African Journal of Biotechnology Vol. 11 (16), pp. 3758-3765, 23 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1337
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effects of rhizobia and plant growth promoting bacteria inoculation on germination and seedling vigor of lowland rice

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Accepted 19 May, 2010

Plant growth promoting rhizobacteria (PGPR) stimulate plant growth by producing phytohormone which enhances the growth and physiological activities of the host plant. Recently, legume bacteria (*Rhizobium* spp.) have been considered as a PGPR for legume as well as non-legumes and have the potential for growth stimulation. A laboratory experiment was conducted to observe the effect of PGPR and Rhizobial inoculation on seed germination, seedling emergence, growth and development of lowland rice variety MR219. The experiment was conducted under laboratory condition using filter paper in Petri dish. The design of the experiment was completely randomized (CRD) with six replicates. The PGPR strains UPMB10 (*Bacillus sphaericus*), *Rhizobium* strains SB16, UPMR1006 and UPMR1102 were used in the experiment. Seeds inoculated with those PGPR and *Rhizobium* strains and the Petri dish were kept in an incubator at 30 ± 2°C for 120 h. The seeds germination and other related attributes were measured. The results suggested that inoculation significantly increased the seedling emergence, seedling vigor, root growth namely root length, root surface area and volume. Among the strains, UPMB10 performed better in seedling growth and strain UPMR1006 produced profuse hair in the radical. The results concluded that PGPR and Rhizobia strain can promote seed emergence and seedling attributes which benefits the early seedling establishment and consequently the crop growth and development.

Key words: *Rhizobium*, PGPR, seedling vigor, germination, rice.

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple diet of over 40% of the world's population, making it the most important food crop (Hossain and Fischer, 1995). It is difficult to increase the production area as the cultivable lands in many countries are decreasing due to rapid urbanization. Therefore, it is necessary to increase the production capacity per unit area which currently requires high input. The seed is one of the most important inputs for higher grain production and the necessity of quality seed is not to be eluded. Quality seed is required for rapid and synchronous seedling emergence, a pre-requisite for successful stand, establishment and uniform plant growth and development. Vigorous seedling growth is important for the successful establishment of rice and other crops. Plant growth promoting rhizobacteria, microbial inoculants have been used for plant growth and development in different leguminous and non-leguminous crops for several decades (Bashan, 1998). Investigations in several

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Abbreviations: PGPR, Plant growth promoting bacteria; **GA,** gibberellins; **GEA,** growth enhancing activities; **IAA,** indole-3-acetic acid; **BNF,** biological nitrogen fixation; **CSE,** coefficient of seedling emergence.

Table 1. Effect of different rhizobial and PGPR inoculation on the percentage of seed germination of lowland rice variety MR219 after 120 h of inoculation.

Treatments	% Germination after 120 h
Control	86.67 a
B. sphaericus (UPMB10)	84.00 a
Rhizobium sp (SB16)	86.67 a
Rhizobium sp. (UPMR1006)	86.00 a
Rhizobium sp.(UPMR1102)	85.33 a

Means having same letter in a column are not significantly different at 5% level of significance.

countries have shown that growth of rice seedling was enhanced by inoculation with plant growth promoting microorganisms which led to increased grain and straw yield and increased the efficiency of fertilizer-N. The capacity of microorganisms to stimulate germination and improve development of plants has been adapted for *in vitro* and *in vivo* conditions of some agricultural and ornamental plants (Ayyadurai et al., 2006; Biswas et al., 2000; Noel et al., 1996; Wilkison et al., 1994; Tsavkelova et al., 2007).

Plant growth stimulators are applied to improve seed germination, seedling vigor and plant development. Investigations in several countries have shown that rice seedling growth was enhanced by following inoculation with plant growth promoting microorganisms, leading to increased grain, straw yield and enhancement of the efficiency of fertilizer-N. The inoculation of seeds or seedlings with microorganisms has been adopted as a method of modifying microbial populations around crop plants to promote both development and yield. The stimulation of seedling development by bacteria has been attributed to the production of biologically active compounds. The major limitation to a more widespread use of seed germination has generally been the variability in effects in both field and laboratory studies.

