Optimization of hydroponic technology for production of mycorrhiza biofertilizer

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Abstract. Mycorrhiza has been known could increase plants’ nutrient uptake through promoting root zone which resulted in higher yield production. A glasshouse experiment was conducted to optimize the technology for arbuscular mycorrhiza biofertilizer production through hydroponic system with automatic watering system and nutrient solution modification. Sorghum bicolor was used as host plants, grown for six weeks on medium contained mixture of zeolite and rice husk charcoal with the addition of arbuscular mycorrhizal fungi inoculum. Different hydroponic techniques (Deep Flow and Ebb Flow) and phosphorus content in nutrient solutions (0, 20, 40, 80 ppm) were tested. Results showed that different types of hydroponic system and rates of P in nutrient solution gave significant differences in increasing mycorrhizal colonization in roots, number of mycorrhizal spores, P uptake and biomass of plants. The best hydroponic technique was Ebb Flow with P content 40 ppm which was 50% lower than the standard nutrient solution for hydroponic. These findings suggest that the production of mycorrhiza biofertilizer through optimization of nutrient solution and growing medium in hydroponic system was potential for future practice. This technology could be used for mass production of “clean” and good quality of mycorrhizal biofertilizer.

1. Introduction
Arbuscular mycorrhizal fungi play a significant role in maintaining soil and plant health [1, 2]. Increased plant growth in the presence of mycorrhizal infection has been attributed mainly to the enhanced uptake of phosphorus. Previous studies showed that AMF occurred in many areas and in symbiosis with more than 90% terrestrial plants] and these fungi were effective as microbial inoculants or biofertilizer for plant production system [3]. Beside reduce the chemical fertiliser application and nutrient uptakes, AMF can also improve the quality of vegetables [4].

Demand of AMF biofertilizer is increasing in Indonesia, nevertheless the source of AMF inoculum is relatively limited [4]. Difficulties in producing AMF inoculum in large quantities was one of the issues [5]. Some factors that limit the availability of inoculum are long duration of production (up to three months), manual laborious of watering and nutrition addition, and small capacity of pot culture production.

One of the alternative solution to improve the production of AMF biofertilizer is automatization of watering and nutrition addition to mycorrhizal plants. The technique include hydroponic system which operate automatization of nutrient solution addition. Hydroponic production provides a controlled nutrient environment and facilitate robust root development, efficient water and nutrient absorption,
avoidance of diseases caused by soil-borne pathogens [5]. From several types of hydroponic system, such as deep flow technique (DFT) and ebb flow technique (EFT) there is a need to test their effectiveness to support AMF biofertilizer production. Previous studies have shown that mycorrhizal plants can be cultured in aeroponics and in a nutrient flow system [6].

Arbuscular mycorrhizal fungi known work properly in soil with low phosphorus concentration [3]. To optimize the production system and to identify whether P concentration from nutrient solution in hydroponic soilless system affect mycorrhizal growth, different rate of P concentration in nutrient solution need to be applied. Furthermore, the objective of this study was to determine the best hydroponic system and rates of P concentration in nutrient solution in improving growth of mycorrhizal plants to produce good quality of mycorrhizal biofertilizer.

2. Materials and Methods

A glasshouse experiment was carried out in hydroponic system area, in Lembang, West Java, Indonesia (-6.820400, 107.627180). The hydroponic system tested were Deep Flow Technique (DFT) and Ebb-Flow Technique (EFT). Different rates of P concentration in nutrient solution was 0, 20, 40, 80 ppm P. The experiment was set up in split plot design with five replications.

Plants used for production AMF biofertilizer was Sorghum bicolor. The mycorrhiza inoculum was mixture of spores and infected sorghum roots by Glomus sp. and Gigaspora sp. These fungi were culture collections of Soil Biology Laboratory Faculty of Agriculture Universitas Padjadjaran.

Seeds of sorghum were pre-germinated overnight and planted into seed tray containing zeolite and mycorrhiza inoculum (100 g or 10% of zeolite weight). Zeolite (1-3 mm) was prepared by washing in tap water over 2 mm mesh sieve and sun-dried. After one week, plants were carefully transferred into net pots (Ø 5 cm, height 6 cm) filled with zeolite:rice husks biochar (3:1) and rock wool in hydroponics systems to be cultivated for another six weeks.

Nutrient solution for hydroponic comprises of solution A and solution B. Nutrient solution A contained (g L⁻¹) Ca(NO₃)₂ (250) and KNO₃ (76), while solution B contained KNO₃ (54), ZA (17), ZK (17), MKP (70), MgSO₄ (166) and trace elements of Fe, Cu, Mn, Zn, B, Mo. The pH of nutrient solution was 6.0. For arrangement of different P concentrations, the Mono Kalium Phosphate (MKP) in nutrient compositions were adjusted according to the treatments to reach 20, 40, or 80 ppm of P. Zero ppm P was the solution without P addition. The nutrient solutions were changed every three days. Aeration of the nutrient solutions was supplied from an air pump which connected to a time switch for 15 minutes two times per day for EFT and continuous flow for DFT.

