Variation and Phenetic Relationship of Tobacco (*Nicotiana tabacum* L.) In Central Java and Yogyakarta Based on Morphological Characters

Agung Dwi Santoso[1]*, Purnomo[2]

[1]Faculty of Biology, Universitas Gadjah Mada
[2]Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada
Jln. Teknika Selatan, Senolowo, Sinduadi, Mlati, Sleman, Yogyakarta 55281
*Corresponding Author:
e-mail: agung.dwi.santoso@mail.ugm.ac.id

ABSTRACT

Tobacco (*Nicotiana tabacum* L.) is a plant used as a mixture of cigarettes, and recreational media especially for men. This study aimed to identify variations, and determine the relationship between tobacco cultivars in Central Java and Yogyakarta based on macromorphological and micromorphological characters. Sampling locations are determined by surveying locations in both regions. Tobacco samples found include 5 cultivars in Central Java namely 'Mantili', 'Uler Magetan', 'Garut', 'Gober Boyolali', 'Manila', and 3 cultivars in Yogyakarta namely 'Siluk', 'Java', and 'Virginia'. Characterization with 23 qualitative macromorphological characters including leaves, and stems, with 9 qualitative and quantitative micromorphological characters including trichome and stomata. Descriptive data analysis is done to obtain the typical character of each cultivar, followed by numerical analysis including scoring characters processed with MVSP (Multi Variate Statistical Package), clustering with UPGMA (Unweighted Pair Group Method with Averages), and calculation of similarity coefficients with Simple matching formula. The results showed variations in the macromorphological characters including the shape of the leaf lamina, the base of the leaf, the absence of leaf stalks, and type of leaf venation. Tobacco has anisositic stomata, and varies in terms of length, width, and density of stomata. Tobacco trichomes are glandular. The result dendrograms form two clusters (A and B) with the similarity index of each cluster above 0.80. Cultivars with close relationships such as 'Siluk'-'Java', and far relationship like 'Java'-'Manila'.

INTRODUCTION

Tobaccos are commercial plant from the family Solanaceae, used widely as offerings in traditional ceremonies, herbal medicine ingredients, and main ingredient of cigarettes for personal entertainment. In Indonesia tobaccos could grow around 15 provinces ranging from lowland such as Nusa Tenggara to highland such as Wonosobo (Djajadi, 2015). Various cultivars appear in Indonesia, making tobacco plants widespread and adaptable. Many tobacco cultivars appear because of cultivation by crossing various inductees in order to obtain superior offspring with good nicotine levels suitable for cigarette mixtures (Amelia, 2012). Cultivars are individuals with similar character within species, and are distinguishable from other cultivars (Federizzi et al., 2012). The development of cultivars makes identification and characterization become the first step to introduce and developing of tobacco's cultivation in the community (Korir et al., 2012). Research of tobacco cultivars such as Djumali (2011) about identification

and characterization of tobacco cultivars found in Temanggung. This research has found various tobacco cultivars such as 'Kemloko-1','2', and '3', 'Dorowati,' 'Mantili', etc. According to this study variations present from leaf morphology such as different sizes and shapes, leaf color, harvest age, and flowers morphology typical in each type of cultivars.

The variety character of tobacco cultivars is quite diverse and specific in certain habitats. This is the result of environmental adaptation and the cultivation process carried out by farmers (Amelia, 2012; Prasetiyo et al., 2016). These adaptive variations are reflected through the morphological and anatomical characters of the plant. Variations in morphological characters are still commonly used for the identification process, and grouping different types of cultivars through phenetic approach. The phenetic analysis is an activity that grouping organisms into specific groups based on visible character equations, which exist in organisms regardless of their evolution (Arrijani, 2003). This grouping is expected to make it easier for researchers and cultivators to know the genealogy and cultivars' relationship. Variations in organisms become the guideline for choosing inductees with the best quality to produce better offspring. Thus, this study was conducted to determine the variation, and relationship of tobacco cultivars in Central Java and Yogyakarta using morphological characters through phenetic analysis.

This study held from August 2020 to April 2021 in Temanggung and Yogyakarta. The samples used were leaves, stems, and flowers from eight tobacco cultivars listed in Table 1 and pictured in Figure 1.

The observations are using 23 macromorphological characters and 7 micromorphological characters of qualitative and quantitative chosen from previous study about tobacco. Characterization of macromorphological characters using leaves (adaxial side), flowers, fruits, and stems. Leaves samples are also taken to be preserved. The leaves are then coated by nail polish to print the leaves surface. The print observed with microscope to characterize the micromorphological characters.