Some reports have demonstrated the role of rhizobia in promoting growth of non-legumes following inoculation but little is known about the mechanism(s) involved. Probable mechanisms increase root growth that favor higher nutrient uptake. Enhanced seed emergence and consequently vigor seedlings are also influenced by PGPR inoculation. Inoculated PGPR produced IAA in the presence of seed exudates that might have triggered faster germination. Significant increases in crop yields following application of PGPR have been documented under diverse field conditions (Bashan, 1998; Mia et al., 2005). Recent advances in symbiotic *Rhizobium*-legume interactions at the molecular level and the ability to introduce new genes into rice through genetic transformations have created an excellent opportunity to investigate the possibilities for growth promoting activities in rice. Yanni et al. (2001) and Sheng and Huang (2001) found that the benefits of this association which leads to greater production of vegetative and reproductive biomass was more likely due to rhizobial modulation of the plant root architecture. Recent research identified the existence of true rhizobial endophytes of rice plants. Research should not ignore the potential to improve rice production through rhizobial inoculation via mechanisms that do not involve biological nitrogen fixation (BNF). It is well-established that many soil and plant-associated bacterial groups are able to synthesize phytohormones (Salmeron et al., 1990; Atzorn et al., 1988; Bastian et al., 1998). Many of these bacteria can produce and excrete in their cultures more than one hormone type; *Rhizobium* isolates synthesize gibberellins (GA) and auxin (Atzorn et al., 1988), *Azotobacter* spp. synthesizes GA, auxin and cytokinins (Salmeron et al., 1990) and *Acetobacter* and *Herbaspirillum* isolates synthesize indole-3-acetic acid (IAA) and GA (Bastian et al., 1998). The growth enhancing activities (GEA) of bacterial inoculants on crop plants may be performed in various ways; as bio-control, induction of the inoculated plant as disease resistance N_2 fixation, solubolization of precipitated chemicals (Subba Rao, 1985) and/or production of phytohormone (Tien et al., 1979). Zahir et al. (2004) found increased root elongation, root dry weight, shoot elongation and shoot dry weight in wheat by the inoculation of *Rhizobium*. Two endophytic strains of *Rizobium leguminosarum* bv. Trifolii increased grain yield of rice by 10 - 45% over a wide range of N supply in a field experiment (Yanni et al., 1997). However, research works have not been undertaken on the effect of rhizobial and PGPR inoculation on germination and seedling vigor of rice. Hence, the present study was undertaken to observe the effect of rhizobial and PGPR inoculation on the germination attributes and seedling vigor of rice.

MATERIALS AND METHODS

The experiment was conducted at the Soil Microbiology Laboratory, Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, Malaysia. The cultivar of rice *(O. sativa* L.) used in this study was MR219. The seeds were collected from the Malaysian Agricultural Research and Development Institute (MARDI), Malaysia.

Plant growth promoting bacteria

The rhizobial and PGPR strains used were collected from the Soil Microbiology Laboratory, Department of Land Management, Universiti Putra Malaysia, Malaysia. The design of the experiment was completely randomized with six replications (Table 1).

Inocula preparation

Strains were grown in yeast manitol broth (YMB) (Summesegaron and Hoben, 1994) and nutrient broth. Exponentially growing cells in shaken broth culture were inoculated. Rice seeds were surface sterilized by 100% ethanol in an Erlenmeyer flask and were treated with 1% Na hypochlorite (Clorox) for 2 min followed by six times washing with sterile water. After that, the seeds were soaked in various rhizobial or PGPR broths. Seeds soaked in normal broth

were treated as control. Twenty five seeds of both inoculated and controls were put in sterilized Petri dishes containing filter paper (Whatman # 102) and the Petri dishes were kept in an incubator at 30°C for 120 h.

