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EFFECTS OF IRON AND MOLYBDENUM WITH DUAL INOCULATION OF NITROGEN FIXING BACTERIA AND PHOSPHATE SOLUBILIZING BACTERIA ON GROWTH OF AEROBIC RICE

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Abstract

Different levels of Fe (0, 2, 10, 50 ppm) and Mo (0, 0.05, 5, 10 ppm) with dual inoculation of N_2-fixing bacteria (NFB) and phosphate solubilizing bacteria (PSB) *Bacillus* sp. were tested in glasshouse on growth of rice in aerobic condition. Combination strains with different levels of Fe and Mo gave higher chlorophyll content (12.65 mg/cm^2) (11.42 mg/cm^2), leaf area index (1.41 cm^2) (1.21 cm^2), total root length (1159.88 cm^2) (15169.19 cm^2) and root volume (0.82 cm^3) (0.68 cm^3) as compared to control. Inoculated plants with combination strains showed better effects on growth of aerobic rice unlike single levels of Fe and Mo applied.

Introduction

Rice (*Oryza sativa* L.) is the staple food crop in Malaysia. Molybdenum plays an important role in metabolism, mainly for its role in nitrogen metabolism and protein synthesis and development of the reproductive parts of the plant. The uptake of molybdenum at high concentrations, however, induces physiological disorders and changes in metabolic pathways in plants (Rout and Das 2002). Molybdenum deficiency results in reduced chlorophyll concentration in the leaves (Das 1977) which leads to decreased photosynthetic efficiency. Iron is also essential for many physiological processes such as photosynthesis, chloroplast development and chlorophyll biosynthesis. It is the major constituent of the cell redox systems such as heme proteins, including cytochromes, catalase, peroxidase and leg-hemoglobin and iron-sulfur proteins, including ferredoxin, aconitase and superoxide dismutase (SOD) (Mehraban *et al.* 2008). Both iron and molybdenum are part of nitrogenase enzyme and abundance or shortage of these elements may affect the enzyme activity as well as the biological N_2-fixation. However, the availability of phosphorus is limited due to its fixation with iron and aluminium, especially in acidic and aerobic condition (Qurban 2012). Currently, insufficient information is available on the role of Mo and Fe in the nitrogen fixation and phosphate solubilizing activities of N_2-fixing and phosphate solubilizing bacteria. Hence, the study was conducted aimed to determine the effect of different levels of Fe and Mo on growth of aerobic rice with dual inoculation of NFB and PSB.

Material and Methods

Aerobic rice seed (*Oryza sativa* MR219-M9) was obtained from Plant Mutation Breeding and Genetics, Malaysian Nuclear Agency and surface sterilized (Panhwar *et al.* 2011). Rice seeds were grown in container moistened with sterilized distilled water for 7 days. NFB strain (*Bacillus* spp.) and PSB strain (*Bacillus* spp.) was grown in N_2-free broth (Nfb) and nutrient broth (NB) on a shaker for 72 and 24 hrs, respectively. The culture was centrifuged at 4000 rpm for 40 min and

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the supernatant was decanted and the bacterial cells were suspended in 0.85% phosphate saline buffer. Ten ml of the bacteria suspension was inoculated to the respective plants. The population of bacteria used as inoculum was confirmed by cell enumeration in drop plate method on nutrient agar (Panhwar *et al.* 2011). Pots were filled with 500 g acid wash and sterilized sand. Three uniform sizes of rice seedlings were transplanted into each pot. Fertilizer application was in the form of nutrient solution modified from Yoshida *et al.* (1976). Four levels of each of Fe (0, 2, 10, 50 ppm) and Mo (0, 0.05, 5.0, 10.0 ppm) were prepared using ferric chloride hexahydrate ($FeCl_3.6H_2O$) sodium molybdate dihydatre ($Na_2MoO_4.2H_2O$), respectively. Each pot was supplied with 100 ml of the nutrient solution (70% N and P) twice per week. After 30 days of transplanting, plants were harvested. Parameters like chlorophyll content, plant biomass, root biomass, leaf area index, total root length and root volume were measured. The experiment was laid out as a factorial experiment with RCBD with three replicates. The treatments used were 0, 2, 10, 50 ppm Fe and; 0, 0.05, 5, 10 ppm Mo and NFB + PSB in presence of 0, 2, 10, 50 ppm Fe and 0, 0.05, 5, 10 ppm Mo separately. Data were analyzed using SAS Software Program (Version 9.4) and treatment means were compared using Tukey's test ($p < 0.05$).

Results and Discussion

Combination of NFB and PSB on aerobic rice plants gave significantly higher chlorophyll content as compared to control (non-inoculated) on both Fe and Mo. Similar information were also stated by (Panhwar *et al.* 2011) where the highest chlorophyll content was obtained from inoculated plants with PSB strains compared to non-inoculated plants. Mutalib *et al.* (2012) reported that inoculated plants with *Bacillus* sp. gave significant effect on chlorophyll content of rice. Fig. 1a and b shows highest chlorophyll content was at each of 10 ppm Fe and Mo in combination NFB and PSB (12.65 mg/cm^2) (11.42 mg/cm^2), respectively.

