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INFLUENCE OF BIOFERTILIZER ON GROWTH IMPROVEMENT OF AEROBIC RICE GENOTYPES

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ABSTRACT

Biofertilizers have emerged as a potential environment friendly inputs that are supplemented for proper plant growth. Two field experiments were conducted at University Putra Malaysia (UPM), Selangor, Malaysia during 1st season (May to September 2015) and 2nd season (September to December 2015) to evaluate the performance of aerobic rice genotypes under different combinations of bio and chemical fertilizer. The experiment consisted of five (5) biofertilizer combinations (chemical fertilizer recommended rate (CFRR) (150 N; 60 P_2O_5; 60 K_2O kg ha^{-1}) 100%, biofertilizer 1 ton + 75% CFRR, biofertilizer 2 tons + 50% CFRR, biofertilizer 3 tons + 25% CFRR, and biofertilizer 4 tons) and three aerobic rice genotypes (MRIA1, MR219-4 and MR219-9) laid out in a split-plot in randomized complete block design (RCBD) with 3 replicates. Results revealed that application of biofertilizer 1 ton + 75% CFRR produced the highest plant height (102.65 cm) and a number of tillers (20.38) in the 2nd season, the highest grain yield (4.30 t ha^{-1}) than the other biofertilizer treatments in the 1st season. MR219-4 produced the highest number of tillers, leaf area and leaf photosynthesis rate in both seasons, grain yield (5.02 t ha^{-1}) in 1st season while MR219-9 produced the highest crop biomass (52.76 g $hill^{-1}$) and crop growth rate (8.00 g $hill^{-1}$ $week^{-1}$) at 12 WAS, grain yield (3.86 t ha^{-1}) than the other genotypes in the 2nd season. The trend in these results indicated that biofertilizer tend to be more effective with limited chemical fertilizer in the 1st season of low rainfall. These results concluded that 1 ton biofertilizer + 75% CFRR and MR219-4 enhances rice growth and grain yield in aerobic condition.

Key words: rice production, eco-friendly, rice varieties, sustainable agricultural system, chemical fertilizer.

INTRODUCTION

Rice is the main food of majority of the people in Asia and likewise is distinct in freshwater usage. Increasing food demand challenge and lessening water availability has become a threat to Asian food security in which almost sixty percent (60%) of the population around the globe lives in the region (Bouman and Tuong 2000). Comparatively, rice is more often cultivated under irrigation conditions as against other field crops, where this practices consumed nearly fifty percent (50%) of the total freshwater meant for irrigation. For which the rice alone consumed nearly eighty percent (80%) of the water used (Farooq *et al.,* 2009; Guerra *et al*., 1998). Relative to the soil, climatic condition of the area and the variety of rice, it is estimated that between 1500 and 5000 liters of water are required to produce one kilogram of unprocessed rice under irrigation system (Tao *et al*., 2006).

Aerobic rice production system is an innovative way of cultivating rice in highly drained, non-puddled, and non-saturated soils without standing water. This method uses specialized rice varieties those respond to inputs and balanced management practice to achieve a minimum of 4 to 6 tons per hectare yield with just fifty percent (50%) to seventy percent (70%) of the water requirement for rice production under irrigation (Akter *et al*., 2016). In this method, the land is prepared under dry condition and brought to a fine tilt, the seeds are sown by dibbling in a determined ratio of rows with adequate spacing and soil moisture condition is retained almost close to field capacity (Shanmuganathan, 2006).

Aerobic rice research is at its infancy stage in Malaysia. Conversely, several studies have been conducted by Malaysian Agricultural Research and Development Institute (MARDI) concerning selection and assessment of potential aerobic rice lines from International Rice Research Institute (IRRI). As such, few lines like AERON1, AERON4 (Habibuddin 2009), MRIA1 (Sariam *et al*., 2013), MR219-4 and MR219-9 (Abdul Rahim *et al*., 2012) are identified as potential aerobic rice varieties. Researchers from MARDI developed a provisional technological package for aerobic rice production system in Malaysia. These technological packages comprise of crop establishment method and nutrients management techniques (Sariam, 2009) and aerobic rice weed management sustainability (Azmi, 2008).

For the last 150 years, the concomitant increase in the application of chemical fertilizer as input has caused damage to the soil and rendered it less productive as well as making farming unsustainable and create serious health and environmental hazards. In this situation, biofertilizer and growth promoting bacteria were recommended in lieu of chemical fertilizer. (Wu *et al*., 2005). Recently, biofertilizer has been identified as a vital constituent of the supply system of integrated nutrient management, this will be a promising device for the improvement of crop yield via eco-friendly supply of nutrients (Wu *et al.,* 2005). These potential biofertilizers play an important function in sustaining the soil and crop production as well as protecting the environment to be eco-friendly and cost-effective input to the farmer. Biofertilizer is less costly, eco-friendly and it is a renewable source of plant nutrient to complement inorganic fertilizer in sustaining agriculture (Khorso and Yousef, 2012).

Biofertilizer is a preparation comprising living cells of various categories of microbes which if applied to soil, plant surfaces or seeds, inhabit the rhizosphere or the internal plant system and stimulating transformation of vital nutrient elements (nitrogen, phosphorus, and potassium) for plant growth from inaccessible to accessible forms via other processes biologically like fixation of nitrogen and solubilization of phosphate rock (Rokhzadi *et al*., 2008).The growth and rice productivity can be enhanced by applying organic- and biofertilizer, hence this can decrease the amount of inorganic nitrogen fertilizer applied (Tran thi *et al.,* 2001). Previous reports showed that biofertilizer could be highly beneficial to the growth of the plant where it is applied along with chemical fertilizers (Panhwar *et al*., 2014). Furthermore, biofertilizer could be an effective technique to lessen the toxic effect from the Aluminum element and ultimately improve the soil fertility (Panhwar *et al.,* 2014).

It is hypothesized that biofertilizer might have significant impacts on the growth of some aerobic rice genotypes. Therefore, the present experiment was conducted to assess the effects of various combinations of bio- and chemical fertilizers on the growth enhancement of aerobic rice genotypes in a tropical environment.

