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GROWTH AND YIELD RESPONSES OF RICE CV. MR219 TO RHIZOBIAL AND PLANT GROWTH-PROMOTING RHIZOBACTERIAL INOCULATIONS UNDER DIFFERENT FERTILIZER-N RATES

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Abstract

Effects of locally isolated plant growth-promoting rhizobacteria (PGPR) and rhizobial inoculations on growth and yield of local Malaysian rice variety MR219 experiments were conducted in glasshouse. The treatments consisted of three PGPR (UPMB10, UPMB19 and UPMB20) and two rhizobial (UPMR30 and UPMR31) inoculations, each with three levels of nitrogen fertilizer (0, 33, 100% of recommended rate), laid in CRD. Results showed that bacterial inoculations significantly improved rice growth and yield parameters, particularly when supplied with minimal N rate (33). Bacterial inoculations increased chlorophyll content at 43 and 67 days after planting (DAP). Similar trend for tiller numbers (except inoculation with UPMB20) at 43 DAP. UPMB10 significantly increased filled spikelet percentage (87.33) and UPMB19 produced highest straw dry weight (114.34 g/plant), spikelet weight (107 g/plant) and biological yield (220.11 g/plant) with minimal fertilizer-N. The positive effects of bacterial inoculations appeared to be due to N_2-fixing ability, solubilize phosphate and potassium, produce IAA, siderophore, cellulase and pectinase. Thus, locally isolated indigenous PGPR and rhizobial strains have the potential for using as liquid biofertilizer to increase growth and yield of rice minimizing N-fertilizer use.

Introduction

The Food and Agriculture Organization of the United Nation has set a goal to double the food production by 2050 in order to meet the ever-increasing demand (FAO 2012). For increasing yield, rice growers tend to use additional nutrient inputs, especially mineral N, to increase yield (Biswas *et al.* 2000) but this indiscriminate practice had a toll on the environment such as eutrophication of water bodies through leaching, volatilization and surface runoff, soil acidification and increased denitrification resulting in higher greenhouse gas emissions. Xiao-Tang *et al*. (2009) reported that in China the current heavy nitrogen fertilizer applications do not significantly increase crop yield but has doubled the N losses to the environment.

These problems have renewed public interest in exploring alternate or supplementary non-polluting sources of N for agriculture (Ladha *et al.* 1997) and one of the methods is by using biofertilizer. This environmentally friendly approach utilizes beneficial microorganisms called plant growth-promoting rhizobacteria (PGPR) that have the capability to promote plant growth and yield through biological nitrogen fixation (BNF), solubilization of inorganic phosphate, and production of phytohormones, siderophore and hydrolyzing enzymes (Alexander and Zuberer 1991, Boddey *et al.* 1995, Chen *et al.* 2007, Reinhold-Hurek and Hurek 1998, Vessey 2003).

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However, there are reports of inconsistencies on the effects of N_2-fixing bacteria as biofertilizer on crop yield (Andrews *et al.* 2003, Cummings 2009). Furthermore, the information regarding the use of PGPR and rhizobia as biofertilizer in rice under tropical conditions especially in Malaysia is still scarce. The present study thus was undertaken to observe the growth and yield responses of rice variety MR219 inoculated with locally-isolated PGPR and indigenous rhizobia with several inorganic fertilizer-N rates under glasshouse conditions.

Material and Methods

The experiment was undertaken in a glasshouse at Field 10, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The paddy soil was collected from a rice farm at Kuala Selangor, Malaysia. The soil was classified as Beriah Series, order Inceptisol, sub group Typic Endoaquept (Paramananthan 2000), chemical properties are shown in Table 1. Soil was air-dried for two weeks and sieved (2 mm) before being transferred into non-drained plastic pots (15 kg each) having 30 cm diameter × 45 cm height.

Liming test was done prior to planting since the pH of the soil was relatively low (pH 3.58, 1:2.5 KCl) for normal rice cultivation. Approximately 34.5 g calcium carbonate ($CaCO_3$) was added and mixed thoroughly in each pot to ensure uniform distribution and the soil was watered and kept at 20% field capacity for the lime to fully react with the soil for two weeks before planting.

