RICE RESPONSE TO INOCULATION WITH P-SOLUBILIZING MICROORGANISMS FROM BRAZILIAN CERRADO

RESPOSTA DE ARROZ À INOCULAÇÃO DE MICRORGANISMOS SOLUBILIZADORES DE FOSFATO DE SOLO DE CERRADO

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ABSTRACT: Two experiments were done under greenhouse conditions to evaluate rice growth promotion through inoculation of P-solubilizing bacteria and fungi isolated from their rhizoplane and rhizosphere. The best inoculum concentration was also determined. In both experiments, plants were grown in tubes (330 mL) containing sterilized soil from Cerrado. In the first trial, each plant was inoculated (1 mL with 10^6 CFU mL^{-1}) with fungal and bacterial isolates and the mixture of both. A completely randomized experimental design with 6 inoculation treatments and 6 replicates was used. In the second trial, plants were inoculated with increasing concentrations (10^6, 10^7, 10^8 and 10^9 CFU mL^{-1}) with two P-solubilizing bacterial and one P-solubilizing fungal isolates. A completely randomized experimental design, as a 3 x 4 factorial (three P-solubilizing isolates and four increasing inoculum concentrations), plus a non inoculated treatment and five replicates were used. In both experiments, plants were harvested 24 days after inoculation with these microorganisms. Plant height, shoot and root dry matter/length/volume were measured. In the first experiment, the height, root dry matter/length/volume on rice increased with the inoculation of the P-solubilizing fungi (PSF 8) as well as the mixture of PSF 8 and the P-solubilizing bacteria PSB 24. In the second experiment, PSF 8 also showed the best performance on rice growth promotion mainly at the highest inoculum concentration.

KEYWORDS: P-solubilizing bacteria and fungi. Rhizosphere. Inoculum. Concentration.

INTRODUCTION

Phosphorus (P) is one of the major limiting factors for crop production mainly in tropical soils (NORMAN et al. 1995) due to the high P fixation problem. This means that P in the soil is tightly bound to the soil particles and relatively unavailable to plants. Thus, the mineral pool of P in the soil can be relatively large but just a little part of it is available to plants. Consequently, high dosages of phosphate fertilizers are required to obtain high yields.

Edaphic P-solubilizing microorganisms (PSMs) can increase P availability to plants improving, therefore, its use efficiency. Moreover, those microorganisms can be inoculated in areas where insoluble P sources, such as Araxá, Patos de Minas and Catalão apatites are used. Hence, the cost of phosphate fertilization can easily decrease if these organisms are applied in association with low soluble P sources. Moreover, these organisms could optimize the fixed P naturally found in soil. Some PSMs can also increase plant growth by mechanisms other than P solubilization, e.g. production of phytohormones such as Indole-acetic acid (ARSHAD; FRANKENBERGAR, 1998). Several authors reported growth promotion through inoculation of rhizobacteria on lettuce (SOTTERO et al. 2006), citrus (FREITAS; VILDOSO, 2004) and wheat (LUZ, 2001). Others related plant growth promotion through simple inoculation of P-solubilizing fungi on wheat (WHITELAW et al. 1997), onion (VASSILEV et al. 1997) and soybean (ABD-ALLA; OMAR, 2001). Besides that, increasing plant disease resistance (LUZ, 2001; PAUL; SHARMA, 2005) and the potential of reducing the use of soluble P sources and, consequently, decreasing chances of environmental contaminations (FREITAS; VILDOSO, 2004) are implicated when these organisms are used. Therefore, P-solubilizing microorganisms can be considered a great option to maximize plant growth promotion, increasing the sustainability of the agroecosystems. However, few studies focusing this group of microorganisms have been done in tropical areas, mainly in Brazil. Naturally, this type of research requires more attention.

The direct effect of PSMs and plant growth promoters are normally observed by increasing on root length, shoot dry matter and improvement on

Received: 22/04/08
Accepted: 12/08/08

plant nutrition (SABINO et al. 2000). NARLOCH et al. (2002) observed greater yield on radish through inoculation of *Aspergillus* sp. and *Penicillium* sp., two types of P-solubilizing fungi. Sabino et al. (2000) also found improvement on rice growth promotion, reporting that the density of inoculated microorganisms associated to the inoculation method is crucial to guarantee the establishment of these organisms in the soil. Then, studies focusing the inoculum concentration of PSMs in order to maximize plant growth promotion are required.