MATERIALS AND METHODS

Experimental site: Two field experiments were conducted at Universiti Putra Malaysia (UPM), (latitude 3° 02'N; longitude 101° 42' E and on the altitude of 31 m above sea level) Selangor, Malaysia during the 1st season (May to September 2015) and 2nd season (September to December 2015). The experimental site was previously planted with sweet corn and was cleared of the debris prior to land preparation. Soil analysis of the experimental site revealed that the soil was clay loam and has a pH value of 6.50 and 6.42, in 1st and 2nd season, respectively (Table 1). The total rainfall received and temperature during the experiments was recorded in 1st and 2nd season (Table 2).

Experimental materials Aerobic rice varieties: Three (3) aerobic rice genotypes namely, MRIA1 obtained from Malaysian Agriculture Research and Development Institute (MARDI) while MR219-4 and MR219-9 obtained from Malaysian Nuclear Agency were used for the experiments.

Preparation of biofertilizer: The biofertilizer used constituted a nitrogen-fixing bacteria conglomeration (*Bacillus sp.* Sb35 and 42) and bacteria for the solubilization of phosphate (*Bacillus sp.* PSB16). One liter of each inoculum was diluted in 4 liters of distilled water (dH_2O) + molasses in soil microbiology laboratory (bacteria). The biofertilizer was then prepared in the biofertilizer processing laboratory glasshouse field 2 using empty fruit bunch (EFB) and peat moss in the ratio of 1:1:1. The prepared biofertilizer was stored at ambient room temperature for one month for the bacteria to multiply prior to application.

Treatments and experimental design: The treatments comprised of five (5) biofertilizer combinations (chemical fertilizer recommended rate (CFRR) (150 N; 60 P_2O_5; 60 K_2O kg ha^{-1}) 100%, biofertilizer 1 ton + 75% CFRR, biofertilizer 2 tons + 50% CFRR, biofertilizer 3 tons + 25% CFRR, biofertilizer 4 tons) and three aerobic rice genotypes (MRIA1, MR219-4 and MR219-9). The experiments were laid out in a split-plot in a randomized complete block design (RCBD) with 3 replicates, with biofertilizer in the main plot and aerobic rice genotypes in the sub-plots.

Crop husbandry practices: Land was ploughed and rotovated to obtain a fine tilt and then marked out as required plot sizes with 1.0 meter space between blocks and 0.5 m spacing between plots. The gross and net plot sizes were 2.5 m × 1.5 m (3.75 m^2) and 2.0 m ×1 m (2.0 m^2), respectively constituting 6 rows in the gross plots and 4 rows in the net plots, respectively. Sown seeds were apparently treated with 70% chlorox (5.25% sodium hypochloride solution) for 30 minutes then rinsed with sterile water (Amin *et al*., 2004). Sowing was done on

24th May 2015 and 3rd September 2015 at an intra and inter-row spacing of 25 cm × 25 cm. Ten (10) dry treated rice seeds were sown hill^{-1} that was later thinned to 5 seedlings hill^{-1} at 14 days after sowing (DAS). 'Butachlor' herbicide (1.2 kg a.i ha^{-1}) was sprayed 2 DAS in 2nd season only. In both seasons, 'Basagran' herbicide (bentazone 0.8 kg a.i ha^{-1} and MCPA 0.12 kg a.i ha^{-1}) was sprayed 21 and 28 DAS in 1st and 2nd season, respectively followed by manual weeding throughout the growing seasons to control weeds. Biofertilizer was incorporated into the soil a day before crop establishment. Compound fertilizer (NPK 15:15:15) was applied at the rate of 400 kg ha^{-1} as basal; and Urea at the rate of 196 kg ha^{-1} in two split doses by side placement at 28 and 56 DAS to supply total recommended nutrients of 150 N; 60 P_2O_5; 60 K_2O kg ha^{-1}. Both were applied as prescribed by the treatments. The crop was grown rain-fed but supplemental irrigation was carried out using a sprinkler to keep the soil at field capacity throughout the growing season. The field was netted to prevent birds' damage. Other pests observed at different growth stages of the crop were rodents and insects, at 4 WAS insect leaf roller (folder) was controlled by application of Lebaycin 550EC, at 8 WAS stem borer (dead heart) was controlled by using Furadon 3G (AGRITOX 3G) @ 17 kg ha^{-1} and at 10 WAS rice bug was controlled by using Hoppergone @ 0.987 l ha^{-1}. Rodents at 14 WAS controlled by Matikus rodenticide (a.i brodifacoum). Diseases viz panicle blast at 14 WAS controlled by Carbendazim (fungicide). All insecticides and fungicide were applied and sprayed at 2 weeks interval. The crop was harvested when the panicles matured, (90% of the grains have turned golden yellow), and grains have hardened enough. A total of sixteen hills equivalent to 1 m^2 from the net plots were cut with a serrated sickle. The panicles were sun-dried, weighed and later threshed and winnowed to obtain clean grains. The grains and straw biomass were recorded.

Parameters measured

Agronomic parameters: Five (5) rice hills from each net plot of all the treatments were randomly sampled and their height was measured at 4, 8 and 12 weeks after sowing (WAS). A number of tillers were counted manually following the same interval.

Leaf area and crop biomass hill^{-1} were determined at 4, 8 and 12 WAS. One rice hill plot^{-1} outside the harvest area was randomly sampled, uprooted and washed to remove all soil particles adhering to the shoot and root. The samples from the field were then brought to the laboratory. Leaf area measurement was done using leaf area meter (MODEL: LI-3100 AREA METER, USA). After the measurement, a constant weight was achieved by oven drying the samples at 70 °C for 72 hours, the plant biomass was then weighed using digital balance and readings were recorded.

Physiological parameters: The chlorophyll content was measured from fully expanded healthy leaves 4 weeks interval starting from 4 weeks after sowing using a portable chlorophyll meter (MINOLTA ™ SPAD-502, Minolta camera Co, Osaka, Japan). The youngest fully expanded leaf (YEL) of each plant an average of 15 leaves plot^{-1} (5 leaves hill^{-1}) SPAD readings were recorded.

A portable photosynthesis system (LI-6400XT, LI-COR Inc. Lincoln, Nebraska, USA) was used for the measurement of single leaf net photosynthesis (µmol CO_2 m^{-2} s^{-1}) at 75 and 65 DAS in 1st and 2nd season respectively. Under full sunlight and at constant CO_2 of 380 µmol CO_2 mol^{-1} in the chamber, the measurements were carried out.