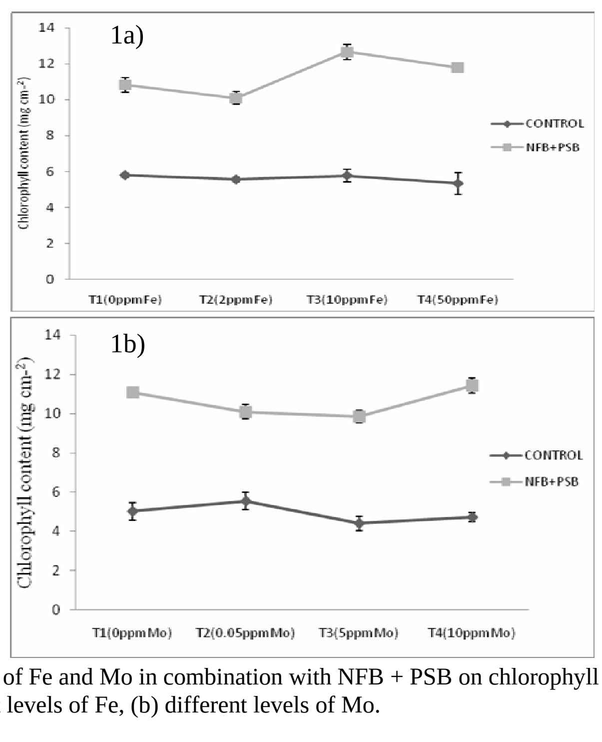

Fig. 1. Effect of Fe and Mo in combination with NFB + PSB on chlorophyll content (a) different levels of Fe, (b) different levels of Mo.

Fig. 2a shows T_2 (2 ppm Fe) and T4 (50 ppm Fe) showed a significant effect on plant biomass of aerobic rice with combined inoculation (NFB + PSB) as compared to non-inoculated (control). Increasing levels of Fe gave a significant increase in root biomass at non-inoculated treatment. Fan *et al.* (2012) stated that application of Fe at 30 kg/ha in aerobic plots of rice significantly increased shoot dry weight when compared with plants with non-Fe treated plants. Highest plant biomass (651.07 mg) and root biomass (112.20 mg) of aerobic rice were recorded at the highest levels of Fe (50 ppm). Fig. 2 shows that there is no significant effect on plant biomass due to different levels of Mo applied. Similar results were stated by Zakikhani *et al.* (2014) where Mo application did not affect shoot dry weight. Application of Mo in non-inoculated increased root biomass plants as of compared to plants with non-Mo application. Results also showed significant effects of all levels of Mo except T_2 on root biomass of aerobic rice with combined inoculation (NFB + PSB) as compared to control. The highest plant biomass (578.63 mg) and root biomass (114.50 mg) due to application of different levels of Mo were recorded at T_2 and T_4, respectively.

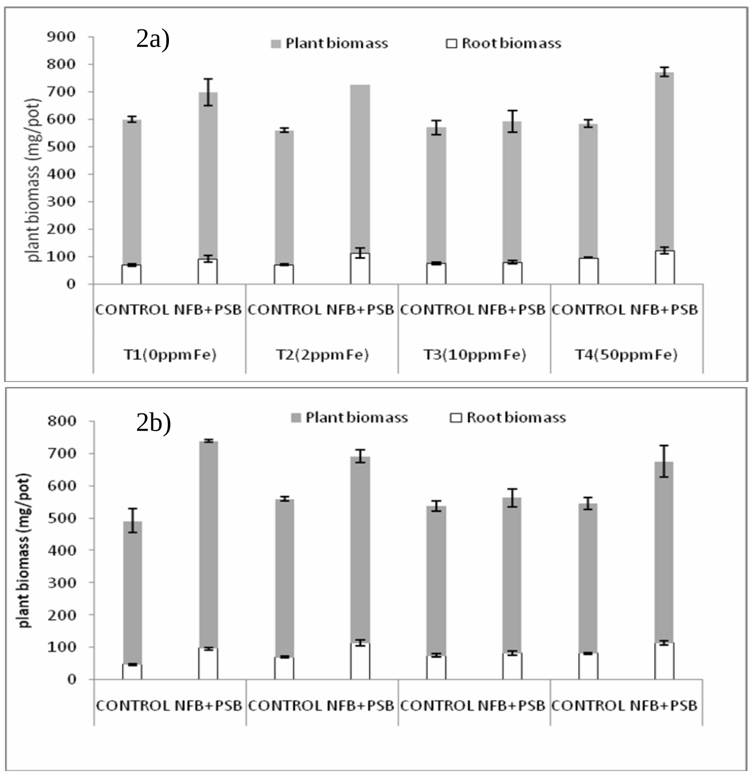

Fig. 2. Effect of Fe and Mo in combination with NFB + PSB on plant biomass (a) different levels of Fe, (b) different levels of Mo.

