Multivariate analysis of *Nypa fruticans* from North Sulawesi based on morphological characters

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Abstract. Nypa grows mainly on the river banks. A number of nypa germplasm accessions were collected from three locations, namely North Minahasa (Wori district), South Minahasa (Paslaten District), and Southeast Minahasa (Tumbak District). The study aimed to determine the morphological differences amongst the collected germplasms and their correlation using multivariate analysis. The research used RCBD with 3 replications using five individual plants. The results showed that characters that have a high correlation for fruit weight are the fruit polar circumference (0.97), the fruit equatorial circumference (0.98), and the rachis length (0.84). An increase in one unit of these characters will increase the fruit weight. The average linkage method and heatmap with a cut-off point of 0.38 divided the accessions into two groups; the first group were Tumbak 1, Tumbak 2, Tumbak 3, Tumbak 4, and Tumbak 5, and the second group were Wori 1, Wori 2, Wori 3, Wori 4, Wori 5, Paslaten 1, Paslaten 2, Paslaten 3, Paslaten 4, and Paslaten 5. Clustergram heatmap showed three groups with a high average of fruit polar circumference and weight per fruit, namely Paslaten 1, Paslaten 3, and Paslaten 5. The multivariate analysis clusters the accessions into groups with similar morphology.

Keywords: correlation, dendrogram, heatmap, quantitative traits

1. Introduction

Nypa is a plant from the Palma family that lives in swamps or riverbanks. Nypa palm is a plant that often acquires mangrove plants so that the mangrove plant population on the banks of the river becomes extinct. Nypa has fibrous roots so it is susceptible to lodging. Nypa palm is a cross-pollinated plant with male and female flowers different from the flower stalk, which causes high diversity. The Minahasa is an area that has a wide river flow so that many nypa plants grow well on the banks of the river. Nypa grows in colonies with the roots submerged in water regularly. The nypa palm population in one hectare is 106 trees with 913 stems. Leaf density is 336 leaves/ha [1]. In industry, the bioethanol generated from nypa palm has low CO\textsubscript{2} emissions [2].

Nypa palm spread widely in North Sulawesi Province, especially in the Minahasa area. The Minahasa region has many rivers, namely North Minahasa, South Minahasa, and Southeast Minahasa. The three locations have agroecological differences that will determine differences in the genetic diversity of nypa accessions. Diversity of agronomic performance is very important for breeding. In addition, with genetic diversity, selection can be made. Nypa is a cross-pollinated plant so that individual performance is different from one another hence a higher genetic diversity. A study [3] stated that genetic diversity can be estimated by the coefficient of genetic diversity tested through
quantitative characters. According to [4], the greatest influence on seed diversity is of genetics and little influence from the environment. Germplasm abundance can be done by grouping individuals in accessions (intra accessions) and between accessions (inter accessions).

Grouping to determine differences in each accession was explained by multivariate analysis. Multivariate analysis consisted of group analysis, correlation analysis, regression analysis, principal component analysis, and path analysis. Multivariate analysis is very useful for characterization and the study on the genetic diversity of germplasm [5]. Correlation analysis has been widely used, one of which is on sorghum plants to determine the characters that affect seed yield per plant [6]. Cluster analysis helps in grouping varieties into the same group [7]. Cluster analysis indicated that 70% of genetically similar groups were close to each other [8]. Cluster analysis helps classify oil palm accessions from 54 accessions [9]. The purpose of this research was to determine the differences in the morphology of nypa palm from the three locations.

