Morphology and isozyme band-profile as sexual determinant of nutmeg plant (*Myristica fragrans* Houtt).

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**Abstract.** Sex information in nutmeg plant is important to distinguish between male and female species. The recommendation of optimum sex-ratio for a nutmeg plantation is 1:10. This research is aimed to discover the morphological characteristic of nutmeg plant and the difference of leaf pattern as sexual determinant between male and female plant with the comparison of isozyme analysis. The result of morphological identification provided from 3 research farm location indicated that female nutmeg was higher, with longer and wider leaf, more vein, bigger diameter of stem and branching angle as compared to male nutmeg. Two parameters which were width of leaf and branching angle showed significant differences, where female nutmeg plants have wider leaves and branching angle. Isozyme analysis suggested that the analysis of peroxidase enzyme (PER) was better and able to provide more information than aspartate amino transferase (AAT) and acid phosphatase (ACP) Enzymes.

1. **Introduction**

Based on the inflorescence, nutmeg plants can be characterized as dioecious, where male and female flowers are located on different trees. In the field, male and female flowers are sometimes also found in the same tree, and therefore there are three types of nutmeg: male, female, and hermaphrodite nutmeg. For the plantation, the information of nutmeg sexuality is important, because the recommended sex-ratio minimum for a nutmeg plantation is 1:10 (male : female). Therefore, it’s required to conduct test to distinguish nutmeg plant sexuality through leaf morphological identification and isozyme analysis.

The aims of this research was to discover nutmeg plant morphological characteristic and isozyme analysis as determinant feature to distinguish male and female nutmeg. The desired target was obtaining nutmeg plant morphological characteristic and that isozyme becomes the sexual determinant of nutmeg plant in the field.

2. **Methods**

Nutmeg plant morphological identification was conducted in 3 (three) nutmeg farm locations which were Kebun Percobaan Balitro located in Cimanggu, Bogor and Cicurug, Sukabumi, and also nutmeg farmer field in Sukamantri area Tamansari sub-district Bogor. This research was conducted by survey method. Sample selection was conducted by purposive sampling. The number of observed sample was
20 female nutmeg plants and 10 male nutmeg plants from each research farm location. The observation was conducted by choosing 10 old and normal leaves that randomly chosen from each male and female nutmeg plant sample in 3 (three) research farm locations. The observed parameters including: length of leaf, wide of leaf, number of veins, length of branch, height of tree measured from the ground to the canopy, diameter of stem on 50 cm height from the ground, and branching angle measured 0° from tree stands. Observation result data were analyzed by Least Significance Difference test.

Isozyme analysis was conducted in PAU Laboratory of Bogor Agricultural University. Samples were provide from young and fresh leaves (buds) of male and female nutmeg plants from the 3 research farm locations. The enzymes analyzed were Peroxidase enzyme (PER), Aspartate Amino Transferase (AAT) and Acid Phosphatase (ACP).

3. Results and Discussions

A. Nutmeg Plant Morphological Identification

Morphological analysis resulted that there were some morphological characteristics that can be used to distinguish the feature of male, female and hermaphrodite nutmeg plants (Figure 1). The Figure 1 shows that female nutmeg was taller, with bigger diameter of stem, longer and wider leaves, had more veins, and had wider branching angle compared to the male ones. This distinction of morphological character was consistent among 3 research farm locations, even though the size of the plants were different (Figure 1). Based on logistic regression analysis by Stepwise method, prediction accuracy level was 85.4% for female and 83.9% for male respectively. It means that the data confidently can be used as a parameter to determine nutmeg plant sex. This result is in accordance to the research carried out by Marzuki et al. (2006), that male nutmeg was featured by smaller habitus, shorter branches, and smaller leaves.
Figure 1. Male, Female, and Hermaphrodite Nutmeg Plant Morphological Characteristics.

Based on the observation, there was significant differences ($\alpha$, 5\%) on width of leaf and branching angle parameters, where the female nutmeg plants had wider leaves and wider branching angle as compared to the male plants (Table 1).
Table 1. Nutmeg Plant Morphological Identification Result

| SEXUALITY  | LENGTH OF LEAF (cm) | LEAF WIDE (cm) | NUMBER OF VEINS (piece) | LENGTH OF BRANCH (cm) | TREE HEIGHT (m) | DIAMETER OF STEM (cm) | BRANCHING ANGLE (°) |
|------------|---------------------|----------------|------------------------|-----------------------|-----------------|-----------------------|-------------------|
| Male       | 11.00 A             | 4.05 A         | 17.98 A                | 1.05 A                | 9.31 A          | 73.08 A               | 47.96 A           |
| Female     | 11.66 A             | 4.89 B         | 18.14 A                | 1.00 A                | 10.32 A         | 75.85 A               | 62.30 B           |
| Hermaphrodite | 11.52 A             | 4.74 AB        | 18.77 A                | 1.20 B                | 10.18 A         | 74.45 A               | 57.83 B           |

Note: t-student analysis (α 5%),

According to Randriani et al. (2007), the characteristic of leaf wide can be used as a simple selection method, because leaf wide positively correlated with nut and mace weight. The results also showed wider female nutmeg branching angle, this is associated to the result provided by Rostiana et al. (2013) that female nutmeg plant branching angle tended to be wider and can be used as sexual determinant between male and female. Similar opinion was also suggested by Wahid (2012) and Plantation General Directorate (1995), that male nutmeg plants have pointing branching angle (acute) while female nutmeg plants have more horizontal/obtuse branching angle.

B. Isozyme Analysis

Isozyme analysis was carried out for 3 different enzymes which were Peroxidase enzyme (PER), Aspartate Amino Transferase (AAT) and Acid Phosphatase (ACP). The result unfortunately did not provide a clear and distinctive feature of sex differentiation of nutmeg plant. Meanwhile, isozyme analysis using PER enzyme relatively provided more information as compared to AAT enzyme and ACP enzyme (data not shown).

Isozyme analysis using PER enzyme showed the number of patterns appeared varied from one to another. Based on dendogram from cluster analysis that was used to discover the similarities of tested varieties, we found three groups obtained with different varies patterns (Figure 2). Sample with the code of 1,2,3,4,5,6,7,10,11,12, and 18 were in the similar level (we call Group 1). Sample with code 8,9, and 13 were in the similar level (Group 2) while sample code 14,15,16,17,19, and 20 were in the similar level (Group 3).

Figure 2. Dendogram calculated from analysis of PER Enzyme
4. Conclusion

The analysis of nutmeg morphology consistently provide sex determination. Female nutmeg typically tended to have higher canopy, bigger diameter of stem, with longer and wider leaf, have more vein and bigger branching angle as compared to male nutmeg. Two parameters which were wide of leaf and branching angle showed significant different. Isozyme analysis suggested that the analysis of peroxidase enzyme (PER) was better and able to provide more information than aspartate amino transferase (AAT) and acid phosphatase (ACP) Enzymes

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