Number of AMF spore, root colonisation by AMF, P uptake, and shoot dry weight were assessed after six weeks. Percentage of root length colonised by mycorrhiza was determined using the gridline-intersect method while spore numbers was counted using wet sieving and decanting method [7]. Phosphorus concentration was determined using the molybdo-P assay on dried- milled shoot and root material. The two ways analysis of variance were applied to test differences between types of hydroponic systems and concentrations of P in nutrient solutions. Percentage of root colonisation were normalised by arcsin square root transformation before performing the analysis of varian.

3. Results and Discussion

There was a significant difference of number of AMF spores when grown on different hydroponic technique and P concentration. However, no significant differences between those treatments on the percentage of mycorrhizal colonization on root of sorghum (Table 1). Although the treatments did not affect the root colonisation, the percentage of mycorrhizal colonisation shown in sorghum root were categorized as high (more than 65%).

The number of spores of AMF on growing medium in Ebb Flow Technique was higher than those grown on Deep Flow Technique. The highest number of spores produced was in EFT system at rate of P 40 ppm followed by 20, 80 and 0 ppm. Similar trend was occurred in Deep Flow Technique even though the numbers were not different.
Table 1. Effect of different concentrations of P in nutrient solutions and hydroponic techniques on mycorrhizal parameters: number of spores of AMF and percentage of root colonisation by AMF.

| Treatments | Number of AMF Spores 10 g medium⁻¹ | Mycorrhizal Root Colonisation (%) |
|------------|-----------------------------------|----------------------------------|
|            | Deep Flow (DFT) | Ebb Flow (EFT) | Deep Flow (DFT) | Ebb Flow (EFT) |
| P Concentrations (ppm) | Hydroponic Techniques | Hydroponic Techniques |
| 0          | 267 a | 391 a | 65 a | 71 a |
|            | A     | A     | A    | A    |
| 20         | 285 a | 863 bc| 69 a | 73 a |
|            | A     | B     | A    | A    |
| 40         | 321 a | 962 c | 77 a | 79 a |
|            | A     | B     | A    | B    |
| 80         | 270 a | 544 ab| 77 a | 76 a |
|            | A     | B     | A    | A    |

Numbers followed by same letters (lower caps vertically and capital horizontally) means no significant difference at p<0.05

Measurements on P uptake of sorghum showed that there was interaction effect between P concentration levels and type of hydroponic systems (Table 2). There was a notable increased in P uptake as the levels of P in nutrient solution increased.

Table 2. Effect of different concentrations of P in nutrient solutions and hydroponic techniques on P uptake of sorghum.

| Treatments | P uptake (mg plant⁻¹) |
|------------|-----------------------|
| P Concentrations (ppm) | Hydroponic Techniques |
|              | Deep Flow (DFT) | Ebb Flow (EFT) |
| 0           | 1643 a | 1572 a |
|             | A     | A     |
| 20          | 7135 b | 4064 b |
|             | B     | A     |
| 40          | 9411 c | 4069 b |
|             | B     | A     |
| 80          | 13218 d| 5758 b |
|             | B     | A     |

Numbers followed by same letters (lower caps vertically and capital horizontally) means no significant difference at p<0.05

When plants grown on zero level of P, both Deep Flow Technique and Ebb Flow Technique systems had lowest P uptake and no statistical different. Furthermore, once P concentration given in the nutrient solutions, Deep Flow Technique system always gave higher P concentration compared to Ebb Flow Technique system. The higher the P levels (20, 40, 80 ppm), the more the P uptake on sorghum in Deep Flow Technique system respectively. Nevertheless, for Ebb Flow Technique system, P uptake as affected by application of P concentration at 20, 40, and 80 ppm did not give significant differences.
Table 3. Effect of different concentrations of P in nutrient solutions and hydroponic techniques on shoot dry weight of sorghum.

| Treatments          | Shoot Dry Weight (g plants⁻¹) | Hydroponic Techniques |
|---------------------|-------------------------------|-----------------------|
| P Concentrations   |                               | Deep Flow (DFT)       |
| (ppm)               |                               | Ebb Flow (EFT)        |
| 0                   | 1.2 a                         | 1.5 a                 |
|                     | A                             | A                     |
| 20                  | 5.8 b                         | 13.2 bc               |
|                     | A                             | B                     |
| 40                  | 11.4 c                        | 16.3 c                |
|                     | AB                            | B                     |
| 80                  | 5.2 b                         | 10.1 b                |
|                     | A                             | B                     |

Numbers followed by same letters (lower caps vertically and capital horizontally) means no significant difference at p<0.05

Measurement on shoot dry weight, showed that there was interaction effect of different P concentrations and hydroponic system (Table 3). No significant difference was observed on shoot dry weight at P level 0 ppm in both Deep Flow Technique and Ebb Flow Technique systems. At P levels 20 and 80 ppm, Ebb Flow Technique was constantly higher than Deep Flow Technique. For both systems, application of P at 40 ppm gave highest shoot dry weight, which was half of recommended dosage.

