IMPROVEMENT OF SPORE PRODUCTION OF AMF: PREPARATION FOR ON-FARM PRODUCTION

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Abstract

Arbuscular mycorrhizal fungi (AMF) are important and beneficial soil fungi which could be potentially used as biofertiliser. Number of spores produced in the AMF cultures is an important indicator for inoculum quality and eventually for the effectiveness of the biofertiliser. Organic materials from animals, such as bone, feather, and blood mills are materials that rich with plant nutrient which have potential to be used for supplement in AMF spore production. Method of AMF culture production used in this experiment was the open pot culture method with zeolite sand and *Pueraria javanica* as plant and fungal growth medium, and host plant respectively. The result showed that supplementing AMF inoculum with bone mills at a rate of 5 and 30% could significantly increase host plant growth, spore production, and percentage of root colonization by AMF.

Key words: mycorrhiza, spore production, biofertiliser, bone mill, blood mill, feather mill

Introduction

Arbuscular mycorrhizal fungi (AMF) was probably the most advance technology and consistently investigated since 1984 (Nuhamara, 1984), and was intensified in early 1990. In the periods of 1990-2000 there were more than 80 research on various aspects of AMF have been conducted in Indonesia (Mansur *et al*., 2001). A culture collection of AMF of Indonesia, i.e. The Bank of Tropical Indigenous Glomales (BTIG) has also been established in 1998 in Bogor, Indonesia, to support the needs of pure AMF inoculum for research purposes. Based on the fact that this group of

mycorrhizae has a wider host species than ectomycorrhiza, the AMF have higher potential for land rehabilitation, which usually involving fast-growing species (Setiadi, 1999, 2001). Besides that, inoculum production of AMF does not need sophisticated facilities and expensive chemical, and in fact, AMF could be produced easily on-site.

Although some mining and forestry companies in Indonesia have adopted AMF technology, the application of AMF in a large scale is still hampered by the impracticality of the current form of inoculum. Since AMF-plant symbiosis is an obligate symbiosis, AMF should be grown together with its host on growth media, such as sand. This made the inoculum is bulky and heavy. Attempts to formulate the inoculum into more practical form of inoculum have not been satisfactory. Besides that, spore density is important to ensure the viability of the inoculum, especially for long storage period.

The objective of this research was to enhance spore production of AMF by supplementing the growth media with bone, blood and feather mills. In this experiment *Pueraria javanica* was used as host plant species.

Materials And Methods

The experiment was conducted at the laboratory of Silviculture, the Faculty of Forestry Bogor Agricultural University. Materials used were zeolite; bone, blood and feather mills; seeds of *P. javanica*; inoculum of AMF containing mixed isolates (*Gigaspora rosea*, *Acaulospora tuberculata*, *Glomus manihotis*, and *Glomus etunicatum*); clay; and plastic cups; staining solutions: 10% KOH, 2% HCl, glycerol, lactic acid, trypan blue, bayclean, and Hiponex with low P content.

Seeds of *P. javanica* were sterilized using bayclean then germinated on zeolite. The germinated seeds were transplanted to plastic cups containing zeolite and at the same time were inoculated with a mixture of AMF inoculum and bone, blood or feather mills according to the designated treatments. The treatments were:

1. Control (M0)
2. 100% AMF inoculum (M100)
3. 30% bone mills + 60% AMF inoculum + 10% clay (TL30M60)
4. 5% bone mills + 50% AMF inoculum + 45% clay (TL5M50)
5. 5% feather mills + 50% AMF inoculum + 45% clay (TB5M50)
6. 5% blood mills + 50% AMF inoculum + 45% clay (TD5M50)

Each cup received 5 g respective treatment and each treatment has 25 replications. The experiment was arranged as randomized complete design.

The experiment was maintained for three months and fertilized weakly using Hiponex with concentration of 2 g per L. The parameters measured were shoot, root, and total dry weight; shoot root ratio, number of root nodules, number of spores produced, and % root colonization.

Results And Discussion

Treatments significantly affected all parameters measured, except for shoot root ratio. **Table 1** shows the results of the experiment. Supplementing inoculum of AMF with bone mills has significantly increased all parameters measured. The addition of bone mills has increased not only plant growth parameters, but also symbiotic relationships with either rhizobium and AMF manifested by number of root nodules, spore numbers, and AMF colonization. The plants receiving mycorrhiza and bone mills grew better than those inoculated with mycorrhiza alone (Fig. 1).