Seedling emergence test

After soaking, the air-dried seeds were used for germination and the seedling percent emergence was calculated with the following formula:

$$\% \text{ Emergence} = \frac{\text{Number of emerged seedlings}}{\text{Number of seeds sown}} \times 100$$

Speed of germination

Copeland (1976) considered both vigor index and co-efficient of germination as measures for speed of germination. The germinated seedlings were counted at an interval of 24 h for 5 days and the speed of germination of seed was monitored. Vigor index, co-efficient of germination and germination index were calculated using following formula (Copeland, 1976):

$$\text{Coefficient of emergence (\%)} = \frac{100(A_1+A_2+A_3+\text{----------}+A_X)}{A_1T_1+A_2T_2+\text{----------}+A_XT_X} \times 100$$

Where, A= number of seed germinated, T = time corresponding to A and x = counting number (1, 2, 3 ...nth).

$$\text{Emergence index} = \frac{\text{Ti Ni}}{\text{S}}$$

Where, Ti = ith number of days after sowing, Ni = ith number of seeds emergence, and S = total no. of seed used.

Plumule and radical length were measured by a foot scale and root surface area, root volume and total root length were measured by a root image scanner (Model: WinRhizon-Analysis for Root, Regent Instruments Inc.). The roots and shoots were separated and kept in an electric oven at 48°C for 72 h to get a constant dry weight. Seedling vigor index was measured according to the modification of Baki and Anderson (1973).

Statistical analysis

Treatments were arranged in a randomized design, the mean, standard error analysis of variance and the LSD were calculated by using SAS package, Version 9.0 (SAS Institute Inc., 2006). Means were compared using the Duncan Multiple Range Test at 5% level.

RESULTS

Effect of different inoculation practices on the seed germination and seedling vigor of rice variety MR219 are described and discussed here.

Seedling emergence percentage

Seedlings that emerged protruding around 2 mm were considered as emergence that was continued for a period of 120 h. It was evident that seedling emergence was influenced by different types of inoculation (Figure 1). Initially, the treatments did not show any differences in emergence however, at 48 to 96 h after inoculation, seeds inoculated with UPMR 1006 and UPMR 1102 showed higher percent emergence compared to others. Finally, both control and inoculated seeds showed similar percent emergence.

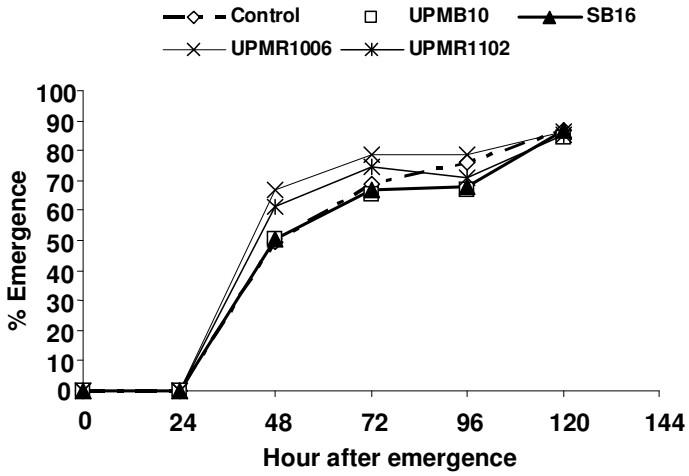

Figure 1. Effect of different rhizobial and PGPR inoculation on % seed emergence of rice.

Emergence index

The seedling emergence index increased with time (Figure 2). There were no significant differences between inoculated and un-inoculated seeds.

Coefficient of seedling emergence (CSE)

The CSE could not show any differences among the treatments (Figure 3). However, *Rhizobium* strains UPMR1006 and UPMR1102 showed slightly higher CSE values.

Vigor index

The seedling vigor index was calculated based on the dry matter production. Inoculation significantly influenced the seedling vigor and the highest value was found in the seeds when it was inoculated with *B. sphaericus* strain UPMB10 (Figure 4). However, the strain SB16 showed the lowest value.

