EFFECTS OFRHIZOBIAL INOCULATION ON THE EARLY GROWTH OF *Acacia mangium* IN THE FIELD

[Pengaruh Inokulasi Rhizobium terhadap Pertumbuhan Awal *Acacia mangium* di Lapangan]

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ABSTRAK

Dalam rangka mendukung program pemerintah dalam mengembangkan Hutan Tanaman Industri (HTI), bibit tanaman hutan berkualitas tinggi dibutuhkan dalam jumlah besar dan berkesinambungan. Untuk tujuan tersebut, 2 isolat Rhizobium terpilih diuji kemampuannya dalam meningkatkan pertumbuhan dan mengefisiensikan pemupukan N pada tanaman *Acacia mangium* yang berasal dari kultur jaringan atau dari benih. Isolat yang kepadatannya 10^9 sel/ml diinokulasikan ke dalam planlet *in vitro* dan bibit *A.mangium* asal biji (masing-masing 30 tanaman). Tanaman yang telah diinokulasi, ditumbuhkan dalam polibag sampai cukup kuat untuk ditanam di lapangan. Percobaan disusun dalam Rancangan Split Plot dengan 3 ulangan. Asal bibit yaitu kultur jaringan dan benih digunakan sebagai main plot sedangkan dosis pemupukan urea (n_0 = 0; n, = 7.5; $n_:$ = 7.5; n_2 = 15, n, = 22,5; n_4 = 30 g/ tanaman) sebagai subplot. Pengamatan yang dilakukan 12 minggu setelah tanam menunjukkan bahwa pertumbuhan kedua jenis bibit tersebut sangat baik meskipun bibit hasil kultur jaringan tumbuh lebih cepat dan lebih seragam. Namun tidak terdapat perbedaan yang nyata pada pertambahan tinggi tanaman, diameter batang dan jumlah cabang antar semua dosis urea yang dipakai berdasarkan uji LSD pada level 5 %. Hasil tersebut menunjukkan bahwa simbiosis antar *A. mangium* dan rhizobium cukup efektif dalam meningkatkan pertumbuhan tanaman selama 3 bulan pertama di lapangan tidak ada perbedaan yang jelas antar tanaman yang diberi dan tanpa pupuk N.

Key Words: mangium *(Acacia mangium),* Rhizobium, simbiosis, urea.

INTRODUCTION

Mangium *{Acacia mangium* Wild.) of the Leguminosae is a fast growing forest tree which is commonly used for reforestration and for providing raw materials for the paper pulp industry. In order to support government programs in developing Industrial Timber Estate (HTI), high quality planting materials of *A. mangium* are needed in large and sustainable quantities.

Mass propagation of mangium has been developed by tissue culture technique (Galliana *et al,* 1991; Lydia *et al.,* 1995). However, the acclimatization and establishment of plantlets under *ex vitro* condition (glass house) is still a major problem. The role of Rhizobial bacterium in increasing survival rate of mangium plantlets during acclimatization process was significant (53%) (Imelda and Sukiman, 1994).

Rhizobium is a potential microbe which has an ability to fix free nitrogen from the air and provides it to the leguminous trees up to 70-80 % of their need (Somasegaran and Hoben, 1985; Sukiman *et al.,* 1996). Besides, the microbe has also an ability to produce IAA (indole acetic acid), a plant growth regulator useful for root induction.

Two Rhizobial isolates namely DBM 5.3 and DCM 2.1.2, have been identified as having high symbiotic capacity and high N2 -fixing ability (Ariani and Sukiman, 1995). In this paper, their potential in enhancing growth and N fertilizer-use efficiency by mangium originating from tissue culture and seedlings was investigated.

MATERIALS AND METHODS

Preparation of selected Rhizobial isolates

Selected rhizobial isolates (DBM 5.3 and DCM 2.1.2) were recultured on YEMA (Yeast Extract Mannitol Agar) medium and incubated at 30 C for 5-7 days. Surviving colonies were transferred to a YEM-Broth medium and shaken at 200 rpm for 5 days at room temperature.

The bacterial masses were then diluted with sterile distilled water to form a cell suspension of 10^9 cells/ml population density or 0.4 optical density. The

concentration of the suspension was measured using a spectrophotometer at 650 nm wavelength.

Preparation of mangium plantlets

High quality seeds from Seed Orchard of the Forest Research Institute, Parung Panjang, were germinated *in vitro* on a sterile wet tissue paper in a petri dish. Apical/lateral shoots were multiplied on MS medium containing 1 mg/1 benzyl aminopurine(BAP). The multiple shoots were then split and subcultured to MS medium without hormones for root induction.