Fertilizer applications were done according to the recommended rate by Department of Agriculture, Kuala Selangor, Selangor, Malaysia for Beriah series. Phosphate and potassium fertilizers were applied as basal dosages and urea was applied during the sowing stage at the rate of 170 kg N/ha and 56.7 kg N/ha for the full and one-third nitrogen treatments, respectively.

Table 1. Chemical properties of the paddy soil from Kuala Selangor, Malaysia.

Soil series	pH (KCl, 1:2.5)	Total N	Available P	Available K	Available Ca	Available Mg
				(%)		
Beriah	3.58	0.55	0.09	0.26	0.27	0.31

Rice seeds were selected physically for uniformity (approximately 0.02 g/seed) and soaked in 20% sodium chloride solution. Floaters were discarded and seeds were surface sterilized by soaking in 95% ethanol for 10 sec (Miche and Balandreau 2001). The seeds were then soaked in sterile distilled water for 24 hrs and decanted and left moist for another 24 hrs. Ten germinated rice seeds were directly seeded to approximately 1 cm depth in each pot at fixed planting points. Plants were watered daily to field capacity for 7 days and subsequently flooded to 5 cm throughout the experiment.

The bacterial inocula used were previously isolated from rice roots (UPMB19 = *Lysinibacillus xylanilyticus*, UPMB20 = *Alcaligenes faecalis)* and leguminous plants (UPMR30 = *Bradyrhizobium japonicum*, UPMR31 = *Rhizobium etli*) grown in rice fields at several main granary areas throughout Peninsular Malaysia. The reference strain UPMB10 (*Bacillus subtilis*) was collected from Soil Microbiology Laboratory, Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia. The biochemical properties and performances of these strains are shown in Table 2.

An amount of 2 ml of the respective bacterial inoculum were inoculated to each rice seed with approximately 10^8-10^9 cfu/ml live bacterial cells. Inoculation was done during the seed sowing stage and repeated at 60 days after planting (DAP).

Treatments applied are as in Table 3. Control pots were inoculated with 2 ml of 0.85% sterile phosphate buffer saline without the bacterial cells.

The leaf chlorophyll contents were determined at 22, 43 and 67 DAP using portable chlorophyll meter (MINOLTA^{TM} SPAD-502). Number of tillers were recorded at 43 and 67 DAP. Yield parameters, namely number of panicles per plant, weight of spikelets per plant, 1000 grain weight, filled spikelet percentage and straw dry weights were recorded during terminal harvest at 115 DAP.

Table 2. Biochemical properties and performances of the selected bacterial strains.

Bacterial isolates	Biological N_2 fixation rate	Phosphate solubiliza-tion rate (µg/ml)	Potassium solubiliza-tion rate (µg/ml)	IAA produc-tion (µg/ml)	Iron siderophore production	Cellulase produc-tion	Pectinase produc-tion
UPMB19	+	30.33	12.67*	23.68***	++	++	-
UPMB20	+	32.11	11.45	23.33***	++	+	-
UPMR30	++	33.50	11.07	13.23*	+	-	-
UPMR31	++	61.87*	10.7	8.93	+	-	-
UPMB10	+	56.59*	14.15*	19.67**	+	+++	+++

Significant at $p < 0.05$; ns = Non significant. All statistical comparison of treatment means is relative to the control. UPMB10 = *Bacillus subtilis,* UPMB19 = *Lysinibacillus xylanilyticus,* UPMB20 = *Alcaligenes faecalis,* UPMR30 = *Bradyrhizobium japonicum,* UPMR31 = *Rhizobium etli.* (Tan *et al.* 2014).

Table 3. Treatment design and description of the respective inoculum.

Treatments	Inoculum	Fertilizer-N rate		
Uninoculated control	-	0% N	33% N	100% N
Inoculated	UPMB10	0% N	33% N	100% N
Inoculated	UPMR19	0% N	33% N	100% N
Inoculated	UPMB20	0% N	33% N	100% N
Inoculated	UPMR30	0% N	33% N	100% N
Inoculated	UPMR31	0% N	33% N	100% N

UPMB10: *Bacillus subtilis,* UPMB19 : *Lysinibacillus xylantilyticus*, UPMB20 : *Alcaligenes faecalis*, UPMR30 : *Bradyrhizobium japonicum* and UPMR31 : *Rhizobium etli* Each treatment had 4 replications.

