Rhizo-inoculation of phosphate solubilizing bacteria strains to improve rice (*Oryza sativa* L. var. FARO 44) growth under ferruginous ultisol conditions.

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Research Article

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Abstract

The research investigated the possibility of phosphate solubilizing bacteria (PSB) with plant growth-promoting (PGP) capabilities to improve growth properties of rice plant under ferruginous ultisol (FU) condition through rhizo-inoculation strategy. The PSB with PGP properties used in this research were *Bacillus cereus* strain GGBSU-1, *Proteus mirabilis* strain TL14-1 and *Klebsiella variicola* strain AUH-KAM-9 that were previously isolated and characterized following the 16S rRNA gene sequencing. The rice seeds were sown in a composite FU soil sample and a humus soil (control) and then rhizo-inoculated along the root region of the growing rice seedling at 16 days after sowing. The rice plant was studied for differences in morphological, physiological and biomass parameters for 16 weeks after rhizo-inoculation. Results showed that the FU soil used in the study had high pH, low bioavailable phosphorus, low water holding capacity and high iron levels which has led to a low growth properties of rice seeds sown in FU soil without rhizo-inoculation. After rhizo-inoculation, a significant increase in plant height and physiological parameters were observed in the rice plant grown in the FU soil as against the control and the rice plant in FU soil without inoculation except for terpenoid which is usually known to signify biotic stress and as part of plant defense mechanism. Generally, rhizo-inoculation of rice seedling with the three PSBs under FU soil condition significantly improved growth properties of the rice plant. This suggest the ability of the PSBs to solubilize and mineralize soil phosphate and improve its availability for plant use in phosphate stressed soil, thereby improving plant growth properties.

1 Introduction

Rice (*Oryza sativa* L.) is an important grain that is consume by over half of the world population. (Maclean et al., 2002). In African, it is the second most consumed food after maize (Ajala and Gana, 2015) and accounts up a large component of the food consumed in most Nigerian families (Ikhajiagbe and Musa, 2020). It is a significant starchy grain for human consumption since it supplies 23% of world human per capita calories and 16% of global human per capita protein (Ojo and Adebayo, 2012). Additionally, it increases national income. Rice consumption per capita in Nigeria is predicted to be more than 100 kg per year (Udemezue, 2018). Rice demand is rising, and more production is required to feed more people while reducing costly imports. Rice production in southern Nigeria has been severely hampered since the 1970s. High soil pH, low nitrogen, phosphorus deficiency, leading to a ferruginous conditions are some of the variables that have been related to this (Doyou et al., 2017). Unfortunately, the majority of soils in southern Nigeria, particularly in Edo State, are ferruginous (Daramola, 2005; Obayelu, 2015). According to Musa and Ikhajiagbe (2021), iron toxicity leading to phosphorus deficiency and acidic pH has been linked to reasons why ferruginous soils are not supporting rice growth. To sustainably improve rice productivity in these P-deficient soils, there is need to consider the use of biological agents such as microorganisms as inoculant, as against the widely used chemical fertilizers (Adnan et al., 2018; Joshis et al., 2007).

Ferruginous soil also known as red soil are iron-rich soil. This kind of soil is usually observed in warm and humid climates (Yu et al., 2016), or in some tropical regions as seen in some parts of Africa (Zhao, 2014). Ferruginous soils have been documented to account for about the average percentage (45.2%) of the Earth's landscape. In Nigeria for instance, it is transcendent in some southern States, for example, Edo state, possessing several regions, including northern region and Benin central (Doyou et al., 2017). Ferruginous soils are known for its unique properties such as high iron levels; which creates complexes with soil phosphate and

making it low available for plant use (Gyaneshwar et al., 2002). Consequently, this condition now brings about low biotic and abiotic properties needed for plant growth (Wang et al., 2014). Hence to enhance soil fertility, some local farmers have tried to use synthetic chemicals which had negative influence on the soil and plants as a results of insufficient beneficial microorganisms to fuel the nutrient cycling and release important metabolites that can improve the soil properties and plant growth (Sharma et al., 2013). Musa and Ikhajiagbe 2021 following 16S rRNA gene sequencing successfully isolated and identified *Bacillus cereus* strain GGBSU-1, *Klebsiella variicola* strain AUH-KAM-9 and *Proteus mirabilis* strain TL14-1 as efficient in solubilizing insoluble phosphate in soils (Musa and Ikhajiagbe, 2020b; Plate 1, 2 and Supplementary table 1). These isolates have significantly improved germination and yield parameters in rice in *in vitro* setup. In a bid to investigate the efficacy of these isolates to influence the iron-phosphate flux in soil and as biofertilizer, there is need to consider a field experiment. According to Saneya and Muhammad (2017), confirmation of efficacy *in vivo* is very important because *in vitro* studies may not necessarily give the real situation when introduced to the ecosystem or may not consider the real environmental conditions. This research considered rhizo-inoculation of rice seedlings with PSB strains in ferruginous ultisol conditions.

Rhizo-inoculation involves introduction of microorganism into the root region of plants to stimulate growth (Ikhajiagbe and Edokpolor, 2019). Rhizo-inoculation of PGPs are known to enhance plants' survival by releasing important plant nutrients as well as plant growth hormones (Glick, 1995). Gupta et al. (2000) and Biswas et al. (2000) have reported significant enhancement in growth and yield of important crops in response to rhizo-inoculation with PGPR. Previous research by Musa and Ikhajiagbe (2021) confirmed the use of PSB with PGP to improve the growth parameters of rice seeds. Increase in physiological parameters of sweet pepper (*Capsicum annum*) as response to rhizo-inoculation with PGP have been severally reported by Backer et al. (2018; 2017). Therefore, the present study aim to investigate the possibilities of these PSBs (*Bacillus cereus* strain GGBSU-1, *Klebsiella variicola* strain AUH-KAM-9 and *Proteus mirabilis* strain TL14-1.) that were previously obtained from FU soils and a control soil to improve the growth parameters of rice plant under FU conditions. The current study will bring about another sustainable strategy of improve agricultural productivity and food security.