The descriptive analysis was done using the existing characters; to obtain the identification key. Then cluster analysis used to generate two dendrogram for each observed macromorphological and micromorphological characters. Dendrogram construction starts from character scoring and coding, calculation of similarity index with Simple Matching coefficient formula.

$$\frac{(a + d)}{(a + b + c + d)}$$

Description : a=number of second properties of data 1, b=number of properties of the first data 1 second data 0, c=number of properties of the first data 0 second data 1, d=number of properties of the second data 0 (Verma and Aggarwal, 2019).

Analysis are continued by constructing similarity matrix using MVSP 3.2A, and clustering with UPGMA algorithm.

MATERIALS AND METHODS

Table 1. Tobacco cultivars studied in this research

No	Cultivar's Name	Location
1	'Mantili'	Parakan, Temanggung, Jawa tengah
2	'Uler Magetan'	Parakan, Temanggung, Jawa tengah
3	'Garut'	Parakan, Temanggung, Jawa tengah
4	'Gober Boyolali'	Parakan, Temanggung, Jawa tengah
5	'Manila'	Parakan, Temanggung, Jawa tengah
6	'Virginia'	Pleret, Bantul, Yogyakarta
7	'Jawa'	Pleret, Bantul, Yogyakarta
8	'Siluk'	Imogiri, Bantul, Yogyakarta

Table 2. Scoring and Coding of Macromorphologicals Characters of Tobacco Cultivars

No	Characters	Score
1	Habit	Herbs = 0, Shrubs = 1
2	Leaf Position in Stem	Alternate = 0, Distichous = 1
3	Leaf Stipule	Absent = 0, Stipula = 1
4	Presence of Petiole	No = 0, Yes = 1
5	Leaves Apex	Acute = 0, Attenuate = 1
6	Leaves Base	Truncate = 0, Rounded = 1
7	Leaves Margin	Entire = 0, Waved = 1
8	Trichomes on Leaves Adaxial Surface	Low = 0, Moderate = 1
9	Lamina Shape	Ovate = 0, Lanceolate = 1
10	Leaves Colour	Lighter shade= 0, Darker shade = 1
11	Petiole	Decurrent = 0, Petiolate = 1
12	Stomata Position	Epistomatic = 0, Amphistomatic = 1
13	Leaves Venation	Camptodromus = 0, Penninerved = 1
14	Stem based of Shoots Growth	Monopodial = 0, Simpodial = 1
15	Stem based of Growth Direction	Erect = 0, Ascendens = 1
16	Flower Arrangement	Raceme = 0, Panicle = 1
17	Flower Type	Simple = 0, Compound = 1
18	Flower Position	Axillar = 0, Terminal = 1
19	Petal Colour	White = 0, Pink = 1
20	Sepal Colour	Light Green = 0, Green = 1
21	Corolla Symmetry	Actinomorphic = 0, Zygomorphic = 1
22	Fruit Shape	Round = 0, Elliptical = 1
23	Fruit Color	Green = 0, Brown = 1

Table 3. Scoring and Coding Micromorphologicals Characters of Tobacco Cultivars

No	Characters	Score
1	Stomatal Type	Anisositic = 0, Parasitic = 1
2	Stomatal Length	<40 µm = 0, >40 µm = 1
3	Stomatal Width	<20 µm = 0, >20 µm = 1
4	Stomatal Density	<40 individual/mm^2 = 0, >40 individual/mm^2 = 1
5	Trichome's Gland Shape	Round = 0, Ellips = 1
6	Glandular Trichome	Yes = 0, No = 1
7	Trichome Length	<400 µm = 0, >400 µm = 1

RESULT AND DISCUSSION
Morphological Character Variations

The characterization results indicate variations in some characters. Macromorphological characters include the shape of the leaf lamina, the base of the leaves, the presence of petiole, and the type of leaf venation. Tobacco has anisocytic stomata and glandular type trichomes. Morphological characters are commonly used in determining the taxon of organisms either genus, species, or more specifically (Awan and Murtaza, 2016). The distinctive macromorphological character of tobacco cultivars, is the presence of petiole in some types of cultivars. Some cultivars have seated leaves, so the base of the leaves have wider size. This part has a distinctive wavy shape like a wing (Figure 2). In contrast to tobacco cultivars with petiolated leaves, where leaf wings are absent. The petiolated leaves will be more rounded because the base of the leaves' form a certain angle before meeting on the stalk. Then the leaves width at the bottom is wider, so it is shaped like an egg. While seated leaves whose the lamina

are elongated because the base the leaves' base does not form a certain angle before meeting on the petiole.

