The use of mycorrhizae in production of clonal seedlings of superior teak, a prospective tree species for increasing agroforestry system productivity

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Abstract. Agroforestry system which consisted of Teak as the main tree component has been widely established in Java. Teak is selected because it produces luxurious wood. The demand of Teak wood increases continuously, therefore Superior Teak becomes fascinating. Seedlings of selected Superior Teaks can be obtained through clonal propagation. The growth of Teak Clonal seedlings can be improved further by application of mycorrhizal fungi which help roots to obtain essential nutrition and water. This study aimed to determine the relationship between root biomass and mycorrhizal fungal colonization with growth of the seedlings. This study used four selected Teak clones (WG1, WG2, F71, and D15). Shoot plantlets were planted on cocopeat: rice husk charcoal mixture (1:1 v/v) in polybags and inoculated with soil containing mycorrhizal spores. Growth parameters were measured fortnightly. Four months after mycorrhizal inoculum application, the plants were harvested and assessed for lateral root number and length and biomass dry weight. Sample of roots were stained with 0.05 % tryphan blue lactoglycerol and assessed for mycorrhizal infection. The results showed that survival and rooting rates varied among clones, ranging from 45-83% and 23-63%, respectively. In the nursery condition, growth of all clonal seedlings was strongly correlated with mycorrhizal colonization varied between positive and negative correlation. The correlation varied among clones, being the greatest at clone WG1, D15, F71 and the least at clone WG 2. The growth and productivity of the selected superior teak in the agroforestry system may be enhanced by application of suitable mycorrhizal inoculum.

Keywords: Superior teak clones, shoot cuttings, mycorrhizal colonization, plant growth, Agroforestry, nursery condition

1. Introduction

Agroforestry is a land-use system integrating agricultural and forestry plants in a particular spatial arrangement. Agroforestry offers opportunity to optimize land use in fulfilling timber and fodder demand [1] and alleviating environmental degradation [2] such as soil erosion, and increasing biodiversity. Agroforestry system has been established in many countries, such as in Indonesia [3], Nepal [4], and Cameroon [5]. In Maluku Island, Indonesia, it has been applied since 17 century [6]. Presently, in Java Island, especially in areas with monsoonal climate and the soil developed from limestone, agroforestry system which consisted of Teak and various plant crops has also been widely practiced.
Teak is a tropical and sub-tropical plant which is categorized as luxurious fancy wood due to its superior strength and durability. It is resistant to termites and fungal attack and can survive up to 500 years [7]. Due to the increasing demand of Teak wood, farmers improve Teak growth by planting selected Superior Teak. Clonal seedlings of this Teak have been developed by Faculty of Forestry UGM through a series of tree improvement programs and vegetative propagation particularly shoot cutting techniques. A study by [8] showed that growth of diameter and height were varied among clonal seedlings. In order to further improve the growth of Teak Clonal seedlings, a study which aimed to determine relationship between mycorrhizal colonization and seedlings growth was conducted.

Understanding this relationship, a strategy for improving Teak Clonal seedlings growth by application of mycorrhizal inoculum might be formulated. Root of Teak can form association with mycorrhizae [9][10] and the Teak growth might be benefited by this association. Mycorrhizae is a mutualistic association between fungi and roots of most terrestrial plants [11] which causes root morphology alteration and increases the absorption area of the root system [12]. Fungal hyphae can penetrate soil pores [13] thus improving the capacity to obtain nutrient from soil especially P. Mycorrhizae also enhances resistant to pathogens and abiotic stress [11].

2. Materials and methods

The experiment was conducted in December 2017. Four Teak clones were used (WG1, WG2, F71, and D15) in this experiment. Shoots (7 - 9 cm length, has minimum 2 nodes, and the bud is not opened yet) were harvested from Superior Teak hedge orchard in the Forest Research and Education (FRE) of Wanagama. The shoots were soaked in 50 mg/L solution of root stimulating hormone mixture (Indole-3-butyric acid, naphthyl acetic acid, indole acetic acid, thiram and filler) for 5 minutes and planted on rooting media which consisted of cocopeat and rice husk charcoal mixture (1:1 v/v) in polybags. Fifteen replication plantlets per Teak clone were established. The plantlets were then arranged at the spaces of 15 cm x 15 cm in a plastic covered compartment under a 70% shading net in the nursery of FRE Wanagama. One and a half month after planting, 20 gram soil inoculum containing 472 spores (mixture of Acaulospora and Glomus) per 100 gram were applied per polybag. Four months after inoculation, the plants were harvested. Observation of plant height, stem diameter, number of leaves and nodes were conducted fortnightly. After harvesting, observation was conducted to find out rooting success, root number and length, and shoot and root biomass.

Approximately 0.1 g root samples (fresh root) were taken and stained with tryphan blue lactoglycerol according to [14]. The roots were cut into 1 cm length and were arranged in a glass slide before analyzing using a dissecting microscope. Forty five pieces of roots per sample were analyzed. The root was regarded as mycorrhizal if hyphae, vesicles, and/or arbuscules were present.

