

GROWTH AND YIELD ASSESSMENT OF RICE (*ORYZA SATIVA*) AFTER RHIZO-INOCULATION WITH SELECTED PLANT GROWTH-PROMOTING RHIZOBACTERIA IN A FERRIC ULTISOL

Beckley Ikhajigbe^{1,2}, Edokpolor Osazee Ohanmu^{1,3*}

¹Environmental Biotechnology and Sustainability Research Group, Department of Plant Biology and Biotechnology, University of Benin, Nigeria

²Applied Environmental Bioscience and Public Health Research Group, Department of Plant Biology and Biotechnology, University of Benin, Nigeria

³Department of Plant Biology and Biotechnology, Faculty of Science, Edo University Iyamho, Edo State, Nigeria

Abstract: The present study investigated the growth and yield responses of rice (*Oryza sativa*) after rhizo-inoculation with selected plant growth promoting rhizobacteria, PGPRs, (*Bacillus subtilis*, *Micrococcus varians* and *Pseudomonas aeruginosa*) in an ultisol. Viable rice (NERICA) seeds were acquired and sown in a nursery. When rice seedlings had attained the 3-leaf stage, they were removed from the nursery to be transplanted unto experimental bowls after immersing their roots in microbial culture of the PGPRs for 25 mins in a 50ml beaker (dilution factor = 10^{-3}). Results showed no effects on chlorophyll contents. Overall plant survival was also not affected ($p > 0.05$) by PGPR-inoculation (88.32 – 98.32%). Significant improvement in rice yield was reported in the stands inoculated with *P. aeruginosa*. There were 49 seeds per plant compared to 25 in the control. Per plant yield was 0.53g in *P. aeruginosa*-inoculated rice stands compared to 0.28g obtained in the control ($p < 0.05$). The yield of stands inoculated with *B. subtilis* and *M. varians* were comparable with the control. There was significant ($p < 0.05$) reduction in leaf loss as well as leaf drying compared to control plants, thus indicating the importance of PGPR in plant development under influence of environmental stress conditions.

Keywords: *Bacillus*, PGPR, *Pseudomonas*, ultisol, upland rice.

INTRODUCTION

Modern crop production systems in many countries are heavily reliant on the use of synthetic pesticides and fertilizers. Intensive use of these agrochemicals has increased human health and environmental concerns (Thakore, 2006; Cummings, 2009). Moreover, over-reliance on the aforementioned would further increase input demand to sustain the current yield levels (Cummings, 2009). In contrast, natural ecosystems are more sustainable in the context of productivity, insect pest resistance and nutritional aspects. Therefore, given the negative environmental impact of synthetic fertilizers, and their increasing costs, the use of beneficial soil microorganisms such as plant growth promoting rhizobacteria (PGPR) for sustainable and safe agriculture has increased globally during the last couple of decades (Glick, 1995; Gupta *et al.* 2000; Podile and Kishore, 2006; Freitas *et al.* 2007). In most cases, PGPR are incorporated into eco-friendly biofertilizers in crop improvement; and this constitutes a sustainable agricultural practice (Freitas *et al.* 2007). PGPR are well-known for their capability to advance plant growth in numerous ways when likened to synthetic fertilizers, insecticides and pesticides. Bacteria of diverse genera have been identified as PGPR, of which *Bacillus* and *Pseudomonas* spp. are predominant (Podile and Kishore, 2006).

In guaranteeing food security, steady availability of a number of staple crops like rice (*Oryza sativa* L.) is considered. Rice is one of the staple foods for more than half of the world's population. It accounts for

about 23% of the world's caloric intake (Bernier *et al.*, 2008; Jeon *et al.*, 2011). In Nigeria, for example, upland rice production accounts for 20% of the total rice produced. The Africa Rice Centre (WARDA) reports that Nigeria is the largest producer of rice in the West Africa sub-region, and the demand for the crop is growing at an annual rate of 5%. Although potentially cultivable with rice area in Nigeria is supposed to be estimated at 4.6 to 4.9 million hectares, only about 1.7 million hectares are presently being cropped to rice. This production margin can be raised not only by occupying vast land space for rice production, but maximizing the fewer spaces available for rice cultivation through enhanced biofertilization. This is even more predicated on the fact that some soils, particularly in southern Nigeria, where rice is best suited ecologically, are either acidic or ferric ultisols (Obi and Salako, 1995; Ekundayo and Obuekwe, 2000; Udom *et al.*, 2004; Imasuen and Onyeobi, 2013; Ikhile, 2016); a soil type that is not favourable for rice cultivation. Iron toxicity in lowland rice, for example, has been reported in various countries such as Sri Lanka, India, Indonesia, Malaysia, Philippines, Senegal, Sierra Leone, Liberia, Nigeria and Colombia (Suresh, 2005). WARDA (1998) reported that iron toxicity is very wide spread in West Africa, particularly throughout the humid forest and savanna zones in about 30 – 40% of all cultivated lowlands. Here, crop yields are reportedly reduced, for example, Prade *et al.* (1993), Sahrawat and Diatta (1996), Audebert and Sahrawat (2000) reported a 12 – 100%

reduction in rice production. Although iron is an essential element for all plants, particularly, its importance in numerous biochemical processes such as photosynthesis, chloroplast development and chlorophyll biosynthesis. At higher levels, iron becomes a physical barrier for the uptake of manganese (Wang and Shuman, 1994) and of zinc (Zhang *et al.*, 1998).

However, it is not yet known if such soils are an advantage for PGPR usage by plants. Although, Mantelin and Touraine (2004) reported that inoculation of PGPR enhanced plant uptake of important nutrients such as Ca, K, Fe, Cu, Mn and Zn during acidification of the soil rhizosphere; they also reported that decreases in soil pH would improve the solubilization of these nutrients.

