PENELITIAN

INTERACTION AMONG PROGENIES/ PROVENANCES OF SENGON, ARBUSCULAR MYCORRHIZAL FUNGI AND RHIZOBIAL ISOLATES GROWN ON ULTISOL*)

by

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ABSTRAK

Penelitian ini bertujuan untuk mengembangkan sengon (*Paraserianthes falcataria*) pada tanah ultisol. Penelitian ini juga bertujuan untuk memperoleh isolat *Rhizobium sp* atau mikorisa yang paling sesuai untuk perkembangan sengon khususnya untuk jenis tanah ultisol. Koleksi *Rhizobium sp* dan mikorisa diutamakan berasal dari tempat-tempat penyebaran sengon alam. Namun pengembangan berikutnya adalah memilih dan menguji bagi tempat-tempat yang paling banyak untuk program HTI yakni tanah ultisol.

Sengon dipilih dari penampilan terbaik tanaman ini di tanah ultisol. Ada 9 progeni yang dipilih dari tiga provenans yakni Morotai-Maluku, Wamena-Irian Barat dan Jasinga land race-Jawa Barat. Ada tiga isolat amf dan *Rhizobium sp* terpilih yang dipergunakan untuk studi ini. Biji-bji tersebut ditanam di pot tanah di dalam rumah kaca. Isolat mikorisa terbaik yang telah diuji di tanah ultisol diutamakan untuk penelitian. Isolat *Rhizobium sp* yang terbukti mempunyai keunggulan di tanah masam (tanah ultisol) juga merupakan pilihan utama untuk isolat.

Hasil penelitian ini menunjukkan bahwa ada perbedaan nyata dalam: (i) seedlot dalam dan di antara provenans sengon. (ii) terdapat interaksi di antara progeni/provenans *P.falcataria*, isolat amf dan rhizobium berpengaruh nyata terhadap pertumbuhan. Pertumbuhan terbaik pada umur 4 bulan diperoleh dari komposisi seedlot provenans Morotai (Maluku), kombinasi *Glomus manihotis* dan isolat *Bradyrhizobium PfLnU 16.2*. Kombinasi terbaik ini dapat meningkatkan pertambahan biomasa sampai lebih dari lima belas kali dibandingkan dengan kontrol, sedangkan kombinasi yang kurang bahkan dapat mengurangi pertumbuhan tanaman inang. Dari hasil tersebut perlu disarankan untuk mempertimbangkan peranan kombinasi terbaik dari progeni unggulan, mikorisa unggulan dan rhizobium unggulan pada tanah ultisol.

Kata kunci: progeni, provenans, mikorisa, rhizobium, ultisol, *paraserianthes falcataria*.

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INTRODUCTION

Background

Indonesia's forest-lands are dominated by acid mineral soils, in particular, the ultisols (red-yellow podzolic). These soils have properties limiting plant growth including: low pH, high exchangeable Al, low available P, low in base-cations (K, Ca, Mg) and are low in some important trace elements (Mo,B, Co). Sudjadi & Effendi (1991) reported that there are 71.48 million ha (37.5%) of ultisols in Indonesia. *Paraserinathes falcataria* has a natural distribution from the eastern parts of Indonesia namely the Moluccas, Irian Jaya, to the eastern regions of Asia Pacific in Solomon and Fiji islands. In its natural habitat, *P. falcataria* grows at high altitudes on soils with porous structure, usually developed under volcanic influence. *P. falcataria* is also cultivated widely in Java as a community forest species due to its economic value and fast-growing properties. Seido *et al.*(1993) has observed the genetic profile of some *P. falcataria* provenances and landraces with the aid of isoenzyme analysis. Their results indicated that *P. falcataria* stands from Java have a narrow genetic-base and they are assumed to have originated from Buru provenance (Moluccas islands). Narrow genetic-base plantations have problems primarily in terms of pest susceptibility. To overcome the narrow genetic-base problems in large plantations of *P. falcataria*, Laboratory of Tree Improvement, Gadjah Mada University, in cooperation with PT Surya Hutani Jaya (a private timber estate company located in East Kalimantan) had explored germ-plasm of naturally-distributed *P. falcataria* in the eastern part of Indonesia such as the Molluccas, Flores and Irian Jaya as well as from some landraces in Java, and subsequently conducted genetic trial tests.