2 Methods

2.1 Preparation of soil used in the experiment

The experiment was carried out at the experimental garden of the Department of Biology and Forensic Science, Admiralty University of Nigeria, Delta State Nigeria. Ferruginous soils that was previously obtained by Musa and Ikhajiagbe (2020a) from six locations around Benin City, Edo State of Nigeria were pooled to obtain a composite sample, whereas non-ferruginous soil (control) was obtained from rich-humus region at the deep underground root of a banana tree at the Botanical garden, University of Benin as reported by Musa and Ikhajiagbe (2020a). The ferruginous soil and the control soil were prepared in experimental bowls (30 x 25 cm) and made in five replicates.

2.2 Soil physiochemical parameter

The ferruginous soil sample and the control soil were air-dried at temperature of 22-25°C and then analyzed for soil organic matter levels (SOM), soil available phosphorus, cation exchange capacity (CEC), pH of the soil,

total nitrogen, organic carbon (OC), exchangeable acidity (EA), available potassium, available micronutrients such as sodium (Na) and Aluminum (Al), electrical conductivity, soil texture class and maximum water holding capacity following Musa and Ikhajiagbe (2020a). The iron levels of the soil was analyzed following the method of Cheng et al. (2013) by using concentrated perchloric acid to digest the soil sample and subjecting it to titration with versanate solution.

2.3 Sowing of rice seeds

An improved rice variety (FARO 44) previously obtained from the Center for Dryland Agriculture, Bayero University, Kano was used in this study. The rice seeds were analyzed for viability according to (AOSA, 2000) and sown at the rate of 10 seeds per pot. The experimental design was at an open environment and as such relied entirely on rainfall. The experimental region experiences a moderate rainfall and humidity (< 120-200 cm) during this study. However, the soil moisture content was maintained periodically as described by USDA (2010) method. This set up was weeded at every two days maintained for 16 days to allow seedling formation.

2.4 Bacterial species

Three phosphate solubilizing bacteria species (*Bacillus cereus* strain GGBSU-1, *Klebsiella variicola* strain AUH-KAM-9 and *Proteus mirabilis* strain TL14-1) that were isolated from FU soil and humus soil in an earlier study in Benin City by Musa and Ikhajiagbe (2021) were prepared in stock cultures for this study. The bacteria species were previously identified using molecular tool of 16S rRNA after biochemical test involving catalase, indole, citrate, nitrogen fixing activity and bromotymol blue test and pH tolerance level test with HCL following (Mondala et al., 2016). PGP capabilities of the isolates were determined by IAA and siderophores production following Gupta et al. (2012a) and Balkar, (2013) respectively and reported in Musa and Ikhajiagbe 2021. The phosphate solubilizing capabilities were determined by formation of a halo-zone region in pikovskaya's media as reported in in Musa and Ikhajiagbe 2021.

2.5 Preparation of inoculum

The pure PSB having PGP traits (*Bacillus cereus* strain GGBSU-1, *Klebsiella variicola* strain AUH-KAM-9 and *Proteus mirabilis* strain TL14-1) were prepared by streaking on to agar plates and incubated at 28°C for 48 hours. After 48 hours growth, the isolates were inoculated in Nutrient broth and then prepared into 0.5 McFarland Standard with Cat. No (TM50) to standardize the approximate number of bacteria in the suspension. Following this process 500 mL of each bacteria isolate was prepared to obtain an average microbial suspension of 1.5×10^8 cfu/mL.

2.6 Rhizo-inoculation of rice seedlings

After 16 days of seedling growth, the prepared McFarland standard (McFarland and Nephelometer, 1944) of 500 mL bacteria inoculum were made in to 10 mL of each bacteria (*Bacillus cereus* strain GGBSU-1, *Klebsiella variicola* strain AUH-KAM-9 and *Proteus mirabilis* strain TL14-1). To obtain the control, 10 mL of distilled water was prepared. All setup were made in five replicates. On to each seedling, the calculated inoculum volume was introduced into the root region of the growing seedling using 10 mL syringe following Etesami et al. (2014). The three bacteria numbered as (A= *Bacillus cereus* strain GGBSU-1, B= *Proteus mirabilis* strain TL14-1 and C= *Klebsiella variicola* strain AUH-KAM-9). The setup was further observed for 16 weeks using randomized

blocked design and wetted with 5 mL distilled water every 3 days. The experimental pots were weeded at every 2 days. Plant growth parameters were measured and recorded.

2.7 Morphological parameters

Morphological parameters that are related to growth and yield of rice were investigated. Fresh shoot length, fresh root length, panicle length and length of the first leaf were calculated in (cm) by using a transparent ruler that was mounted on a white calibrated paper throughout the study. To obtain the dry shoot length and dry root length (cm), seedlings were air dried for 24 hours and the root were measured from day 3 to 16 weeks after rhizo-inoculation. The length of internodes (cm) was measured from the coleoptile to the first node using a sample of 5 best seedlings from all treatments weekly, while the number of secondary roots were carefully observed and counted daily.

2.8 Physiological parameters

Total soluble sugar of fresh leaves were estimated a day before rhizo-inoculation (16th day) and at interval of 2 weeks after rhizo-inoculation till 16th week by drying the tallest leaf in oven at 70°C for 24h as described by Nelson (1944) with some modification by Sankar and Selvaraju (2015). Growth enzymes such as alpha amylase (AA) of the seedling and growing plant extract was determined at day 16 after sowing (before rhizo-inoculation) and at 2 weeks interval (after rhizo-inoculation) till 16th week (harvest day) by DNS method of Lowry et al. (1951) at a pH of 7.5. Terpenoid and lycopene of fresh leaves were determined at similar days as AA following the method of Moran (1982). Chlorophyll content index (CCI) of old and fresh leaves were determined using a non-destructive method by Apogee chlorophyll concentration meter. The CCI were measured as average of the mesocotyl, mid seedling and top seedling at similar days as AA. Chlorophyll *a* and *b* levels were determined following Arnon *et al* (1949); Maxwell and Johnson (2000).