Seedling growth

Application of PGPR did not influence the seedling shoot

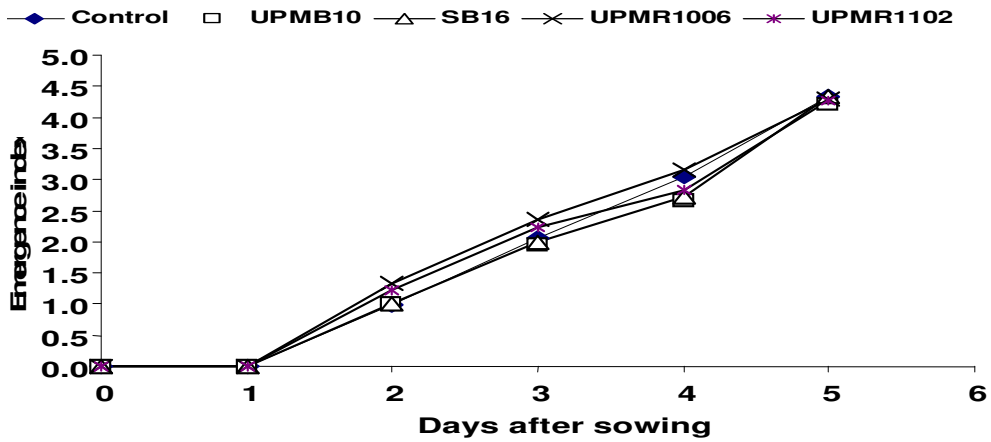

Figure 2. Effect of different rhizobial and PGPR inoculation on seed emergence index of rice.

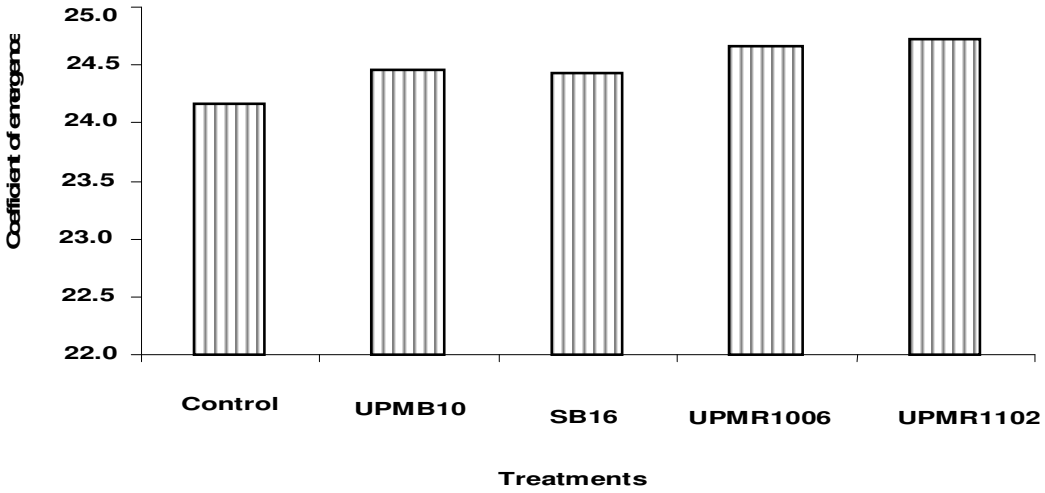

Figure 3. Effect of different rhizobial and PGPR inoculation on coefficient of emergence of rice.