The association of *P.falcataria* with *Rhizobium* sp and amf has a potential of increasing its growth-performance on soils of low fertility. Combined with soil fertility management, symbiotic aspects can be manipulated to support plant growth in such condition. Based on its dual-symbiosis, the manipulation of the symbiotic elements should at least include: (i) host genotype, (ii) amf isolates and (iii) Rhizobial isolates.

With the increasing timber demands for industrial purposes, *P.falcataria* are widely planted by the timber estate companies in large areas dominated by ultisol. However, properties of ultisols are not particularly suitable for nitrogen fixing plants in terms of physical and chemical properties, including Al, Mn, Co,B,pH, P, cations3, porosity, texture and structure (O'Hara *et al.* 1988). Efforts to increase productivity has been carried-out from the host plant aspect as well as its symbionts.

Several studies had been done on fertilization, inoculation but no study conducted on genetic combined with symbiont, micro-macropropagation and fertilization. This experiment constitutes a preliminary study in developing of *P.falcataria* for the ultisols through integrated approach consists of : development in host plant

(tree improvement), symbionts (soil microbiology), micro- and macropropagation (tree physiology), and fertilization (soil chemistry and fertility).

Objectives

The objectives of this study was to determine further design of association involving *P.falcataria* host plant genotype, Rhizobial isolates and amf isolates grown on ultisol. The information from this experiment would be used in the selection of Rhizobial isolates, amf isolates and host plant, in providing further answers to the following questions, (i) could rhizobial isolates be selected separately from amf isolate, or vice versa (ii) if the selection of the symbionts should be used the clone, same seedlot number or same provenance of *P.falcataria*, (iii) how far interaction of the host plant with rhizobium and amf isolates influences the host growth.

REVIEW OF LITERATURE

P.falcataria is one of the most promising fast-growing tree species for development based on their tolerance to marginal soils, fast-growing trait as well as the high-valued timber produced. *P.falcataria* timber can be used as raw material for pulp, plywood, wood working, and home construction (Suhardi *et al*, 1993).

P. falcataria is a leguminous plant capable of associating with *Rhizobium* sp. in fixing atmospheric nitrogen and of associating with arbuscular mycorrhizal fungi (amf). Several studies have been conducted to asses the role of symbiosis in *P.falcataria* growth (de La Cruz *et al.*, 1988; Suhardi *et al.*, 1993; Umali-Garcia et al., 1988). Dual inoculation had been done to increase the growth of *P.falcataria* succesfully (Umali-Garcia et al, 1988).

Galiana *et al.* (1994) investigated interaction between isolates of *Rhizobium* sp. and *Acacia mangium* provenances. They found that there were compatibility requirements between isolate or strain of *Rhizobium* with its host plant, although their earlier experiment *in vitro* did not find this interaction (Galiana *et al.* 1991). The inappropriate combination tended to depress the host-plant growth.

MATERIALS AND METHODS

P.falcataria Seed

Nine seedlot numbers/progenies collected from seed exploration in three provenances were used as the host plants, namely: seedlot numbers 530; 544 and 559 (Morotai provenance-Moluccas island,designated as provenance 1), seedlot numbers 002; 04 and 120 (Jasinga landrace-West Java, designated as provenance 2) and seedlot numbers of 52, 60 and 63 (Wamena provenance-West Irian, desig-

nated as provenance 3) . These nine seedlots were also used as material of progeny test and designed as P1,P2,P3,P4,P5,P6,P7,P8,P9, respectively. All materials were taken from the Lab. of Tree Improvement, Fac. of Forestry, GMU.

Amf Isolates

Three amf isolates from ultisols were selected based on growth performance, namely: *Glomus manihotis (A1), G. fasciculatum (A2)* and *G. geosporum (A3)*. These isolates were obtained from the collection of Lab. of Soil Microbiology, Fac. of Agriculture, GMU. Isolates were aseptically-propagated with corn.