2.9 Biomass parameters

Leaf area (cm²) was determined using an android application (Leaf-IT) following Julian et al. (2017) at 16th day after sowing (before rhizo-inoculation) and at from 2 weeks after rhizo-inoculation to the 16th week with 3 weeks intervals. Number of leaves were measured by counting at 16th day after sowing (before rhizo-inoculation) and at from 2 weeks after rhizo-inoculation to the 16th week with 2 weeks intervals. Leaf tip necrosis was calculated as the percentage of the total number of leaves produced by plants that showed significant signs of necrosis following Ikhajiagbe *et al.* (2017) at similar days as the number of leaves. Weight of fresh leaf (g) was calculated using analytical weighing balance at similar days as the number of leaves.

2.10 General growth characteristics at harvest

Time at which the rice plant matured was measured as the period when more than half the total number of seed began to turn dry following Ikhajiagbe et al., 2021 with slight modification. Average number of panicle per pot, number of tillers, number of reproductive tillers, and number of seeds per panicle were measured by counting. Average panicle weight, weight of husked rice, weight of de-husked rice, weight of peduncle without rice were measured using analytical weighing balance. 100 grains were counted from five plants of each replicate and weighed (g). Plant tissue water content was calculated at the harvest day as:

$$Tissue\ water\ content(\%) = \frac{(Freshweight - Dryweight) \times 100}{Freshweight}$$

2.11 Statistical analysis

Data obtained from the analysis were presented as means and standard errors of five replicates. Data were analyzed following two-way analysis of variance on GENSTAT (8th edition). Significant p-values were obtained, differences between means were separated using Student Newman Keuls Test (Alika, 2006). The ferruginous soil used in the current experiment was homogenized.

3 Results

3.1 Physico-chemical properties of the experimental soil

Table 1 showed the physical and chemical properties of the FU and the control soils used in the current study. The result revealed that originally, the control soil pH was 5.92 ± 0.98 with available phosphorus of 20.21 ± 0.05 mg/kg. This result is significantly higher than the available phosphorus observed in the FU soil 8.01 ± 0.04 mg/kg and the pH (5.01 ± 0.21). However, the iron content in the control soil (51.22 ± 1.48 mg/kg) is significantly lower than the iron content in the FU soil (200.67 ± 2.44 mg/kg).

Table 1

Physical and chemical parameters of the experimental non-ferruginous (control) and ferruginous soil.

Parameters	Non-ferruginous soil	Ferruginous soil
Available phosphorus (mg/kg)	20.21 ± 0.05	8.01 ± 0.04
Electric conductivity (µS/cm)	111.0 ± 1.55	301.09 ± 1.22
pH	5.92 ± 0.98	5.01 ± 0.21
Total organic carbon (%)	0.72 ± 0.10	0.41 ± 0.11
Soil organic matter (%)	17.08 ± 0.09	7.31 ± 0.56
Total Nitrogen (%)	0.20 ± 0.05	0.62 ± 0.01
Exchangeable acidity (meq/100g)	0.21 ± 0.20	0.16 ± 0.13
Cation exchange capacity (cmol/kg)	2.22 ± 0.01	1.70 ± 0.02
Textural class	Loam-silty	Loamy-sandy
Clay (%)	25.24 ± 0.01	10.92 ± 1.42
Silt (%)	40.10 ± 0.09	8.72 ± 2.76
Sand (%)	34.65 ± 0.03	95.10 ± 0.09
Fe (mg/kg)	51.22 ± 1.48	200.67 ± 2.44
Water holding capacity (%)	85.11 ± 0.02	68.89 ± 0.12
Available potassium (mg/kg)	0.11 ± 0.12	0.02 ± 0.08
Mg ²⁺ (meq/100g)	1.63 ± 0.04	4.21 ± 1.12
Na ⁺ (meq/100g)	1.91 ± 0.02	3.09 ± 0.29
Al (meq/100g)	0.74 ± 0.05	6.23 ± 1.22
Fe = iron, Al= aluminum, Na= sodium, Mg= magnesium, F = ferruginous soil, NF= non-ferruginous soil.		

3.2 Influence of inoculated PSBs on rice morphological parameters

3.2.1 Morphological performance of rice seedling before rhizo-inoculation

Significant differences (Table 2; Fig. 1) were observed in rice seedling morphological parameters between the ferruginous soils and the non-ferruginous soil before the rhizo-inoculation. For the 16 DAS study, the non-ferruginous soil showed 55.5% morphological yield compared to the ferruginous soils. However, no significant difference was determined in morphological yield among the seedlings grown in the ferruginous soils throughout the 16 days after sowing.

