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Evaluation of Previously Isolated and Characterized Phosphate Solubilizing Bacteria with Plant Growth-Promoting Potentials for Rice Grown Ferruginous Ultisol Soil in Benin City, Edo State, Nigeria

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ABSTRACT: The objective of this paper is to evaluate the potentials of previously isolated and characterized phosphate solubilizing bacteria (PSB) [*Bacillus cereus* strain GGBSU-1, *Proteus mirabilis* strain TL14-1, and *Klebsiella variicola* strain AUH-KAM-9] with plant growth-promoting (PGP) capabilities on growth properties of rice plants under ferruginous ultisol (FU) conditions through a rhizo-inoculation strategy. 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 and high iron levels which has led to low growth properties of rice seeds sown in FU soil without rhizo-inoculation. After rhizo-inoculation, a significant improvement was 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. This research suggests that PSB rhizoinoculation technique can be used in improving growth properties of rice plants even under FU conditions.

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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).

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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 considered inadequately use of synthetic chemicals which had negative influence on the soil and plants as a result 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). 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 plant growth promoting capabilities. Previous research by Musa and Ikhajiagbe (2021) confirmed the use of specific indigenous bacteria 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 objective of this paper is to evaluate the potentials of previously isolated and characterized phosphate solubilizing bacteria (PSB) [*Bacillus cereus* strain GGBSU-1, *Proteus mirabilis* strain TL14-1, and *Klebsiella variicola* strain AUH-KAM-9] with plant growth-promoting (PGP) potentials for Rice grown on ferruginous ultisol (FU) soil in Benin City, Edo State of Nigeria through rhizoinoculation. The current study will bring about another sustainable strategy of improve agricultural productivity and food security.

MATERIALS AND METHODS

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 were 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.

Soil physiochemical parameter: The ferruginous soil sample and the control soil were air-dried at temperature of 22-25^oC 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 were 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.

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.

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 bromotyhmol 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 holo-zone region in pikovskaya's media as reported in in Musa and Ikhajiagbe 2021.

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^{o}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 $1.5x10^8$ cfu/mL.

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 bacterium (*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 2dyas. Plant growth parameters were measured and recorded.

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.

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^{o}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).

Biomass parameters: Leaf area (cm^2) was determined using an android application (Leaf-IT) following Julian *et al*. (2017) at 16th day after sowing (before rhizo-inoculation) and at from 2weeks 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 2weeks 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.

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). Average number of panicles 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 (Equation 1) following Ikhajiagbe *et al.* (2021)

$$Tissue\ water\ content\ (\%) = \frac{(\text{Fresh weight} - \text{Dry weight})\ \text{X}\ 100}{\text{Fresh weight}} \quad (1)$$

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).

RESULTS AND DISCUSSION

Physicochemical 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). These physicochemical parameters of the FU in the current study are consistent with the observation of Musa and Ikhajiagbe (2020a). FU is defined most especially by the high iron, acidity and low phosphorus levels in soils as observed in table 1.

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.

Influence of inoculated PSBs on rice morphological parameters: Morphological performance of rice seedling before rhizo-inoculation: Significant differences (Table 2a,b and c; 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. This clearly showed the anti-plant- growth nature of the ferruginous soil (Wang *et al.*, 2014). According to Musa and Ikhajiagbe (2020a), the high iron, high pH and low phosphorus levels of ferruginous soils impaired plant growth.

Table 2a: Morphological performance of rice seedling before rhizo-inoculation

	Fresh Shoot length (cm)				Fresh Root length (cm)			
Soil samples	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]

Table 2b: Morphological performance of rice seedling before rhizo-inoculation

	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]

Table 2c: Morphological performance of rice seedling before rhizo-inoculation

	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.