Rice grains and straws were air-dried and weight were taken at 14% moisture. Data were subjected to ANOVA using SAS software. Means were compared by LSD test at a probability level of 0.05.

Results and Discussion

Growth parameters (Chlorophyll content (SPAD value) and tiller numbers): All bacterial inoculations significantly increased the leaf chlorophyll content at the late vegetative stages (43 and 67 DAP), when supplied with 33% of inorganic fertilizer-N (Table 4). The effects of bacterial inoculation on SPAD values were more prevalent at the early growth stage (43 DAP) when supplied with 100% inorganic fertilizer-N. Without fertilizer-N supply, significant differences were observed at the later growth stage which was at 67 DAP.

Generally, all bacterial inoculations increased rice plant tiller production with and without fertilizer-N at 43 and 67 DAP, although some of the increments were not statistically significant (Table 5). Bacterial inoculations (except UPMB20) produced significantly more tillers with minimal fertilizer-N. However, with full fertilizer-N supply, UPMB20, UPMR30 and UPMR31 significantly produced more tillers *viz.* 32, 31, 33, respectively). The highest tiller number (28) produced with minimal fertilizer-N was from rice plant inoculated with UPMB19.

Table 4. Effects of bacterial inoculations on chlorophyll content (SPAD).

Treatment	SPAD reading @ 22 DAP			SPAD reading @ 43 DAP			SPAD reading @ 67 DAP		
	0%N	33%N	100%N	0%N	33%N	100%N	0%N	33%N	100%N
Control	34.76	33.54	37.99	37.30	37.48	40.98	35.78	36.75	38.51
UPMB10	35.03ns	34.77ns	40.69ns	39.00ns	41.18*	45.91*	37.65	39.38*	40.76ns
UPMB19	34.89ns	34.54ns	38.52ns	38.98ns	42.11*	44.58*	37.90*	41.26*	40.21ns
UPMB20	37.01*	35.03ns	39.06ns	40.16ns	43.00*	45.04*	38.60*	39.64*	41.49*
UPMR30	35.09ns	36.78*	41.20*	38.25ns	41.68*	45.05*	38.10*	40.84*	40.56ns
UPMR31	34.51ns	35.20ns	38.61ns	40.13ns	43.78*	46.03*	38.14*	39.70*	38.58ns

*Significant at $p < 0.05$; ns = Non significant. All statistical comparisons of treatment means are relative to the control.

Data on chlorophyll content and tiller numbers have emphasized the importance of minimal fertilizer-N as a starter fertilizer for the rice plants and bacteria to grow and interact beneficially. These increased parameters indicate the beneficial interactions of these bacterial inocula over the control plant with higher level of inorganic fertilizer-N. These could be due to the higher efficiency of inoculated bacteria to supply N from the BNF processes, particularly when the plants were lacking in nutrient-N, and also due to combined beneficial characteristics such as solubilization of phosphate and production of phytohormone as has been shown in Table 2 and also as reported by other researchers (Richardson *et al*. 2009). BNF process is not the sole supplier of nitrogen especially during the crucial early growth stage of the plant. This is in agreement with Becker *et al*. (1991) which have stated that addition of mineral N fertilizer (30 kg N/ha) as urea increased total N accumulation (40%) in *Sesbania rostrata* legume and yield of the subsequent rice crop. Mia *et al*. (2010) also have demonstrated the effectiveness of PGPR inoculations particularly with minimal fertilizer-N supply (33%) to increase growth, nutrient uptake and yield of banana. In addition, the reference strain UPMB10 has also been inoculated to oil palm seedlings and shown to contribute as much as 63% N in the form of Ndfa through ^{15}N isotope-aided experiment (Zakry *et al*. 2012). It is known that nitrogen application has a major effect on plant growth as well as the bacteria which also need nitrogen as a nutrient source, although they are nitrogen fixers (Yaser *et al*. 2011). Tiller production is a good indicator of rice plant fertility status and always produce a positive correlation to grain yield. Similar findings have been reported by Nayak *et al*. (1986) who found that *Azospirillum lipoferum* inoculation increased rice tiller numbers and subsequently the grain yield. Thus it is hypothesized that these bacterial inoculations could also lead to a significant boost on the grain yield.

