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Role of phosphate solubilizing bacteria on rock phosphate solubility and growth of aerobic rice

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Abstract

Use of phosphate-solubilizing bacteria (PSB) as inoculants has concurrently increased phosphorous uptake in plants and improved yields in several crop species. The ability of PSB to improve growth of aerobic rice (*Oryza sativa* L.) through enhanced phosphorus (P) uptake from Christmas island rock phosphate (RP) was studied in glasshouse experiments. Two isolated PSB strains; *Bacillus* spp. PSB9 and PSB16, were evaluated with RP treatments at 0, 30 and 60 kg ha^{-1}. Surface sterilized seeds of aerobic rice were planted in plastic pots containing 3 kg soil and the effect of treatments incorporated at planting were observed over 60 days of growth. The isolated PSB strains (PSB9 and PSB16) solubilized significantly high amounts of P (20.05-24.08 mg kg^{-1}) compared to non-inoculated (19-23.10 mg kg^{-1}) treatments. Significantly higher P solubilization (24.08 mg kg^{-1}) and plant P uptake (5.31 mg plant^{-1}) was observed with the PSB16 strain at the highest P level of 60 kg ha^{-1}. The higher amounts of soluble P in the soil solution increased P uptake in plants and resulted in higher plant biomass (21.48 g plant^{-1}). PSB strains also increased plant height (80 cm) and improved root morphology in aerobic rice. The results showed that inoculation of aerobic rice with PSB improved phosphate solubilizing activity of incorporated RP.

Key words

Aerobic rice, *Bacillus* spp., Christmas island, Rock phosphate (RP), Inoculation, Phosphate solubilizing bacteria (PSB)

Introduction

Phosphorus (P) is an essential plant nutrient for plant growth. Most soils contain considerable reserves of total P, but a major portion of it remains comparatively immobile and only less than 10% of soil P enters the plant-animal cycle (Kucey *et al*., 1989). P fertilizers are required for crop production, but only a small part of P is utilized by plants, while the rest is converted into insoluble fixed forms (Rodriguez and Fraga, 1999). P deficiency is usually the consequence of low intrinsic P fertility due to weathering, in combination with intensive, nutrient-extracting agricultural practices (Sanchez *et al*., 1997). Moreover, phosphate diffusion to plant roots may be too low to meet the requirements of the crop if soils have low P solubility and a high P fixation capacity (Hoberg *et al*., 2005). P solubilization mechanism is different in aerobic condition compared to anaerobic rice cultivation system. Nutrient deficiencies have been studied in upland rice systems where rice is grown in aerobic soils. P deficiency has been recognized as one of the main limiting factors in upland rice production in many parts of the world (Sahrawat *et al*., 2001). Deficiency in N and P in upland rice is quite common (Fageria and Breseghello, 2001). The demand for P fertilizer application can be more serious for aerobic rice than for flooded lowland rice as higher P fixation occurs in aerobic soils. Almost 75 to 90% of added P fertilizer in agricultural soils is precipitated by iron, aluminum and calcium complexes present in soils (Turan *et al*., 2006). Furthermore, phosphatic fertilizers are expensive, and excessive use of rock phosphate (RP) is potentially and environmentally undesirable.

There are substantial deposits of cheaper low grade RP in many countries of the world (Sharma and Prasad, 1996). Hence direct use of such RP could minimize pollution and decrease the cost of chemical treatment. However, there is growing interest in finding ways of manipulating such rocks into more value added products. Common efforts include the use of chemicophysical means, such as partially acidulating RP with synthetic and natural organic acids and decreasing particle size (Singh and Amberger, 1998). These approaches involve additional costs.

Rock phosphate application is also not economically feasible under soil conditions characterized by high P sorption capacity, low cation exchange capacity, high pH, low rainfall, low organic matter content, and low microbial activity. Hence, there is growing interest in manipulating RP by biological methods in order to enhance its agronomic effectiveness. Currently, there is increasing emphasis on application of P-solubilizing microorganisms for RP solubilization in soils (Rodriguez and Fraga, 1999; Whitelaw, 2000; Vassilev *et al*., 2001).

Among microbial populations in soils, phosphate solubilizing bacteria (PSB) constitute solubilization potential of between 1 to 50%, while phosphorus solubilizing fungi (PSF) exhibit only 0.1 to 0.5% solubilization potential (Chen *et al.,* 2006). The mechanism involves solubilization of the phosphate in the presence of organic acids released by microorganisms (Goldstein, 1995). The most powerful P solubilizers were reported to be bacterial strains from the genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* along with *Penicillium* and *Aspergillus* fungi (Whitelaw, 2000).