At 4, 8 and 12 WAS, crop growth rate (CGR) was calculated using the following equation

$$CGR = \frac{w2 - w1}{t2 - t1} \text{ (g hill}^{-1}\text{ week}^{-1}\text{)}$$

Where w_2 and w_1 are dry shoot weight taken at two consecutive harvest time's interval t_2 and t_1

Only the data at 12 WAS for plant height, a number of tillers, SPAD value, leaf area, crop biomass and crop growth rate were presented.

Rice grain yield (t ha^{-1}): Panicles from each net plot (1 m^2) were harvested, threshed, winnowed to remove the chaff, weighed using weighing balance and the grain yield was converted to ton ha^{-1}.

Rice straw yield (t ha^{-1})**:** At harvest, five (5) rice hills per net plot were randomly sampled and cut from the above-ground portion of the plant. These were oven dried at 70 °C for 72 hours and the plant biomass was recorded to obtain the rice straw yield**.**

Harvest index (HI): Harvest index is the proportion of grain in the total biomass of the plant and was obtained by taking the ratio of the weight of grains to the total dry plant materials.

$$H1 = \frac{Grain\ weight}{Total\ dry\ matter}$$

Data analysis: Collected data were subjected to analysis of variance (ANOVA) in order to determine any significant difference among treatments using the SAS Software Program (Version 9.4), and treatment means were compared using Duncan's New Multiple Range Test (DNMRT).

RESULTS

Plant height: Application of biofertilizer significantly ($p<0.05$) affected plant height in 2nd season only (Figure 1). The tallest plants (102.93 cm) were significantly produced from CFRR 100% and the shortest plants (89.28 cm) were from 4 tons biofertilizer at par with 2 tons biofertilizer + 50% CFRR and 3 tons biofertilizer + 25% CFRR. Similarly, plant height was significantly affected by genotypes (Figure 1) in both seasons. The tallest plants (93.85 cm and 111.47 cm) were produced by MRIA1. MR219-4 and MR219-9 were different in the 1st season but at par in the 2nd season. In both seasons, there was no significant ($p>0.05$) interaction between biofertilizer × genotypes on plant height.

A number of tillers: In both seasons, there was significant ($p<0.05$) effect of biofertilizer on a number of tillers (Figure 2). The maximum number of tillers (21.64 and 22.22) were significantly produced from CFRR 100%. The least number of tillers (15.46 and 16.97) were produced from 4 tons biofertilizer at par with 2 tons biofertilizer + 50% CFRR and 3 tons biofertilizer + 25% CFRR in the 2nd season. A number of tillers were significantly affected by genotypes (Figure 2) in both seasons. MR219-4 significantly produced the highest number of tillers (23.85 and 23.80) at par with MR219-9. In both seasons, there was no significant ($p>0.05$) interaction between biofertilizer × genotypes on a number of tillers.

Leaf chlorophyll content (SPAD value): In both seasons, leaf chlorophyll content was not significantly ($p>0.05$) affected by biofertilizer (Figure 3) but it was significantly ($p<0.05$) affected by genotypes (Figure 3). In both seasons, MRIA1 significantly produced the highest (38.78 and 34.82) SPAD value different from the other genotypes. In both seasons, there was no significant ($p>0.05$) interaction between biofertilizer × genotypes on SPAD value.

Leaf area and crop biomass: In both seasons, there was significant ($p<0.05$) effect of biofertilizer and genotypes, the interaction between biofertilizer × genotypes on leaf area and crop biomass. In the 1st season, in the biofertilizer treatments, MR219-4 significantly produced the highest leaf area that ranged from 872.75 - 1292.69 cm^2 $hill^{-1}$ different from the other genotypes except in 2 tons biofertilizer + 50% CFRR and 3 tons biofertilizer + 25% CFRR. In all biofertilizer treatments, MRIA1 significantly produced the least leaf area that ranged from 300.75 - 648.66 cm^2 $hill^{-1}$ except in 3 tons biofertilizer + 25% CFRR (Table 3).

In the 2nd season, in all the biofertilizer treatments, MR219-4 significantly produced the highest leaf area that ranged from 1670.42 - 3275.14 cm^{-2} $hill^{-1}$ at par with MR219-9 except in 1 ton biofertilizer + 75%. MRIA1 significantly produced the least leaf area that ranged from 318.67 - 627.19 cm^{-2} $hill^{-1}$ (Table 4).

In 1st season, there was significant ($p<0.05$) effect of biofertilizer and genotypes; and interaction between biofertilizer × genotypes on crop biomass (Table 5). In all the biofertilizer treatments, MR219-4 significantly produced the heaviest crop biomass that ranged from 40.76 - 52.79 g $hill^{-1}$ different from the other genotypes except in 2 tons biofertilizer + 50% CFRR. MRIA1 significantly produced the lightest crop biomass that ranged from 18.51 - 33.33 g $hill^{-1}$ in all the biofertilizer treatments that were significant. There was no significant effect ($p>0.05$) of genotypes on crop biomass in 1 ton biofertilizer + 75% CFRR and 3 tons biofertilizer + 25% CFRR.

In the 2nd season, there was significant ($p<0.05$) effect of biofertilizer and genotypes; and interaction between biofertilizer × genotypes on crop biomass (Table 6). Plants in CFRR 100% significantly produced the highest crop biomass (55.10 g $hill^{-1}$) different from the other biofertilizer treatments and the least crop biomass (36.28 g $hill^{-1}$) was produced in 4 tons biofertilizer. MR219-4 significantly produced the highest crop biomass (52.59 g $hill^{-1}$) while MRIA1 significantly produced the least crop biomass (28.30 g $hill^{-1}$). In all the biofertilizer treatments except in 3 tons biofertilizer + 25% CFRR and in 4 tons biofertilizer, MR219-9 significantly produced the highest crop biomass that ranged from 48.72 - 79.35 g $hill^{-1}$. In all the biofertilizer treatments MRIA1 significantly produced the least crop biomass that ranged from 19.18 - 32.93 g $hill^{-1}$.