In pristine and/or unfavourable environments, PGPRs are known to enhance plants' survival through the production of relevant plant nutrients as well as plant growth hormones (Glick, 1995). Significant increases in growth and yield of agronomically important crops in response to inoculation with PGPR have been repeatedly reported (Gupta *et al.* 2000; Biswas *et al.* 2000; Mariano and Kloepper 2000; Vessey 2003; Gray and Smith 2005; Silva *et al.* 2006; Figueiredo *et al.* 2008; Araujo 2013). Previous research by Mayak *et al.* (2004) confirmed the use of PGPR to confer resistance to water stress in tomatoes and peppers. Herman *et al.* (2008) also reported improved physiological responses of sweet pepper with inoculation of PGPR and mycorrhiza. Studies have also shown that the growth-promoting ability of some bacteria may be highly specific to certain plant species, cultivar and genotype (Bashan and Holguin 1997; Gupta *et al.* 2000; Lucy *et al.* 2004). It is therefore the aim of this study to investigate possible impact of rhizo-inoculation of rice with selected PGPR on selected growth and yield parameters of the test plant in an ultisol.

MATERIALS AND METHODS

Collection and preparation of materials for the experiment

The experiment was conducted at the Department of Plant Biology and Biotechnology (Dept.PBB) Screen House, University of Benin, Benin City, Nigeria (South-south Nigeria). Top soil (1 - 10 cm) was obtained from the Dept.PBB Botanic Garden, and sun dried to constant weight. Samples of this soil (an ultisol) were taken to the Lab for determination of selected physical and chemical characteristics prior to use, and according to methods described by APHA (1985). Selected soil heavy metal composition was determined by atomic absorption spectrometry. Total organic carbon (TOC) and total organic matter (TOM) contents were determined according to Nelson and Sommers (1982) and Osuji and Nwoye (2007). Soil textural components as well as elemental composition of the materials used were determined according to the methods of Bray and Kurtz (1945 a,b). Culturable microbial composition of top soil to be used was also determined following laid down procedure

(Cheesebrough, 2001). The soils were eventually measured into holding plastic experimental bowls measuring 68 cm diameter and 26 cm deep, and adequately moistened. There were 20 bowls.

Sowing of Rice Seedling in Nursery

Viable rice (NERICA) seeds were acquired and sown in a nursery. After seedlings had attained the 3-leaf stage, they were taken to the screen house for transplanting.

Bacterial species

The bacteria species used in this study (*Bacillus subtilis*, *Micrococcus varians* and *Pseudomonas aeruginosa*) were obtained from the Graduate Research Laboratory Culture Collection Unit of the Department of Microbiology, University of Benin, Benin City, Nigeria. They were initially isolated from plant rhizospheres of *Chromolaena odorata* during a previous study (Ikhajiagbe and Akendolor, 2016). The bacterial cultures were grown on nutrient agar and immediately put to use.

Rhizo-inoculation of Rice Transplants

Stock cultures of the PGPR were prepared and kept in McCartney bottles. Cultures of each PGPR was serially diluted with distilled water by a factor of 10^{-3} , and transferred into a 50 ml beaker. Prior to inoculation, the roots of each rice seedling were carefully washed in several changes of distilled water – to remove all soil attachments on the roots. Thereafter, the roots-washed seedling was immersed (root-deep) into the already prepared diluted microbial culture in the beaker for a period of 25 minutes each, before the inoculated seedling was eventually transplanted into newer experimental bowls. This was done separately for *B. subtilis* culture, as well as for *M. varians* and *P. aeruginosa* respectively. Control plants were not inoculated. Both inoculated and control plant bowls were kept in a well-ventilated screen house for growth monitoring.

Husbandry

The transplanted experimental bowls were constantly weeded, and carefully irrigated every other day with 500 ml of water (pH 6.6 – 6.8) especially during dry and hot days. Care was taken to ensure that soil moisture level was adequate for plant development, following procedures laid out by USDA (1998).

Parameters Considered and Experimental Design

The plants were physically observed for any growth anomaly as compared against the control. Leaf chlorophyll content was determined according to Arnon *et al.* (1949). Other parameters determined were plant height, leaf number, number of dried-out leaves, leaf length, number of additional tillers, as well as percentage senescence. Percentage plant survival was determined as percentage of plants that grew till maturity out of the totality of transplants initially introduced into experimental bowls, following rhizo-

inoculation. Leaf colour was recorded, and thereafter presented as colour codes. These colour codes are available online on the Google App Store®. The yield characteristics were also taken to note.

The experimental design chosen was the completely randomized design (CRD) following assumption of homogeneity of the experimental plot in use. As a result, treatments were randomized over the whole plot in the screen house. Each treatment consisted of 5 replicates. In order to avoid bias and misidentification, treatment bowls were properly labelled.

Statistical Analysis

Results were therefore analysed using SPSS-20 statistical software for one-way Analysis of Variance (ANOVA), principal components, as well as hierarchical cluster analyses where necessary.