Application of potential Rhizobium into mangium plantlets

Rooted mangium plantlets about 5 cm high were dropped with 1 ml of the prepared rhizobium suspension. Two selected isolates with high effectiveness for symbiosis with mangium plants namely DCM 2.1.2 and DBM 5.3 (Ariani and Sukiman, 1995) were used in this experiment. Each isolate was inoculated on 10 plantlets.

After 4-6 weeks, the plantlets were acclimatized in pots containing sterilized vermiculite (autoclaved at 120 ° C at 1 atm for 40 minutes), then watering by nutrient solution (1/2 N) until soil saturation and covered with plastic bag until new leaves appeared. After 2 weeks, surviving plantlets were transferred to a mixed medium of sterilized soil and compost (1:1). All plants were placed in the green house with high humidity (80%).

Inoculation of potential Rhizobium on mangium seedlings

Mangium seeds from the Seed Orchard of Seed Technology Division, Forest Research Institute, Parung Panjang (Lot 1-11) with 4.57% water content were germinated in a mixed medium of soil and rice husks (1:1).

Seedlings about 1 month were dipped in the prepared Rhizobial suspension of 10^9 cells/ml density for 30 minutes. Seedlings were then grown in polybags and placed in the nursery until strong enough to be grown in the field.

Planting in the field

Inoculated planting materials were planted in the Experimental Garden of the Forest Research and Development Agency, at Rumpin, Parung, using a Split Plot Design with 3 replications. Micropropagated and seedling plants were put in the main plot while 5 dosages of urea (n_0 = 0, n, = 7.5, n, = 7.5, n_2 = 15, n_3 = 22.5, n_4 = 30 g/plant) as sub plot.

Observation was done on the increment of plant height, stem diameter and number of branches every 2 weeks.

RESULTS

Evaluation on ten Rhizobial isolates originating from several sources in Java and Sumatera shows that isolate DCM 2.1.2 possessed higher symbiotic capacity and effectiveness in fixing N from the air than DBM 5.3 (Table 1). Therefore, only isolate DCM 2.1.2 was selected for use in this research.

In vitro propagation of *A. mangium* through shoot multiplication on MS medium containing 1 mg/1 BAP was capable of producing 20 shoots within 8 weeks (Lydia *et al.*, 1995). Rooting of plantlets was readily inducible on MS medium without hormone.

Inoculation of selected Rhizobium into plantlet roots (photo 1 .a) will produce nodules (photo 1 .b) within 4-6 weeks, with DBM 5.3 producing an average of 5 and DCM 2.1.2, 3 nodules/plantlet (Table 1, photo 1.c)

Table 1. Ability of two rhizobial isolates in forming root nodules.

Plantlet no.	Number of root nodules	
	DCM 2.1.2	DBM S.3
1	4	3
2	1	1
3	3	1
4	5	1
5	5	3
6	4	3
7	3	3
8	4	1
9	1	1
10	1	1
Average	3.1	1.8

Twelve weeks after planting, growth in both tissue culture and seedling-derived plants was remarkable, althought the former was faster and more uniform (fig. 1, photo 1 .e, 1 .f). However, tissue culture-derived plants showed less increment in stem diameter than seedling-derived plants (fig. 2), even though their dif-

Table 2. Effects of plant source and urea dosages on the increment of *A. mangium* plant height, stem diameter and number of branches.

Growth increment	Plant source	Urea dosages (g/plant)				
		n_0	ni	n_2	n_3	n_4
Plant height	S	14.52 abc	9.83 a	16.53 be	11.58 ab	16.89 c
(cm)	TC	22.23 b	18.35 ab	22.85 b	15.65 a	22.28 b
Plant diameter	S	2.33 ab	1.78 a	2.32 ab	1.62 a	3.66 b
(cm)	TC	1.78 ab	1.08 a	2.43 b	0.82 a	2.42 b
Number of	**S**	2.17 a	1.17a	3.33 a	2.17a	2.17a
branches	TC	2.50 ab	0.50 a	3.00 b	3.00 b	4.17b

Note : S = Seedling
TC = Tissue culture
Means within rows followed by the same letter are not significantly different according to LSD test at 5 % level

ferences were not significant. At high levels of N (n2, n3, n4) tissue culture-derived plants produced significantly more branches than seedling-derived plants (fig. 3). No significant differences were observed among increment plant height, stem diameter and the number of branches from applicatiions of other urea dosages according to LSD test at 5 % level (Table 2)