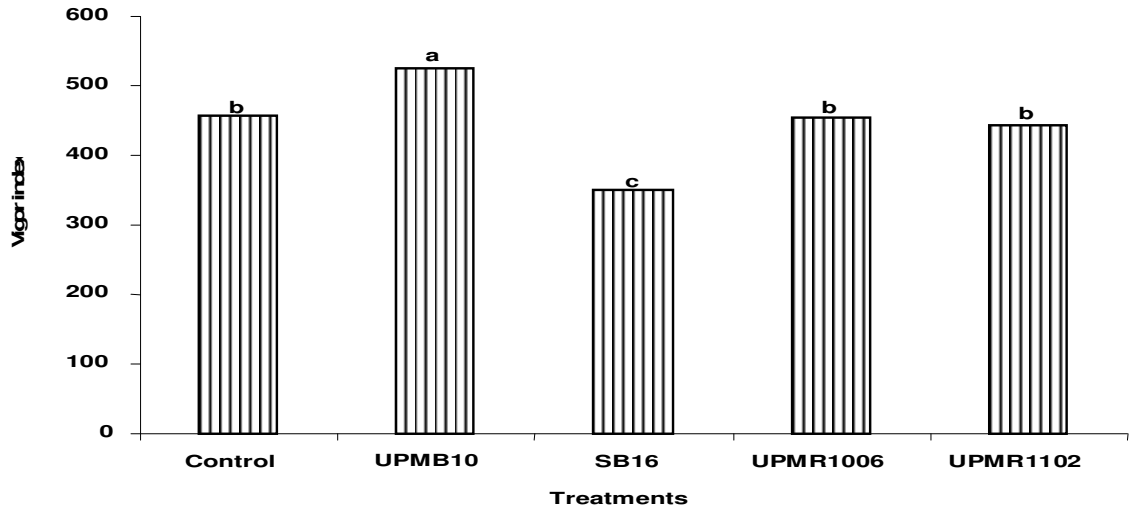

Figure 4. Effect of different rhizobial and PGPR inoculation on seedling vigor of rice.

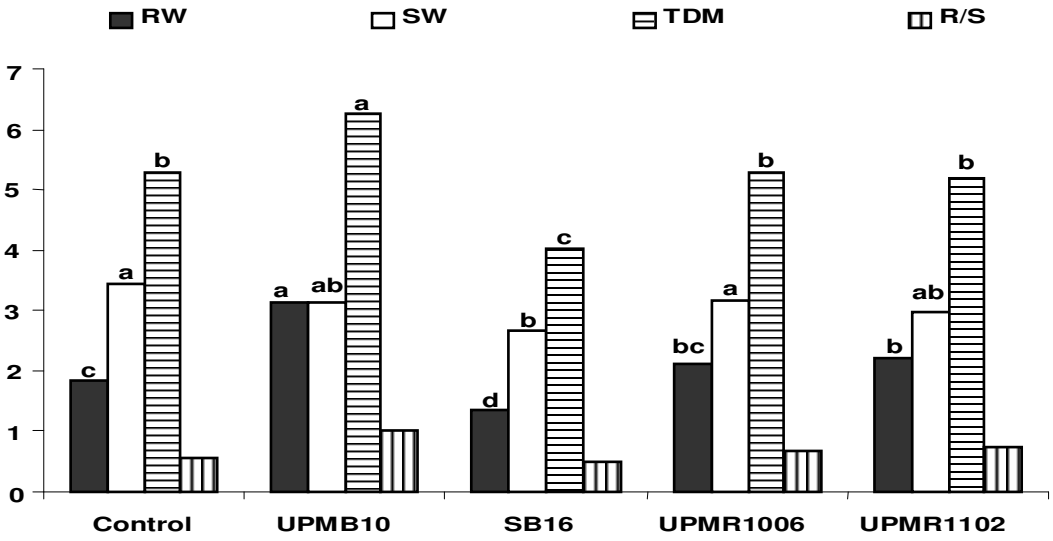

Figure 5. Effect of different rhizobial and PGPR inoculation on root and shoot growth of rice.

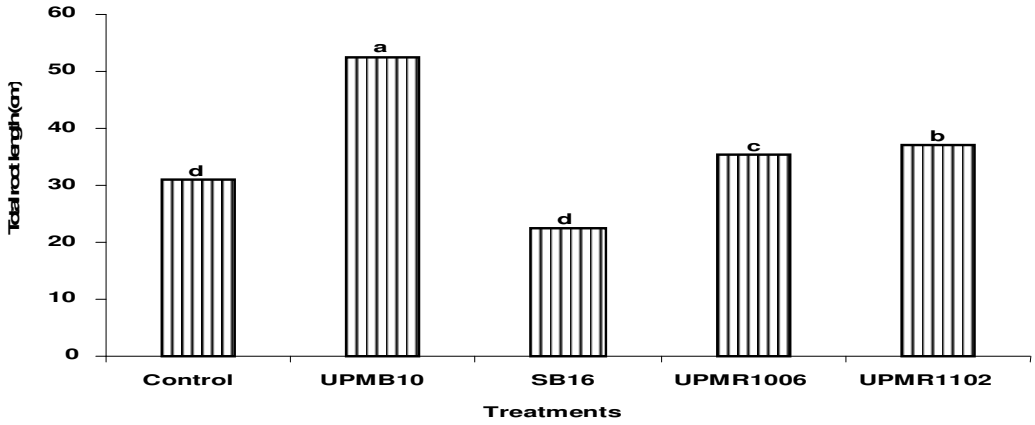

Figure 6. Effect of different rhizobial and PGPR inoculation on total root length of rice.