Crop growth rate (CGR): In 1st season at 12 WAS, there was no significant ($p>0.05$) effect of biofertilizer on crop growth rate but genotypes and interaction between biofertilizer × genotypes had significant ($p<0.05$) effect on crop growth rate. MR219-4 significantly produced the highest crop growth rate (3.66 g $hill^{-1}$ $week^{-1}$) while MR219-9 significantly produced the least crop growth rate (2.67 g $hill^{-1}$ $week^{-1}$). In the case of interaction effect, in 1st season, at 12 WAS in CFRR 100%, 1 ton biofertilizer + 75% CFRR and 3 tons biofertilizer + 25% CFRR,, there was no significant ($p>0.05$) effect of genotypes on crop growth rate. In 2 tons biofertilizer + 50% CFRR, MR219-4 significantly produced the highest crop growth rate (3.29 g $hill^{-1}$ $week^{-1}$). MRIA1 significantly produced the least crop growth rate (1.13 g $hill^{-1}$ $week^{-1}$). In 4 tons biofertilizer, MR219-4 significantly produced the highest crop growth rate (3.89 g $hill^{-1}$ $week^{-1}$). MRIA1 significantly produced the least crop growth rate (1.49 g $hill^{-1}$ $week^{-1}$) Table7.

In 2nd season at 12 WAS, there was significant ($p<0.05$) effect of biofertilizer and genotypes; and interaction between biofertilizer × genotypes on crop growth rate. In the biofertilizer treatments, plants in 3 tons biofertilizer + 25% CFRR significantly produced the highest crop growth (5.27 g $hill^{-1}$ $week^{-1}$) while plants in 4 tons biofertilizer significantly produced the least crop growth rate (2.81 g $hill^{-1}$ $week^{-1}$). MR219-9 significantly produced the highest crop growth rate (8.00 g $hill^{-1}$ $week^{-1}$) while MRIA1 significantly produced the least crop growth rate (0.10 g hill^{-1} week^{-1}). In the case of interaction effect, at 12 WAS in CFRR 100% and 1 ton biofertilizer + 75% CFRR, MR219-9 significantly produced the highest crop growth rate (12.28 and 10.54 g hill^{-1} week^{-1}), respectively. MRIA1 and MR219-4 significantly produced negative (-) crop growth rate (-4.64 and -0.47 g hill^{-1} week^{-1}), respectively. In 2 tons biofertilizer + 50% CFRR, MR219-9 significantly produced the highest crop growth rate (5.79 g hill^{-1} week^{-1}). MRIA1 significantly produced the least crop growth rate (2.57 g hill^{-1} week^{-1}). In 3 tons biofertilizer + 25% CFRR, MR219-4 significantly produced the highest crop growth rate (9.74 g hill^{-1} week^{-1}). MRIA1 significantly produced negative (-) crop growth rate (-0.55 g hill^{-1} week^{-1}). In 4 tons biofertilizer, MR219-9 significantly produced the highest crop growth rate (4.81 g hill^{-1} week^{-1}) different from other genotypes that differed significantly. MR219-4 significantly produced the least crop growth rate (0.67 g hill^{-1} week^{-1}) Table 8.

Leaf photosynthesis: There was significant ($p<0.05$) effect of biofertilizer and genotypes on leaf photosynthesis in 1st season only and there was a significant effect of interaction between biofertilizer × genotypes on leaf photosynthesis in 2nd season only (Table 9). In 1st season, 1 ton biofertilizer + 75% CFRR significantly produced the highest photosynthetic rate (14.06 µmol CO_2 m^{-2} s^{-1}). The least photosynthetic rate (9.91 µmol CO_2 m^{-2} s^{-1}) was significantly produced by 4 tons biofertilizer MR219-4 significantly produced the highest photosynthetic rate (14.09 µmol CO_2 m^{-2} s^{-1}) while MR219-9 significantly produced the least photosynthetic rate (10.23 µmol CO_2 m^{-2} s^{-1}).

In 2nd season interaction between biofertilizer × genotypes (Table 10), the genotypes significantly differed in CFRR 100% and in 4 tons biofertilizer. MR219-9 significantly produced the highest photosynthesis rate (20.68 µmol CO_2 m^{-2} s^{-1}) in CFRR 100% different from MRIA1 that significantly produced the least photosynthesis rate (13.09 µmol CO_2 m^{-2} s^{-1}). MR219-4 significantly produced the highest photosynthesis rate (18.80 µmol CO_2 m^{-2} s^{-1}) in 4 tons biofertilizer different from the other genotypes that differed from each other. MR219-9 significantly produced the least photosynthesis rate (16.52 µmol CO_2 m^{-2} s^{-1}). There was no significant effect of genotypes on leaf photosynthesis in 1 ton biofertilizer + 75% CFRR, 2 tons biofertilizer + 50% CFRR, and 3 tons biofertilizer + 25% CFRR.

Grain yield: Biofertilizer had significant ($p<0.05$) effect on grain yield in 1st season (Table 9). Biofertilizer 1 ton + 75% CFRR produced the highest grain yield (4.30 t ha^{-1}) while 4 tons biofertilizer produced the lowest grain yield (2.95 t ha^{-1}). MR219-4 produced the highest grain yield (5.02 t ha^{-1}) in the 1st season while MR219-9 produced the highest grain yield (3.86 t ha^{-1}) in the 2nd season. MRIA1 produced the lowest grain yield (1.85 and 2.47 t ha^{-1}) in 1st and 2nd season, respectively.

Straw yield: Biofertilizer significantly ($p<0.05$) affected straw yield in both seasons (Table 9). CFRR 100% produced the highest straw yield (8.39 and 9.16 t ha^{-1}) in 1st and 2nd season, respectively while 4 tons biofertilizer produced the lowest straw yield (6.41 and 6.57 tons ha^{-1}) in 1st and 2nd season, respectively. MR219-4 produced the highest straw yield (9.25 t ha^{-1}) in the 1st season while MR219-9 produced the highest straw yield (8.72 t ha^{-1}) in the 2nd season. MRIA1 produced the lowest straw yield (5.06 and 4.98 t ha^{-1}) in 1st and 2nd season, respectively.

Harvest index: Biofertilizer had significant ($p<0.05$) effect on harvest index in 2nd season (Table 9). CFRR 100% produced the lowest harvest index (0.40) while MRIA1 produced the lowest harvest index (0.38) in the 1st season.

