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Contribution of phosphate-solubilizing bacteria in phosphorus bioavailability and growth enhancement of aerobic rice

Q. A. Panhwar[1]*, O. Radziah[1], A. Zaharah Rahman[1], M. Sariah[2], I. Mohd Razi[3] and U. A. Naher[2]

[1] *Department of Land Management*
[2] *Institute of Tropical Agriculture*
[3] *Department of Crop Science. Universiti Putra Malaysia. Selangor DE. Malaysia*

Abstract

The phosphate-solubilizing bacteria (PSB) can solubilize insoluble forms of phosphorous (P) into simple soluble forms that can be taken up by plants. The main focus of this study was to determine the effect of PSB on P availability in presence of different P rates for improved and sustainable rice (*Oryza sativa* L.) production under aerobic conditions. Triple superphosphate (TSP) at three levels (0, 30 and 60 kg ha^{-1}) and two isolated PSB (*Bacillus* sp.) strains (PSB9 and PSB16) were tested in glasshouse conditions. Surface sterilized seeds of aerobic rice (M9 variety) were planted in plastic pots containing 3 kg of soil for 60 days. PSB strains exhibited capability of producing organic acids from soil and plant roots and increased yield of aerobic rice. Significantly, high P solubilization (28.7 mg kg^{-1}) and plant uptake (7.94 mg kg^{-1}) was found in PSB16 inoculated treatments at 30 kg ha^{-1} of P_2O_5. In this treatment were also observed high leaf chlorophyll content (34.57), photosynthesis rate (7.59 µmol CO_2 m^{-2} s^{-1}) and root development. Isolated strains showed potential to make higher availability of P and increase content of organic acids from soil and roots at lower doses of TSP in aerobic rice. With the production of organic acids (oxalic, malic, succinic and propionic) higher amounts of P in the soil solution increased plant P uptake and resulted in higher plant biomass. The application of these potential inoculants in an appropriate combination with chemical fertilizers could be considered in organic and sustainable aerobic rice cultivation system.

Additional key words: organic acids; solubilization; strains; triple superphosphate; uptake.

Resumen

Contribución de las bacterias solubilizadoras de fosfato a la biodisponibilidad del fósforo y a la mejora del crecimiento del arroz aeróbico

Las bacterias solubilizadoras de fosfato (PSB) son capaces de solubilizar formas insolubles de fósforo (P), para que puedan ser absorbidas por las plantas. Se estudió el potencial de dos cepas de PSB (*Bacillus* sp.), PSB9 y PSB16, para la solubilización del P y el aumento del crecimiento del arroz aeróbico (*Oryza sativa* L.) en condiciones de invernadero, utilizando superfosfato triple (TSP) a tres niveles (0, 30 y 60 kg ha^{-1}). Se sembraron semillas de arroz aeróbico (variedad M9) en macetas de plástico con 3 kg de suelo. Las cepas PSB fueron capaces de producir ácidos orgánicos a partir del suelo y de las raíces de las plantas y aumentaron el rendimiento del arroz aeróbico. Se encontró una alta y significativa solubilización de P (28,7 mg kg^{-1}) y de absorción por la planta (7,94 mg kg^{-1}) en los tratamientos inoculados con PSB16 utilizando 30 kg ha^{-1} de P_2O_5. En este tratamiento también se observó un alto contenido de clorofila (34,57), tasa de fotosíntesis (7,59 mmol CO_2 m^{-2} s^{-1}) y desarrollo de raíces. Las cepas mostraron potencial para una mayor disponibilidad de P y aumento de ácidos orgánicos del suelo y las raíces a bajas dosis de TSP en el arroz aeróbico. Con la producción de ácidos orgánicos (oxálico, málico, succínico y propiónico), mayores cantidades de P solubles en el suelo incrementaron la captación de P por la planta y como resultado, aumentó su biomasa. Estos potenciales inoculantes combinados adecuadamente con fertilizantes químicos pueden ser aplicados en un cultivo orgánico y sostenible del arroz aeróbico.

Palabras clave adicionales: absorción por la planta; ácidos orgánicos; captación de P; cepas; solubilización; superfosfato triple.

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Abbreviations used: LAI (leaf area index); P (phosphorus); PGPR (plant growth promoting rhizobacteria); PSB (phosphate-solubilizing bacteria); SPAD (Soil Plant Analysis Division: chlorophyll meter); TSP (triple superphosphate); YEL (youngest expanded leaf).

