Lampiran 1

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Effects of Diazotrophic Microorganisms on Growth of Paddy Seedlings under Aseptic Conditions

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Abstract

Diazotrophic microorganisms both as a free-living or in association with the non-leguminous plants can fix nitrogen from the atmosphere and supply the fixed N_2 required by the host. Two experiments were conducted with the following objectives; **1st experiment**: to observe the ability of locally isolated diazotrophic microorganisms to fix N_2 and to quantify the fixation rates by acetylene reduction assay. The results successfully showed that the free-living diazotrophic microorganisms have the ability to fix N_2 from the air. The highest nitrogenase activities were derived from *Azospirillum* sp. (0.4 – 2.0 µmol/cfu/h) followed by locally isolated *Bacillus* sp. (0.05 – 0.8 µmol/cfu/h). **2nd experiment**: to observe effects of inoculation on growth of paddy seedlings under aseptic conditions after 23 days of growth (D_{23}). Newly germinated paddy seedlings were grown at one seedling per test tube (50 ml) with 7 inoculation treatments; + *Azospirillum brasilense* (Sp 7), + *Herbaspirillum seropedicae* (Z 78) and + locally isolated rhizobacteria; + USMR 8, + USMR 9 and + USMR 12; Control 1 (+ Sp 7 killed) and Control 2 (+ full applications of inorganic nitrogen fertilizer; N_i). The results showed that inherent inoculated seedlings (+ Sp 7 and + Z 78) had enhanced the roots dry weight by 40% increment and higher root length up to 50% increment compared to the control (+ Sp 7 killed).

Introduction

In recent years, some studies have shown that significant quantities of nitrogen can be derived from associated biological nitrogen fixation process for certain forage grasses, wetland rice and sugar cane (Boddey *et al.*, 1994). It has been recognized that associative nitrogen fixation by *Azospirillum* sp. in association with rice is of utmost importance (Kanungo *et al.*, 1998). Nitrogen fixation represented as the nitrogenase activities could be determined by the assay of Acetylene reduction to observe the ability of diazotrophic microorganisms in fixing N_2. Besides that, the plant growth-promoting rhizobacteria (PGPR) like *Azospirillum* and *Bacillus* could also stimulate plant growth through excretion of plant growth hormones (Kloepper, 1994). Thus, the objectives for these experiments were; (1) to observe the ability of locally isolated diazotrophic microorganisms in fixing N_2 by acetylene reduction assay and (2) to observe the effects of inoculation on growth of paddy seedlings root growth and chlorophyll content under aseptic conditions.

Materials and Methods

Experiment 1: N_2 fixation activities

The experiment was conducted to quantify the N_2 fixation rates of *Azospirillum brasilense* (Sp 7), *Azospirillum lipoferum* (Sp 59b), *Herbaspirillum seropedicae* (Z 78),

and 3 locally isolated *Bacillus cereus* (BC), *Bacillus* spp. UPMB 10 and *Bacillus* spp. UPMB 13. The nitrogenase activities for each of the bacterial strains were measured under liquid culture conditions. Each of the bacterial culture tested (10 ml) was incubated in an air tight bottles (64 ml). 10% of air from the air tight bottle was withdrawn by an air-tight syringe (1 ml) and replaced with purified D. acetylene gas (99.8%). 30 min after incubation, 1 ml of the gas mixture was withdrawn from the bottle and transferred into vacuum tube, before analyzing the reduction of C_2H_2 to C_2H_4 by gas chromatography (Hitachi G-3000 fitted with a Supelco stainless steel column)(Somasegaran and Hoben, 1985). Nitrogen gas was used as the carrier gas at flow rate of 3.5kgf/cm^2. The column and injector/detector temperature were 165°C and 230°C respectively.

Experiment 2: Effects of rhizobacterial inoculation on growth of paddy seedlings
Newly germinated paddy seedlings (MR 220, provided by Malaysian Agriculture Research & Development Institute, MARDI) were inoculated with *A. brasilense* (Sp 7), *H. seropedicae*, (Z 78) and three locally isolated rhizobacteria namely USMR 8, USMR 9 and USMR 12. The control treatments were Control 1: + Sp 7 killed (autoclaved at 121°C, 15 min) and Control 2: applications of inorganic N as recommended for MS media (+ N_i). The treated seedlings were grown for 23 days (D_{23}) before harvesting and the parameters observed were chlorophyll content, root dry weight, root numbers and root length.

Results and Discussion

Experiment 1

A. lipoferum (Sp 59b) showed the highest N_2 fixation capacity (highest nitrogenase activities) (1.940 x 10^{-3} µmol C_2H_4/c.f.u./ml) compared to the other inocula tested. Locally isolated *Bacillus* spp. UPMB 13 also showed the highest nitrogenase enzyme activities (0.761 x 10^{-3} µmol C_2H_4/c.f.u./ml) as shown in **Figure 1**.

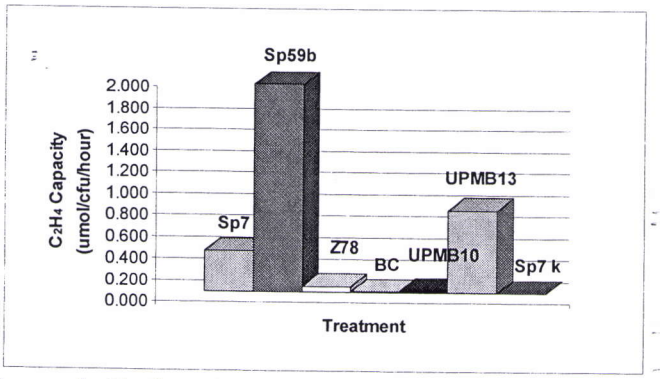

Figure 1: Estimation on N_2 fixation capacities for diazotrophic microorganisms.