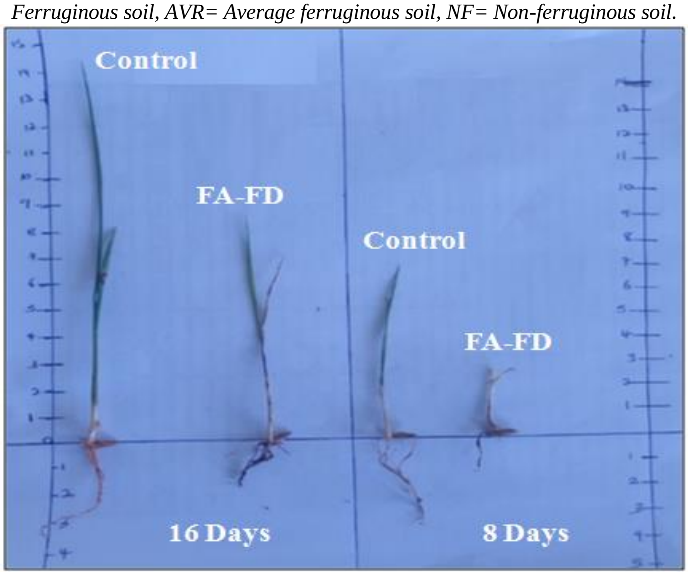

Fig. 1: Rice seedling performance at day 8 and 16 after sowing. Control soil = Non-ferruginous soil. DAS= Days after sowing

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. This has availed the ability of bacterial to improve plant morphology. 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). 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. 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.

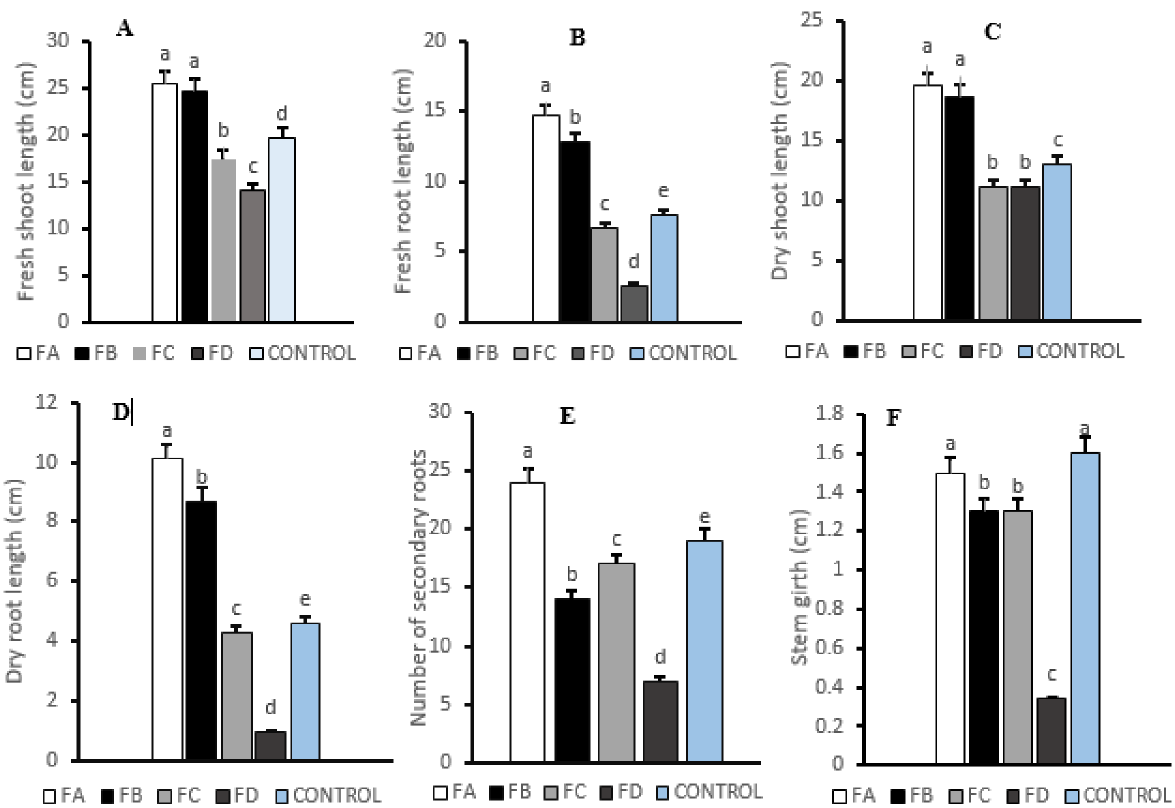

Fig. 2a-f: Morphological parameters of rice seedling at 2 weeks after rhizo-inoculation (30 days after sowing). Here, FA= rice seedling in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice seedling in ferruginous soil, rhizo-inoculated with *Proteus mirabilis* strain 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.