Table 1. Physico-chemical properties of the soil (0 - 20 cm) at the site collected prior to the onset of the experiment for the 1st and 2nd season.

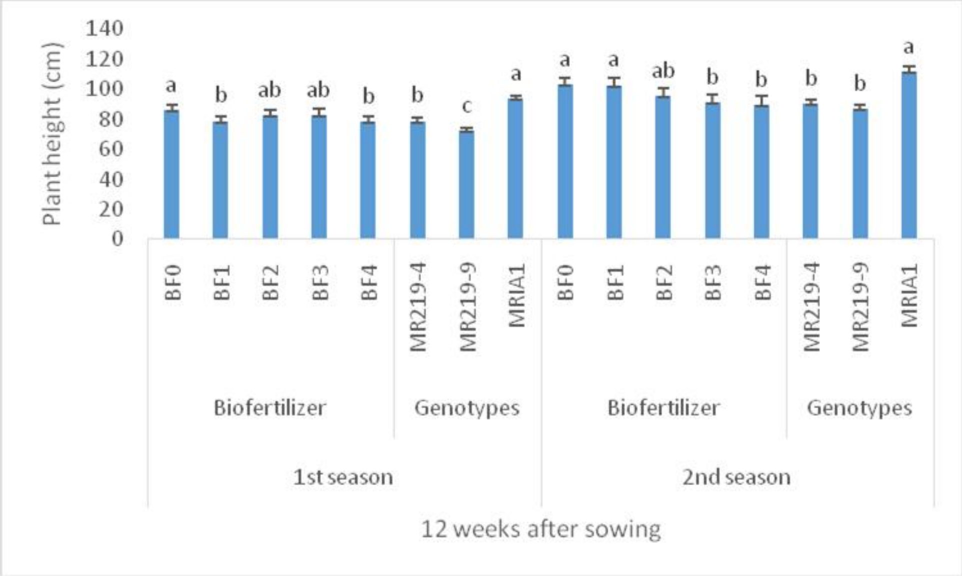
Properties	1st season	2nd season
Physical properties		
Sand	37.36	40.46
Silt	37.36	40.46
Clay	29.88	28.99
Soil Texture Class	clay loam	clay loam
Chemical properties		
pH	6.5	6.42
EC (µS/cm)	58	113.9
CEC (cmol/kg)	5.05	5.01
Total C (%)	0.79	0.77
Total N (%)	0.06	0.07
Total S (%)	0.02	0.02
Extractable (µg/g)		
P	84.7	165.2
K	41.24	55.07
Ca	876.7	955.9
Mg	62.7	106.6
Cu	1.60	1.54
Fe	168	145.4
Mn	5.95	11.1
Zn	1.72	2.54

Table 2. Monthly temperature and rainfall at the experimental site from May- December 2015.

1st season					2nd season				
	Temperature (°C)			Rainfall (mm)		Temperature (°C)			Rainfall (mm)
Month	Max.	Min.	Ave.		Month	Max.	Min.	Ave.	
May	34	25	29	34	September	33	25	29	98
June	33	26	29	125	October	33	25	29	411
July	34	25	29	65	November	33	24	28	426
August	33	25	29	324	December	33	24	28	503
Average	33.5	25.25	29		Average	33	24.5	28.5	
Total				548	Total				1438

Source: http://www.accuweather.com/en/my/salak-selatan/228560/august-weather/228560?monyr=8/1/2015&view=table

(1A)



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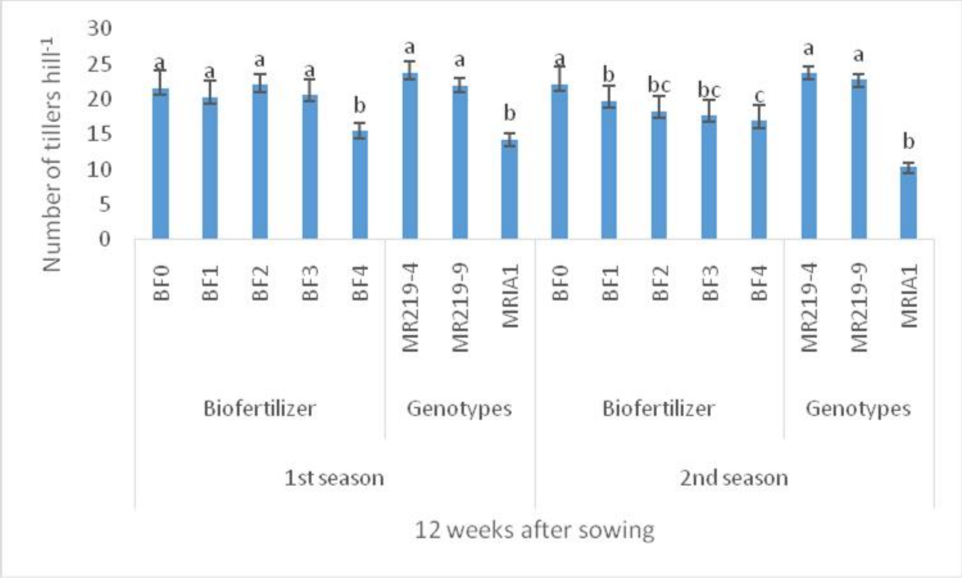

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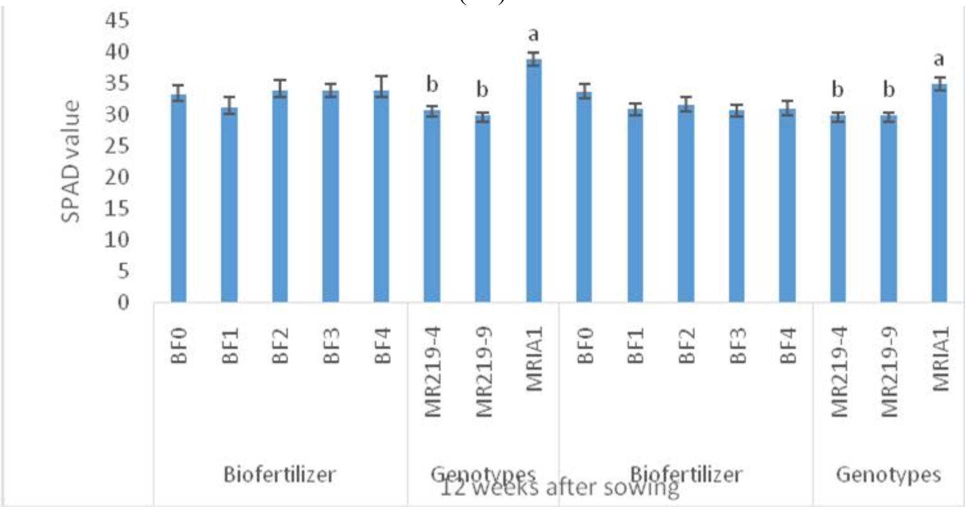