This work evaluated rice growth promotion through inoculation of P-solubilizing bacteria and fungi isolated from their rhizoplane and rhizosphere. The effect of increasing PSMs concentrations on plant growth promotion was also evaluated.

MATERIALS AND METHODS

This work was done under greenhouse conditions at Centro Federal de Educação Tecnológica de Rio Verde - GO (CEFET - Rio Verde). The first step of this work was to isolate P-solubilizing bacteria (PSB) and fungi (PSF) from the rice rhizoplane and rhizosphere. Rice (Primavera variety) was sown in tubes (330 mL) containing soil classified as Latosol collected from 10–40 cm layer at experimental area of CEFET - Rio Verde. This soil was used for Experiments 1 and 2. Chemical and textural analysis were done (EMBRAPA, 1997) obtaining the following results: pH $_{(in\ water)}$ = 5.6; $P_{(Mehlich\ 1)}$=1.5 mg dm^{-3}; K = 0.08 mmol dm^{-3}; Ca = 10.4 mmol dm^{-3}; Mg = 3.2 mmol dm^{-3}; Al = 0.0 mmol dm^{-3}; Organic matter = 22.8 g dm^{-3}; sand = 39%; silt=11% and clay=50%.

Three days after emergence, seedlings were thinned to two plants per tube. Twenty two days after plant emergence, PSMs isolation was done. Twenty plants were used. Soil samples (10g) from the rhizoplane and rhizosphere of each plant were mixed in flasks (125 mL) containing 90 mL of saline solution (0.85%) following the successive dilution technique till 10^{-4}. The dilutions 10^{-3} and 10^{-4} were chosen for plating (triplicates), using the GL solid medium (glucose, 10g; yeast extract, 2g and agar-agar, 15g L^{-1}). Before plating, this medium was maintained at 45 °C while 100 mL of $CaCl_2$ (10%) and 50 mL of K_2HPO_4 (10%) L^{-1} were sterilized separately, and then added to the GL medium to form $CaHPO_4$ (10%), according to Sylvester-Bradley et al. (1982). After incubation (four days at 32 ºC, darkness) of the Petri dishes, direct counting of PSB and PSF was done. Microorganisms able to solubilize this phosphate showed a transparent halo around their colonies, according to DI SIMINI et al. (1998). These organisms were purified and stored in tubes (8 mL) containing GL medium plus glycerol, under room temperature.

Experiment 1: Inoculation of P-solubilizing bacteria and fungi isolates in rice grown in soil from Cerrado

This experiment was done to evaluate rice growth promotion through inoculation of P-solubilizing bacteria and fungi isolated from their rhizoplane and rhizosphere. Rice seeds (Primavera variety) were sterilized according to Rumjanek (1999) and sown in tubes (330 mL) also sterilized (immersion in sodium hypochlorite, 1% for 10 min). Plants were grown in autoclaved soil (120 ºC for 15 min) and irrigated every other day. Three days after emergence, seedlings were thinned to one plant per tube.

A completely randomized design with six inoculation treatments (PSB 24, PSB 36, PSF 8, PSF 8 + PSB 24, PSF 8 + PSB 36 and non inoculated treatment) and six replicates were used.

Four days after emergence, seedlings received 1 mL of GL liquid medium (glucose, 10g and yeast extract, 2g L^{-1}) containing 10^6 CFU mL^{-1} of each inoculum treatment. PSB isolates were inoculated in flasks (125 mL Erlenmeyers) containing 20 mL of GL liquid medium and incubated (24 h, 28 °C, darkness). From this suspension, 1 mL was pipetted, quantified using successive dilution and direct counting techniques and standardized to 10^6 CFU mL^{-1}. In relation to the PSF isolates, spore suspension was prepared by the following procedure: each isolate was incubated in Petri dishes (four days, 28 ºC, darkness), in GL solid medium, until the Petri dishes were covered. The spores were suspended in water plus tween (1%), using a Drigalsky spatula following the same techniques to standardize the inoculum to 10^6 CFU mL^{-1}, as used with PSB isolates. The non inoculated treatment consisted of inoculation of 1 mL of sterilized GL liquid medium without any PSMs.