Influence of inoculated PSBs on rice physiological parameters: 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 FU soils and the non-ferruginous soil. The non-ferruginous soil showed greater physiology yield than the FU soils. This shows the low productivity of FU soil. Terpanoids activity in rice seedling from the non-ferruginous soil was observed to be significantly lower ($p > 0.5$; 24%) than from the FU soil before rhizo-inoculation. 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 the FU soil before rhizo-inoculation showed some possible biotic stressors, which may be linked to the poor physico-chemical conditions of the soil (Table 1) that signifies nutrient stress. Significantly high ($p > 0.5$; 20%) lycopene content was observed in the non-ferruginous soil compared to the FU soil, which further signifies biotic stress (Musa and Ikhajiagbe, 2024). 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 FU soils also signifies the negative effect of the FU 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.

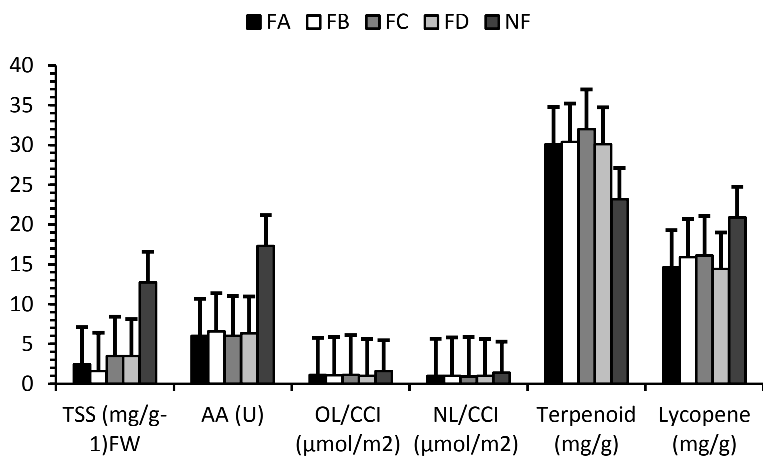

Fig. 3: Total soluble sugar contents (TSS), Alpha amylase (AA), chlorophyll context index (CCI) of old (OL) and new (NL) leaf terpanoid and lycopene at 16th day after sowing. FA-FD= Ferruginous soil, NF= Non-ferruginous soil.

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.

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. Plant productivity can be determined using TSS and AA (Musa *et al.*, 2024). 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. This significant increase in plant physiological parameters after bacteria rhizo-inoculation signifies nutrient availability and improvement in biotic properties. As reported by comfort *et al*. (2024), availability of increased PGP bacteria community in the rhizoid improves nutrient availability and assimilation by plant tissues. Also, the increase in all photosynthetic pigments, may be that the bacteria have improved the plant chlorophyll metabolism pathways (Musa and Ikhajiagbe, 2021; Ikhajiagbe *et al.*, 2021).

Furthermore, Fig. 4b showed that terpenoid was higher in the growing rice seedling under FU 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 were observed to show lowest lycopene levels.

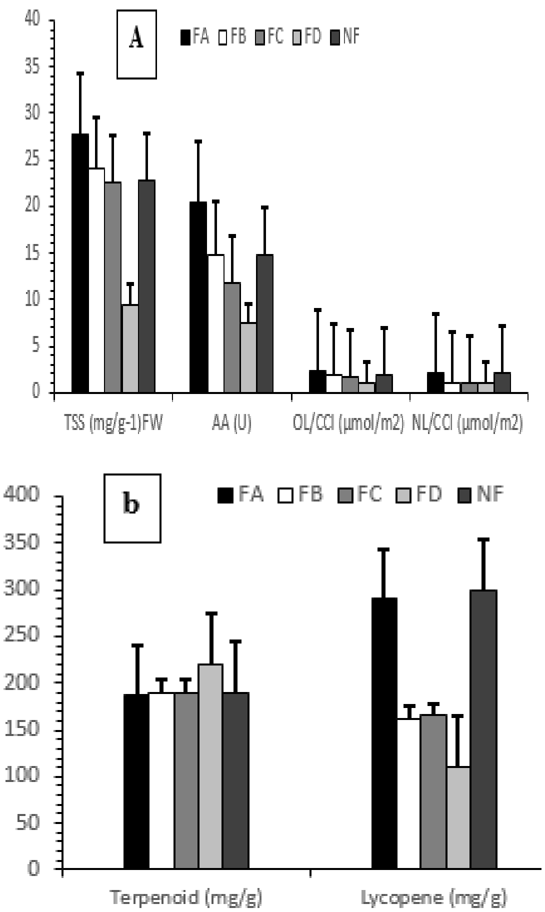

Fig. 4a and b: Total soluble sugar contents (TSS), Alpha amylase (AA), chlorophyll context index (CCI) of old (OL) and new (NL) leaf terpanoid and lycopene at 2 weeks after rhizo-inoculation. Here, FA= rice seedling in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice 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. 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). This 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 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.