2. Materials and methods
The study was conducted in North Sulawesi in three locations: North Minahasa in the Wori area, South Minahasa in the Paslaten area, and Southeast Minahasa in the Tumbak area (Figure 1). The three locations have different agroecosystems. The genetic materials used were Wori accession, Paslaten accession, and Tumbak accession. Each accession was observed for 5 trees and each tree was observed for vegetative and generative characters. Observations of vegetative characters include:

1. Petiole length (cm), measured from the length of the petiole attached to the stem until the first leaf appears
2. The length of the rachis (cm), the part of the petiole is measured from the beginning of the leaf appearance to the end point of the petiole

Other generative character observations:
1. Polar circumference of fruit, measured in a circle from the tip to the base of the fruit
2. Equatorial circumference of fruit, measure in the middle of the fruit as long as it's circular
3. Weight per fruit, weighing from each fruit with digital measurement
4. Count fruit bunch\(^1\), counting the sum of fruits in one bunch

The data obtained were then processed using analysis of variance. If there is a significant difference between treatments, the Duncan Multiple Range Test (DMRT) is carried out. Data analysis includes visualization of the difference in the mean value and circular barplot, the correlation between characters, and grouping of germplasm accessions and characters using principal component analysis with the package. Software used to process data and visualize data was R. 4.0.5.

Figure 1. Distribution of nypa population in North Sulawesi (source: www.kemendagri.go.id).
3. Results and Discussion

3.1. Petiole length, length of rachis, polar circumference of fruit

The three accessions tested showed no significant difference in the petiole length character. The length of rachis character showed that the accession of Paslaten 1 was significantly higher than Tumbak 1, Tumbak 2, Tumbak 3, Tumbak 4, and Tumbak 5. Accessions Paslaten 1, Paslaten 3, Paslaten 4 and Paslaten 5 showed significantly different lengths of polar circumference of fruit compared to accessions Wori 1, Wori 5, Tumbak 1, Tumbak 2, Tumbak 3, Tumbak 4, and Tumbak 5 (Table 1).

Table 1. Mean petiole length, length of rachis, polar circumference of fruit, equatorial circumference of fruit, weight per fruit, and number of fruits per bunch.

| Accession | PTL | RCH | FP  | FE  | WF  | NF  |
|-----------|-----|-----|-----|-----|-----|-----|
| Wori 1    | 214.33a | 619.67abc | 25.60c | 20.33abc | 122.30d | 87.67ab |
| Wori 2    | 191.33a | 609.67abc | 27.56ab | 19.43abcde | 124.30d | 59.00bc |
| Wori 3    | 164.67a | 544.67abc | 26.57abc | 20.30abc | 134.67cd | 94.33a |
| Wori 4    | 220.67a | 579.00abc | 27.17abc | 20.13abcd | 137.93bcd | 54.33c |
| Wori 5    | 205.00a | 533.33abc | 26.00bc | 20.67abc | 136.60bcd | 81.00abc |
| Tumbak 1  | 242.67a | 513.33bc | 20.43d | 17.17cdef | 67.0e | 75.33abc |
| Tumbak 2  | 148.67a | 517.33bc | 20.67d | 14.93f | 58.90e | 87.33ab |
| Tumbak 3  | 128.67a | 431.67c | 21.07d | 15.60ef | 59.10e | 78.67abc |
| Tumbak 4  | 240.00a | 531.67bc | 22.13c | 17.00bcdef | 80.33e | 68.00abc |
| Tumbak 5  | 204.00a | 532.67bc | 20.83d | 15.60ef | 59.10e | 78.67abc |
| Paslaten 1| 270.00a | 801.67a | 28.23a | 22.00abdef | 173.70a | 98.00a |
| Paslaten 2| 250.00a | 730.00ab | 27.93ab | 20.76abc | 155.03abc | 88.67ab |
| Paslaten 3| 251.33a | 684.33abc | 27.93a | 22.50a | 165.90a | 90.3ab |
| Paslaten 4| 233.67a | 747.33ab | 28.03a | 22.00ab | 157.00ab | 66.33abc |
| Paslaten 5| 197.33a | 711.33ab | 27.93a | 23.20a | 170.73a | 67.67abc |

Note: Means in the same column followed by the same letter are not significantly different based on DMRT at 5% level, PTL=Petiole length, RCH= length of rachis, FP=polar circumference of fruit, FE=Equatorial circumference of Fruit, WF=Weight per fruit, NF=Number of fruits per bunch.