Table 2
Morphological performance of rice seedling before rhizo-inoculation

Soil samples	Fresh Shoot length (cm)				Fresh Root length (cm)			
	3DAS	7DAS	14DAS	16DAS	3DAS	7DAS	14DAS	16DAS
FA	1.9 ± 0.10 ^a	2.7 ± 0.14 ^a	5.7 ± 0.61 ^a	8.2 ± 3.11 ^a	0.7 ± 0.02 ^a	1.1 ± 0.64 ^a	1.4 ± 1.20 ^a	2.1 ± 0.215 ^a
FB	1.9 ± 0.17 ^a	2.5 ± 0.03 ^a	5.5 ± 0.11 ^a	8.0 ± 1.41 ^a	0.7 ± 0.38 ^a	1.2 ± 0.16 ^a	1.4 ± 1.16 ^a	2.1 ± 2.16 ^a
FC	1.9 ± 0.16 ^a	2.6 ± 0.22 ^a	5.5 ± 0.21 ^a	7.9 ± 0.34 ^a	1.0 ± 0.35 ^a	1.2 ± 0.10 ^a	1.6 ± 1.13 ^a	2.1 ± 1.22 ^a
FD	1.9 ± 0.72 ^a	2.7 ± 0.25 ^a	4.9 ± 0.11 ^b	8.2 ± 1.12 ^a	0.7 ± 0.14 ^a	1.1 ± 0.23 ^a	1.5 ± 1.13 ^a	1.9 ± 8.12 ^a
AVR	1.9 ± 0.13 ^a	2.6 ± 0.10 ^a	5.4 ± 0.06 ^a	8.0 ± 0.12 ^a	0.8 ± 0.19 ^a	1.1 ± 0.27 ^a	1.5 ± 0.43 ^a	2.0 ± 0.22 ^a
NF	3.2 ± 0.16 ^b	7.4 ± 0.02 ^b	9.0 ± 0.63 ^c	14.2 ± 1.00 ^c	2.7 ± 1.07 ^b	3.3 ± 0.11 ^b	3.4 ± 0.18 ^b	4.0 ± 0.10 ^b
Soil samples	Dry Shoot length (cm)				Dry Root length (cm)			
	3DAS	7DAS	14DAS	16DAS	3DAS	7DAS	14DAS	16DAS
FA	1.3 ± 0.12 ^a	1.8 ± 1.33 ^a	2.7 ± 0.12 ^a	5.1 ± 0.16 ^a	0.4 ± 0.11 ^a	0.6 ± 0.64 ^a	0.6 ± 0.12 ^a	1.0 ± 0.15 ^a
FB	1.4 ± 1.10 ^a	1.9 ± 1.24 ^a	2.9 ± 0.11 ^b	4.9 ± 0.11 ^a	0.2 ± 0.02 ^b	0.3 ± 0.26 ^b	0.3 ± 0.11 ^b	0.9 ± 0.53 ^a
FC	1.2 ± 2.36 ^a	1.7 ± 1.25 ^a	3.0 ± 0.14 ^b	5.1 ± 0.48 ^a	0.6 ± 0.10 ^c	0.8 ± 0.00 ^c	0.8 ± 0.03 ^c	1.1 ± 0.54 ^a
FD	1.2 ± 0.22 ^a	1.8 ± 0.18 ^a	3.0 ± 1.23 ^b	4.8 ± 0.44 ^a	0.2 ± 0.14 ^b	0.3 ± 0.25 ^b	0.3 ± 0.11 ^b	0.8 ± 0.11 ^a
AVR	1.2 ± 0.04 ^a	1.8 ± 0.23 ^a	2.9 ± 1.11 ^b	4.9 ± 0.11 ^a	0.3 ± 0.12 ^b	0.5 ± 0.37 ^a	0.5 ± 0.32 ^a	0.9 ± 0.14 ^a
NF	2.1 ± 0.23 ^b	5.9 ± 0.45 ^b	6.4 ± 0.23 ^c	9.8 ± 0.65 ^b	0.6 ± 0.71 ^c	1.4 ± 0.11 ^e	2.0 ± 0.09 ^d	2.8 ± 0.45 ^b

Soil samples	Length of first leaf (cm)				Number of secondary roots			
	3DAS	7DAS	14DAS	16DAS	3DAS	7DAS	14DAS	16DAS
FA	Not Present	1.0 ± 0.11 ^a	1.2 ± 0.11 ^a	3.2 ± 0.12 ^a	4.0 ± 0.21 ^a	4.0 ± 0.14 ^a	5.0 ± 0.12 ^a	6.0 ± 0.44 ^a
FB	Not Present	0.9 ± 0.34 ^a	1.1 ± 0.24 ^a	3.1 ± 0.11 ^a	2.0 ± 0.12 ^a	4.0 ± 0.61 ^a	5.0 ± 0.11 ^a	5.0 ± 0.11 ^b
FC	Not Present	0.9 ± 0.11 ^a	1.1 ± 0.68 ^a	3.4 ± 0.68 ^b	2.0 ± 2.12 ^a	5.0 ± 0.11 ^b	5.0 ± 0.31 ^a	5.0 ± 0.61 ^a
FD	Not Present	0.6 ± 1.22 ^b	0.8 ± 0.61 ^b	2.5 ± 0.22 ^c	2.0 ± 0.24 ^a	4.0 ± 0.15 ^a	5.0 ± 0.32 ^a	5.0 ± 0.55 ^a
AVR	Not Present	0.8 ± 0.43 ^b	1.1 ± 2.01 ^a	3.0 ± 0.12 ^a	2.0 ± 0.02 ^a	4.2 ± 0.57 ^a	5.0 ± 0.13 ^a	5.2 ± 0.34 ^a
NF	Not Present	1.7 ± 0.22 ^c	2.8 ± 0.22 ^c	4.4 ± 0.13 ^c	4.0 ± 0.09 ^b	9.0 ± 0.81 ^c	11.0 ± 0.21 ^b	16.0 ± 0.64 ^c

DAS= Days after sowing. Results showing similar superscripts on same column did not differ from each other at (p>0.05). FA-FD= Ferruginous soil, AVR= Average ferruginous soil, NF= Non-ferruginous soil.

3.2.2 Morphological performance of rice after rhizo-inoculation

After rhizo-inoculation of the rice seedling with the PSBs at day 16th after sowing, the setup was allowed to grow for two weeks. At the two weeks, significant increases were observed in fresh shoot length, dry shoot length, fresh root length, dry root length, length of internodes, number of secondary roots and stem girth in the rhizo-inoculated setup. The seedling inoculated with *Bacillus cereus* strain GGBSU-1 was observed to show highest morphological yield (80%) compared to the non-ferruginous soil (65%) (Fig. 2 and Plate 3). The ferruginous soil with no bacteria inoculation was observed to show lowest yield. More increase in morphological properties were observed in the inoculated seedling with increasing days after rhizo-inoculation (Supplementary table 2) however, the ferruginous soil without inoculation was observed to show no morphological improvement since week 6 after rhizo-inoculation.