Experiment 2

The host seedlings inoculated with USMR 8, USMR 9, USMR 12 and Sp 7 showed the highest leaf chlorophyll content (3.53 – 4.05 Minolta® SPAD meter values) as compared to the control inoculated with Sp 7 killed (**Figure 2**). Similar response of inoculation was

also shown for the root growth and development of the host plants. The highest root dry weight was recorded for Sp 7 (0.70 g) and USMR 8 (0.71 g)(**Figure 3**). Similar response of bacterial inoculation was also shown for enhanced root numbers especially for Sp 7 and Z 78 (**Figure 4**). The root length had increased up to 50% for the seedlings inoculated with USMR 12 (**Figure 5**).

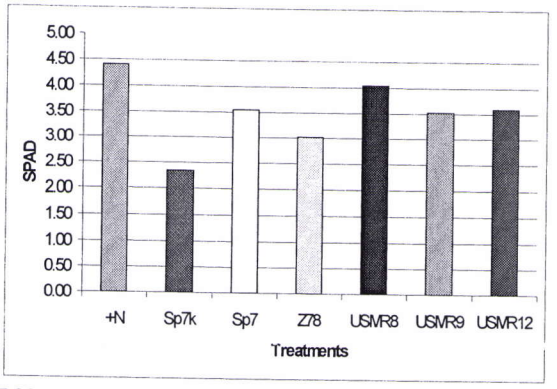

Figure 2: Effects of rhizobacterial inoculations on chlorophyll content of paddy seedlings at D_{23}

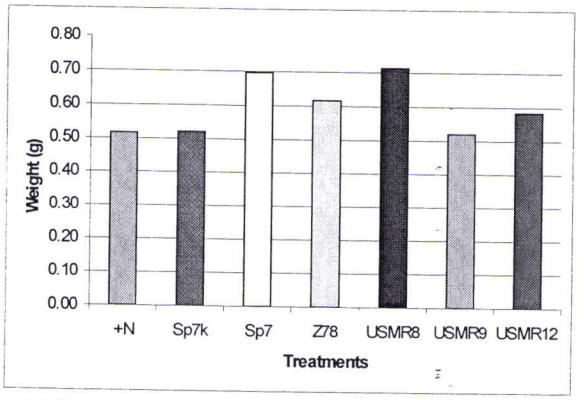

Figure 3: Effects of rhizobacterial inoculations on root dry weight of paddy seedlings at D_{23}

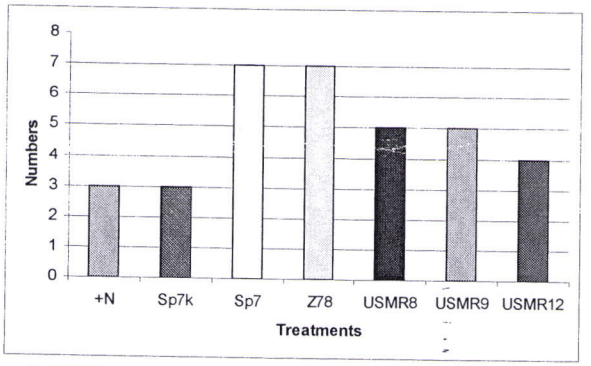

Figure 4: Effects of rhizobacterial inoculations on root numbers of paddy seedlings at D_{23}

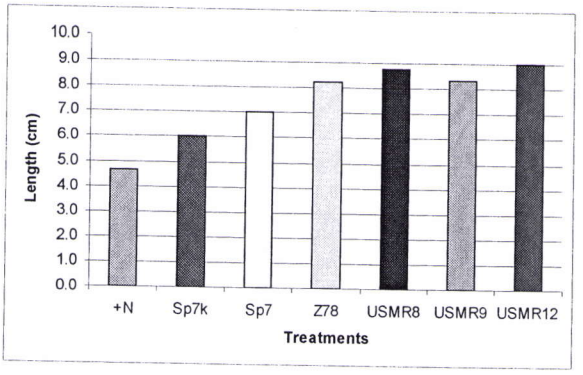

Figure 5: Effects of rhizobacterial inoculations on root length of paddy seedlings at D_{23}

Conclusion

The experiment had successfully shown that *Azospirillum* Sp 7 is the most effective N_2 fixer together with locally isolated UPMB 13. The inocula tested (Sp 7 & USMR 13) could also enhanced growth of the host plants (paddy seedlings).

Acknowledgements

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Gantt chart/milestone (Lampiran 2)

Research Activities	2005									2006		
	A	M	J	J	A	S	O	N	D	J	F	M
1. a) Ammonium Assimilation Assays & N2 Fixation (ARA) with free-living rhizobacteria.	11 - 16 ↔											
1. b) Phytohormone study & N_2 fixation in free-living rhizobacteria; HPLC & Salkowsky Assay.	25 - 30 ↔											
2. a) Phytohormone study & N_2 fixation in associated with Oil palm plantlets; HPLC & Salkowsky Assay		16 - 20 ↔										
2. b) Ammonium Excretion association with plantlets.				↔								
3. a) Ammonium Assimilation Assays & N2 Fixation (ARA) associated with Oil palm seedlings.		**Preparation in 1 month before transfer into soil in polybags 6/5 – 6/11** ↔										
3. b) Phytohormone study & N_2 fixation associated with Oil palm seedlings.		**Preparation in 1 month before transfer into soil in polybags 13/5 – 13/11** ↔										
3. c) Ammonium Excretion association with Oil palm seedlings.					↔							
Thesis writing	↔											