Introduction

Phosphorus (P) is an important major nutrient for the growth and development of plants. Chemical P fertilizer is the main source of plant available P in agricultural soils, but almost 75 to 90% of added P fertilizer is precipitated by iron, aluminum and calcium complexes present in the soils (Turan *et al.,* 2006). The P diffusion to plant roots may be too low to acquire the requirements of crops if soils have less P solubility and a high P fixation capacity (Hoberg *et al.,* 2005). Worldwide, acid-soil-related infertility with P deficiency are the main restrictions to low yielding crops like upland rice (*Oryza sativa* L.) in the humid and semi humid tropical regions (Bationo *et al.,* 2008). In addition the most common problems in acid upland soils are aluminum and manganese toxicity, and phosphorus, potassium, calcium and magnesium deficiency, and interaction between various toxicities and deficiencies (Bationo *et al.,* 2008).

In aerobic condition, P solubilization mechanism is different compared to anaerobic rice cultivation system. Mostly aerobic soils have stable inositol hexaphosphates due to their strong complexation with metals and clay surfaces (Celi and Barbaris, 2005). Nutrient deficiencies have been studied in upland rice system where rice is grown in aerobic soils. Deficiency of P has been recognized as one of the main limiting factors for upland rice production in many parts of the world (Sahrawat *et al.,* 2001). The native supply of P can be decreased due to P fixation. Hence, the demand for P fertilizer application can be more serious for aerobic rice than for flooded lowland rice. It is predicted that with a population growth up to 8-9 billion people by 2040 and an extra 40 and 20 million metric tons of N and P fertilizers will be required respectively, to meet the food production needs (Vance, 2001).

The microorganisms perform an important role in agriculture by supplying nutrients to plants and reduce the demand of chemical fertilizers (Cakmakci *et al.,* 2006). Microorganisms participate in different processes that affect the transformation of soil P and play an essential part of the soil P cycle (Chen *et al.,* 2006). Particularly, phosphate-solubilizing micro-organisms are able to solubilize unavailable soil P and enhance the yield of crops (Adesemoye and Kloepper, 2009). Microorganisms, especially phosphate-solubilizing bacteria (PSB) and arbuscularmycorrhizal fungi, have ability to solubilize P in low soil and reduce inputs of chemical fertilizers (Arpana and Bagyaraj, 2007). The performance of PSB particularly their interactions with plants can enhance plant growth by solublizing P from different fractions of soil (Ahmed *et al.,* 2008).

Sundara *et al.* (2002) reported that the application of the PSB *Bacillus megatherium* increased the PSB population in the rhizosphere and P availability in the soil. It also enhanced sugarcane growth, yield and quality when used in conjunction with P fertilizers, and reduced the required P dosage by 25%. Chung *et al.* (2005) reported that the efficient PSB dissolved poorly soluble P and convert these insoluble P into soluble forms by the process of acidification, chelation, exchange reactions and production of organic acids. Whitelaw (2000) stated that the bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter,* along with *Penicillium* and *Aspergillus* fungi, are the most powerful P solubilizers. The use of these PSB as bio-inoculants will increase the available P in soil to minimize P fertilizer applications, production costs and at the same time reduce environmental pollution and promotes sustainable agriculture (Galal *et al.,* 2001). The rhizospheric microorganisms can positively play a role in plant growth promotion and enhance N and P in green gram (*Phaseolus aureus*) (Zaidi and Khan, 2006).

The combination of chemical and biological methods as sources of plant P can positively enhance the efficiency of naturally and synthetically-produced P resources and thus optimize the chemical fertilizer application for crop production (Salimpour *et al.,* 2010). Microbial solubilization of P and its use in agriculture is receiving a great attention. Potential PSB could play an important role in supplying P to plants in a more environmental friendly and in a sustainable manner, with the optimum dosage of P fertilizer. Hence the main focus of study was to determine the effect of PSB on P availability in presence of different P rates for improved and sustainable rice production under aerobic conditions.

Material and methods

Location, soil selection and treatments

A glasshouse experiment was conducted at the Universiti Putra Malaysia, Malaysia (2° 59’ N and 101° 42’ E) to study the effect of PSB (*Bacillus* sp.) strains (PSB9 and PSB16) on P solubilization from difference levels of P in aerobic rice. The bacterial strains were originally isolated from an aerobic rice field in Kepala Batas Penang, Malaysia. Before starting the experiments, the strains were tested under *in vitro* conditions to evaluate their beneficial characteristics, such as: indole acetic acid, P solubilization activity and organic acids. The soil used was an alluvial sandy clay loam with 0.12% of total N, 9.6 mg kg^{-1} of P, 0.29 cmol (+) kg^{-1} of K, 0.12 cmol (+) kg^{-1} of Ca, 0.25 cmol (+) kg^{-1} of Mg, 1.01% of organic carbon and soil pH 5.6. Air dried soil was ground and passed through a 2 mm sieve and 3 kg of the sieved soil was packed into plastic pots (17 cm diameter × 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 muriate of potash (MOP), respectively. Triple superphosphate (TSP) was applied at 0, 30 and 60 kg P_2O_5 ha^{-1}. Three seedlings of 7-d old aerobic rice (cv. M9) were transplanted to each plastic pot and then grown for 60 days.