3.3 Influence of inoculated PSBs on rice physiological parameters

3.3.1 Physiological performance of rice seedling before rhizo-inoculation

Physiological parameters relating to rice seedling yield at 16th DAS (before rhizo-inoculation) was investigated. Significant differences (Fig. 3) were witnessed in total soluble sugar (TSS), alpha amylase (AA) and chlorophyll content index (CCI) between the ferruginous soils and the non-ferruginous soil. The non-ferruginous soil showed greater physiology yield than the ferruginous soils. Terpanoids activity in rice seedling

from the non-ferruginous soil was observed to be significantly lower ($p > 0.5$; 24%) than from the ferruginous soil before rhizo-inoculation, while lycopene content was observed to be significantly higher ($p > 0.5$; 20%) in the non-ferruginous soil compared to the ferruginous soil.

Chlorophyll-a and Chlorophyll-b content of rice seedlings at day 8 and 16 after sowing (Table 3) also showed significant difference with the non-ferruginous soil, showing 50% greater than the chlorophyll levels in the ferruginous soils.

Table 3
Chlorophyll content of rice seedling before rhizo-inoculation

Soil samples	Chlorophyll-a (mg/cm ²) FW on		Chlorophyll-b (mg/cm ²) FW on	
	8DAS	16DAS	8DAS	16DAS
FA	6.4	8.9	2.9	3.6
FB	6.1	8.1	2.6	3.2
FC	6.5	8.5	2.6	3.0
FD	6.0	8.2	3.1	3.6
AVR	6.2	8.4	2.8	3.3
NF	9.9	16.1	8.8	11.9
DAS= Days after sowing, FW=fresh weight, FA-FD= Ferruginous soil, AVR= Average ferruginous soil, NF= Non-ferruginous soil.				

3.3.2 Physiological performance of rice after rhizo-inoculation

Results of the rice yield physiology at 2 weeks after rhizo-inoculation are presented in Fig. 4. Plants inoculated with the three bacterial species were observed to show significant increase (Fig. 4a) in TSS, AA, old and new leaf CII as compared with the seedling without rhizo-inoculation. The seedling rhizo-inoculated with *Bacillus cereus* strain GGBSU-1 was observed to show improved physiological yield properties, even though a not significant difference was witnessed in the both leaf CCI with the control.

Furthermore, Fig. 4b showed that terpenoid was higher in the growing rice seedling under ferruginous soil without inoculation, while the rice seedling from the non-ferruginous soil and those rhizo-inoculated with *Proteus mirabilis* strain TL14-1 and *Klebsiella variicola* strain AUH-KAM-9 showed no significant difference. Lycopene; a protein that protects plants from excessive light damage was observed to show significant increase in all rhizo-inoculated seedlings as compared to the non-inoculated seedlings (Fig. 4b). Also, the lycopene level at 2 weeks after rhizo-inoculation was observed to show no significant difference between the *Bacillus cereus* strain GGBSU-1 inoculated seedling and the control. However, the rice seedlings from non-inoculated ferruginous soil was observed to show lowest lycopene levels.

Supplementary table 3 showed the physiological performance of rice plant from 5 weeks after rhizo-inoculation to 16 weeks after rhizo-inoculation. The TSS and AA of rice plants inoculated with bacterial isolates were observed to show significant increases as compared to the non-inoculated plant. These

metabolites were observed to keep increasing with increasing weeks till harvest day (16WAI). The CCI of the old and new leaf were also witnessed to follow similar trend. However, the old and new leaf CCI of the ferruginous soil without inoculation was observed to remain the same with no significant increase. There was no significant difference between the old and new leaf CCI between the *Bacillus cereus* strain GGBSU-1 inoculated rice plant (FA) and the control (NF). However, a significant difference was witnessed across the three species of bacteria inocula.

The chlorophyll content of rice leaves from 5WAI to 16 WAI as observed in the (Supplementary table 3) showed significant changes in the chlorophyll pattern. In chlorophyll a, the plant from the ferruginous soil without inoculation was observed to be lowest as compared to the one from inoculated ferruginous soil and the control. A similar observation was seen in chlorophyll b. The rice leaves from the seedling rhizo-inoculated with *Bacillus cereus* strain GGBSU-1 (FA) in a ferruginous was seen to show highest levels of chlorophyll contents.

Results of terpenoids levels in the growing rice plants were analyzed and presented in (Supplementary table 3). Plants sown in FU soil without inoculation had significantly higher terpenoid levels and this keep increasing with increasing WAI. The control soils and the FU soils inoculated with species of bacterial showed no significant increase in terpenoids activities. The rice seedlings rhizo-inoculated with *Bacillus cereus* strain GGBSU-1 (FA) had reduction in terpenoid activities at their leaf-mid as compared to the other bacterial influenced plants. The Lycopene activity (Supplementary table 3) in rice plants showed that lycopene levels in rice leaves grown in FU soils was generally lower as against to the control. Meanwhile, the rice seedling that was rhizo-inoculated with the three PSBs showed significant increase in lycopene.

3.4 Influence of inoculated PSBs on rice biomass parameters

3.4.1 Biomass performance of rice seedling before rhizo-inoculation

Table 4 showed the performance of rice seedling at 16th DAS (before rhizo-inoculation). Rice seedlings from the non-ferruginous soil were observed to have higher weight of fresh leaf (0.09), leaf length area (2.0) and no leaf tip necrosis was observed as against the seedlings from ferruginous soils, where all the seedlings showed signs of necrosis.

Table 4
Biomass performance of rice seedling before rhizo-inoculation

Inoculum	WFP(g ⁻¹)	LLA (cm ²)	LIN (cm)	NL	LTN
FA	0.21 ± 0.11 ^a	1.77 ± 0.14 ^a	Not present	2.0 ± 0.10 ^a	2.0 ± 0.10 ^a
FB	0.46 ± 0.13 ^b	1.50 ± 0.22 ^b	Not present	2.0 ± 0.17 ^a	2.0 ± 0.17 ^a
FC	0.36 ± 0.11 ^c	1.50 ± 0.11 ^b	Not present	2.0 ± 0.16 ^a	2.0 ± 0.16 ^a
FD	0.33 ± 0.43 ^c	1.75 ± 0.48 ^a	Not present	2.0 ± 0.72 ^a	2.0 ± 0.72 ^a
AVR	0.34 ± 0.33 ^c	1.63 ± 0.33 ^a	Not present	2.0 ± 0.13 ^a	2.0 ± 0.13 ^a
NF	0.90 ± 0.67 ^d	2.00 ± 1.22 ^c	0.9 ± 0.16	2.0 ± 0.16 ^a	Not present
WFP= Weight of fresh plant, LA= Largest leaf area, LIN= Length of internode, NL= Number of leaf, LTN= Leaf tip necrosis. FA-FD= Ferruginous soil, AVR= Average ferruginous soil, NF= Non-ferruginous soil.					