Rhizobium sp. Isolates

The best isolates were selected from *P.falcataria* grown on ultisols. Nodules were excised, cultured, isolated and selected. The isolates were collected from Pulau Laut, South Kalimantan and from Jasinga, West Java which were typically ultisol. Biphasic-cultured isolates were slurry-inoculated to the *P.falcataria* seedlings grown on modified Leonard-jar containing ultisol. Isolates were selected based on its association to support host-plant growth. Three isolates were further used as inoculants,i.e. *Rhizobium Pf*LnU 9.2 as R1(fast growing), *P*LnU 16.2 (slow-growing) as R2 and *Pf*UJ (fast growing) as R3.

Soil

Ultisol soil was collected from Jasinga, West Java. The soil properties as shown in Table 1 are with very low available P. The soil then was air-dried, sieved through 0.5 mm sieve, homogenized, filled into pots at 2 kg each and autoclaved at 121° C for 2 hrs. Basal fertilizers were applied prior to the planting of each seedlot of aseptic seedling. Arbuscular mycorrhizal fungi inoculants were applied as layer inoculation, at 5 cm depth and the surface of potted soil, where *Rhizobium* were applied as slurry inoculant. The soil was maintained at near field capacity by watering daily with sterilized-deionized water.

Table 1. Results of soil analysis used for experiment

Paramater	Value	
Total N	0.11%	
Total P	237.83	ug.g-1
Available- P	9.1	ug.g-1
Exchangeable Al	11.63	cmol(+)kg-1

Data Analysis

A factorial experiment in completely randomized design was employed used. Total number of pots was 630. Plants height and stem diameter were measured at 2 -week- intervals. The plants were harvested after 4 months and the following parameters measured: (i) height, (ii) diameter, (iii) root dry weight, (iv) shoot dry weight, (v) number of nodules, (vi) nodule dry weight, (vii) specific nitrogenase (acetylene reduction activity). The data were analyzed by ANOVA and Duncan's Multiple Range Test with SAS program.

RESULTS AND DISCUSSIONS

Tabel 2 showed the summary of anova of all parameters which were used in this experiment. The result showed that most of the treatments had a significant effect on the parameters. For example *P.falcataria* provenances were significantly different in the following parameters: (i) height, (ii) diameter and (iii) number of nodules (Table 2) . The Wamena provenance showed the best growth followed by the Morotai provenance and the Jasinga landrace.

Biomass

Mycorrhizal inoculation resulted in significant increase in all parameters measured at the 99% level. *Glomus manihotis* (A1) proved to be the best isolate tested and it increased host biomass 16 times over the control (Table 3 and Table 5). It clearly showed the important role of mycorrhizae in plant growth on soils with limited available P, with Al toxicity problem.

Two other isolates (*G. fasciculatum* and *G. geosporum*) did not give significant growth increase, but DMRT significant showed differences in acetylene reduction activity (ARA) (Table 2).The high level of exchangeable Al have inhibited their growth and interaction with the host plant. On the other hand, *G. manihotis* was apparently well adapted to such a condition.

Table 3 Percent biomass increase with the combination of *P.falcataria* provenance and arbuscular mycorrhizal isolate over the uninoculated plants

Provenances	A1 *Glomus manihotis*	A2 *Glomus fasciculatum*	A3 *Glomus geosporum*
Morotai	450	19.3	58.7
Jasinga	583	37.7	11
Wamena	1170	85.4	61.9