Rice seedlings transplanting and preparation of inocula

Rice seeds were shaken in 70% ethanol for 5 min; ethanol was discarded and the seeds were agitated in hypochlorite solution comprising 3% of Chlorox™ (2.6% NaOCl) and washed with sterilized distilled water (Amin *et al.,* 2004). After the treatment, aerobic rice genotype seeds were grown in axenic conditions on sterile plastic tray containing filter paper. Seven days old seedlings were uprooted and transplanted into the pots (3 per pot). The *Bacillus* sp. strains PSB9 and PSB16 were grown in National Botanical Research Institute Phosphate (NBRIP) growth medium (Nautiyal, 1999) for 72 h. The bacterial cells were harvested by centrifugation at 13,500 rpm for 10 min in centrifuge tubes and washed with distilled water. Three days after transplanting, approximately 5×10^8 live washed bacterial cells mL^{-1} were used as inoculum in each bacterial treatment. The population was confirmed by cell enumeration using drop plate method (Somasegaran and Hoben, 1985).

Determination of organic acids from soil solution

Organic acids were extracted from 150 g of rhizosphere soil and kept at –80°C until analysis. Aliquots of soil containing 40% of water were centrifuged at 3,000 rpm and filtered through a membrane filter (0.2 µm). The organic acids were determined using a high performance liquid chromatography (HPLC, Jasco Borwin software) with a UV detector set at 210 nm, using a Rezex ROA-organic acid H+ (8%) column (250 × 4.6 mm) from Phenomenex Co.; 0.005 N H_2SO_4 was the mobile phase with a flow rate of 0.17 mL min^{-1}; organic acid standards of oxalic, malic, succinic and propionic acids were prepared.

Determination of organic acids from plant roots

About 10 g of roots from plants were cut off, rinsed with distilled water, dried with paper towel, weighed and immediately frozen in liquid nitrogen, and stored at –20°C for organic acid determination. The frozen roots were ground in a mortar with 70% ethanol and acid-washed sand. The mixture was centrifuged at 4.000 rpm for 10 min, and the pellet was extracted twice with boiling water. The supernatant from each of these extractions were transferred to a rotary evaporator and concentrated at 35°C under vacuum. The dried residues were dissolved in bi-distilled water and filtered through a membrane filter (0.2 µm). For Organic acids, 20 µL of samples were injected in HPLC with a UV detector set at 210 nm. Rezex ROA-organic acid H+ (8%) column (250 × 4.6 mm) from Phenomenex Co. was used, the mobile phase being 0.005 N H_2SO_4 with a flow rate of 0.17 mL min^{-1}.

Determination of leaf chlorophyll and photosynthesis rate

The leaf chlorophyll content was determined at 60 days after planting using portable chlorophyll meter (MINOLTA™ SPAD-502) (Peterson *et al.,* 1993). The SPAD reading was taken from the youngest expanded leaf (YEL) of each plant. Each value was the mean of 6 replications. The single-leaf net photosynthesis rate (A_{max}) was determined after 60 days of transplanting from YEL of each treatment using LI-6400XT Portable photosynthesis system, LI-COR Inc. (Lincoln, NE, USA). Measurements were done under full sunlight and at constant CO_2 of 380 µmol CO_2 mol^{-1} in the chamber.

Determination of leaf area index and plant biomass

The leaf area of the plant was determined at 60 days after inoculation when the plants were harvested. After harvest, randomly one plant's all leaves per treatment were carried into the laboratory in plastic bags and leaf areas measured using leaf area meter (LI-3100 area meter, LI-COR. Inc. (Lincoln, Nebraska NE, USA). Leaf area index (LAI) was calculated using the following formula:

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

After harvest, plant samples were carefully washed to remove all soil particles and dried in oven at 70°C for 3 days until constant weight was achieved.

Determination of soil available P, plant tissue P and root morphology

The soil available P was determined by the Bray II (Bray and Kurtz, 1945) extraction method and P in the tissue was analyzed by wet digestion method. The root morphology was studied at harvest using root scanner, model Epson Expression 1680 with root scanning analysis software, version Win-Rhizo 2007d. Fresh roots were washed with distilled water and placed in the root scanner. Total root length (cm), total surface area (cm^2) and total volume (cm^3) were quantified using a scanner (Expression 1680, Epson) equipped with a 2 cm depth plexiglass tank (20 × 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).