After 24 days of inoculation, plants were harvested and height, shoot and root dry matter, root length and volume were evaluated. The root length was calculated according to Tennant (1975) while the root volume was measured after root immersion in graduate cylinder containing water, and determining the volume difference.

The data were submitted to ANOVA and means compared by Scott-Knott test ($p \leq 0.05$), using the SISVAR statistical package (FERREIRA, 1999).

Experiment 2: Inoculation of increasing concentrations of P-solubilizing bacteria and fungi in rice grown in soil from Cerrado

This experiment was done to identify the best P-solubilizing bacteria and fungi inoculum concentration in order to improve plant growth. Rice seeds (Primavera variety) were sterilized according to Rumjanek (1999) and sown in tubes (330 mL) also sterilized (immersion in sodium hypochlorite, 1% for 10 min). Plants were grown in autoclaved soil (120 ºC for 15 min) and irrigated every other day. Three days after emergence, seedlings were thinned to one plant per tube.

The experimental design was disposed in a completely randomized design, in a 3 x 4 factorial (three types of PSMs and four increasing inoculum concentration), plus an additional treatment (absence of any PSMs inoculation) and five replicates. The inoculation treatments were: PSB 24, PSB 36 and PSF 8.

After four days of emergence, seedlings received 1 mL of GL liquid medium containing 10^6, 10^7, 10^8 or 10^9 CFU mL^{-1} of each inoculum treatment. The procedure for standardization of the PSB and PSF increasing inoculum treatments was the same described for Experiment 1.

After 24 days of inoculation, plants were harvested and height, shoot and root dry matter, root length and volume were evaluated. The root length was calculated according to Tennant (1975) while the root volume was measured after root immersion in graduate cylinder containing water, determining the volume difference.

The data were submitted to ANOVA and means compared by Scott-Knott test ($p \leq 0.05$) and regression analysis ($p \leq 0.05$) using the SISVAR statistical package (FERREIRA, 1999).

RESULTS AND DISCUSSION

In the first part of this work, four PSB isolates and only one PSF were identified as phosphate solubilizers due to their ability to form a transparent halo around their colonies. Sylvester-Bradley et al. (1982) suggested the possibility of inhibition of fungal growth due to the metabolites produced by bacterial isolates under in vitro conditions. These results are in agreement with those authors, who also detected a smaller number of PSF compared to PSB in soil from the Brazilian Amazon. Similar result was also found by Souchie et al. (2006) who detected a greater number of PSB using the GL and GELP media for soil from an area of Secondary Forest in Paraty, Rio de Janeiro. These authors suggested that it might be due to the presence of yeast extract in the growth medium composition.

The PSMs inoculated in the Experiment 1 showed different behavior in relation to rice growth promotion (Figure 1). For instance, height and root dry matter were increased when PSF 8, PSF 8 + PSB 24, PSF 8 + PSB 36 and PSB 36 were inoculated, surpassing the non inoculated treatment (Figure 1A and B). Similar results were found by Freitas e Vildoso (2004), who detected increased citrus growth through inoculation of *Bacillus* and *Pseudomonas* rhizobacteria.

Otherwise, no effect of these inocula were found on the shoot dry matter, where plants inoculated with PSF 8 recorded highest values (32 mg $plant^{-1}$), not differing, however, of the non inoculated treatment (22 mg $plant^{-1}$). The inoculation with PSF 8, PSF 8 + PSB 24 and PSF 8 + PSB 36 increased root length (Figure 1C). It could be also due to the possibility of phytohormones production by these isolates. For instance, the inoculation with Azospirillum brasiliensis increased the root length and formation of lateral roots in wheat by indol acetic acid production (SABINO et al. 2000). Similarly, the root volume in rice was increased when PSF 8 and PSF 8 + PSB 24 were inoculated (Figura 1D). It also could have occurred due to the same reason reported above. According to Barea et al. (1997) and Bowen e Rovira (1999), mechanisms as phytohormone, vitamin and amino acid production can be involved on plant growth promotion by PSMs. Hence, PSF 8 and PSF 8 + PSB 24 certainly behaved as plant growth promoters since height, root dry matter/length/volume were increased with their inoculation. Rice growth promotion also might have occurred due to the solubilization of the native P from the soil. Naturally, if plants had more P availability, better growth would be found.