Microbial solubilization of RP and its use in agriculture has received much attention. However, it is important to select efficient indigenous PSB strains which can increase P solubilization. The potential of indigenous PSB strains for enhancing growth performance in aerobic rice has not been investigated. Hence, the present study was undertaken to examine the effect of selected indigenous PSB (*Bacillus* spp. PSB9 and PSB16) on P solubilization of RP and growth improvement of aerobic rice.

Materials and Methods

Treatments and experimental design: The experiment was conducted in a glasshouse at University Putra Malaysia, Serdang, Malaysia. The treatments comprised of a factorial combination of two *Bacillus* spp. (PSB9 and PSB16) strains and three RP levels arranged in a completely randomized design with 6 replications. The two bacterial strains used were originally isolated from an aerobic rice field in Kepala Batas, Penang, Malaysia. Prior to the experiment strains were tested, *in vitro* condition for their beneficial characteristics such as; Indoleacetic acid, P solubilization activity, organic acid and siderophore production. The soil medium used was a sandy clay loam. The air dried soil was ground and passed through a 2 mm sieve and 3 kg of sieved soil was packed into plastic pots (17 cm diameter x 23 cm height) lined with perforated plastic bags. All pots received N and K at the rates of 60 and 40 kg ha^{-1} in the form of Urea and MOP, respectively. Christmas Island rock phosphate(Table. 1) was applied at 0, 30 and 60 kg P_2O_5 (equivalent to 0, 13, 26 kg P ha^{-1}). Three seven-day old aerobic rice (*Oryza sativa* L. line MR219-9 Mutant) seedlings obtained from the Malaysian Agricultural Research and Development Institute (MARDI) were transplanted into each pot and plants were grown for 60 days.

Table - 1: Characteristics of Christmas island rock phosphate

Size of CIRP	Total P %	P_2O_5 %	Total Ca%	Solubility (% P_2O_5)			
				H_2O	2% Formic acid	2% Citric acid	2% Nent Amm. citrate
<100µm	14.0	32.2	24.2	0.01	11.6	9.3	3.6

Preparation of rice seedlings and transplanting: The aerobic rice seeds were surface sterilized by the method of Amin *et al.* (2004). The seeds were sown in a plastic tray lined with filter paper. Sterile distilled water was added daily to moisten the seeds and seedlings were grown for 7 days. The efficacy of sterilization was monitored by sowing a duplicate set of seeds on nutrient agar plates. After 7 days three seedlings were transplanted into each of the pots prepared as described above.

Bacterial strains *Bacillus* spp. PSB9 and PSB16 were cultured in growth medium for 72 hrs. The bacterial cells were harvested by centrifugation at 13500 rpm for 10 min in centrifuge tubes and washed with distilled water. Three days after transplanting approximately 5 x 10^8 cfu ml^{-1} live washed bacterial cells were used as inoculum in each bacterial treatment and population was confirmed by cell enumeration using drop plate method (Somasegaran and Hoben, 1985). The non-inoculated pots received the same amount of dead cell.

Leaf chlorophyll content (SPAD value): The leaf chlorophyll content as well as leaf greenness was determined at 60 days after transplanting, using a portable chlorophyll meter (MINOLTATM SPAD-502) (Peterson *et al.*, 1993). The SPAD readings were recorded on the youngest fully expanded leaf (YEL) of each plant.

Photosynthesis (A_{max}): The single-leaf net photosynthesis (A_{max}) was determined at 60 days after transplanting on the youngest fully expanded leaf (YEL) of each treatment using a portable photosynthesis system (LI-6400XT, LI-COR Inc. Lincoln, Nebraska, USA). Measurements were carried out under full sunlight and at constant CO_2 of 380 µmol CO_2 mol^{-1} in the chamber.

Leaf area index (LAI): After harvest at 60 days leaf samples were placed in plastic bags and brought to the laboratory for leaf area measurement using a leaf area meter (Model LI-3100, Leaf Area Meter, LI-COR. Inc. Lincoln, Nebraska USA). Leaf area index was calculated using the following formulae:

$$LAI = \frac{\text{Mean leaf area of whole plant}}{\text{Surface area of pot (cm}^2\text{)}}$$

P in soil and plant tissue: The soil available P was determined by the method of Bray 2 (Bray and Kurtz, 1945) and total plant tissue P was analysed by wet digestion method (Havlin and Soltanpour, 1980).