Data analysis

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

Effect of PSB strains on P solubilization and P uptake in aerobic rice

After 60 days, the application of PSB significantly increased soluble P and plant P uptake in aerobic rice genotype (Fig. 1). The amounts of P dissolved and P in the plant tissue differed according bacterial strains and P fertilizer rates. Significantly, high P solubilization (28.7 mg kg^{-1}) and plant uptake (7.94 mg $plant^{-1}$) were found in the treatment with 30 kg P_2O_5 ha^{-1} and inoculated with strain PSB16, followed by treatment PSB9 strain at 60 kg P_2O_5 ha^{-1}.

Production of organic acid from soil and roots

The study showed that the concentration of organic acids (oxalic, malic, succinic and propionic) in roots was higher than in soil (Fig. 2). The content of organic acids was significantly higher with PSB inoculation. Among the four organic acids, oxalic acid was found at the highest concentration. The highest oxalic acid content (170.10 µM) was produced by PSB16 at 30 kg P_2O_5 ha^{-1} levels, followed by PSB9 (87.69 µM) at the level of 60 kg P_2O_5 ha^{-1} from roots of aerobic plants, while propionic acid production (0-3.96 µM) was found in lower values from soil and roots.

PSB effect on dry matter yield of aerobic rice

In general, dry matter yields of aerobic rice obtained from inoculated treatments were significantly higher than those from control (Fig. 3). There was signifi-

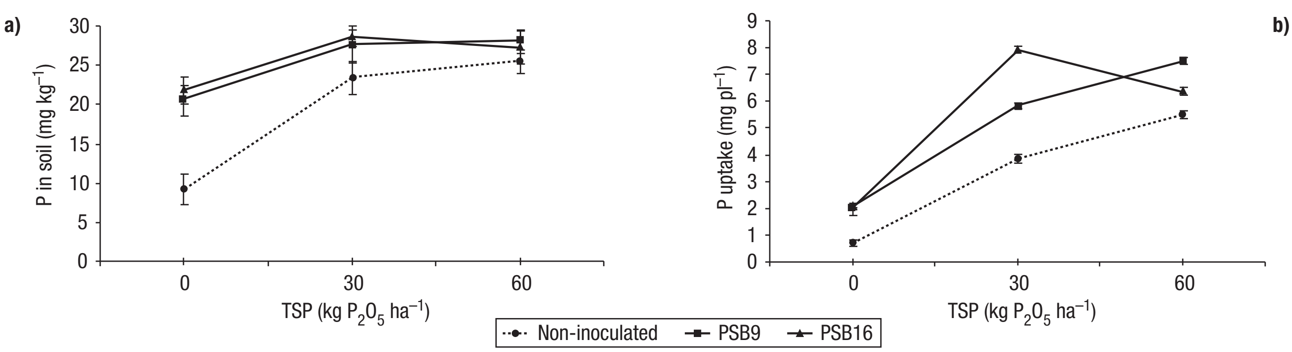

Figure 1. Effect of bacterial strains PSB9 and PSB16 inoculation with different levels of fertilizer applications on: a) P available in soil, b) plant P uptake. Bars indicate standard error, n = 5.