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 FU soil without inoculation was observed to be lowest as compared to the one from inoculated ferruginous soil and the control, indicating the photosynthetic modifications resulting from the rhizo-inoculation. 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, indicating poor soil condition.

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.

Influence of inoculated PSBs on rice biomass parameters: 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 FU soils, where all the seedlings showed signs of necrosis. This further showed the anti-growth properties of the FU soil.

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.

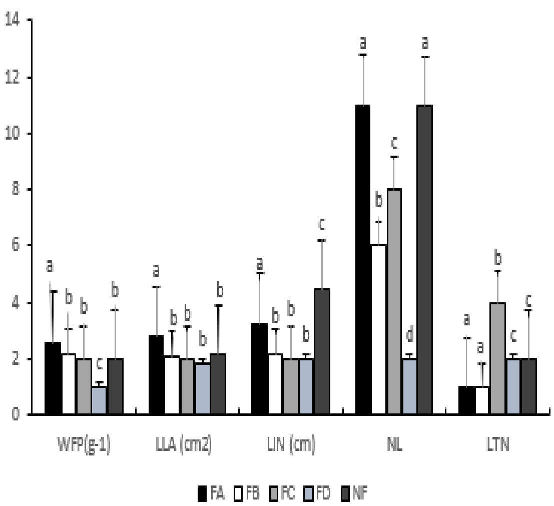

Fig. 5: Weight of fresh plant (WFP), Largest leaf area (LLA), Length of internode (LIN), Number of leaves (NL) and Leaf tip necrosis (LTN) at 2 weeks after rhizo-inoculation. Results showing same alphabets on same bars did not differ from each other ($p>0.05$). Here, FA= rice seedling in ferruginous 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.

This result is consistent with the work of Sarma and Saikia (2014) who observed that *Bacillus lentimorbus* and *Pseudomonas aeruginosa* strains improved biomass properties of *Vigna radiata* plants under drought stress. A significant increase in number of leaves was observed with the inoculation of all bacterial species. The non-ferruginous soil and the FU 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 (2) of leaf of which all showed observable sign of leaf tip necrosis. Deficiency in photosynthetic pigments usually brings about necrosis (Ann, 2009). 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.

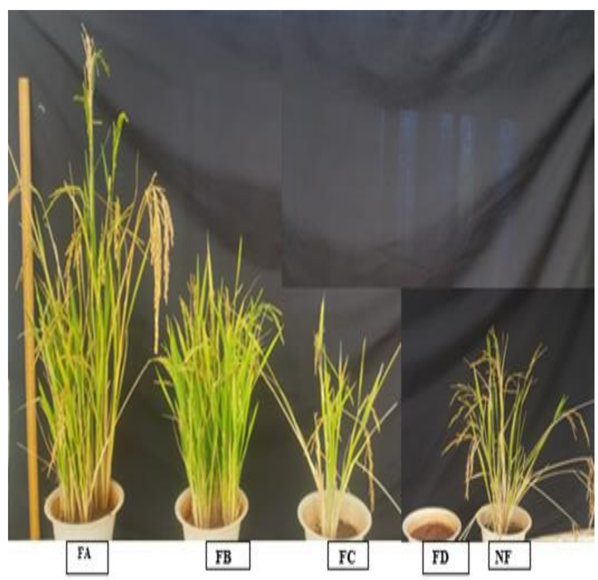

Fig. 6: Growth performance of rice at week 15th after sowing. Here, FA= rice plant in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice plant in ferruginous soil, rhizo-inoculated with *Proteus mirabilis* strain TL14-1; FC = rice plant in ferruginous soil, rhizo-inoculated 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.

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.