Fig. 3(a), showed that significant effects were recorded on leaf area index of aerobic rice treated with different levels of Fe in dual inoculation plants. Different levels of Mo also gave significant effects on leaf area index at non-inoculated (control) plants as presented in Fig. 3 (b). Significantly higher plant's leaf area indices were recorded with dual inoculated (NFB + PSB) plants as compared to control at different levels of Fe and Mo treatments. Similar findings were mentioned by Panhwar *et al.* (2011), where plants inoculated with PSB strains produced higher leaf area index as compared to control (non-inoculated).

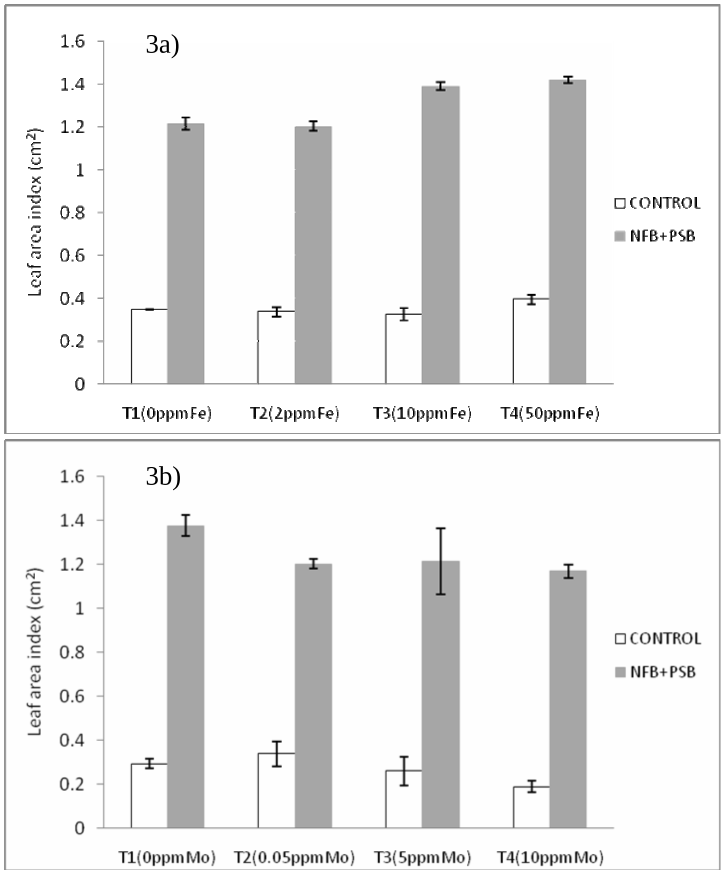

Fig. 3. Leaf area index (a) different levels of Fe, (b) different levels of Mo inoculated with NFB + PSB.

Table 1. Total root length and root volume of rice at different levels of Fe.

	Total root length (cm)		Root volume (cm^3)	
Treatments	Control	NFB+PSB	Control	NFB+PSB
T1 (0 ppm Fe)	671.35 A b	1034.48 A a	0.39 A b	0.64 B a
T2 (2 ppm Fe)	834.74 A a	1049.39 A a	0.51 A b	0.69 AB a
T3 (10 ppm Fe)	690.36 A b	1040.79 A a	0.52 A a	0.61 B a
T4 (50 ppm Fe)	742.33 A b	1159.88 A a	0.44 A b	0.82 A a

Table 2. Total root length and root volume of rice at different levels of Mo.

Treatments	Total root length (cm)		Root volume (cm^3)	
	Control	NFB+PSB	Control	NFB+PSB
T1 (0 ppm Mo)	790.40 A b	1221.79 B a	0.50 A b	0.59 A a
T2 (0.05 ppm Mo)	834.74 A b	1049.39 C a	0.51 A b	0.69 A a
T3 (5 ppm Mo)	764.82 A b	1325.54 B a	0.520A a	0.55 A a
T4 (10 ppm Mo)	855.38 A b	1516.19 A a	0.49 A b	0.68 A a

Fe did not give significant effect on total root length of aerobic rice except T_2 treatment (Table 1). Similarly, the combination of NFB and PSB strains of plants failed to produce significantly higher total root length of aerobic rice as compared to control. Application of dual strains (NFB + PSB) to plants resulted in significant effect on root volume of aerobic rice as compared to control. The highest value of total root length (1159.88 cm) and root volume (0.82 cm^3) were measured at the highest level of Fe (50 ppm) due to dual inoculation of plants. Table 2 shows, combined effect of NFB + PSB with Mo gave non significant identical effects on total root length and root volume as compared to control. The highest total root length (1516.19 cm) and root volume (0.699 cm^3) for Mo treatments were recorded at T_4 (10 ppm Mo) and T_2 (0.05 ppm Mo) treatments, respectively.

In conclusion, it could be assessed that inoculated plants with combination strains enhanced plant growth of aerobic rice. Application of different levels of Fe and Mo did not give much effect on plant growth. However, it can be done with more details to evaluate the effects of these two micronutrients on aerobic rice growth.

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