Plant biomass: After harvest plant samples were washed to remove all soil particles and dried in an oven at 70 ^{0}C for 3 days until constant weight was achieved.

The adhering soil was rinsed off from root samples with distilled water and total root length (cm), total surface (cm^2), and root volume (cm^3) were quantified using a scanner (Expression 1680, Epson) equipped with a 2 cm depth plexiglass tank (20 x 30 cm) filled with UP H_2O (Hamdy *et al*., 2007). The scanner was connected to a computer and scanned data were processed by Win-Rhizo© software (Regent Instruments Inc., Québec, Canada).

All data were statistically analyzed using the SAS Software Program (Version 9.1), and treatment means were compared using Tukey's test ($p \leq 0.05$).

Results and Discussion

P solubilization and P uptake: The application of PSB significantly increased soluble P and plant P uptake in aerobic rice (Table. 2). The amount of P solubilization and P uptake in plant tissue differed significantly between bacterial strains and P fertilizer rates. Highest P solubilization (24.8 mg kg^{-1}) and plant uptake (5.31 mg $plant^{-1}$) were observed in PSB16 inoculated treatments with 60 kg, followed by PSB9. Results of this study indicate the highly beneficial effect of PSB inoculation on P release from rock phosphate and the consequent significant increase in P uptake in aerobic rice plants compared to non-inoculated treatments. The PSB strains tested showed significant potential for P solubilization and offers an alternative of cheaper fertilizer for crops.

Gull *et al.* (2004) reported that PSB have potential to solubilize fixed P resulting higher crop yields. The rate of P solubilization and P uptake in plant tissues varied with the bacterial strain and P fertilizer used. The Increase in soluble P with applications of PSB to insoluble P has been demonstrated (Subba Rao, 1984) and PSB has been used as inoculants to increase P uptake in several plants (Rodriguez and Fraga, 1999; Gulati *et al*., 2007).

Dry matter yield: The dry matter yields of aerobic rice in inoculated treatments were higher than non-inoculated treatments (Table. 3). There was significantly higher dry matter (21.48 g) found in treatments with 60 kg P_2O_5 ha^{-1} inoculated with PSB16, while the response in the control treatment was very low. Dry matter response curves for

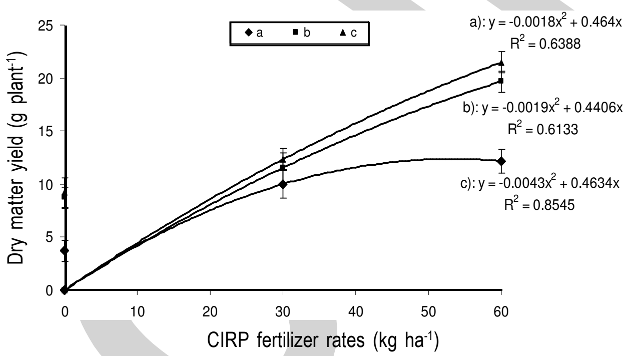

Fig. 1: Yield response curves for CIRP with and without PSB inoculation (a) Control, (b) PSB9+RP, (c) PSB16+RP in aerobic rice. (RP = Rock phosphate)