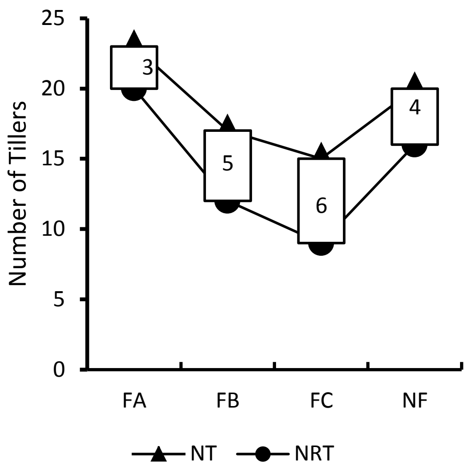

Fig. 7: Number of tillers (NT) and number of reproductive tillers (NRT) at harvest day. Here, FA= rice plant in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice plant in ferruginous soil, rhizo-inoculated with *Proteus mirabilis* strain TL14-1; FC = rice plant in ferruginous 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.

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 FU 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). 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. Fig. 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.

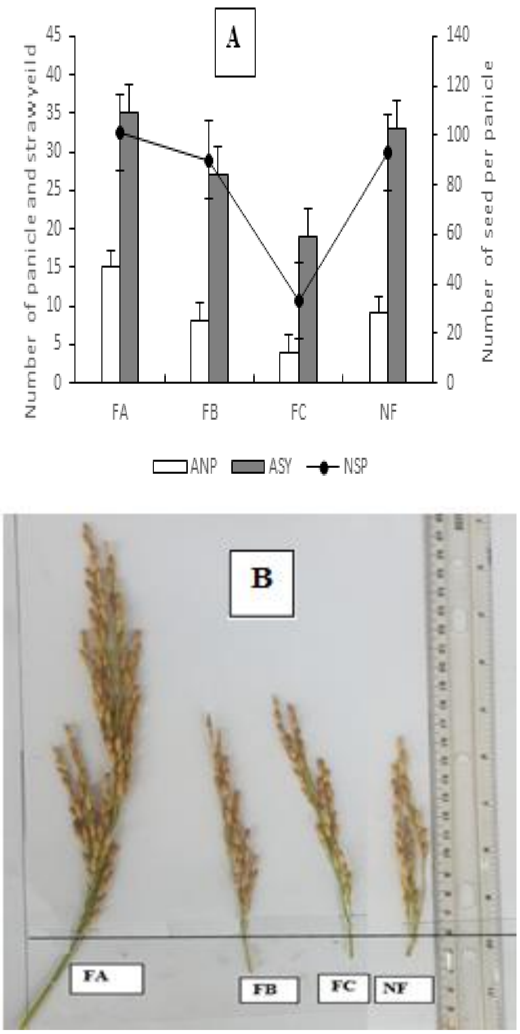

Fig. 8a and b: Number of panicle per pot (ANP), straw yield (ASY) and number of seed per panicle (NSP) at harvest day. Results are average of five replicates. Here, FA= rice plant in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice plant in ferruginous soil, rhizo-inoculated 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.

Similarly, the FA was observed to show improved number of panicles, straw yield and seed per panicle (Fig. 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>00.05). A similar result was obtained in the plant tissue water content (Table 5).

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.

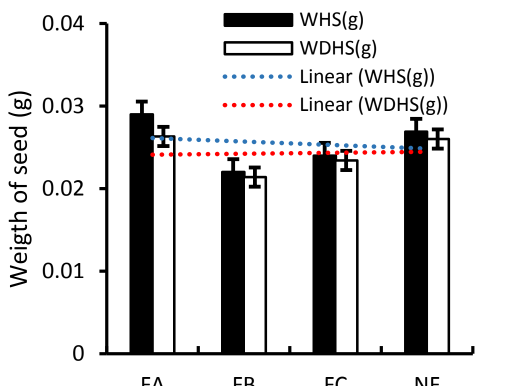

Fig. 9: Weight of husked seed (WHS) and weight of dehusked seed (WDHS) at harvest day. Results are average of five replicates. Here, FA= rice plant in ferruginous soil, rhizo-inoculated with *Bacillus cereus* strain GGBSU-1; FB = rice plant in ferruginous soil, rhizo-inoculated with *Proteus mirabilis* 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.

Fig. 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.

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). 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). 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). A more developed root architecture that was observed in the FA showed the positive influence of the PSB, leading to more establishments of new micro-environments and ecological niches for different microbial species, in order to bring about beneficial plant-bacteria interaction at the rhizospheric regions

Conclusions: The current study has established that rhizo-inoculation of rice seedling with phosphate solubilizing bacteria with plant growth-promoting capabilities have the ability to improve growth parameters of rice plant grown in ferruginous soil. From this study, ferruginous soil has shown to be a low nutrient and high iron 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.

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.

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Availability of data: All data are available upon request from the first author

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