3.4.2 Biomass performance of rice after rhizo-inoculation

At 2 weeks after rhizo-inoculation, the biomass performance of rice seedling (Fig. 5) was observed to show significant increase compared to the rice seedling without inoculation (FD). The *Bacillus cereus* strain GGBSU-1 inoculated seedling (FA) was seen to have higher weight of fresh plant, while the plant from ferruginous soil without any bacterial inoculation (FD) showed lowest weight of fresh plant. Higher leaf internode was observed in the non-ferruginous soil as there was no significant difference between the lengths of internode in the ferruginous soil without inoculation and those with inoculation. A significant increase in number of leaf was observed with the inoculation of all bacterial species. The non-ferruginous soil and the ferruginous soil inoculated with *Bacillus cereus* strain GGBSU-1 showed no significant difference. However, the non-ferruginous soil showed more signs of leaf tip necrosis compared to the *Bacillus cereus* strain GGBSU-1 inoculated seedling. The rice plant from ferruginous soil with no bacterial inoculation showed the lowest number of leaf (2) of which all showed observable sign of leaf tip necrosis.

Supplementary table 4 showed the biomass parameters in terms of weight of fresh plants, largest leaf area, number of leaves and leaf tip necrosis. A significant increase (29%) was observed in the FA as against the control (non-ferruginous soil without inoculation). The ferruginous soil without bacterial inoculation (FD) was observed to show the least plant weight at all the assayed days. Before rhizo-inoculation, the control seedlings were observed to show highest leaf area (Table 4), while seedlings from ferruginous soil showed least, this trend was changed as significant increases were observed in the bacterial inoculated seedlings with the FA showing the highest leaf area while the plant from (FD) showed the least. Also, no increase was observed in the (FD) leaf area with increasing days after inoculation. A significant increase was observed in the number of leaves in the FA as against the control. However, the FD showed least leaf number and all the leaves were observed to show signs of necrosis. At 16th WAI, FA showed 11% leaf necrosis, the FB showed 23% leaf necrosis, while the FC was observed to show 36% necrosis. The plant from NF without bacterial inoculation showed 34% necrosis while the FD 100% necrosis.

3.5 General growth characteristics at harvest day

The time at which the rice matured was observed to be the 13th week after sowing for plants inoculated with bacterial seedlings in ferruginous soil. However, the non-ferruginous soil was observed to mature at 14th week. For the ferruginous soil without bacterial inoculation (FD) growth and yield seized at the 3rd week after sowing (Fig. 6).

Figure 7 showed the number of tillers and reproductive tillers observed at harvest day. A significant difference was observed among all the rice plat. The FA was observed to show highest NT and NRT while the FC was observed to show the least. In the FA, only 3 of 23 tillers did not produce panicle and seed while, while in the FC, 6 of 15 tillers did not produce panicle and seed. Similarly, the FA was observed to show improved number of panicle, straw yield and seed per panicle (Figure 7 and Fig. 8). However, there was no significant difference in the straw yield and number of seed per panicle between the FA and the non-ferruginous soil (NF).

The 100-grain weight at harvest was observed to be significantly lower in the FC than other inoculation treatments. About 15% increase in the 100-grain weight was observed in the FA which showed no significant difference with the NF ($p>0.05$). A similar results was obtained in the plant tissue water content (Table 5). The FA was observed to have highest yield in terms of weight of rice panicle, weight pf peduncle without rice, weight of husked and de-husked rice seed (Table 5 and Fig. 9).

Table 5
Rice yield parameters at harvest

Soil samples	WRP (g)	WPWR (g)	100 grains (g)	PTW
FA	2.61 ± 0.26^a	0.58 ± 1.34^a	2.7 ± 0.97^a	0.71 ± 2.11^a
FB	2.38 ± 0.11^b	0.36 ± 0.22^b	2.5 ± 0.55^c	0.64 ± 0.44^b
FC	0.74 ± 0.16^c	0.07 ± 0.34^c	2.3 ± 0.76^b	0.64 ± 0.98^b
NF	2.42 ± 0.34^b	0.393 ± 0.87^b	2.66 ± 0.25^a	0.7 ± 0.66^a

WRP= Weight of rice panicle, WPWR = weight of peduncle without rice, 100 grains = weight of 100 grains and PTW= plant tissue water. Here, FA= rice plant in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice plant in ferruginous soil, rhizo-inoculated with *Proteus mirabilis* strain TL14-1; FC = rice plant in ferruginous soil, rhizo-inoculated with *Klebsiella variicola* strain AUH-KAM-9; FD = rice seedling in ferruginous soil without rhizo-inoculation; NF = (control) rice seedling in non-ferruginous soil without rhizo-inoculation.

Root development and architecture at harvest showed a more developed nature at the FA compared to FB, FC and NF, even though the NF showed more root hair (Fig. 10)

4 Discussion

The influence of bacterial inoculum on growth parameters of rice in FU soil comparative to the control (non-ferruginous soil) have been analyzed. Ferrugenicity is defined most especially by the high iron, acidity and low phosphorus levels in soils (Musa and Ikhajiagbe, 2020a) as observed in Table 1.