In Experiment 2, no effect of increasing inoculum concentration on height and shoot dry matter was found. Nevertheless, an effect of PSMs inoculation was detected where PSF 8 showed the best performance, increasing the values of these variables (Figure 2A and B).

An interaction between PSMs and increasing inoculum concentration was observed in relation to root dry matter production. A linear effect of the increasing concentration of PSF 8 was observed (Figure 3A). There was no effect of the inoculation with PSB 24 and PSB 36. As to the root dry matter, PSF 8 induced similar results when root length/volume was measured (Figure 3B and C).

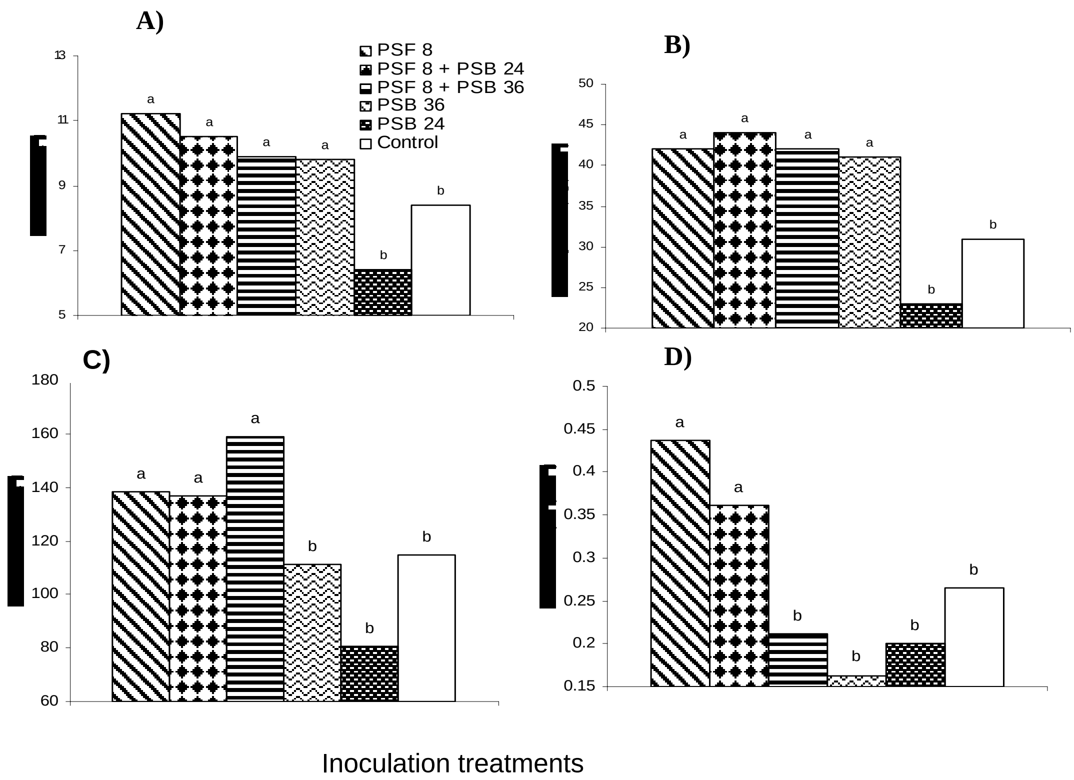

Figure 1. Height (A), root dry matter (B), root length (C) and root volume (D) of rice plants inoculated with P-solubilizing bacterial (PSB) and fungal (PSF) isolates, after 24 days grown in soil from Cerrado. Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$).