Table 1 Effects of bone, feather and blood mills on growth parameters of *Pueraria javanica*

Treatments	TDW	SDW	RDW	SRR	RN	SN	%AMF
M0	0.2bc	0.16bc	0.04c	3.67a	1c	0c	0
M100	0.25bc	0.18bc	0.07bc	2.53a	5bc	5bc	7.28bc
TL30M60	2.13ab	1.61ab	0.52b	3.2a	34a	10ab	28.08a
TL5M50	2.82a	2.14a	0.68a	4.26a	23ab	13a	12.36b
TB5M50	0.24bc	0.17bc	0.07bc	2.26a	9.33b	6b	12.2b
TD5M50	0.1c	0.1c	0.03c	2.84a	1c	0c	8.61bc

Notes: TDW= total dry weight, SDW= shoot dry weight, RDW=root dry weight, SRR=shoot root ratio, RN= number of root nodules, SN=spore number, %AMF= percentage of root colonized by AMF. Values in the same column followed by the letter do not significantly different ($p \geq 0.05$).

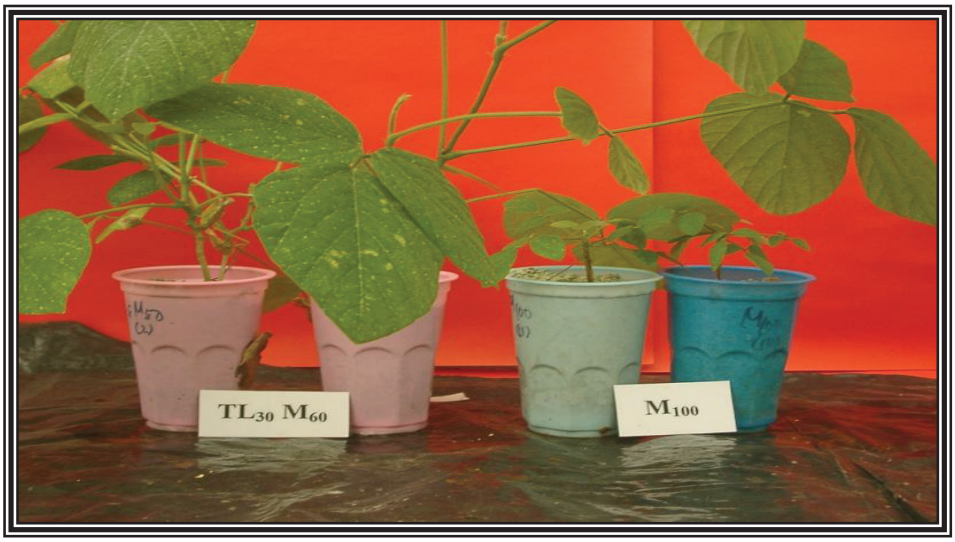

Figure 1 The performance of *Pueraria javanica* inoculated with AMF supplemented with 30% bone mills (TL30M60) compared with those received AMF alone (M100).

Bone is an organic waste which has been regarded as no value, where people could collect the bone from slaughter houses, traditional markets or restaurants. The fact that the addition of bone mills has increase plant growth (1100% and 1400% increased from conventional AMF production technique and with no-AMF inoculation, respectively) and number of spore produced (200-260% higher than conventional AMF production technique) has open an opportunity for farmers to produce their own high quality AMF based biofertiliser.

Bone contains phosphorous (P) and calcium (Ca) in substantial amount but not in available state. This means that plant could not utilise the nutrients, especially P. With the help of AMF, the P could be released from the complex and absorb by the fungal hyphae and eventually transferred to the host plant. Therefore, bone mills could be regarded as slow release fertliser (SRF) and AMF is an agent to facilitate the utilisation the SRF by plants.

Conclusion

From this experiment it could be concluded that among organic waste tested (bone mills, blood mills, feather mills), bone mills is the most promising material to be used to increase spore production. A concentration of 5% bone mills is sufficient to increase host plant growth, spore production and root infection by AMF.

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