DISCUSSION

Acclimatization of mangium plantlets gave a survival rate of 100 % with rhizobial inoculation and 0 % without inoculation (Imelda and Sukiman, 1994). The significantly higher survival rates of Rhizobium inoculated plantlets after acclimatization (photo l.d) was probably due to root formation induced by IAA production of Rhizobium. IAA has an important role in root induction (George and Sherrington, 1984). The presence of more functional roots will facilitate acclimatization from the *in vitro* to the *ex vitro* condition. *In vitro* plantlets of woody species are often difficult to adapt to *ex vitro* conditions due to their physiological, morphological and anatomical abnormalities induced during the *in vitro* culture (Ziv, 1994). Therefore, symbiosis between mangium and appropriate Rhizobium will benefit both in enhancing the survival of acclimatized plantlets and/or in the fixation of free N from the air.

Gales (1995) recommended an application of 30 g urea per plant for Rhizobium non-inoculated mangium seedlings based on cultivation trial at Subanjeriji, Palembang. In this experiment, the highest level of urea dosage (30 g/plant) did not show any difference in plant growth compared with control treatment (no= 0 g/plant urea). It means that while the other major elements like P (phosphor) and K (potasium) were provided sufficiently, N (nitrogen) that was needed for growth during their early growth in the soil could be fixed from the air by inoculated plants.

Among three dosages of N fertilizer, n_2 is the best for plant height, stem diameter of tissue culture-derived plants. Probably half recommended dosage of N (n_2) combined with appropriate rhizobial inoculation could reach almost the same result with the optimal growth of non-inoculated mangium (n_4). Even without N fertilixer (n_o) inoculated plants still showed good results in growth (Table 2).

CONCLUSION

Symbiosis between *A. mangium* and *Rhizobium* was effective in enhancing plant growth during the first three months with no apparent requirements for N fertilizer application.

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Photo.l a. *In vitro* plantlet of mangium inoculated with Rhizobium; b. Root nodules on *in vitro* plantlet of mangium; c. Mangium plants originating from tissue culture; d. Root nodules induced by Rhizobium isolates DBM 5.3 and DCM 2.1.2; e. Mangium plantlet from tissue culture 4 weeks after planting; f. Mangium plants from seed 4 weeks after planting

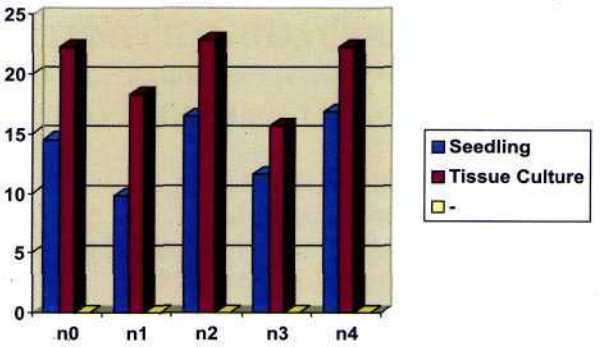

Fig. 1. Effects of Rhizobium inoculation on the increment of plant height.

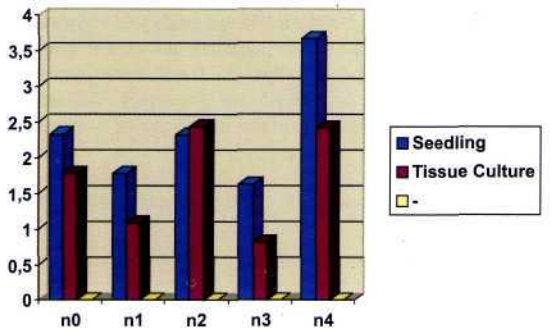

Fig. 2. Effects of Rhizobium inoculation on the increment of stem diameter

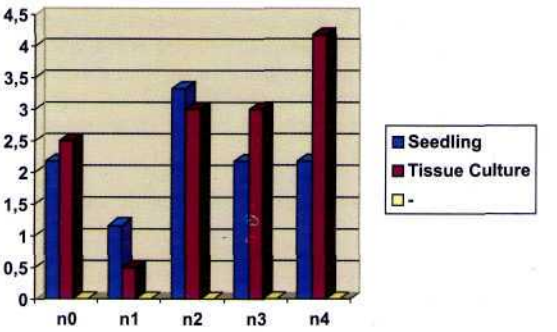

Fig. 3. Effects of Rhizobium inoculation on the number ofbranches

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