Table 2. Results of analysis of variances

Sources of variation	df	biomass (gram)	height (cm)	dia-meter (mm)	number of nodule	nodule dry weight (gram)	specific ARA *a)*	total ARA *b)*
Treatments	143	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***
P	2	0.0766ns	0.0001***	0.005**	0.0001***	0.1371ns	0.0183**	0.0144**
p	8	0.003***	0.0001***	0.004**	0.0001***	0.2082ns	0.1354ns	0.1047ns
p (P)	6	0.0005***	0.2962ns	0.0574ns	0.0006***	0.362ns	0.6172ns	0.5671ns
R	3	0.5444ns	0.6509ns	0.3856ns	0.0008***	0.0795ns/** /0.0001***	0.0174NS*	0.0001***
P X R	6	0.009**	0.6172ns	0.048*	0.0283*	0.0434*	0.5085ns	0.6003ns
p X R	24	0.0001***	0.8551ns	0.2288ns	0.0001***	0.0014**	0.7836ns	0.8514ns
p (P)X R	18	0.0002***	0.8296ns	0.5780ns	0.0001***	0.0040**	0.7251ns	0.7574ns
A	3	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***
P X A	6	0.0001***	0.099ns	0.0064**	0.0001***	0.7867ns	0.0159**	0.0102**
p (P)X A	18	0.0002***	0.6657ns	0.0555ns	0.0001***	0.0552ns	0.1336ns	0.0932ns
pX A	24	0.0001***	0.3758ns	0.005**	0.0001***	0.1338ns	0.0247**	0.0131**
R X A	9	0.1147ns	0.3184ns	0..3104ns	0.0292*	0.1373ns	0.0052ns/**	ns/0.0012**
p(P) X AX R	72	0.0001***	0.3581ns	0.0081**	0.0001***	0.0172**	0.1019ns	0.1059ns
p X A X R	72	0.0001***	0.3581ns	0.0081**	0.0001***	0.0172**	0.1970ns	0.2199ns

P = provenance
p = progeny among and within provenance
p(P)= progeny within provenance
A = arbuscular mycorrhizal isolates
R = rhizobial isolates
a = analysis based on among progeny

b = analysis based on among provenance
a) = nmol C_2H_4 produced per gram dry weight nodules per hour
b) = nmol C_2H_4 produced per plant per hour
* = significant at ó 0.05
** = significant at ó 0.01
*** = significant at ó 0.001
ns = not significant

There were strong interactions between *P.falcataria* progenies and arbuscular mycorrhiza fungi isolates, both among provenances and within provenance. P3 (seedlot no. 559. Prov. Morotai) gave the best result (biomass 6.129 g) in association with *G. manihotis as* compared with P3 in association with A2 (*G. fasciculatum*) which produced 0.622 g (Figure 1, Table 4). The occurrence of significant interaction between progenies within provenance and amf isolates indicated that progenies (seedlots) within provenance constituted an important source of variance in these symbiosis processes. Significant interaction between *P.falcataria* provenance and amf isolate was also noted in the above ANOVA. These interactions may be contradictive since amf has so far been known to be a broad-host range symbiont, which means it does not have restricted compatibility.

Table 4. Best and worst combination of progeny and arbuscular mycorrhizal fungi to the biomass

Combination	Mean of biomass (g)	Duncan Grouping
Progeny number 3 X *G. manihotis*	6.129	A
Progeny number 7 x *G.manihotis*	5.435	AB
Progeny number 8 x *G.manihotis*	4.907	B
Progeny number 9 x *G.manihotis*	4.701	BC
Progeny number 1 x *G.manihotis*	4.033	CD
Progeny number 3 x *G.fasciculatum*	0.622	E
Progeniy number 8 X *G. geosporum*	0.567	E
Progeny number 9 x *G.geosporum*	0.545	E
Progeny number 8 x no mycorrhiza	0.529	E
Progeny number 9 x no mycorrhiza	0.521	E
Progeny number 1 x no mycorrhiza	0.496	E

There was a strong indication interaction among the three components of the symbiosis. Fig. 1 shows that interaction of *P.falcataria with* amf and Rhizobial isolates in terms of growth response (appear spotted), depended on the components of the symbiosis. A large growth response resulted from a compatible interaction (Table 5, Figure 3). It showed that interaction among the five best and the five worst, 7.884 g compared to 0.277 g. Results of ANOVA (Table 2) supported the evidence of tripartite interaction.