RESULTS AND DISCUSSION

The physico-chemical characteristics of the soil prior to use have been presented on Table 1. The soil, which was slightly acidic, had a total organic carbon content of 0.92% and a total nitrogen content amounting to 2.13% (Table 1). Also presented (Table 2) is culturable microbial composition of the soil prior to use. Bacterial counts (1.52×10^5 cfu/g) significantly outnumbered the available fungi species by a factor of 10 (2.03×10^4 cfu/g). *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Micrococcus varians*, *Staphylococcus epidermidis* were the bacterial isolates available. The presence of *Pseudomonas aeruginosa* and *Micrococcus varians* in the bulk soil may eventually be beneficial for plant development as these bacteria, when found within plant rhizosphere, generally enhance plant growth (Mayak *et al.*, 2004; Bernier *et al.*, 2008; Nautiyal *et al.*, 2008; Martinez-Viveros *et al.*, 2010; Jeon *et al.*, 2011; Vacheron *et al.*,

2013; Sarma and Saikia, 2014). Suggestively, soil acidic condition is a possible reason for microbial proliferation (Ikhajiagbe *et al.*, 2013). The authors also reported the presence of the *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Micrococcus varians* in soils with pH of as low as 5. This may however not always be true; as there are several other factors that affect microbial proliferation in the soil – moisture, temperature, nutrient composition, total organic content, soil oxygen, soil type, and presence of contaminants; the effects of these factors are also synergistic and not entirely isolated (Vidali, 2001; Ikhajiagbe, 2010).

Physical observation about the rice plants during the 4-8th week after inoculation were reported (Table 3). In the control, the only anomaly reported was the presence of dried leaves. Leaf dryness progressed from the leaf tip inwardly to other leaf parts. However, this drying out of leaves was exclusively restricted to only older leaves in both control and inoculated plants, but reportedly less frequent ($p < 0.05$) in the latter. Leaf yellowing was observed at middle of a few leaves in the *Bacillus*-inoculated plants. Generally however, leaf yellowing (chlorosis) was common to the rhizo-inoculated plants, although this occurrence was not very significant. Leaf color was mainly green. However, there was presentation of the green leaf colour in different shades of green, including Lime green (#32CD32), Olive drab (#6B8E23), Green yellow (#ADFF2F), Sea green (#2E8B57) and Dark green (#006400). There were no significant differences in chlorophyll contents of leaves of the rhizo-inoculated and control rice plant stands. Total chlorophyll ranged from 0.253 – 0.293 U/mg (Table 3). Sharma *et al* (2014) however reported increases in chlorophyll contents of rice plants inoculated with *Pseudomonas putida* and *Pseudomonas fluorescens*.

Table 1.

Physical and chemical properties of soil before contamination

Parameters	Mean value (n = 5)
Ph	5.87 ± 0.52
Total organic carbon (%)	0.92 ± 0.09
Total Nitrogen (%)	2.13 ± 1.01
Exchangeable acidity (meq/100g)	0.25 ± 0.06
Electric conductivity (µs/cm)	302.33 ± 21.42
Ca (meq/100g)	13.96 ± 3.02
K (meq/100g)	1.61 ± 0.73
Mg (meq/100g)	12.05 ± 3.14
Na (meq/100g)	10.14 ± 2.52
NO ₂ (mg/kg)	157.34 ± 31.55
NO ₃ (mg/kg)	250.21 ± 22.14
Fe (mg/kg)	1034.12 ± 66.34
Cd (mg/kg)	<0.001
Cu (mg/kg)	3.62 ± 0.14
Zn (mg/kg)	28.96 ± 4.86
Mn (mg/kg)	19.22 ± 5.23
Pb (mg/kg)	0.02 ± 0.01
Clay (%)	5.23 ± 0.48
Silt (%)	7.50 ± 1.11

Sand (%)	84.87 ± 12.01
----------	---------------

These are background mean concentrations (n = 5)

Table 2.

Microbial composition of soil prior to use in the experiment

	Bacteria (x 10 ⁵ cfu/g)	Fungi (x 10 ⁴ cfu/g)	p-value
*Heterotrophic counts	1.52a	2.03b	0.017
Culturable isolates	<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Micrococcus varians</i> , <i>Staphylococcus epidymis</i> .	<i>Aspergillus flavus</i> , <i>Trichoderma harzianum</i> , <i>Mucor mucedo</i> , <i>Fusarium solani</i> , <i>Penicillium sp.</i> , <i>Aspergillus niger</i>	Not applicable

*Means on the same row with similar accompanying alphabets do not differ from each other (p>0.05)

Table 3.

Physical observation of test plant during exposure in the field as well as foliar chlorophyll contents

Treatments	Observation on field between 4 – 8 WAI	Prominence of foliar colour between 4 – 8 WAI	*Total chlorophyll content (U/mg protein) at 8 WAI
<i>B. subtilis</i>	Leaves drying from tip inwardly. Also, drying from stem tip outwardly. Leaf yellowing observed at middle of a few leaves. Plants were generally the shortest.	The most prominent leaf colour was Green (#008000); other foliar colours identified were Sea green (#2E8B57) and Dark green (#006400). Green yellow (#ADFF2F) was reported in only 2 leaves with severe chlorosis.	0.2534a
<i>M. varians</i>	Chlorosis at tip present in few leaves; Leaves drying from tip inwardly. Foliar anomaly was mostly common to older leaves.	Most prominent colour was Green (#008000); other foliar colours identified were Sea green (#2E8B57), Lime green (#32CD32), and Dark green (#006400). Most leaves that eventually dried out turned from green through Olive drab (#6B8E23) to brown before drying out.	0.2634a
<i>P. aeruginosa</i>	Leaves drying from tip inwardly. Yellowish leaf tip present in few leaves. Foliar anomaly was mostly common to older leaves.	Most prominent colour was Green (#008000); Dark green (#006400) was also prominently observed. Sea green (#2E8B57) was least observed. A few insignificant number of older leaves showed color deterioration from green to Green yellow or lime green	0.2712a
Control	Leaf drying occurred from tip and then inwardly to entire leaf. Foliar anomaly was mostly common to older leaves.	Colour presentation nearly similar to <i>Pseudomonas</i> -inoculated plant leaves.	0.2934a
p-value	Not applicable	Not applicable	0.512

WAI - weeks after inoculation. *Means on the same column with similar accompanying alphabets do not differ from each other (p>0.05)

Key:

	Lime green (#32CD32)		Olive drab (#6B8E23)
	Green (#008000)		Green yellow (#ADFF2F)
	Sea green (#2E8B57)		Dark green (#006400)

Table 4.