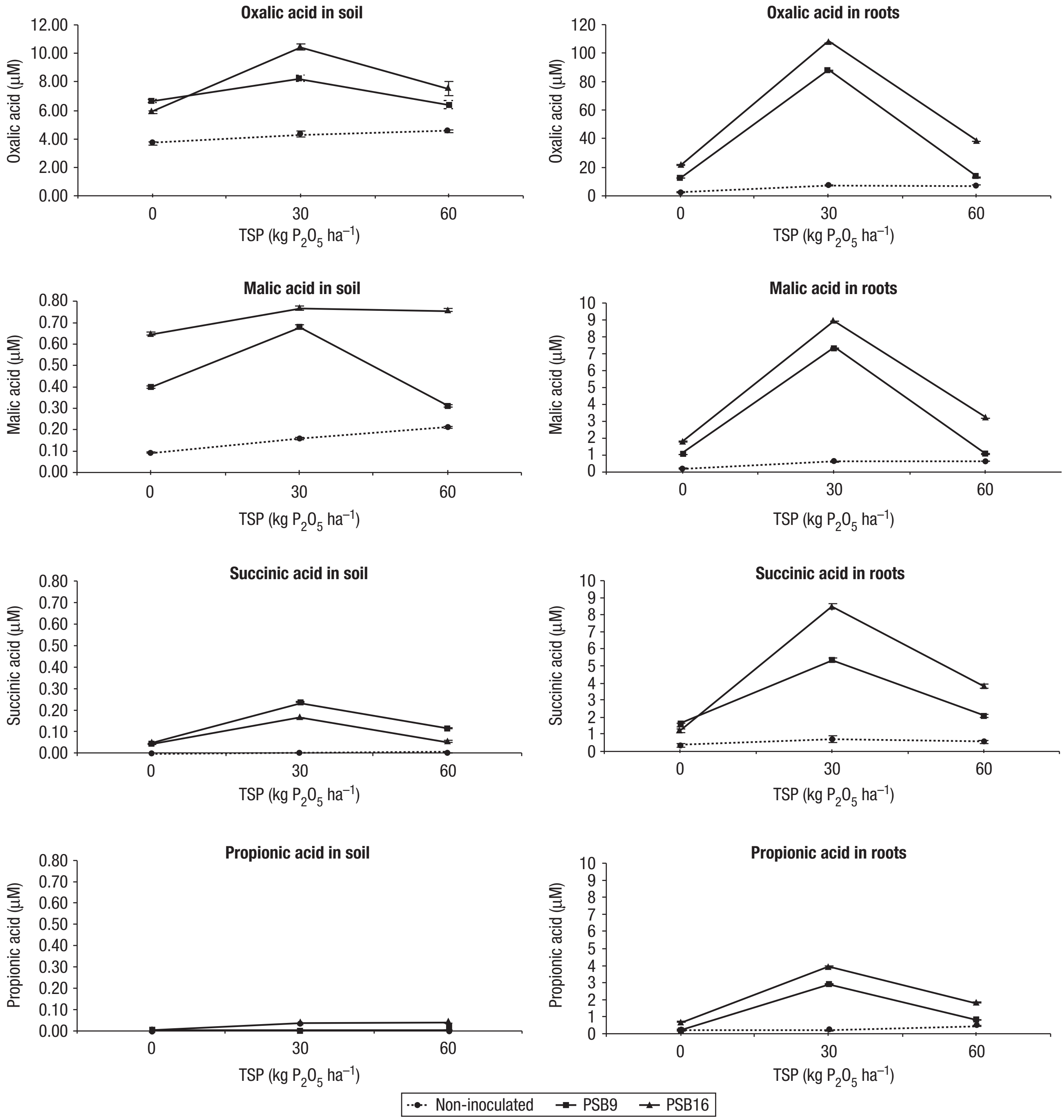

Figure 2. Production of organic acids in soil and roots of aerobic rice inoculated with PSB strains at different rates of triple superphosphate (TSP). Bars indicate standard error, n = 5.

cantly higher dry matter (30.08g) at the 30 kg P_2O_5 rate with PSB16 strain, while lowest dry weight was observed in control treatments. Dry matter response curve for TSP with inoculation and non-inoculation of PSB shows different responses in aerobic rice (Fig. 3). The inoculation of PSB with TSP resulted in higher dry matter yield than those without PSB inoculation. The PBS16 strains fertilized with 30 kg showed better results as compared to those with 60 kg P_2O_5 ha^{-1} with PSB9 strain. This indicated that lower doses of TSP with PSB inoculation could be more effective and reduce phosphate fertilizers.

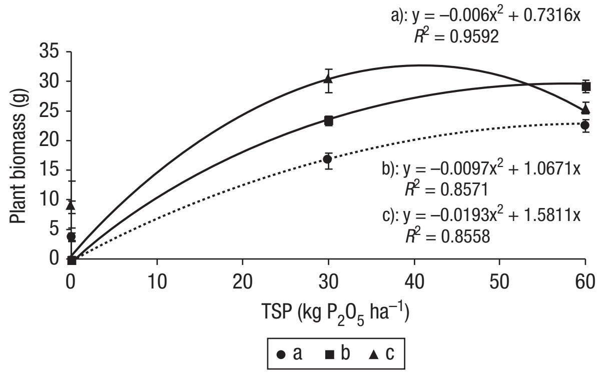

Figure 3. Plant biomass yield response curves for triple superphosphate (TSP) with inoculation and non-inoculation of bacterial strains PSB9 and PSB16 in aerobic rice. a: non-inoculated, b: PSB9+TSP, c: PSB16+TSP.

Effect on leaf chlorophyll content (SPAD value) and leaf photosynthesis rate

Application of PSB inoculation increased SPAD chlorophyll values and leaf photosynthesis rates compared to non-inoculated treatments in aerobic rice genotype (Fig. 4). Significantly ($p < 0.05$) high chlorophyll value (34.57) and net photosynthesis rate (7.59 µmol CO_2 $m^{-2}s^{-1}$) were observed at 30 kg P_2O_5 ha^{-1} with PSB16 inoculated treatments.

Effect on leaf area index and plant tillers

Inoculation of PSB significantly ($p < 0.05$) increased LAI and tillers of aerobic rice genotypes (Fig. 5 a-b).The highest LAI (2.14 cm^2) and plant tillers $hill^{-1}$ (5.6) were obtained in plants inoculated with the PSB16 strain at 30 kg P_2O_5 ha^{-1}, while the lowest LAI and number of tillers were observed in control treatments.

