Summary of Doctoral Thesis

Enrollment year: 2015 y 4 m
UGAS Specialty: Bioproduction Sciences
Name Ahdiar Fikri Maulana

Title	Characterization of arbuscular mycorrhizal and endophytic fungi isolated from forest soils in Indonesia and its effect on plant growth

Introduction and purpose

Deforestation by land-use conversion into agricultural fields and plantations, open cast mining, and illegal logging is increasing in Indonesia. Reforestation of tropical forests, such as those in Indonesia, requires human assistance to recover forest structure and species composition. There are few methods to remediate degraded forest in Indonesia. Utilization of beneficial symbiotic microorganisms is an environmentally safe way to ensure the plant survival rate after transplantation to the field. Arbuscular mycorrhizal fungi (AMF) and endophytic fungi (EPF) are groups of root symbiotic fungi which reported to be able to promote plant growth. AMF support plant growth through several mechanisms and is beneficial for plant survival in a degraded forest. However, the effectiveness of AMF in promoting plant growth has been shown to vary. AMF isolate and plant species are the main factors determining plant response to inoculation with AMF. Selection of the appropriate AMF isolate for a certain plant species is a strategy that would guarantee the success of reforestation efforts. There is no report yet about utilization of EPF for tree species in Indonesia. Study about AMF and EPF in the tropics is less than in temperate region despite higher biodiversity in the tropics. The importance is not limited to as an effort to support reforestation of Indonesian forest, but also for wider knowledge for example about role and mechanism of those fungal group in affecting plant growth. The objectives of this study were (1) to isolate AMF and EPF from forest soil in Indonesia, (2) to determine condition of screening of EPF, and (3) to screen effective isolates of AMF and EPF with tropical tree species.

Materials and methods

Five AMF isolated from forest soils in Indonesia were propagated by inoculating to *Trifolium repens* (soil culture) and hairy root of *Linum usitatissimum* (root organ culture). Spores were mounted in PVLG and a mixture of PVLG and Melzer's reagent and their morphology was observed. The rDNA LSU region of the fungi was amplified from DNA of spores and identified. The spores were inoculated to four leguminous trees (*Calliandra calothyrsus*, *Paraserianthes falcataria*, *Cassia siamea*, *Sesbania grandiflora*) and grown in growth chamber with the application of low phosphorus (P) (1 mg L^{-1}) nutrient solution for screening of effective AMF isolates. EPF isolated from forest soils in Indonesia in the previous study was used to determine methods to clarify the factors affecting relationship between EPF and plant. *Brassica campestris* was used as the host plant. *B. campestris* was transplanted to pre-grown EPF colony supplied with different concentration of liquid malt extract. *B. campestris* was also inoculated with EPF and grown on different concentration of MS (Murashige and Skoog) medium. In other experiments, EPF were isolated from forest soils in Indonesia by trap culture using *P. falcataria* and *S. bicolor*

as host plants. EPF were identified by extracting the rDNA ITS region. EPF were inoculated to *P. falcataria* and *B. campestris* grown on 1/100 MS and 1/10 MS medium to clarify the effect of nutrient concentration on relationship between EPF and host plant. Effective EPF were inoculated to *B. campestris* and grown on 1/100 MS and 1/10 MS medium with modified carbon, nitrogen (N) and P concentration. EPF were also isolated from roots of *Santalum album* and *Swietenia macrophylla* and screened using *B. campestris* on 1/100 MS medium.

Results

Five AMF isolates were propagated using *T. repens*. Two isolates were propagated using *L. usitatissimum*. Five AMF isolates were identified as *D. gibbosa* (M10-2), *Acaulospora* sp. (M11-1), Glomeromycota sp. (M44-3), *A. appendicula* (M60-3), and *Glomus* sp. (S6-4). In contrast to trees inoculated with other AMF, trees inoculated with S6-4 showed similar or higher values of shoot fresh weight (SFW), shoot P content, shoot: root ratio, and plant height. Colonization rate of S6-4 was 84–99% and higher than that of another AMF. Isolate M44-3 belongs to the same Glomaceae family as S6-4 and showed the opposite result. M11-1, increased number of leaves of *P. falcataria* and *C. calothyrsus*, and shoot fresh weight SFW, shoot P content, and shoot: root ratio of *P. falcataria*. M10-2, increased shoot P content of all leguminous species. Mean mycorrhizal dependency (MD) of *C. calothyrsus* (51%) was not different from that of *P. falcataria* (27%) but was higher than that of *S. grandiflora* (19%) and *C. siamea* (11%), irrespective of AMF isolate. Positive correlations between colonization rate and shoot P content were observed for *P. falcataria* ($R^2 = 0.87$, $P < 0.001$), *C. calothyrsus* ($R^2 = 0.41$, $P < 0.001$), *C. siamea* ($R^2 = 0.39$, $P = 0.0011$), and *S. grandiflora* ($R^2 = 0.13$, $P = 0.048$). *P. falcataria* showed higher correlation between colonization rate and shoot P content than the other leguminous species. Higher concentration of malt extract increased growth of two EPF. External colonization on *B. campestris* of both EPF in higher concentration of malt extract were higher than that in control. Higher colonization decreased the survival rate and growth of *B. campestris* inoculated with EPF. Sixteen of the 33 EPF isolates had the closest match to fungi identified to the species level. Three isolates were specific to certain forest sites shared by *P. falcataria* and *S. bicolor*. Some isolates were specific to certain forest sites but were not shared by the two host plants; *Dictyosporium heptasporum* in *T. grandis* monoculture, *Mariannaea camptospora* in *Gmelina arborea*, *Artocarpus champeden*, and Dipterocarp mixed, and *Mycoleptodiscus* sp. in *Macaranga* sp. secondary forest. Two inoculated *B. campestris* and one inoculated *P. falcataria* exhibited increased shoot dry weight (SDW) when grown on 1/100 MS medium but not on 1/10 MS medium. The number of inoculated *B. campestris* and *P. falcataria* with higher positive PR was larger when grown on 1/100 MS medium than 1/10 MS medium. The increase of N and P in 1/100 MS medium decreased the PRs of *B. campestris* inoculated with four EPF. These results showed that N and Ps were the driver of the decrease of PRs in 1/10 MS medium compared to 1/100 MS. EPF isolated from roots of *S. album* and *S. macrophylla* using *B. campestris* as host plant and 1/100 MS medium as medium yielded negative to positive plant response.

Conclusion and consideration

These results suggest that (1) different leguminous species have different MD, (2) inoculation of AMF *Glomus* sp. (S6-4) promote growth of *P. falcataria* and *C. callothyrsus*, (3) 1/100 MS medium is a reliable medium for screening of EPF with *B. campestris* as host plant, and (4) concentration of N and P in medium affect the relationship between EPF and host plant.

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