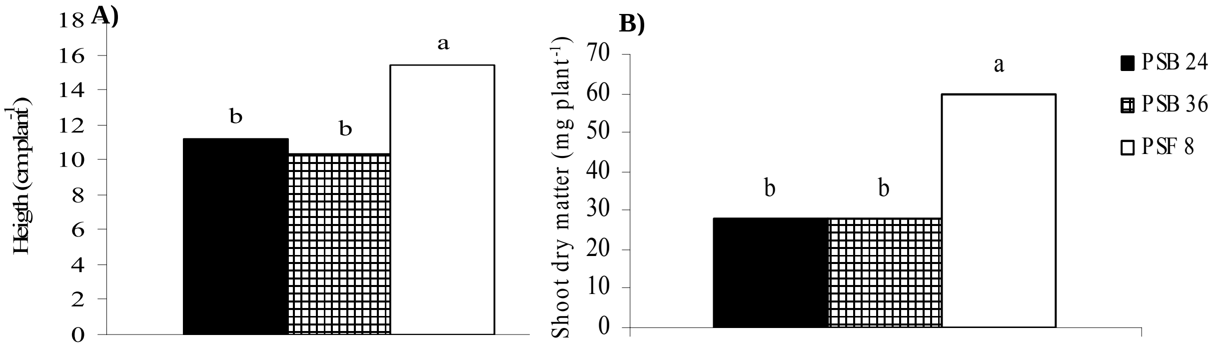

Figure 2. Height (A) and shoot dry matter (B) of rice plants inoculated with P-solubilizing bacterial (PSB) and fungal (PSF) isolates. Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$).

