Composition of media that promotes seedling growth and root nodules of dog fruit (*Pithecellobium lobatum* Benth)

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**Abstract.** A study aimed to find out the best medium to promote plant growth and the presence of root nodules of dog fruit seedlings was conducted at Indonesian Tropical Fruit Research Institute (ITFRI) from September 2017 to April 2018. This research was arranged in a randomized block design with 10 treatments and 3 replications. The treatments were 10 compositions of media that consisted of a control medium (soil:cow manure = 1:1 v/v) and 9 other combinations of two or three materials, namely: soil, cow manure, compost, and rice husk charcoal with a ratio of 1:1 (v/v) without and with the addition of mycorrhizae. The results showed that the best media to promote dog fruit seedling growth was soil:rice husk charcoal 1:1 (v/v) and the highest number of root nodules was also found in this medium composition. There was no significant growth difference in dog fruit seedlings at 6 months after planting due to the addition of mycorrhizae. The result of this study can be considered in the selection of medium for better seedling growth of dog fruit.

1. Introduction

Dog fruit (*Pithecellobium lobatum* Benth, synonyms *P. jiringa*, *Archidendron pauciflorum*) is one of the popular tropical plants. The level of dog fruit consumption in Indonesia is quite high. The fruit that also known as Jengkol or Jering has many health benefits such as an anti-cancer substance [1], an alternative food supplement to reduce the risk of obesity [2] and its rind extract has to benefit for wound healing process [3].

Dog fruit is one of the commodities that will be developed in Indonesia, especially in West Sumatra. Generally, dog fruit is produced from forest plants that have not been seriously cultivated. However, given the high market demand for this commodity, several regions in West Sumatra province wish to become the centers of dog fruit production [4].

Fabaceae is well-known as a plant group that many of its members form a symbiosis with beneficial bacteria in their roots. The symbiosis between Rhizobia and plant roots causes the formation of new organs called nodules, where these micro symbionts convert atmospheric nitrogen into an available form to use by plants, namely Ammonia [5]. Dog fruit is also included in this legume family. However, no information was found about the root nodules of dog fruit.

Recently, the agricultural sector is directed towards sustainable agriculture. One of the efforts that can be done is by utilizing natural materials and symbiotic microbes to support crop production. The effect of mycorrhizae and symbiotic bacteria to enhance the growth of various plants has been widely reported [6–9]. Arbuscular mycorrhizal fungi have been known as plant symbionts which can improve nutrient uptake such as phosphorus and nitrogen and increase plant yield [9]. However, there is no information available about the media which are capable to support the development of these fungi.
symbioses and their effect on the growth of dog fruit seedlings. Therefore, we conducted research which aimed to find out the effect of several media compositions to promote seedling growth and root nodules of dog fruit.

2. Materials and methods

2.1. Materials
Dog fruit seeds used in this research have an appropriate level of age for cultivated and derived from the dog fruit tree in Limau Manis, Padang. The mycorrhizae formulation used in this study was produced by the Indonesian Tropical Fruits Research Institute, Solok, West Sumatra.

2.2. Methods
This research was conducted from September 2017 to May 2018 at Sumani Experimental Station, Indonesian Tropical Fruits Research Institute (ITFRI), Solok, West Sumatra. Nutrient contents of media were assessed in Chemical and Postharvest Laboratory, ITFRI.

This study used a Randomized Block Design with 10 treatments and 3 replications which each treatment unit consisted of 20 seedlings. The treatments were media compositions as follow:

1. Soil : Cow Manure = 1:1 (v/v) as control (medium composition that frequently used for nursery),
2. Soil : Compost = 1:1 (v/v),
3. Soil : Rice Husk Charcoal = 1:1 (v/v),
4. Soil : Cow Manure : Rice Husk Charcoal = 1:1:1 (v/v/v),
5. Soil : Compost : Rice Husk Charcoal = 1:1:1 (v/v/v),
6. Soil : Cow Manure = 1:1 (v/v) + Mycorrhizae,
7. Soil : Compost = 1:1 (v/v) + Mycorrhizae,
8. Soil : Rice Husk Charcoal = 1:1 (v/v) + Mycorrhizae,
9. Soil : Cow Manure : Rice Husk Charcoal = 1:1:1 (v/v/v) + Mycorrhizae,
10. Soil : Compost : Rice Husk Charcoal = 1:1:1 (v/v/v) + Mycorrhizae.

Mycorrhizae product containing Glomus sp. was formulated by ITFRI and applied 5 g per seedlings before the seeds were cultivated on media. Selected dog fruit seeds were soaked in water for ±3 hours and drained afterward. The seeds were sown on polybags with size 15 x 21 cm which containing media as previously described. Later, the seedlings were transferred into a bigger polybag containing the same media without the addition of mycorrhizae anymore so the application of mycorrhizae was only applied once.