Table 5. The best five and the worst five combinations of symbiosis components to biomass

Combination	biomass average (gram) with Duncan grouping
P3 R1 A1	7.884[a]
P7 R2 A1	6.097[b]
P3 R3 A1	6.059[b]
P1 R2 A1	5.969[b]
P8 R1 A1	5.925[b]
P9 R1 A3	0.330[s]
P8 R0 A3	0.327[s]
P3 R0 A2	0.327[s]
P9 R0 A0	0.295[s]
P5 R0 A0	0.277[s]

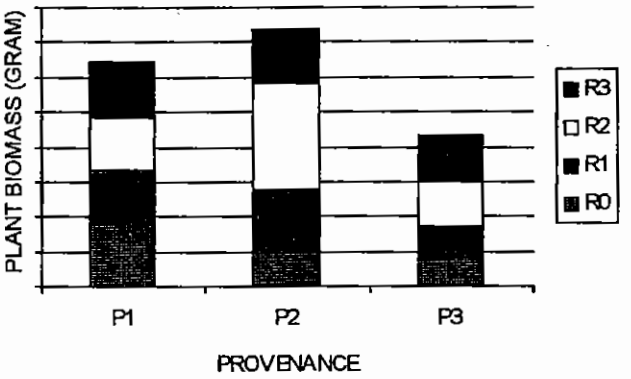

Figure 1. Responses of *P.falcataria* provenances to *Rhizobium sp* inoculation

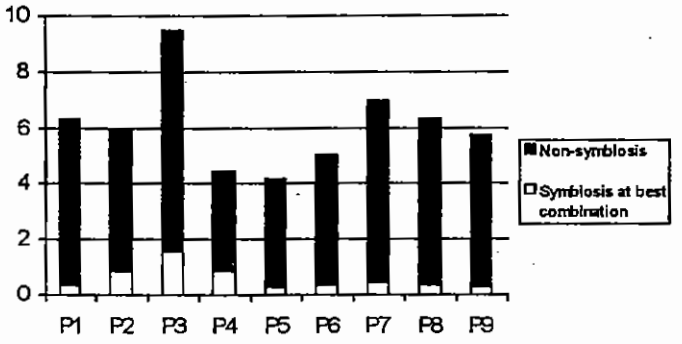

Figure 2. Response of *P.falcataria* progeny to dual symbiosis

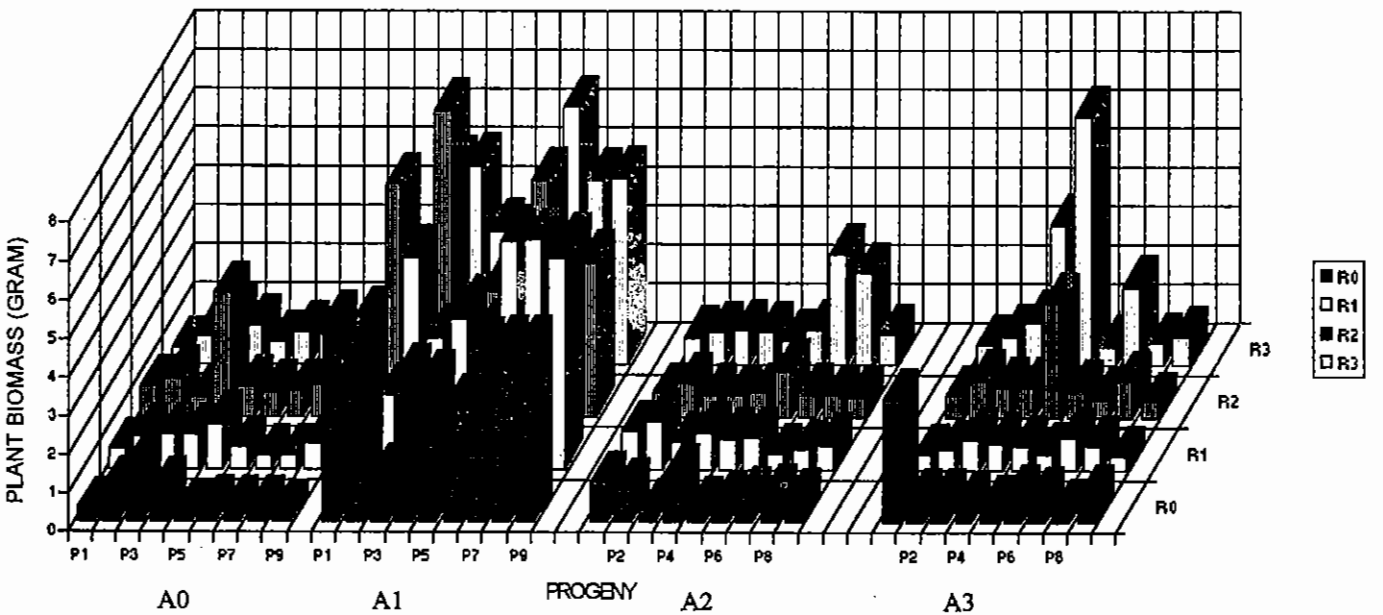

Figure 3. Tripartite association among *P.falcataria* progeny, arbuscular mycorrhizal fungi and Rhizobial isolates grown on ultisol soil.

Height

Height was significantly affected by treatment, provenances, progeny, and mycorrhiza but no interactions were found among other parameters (Tabel 2). It seemed that provenances and progeny had strong genetic effect on height. However *Rhizobium sp* inoculation did not have significant effect on the height parameter (Table 2). All combination with Rhizobium did not have a significant effect on height growth.

Rhizobium sp effect mostly in high pH soil while in ultisol soil with is low pH was not suitable to the *Rhizobium sp* association. It was also possible that *Rhizobium sp* isolates were not suitable in ultisol soil .

Wamena provenances showed the best perfomances in ultisol soil (Table 6). Wamena provenances found in natural distribution as same as Morotai provenances. Mycorrhiza has also strong effect to the height parameter probably due to the release of phosphate available to the soil.