Some growth characteristics of rice at 13 weeks after exposure to selected PGPR

Rhizo-inoculants	Percentage plant survival (%)	Height of Plants (cm)	length of Plants leaf (cm)	Additional tillers*
<i>Bacillus subtilis</i>	88.33a	52.50a	44.00a	0a
<i>Micrococcus varians</i>	89.15a	64.25ab	50.50ab	1ab
<i>Pseudomonas aeruginosa</i>	93.33ab	82.25bc	52.25ab	2b
Control	98.325b	93.75c	65.00b	2b
p-value (0.05)	0.177	0.002	0.149	0.041

*Presented to the nearest whole number. Means on the same column with similar accompanying alphabets do not differ from each other ($p > 0.05$)

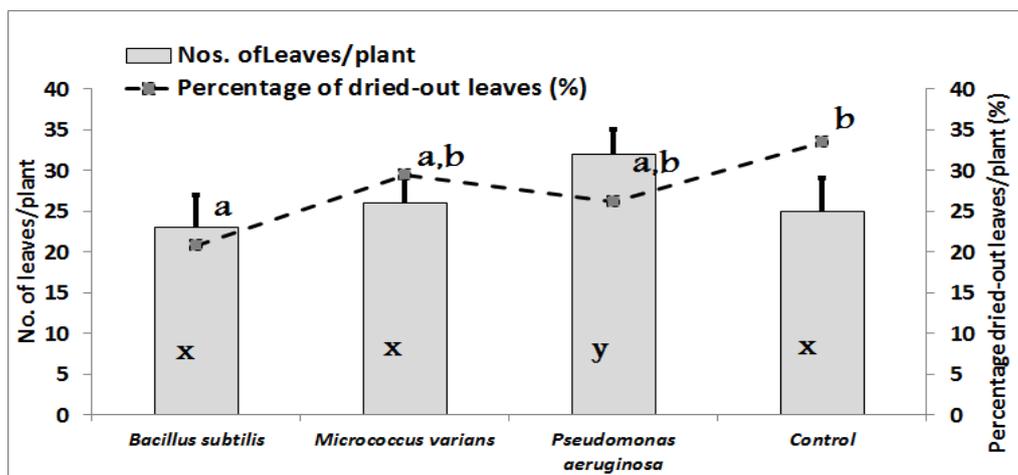


Fig. 1. Amelioration of plant senescence and leaf drying incidents by inoculated rhizo-bacteria reported 13 weeks after inoculation. Means on the same graph types with similar accompanying alphabets do not differ from each other ($p > 0.05$).

Plant overall survival after 13 weeks was not affected by PGPR-inoculation when compared with the control (88.32 – 98.32%) (Table 4). However, control rice plants were taller (ht. = 93.75 cm) than those plants that were rhizo-inoculated with *B. subtilis* and *M. varians* (ht. = 88.33 – 89.15 cm), but comparable with *Pseudomonas*-inoculated plant stands (ht. = 93.33 cm). There were not significant increases in plant height compared with the control as a result of *pseudomonas* inoculation (82.25 – 93.75 cm). Ahmed *et al.* (2013) reported increases in plant height of a cold area rice variety ‘Fakre Malakand’ upon inoculation with *Pseudomonas* Ky1 strain. Significant reduction was however reported in rice stands inoculated with *Bacillus* (52.50 cm). *Bacillus*-inoculated plants stands also had shorter leaves.

It was observed that plants that were rhizo-inoculated were less prone to stress measured herein as number of dried-out leaves (Fig. 1). There were more dried-out leaves in the control (33.4 %) than in bacterial-inoculated rice plants (20.74 – 29.41%). Generally however, the *pseudomonas*-inoculated rice stands had more leaves (32 leaves per plant) compared to the control plants (25 leaves per plant). Abiotic stresses are known to cause drastic yield reductions in many farms this however depends on the intensity of

the stress as well as the soil type, not forgetting plant factors (Nadeem *et al.*, 2010). It is perhaps suggested that inherent soil conditions may be one of the factors that triggered such plant morphological stress responses like foliar drying and senescence common to both inoculated and control plants. The soil is a ferric ultisol, having reported phytotoxic impact on rice cultivation. This condition ordinarily would pose serious challenge for rice growth and development (Prade *et al.*, 1993; Sahrawat and Diatta, 1996; WARDA, 1998; Zhang *et al.*, 1998; Suresh, 2005). However, as reported in the present study, the effects on rice plants may have been ameliorated with increased presence of PGPR in plant rhizosphere.

Some of the plants are able to withstand the effects of the stress by their ability to utilize necessary antioxidant defense mechanisms. Nautiyal *et al.* (2008) demonstrated that the *Bacillus lentimorbus* strain enhanced the plant antioxidant capability as well as increasing growth. *Pseudomonas aeruginosa* strain was reported to enhance the growth of *Vigna radiata* plants under drought stress (Sarma and Saikia, 2014). The enhancement of survival of rice plants under salt stress after inoculation with *Pseudomonas* Ky1 strain has also been reported (Sen and Chandrasekhar, 2014).

