Correlation between morphological characters and the sex phenotypes of *Myristica fragrans* Houtt Trees

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Abstract. Nutmeg is a dioecious plant where male and female flowers are generally located on different trees. Identification of early tree sex phenotypes in the vegetative phase will help grower optimize land productivity. This aim of this study was to identify morphological characters that are associated with sex expression. In this research 188 accessions numbers of nutmeg were used as genetic material consisting of 104 female and 84 of male trees. The morphological characters observed were habitus, leaves, flowers, fruit, seeds, and mace. The relationship between sex expression and morphological characters was tested by the individual proportion on each character and Pearson correlation. The results showed that morphological characters could not predict sex expressions. Male tree habitus and corolla size were generally bigger than female but female pedicellus size was bigger than male ones. There were no growth and yield component characters correlated to their yield.

Keywords: Dioecious plant, spice, Myristica sp., flower expression, sex phenotypes.

1. Introduction

As the center of origin of nutmeg (*Myristica fragrans* Houtt.), Indonesia must take a key role in the management, development and utilization of this species. Phenotypic character identification must be elucidated for the wider purposes of germplasm use to support nutmeg breeding program. Therefore, identification and characterization of many important and valuable phenotypes of nutmeg germplasm should be the major undertaking for researchers working with this crop.

Nutmeg identification and characterization can be done either based on morphological and agronomic characters or using isoenzyme and molecular markers. Observations on morphological and agronomic characters must use the applicable, systematic and adopted methods for each plant species. The observed morphological and agronomic characters could include plant statue (habitus), roots, stems, leaves, flowers, fruit and seeds.

Nutmeg (*Myristica fragrans* Houtt.) belongs to the Myristicaceae family. According to Flach [1], based on the sex phenotype expression of the tree - the Myristicaceae can be divided into two groups namely dioecious and monoecious trees. The dioecious trees consist of trees having either only male flowers (androecious) or female flowers (gynoecious). On the other hand, the monoecious trees have both male and female flowers in a single Myristicaceae tree.
Flach [1] showed that there were only four groups of trees based on their sex expression, namely unisexual males, bisexual males, pure bisexuals and bisexual females, based on the number of predominance flower types. The percentage of gynoecious and androecious trees formed from seeds is almost the same. Janse (1898) in [1] said that of 95 nutmeg trees planted from seeds, 60 gynoecious trees were obtained and the rest were androecious ones. The statement was also supported by Guenther (1960) in [1] which stated that in Grenada about 50% of trees grew into androecious trees if using seed for planting materials.

The results of Flach's research in 1966 [1], also showed that of the 93 trees planted, 46 gynoecious trees were formed and the remaining 47 trees were androecious. Deinum (1949) in [1] stated that according to an Agricultural Extension Officer in West Irian, planting from seed would produce 55% unisexual (gynoecious) female flowering trees, 40% unisexual (androecious) male flowering trees and 5% bisexual flowering trees.

Identification of *M. fragrans* morphological characters were carried out on several nutmeg populations in the Banda Islands, Seram Island and Ambon Island [2], North Maluku [3], Ambon Island [4], Siau Island [5], Sangihe Islands and Sitaro, Central Maluku District [6], Maluku [7] and Papua [8]. Besides being used to identify accessions and observe genetic relationships, morphological characters can also be used to identify the sex expression of trees in dioecious plants [9].

Identification of the sex expression of nutmeg trees using the structure of flowers and fruit is time consuming, since it requires about 5-7 years to flower. Since that period is quite long, it will be costly to keep the androecious plants in the field. The sooner the identification of nutmeg sex expression, the better it is to optimize land productivity. Therefore, early selection of nutmeg sex expression using vegetative morphological characters is needed for maintenance efficiency and land use costs.

In papaya plants [10] black and brown papaya seed colors can be used to predict female and hermaphrodite plants. To predict females and males in zalacca can use belt on seed, “belted seed” will produce female plants and otherwise will produce male plants. Morphological characters has been used to predict sex expression of nutmeg populations of Banda Island [11], North Maluku Province [3], and several nutmeg individuals at Cicurug research station [12].

2. Materials and methods

The study was conducted at the Cicurug research station (550 m asl), Sukabumi, from January 2015 to January 2018 using 26-year-old nutmeg trees. The genetic material used was 188 nutmeg accessions consisting of 104 gynoecious trees and 84 androecious trees. The sex expression of the nutmeg tree is determined based on the dominance of the existing flower types [1]. A tree dominated by female flowers is called a female tree. If the male flower is dominant. The tree is called a male tree. Female flowers are flowers having only pistils (female genitalia) while male flowers are one having stamens (male genitalia).

Plant morphological characterization refers to modified Tropical Fruits Descriptors ([13]; [11]; [14]; [15]). Observations were made on all parts of the plant (habitus, leaves, flowers, fruit, seeds, and mace) in the vegetative and generative growth phases. Habitus data were obtained from each tree, leaves from 10 mature leaf samples on each tree, flowers from 10 bloomed flowers, fruit, seeds and mace from 10 fruit on each tree that had a same level of maturity and uniform in size.

The relationship between morphological characters and sex expressions is shown in the form of a bar chart of the observe frequency of the evaluated characters in male and female tree populations. The observed morphological characters were divided into two groups, namely qualitative and quantitative.