Table 6 Effect of provenances to the height of *Paraserianthes falcataria* in ultisol

Progeny	Mean	Duncan Grouping
P7 (Wamena)	16.10	A
P9 (Wamena)	15.927	A
P8 (Wamena)	15.798	A
P4 (Jasinga)	15.098	AB
P5 (Jasinga)	14.083	ABC
P3 (Morotai)	1.471	BC
P6 (Jasinga)	12.69	C
P1 (Morotai)	12.31	C
P2 (Morotai)	12.298	C

Diameter

Provenance, progeny among and within provenance, progeny within provenance, and mycorrhiza affect significantly diameter growth. However *Rhizobium sp* inoculation did not affect significantly the diameter. Diameter responses more to mycorrhiza inoculation than to *Rhizobium sp* inoculation.

The combination effect (Table 7) showed that Morotai provenance combined with *G. manihotis* showed was the best combination followed by combination of Wamena and *G. manihotis*.

Table 7. The best five and the worst five combinations of symbiosis components to diameter

Combination	diameter average (mm) with Duncan grouping
P2 R2 A0	7.993 a
P3 R1 A1	6.153 b
P9 R0 A1	5.703 bc
P7 R3 A1	5.463 bcd
P9 R3 A1	5.437 bcd
P5 R0 A2	1.440 vwx
P6 R1 A3	1.437 vwx
P9 R0 A0	1.410 wx
P2 R0 A0	1.400 wx
P5 R0 A0	1.353 x

Diameter seemed to be affected by mycorrhiza and not by *Rhizobium sp* inoculation.. The best combination could increase the diameter up to 590 %. Mycorrhiza activities will increase the release of some nutrient that will become available, therefore stimulate the metabolism and affect the diameter growth.

Number of Nodules, Nodule Dry Weight and Specific ARA

All treatments showed that number of nodules were significantly affected by treatments, provenances, progeny, rhizobium, mycorrhiza, and their combination. Without inoculation of *Rhizobium sp* the seedlings produced the smallest number of nodules.