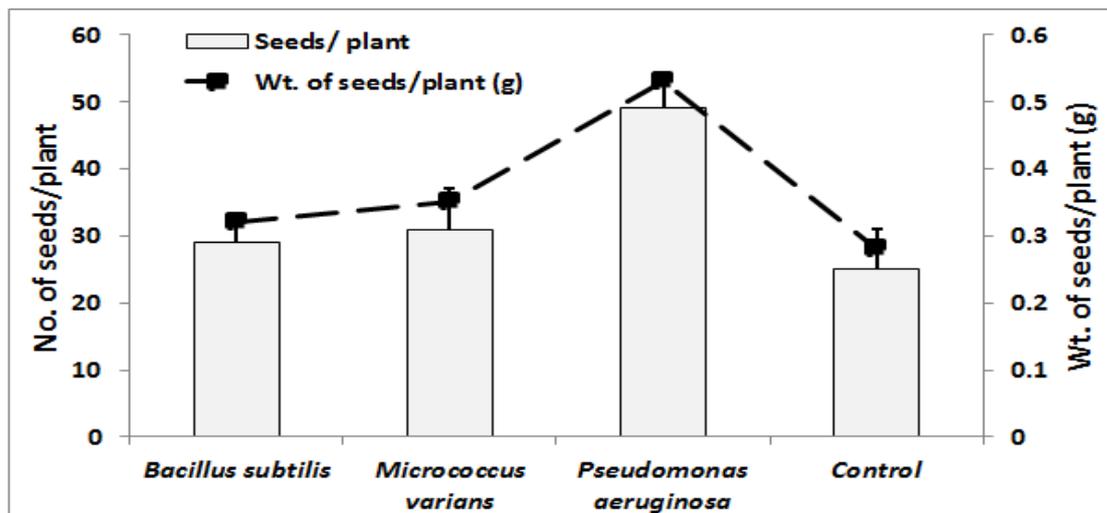


Fig. 2. Yield characteristics of rice 13 weeks after rhizo-inoculation with selected PGPR. Means on the same graph types with similar accompanying alphabets do not differ from each other ($p>0.05$).

Significant improvement in rice yield was reported in the stands inoculated with *P. aeruginosa*. There were 49 seeds per plant compared to 25 in the control (Fig. 2). Per plant yield was 0.53g in *P. aeruginosa*-inoculated rice stands compared to 0.28g obtained in the control ($p<0.05$). The yield of stands inoculated with *B. subtilis* and *M. varians* were comparable with the control. Ahmed *et al.* (2013) reported increase in yield of a cold area rice variety, Fakre Malakand, when inoculated with *Pseudomonas* Ky1 strain. In a bid to find out possible correlation between any 2 selected growth and yield parameters, a bivariate correlation was computed (Table 5). Results showed that plant height highly positively correlated with additional tillers ($r = 0.953, p<0.05$), as well as with percentage plant survival ($r = 0.964, p<0.05$). The implication of this relationship is that increases in plant height, with regards to the present study only, would favour expression of the aforementioned growth and yield parameters (Table 5). Similarly, per plant seed weight

was absolute a factor of the number of harvested seeds per plant ($r = 0.999, p<0.01$).

As a follow up to the aforementioned, a principal component analysis was carried out with a view to determining which of the assessed parameters was the majorly critical to influencing the outcome of the study (Table 6). The Principal component analysis output showed 2 components presenting a set of 2 possible parametric groups that were most likely influenced by the experimental condition. Component-1 majorly relied on morphological parameters (leaf length, percentage plant survival, plant height, additional tiller, and percentage dried out leaves), whereas component-2 relied basically on yield parameters (number of seeds per plant and seed weight per plant). The PCA plot (Fig. 3) shows that plant inoculation with *P. aeruginosa* was most likely associated with plant height, number of leaves as well as number of seeds per plant.

Table 5.

Bivariate correlation between selected growth and yield parameters of rice exposed to rhizo-inoculation with selected PGPRs at 2-tailed

		No of seeds per plant	Seed wt. per plant	No. of leaves per plant	Percentage dried out leaves	Percentage plant survival	Plant height	Leaf length
No of seeds per plant	R	1						
	P-value							
Seed wt. per plant	R	0.999**	1					
	P-value	7.00E-04						
No of leaves per plant	R	0.931	0.935	1				
	P-value	0.069	0.065					
Percentage dried out leaves	R	-0.27	-0.25	0.101	1			
	P-value	0.731	0.745	0.899				
Percentage plant survival	R	-0.07	-0.08	0.227	0.746	1		
	P-value	0.935	0.924	0.773	0.254			

Plant height	R	0.138	0.133	0.449	0.785	0.964*	1	
	P-value	0.862	0.867	0.551	0.215	0.036		
Leaf length	R	-0.24	-0.24	0.104	0.906	0.952	0.926	1
	P-value	0.761	0.76	0.896	0.094	0.048	0.074	
Additional tiller	R	0.377	0.378	0.674	0.739	0.839	0.953*	0.805
	P-value	0.623	0.622	0.326	0.261	0.161	0.047	0.195

*correlation is significant at 0.05 (2-tailed)

**correlation is significant at 0.01 (2-tailed)

Table 6.

Principal component analysis output showing component score coefficient matrix for some growth and yield parameters of rice exposed to rhizo-inoculation with selected PGPRs

	Component-1	Component-2
Leaf length	0.987	-0.157
Percentage plant survival	0.977	0.023
Plant height	0.974	0.222
Additional tiller	0.881	0.449
Percentage dried out leaves	0.867	-0.208
No of seeds per plant	-0.084	0.996
Seed wt per plant	-0.088	0.994
No. of lvs per plant	0.25	0.951

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

^aCoefficients are standardized.