Effect on root morphology of aerobic rice

The PSB inoculation significantly ($p < 0.05$) increased root morphology of aerobic rice plants (Fig. 5 c-e). The highest root length (1,270 cm), root surface area (858 cm^2) and root volume (30 cm^3) were observed after inoculation with PSB16 at 30 kg of P_2O_5 ha^{-1} treatments.

Influence of PSB inoculation on biomass yield increment (%)

Inoculation of PSB along with P fertilizers application significantly increased plant biomass production in aerobic rice genotype at 60 days in glasshouse (Table 1). Inoculation with PSB9 increased biomass yield 57.66% in control, 84.12% in 30 kg and 87.33% in 60 kg P_2O_5 ha^{-1} treatment, over non-inoculated treatment respectively. On the other hand PSB16 (*Bacillus* sp.) inoculation produced 59.87 biomass in control, 85.47% in 30 kg and 85.47% in 60 kg P_2O_5 ha^{-1} treatment over non-inoculated treatment. The highest yield increment (87.7%) was obtained with PSB16 at 30 kg of P_2O_5 ha^{-1}.

Correlation coefficient of P solubility with organic acids and other agronomic variables

There was significant positive correlation between P solubilization and organic acids concentrations

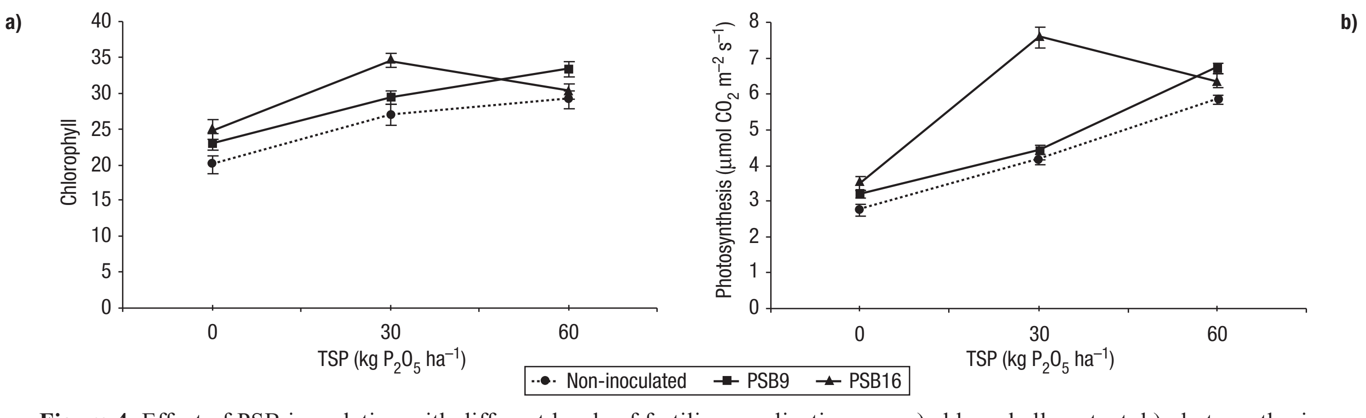

Figure 4. Effect of PSB inoculation with different levels of fertilizer applications on: a) chlorophyll content, b) photosynthesis. Bars indicate standard error, n = 5.