In most cases *Rhizobium sp* inoculation had only a significant effect on number of nodules and specific ARA (Table 2). It might be that especially in ultisol soil *Rhizobium sp* activities could not increase growth as good as in alkaline condition.

Rhizobium inoculation resulted in a significant difference in number of nodules formed, nodule dry weight, and nitrogenase activity (Table 2), There were interactions between *P.falcataria* provenances and Rhizobial isolates as well as between *P.falcataria* progenies (among and within provenances) and Rhizobial isolates. Negative interactions were found between Morotai provenances and all Rhizobial isolates indicating that there were incompatibilities in the association (Table 8). It is not as yet known what caused this incompatibility. It could be due to geographical difference (host from Moluccas islands), whereas *Rhizobium* were from South Kalimantan and West Java. Incompatibility was caused by undetectable Acetylene Reduction Activity. The nodules were blackish and small, even

though the number was high (see Figure 1). Later, in tripartite symbiosis of seedlots of the Morotai provenance, there was a strong correlation (r = 0.89) between root nodule's dry weight and biomass which might be an indication of good correlation between nitrogen fixation activity and plant growth. This phenomenon was not found in the other provenances.

Under the non-symbiotic conditions (Table 2), progenies among and within provenance were significantly different not only in number of nodules but also in the following parameters; (i) biomass, (ii) height and (iii) diameter. The fact that there were significant variation among progenies within provenances would indicate that *P.falcataria* progeny or seedlot (even within the same provenance) was the important source of *P.falcataria* variation beside provenance. Naturally half-sib fertilization may explain the occurrence of variation within the same provenance and even in same number of seedlot.

Another result showed that :

1. *Rhizobium* isolate PfUJ when associated with *P.falcataria* randomly increased acetylene reduction activity 10X as compared with *Rhizobium Pf*LnU 16.2. Dual-symbiosis with *G. manihotis* and *P.falcataria* randomly increased ARA by 32.6%, and if associated with progeny 8 (seedlot from Wamena provenance), ARA will be increased by 93.67% (ARA value 106 nmol C_2H_4/ plant/hour). Association with *P.falcataria* from Morotai provenance will decrease growth, including ARA value.
2. *Rhizobium* isolate PfUJ increased number of nodules formed by 80.9% when associated with *P.falcataria* randomly. Dual-symbiosis with *G. manihotis* will increase nodules formed into 281%, and when associated with progeny it will increase up to 696.9%. The maximum number of nodules occurred in the combination of progeny 8, *G. manihotis* and *Bradyrhizobium* PfLnU 16.2, with nodules formed 276.3 pcs per plant (1250%).

Table 8 Percent biomass increase with the combination of *P.falcataria* provenance and *Rhizobial* isolate relative to the uninoculated plants

Provenances	R1 (*Rhizobium Pf*LnU 9.2)	R2 (*Bradyrhizobium Pf*Ln 16.2)	R3 *Rhizobium Pf*UJ
Morotai	-19.3	-18.8	-13.6
Jasinga	70	200	52.4
Wamena	28.6	80	88

CONCLUSIONS

This research entitled " Interaction among Progenies /Provenances of Sengon (*P. falcataria*), Arbuscular Mycorrhizal Fungi and Rhizobial Isolates Grown on Ultisol "showed the following results.

1. There was a significant growth variation of seedlot among and within sengon provenances,
2. There was a positive interaction among *P.falcataria* progenies/provenances, amf isolates and rhizobial isolates in terms of host plant growth.
3. The best growth up till 4 months was achieved in the combination seedlot of *Morotai* provenance (Moluccas), *Glomus manihotis*, and Bradyrhizobium isolate *Pf*LnU 16.2. This combination increased biomass more than fifteen times over the control .
4. The inappropriate combination of symbiotic components could not increase the host growth, to the contrary it reduced the host plant growth.

The results suggested that further exploitation of the symbiotic aspects in the development of *P.falcataria* on acid soils should consider interaction of three symbiosis components. Selection methods of symbionts should refer to the host plant genotype.

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