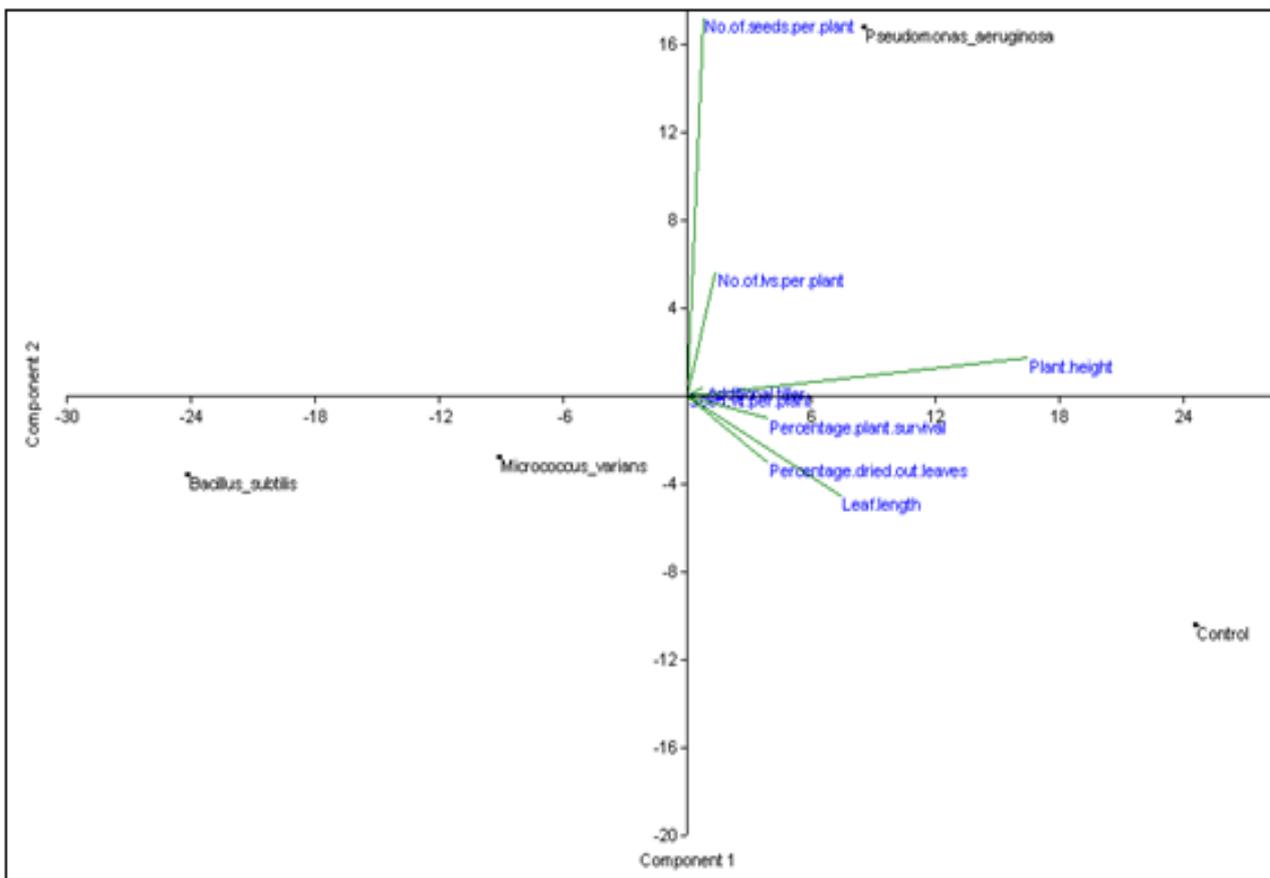


Fig. 3. Component plot as output of principal component analysis conducted on some growth and yield parameters of rice exposed to rhizo-inoculation with selected PGPRs.

When compared with other referred studies earlier presented, improved yield was reported in contrary to the report of this study. One thing was common with these reported studies; none provided information on physicochemical condition of soil on which rice was sown. Soil properties have been reported to greatly influence microbial activity (Vidali, 2001; Ikhajiagbe, 2010; Ikhajiagbe *et al.*, 2013). PGPR are generally known as growth enhancers. However, there have been a number of reports that describe them as also having anti-growth attributes. Certain *Pseudomonas* species, for example, produce cyanide as a growth-promotion factor, where it acts as a biocontrol agent against certain plant pathogens (Martinez-Viveros *et al.*, 2010); but cyanide also has a growth inhibition characteristic (Bakker and Schippers, 1987). Plant growth-promoting auxins produced by certain PGPR can also impede overall plant development (Vacheron *et al.*, 2003). It is important to note that the effectiveness of auxin relies upon its concentration. (Xie *et al.*, 1996) also links over production of these plant growth factors by PGPR as possible reasons for overall growth suppression. For example, auxins enhance plant growth at low concentrations, but inhibit root development at higher concentrations (Xie *et al.*, 1996). As important as the root is, whatever negatively impacts on plant root development, invariably would affect the overall growth performance of the plant.

CONCLUSION

PGPR are important growth enhancer of plant in an environmentally stressed condition, however, this varies with plant type and species. From the study, *P. aeruginosa* is suitable in the rhizo-inoculation of rice stands in a ferric ultisol for increased yield productivity and food security. However, molecular studies is needed to identify the genetic strain that is able to stimulate the plant inherent resistance in such soils.