2.3. The effect of media composition on plant growth and the number of root nodules of dog fruit seedlings
We randomly selected five seedlings per each treatment unit to assess the effect of media compositions on plant growth and the number of root nodules. We observe seedling height, stem diameter, number of branches, leaves, lateral roots and root nodules, above-ground plant parts dry weight, below-ground plant parts dry weight and total dry weight. Root nodules with size more than 1 mm were counted.

2.4. Data analysis
The data were analyzed using arithmetic and analysis of variance. Duncan New Multiple Range Test (DNMRT) at α 0.05 was carried out when we found a significant difference between treatments. To measure whether two variables observed have an association between them, Pearson’s correlation among observed variables was also provided.
3. Results and discussion

3.1. Characteristic of media
The characteristics and nutrient contents of the media used in this study were stated in Table 1. All media composition increased the media acidity (pH), N, P, K and Mg contents (Table 1). It means the addition of organic matters into soil enhances the nutrient contents of the media. In line with our findings, other study reports organic matters amendment also increase pH and available nutrients contents [10].

The best media for dog fruit seedling growth was soil : rice husk charcoal = 1:1 (v/v). This medium composition had a very low level of Calcium (Ca), a low level of Carbon (C) and Nitrogen (N), a medium level of Magnesium (Mg) and a very high level of Phosphor (P) and Potassium (K) contents. Media containing rice husk charcoal was also reported to increase nutrient and media porosity. In line with the results of this study, another study reported that the addition of rice husk charcoal increased pH, CEC, available Nitrogen, available Phosphor in the paddy field and also increased the growth of maize [11]. Besides, using rice husk charcoal in media has the advantage that the media has a light weight making it easier to be transported.

Table 1. Characteristics and nutrient contents of media used in this study.

| Media*          | Acidity | CEC** | C   | N       | P        | K      | Ca     | Mg     |
|-----------------|---------|-------|-----|---------|----------|--------|--------|--------|
| Soil            | very acidic | low   | high | very low | medium   | very low | very low | very low |
| 1 and 6         | acidic   | low   | medium | medium   | very high | very high | medium | high   |
| 2 and 7         | neutral  | low   | high  | medium   | very high | very high | high   | high   |
| 3 and 8         | acidic   | low   | low   | low      | very high | very high | very low | medium |
| 4 and 9         | acidic   | low   | medium | medium   | very high | very high | low    | high   |
| 5 and 10        | neutral  | low   | high  | medium   | very high | very high | medium | high   |

Notes: *1. Soil : cow manure = 1:1 (v/v) as control, 2. Soil : compost = 1:1 (v/v), 3. Soil : rice husk charcoal = 1:1 (v/v), 4. Soil : cow manure : rice husk charcoal = 1:1:1 (v/v/v), 5. Soil : compost : rice husk charcoal = 1:1:1 (v/v/v), 6. Soil : cow manure = 1:1 (v/v) + mycorrhizae, 7. Soil : compost = 1:1 (v/v) + mycorrhizae, 8. Soil : rice husk charcoal = 1:1 (v/v) + mycorrhizae, 9. Soil : cow manure : rice husk charcoal = 1:1:1 (v/v/v) + mycorrhizae, 10. Soil : compost : rice husk charcoal = 1:1:1 (v/v/v) + mycorrhizae. **Cation exchange capacity.

3.2. The effect of media composition on dog fruit seedling growth
ANOVA test showed that the treatments gave a significant effect on dog fruit seedling growth at six months after planting. The highest plant height, number of some branches and leaves were obtained from a medium composition containing soil : rice husk charcoal = 1:1 (v/v) with an average 83.24 cm height, 8.47 branches and 68.08 leaves (Table 2). The same medium composition with the addition of mycorrhizae produced seedlings with a lower number of leaves compared to the treatment without mycorrhizae whereas the plant height and number of branches were almost the same.
Table 2. Plant height, stem diameter, number of branches, number of leaves, percentage of live seedlings of dog fruit at 6 months after sowing in 10 treatment media.