Figure 1. (A) plant height at 12 weeks after sowing, (B) number of tillers at 12 weeks after sowing and (C) SPAD value at 12 weeks after sowing as influenced by biofertilizer and rice genotypes for 1st and 2nd season. Different letter(s) above bars indicate a significant difference at p≤0.05 according to Duncan's multiple range test (DMRT). BF0 = CFRR 100%, BF1 = biofertilizer 1 ton + 75% CFRR, BF2 = biofertilizer 2 tons + 50% CFRR, BF3 = biofertilizer 3 tons + 25% CFRR, BF4 = biofertilizer 4 tons, CFRR = chemical fertilizer recommended rate

Table 3. Leaf area at 12 weeks after sowing as influenced by the interaction between biofertilizer × genotypes for the 1st season.

	Biofertilizer (ha^{-1})					
Genotypes	**BF0**	**BF1**	**BF2**	**BF3**	**BF4**	**Mean**
			cm^2 hill^{-1}			
MR219-4	1292.74a	1095.69a	1042.55a	773.75b	872.75a	1015.50a
MR219-9	1126.91b	1031.07a	1131.94a	1051.29a	652.52b	998.75a
MRIA1	548.45c	648.66b	300.75b	789.17b	363.70c	530.15b
Mean	989.36A	925.14B	825.08C	871.40C	629.66D	
Significance level			***			
SEM			34.486			

Means in a column followed by the same letter are not significantly different at P=0.05 using Duncan's new multiple range test (DNMRT), BF0 = CFRR 100%, BF1 = biofertilizer 1 ton + 75% CFRR, BF2 = biofertilizer 2 tons + 50% CFRR, BF3 = biofertilizer 3 tons + 25% CFRR, BF4 = biofertilizer 4 tons, CFRR = chemical fertilizer recommended rate, SEM = standard error of means

Table 4. Leaf area at 12 weeks after sowing as influenced by the interaction between biofertilizer × genotypes for the 2nd season.

	Biofertilizer (ha^{-1})					
Genotypes	**BF0**	**BF1**	**BF2**	**BF3**	**BF4**	**Mean**
			cm^2 hill^{-1}			
MR219-4	3275.14a	1725.05b	1670.42a	2160.47a	2201.45a	2206.51a
MR219-9	3261.75a	2376.14a	1450.85a	1565.48b	1327.72b	1996.39b
MRIA1	318.67b	364.69c	491.07b	627.19c	561.01c	472.53c
Mean	2285.19A	1488.63B	1204.12C	1451.05B	1363.39B	
Significance level			***			
SEM			51.021			

Means in a column followed by the same letter are not significantly different at P=0.05 using Duncan's new multiple range test (DNMRT), BF0 = CFRR 100%, BF1 = biofertilizer 1 ton + 75% CFRR, BF2 = biofertilizer 2 tons + 50% CFRR, BF3 = biofertilizer 3 tons + 25% CFRR, BF4 = biofertilizer 4 tons, CFRR = chemical fertilizer recommended rate, SEM = standard error of means

Table 5. Crop biomass at 12 weeks after sowing as influenced by the interaction between biofertilizer × genotypes for the 1st season.

	Biofertilizer (ha^{-1})					
Genotypes	**BF0**	**BF1**	**BF2**	**BF3**	**BF4**	**Mean**
			g hill^{-1}			
MR219-4	52.79a	40.05a	40.76a	38.86a	42.20a	42.93a
MR219-9	48.78ab	32.66a	36.81a	41.29a	20.45b	36.00b
MRIA1	33.33b	37.72a	18.51b	44.17a	19.58b	30.66c
Mean	44.97A	36.81AB	32.03BC	41.44A	27.41C	
Significance level			***			
SEM			3.207			

Means in a column followed by the same letter are not significantly different at P=0.05 using Duncan's new multiple range test (DNMRT), BF0 = CFRR 100%, BF1 = biofertilizer 1 ton + 75% CFRR, BF2 = biofertilizer 2 tons + 50% CFRR, BF3 = biofertilizer 3 tons + 25% CFRR, BF4 = biofertilizer 4 tons, CFRR = chemical fertilizer recommended rate, SEM = standard error of means

Table 6. Crop biomass at 12 weeks after sowing as influenced by the interaction between biofertilizer × genotypes for the 2nd season.

	Biofertilizer (ha^{-1})					
Genotypes	**BF0**	**BF1**	**BF2**	**BF3**	**BF4**	**Mean**
			g hill^{-1}			
MR219-4	66.78b	35.74b	47.25a	66.77a	46.43a	52.59a
MR219-9	79.35a	54.51a	48.72a	47.13b	34.09b	52.76a
MRIA1	19.18c	28.96c	32.93b	32.10c	28.33c	28.30b
Mean	55.10A	39.74CD	42.96C	48.67B	36.28D	
Significance level			***			
SEM			1.426			

Means in a column followed by the same letter are not significantly different at P=0.05 using Duncan's new multiple range test (DNMRT), BF0 = CFRR 100%, BF1 = biofertilizer 1 ton + 75% CFRR, BF2 = biofertilizer 2 tons + 50% CFRR, BF3 = biofertilizer 3 tons + 25% CFRR, BF4 = biofertilizer 4 tons, CFRR = chemical fertilizer recommended rate, SEM = standard error of means

Table 7. Crop growth rate at 12 weeks after sowing as influenced by biofertilizer × genotypes interaction for the 1st season.