AUTHORS CONTRIBUTIONS

Conceptualization: Dr. Beckley Ikhajiabe, Dr. Edokpolor Osazee Ohanmu. Methodology: Dr. Beckley Ikhajiabe. Data collection: Dr. Beckley Ikhajiabe. Data validation: Dr. Edokpolor Osazee Ohanmu, Dr. Beckley Ikhajiabe. Data processing: Dr. Edokpolor Osazee Ohanmu, Dr. Beckley Ikhajiabe. Writing: original draft preparation - Dr. Beckley Ikhajiabe; review and editing - Dr. Edokpolor Osazee Ohanmu, Dr. Beckley Ikhajiabe.

The researchers are grateful to interns at the Environmental Biotechnology and Sustainability Research Group (EBSRGroup) Laboratory for assistance during this study.

FUNDING

This research received no specific grant from any funding public agency, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Ahmed B, Midrarullah M, Sajjad-Mirza, M, Effects of inoculation with plant growth promoting rhizobacteria (PGPRs) on different growth parameters of cold area rice variety, Fakre Malakand. *African Journal of Microbiology Research*, 7(17), 1651-1656, 2013.
- APHA, Standard method for the examination of water and waste water. American Public Health Association, Washington DC, 256p, 1985.
- Araújo AES, Baldani VLD, Galisa PS, Pereira JA, Baldani JI, Response of traditional upland rice varieties to inoculation with selected diazotrophic bacteria isolated from rice cropped at the Northeast region of Brazil. *Applied Soil Ecology*, 64, 49–55, 2013.
- Arnon DI, Copper enzyme in isolated chloroplast. *Plant Physiology*, 24, 1-15, 1949.
- Audebert A, Sahrawat KL, Mechanisms for iron toxicity tolerance in lowland rice. *Journal of Plant Nutrition*, 23, 1877–1885, 2000.
- Bakker AW, Schippers P, Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp.-mediated plant growth-stimulation. *Soil Biology and Biochemistry*, 19, 451–457, 1987.
- Bashan Y, Holguin G, Azospirillum-plant relationships: environmental and physiological advances (1990–1996). *Canada Journal Microbiology*, 43, 103–121, 1997.
- Bernier J, Atlin GN, Serraj R, Kumar A, Spaner D, Breeding upland rice for drought resistance. *Journal of Science, Food and Agriculture*, 88, 927–939, 2008.
- Biswas JC, Ladha JK, Dazzo FB, Rhizobial inoculation influences seedling vigor and yield of rice. *Journal of Agronomy*, 92,880–886, 2000.
- Bray RH, Kurtz LT, Soil chemical analysis. *Soil Science*, 59, 39-45, 1945a
- Bray RH, Kurtz LT, Determination of total organic and available form of phosphorus in soils. *Soil Science*, 59,45-49, 1945b
- Cheesebrough M, District Laboratory Practice in Tropical Countries, part 2. Cambridge University Press, Cambridge, 355p, 2001
- Cummings SP, The application of plant growth promoting rhizobacteria (PGPR) in low input and organic cultivation of graminaceous crops, potential and problems. *Environmental Biotechnology*, 5 (2), 43-50, 2009.
- Ekundayo EO, Obuekwe CO Hydrocarbons utilization in yeast isolates found to grow in association with petroleum in a polluted ultisol of Midwest Nigeria. *Environmental Monitoring and Assessment*, 63, 381-87, 2000.
- Figueiredo MVB, Burity HA, Martinez CR, Chanway CP, Alleviation of water stress effects in common bean (*Phaseolus vulgaris* L.) by co-inoculation *Paenibacillus* x *Rhizobium tropici*. *Applied Soil Ecology*, 40, 182–188,