growth; length and dry weight. The control possessed higher shoot weight compared to strain SB16 (Figure 5). However, root length and weight were significantly influenced due to inoculation by PGPR (Figure 6). Seeds inoculated with UPMB 10 resulted in the highest total root length followed by UPMR 1102 and UPMR1006. Similarly, the earlier strain influenced the production of the highest root weight and consequently the total dry matter content. The ratio of root to shoot was also influenced due to PGPR inoculation where UPMB10 produced the highest root shoot ratio as compared to others.

Root volume and surface area

The root volume and surface area were determined by root image scanner. The results revealed that inoculation had positive effect on the production of root surface area and volume (Figures 7 and 8). The strain UPMB10 produced the highest surface area and similarly, the root volume. However, the strain SB16 recorded the lowest root volume and surface area.

DISCUSSION

The present study revealed that significant improvement was made on seedling emergence and seedling vigor due to PGPR inoculation. Higher vigor seeds are pre-requisite for better establishment of seedling. Seed vigor and viability are important components influencing seedling establishment, crop growth and productivity (McDonald and Copeland, 1997). Although, percent germination was not I nfluenced but other parameters

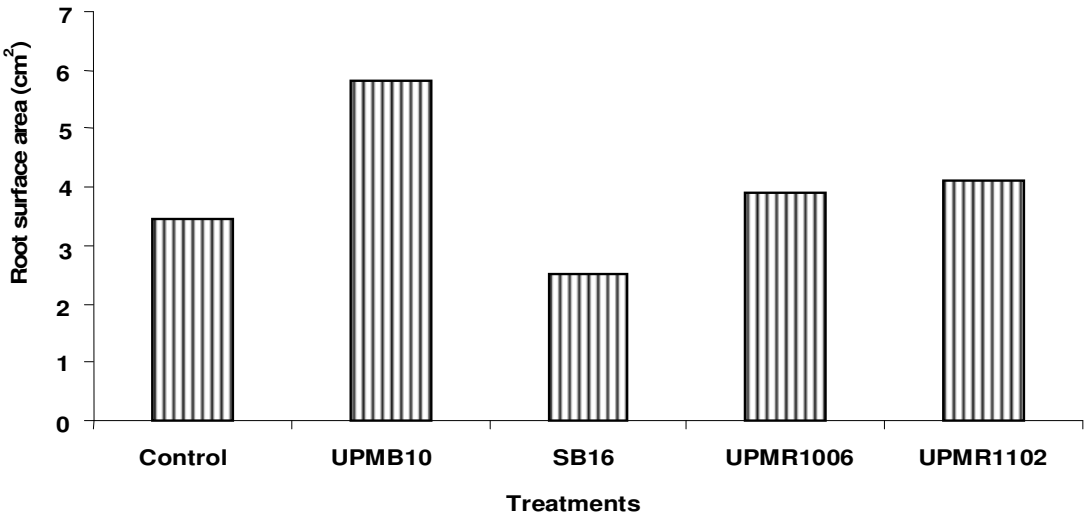

Figure 7. Effect of different rhizobial and PGPR inoculation on root surface area of rice.