Inoculated plants without fertilizer-N supply produced significantly higher 1000-grain weight and straw dry weight (except UPMR31) compared to uninoculated control without fertilizer-N (Table 6). Inoculation with UPMR30 produced significantly highest panicle numbers (25), straw dry weight (113.1 g), spikelet weight (103.6 g), and biological yield (216.6 g). Inoculation with UPMR31 resulted in an increase of 1000-grain and spikelet weight, without increasing the straw dry weight. This has resulted in a 10% increase of the harvest index which was significantly higher than the uninoculated control.

Table 5. Effects of bacterial inoculations on tiller numbers.

Treatment	Tiller Numbers @ 43 DAP			Tiller Numbers @ 67 DAP		
	0 % N	33 % N	100 % N	0 % N	33 % N	100 % N
Control	21.63	23.63	27.00	23.88	27.63	29.25
UPMB10	23.88ns	26.50*	30.75	25.88ns	27.75ns	31.25ns
UPMB19	24.25ns	28.38*	30.25	25.75ns	29.00ns	32.38ns
UPMB20	23.25ns	25.88ns	31.88*	24.75ns	26.75ns	32.88ns
UPMR30	23.63ns	27.50*	31.13*	28.13ns	27.88ns	31.38ns
UPMR31	24.38ns	27.63*	32.63*	26.00ns	28.38ns	33.50ns

*Significant at $p < 0.05$; ns = Non significant. All statistical comparisons of treatment means are relative to the control.

Table 6. Effects of bacterial inoculations with 0% inorganic fertilizer-N application on yield parameters of rice.

Treatment	Number of panicles/ plant	Filled spikelet/ plant (%)	1000-grain weight (g)	Straw dry weight/plant (g)	Spikelet weight/plant (g)	Biological yield (g)	Harvest index
Control	22.14	72.88	22.6	91.20	85.98	177.18	0.48
UPMB10	23.33ns	75.67ns	25.34*	103.44*	74.66ns	178.10ns	0.42ns
UPMB19	23.41ns	77.00ns	25.04*	104.09*	74.67ns	178.76ns	0.42ns
UPMB20	23.25ns	84.17*	26.13*	106.32*	89.6ns	195.92*	0.46ns
UPMR30	25.18*	82.00ns	26.08*	113.07*	103.57*	216.64*	0.48ns
UPMR31	23.33ns	80.83ns	25.35*	90.35ns	103.06*	193.41ns	0.53*

*Significant at $p < 0.05$; ns = Non significant. All statistical comparisons of treatment means are relative to the control

Effects of the bacterial inoculations on rice yield parameters, especially on panicle numbers, straw dry weight, spikelet weight and biological yield, were clearly observed on plants supplied with 33% inorganic fertilizer-N (Table 7). With full fertilizer-N, bacterial inoculations (except UPMR31) resulted in a significant increase of the 1000 grain weight values (Table 8). All inoculated plants without fertilizer-N produced significantly higher 1000 grain and straw dry weight (except UPMR31) when compared to the control with 33% fertilizer-N. Inoculation with UPMB19 with minimal fertilizer-N (33% N) resulted in a slightly higher spikelet weight compared to the control with full fertilizer-N (100% N). The spikelet weight value is 107 g with the bacterial inoculation as compared to 102.86 g obtained with the full control, an increment of 4%.

Without fertilizer-N addition, all bacterial inoculations still produced higher 1000-grain weight than the control and some of these values were higher than findings by Morteza *et al*.