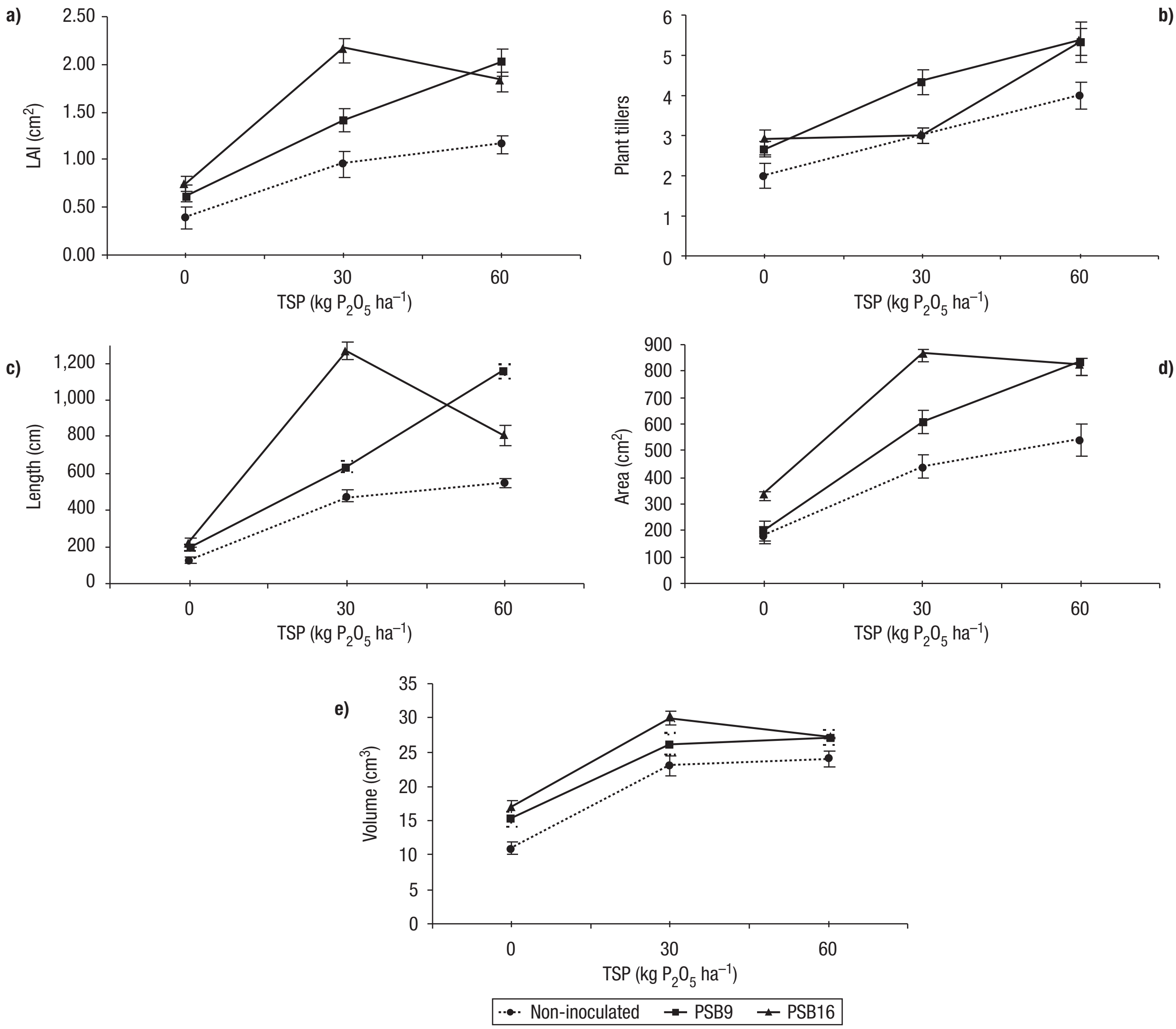

Figure 5. Effect of PSB inoculation with different levels of fertilizer applications on a) leaf area index (LAI), b) plant tillers, c) root length, d) root surface area, e) root volume. Bars indicate standard error, n = 5.

(Table 2) as well as other plant variables including P uptake, dry biomass, photosynthesis, chlorophyll and root length (Table 3). With the increase of special oxalic and malic acids content, the solubility of P improved.

Discussion

The results of this study suggest high positive effect of PSB strains with fertilizer treatments and PSB have enormous potential in providing soil P for the plant growth. PSB can help in increasing the availability of insoluble P for the growth of aerobic rice. In addition, the PSB strains significantly increased soluble P and plant P uptake compared to non-inoculated treatments in aerobic rice. There have been a number of reports on plant growth promotion by bacteria (Kucey *et al.,* 1989) that have the ability to solubilize inorganic or organic P from soil after their inoculation in soil. Wani *et al.* (2007c) reported a synergistic effects of PSB and N_2-fixing bacteria on chickpea plants with the significant increase of grain yield and uptake of P and N. Larger quantities of the insoluble P became soluble after the addition of PSB (Subba Rao, 1984) and concurrently use of PSB as inoculants enhances plant P

Table 1. Influence of PSB on biomass yield increment (%) on aerobic rice

Strains	Biomass increment (%)		
	Over control	Over 30 kg P_2O_5 ha^{-1}	Over 60 kg P_2O_5 ha^{-1}
Without PSB	0	77.68	83.51
PSB9	57.66	84.12	87.33
PSB16	59.87	87.70	85.47

uptake and crop yield (Rodriguez and Fraga, 1999; Gulati *et al.,* 2007). Strains were able to produce organic acids such as oxalic acid, malic acid, succinic acid and propionic acid from both soil and roots of aerobic rice. The production of organic acids might be the reason for the solubilization of P. Excretion of organic acids from roots is thought to be one of the main mechanisms by which plants mobilize less readily available soil phosphates (Marschner, 1995), moreover changes in pH, chelations by organic acids which bind phosphate anions also make availability of phosphate in soil solution (Jones and Darah*,* 1994).