3. Results and Discussion

Successful shoot cutting is indicated by formation of new leaves (known as survive shoot), followed by formation of adventitious root and further growth of leaves and stem. Survival and rooting rates of shoots (plantlets), therefore, are important processes in vegetative propagation. High survival rates, however, is not necessarily followed by high rooting rates because the shoot cuttings could survive by forming callus without forming root. Results of the experiment showed that survival and rooting rates varied among clones (Table 1) but in general, clone WG2 had the greatest survival rates, rooting rates, root growth and mycorrhizal colonization. Previous research by [15] also showed that clone WG2 had the greatest rooting rates which was 85%.
Table 1. Survival rates, rooting rates, root growth and mycorrhizal colonization of clonal seedlings of four superior teak clones in the nursery of FRE Wanagama at 5.5 months after planting

| Clones | Survival rates (%) | Rooting rates (%) | Number of lateral root | Length of lateral root (cm) | Root dry weight (gr) | Mycorrhizal colonization rates (%) |
|--------|-------------------|------------------|-----------------------|-----------------------------|---------------------|----------------------------------|
| WG1    | 53.33             | 43.75            | 2.0 b                 | 18.03 b                     | 0.64                | 81.11                            |
| WG2    | 83.33             | 76.00            | 4.8 a                 | 50.93 a                     | 1.64                | 83.33                            |
| F71    | 70.00             | 42.86            | 1.5 b                 | 26.03 b                     | 0.69                | 57.78                            |
| D15    | 43.33             | 76.92            | 3.8 ab                | 22.23 b                     | 0.53                | 72.78                            |

Note: Figures followed by different alphabets are significantly different

Clone WG2 also showed the greatest above ground growth (Table 2), while the growth of other clones were approximately half of the WG2. This data indicated that root growth performance will affect above ground growth. This is an accordance with the study by [16] showed that inoculated Teak with the greatest root growth also has the greatest above ground growth.

Table 2. Above ground growth of clonal seedlings of four superior teak clones in the nursery of FRE Wanagama at 5.5 months after planting

| Clones | Height (cm) | Diameter (cm) | Number of leaves | Number of nodes | Shoot dry weight (gr) |
|--------|-------------|---------------|------------------|----------------|-----------------------|
| WG1    | 6.95        | 0.079         | 1.96 b           | 1.93 b         | 2.42                  |
| WG2    | 11.96       | 0.15          | 4.06 a           | 4.4 a          | 5.05                  |
| F71    | 5.33        | 0.068         | 2.23 b           | 2.4 b          | 2.47                  |
| D15    | 6.21        | 0.07          | 2.05 b           | 2.38 b         | 1.97                  |

Note: Figures followed by different alphabets are significantly different

Correlation analysis to find out the relationship between root dry weight and above ground growth (Table 3) and between mycorrhizal colonization and above ground growth (Table 4) of four Superior Teak clones revealed that height and diameter growth of all clones (except diameter of clone D15) correlated strongly with root growth. In contrast, only growth of clone WG1 had strong positive correlation with mycorrhizal colonization (Table 4). This data indicated that root formation and growth is the more important factor determining growth of teak clonal seedlings.

Table 3. R value (correlation analysis) of relationship between root dry weight and above ground growth of four superior teak clones

| Clones | Height | Diameter | Number of leaves | Number of nodes |
|--------|--------|---------|-----------------|----------------|
| WG1    | 0.7385 | 0.9942  | -0.2122         | -0.0135        |
| WG2    | 0.8126 | 0.8104  | 0.7326          | 0.6581         |
| F71    | 0.9835 | 0.8218  | 0.6699          | 0.6212         |
| D15    | 0.9125 | -0.3337 | 0.7088          | 0.5649         |

Relationship between mycorrhizal colonization and above ground growth which varied from strong positive correlation, moderate negative correlation and only weakly correlated (Table 4) can be caused by various factors. [17] found that mycorrhizal colonization in acclimatization phase of two Teak clones varied depending on fungal inoculum and host genotypes. The Effect of fungi and host plant suitability on mycorrhizal colonization and its effect on plant growth have been reported before by many researchers (e.g. [18], [19]). Some researches showed that Acaulospora [20] and Glomus [21] [16] [22] were used for inoculation on teak. Plant growth and mycorrhizal efficiency index (MEI) of teak
seedlings inoculated with *Glomus proliferum* was higher than inoculated with *Funelliformis mosseae* and *Glomus intradices* [16]. Suitable fungal inoculum for a certain teak clone needs to be discovered.

### Table 4. R value (correlation analysis) of relationship between mycorrhizal colonization and above ground growth of four superior teak clones

| Clones | Height | Diameter | Number of leaves | Number of nodes |
|--------|--------|----------|-----------------|----------------|
| WG1    | 0.808  | 0.771    | 0.54054         | 0.56761        |
| WG2    | -0.0704| -0.15406 | -0.24025        | -0.29319       |
| F71    | -0.791 | -0.59914 | -0.2785         | -0.26          |
| D15    | -0.6082| 0.49989  | -0.7283         | -0.5518        |

Various correlation between mycorrhizal colonization and above ground growth can be caused by some factors which influence mycorrhizal inoculation. [23] revealed that there is range factors affecting in mycorrhizal inoculation in a micropropagation system. These factors were inoculation timing (*in vitro* and *ex vitro*), host-fungus interactions, physical and chemical composition of the growth substrate, and the condition of greenhouse and growth chamber.

### 4. Conclusion

The growth and productivity of Teak in agroforestry system may be enhanced by application of suitable mycorrhizal inoculum and inoculation timing. Further experiments are needed to clarify the suitable mycorrhizal inoculum for Superior Teak clones and also inoculation timing.

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