| Treatments*       | Plant height (cm) | Stem diameter (mm) | Number of branches | Number of leaves | Percentage of live seedlings (%) |
|-------------------|-------------------|--------------------|--------------------|-----------------|----------------------------------|
| 1                 | 63.56 cd**        | 9.15 bc**          | 6.97 c*            | 51.90 cde*      | 100.00 ns*                       |
| 2                 | 56.29 e           | 9.36 b             | 6.83 c             | 50.27 de        | 98.89                            |
| 3                 | 83.24 a           | 9.37 b             | 8.47 a             | 68.08 a         | 97.78                            |
| 4                 | 64.42 c           | 9.16 bc            | 6.93 c             | 51.83 cde       | 100.00                           |
| 5                 | 57.46 e           | 9.28 b             | 7.00 bc            | 50.73 de        | 95.56                            |
| 6                 | 71.30 b           | 9.86 a             | 7.45 b             | 55.75 c         | 98.89                            |
| 7                 | 58.07 e           | 9.35 b             | 6.83 c             | 51.53 cde       | 100.00                           |
| 8                 | 80.40 a           | 9.09 bc            | 8.12 a             | 62.82 b         | 98.89                            |
| 9                 | 58.15 e           | 8.64 d             | 7.17 bc            | 53.17 cd        | 98.89                            |
| 10                | 59.31 de          | 8.73 cd            | 6.75 e             | 48.43 e         | 100.00                           |
| Without mycorrhizae | 64.99 ns**       | 9.26 ns**          | 7.24 ns            | 54.56 ns**      | 98.45 ns**                       |
| With mycorrhizae  | 65.44             | 9.13               | 7.26               | 54.34           | 99.33                            |

Notes: *1. Soil : cow manure = 1:1 (v/v) as control, 2. Soil : compost = 1:1 (v/v), 3. Soil : rice husk charcoal = 1:1 (v/v), 4. Soil : cow manure : rice husk charcoal = 1:1 (v/v/v), 5. Soil : compost : rice husk charcoal = 1:1 (v/v/v), 6. Soil : cow manure : rice husk charcoal : mycorrhizae = 1:1:1 (v/v/v), 7. Soil : compost : rice husk charcoal : mycorrhizae = 1:1:1 (v/v/v), 8. Soil : rice husk charcoal = 1:1 (v/v) + mycorrhizae, 9. Soil : cow manure : rice husk charcoal = 1:1 (v/v/v) + mycorrhizae, 10. Soil : compost : rice husk charcoal = 1:1:1 (v/v/v) + mycorrhizae. **Numbers followed by the same letters on the same column are not significantly different each other based on DNMRT α 5%. Numbers followed by ns mean the average numbers are not significantly different among treatments based on ANOVA test α 5%.

3.3. The effect of media composition on dog fruit dry weight and number of root nodules

Dry weight is a parameter that reflects the rate of nutrient uptake by plants where the better nutrient absorption, the higher the plant dry weight [6]. Based on the data in table 3, it can be seen that the best medium for dog fruit seedling growth was soil : rice husk charcoal = 1:1 (v/v). This treatment resulted in the highest total dry weight compared to other treatments, namely 43.26 g. The addition of rice husk charcoal was reported to increase the fresh weight of patchouli [12]. Despite much literature states that the addition of mycorrhizae can increase the growth of various plants [6,13], however this effect on dog fruit seedling had not been observed since the beginning of seedling grow until 6 months after planting. The effect of mycorrhizae on dog fruit seedlings probably will be seen for a longer time or this kind of mycorrhizae did not establish symbiosis with dog fruit seedlings.

Soil : rice husk charcoal = 1:1 (v/v/v) without or with the addition of mycorrhizae is the best medium for the growth of dog fruit root nodules. In line with other studies [7], the result of this research also confirmed that symbiotic bacteria on root nodules can support plant growth.
Table 3. The root length, total fresh weight, dry weight (below-ground part, above-ground part, total) and number of root nodules of dog fruit at 6 months after sowing in 10 treatment media.

| Treatments* | Total fresh weight (g) | Dry weight (g) | Number of root nodules*** |
|-------------|------------------------|----------------|--------------------------|
|             |                        | Below-ground part | Above-ground part | Total |
| 1           | 78.22 bc**             | 15.12 ns***     | 23.18 b**             | 38.30 ab** | 2.57 bcd** |
| 2           | 91.20 ab               | 15.52           | 22.95 b               | 38.46 ab   | 2.37 bcd   |
| 3           | 107.15 a              | 14.19           | 29.08 a               | 43.26 a    | 6.91 a     |
| 4           | 75.60 bc              | 11.37           | 20.99 b               | 32.36 b    | 1.69 ed    |
| 5           | 81.45 bc              | 15.69           | 19.77 b               | 35.46 ab   | 1.68 ed    |
| 6           | 69.65 c               | 11.81           | 20.87 b               | 32.68 b    | 3.37 b     |
| 7           | 93.32 ab              | 14.52           | 22.44 b               | 36.96 ab   | 1.92 bcd   |
| 8           | 77.33 bc              | 10.14           | 20.10 b               | 30.23 b    | 6.56 a     |
| 9           | 86.58 bc              | 15.60           | 22.82 b               | 38.41 ab   | 3.05 bc    |
| 10          | 84.61 bc              | 13.22           | 23.31 b               | 36.96 ab   | 1.14 d     |
| Without mycorrhizae | 86.72 ns** | 14.38 ns**     | 23.19 ns**            | 37.57 ns** | 3.04 ns**  |
| With mycorrhizae   | 82.30             | 13.06           | 21.91                 | 34.96      | 3.21       |