Finding from this experiment showed that production of arbuscular mycorrhizal fungi on hydroponic system especially Ebb Flow Technique was potentially efficient since the highest arbuscular mycorrhizal fungi spore numbers and biomass reached at reduced rate of P content in nutrient solution. The potential uses of hydroponic system for culturing and maintaining mycorrhizal fungus was also suggested by several researchers [8].

The percentages of root colonisation in this experiment were also found to be high (Table 1) and Ebb Flow Technique showed slightly higher than Deep Flow Technique eventhough no statistical difference between the two systems. This finding confirm that mycorrhizal fungi was functioning well and could colonize plants in hydroponic system. This was comparable with other research which reported that new infections ascended within the fast growing root system, and hyphae spread out into the liquid then infected the plants[8]. Moreover, the fungal structures in the mycorrhizal roots of sorghum found to be active as shown by high total colonisation rates of roots even after vigorous root growth in nutrient solution in hydroponic system [6]. It seems that the aeration conditions in these two systems had no negative effect on root colonisation by the fungus.

In production of arbuscular mycorrhizal system, the high number of spores are needed. According to the standard of mycorrhizal biofertilizer revealed by [9] the minimum number of spores is 50 in each gram of inoculant. This was achieved in Ebb Flow Technique when phosphorus applied (Table 1), especially when P levels applied at 20 ppm and 40 ppm. When P levels applied at 80 ppm, the number of spores decreased. As previously reported [6, 10], nutrient medium contained a high P concentration did not produce viable cultures of mycorrhizal colonisation. In contrast, the number of spores in Deep Flow Technique were all found to be under the minimum requirements for mycorrhizal biofertilizer.

Besides the use of low P concentration, the use of a longer aeration period also influenced the growth of mycorrhizal fungi [6]. In Deep Flow Technique system, the nutrient continuously circulated for 24 h, while in Ebb Flow Technique the nutrition aerated for short periods (10 min daily) and the nutrient solution in this system was hold by rock wool block which can provide sufficient water and nutrition for a day. The Ebb Flow Technique was produced more spores and higher root colonisation which may be due to reduce the amount of movement in the nutrient medium that would disturb fungal growth [6].

Phosphorus uptake observed in this study significantly different between Deep Flow Technique and Ebb Flow Technique. This could also related to the previous reason where longer aeration period
applied to Deep Flow Technique (24 h) compared to intermittent aeration (10 min daily) in Ebb Flow Technique. The P uptake remarkably increased as P levels increased in Deep Flow Technique. No differences in the P concentration of mycorrhizal plants were observed in Ebb Flow Technique at 20, 40, and 80 ppm, possibly due to the lack of diffusion restrictions for P in hydroponic solution [6]. Phosphorus in nutrient solutions of hydroponic was easily available and this would possibly make the growth of external hyphae terminated in terms of P acquisition of the mycorrhizal root [6].

The biomass of plants in term of shoot dry weight were showed similar trends in both systems. The lowest were at zero P levels, increased at 20 and 40 ppm but then decreased when P at 80 ppm. In general, Ebb Flow Technique had higher shoot dry weight compared to Deep Flow Technique. The growth stimulation in the plants grown on Ebb Flow Technique was conceivably not related to increased P uptake but to non-nutritive fungus-mediated effects since they had lower P uptake but higher number of spores and root colonisation. This fungus-mediated effect influence for instance shoot:root carbohydrate allocation [6]. The highest biomass that achieved at lower levels of P had also been explained by other researchers which stated the importance of providing hydroponic AM fungal substrates with lower amounts of nutrients (i.e. fertilizer containing N and P) to improve the quality of the crops [11, 12] and reduce the use of chemical fertilizers.

4. Conclusion
Hydroponic culture of mycorrhizas biofertilizer is of potential use for the purposes of harvesting roots and fungal propagules without contamination from a solid substrate. Ebb Flow Technique with intermittent aeration of the plant roots gave adequate aeration and minimal disturbance of the fungus, hence produced more spores and colonised roots which were important parts of the biofertilizer. The use of phosphorus in hydroponic nutrient solution could be reduced up to 50% in the production of arbuscular mycorrhiza biofertilizer, thus offering a more sustainable farming system that was respectful of the environment.

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