Consequently the PSB inoculants provided better results in aerobic rice at lower doses of P fertilizer and it shows high effectiveness of the strains. Hence the chemical application of fertilizers could be minimized. Young *et al.* (2003) reported that when different PSB strains were applied with half dose of chemical fertilizer on lettuce (*Lactuca sativa*), there was a 25% growth increase as compared with only chemical fertilizer treatments and at least 50% of chemical fertilizers can be reduced by using biofertilizer application. The PSB inoculation with mineral P increased the efficiency of P fertilizer and would decrease about 25% of the required P to plants (Attia *et al.,* 2009).

The inoculation of PSB in aerobic rice genotypes proved the potential to increase plant height and numbers of tillers. Afzal and Bano (2008) reported that a 30-40% more efficiency of PSB strains with P fertilizer for improving grain yield of wheat (*Triticum aestivum*) and dual inoculation of microorganisms without P fertilizer improved 20% more grain yield. The results in the present study may be explained with P-dissolution capabilities of PSB strains, while PSB may solubilize inorganic P due to excretion of organic acids (Hoberg *et al.,* 2005). Plant response to these PSB could be associated with other mechanisms, rather than only P solubilization. However, PSB can facilitate growth and development of plants by producing essential nutrients or by varying the concentration of plant growth promoting substances including phytohormones, such as indoleacetic acid (Wani *et al*., 2007a,b).

There were significant differences in dry biomass yield, LAI and plant tillers with PSB inoculated treat-

Table 2. Correlation coefficients between P solubilization and organic acid production

	P solubilization	Oxalic acid	Malic acid	Succinic acid	Propionic acid
P solubilization	—	0.802**	0.435*	0.427*	0.490*
Oxalic acid		—	0.772**	0.833**	0.780**
Malic acid			—	0.975**	0.904**
Succinic acid				—	0.961**
Propionic acid					—

*: 0.05, and **: 0.01, at $p \leq 0.05$.

Table 3. Correlation coefficient between photosynthesis, P solubilization, P uptake, dry weight, root length and chlorophyll

	Photosynthesis	P solubilization	P uptake	Dry weight	Root lenth	Cholophyll
Photosynthesis	—	0.426*	0.425*	0.432*	0.93**	0.960**
P solubilization		—	0.982**	0.984**	0.498**	0.548**
P uptake			—	0.999**	0.536**	0.575**
Dry weight				—	0.540*	0.581**
Root length					—	0.979**
Chlorophyll						—

*: 0.05, and **: 0.01, at $p \leq 0.05$.

ments to non-inoculated ones in aerobic rice. It showed the high effectiveness of PSB inoculants in aerobic rice and these significantly affected different plant variables. The higher values of chlorophyll contents and leaf photosynthesis rates were obtained from inoculated aerobic rice plants with PSB strains. Similar results showing increase in chlorophyll content and photosynthesis rates with inoculation of PSB were found by Mehrvarz *et al.* (2008). On the other hand, in PSB inoculated treatments, significant differences were found in the root morphology variables like root length, surface area as well as in root volume of aerobic rice plants. Similar results were revealed by Trivedi *et al.* (2003) which showed that PSB (*Bacillus subtilis*) significantly increased rice root length and yield in contrast to the control in both pot and field experiments in a Himalayan soil. PSB share in the rhizosphere with other agronomically useful microbes and could play preservative or synergistic role in plant growth. Thus, significant crops yield increases were recorded in different plant inoculated with PSB and other plant-growth promoting rhizobacteria (Afzal and Bano, 2008).

The microorganisms have enormous potential in providing soil P for plant growth. The inoculation of PSB and plant growth-promoting rhizobacteria (PGPR) together could reduce 50% of P fertilizer application without any significant decrease of crop yield (Jilani *et al.,* 2007; Yazdani *et al.,* 2009). The Bradyrhizobia and PSB (*Pseudomonas* spp.) application in soybean crop improved the number of nodules, dry weight of nodules, yield components, grain yield, soil nutrient availability and uptake. In addition, the economic efficiency could be increased in term of reducing the production cost for soybean (Tran *et al.,* 2006). According to Shaharoona *et al.* (2008) it was observed that PSB (Pseudomonads) might be used in combination with appropriate doses of fertilizers for better plant growth thus saving the cost of chemical fertilizers.

In conclusion, the result shows that PSB strains produced significant amount of organic acids and have enormous potential for increasing available P to the plant vicinity, simultaneously enhanced P uptake (7.94 mg plant^{-1}). Bacterial inoculants (PSB16) increased 87.7% of plant biomass and have great prospects for sustaining crop production with optimal P fertilization. Hence PSB biofertilizer can be used for the better P fertilization to enhance productivity, maintenance of soil P pool and sustainability of aerobic rice.

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