Notes: *1. Soil : cow manure = 1:1 (v/v) as control, 2. Soil : compost = 1:1 (v/v), 3. Soil : rice husk charcoal = 1:1 (v/v), 4. Soil : cow manure : rice husk charcoal = 1:1 (v/v/v), 5. Soil : compost : rice husk charcoal = 1:1 (v/v/v), 6. Soil : cow manure = 1:1 (v/v) + mycorrhizae, 7. Soil : compost = 1:1 (v/v) + mycorrhizae, 8. Soil : rice husk charcoal = 1:1 (v/v) + mycorrhizae, 9. Soil : cow manure : rice husk charcoal = 1:1 (v/v/v) + mycorrhizae, 10. Soil : compost : rice husk charcoal = 1:1 (v/v/v) + mycorrhizae. **Numbers followed by the same letters on the same column are not significantly different each other based on DNMRT α 5%. ***Numbers followed by ns mean the average numbers are not significantly different among treatments based on ANOVA test α 5%. These data were transformed with \( \sqrt{x+1} \).

3.4. Correlation between the number of root nodules and plant growth parameters

Based on our observation, most dog fruit seedlings with a good grow performance had many root nodules on their roots whereas the poor grow seedling rarely had them. Sometimes, the root nodules existed at poor grow seedlings but the number of root nodules was low (figure 1). Therefore, we performed Pearson’s correlation to check the relation between the number of root nodules and other growth parameters. The results as stated in table 4.

The root nodules of dog fruit were found at primary and lateral roots, they distributed singly or in clusters as reported for its relative, *P. dulce* (Roxb.) Benth [14]. The shapes of dog fruit root nodules were irregular, like small capsules or sometimes found in clusters with coralloid forms (figure 1d). However, we did not identify the species of bacteria that formed a symbiosis with dog fruit roots in this study.
Dog fruit root nodules significantly positively correlated with seedling height, above-ground dry weight and total dry weight of dog fruit. It means if the number of root nodules increase, the dog fruit seedling height, above-ground dry weight and total dry weight also increase. The number of lateral roots was also significantly positively correlated with stem diameter, above-ground dry weight and total dry weight of dog fruit seedlings (table 4).

### Table 4. Correlation values between growth parameters of dog fruit seedlings.

|                      | Height       | Stem diameter | Σ branches | Σ leaves | Σ lateral roots | Σ root nodules | Below-ground dry weight | Above-ground dry weight | Total dry weight |
|----------------------|--------------|---------------|------------|----------|----------------|-------------------|-------------------------|-------------------------|------------------|
| Height               | 1            |               |            |          |                |                   |                         |                         |                  |
| Stem diameter        | 0.843**      | 1             |            |          |                |                   |                         |                         |                  |
| Σ branches           | 0.116        | 0.381         | 1          |          |                |                   |                         |                         |                  |
| Σ leaves             | 0.339        | 0.601         | 0.820**    | 1        |                |                   |                         |                         |                  |
| Σ lateral roots      | 0.622        | 0.712**       | -0.023     | 0.399    | 1              |                   |                         |                         |                  |
| Σ root nodules       | 0.655**      | 0.603         | -0.165     | 0.188    | 0.648          | 1                 |                         |                         |                  |
| Below-ground dry weight | 0.704*     | 0.655*        | 0.004      | 0.105    | 0.364          | 0.594             | 1                       |                         |                  |
| Above-ground dry weight | 0.775**    | 0.909**       | 0.390      | 0.744*   | 0.819**        | 0.668*            | 0.472                   | 1                       |                  |
| Total dry weight     | 0.864**      | 0.937**       | 0.278      | 0.574    | 0.743*         | 0.739*            | 0.781**                 | 0.919**                 | 1                |

Notes: * Numbers followed by one asterisk sign means the correlation coefficients were significantly different from zero based on Pearson’s correlation with α 5%.

** Numbers followed by two asterisk signs means the correlation coefficients were significantly different from zero based on Pearson’s correlation with α 1%.

### 4. Conclusions

Based on the results of this study, the following conclusions can be drawn. The best medium for dog fruit seedling growth until six months after planting was soil : rice husk charcoal = 1 : 1 (v/v). The highest number of dog fruit root nodules were produced on media containing soil:rice husk charcoal = 1:1 (v/v) without or with the addition of mycorrhizae. No significant effect of mycorrhizae to enhance dog fruit seedling growth at six months after planting and there was a significant positive correlation between some root nodules and growth parameters of dog fruit seedlings, namely plant height, above-
ground part and total dry weight. The results of this study can be used as reference for the farmer to select media that be able to promote dog fruit seedling growth at the nursery stage.

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