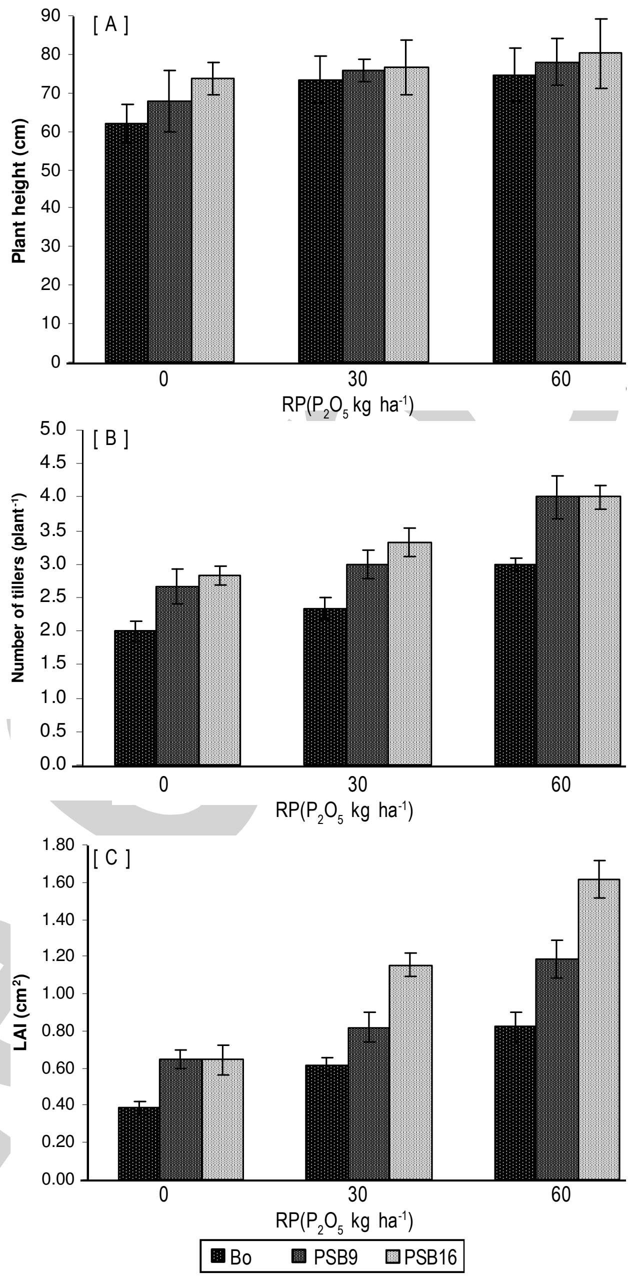

Fig. 2: Effect of PSB inoculation and P fertilizer application on (A) Plant height (B) Tiller production and (C) Leaf area index (LAI), of aerobic rice. (B0= without PSB, RP=Rock phosphate). Bars indicate S.E.(n=6)

RP with inoculation and non-inoculation of PSB showed different responses in aerobic rice (Figure 1). Inoculation with both PSB strains showed biomass increment over non-inoculated controls at all levels of RP applied (Table. 3). The highest increment was obtained with PSB16 application. The importance of PSB was also shown in other crops. The application of bradyrhizobia (*Bradyrhizobium japonicum*) and PSB (*Pseudomonas* spp.) enhanced the number of nodules, dry weight of nodules, yield components, grain yield, soil nutrient availability and uptake of soybean crop. Additionally, the use of PSB can increase the economic efficiency in terms of reduced

Table - 2: Effect of PSB strains on P solubilization (mg kg^{-1}) and plant P uptake (mg plant^{-1}) of aerobic rice at different doses of rock phosphate fertilizer

Treatments	Rock phosphate (P_2O_5 kg ha^{-1})					
	P solubilization			P uptake		
	0	30	60	0	30	60
Non-inoculated (control)	19.2^c	22.2^c	23.1^c	0.71^c	2.25^c	2.86^c
PSB9	20.5^b	22.9^b	24.1^b	1.90^b	2.65^b	4.76^b
PSB16	21.8^a	23.7^a	24.8^a	2.07^a	2.94^a	5.31^a

Different letters in the columns are significantly different at $p<0.05$

Table - 3: Effect of PSB inoculation and P fertilizer application on plant dry mass (g plant^{-1}) and biomass increment (%) of aerobic rice

Treatments	Rock phosphate (P_2O_5 kg ha^{-1})					
	Dry matter yield			Biomass increment over control		
	0	30	60	0	30	60
Non-inoculated (control)	3.70^c	9.99^c	12.16^c			
PSB9	8.74^b	11.53^b	19.68^b	136.2	15.4	61.8
PSB16	9.22^a	12.33^a	21.48^a	149.2	23.4	76.6

Different letters in the columns are significantly different at $p<0.05$

Table - 4: Effect of PSB strains and P fertilizer levels on chlorophyll content (SPAD value) and leaf photosynthesis (µmol CO_2 m^{-2} s^{-1}) of aerobic rice

Treatments	Rock phosphate (P_2O_5 kg ha^{-1})					
	Chlorophyll			Leaf photosynthesis rate		
	0	30	60	0	30	60
Non-inoculated (control)	20.03^c	25.20^b	27.17^c	2.74^c	3.63^c	3.96^c
PSB9	23.20^b	26.70^a	28.47^b	3.20^b	3.75^b	4.55^b
PSB16	24.87^a	27.43^a	29.30^a	3.50^a	3.90^a	5.71^a