	Biofertilizer (ha^{-1})					
Genotypes	**BF0**	**BF1**	**BF2**	**BF3**	**BF4**	**Mean**
	g hill^{-1} week^{-1}					
MR219-4	4.13a	3.18a	3.29a	3.79a	3.89a	3.66ba
MR219-9	4.61a	1.66a	2.09b	3.92a	1.10b	2.67b
MRIA1	3.37a	4.37a	1.13c	3.34a	1.49b	2.74b
Mean	4.04A	3.07A	2.17A	3.68A	2.16A	
Significance level			*			
SEM			0.598			

Means in a column followed by the same letter are not significantly different at P=0.05 using Duncan's new multiple range test (DNMRT), BF0 = CFRR 100%, BF1 = biofertilizer 1 ton + 75% CFRR, BF2 = biofertilizer 2 tons + 50% CFRR, BF3 = biofertilizer 3 tons + 25% CFRR, BF4 = biofertilizer 4 tons, CFRR = chemical fertilizer recommended rate, SEM = standard error of means

Table 8. Crop growth rate at 12 weeks after sowing as influenced by biofertilizer × genotypes interaction for the 2nd season.

	Biofertilizer (ha^{-1})					
Genotypes	**BF0**	**BF1**	**BF2**	**BF3**	**BF4**	**Mean**
			g hill^{-1} week^{-1}			
MR219-4	4.84b	-0.47c	5.05a	9.74a	0.67c	3.97b
MR219-9	12.28a	10.54a	5.79a	6.62a	4.81a	8.00a

MRIA1	-4.64c	0.18b	2.57b	-0.55	2.93b	0.10c
Mean	4.16B	3.41C	4.47B	5.27A	2.81C	
Significance level			***			
SEM			0.608			

Means in a column followed by the same letter are not significantly different at P=0.05 using Duncan's new multiple range test (DNMRT), BF0 = CFRR 100%, BF1 = biofertilizer 1 ton + 75% CFRR, BF2 = biofertilizer 2 tons + 50% CFRR, BF3 = biofertilizer 3 tons + 25% CFRR, BF4 = biofertilizer 4 tons, CFRR = chemical fertilizer recommended rate, SEM = standard error of means

Table 9. Photosynthesis, grain yield, straw yield and harvest index (above ground) of aerobic rice as influenced by biofertilizer, genotypes and their interactions for the 1st and 2nd season.

Treatments	Photosynthesis (μmol CO_2 m^{-2} s^{-1})	Grain yield (t ha^{-1})		Straw yield (t ha^{-1})		Harvest index	
	1st season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Biofertilizer (ha^{-1}) (B)							
CFRR 100%	13.21ab	4.25a	3.57a	8.39a	9.16a	0.49a	0.40b
Biofertilizer 1 ton + 75% CFRR	14.06a	4.30a	3.77a	8.09ab	7.84ab	0.52a	0.49a
Biofertilizer 2 tons + 50% CFRR	13.71a	3.73a	3.23a	7.36ab	6.89b	0.49a	0.48a
Biofertilizer 3 tons + 25% CFRR	10.63bc	3.93a	3.29a	7.68ab	6.77b	0.53a	0.49a
Biofertilizer 4 tons	9.91c	2.95b	3.08a	6.41c	6.57b	0.44a	0.48a
Genotypes (G)							
MR219-4	14.09a	5.02a	3.84a	9.25a	8.65a	0.55a	0.45a
MR219-9	10.23b	4.63a	3.86a	8.45a	8.72a	0.56a	0.45a
MRIA1	12.64ab	1.85b	2.47b	5.06b	4.98b	0.38b	0.50a
Significance level							
B	*	*	ns	**	*	ns	**
G	*	***	***	***	***	***	ns
B G	ns	ns	ns	ns	ns	ns	ns
SEM							
B	0.920	0.238	0.284	0.037	0.012	0.281	0.556
G	0.880	0.156	0.161	0.028	0.016	0.332	0.344
B × G	1.968	0.349	0.361	0.063	0.036	0.743	0.769

Means in a column for each factor followed by the same letter(s) are not significantly different at P=0.05 using Duncan's new multiple range tests (DNMRT), *, **, *** represent significant at P≤0.05, P≤0.01 and P≤0.001 respectively, ns = not significant at P>0.05, CFRR = chemical fertilizer recommended rate, SEM = standard error of means

Table 10. Leaf photosynthesis at 65 days after sowing as influenced by biofertilizer × genotypes interaction for the 2nd season.

	Biofertilizer (ha^{-1})					
Genotypes	**BF0**	**BF1**	**BF2**	**BF3**	**BF4**	**Mean**
			μmol CO_2 m^{-2} s^{-1}			
MR219-4	18.33a	19.38a	19.18a	16.68a	18.80a	18.49a
MR219-9	20.68a	17.60a	19.72a	20.17a	16.52c	18.94a
MRIA1	13.09b	18.90a	21.33a	21.40a	17.14b	18.38a
Mean	17.37A	18.63A	20.08A	19.42A	17.52A	
Significance level			***			
SEM			0.894			

Means in a column followed by the same letter are not significantly different at P=0.05 using Duncan's new multiple range test (DNMRT), BF0 = CFRR 100%, BF1 = biofertilizer 1 ton + 75% CFRR, BF2 = biofertilizer 2 tons + 50% CFRR, BF3 = biofertilizer 3 tons + 25% CFRR, BF4 = biofertilizer 4 tons, CFRR = chemical fertilizer recommended rate, SEM = standard error of means