Before rhizo-inoculation, the non-ferruginous soil (NF) was observed to show higher morphological parameters than the ferruginous soil. This clearly showed the anti-growth properties of the ferruginous soil (Wang et al., 2014). At 2 weeks after inoculation, the morphological properties were improved especially in the *Bacillus cereus* strain GGBSU-1 inoculated seedling, even more than the control. This has avail the ability of bacterial to improve plant morphology. This set up was continuously monitored for 16 weeks and similar trend was observed. This is likely as a result of the ability of these bacteria to interact with the root architecture and improving water use efficiency and physico-chemical parameters of the rhizospheric soil, thereby improving rice plant morphology. This is consistent with the work of Gupta et al. (2012b) who observed that treatment of plants with certain PSB bacteria consequently improved plant morphological parameters.

Plant productivity can be determined using total sugars. Before inoculation, a low plant physiological parameters was observed in the ferruginous soil as against the non-ferruginous soil. This shows the low productivity of ferruginous soil. Terpenoid is usually known to signify biotic stress and as part of plant defense mechanism (Bharat and Ram, 2015), the high level of terpenoid observed in the seedlings from ferruginous soil showed some possible biotic stressors, this stress may be linked to the poor physico-chemical conditions of the soil (Table 1) or under nutrient stress. However, the low terpenoid observed in the non-ferruginous soil indicate low biotic stress. The significant reduction in terpenoid levels observed in the ferruginous soils with the introduction of all bacterial inoculum signifies nutrient availability and improvement in biotic properties. Furthermore, low photosynthetic pigments chlorophyll-a and chlorophyll-b that were observed in the ferruginous soil as against the non-ferruginous soil signifies the negative effect of the ferruginous soil on rice seedlings. Since poor soil condition can damage photosynthetic pigments and bring negative effects on gas exchange (Lin et al., 2018), it is most likely the reason why plants physiological parameters in the ferruginous soil was influenced. But from two weeks after rhizo-inoculation of bacterial species, a significant increase was observed in all photosynthetic pigments, this may be that the bacteria have improved the chlorophyll metabolism pathways (Musa and Ikhajiagbe, 2021; Ikhajiagbe et al., 2021).

Rice seedlings with higher sugar contents and AA may signify improved growth and yield (Lucas et al., 2014; Ikhajiagbe et al., 2020), the low sugar contents in ferruginous soil may imply the opposite. The significant increase in AA and TSS witnessed with the introduction of the PSBs indicated the enzymatic roles of the PSB isolates. Different bacterial stains modify different innate plant mechanisms such as total soluble sugar at different levels (Xia et al., 2019).

The low weight of fresh plant observed in the ferruginous soil as against the non-ferruginous further showed the anti-growth properties of the soil. However, an improved weight was observed from 2 weeks after PSBs inoculation till harvest. This result is consistent with the work of Sarma and Saikia (2014) who suggested that *Bacillus lentimorbus* and *Pseudomonas aeruginosa* strains improved biomass properties of *Vigna radiata* plants under drought stress. All the leaves observed in the ferruginous soils turned to show signs of leaf tip necrosis. Deficiency in photosynthetic pigments usually brings about necrosis (Ann, 2009) and this was observed in the ferruginous soil before inoculation. However, photosynthetic pigments were improved with the rhizo-inoculation which may be the reason why a low necrosis rate was observed in the PSB inoculated seedlings compared to the non-inoculated seedling. The effect of the bacteria inocula on leaf tip necrosis of rice showed the beneficial influence of the growth-promoting bacteria on rice performance. The present result

agreed with the work of Beckley and Edokpolor (2019) who investigated the influence of growth promoting rhizobacteria on rice where they found reduced necrosis rate with the introduction of different bacteria isolates.

The FA showed fastest time at which the rice matured. This signifying the effectiveness of the *Bacillus cereus* strain GGBSU-1 strain as against the other isolates. This effect is also observable when higher tillers and reproductive tillers were produced in the FA compared to others. All harvest parameters showed significant increase in the FA as against other isolates. Root systems influences plant fitness, health and productivity (Adnan et al., 2018). A more developed root architecture that was observed in the FA showed the positive influence of the PSB, leading to more establishment of new micro-environments and ecological niches for different microbial species, in order to bring about beneficial plant-bacteria interaction at the rhizospheric regions.

5 Conclusions

The current study has been established that rhizo-inoculation of rice seedling with phosphate solubilizing bacteria having plant growth-promoting capabilities have the ability to improve growth parameters of rice plant grown in ferruginous soil. Results of this study showed the ferruginous soil to be having low nutrients with high iron as against the non-ferruginous soil. Before rhizo-inoculation, rice growth parameters were observed to be low in the ferruginous soil setup, which increase significantly after rhizo-inoculation with the three PSB strains. The three PSB bacteria showed different levels of growth and yield properties on the rice plant. Generally, the setup inoculated with *Bacillus cereus* strain GGBSU-1 (FA) was observed to show the highest growth influence on the rice seeds, followed by *Proteus mirabilis* strain TL14-1 (FB) while *Klebsiella variicola* strain AUH-KAM-9 (FC) was observed to show the least rice growth parameters. Since these bacteria proved effective in improving growth and yield parameters of rice plant in ferruginous ultisol, further studies should be conducted to trail the effectiveness of these bacteria on other important crops in nutrients deficient soils. This would provide a sustainable way of enhancing crop production to serve increasing world population.

6 List Of Abbreviations

FU: Ferruginous ultisol; CFU/mL: Colony forming unit per milliliter; FA: Rice growing in ferruginous soil, Which was rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB: Rice growing in ferruginous soil, Which was rhizo-inoculated with *Proteus mirabilis* strain TL14-1; FC: Rice growing in ferruginous soil, Which was rhizo-inoculated with *Klebsiella variicola* strain AUH-KAM-9; FD: Rice growing in ferruginous soil without rhizo-inoculation; 16S rRNA: 16S ribosomal RNA; TSS: Total soluble sugar contents; AA: Alpha amylase; CCI: Chlorophyll Context Index; OL: Old Leaf; NL: New Leaf; WFP: Weight of Fresh Plant; LA: Largest Leaf Area; LIN: Length of Internode; NL: Number of Leaf; LTN: Leaf Tip Necrosis; WFP: Weight of Fresh Plant; LLA: Largest Leaf Area; LTN: Leaf Tip Necrosis; NT: Number of tillers; NRT: Number of Reproductive Tillers at harvest day; ANP: Number of Panicle per pot; ASY: Straw Yield; NSP: Number of Seed per Panicle at harvest day; WRP: Weight of Rice Panicle; WPWR: Weight of Peduncle without Rice; PTW: Plant Tissue Water. WHS: Weight of Husked Seed; WDHS: Weight of Dehusked Seed at harvest day; DAS: FW: Weight of Fresh Plant; WAI: Weeks After Rhizo-inoculation.