Different letters in the columns are significantly different at $p<0.05$

Table - 5: Correlation coefficient between photosynthesis, P solubilization, P uptake, dry weight, root length and chlorophyll

	Photosynthesis	P solubilization	P uptake	Dry wt	Root length	Chlorophyll
Photosynthesis	-	0.158**	0.646**	0.652**	0.950**	0.930**
P solubilization		-	0.924**	0.917**	0.325*	0.489**
P uptake			-	0.999**	0.427*	0.568**
Dry wt				-	0.432*	0.579**
Root length					-	0.903**
Chlorophyll						-

Significance levels are * = 0.05, and ** = 0.01, at $p<0.05$

production cost of P fertilizers (Ngoc Son *et al*., 2006). In wheat, inoculation of PSB strains along with P fertilizer were 30 - 40% more efficient than P fertilizer alone in improving grain yields, and dual inoculation of the microorganisms without P fertilizer improved yields up to 20 % against sole P fertilization (Afzal and Bano, 2008).

Leaf chlorophyll content and photosynthesis: PSB inoculated treatments increased SPAD values and resulted in higher leaf photosynthesis compared to non-inoculated treatments (Table. 4). Highest chlorophyll content (29.30) and photosynthetic rate (5.71 µmol CO_2 m^{-2} s^{-1}) was obtained in treatments with P at 60 kg ha^{-1} inoculated with PSB16 (*Bacillus* spp.). The PSB inoculated treatments in the present study on aerobic rice demonstrated a significant increase in chlorophyll content and leaf photosynthesis compared to non-inoculated treatments. Mehrvarz *et al*. (2008), also reported similar results with mycorrhiza along with PSB (*Pseudomonas putida*) which showed an increase in leaf chlorophyll content in barley. These effects may also influence other physiological functions and agronomical parameters in the plant.

Plant growth: PSB inoculation significantly ($p<0.05$) increased plant height, leaf area index and tiller numbers in the aerobic rice genotype tested (Fig. 2A, B, C). The highest plant height (80 cm) and leaf area index (1.61 cm^2) were obtained in PSB16 inoculated