The frequency of qualitative characters is shown based on the criteria of the intended characters, whereas the quantitative characters are grouped based on the quartile values of either 1, 2 or 3. The association between sex expression and morphological characters was tested by the individual proportion on each character and Pearson correlation analysis. The correlation analysis was performed using MINITAB version 17 software.
3. Results and Discussion

Estimation of sex expression using vegetative morphological characters is carried out to identify useful characters for early selection. If the sex expression of nutmeg trees can be predicted at the vegetative growth phase, nutmeg farmers can increase their land productivity because the planting of male trees can be minimized. Based on the qualitative morphological characters in the vegetative phase (Figure 1), there are no characters that can distinguish the sex expression (male or female) in nutmeg tree. The percentage of female and male trees having certain observed morphological characters was almost the same. There are no specific dominant characters which are associated with the male or female nutmeg trees. Das et al. [3] research showed that habitus of female tree is dominantly pyramid, while the male tree is semi-pyramid.

When using quantitative characters of the vegetative and generative growth phases, the width of the canopy and the circumference of the stem (Figure 2), the length of the corolla and the diameter of the corolla (Figure 3) on female trees tend to be smaller than in male trees. The length of the pedicels and the diameter of corolla on female trees tend to be larger than in male trees (Figure 3). Male and female tree leaves show no differences in size (length, width and thickness), as well as the length of the petiole (Figure 4). The results of this study are in contrast to the results of the Marzuki et al. [2] study which states that male trees are characterized by smaller habitus than females, straighter branches, and smaller leaves. The difference between male and female flowers, male flowers grow straighter on the fruit branches, with the smaller size of the flower stalks, and flower sizes, and thinner flower shape than female flowers. Leaf morphology for male trees were generally smaller with oval or elliptical shapes, tapered leaf base and tip. Despite these tendencies, these characters cannot yet be used as a method for predicting the sex expression phenotype of a nutmeg tree because the character's expression appears when the tree entered the maturity stage. Therefore, they are less useful for early selection.

Because the leaf character could be evaluated as early as at the seedling stage. Therefore, leaf characters can be used as characters for early selection for sex expression phenotypes of the nutmeg
trees. However, results of this study showed leaf characters (length, width and thickness) and leaf stalks did not show a positive correlation to the specific sex expression phenotypes and did not show positive correlation to other characters associated with sex expression either. Results of this study confirm those of Rostiana and Heryanto [16].

The width of the canopy is positively and significantly correlated with the diameter of the flower stalk. Therefore, the canopy width may be used as the early indication for selecting certain sex expression phenotype.

**Figure 2.** Frequency of plant canopy width, and stem circumference among populations of different sex phenotypes (male or female) of *Myristica fragrans* Houtt. trees at Cicurug research station. (■) – male trees and (□) – female trees

**Figure 3.** Frequency of corolla length, corolla diameter, pedicel length and pedicel diameters at generative growth stage among populations of different sex phenotypes (male or female) of *Myristica fragrans* Houtt. trees at Cicurug research station. (■) – male trees and (□) – female trees
Figure 4. Ranges of leaf width, leaf length, leaf diameter and petiole length among populations of different sex phenotypes (male or female) of *Myristica fragrans* Houtt. trees at Cicurug research station. (■) – male trees and (□) – female trees

Table 1. Correlation between morphological characters on *Myristica fragrans* Houtt. At Cicurug research station

| Characters | CW  | SC  | LL  | LW  | LT  | PL  | CL  | PeL | CD  | PeD |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| CW         | -   | 0.53|-     |     |     |     |     |     |     |     |
| SC         | 0.04| -0.16|     |     |     |     |     |     |     |     |
| LL         | 0.14| -0.02| 0.52|     |     |     |     |     |     |     |
| LW         | -0.03| 0.12| -0.05| 0.25|     |     |     |     |     |     |
| LT         | 0.11| 0.02| 0.09| 0.18| 0.15|     |     |     |     |     |
| PL         | 0.12| 0.13| 0.03| 0.02| -0.03| 0.08|     |     |     |     |
| CL         | -0.03| -0.04| 0.10| 0.06| -0.02| 0.04| 0.01|     |     |     |
| PeL        | 0.06| 0.13| -0.01| 0.09| 0.05| 0.10| 0.54| 0.19|     |     |
| CD         | 0.15| 0.08| -0.04| 0.06| -0.04| 0.06| 0.14| 0.24| 0.07|     |

Noted: Bold number: significant at 5% with Pearson correlation

In addition to endogenous factors, flower appearance and expression of plant sex phenotypes is also influenced by either exogenous or environmental factors. The sex expression of a tree may be affected by genes that regulate sex expression phenotypes located in certain chromosomes. Sex expression phenotypes are strongly influenced by hormone concentration, transport and sensitivity, while hormonal activity is strongly influenced by environmental conditions including light quality, photoperiodicity, nutrition and temperature [17] [12]. Das et al. [3] states that the pattern of acquired segregation ratio, the nature of nutmeg sex expression may be controlled by two genes that are fully dominant at both loci, or the presence of duplicative interactions of genes controlling the nature of nutmeg sex expression phenotypes.
4. Conclusions
Most of the morphological characters cannot be used to predict the expression of sex phenotypes in nutmeg. However, there is a correlation between the tree size to the sex phenotype since generally the male nutmeg trees are bigger than female ones, while the opposite is shown in the character of flower sizes.

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