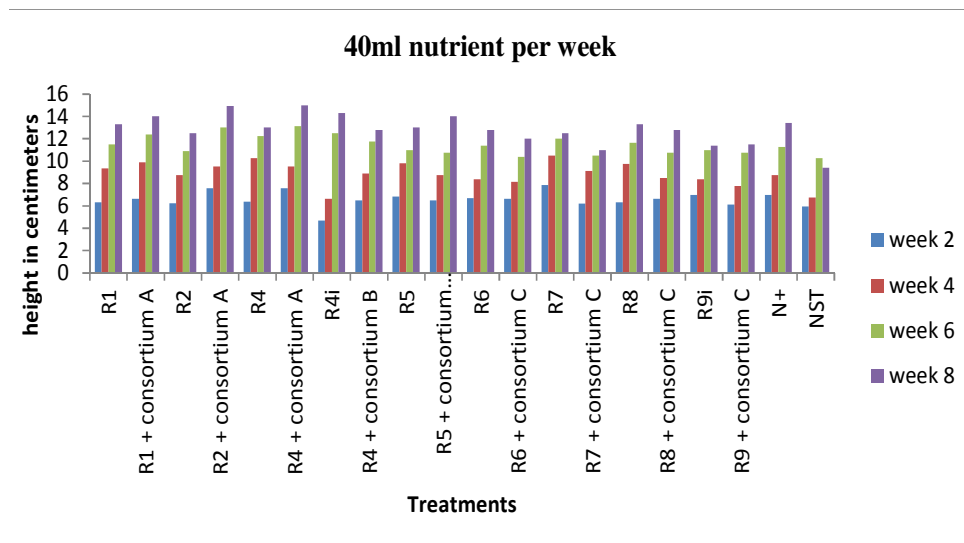


Fig 3.2: 40 ml Nutrient per Week Height in Centimetre Versus Treatments



Table1. 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 A	-	-	75%	-	100%	100%
R4	-	-	100%	-	100%	100%
Rh 4+ 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	-	-	-	-	-	-

KEY - NST- non sterile soil, N+ - Nitrogen treatment, Wap- week after planting

Consortium A: *Agrobacterium spp.*, *Azotobacter agilis*, *Panibacillus wynii*

Consortium B: *Panibacillus wynii*, *Pseudomonas aeruginos*

Consortium C: *Azotobacter sp.*, *Azotobacter indicus*

4. DISCUSSION

The heights of plants with 40ml nutrient per week were higher than those with 20ml nutrient per week and all the treatments were taller than the plant from potted non sterile soil this similar to the result of *Eutropia et al.*, 2013 (30). At 20ml nutrient per week, 22% were shorter than the N+ treatment while at 40ml 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 20ml and 40ml nutrient per week than their counterpart treatment containing only rhizobia this agreed with the work of Verma *et al.*, 1986 (31) and Yahalom *et al.*, 1987 (7) where co-inoculation of *Bradyrhizobium* 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.

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5. 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 NRM. 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.

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