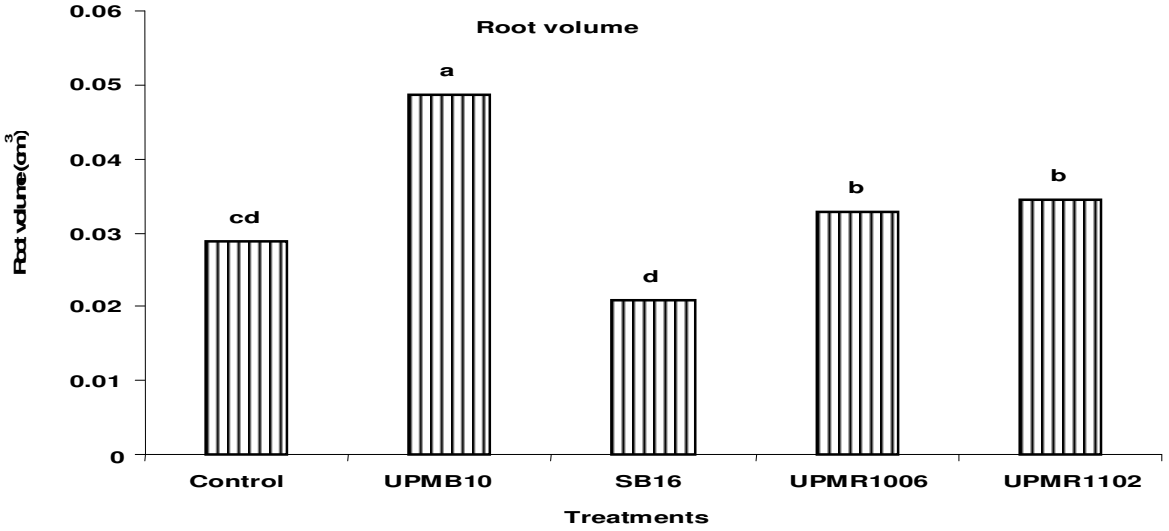

Figure 8. Effect of different rhizobial and PGPR inoculation on root volume.

were affected due to inoculation. The reason behind this is that rice seed actually do not have any germination problem, after a certain period the seeds were germinated with a minimum failure. The increases of seed germination percentage and seedling shoot length were considered typical gibberellins-like responses. They mimic the effect of exogenous GA_3 application. Initially, inoculated plants showed higher emergence which might be due to the production of phytohormone as phytohormone influences seed germination. During cereal seed germination, α-amylase in the aleurone layer plays an important role in hydrolyzing the endosperm starch into metabolizeable sugars, which provide the energy for growth of roots and shoots (Akazawa and Hara-Mishimura, 1985; Beck and Ziegler, 1989). Physiological and biochemical studies have revealed that α-amylase expression in the aleurone layers occurs as follows: first, active gibberellins (GA) biosynthesis commences in the embryo and the gas is transported from the embryo to the aleurone layer (Fincher, 1989). Consistent with this observation, Barbieri et al. (1991) described the ability of PGPR to modify root growth in grasses through phytohormone production and Burdman et al. (1996) reported a similar effect by inoculation of legume seedlings with *Azospirillum* sp. This interpretation is in line with well-known characteristics of certain phytohormone to elicit stimulatory plant growth response within a narrow window of low concentration, outside of which the plant is

either unresponsive or inhibited (Esashi, 1991; Jackson, 1991). Induction of longer roots with increased number of root hairs and root laterals is a growth response attributed to IAA production by other rhizobacteria. PGPR inoculation produced high quality seedling and ultimately had influence on higher growth and development of rice plant. Noel et al. (1996) reported significant enhancement of early seedling root growth in non-legumes by seed inoculation with *R. leguminosarum* and attributed this effect to bacterial phytohormone production. The consistently higher DM accumulation by rice seedling due to inoculation with rhizobial strains UPMR1006 and UPMR1102 on rice roots, their longevity is nevertheless adequate to trigger plant growth stimulation and vigor of young seedlings that they carryover to produce more productive plants, resulting in higher yields at maturity (Biswas et al., 2000). It has been demonstrated that strains *R. leguminosarum* bv. Trifolli strain E-11 has been reported to have a good effect on rice (Yanni et al., 2001) due to production of IAA (Dazzo et al., 2000). Vigor index determines the state of the health of seedling and ultimately the state of the productivity of the plant. The higher the vigor index the better will be the yield of the plant.

Conclusions

The results of this study concluded that PGPR and rhizobial inoculation increased the seed emergence, seedling vigor and seedling root attributes. Seeds inoculated with UPMB10 showed better performance in respect of seedling growth although percent germination was not influenced. Similarly, seeds inoculated with UPMR 1006 produced plenty of root hairs after 48 h of inoculation. In conclusion, the results suggested that simultaneous screening of PGPR for seedling establishment under pot and field experiment is a good tool to select effective PGPR for biofertilizer development biotechnology.

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