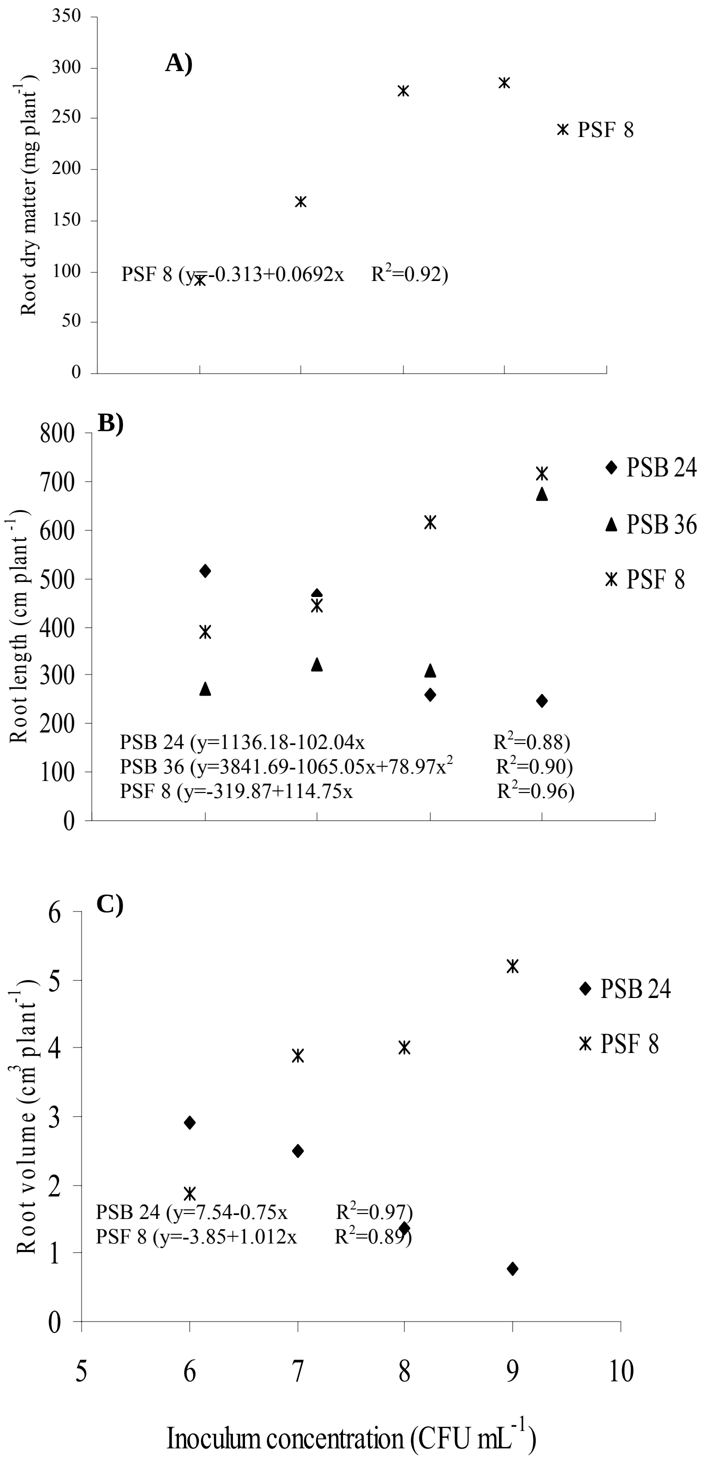

Figure 3. Root dry matter (A), root length (B) and root volume (C) of rice plants inoculated with increasing concentrations of P-solubilizing bacterial (PSB) and fungal (PSF) isolates.

The isolate PSF 8 showed the best performance increasing all plant growth variables measured. The establishment ability of fungal isolates is normally higher than bacterial isolates especially due to their capacity to produce spores. In addition, due to their ability to produce higher biomass, a greater P-solubilization and, consequently, higher plant growth promotion capacity would be encountered. However, according to Wakelin et al. (2004), P-solubilizing activity is related not only to biomass production, but the physiological state of the biomass. Moreover, since P-solubilizing bacteria generally out-number P-solubilizing fungi in soil by 2-150 fold (KUCEY,

1983) their contribution to the plant P availability can not be minimized.

The isolate PSB 24 apparently showed low functional compatibility with rice. Considering the increasing inoculum concentration, decreasing root length / volume was detected (Figure 3B and C). Souchie et al. (2005) reported certain incompatibility between P-solubilizing bacteria and *Enterolobium contortisiliquum*. According to these authors, lower shoot and root dry matter were found when a mix of three P-solubilizing isolates were inoculated.

Hence, efforts are required to elucidate the specific mechanisms from these microorganisms in order to maximize their plant growth promotion potential. Moreover, further studies in order to testing the efficiency of these isolates under natural soil conditions are also interesting.

CONCLUSIONS

Height, root dry matter/length/volume of rice plants were increased in the presence of PSF 8 and PSF 8 + PSB 24.

The single inoculation with the PSB 24 was not efficient, showing a performance similar to the non inoculated treatment.

The isolate PSF 8 showed the best performance on rice growth promotion in the highest inoculum concentration.

RESUMO: Dois experimentos foram conduzidos em condições de casa de vegetação para avaliar a capacidade promotora de crescimento de arroz (*Oryza sativa* L.) por bactérias e fungos solubilizadores de fosfato isolados de sua rizosfera, além de identificar a melhor concentração de inoculante que maximizasse o crescimento vegetal. Em ambos os experimentos, as plantas foram cultivadas em tubetes (330 mL) contendo solo de Cerrado autoclavado. No primeiro experimento, cada planta foi inoculada com 1 mL contendo 10^6 UFC mL^{-1} com isolados fúngicos, bacterianos e a mistura de ambos. Utilizou-se um delineamento inteiramente casualizado com 6 tratamentos de inoculação e 6 repetições. No segundo experimento, as plantas foram inoculadas com concentrações crescentes (10^6, 10^7, 10^8 e 10^9 UFC mL^{-1}) com dois isolados bacterianos e um fúngico, utilizando-se um delineamento inteiramente casualizado, esquema fatorial 3 x 4 (três tipos de microrganismos inoculados e quatro concentrações de inoculante), mais um tratamento não inoculado e 5 repetições. Em ambos os experimentos, as plantas foram colhidas 24 dias após a inoculação com os solubilizadores, avaliando-se a altura, matéria seca de parte aérea, matéria seca de raiz, extensão e volume radicular. No primeiro experimento, a altura de parte aérea, matéria seca, extensão e volume das raízes das plantas de arroz foram incrementadas com a inoculação do fungo solubilizador de fosfato FSF 8 e sua mistura com a bactéria solubilizadora de fosfato BSF 24. No segundo experimento, o FSF 8 demonstrou maior potencial de estímulo ao crescimento de arroz que os isolados bacterianos, principalmente nas concentrações de inóculo mais altas.

PALAVRAS-CHAVE: Bactérias e fungos solubilizadores de fosfato. Rizosfera. Concentração de inoculante.

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