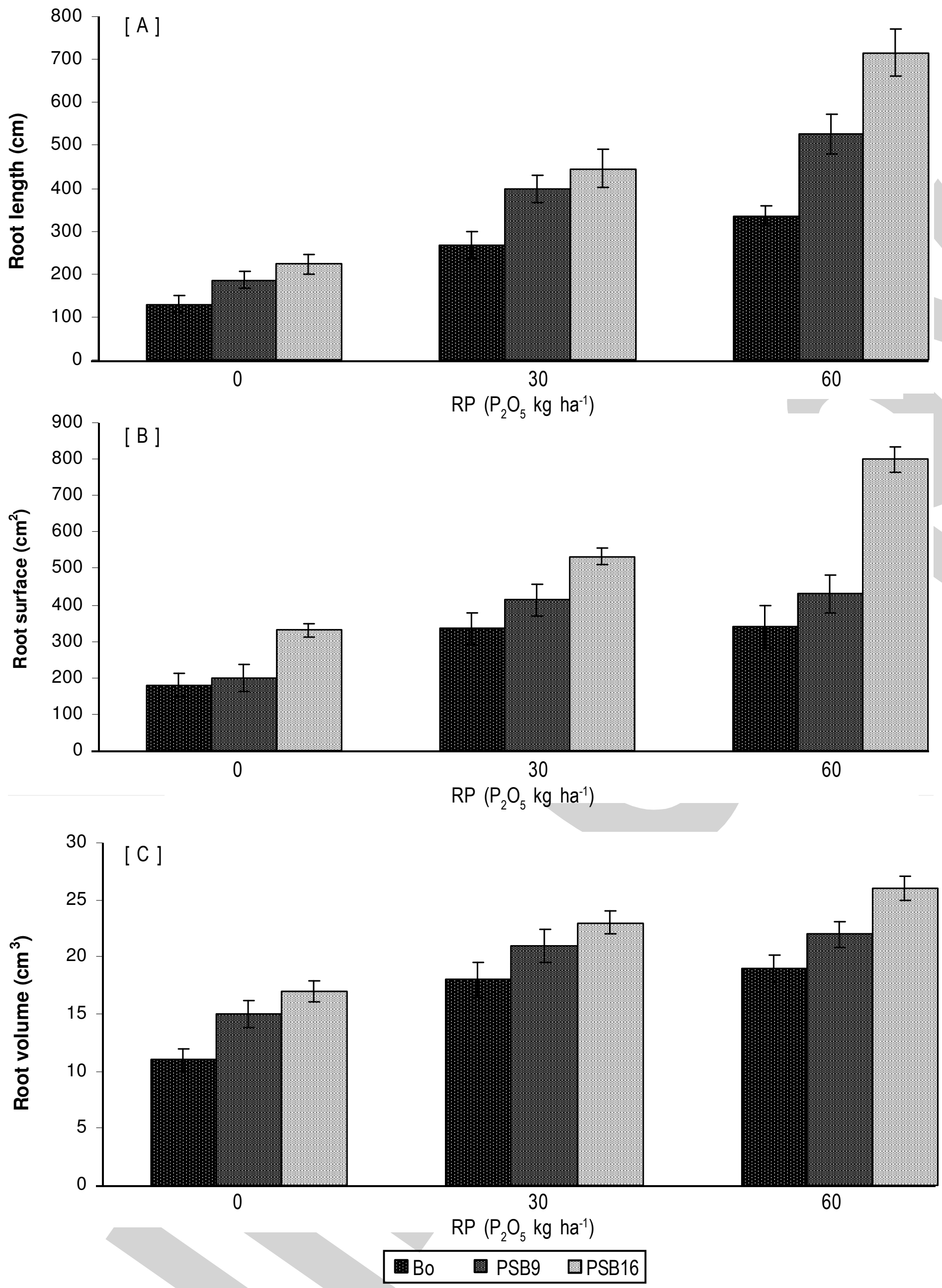

Fig. 3: Effect of PSB inoculation on (A) root length, (B) root surface area and (C) root volume of aerobic rice. (B0= without PSB, RP=Rock phosphate). Bars indicate S. E. (n=6)

treatments with P_2O_5 at 60 kg ha^{-1}, while equally high number of tillers were found in both *Bacillus* spp. inoculated treatments receiving P_2O_5 at 60 kg ha^{-1}. Plant height, leaf area index, tiller numbers and dry biomass yield increased with increase in P solubilization and plant P uptake due to the influence of PSB. In the present study, RP treatments with PSB16 inoculation showed better results in all plant parameters measured, compared to control treatments. Sharma (2006), reported similar results in wheat, where the rock phosphate treatment with PSB (*Pseudomonas striata*) inoculation gave higher results and enhanced the crop yields.

Root development: PSB inoculations significantly ($p<0.05$) increased root length, root surface area as well as root volume in the rice variety tested (Fig. 3A, B, C). Maximum root length (716 cm), root surface area (800 cm^2) and root volume (26 cm^3) were observed in plants treated with PSB16 and 60 kg of P_2O_5 ha^{-1}. The non-inoculated treatments showed lower responses. PSB strains used in the present study proved to increase P solubility and enhanced P uptake in aerobic rice in the P deficient soil. Previous study with tomato plants shown that, PSB (*Bacillus* FS-3) inoculations solubilized about 20% more soluble P from the insoluble forms and resulted in significantly higher shoot and root dry weight of tomato plants (Turan *et al*., 2006). Dual inoculation of arbuscular mycorrhiza and PSB improved uptake of both native P from the soil and P from the phosphatic rock (Cabello *et al*., 2005).

Correlation coefficient of P solubility with agronomic parameters: There was significant positive correlation found between P solubilization with plant P uptake, dry biomass, photosynthesis, chlorophyll and root length (Table 5). This indicated that with addition of PSB more P was solubilized and positively affected plant growth.

There were significant improvements found in shoot and root development with PSB inoculation and plants were able to obtain more nutrients and simultaneously affected growth of aerobic rice. The results indicate the tremendous ability of the PSB strains on P solubility from rock phosphate, and hence reduce usage of P fertilizers. Sharma *et al*. (2010) also reported similar findings with Mussoorie rock phosphate in the presence of PSB and suggested the potential of PSB as a useful substitute to DAP fertrliser in the rice–rapeseed–mungbean cropping system.

In conclusion, PSB inoculation with *Bacillus* strains PSB 9 and PSB 16 can effectively enhance P solubility of applied RP fertilizers, maintain a favourable soil P pool and increase productivity of aerobic rice. In the long term, this approach would ensure a cost-effective, sustainable and environmental friendly production system for aerobic rice.

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