DISCUSSION

Results of these experiments showed that use of biofertilizer not only increase the growth of rice under aerobic condition but also reduces the consumption of inorganic fertilizer. The helpful influence of biofertilizer on the growth and productivity of soybean, maize, sugar beet, and wheat were emphasized by nitrogen fixation ability, phosphorus solubilization, and production of phytohormones (Naseri and Mirzaei, 2010; Naseri *et al.,* 2013).These potential inoculants, its' application properly combined with inorganic fertilizer could be deliberated in the sustainable and organic cultivation of aerobic rice (Panhwar *et al*., 2011a). The considerable increase in the height of plant, number of tillers and leaf photosynthesis in 1 ton biofertilizer + 75% CFRR applied plots might be due to microorganisms contained in the biofertilizer helped in the fixation of atmospheric nitrogen and solubilized the insoluble forms of phosphates into available forms thereby making these nutrients available for the crop growth. Panhwar *et al.* (2011b) reported PSB strains increased plant height in aerobic rice. Their findings revealed that aerobic rice inoculation with PSB enhanced microbial phosphate solubilization activity of applied treatments. Similarly, Tambekar *et al.* (2009) reported that symbiotic nitrogen fixing and phosphate solubilizing microorganisms play a significant role in complementing plant's nitrogen and phosphorus requirement, permitting a nitrogen and phosphate fertilizers usage sustainably. From the findings of this study, about twenty-five percent (25%) of the recommended chemical fertilizer is saved. This is contrary to findings of Habibi *et al.* (2011) where they reported fifty percent (50%) of needed nitrogen and phosphorus fertilizers may possibly be substituted by bio- and organic fertilizers, since bio-and organic fertilizers enriched the efficacy of suggested nitrogen and phosphorus fertilizers and decrease chemical fertilizers costs, similarly prevent contamination of the environment from wide use of inorganic fertilizers. Naher *et al.* (2016) stated the application of chemical N and P can be reduced by fifty percent (50%) and increase rice yield with the supplement of 5 tons ha^{-1} bio-organic fertilizer. However, it was reported that when various PSB strains were used along with a half-dose of inorganic fertilizer on lettuce (*Lactuca sativa*), twenty-five percent (25%) growth increase was achieved in contrast with sole chemical fertilizer treatments and by applying biofertilizer at least fifty percent (50%) of chemical fertilizer could be saved (Young *et al*., 2003).

With regards to the increase in chlorophyll contents, leaf photosynthesis rate, leaf area, crop biomass and crop growth rate, 1 ton biofertilizer + 75% CFRR at par with CFRR 100% seemed to be more effective than the other biofertilizer treatments. The higher values obtained on these parameters in 1 ton biofertilizer + 75% CFRR showed the effectiveness of N-fixing and PSB inoculants in aerobic rice. The biofertilizer used in this study contains N2-fixing and phosphate solubilizing bacteria (PSB) with the capability to produce growth promoting phytohormones (IAA), organic acids and enzymes (Naher *et al*., 2009). The beneficial features of the biofertilizer directly opinionated the physiology of rice (Panhwar *et al*., 2015) and improved the chlorophyll contents in its leaves. Application of biofertilizer will, therefore, give rise to the fixation of some atmospheric nitrogen, which else required to be supplied from chemical N fertilizer and bio-available P. Hence, using this biofertilizer, to a certain degree, enhanced the rice growth and yield (Radziah and Panhwar, 2014). Mehrvarz *et al.* (2008) reported an increase in chlorophyll content and photosynthesis rates with inoculation of PSB. Other studies established that biofertilizer promptly enhances crop growth (Kachrooc and Razdiah, 2006; Son *et al.,* 2007).The highest grain yield recorded in biofertilizer 1 ton + 75% CFRR might also be that in this treatment major nutrients like N, P and K were readily available to plants at earlier stages of plant growth then followed by atmospheric N fixation and solubilization of insoluble P by microorganisms contained in the biofertilizer. Habibi *et al.* (2011) strongly suggested that using biofertilizers (combined strains) plus half a dose of organic and chemical fertilizers have resulted in the greatest grain yield and oil yield in medicinal pumpkin. They revealed that 50% of required nitrogen and phosphorus fertilizers could be replaced by bio and organic fertilizers because bio and organic fertilizers improved the use efficiency of recommended nitrogen and phosphorus fertilizers and reduced the cost of chemical fertilizers, also prevented the environment pollution from extensive application of chemical fertilizers. Beans inoculation with *R. leguminosarum* and *P. putida* R 105 increased the number of nodules and acetylene reduction activity (ARA) significantly (De Freitas *et al*., 1993). A significant positive effect on grain yield and ARA in roots of barley was obtained due to combined inoculation of nitrogen fixer's *Azospirillum lipoferum*, *Arthrobacter mysorens* and the phosphate solubilizing strain *Agrobacterium radiobacter* by Belimov *et al.* (1995). Tran *et al.* (2006) reported that application of Bradyrhizobia and PSB (*Pseudomonas* sp) resulted in substantial development in a number and dry weight of nodules, yield components, grain yield, soil nutrient availability and uptake in the soybean crop. The inoculation of plant growth-promoting rhizobacteria (PGPR) and PSB jointly could help decrease fifty percent (50%) of the required P fertilizer with little decrease in crop yield (Jilani *et al*., 2007; Yazdani *et al*., 2009). The greater performance of MR219-4 genotype in respect to a number of tillers, leaf area, crop biomass; crop growth rate at all sampling period, leaf photosynthesis rate, as well as grain yield, straw yield and harvest index and MRIA1 superior performance in plant height could be attributed to their genetic makeup and adaptability to growing conditions. MR219-4 plant height ranged from 80 - 90 cm and a number of tillers 4.6 - 4.8 hill^{-1}. These findings were contrary to what was reported by (Abdullah *et al.* 2009) that plant height (100.1 cm) and a number of tillers (9.1) under aerobic soil condition. MRIA1 plant height ranged from 90 - 110 cm and a number of tillers 2-2.8 hill^{-1}. Sariam *et al*. (2013) reported plant height (92 - 99 cm) and a number of tillers 5 - 7 hill^{-1}. Different genotypic responses to biofertilizers have been discussed by researchers. Adeline *et al*. (2014) assess two different genotypes (CD 104 and CD 120) under greenhouse conditions. They discover that CD 120 cultivars activated with *Herbaspirillum seropedicae* have advanced yield when urea was not added to the plants. In another study conducted by Antonio *et al*. (2013) in the northeastern Brazil region, reaction to the commercial type of Bonanca to turn with a much lower AR1122 strain suggested that biofertilizer inoculation programs for traditional rice varieties need to take note of genetic interactions between strains and variety of rice. The strain of *B. vietmaniensis* diazotrophic AR1122 is a good biofertilizer candidate for traditional rice varieties inoculation

Conclusion: Rice growth and yield could be improved by the application of 1 ton biofertilizer + 75% CFRR on MR219-4 genotype in aerobic condition and thus minimizes the inorganic fertilizer application. It was concluded that 25% reduced dose of chemical fertilizer and its combination with biofertilizer was optimum for most of the parameters studied as compared to the sole inorganic or chemical fertilizer on both crop growth and yield parameters measured.

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