2008

- Freitas ADS, Vieira CL, Santos CERS, Stamford NP, Lyra MCCP, Caracterizaçãõ de rizo´ bios isolados de Jacatupé´ cultivado em solo salino no Estado de Pernanbuco, Brasil. *Bragantia*, 66:497–504, 2007
- Glick BR, The enhancement of plant growth by free living bacteria. *Canada Journal of Microbiology*, 41, 109-114, 1995.
- Gray EJ, Smith DL, Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. *Soil Biology and Biochemistry*, 37:395–412, 2005.
- Gupta A, Gopal M, Tilak KV, Mechanism of plant growth promotion by rhizobacteria. *Indian Journal of Experimental Biology*, 38,856–862, 2000.
- Herman MAB, Nault BA, Smart CD, Effects of plant growth-promoting rhizobacteria on bell pepper production and green peach aphid infestations in New York. *Crop Protection* 27, 996-1002, 2008.
- Ikhajiagbe B, Akendolor A, Comparative effects of pretreatment of stem cuttings of *Chromolaena odorata* (Siam weed) with sodium azide and hydroxylamide on the survival and phyoremediative performance in an oil-polluted soil. *Nigeria Journal of Biotechnology*, 30(1), 27 – 39, 2016.
- Ikhajiagbe B, Anoliefo GO, Oshomoh EO, Airhienbuwa N, Changes in heavy metal contents of a waste engine oil polluted soil exposed to soil pH adjustments. *British Biotechnology Journal*, 3(2), 158-168, 2013.
- Ikhajiagbe B, Synergism in Bioremediation: Phytoassessment of Waste Engine Oil Polluted Soils after Amendment and Bioaugmentation. LAP Lambert Academic Publishing, Köln, Germany, 2010
- Ikhile CI, Geomorphology and Hydrology of the Benin Region, Edo State, Nigeria. *International Journal of Geosciences*, 7, 144-157, 2016.
- Imasuen OI, Onyeobi TUS, Chemical compositions of soils in parts of Edo state, southwest Nigeria and their relationship to soil productivity. *Journal of Applied Science and Environmental Management*, 17 (3), 379- 386, 2013.
- Jeon JS, Jung KH, Kim HB, Suh JP, Khush GS, Genetic and molecular insights into the enhancement of rice yield potential. *Journal of Plant Biology*, 54, 1-9, 2011.
- Lucy M, Reed E, Glick BR, Applications of free living plant growth-promoting rhizobacteria. *Review Antonie Van Leeuwenhoek* 86, 1–25, 2004.
- Mantelin S, Touraine B, Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. *Journal Experimental Botany*, 55, 27–34, 2004
- Mariano RLR, Klopper JW, Me´todo alternativo de biocontrole: Resistência sistêmica induzida por rizobactérias. *Revisão*
- Annual de Patologia de Plantas*, 8, 121–137, 2000
- Martinez-Viveros O, Jorquera MA, Crowley DE, Gajardo G, Mora ML, Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *Journal of Soil Science and Plant Nutrient*, 10, 293–319, 2010
- Mayak S, Tirosh T, Glick BR, Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Science*, 166(2), 525-530, 2004.
- Nadeem SM, Zahir ZA, Naveed M, Ashraf M, Microbial ACC-deaminase, prospects and applications for inducing salt tolerance in plants. *Critical Review in Plant Science*, 29, 360–393, 2010.
- Nautiyal CS, Govindarajan R, Lavania M, Pushpangadan P, Novel mechanisms of modulating natural antioxidants in functional foods: Involvement of plant growth promoting rhizobacteria NRRL B-30488. *Journal of Agriculture, Food and Chemistry*, 56: 4474–4481, 2008
- Nelson DW, Sommers LE, Total carbon, organic carbon and organic matter. In: *Methods of soil analysis, Part 2. ASA/SSSA. Madison WI. pp 539 – 579, 1982.*
- Obi ME, Salako FK, Rainfall parameters influencing erosivity in Southeastern Nigeria. *CATENA* 24, 275-280, 1995.
- Osuji LC, Nwoye I, An appraisal of the impact of petroleum hydrocarbon on soil fertility: The Owaza experience. *African Journal of Agricultural Research* 2, 318-324, 2007
- Podile AR, Kishore GK, Plant growth-promoting rhizobacteria. In *Plant-Associated Bacteria*. Springer, Dordrecht, Netherlands, pp 195–230, 2006.
- Prade K, Ottow JCG, Jacq VA, Excessive iron uptake (iron toxicity) by wetland rice (*Oryza sativa* L.) on an acid sulphate soil in the Casamance /Senegal. *International Institute for Land Reclamation and Improvement, Wageningen, Netherlands. ILRI Publication* 44, 150–162, 1993
- Sahrawat KL, Diatta S, Nutrient management and season affect soil iron toxicity, in West Africa Rice Development Association. *Annual Report*, pp 34–35, 1996.
- Sarma RK, Saikia RR, Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRK21. *Plant Soils*, 377, 111–126, 2014
- Sen S, Chandrasekhar CN, Effect of PGPR on growth promotion of rice (*Oryza sativa* L.) under salt stress. *Asian Journal of Plant Science and Research*, 4(5), 62-67, 2014
- Sharma A, Shankhdhar D, Sharma A, Shankhdhar SC, Growth promotion of the rice genotypes by PGPRs isolated from rice rhizosphere. *Journal of Soil Science and Plant Nutrition*, 14 (2), 505-517, 2014

- Silva VN, Silva LESF, Figueiredo MVB, Atuacão de rizo´ bios com rizobactérias promotoras de crescimento em plantas na cultura do caupi (*Vigna unguiculata* L. Walp). *Acta Science Agronomy*, 28, 407–412, 2006.
- Suresh S, Characteristics of soils prone to iron toxicity and management: A review. *Agriculture Review*, 26 (1), 50 – 58, 2005.
- Thakore Y, The biopesticide market for global agricultural use. *Industry Biotechnology*, 2, 194-208, 2006.
- Udom BE, Mbagwu JSC, Adesodun JK, Agbim, Distributions of zinc, copper, cadmium and lead in a tropical Ultisol after long-term disposal of sewage sludge. *Environmental International*, 30, 467 – 470, 2004
- USDA, Estimating Soil Moisture by Feel and Appearance. United States Department of Agriculture, Natural Resources Conservation Service, Program Aid No. 1619, April, 1998. 6p, 1998.
- Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moëgne-Loccoz Y, Muller D, Plant growth-promoting rhizobacteria and root system functioning. *Frontier Plant Science* 4, 356, doi: 10.3389/fpls.2013.00356, 2013.
- Vessey JK, Plant growth-promoting rhizobacteria as biofertilizers. *Plant Soil*, 255:571–586, 2003.
- Vidali M, Bioremediation: An overview. *Pure Applied Chemistry*, 73, 1163-1172, 2001.
- Wang J, Shuman LM, Transformation of phosphorus in rice (*Oryza sativa* L.) rhizosphere and its influence on phosphorous nutrition of rice. *Journal of Plant Nutrient* 17,1803-1815, 1994
- WARDA, West Africa Rice Development Association, Bouake, Côte d'Ivoire. 1997 Annual Report. 1998.
- Xie H, Pasternak JJ, Glick BR, Isolation and characterization of mutants of the plant growth promoting rhizobacterium *Pseudomonas putida* CR12–2 that overproduce indoleacetic acid. *Current Microbiology*, 32, 67–71, 1996.
- Zhang X, Zhang F, Mao D, Effect of iron plaque outside roots on nutrient uptake by rice (*Oryza sativa* L.): II. Zinc uptake by Fe-deficient rice. *Plant Soil*, 202, 33- 39, 1998.