3.2. Equatorial circumference of fruit, weight per fruit, number of fruits per bunch

Accession Paslaten 3 and Accession Paslaten 5 showed a significantly longer equatorial circumference of fruit than accessions Tumbak 1, Tumbak 2, Tumbak 3, Tumbak 4, and Tumbak 5. Accession Paslaten 1 with Paslaten 2, Paslaten 3, Paslaten 4, and Paslaten 5 had significantly higher weight per fruit than Wori and Tumbak. Paslaten Accession and Tumbak Accession were not significantly different in the number of fruits per bunch, but Paslaten 1 and Wori 3 were significantly higher than other accessions in the number of fruits per bunch (Table 1).

3.3. Agronomic Characters Correlation

The agronomic characters tested showed a strong correlation between weight per fruit and polar circumference of fruit (0.97), the length of rachis (0.84), equatorial circumference of fruit (0.98), and petiole length (0.49). The strong correlation between characters shows a relationship between characters. The higher the correlation coefficient value, the more influence it will have on other characters. High correlation values with other characters can be used as a simultaneous selection with high yielding characters [10] (Figure 2). Correlations between genotypes that affect fruit indicate a true relationship; thus the environmental influences are ruled out [11]. Fruit weight on millet plants has a significant correlation to yield [12].
Figure 2. Correlation of the agronomic characters of the three nypa palm accessions tested.

3.4 Dendrogram of nypa accessions

The results of the average linkage dendrogram analysis with a cut point of 0.38 indicate that there are 2 groups. The first group consisted of Tumbak 1, Tumbak 2, Tumbak 3, Tumbak 4, and Tumbak 5 while the second group consisted of Wori 1, Wori 2, Wori 3, Wori 4, Wori 5, Paslaten 1, Paslaten 2, Paslaten 3, Paslaten 4, and Paslaten 5. Figure 3 shows that the accession to Tumbak tree numbers and the accession to Wori and Paslaten tree numbers are different. The dissimilarity of the Tumbak accession tree numbers with the Paslaten accession tree numbers was above 0.50 except for the Tumbak 4 with the Paslaten 2 (0.47), and the Tumbak 4 with the Paslaten 4 (0.48). This shows that the accession of Paslataen with Tumbak is different, but compared with Wori accession the agronomic characters are considered the same because the coefficient of dissimilarity is below 0.50 (Table 2). A study [13] demonstrated the dendrogram obtained from cluster analysis divided 36 tomato genotypes into 6 different groups. In addition, [14] showed that cluster analysis succeeded in classifying 60 maize lines based on their agromorphological characteristics.

Figure 3. Dendrogram of the three tested nypa accessions.
3.5. Heatmap clustergram analysis

Analysis of clustergrams is a method to unite two types of dendrograms into the same dimension [15]. Heatmap is used to simplify the visualization and explain the relationship between clusters [16]. Clustergram analysis shows that three groups have a high average of polar circumference of fruit and weight per fruit, namely Paslaten 1, Paslaten 3, and Paslaten 5 (Figure 4).

![Figure 4: Clustergram heatmap analysis of 15 accession numbers based on PTL=Petiole length, RCH=Length of rachis, FP=Polar circumference of fruit, FE=Equatorial circumference of Fruit, WF=Weight per fruit, NF=Number of fruits bunch](image-url)
4. Conclusion
Characters that have a high correlation with fruit weight are fruit polar circumference (0.97), fruit equatorial circumference (0.98), and rachis length (0.84). An increase of fruit polar circumference, fruit equatorial circumference, and rachis length will increase the fruit weight. The average linkage method with a cut point of 0.38 dividing the accessions into 2 groups, namely group 1 (Tumbak 1, Tumbak 2, Tumbak 3, Tumbak 4, and Tumbak 5) and group 2 (Wori 1, Wori 2, Wori 3, Wori 4, Wori 5, Paslaten 1, Paslaten 2, Paslaten 3, Paslaten 4, and Paslaten 5). Clustergram heatmap divided the accessions into three accessions that have a high average of Polar Circumference of Fruit and Weight per fruit, namely Paslaten 1, Paslaten 3, and Paslaten 5.

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