(2011) which recorded a maximum 1000 grain weight of 25.42 g in plants treated with 2 t/ha organic fertilizer. UPMR30 performed better with no fertilizer-N supply which was probably due to its rhizobial characteristics as a good nitrogen fixer with legumes (Table 2). This is in agreement with Wu *et al.* (2005) and Wani *et al.* (2007) who noted that low levels of fertilizer-N resulted in a higher level of microbial population in the rhizosphere.

Table 7. Effects of bacterial inoculations with 33% inorganic fertilizer-N application on yield parameters of rice.

Treatment	Number of panicles/ plant	Filled spikelet/plant (%)	1000-grain weight (g)	Straw dry weight/ plant (g)	Spikelet weight/plant (g)	Biological yield (g)	Harvest index
Control	21.60	81.60	25.10	84.77	88.29	173.06	0.51*
UPMB10	22.71ns	87.33*	27.02*	100.44*	100.94*	201.38*	0.50ns
UPMB19	26.68*	81.00ns	26.58ns	114.34*	107.00*	221.34*	0.48ns
UPMB20	24.83*	84.17ns	26.06ns	109.82*	96.93ab	206.74*	0.47ns
UPMR30	24.00ns	81.67ns	27.17*	106.50*	101.10*	207.60*	0.49ns
UPMR31	25.33*	80.80ns	25.86ns	112.18*	95.80ab	207.98*	0.46ns

*Significant at $p < 0.05$; ns = Non significant. All statistical comparisons of treatment means are relative to the control.

Table 8. Effects of bacterial inoculation with 100% inorganic fertilizer-N application on yield parameters of rice.

Treatment	Number of panicles/ plant	Filled spikelet/ plant (%)	1000-grain weight (g)	Straw dry weight/plant (g)	Spikelet weight/ plant (g)	Biological yield (g)	Harvest index
Control	27.43	80.14	25.55ns	109.39ns	102.86	212.26	0.49
UPMB10	28.83ns	83.17ns	28.18*	129.56*	104.93ns	234.50*	0.45ns
UPMB19	28.68ns	89.20*	27.34*	128.29*	116.31*	244.59*	0.48ns
UPMB20	28.00ns	83.67ns	27.53*	117.02ns	113.76ns	230.78ns	0.49ns
UPMR30	27.33ns	83.40ns	27.59*	112.20ns	119.06*	231.26ns	0.51ns
UPMR31	30.71ns	86.20ns	26.57ns	134.23*	100.62ns	234.93*	0.43ns

*Significant at $P < 0.05$; ns = Non significant. All statistical comparisons of treatment means are relative to the control.

The UPMR31 inoculation also produced a beneficial effect on rice plant as this particular rhizobial strain enhanced the spikelet weight without any significant increment in the vegetative part of the plant (straw). It contributed to a significantly higher harvest index value at 0.53, much higher than findings by Khorshidi *et al*. (2011) which reported a harvest index value of 0.50 with *Pseudomonas* inoculation and nitrogen fertilization at 75 kg N/ha. Low harvest index value could lead to a detrimental lodging, often associated with excessive application fertilizer-N (Yanni and Dazzo 2010).

The rhizobial isolate from soybean, UPMR30, could also enhance the yield parameters of the rice plant, with minimal fertilizer-N which suggests that it may perform well under conditions of zero and minimal fertilizer-N. This is in agreement with findings by Yanni and Dazzo (2010) which recorded similar trends of rice grain yield when inoculated with rhizobial strain *Rhizobium leguminosarum* bv. trifolii at minimal doses of fertilizer-N. These symbiotic effects were greater than the effect of sole application of inorganic fertilizer which have been known to exhibit high nutrient losses and low plant uptake ratio (Choudhury and Kennedy 2005, Peng *et al*. 2011). The significant enhancement in spikelet weight upon bacterial inoculations (UPMB19 and UPMR30) can be directly translated to the increment in plant grain yield. Thus, these PGPR and rhizobial strains could be applied during the conventional rice cultivation to further increase the maximum yield potential of the plant. The average yield of 3.75 t/ha obtained in Malaysia (DOA Malaysia 2012) could be increased by approximately 13 and 16%, respectively, through incorporation of these two bacterial strains in the current conventional practices.

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