7 Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not Applicable

Availability of data and material

The datasets generated and/or analyzed during the current study are included in this published manuscript and as supplementary materials.

Competing interests

The authors declare no conflict of interests

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Authors' contributions

BI and MSI designed the study, MSI carried out the research under the supervision of BI. MSI carried out the statistical analysis and interpretation of data. MSI wrote the first draft. BI edited the final draft of the manuscripts. The authors read and approved the final manuscript.

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Addendum

The Addendum is available in the Supplementary Files section.

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Figures

Figure 1

Rice seedling performance at day 8 and 16 after sowing. Control soil = Non-ferruginous soil. DAS= Days after sowing

Figure 2

Morphological parameters of rice seedling at 2 weeks after rhizo-inoculation (30 days after sowing). Here, FA= rice seedling in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice seedling in ferruginous soil, rhizo-inoculated with *Proteus mirabilis* strain TL14-1; FC = rice seedling in ferruginous soil, rhizo-inoculated with *Klebsiella variicola* strain AUH-KAM-9; FD = rice seedling in ferruginous soil without rhizo-inoculation; Control = rice seedling in non-ferruginous soil without rhizo-inoculation.

Figure 3

Total soluble sugar contents (TSS), Alpha amylase (AA), chlorophyll context index (CCI) of old (OL) and new (NL) leaf terpanoid and lycopene at 16th day after sowing. FA-FD= Ferruginous soil, NF= Non-ferruginous soil.

Figure 4

a and b: Total soluble sugar contents (TSS), Alpha amylase (AA), chlorophyll context index (CCI) of old (OL) and new (NL) leaf terpanoid and lycopene at 2 weeks after rhizo-inoculation. Here, FA= rice seedling in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice seedling in ferruginous soil, rhizo-inoculated with *Proteus mirabilis* strain TL14-1; FC = rice seedling in ferruginous soil, rhizo-inoculated with *Klebsiella variicola* strain AUH-KAM-9; FD = rice seedling in ferruginous soil without rhizo-inoculation; NF = rice seedling in non-ferruginous soil without rhizo-inoculation.

Figure 5

Weight of fresh plant (WFP), Largest leaf area (LLA), Length of internode (LIN), Number of leaves (NL) and Leaf tip necrosis (LTN) at 2 weeks after rhizo-inoculation. Results showing same alphabets on same bars did not differ from each other ($p > 0.05$). Here, FA= rice seedling in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice seedling in ferruginous soil, rhizo-inoculated with *Proteus mirabilis* strain TL14-1; FC = rice seedling in ferruginous soil, rhizo-inoculated with *Klebsiella variicola* strain AUH-KAM-9; FD = rice seedling in ferruginous soil without rhizo-inoculation; NF = (control) rice seedling in non-ferruginous soil without rhizo-inoculation.

Figure 6

Growth performance of rice at week 15th after sowing. Here, FA= rice plant in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice plant in ferruginous soil, rhizo-inoculated with *Proteus mirabilis* strain TL14-1; FC = rice plant in ferruginous soil, rhizo-inoculated with *Klebsiella variicola* strain AUH-KAM-9; FD

= rice seedling in ferruginous soil without rhizo-inoculation; NF = (control) rice seedling in non-ferruginous soil without rhizo-inoculation.

Figure 7

Number of tillers (NT) and number of reproductive tillers (NRT) at harvest day. Here, FA= rice plant in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice plant in ferruginous soil, rhizo-inoculated with *Proteus mirabilis* strain TL14-1; FC = rice plant in ferruginous soil, rhizo-inoculated with *Klebsiella variicola* strain AUH-KAM-9; FD = rice seedling in ferruginous soil without rhizo-inoculation; NF = (control) rice seedling in non-ferruginous soil without rhizo-inoculation.

Figure 8

a and b: Number of panicle per pot (ANP), straw yield (ASY) and number of seed per panicle (NSP) at harvest day. Results are average of five replicates. Here, FA= rice plant in ferruginous soil, rhizo-inoculated with *Bacillus*

cereus strain GGBSU-1; FB = rice plant in ferruginous soil, rhizo-inoculated with *Proteus mirabilis* strain TL14-1; FC = rice plant in ferruginous soil, rhizo-inoculated with *Klebsiella variicola* strain AUH-KAM-9; FD = rice seedling in ferruginous soil without rhizo-inoculation; NF = (control) rice seedling in non-ferruginous soil without rhizo-inoculation.

Figure 9

Weight of husked seed (WHS) and weight of dehusked seed (WDHS) at harvest day. Results are average of five replicates. Here, FA= rice plant in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice plant in ferruginous soil, rhizo-inoculated with *Proteus mirabilis* strain TL14-1; FC = rice plant in ferruginous soil, rhizo-inoculated with *Klebsiella variicola* strain AUH-KAM-9; FD = rice seedling in ferruginous soil without rhizo-inoculation; NF = (control) rice seedling in non-ferruginous soil without rhizo-inoculation.

Figure 10

Root architecture of rice plants at harvest day (16 WAI). Here, FA= rice plant in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice plant in ferruginous soil, rhizo-inoculated with *Proteus mirabilis* strain TL14-1; FC = rice plant in ferruginous soil, rhizo-inoculated with *Klebsiella variicola* strain AUH-KAM-9; FD = rice seedling in ferruginous soil without rhizo-inoculation; NF = (control) rice seedling in non-ferruginous soil without rhizo-inoculation.

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