Micromorphological character variations are quantitative such as stomata density. Quantitative character measurements are taken in a certain range as the score. Qualitative characters include stomata type, and the trichomes on the sample are likely uniform. Stomata belong to anisocytic type where the stoma is surrounded by several neighbouring cells, that can be distinguished by the surrounding epidermis cells (Dewi et al., 2015) (Figure 3). This type of stomata is also commonly found in other Solanaceae (Awan and Murtaza, 2016; Fajri, 2013). The sample also has a uniform type of trichomes which is glandular (Figure 4).
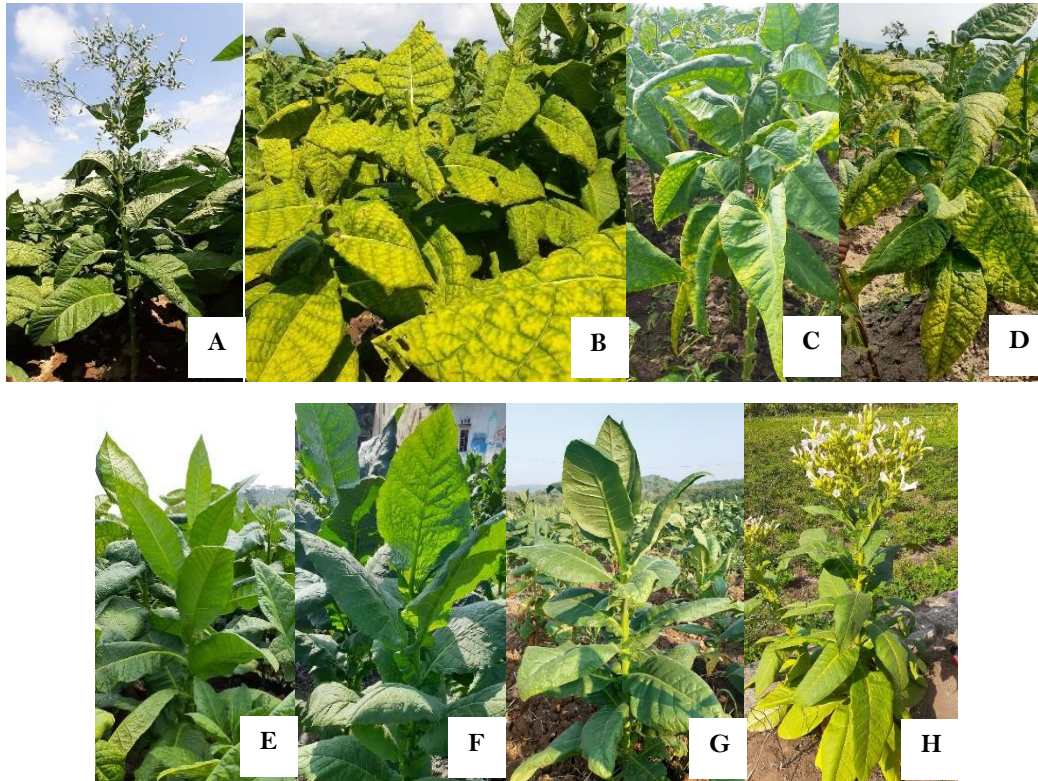

Figure 1. A) Tobacco 'Mantili', B) Tobacco 'Uler Magetan', C) Tobacco 'Garut', D) Tobacco 'Gober Boyolali', E) Tobacco 'Manila', F) Tobacco 'Virginia', G) Tobacco 'Jawa', H) Tobacco 'Siluk'
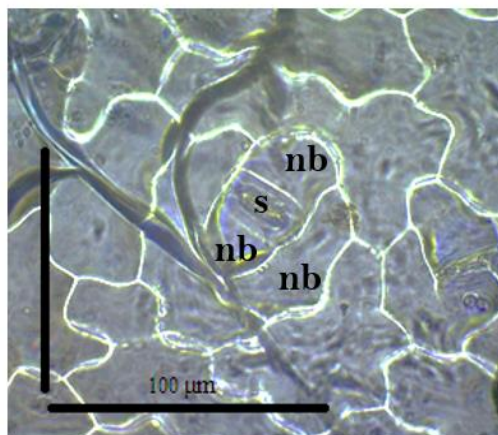

Figure 2. Leaves Wing on Tobacco. W=wing.

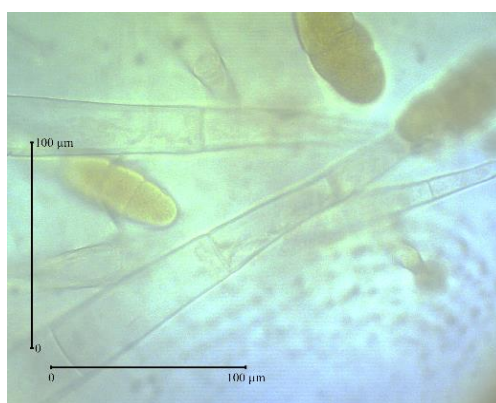**Figure 3.** Anisocytic Stomata. s : stoma, nb : neighboring cell.

Figure 4. Glandular Trichomes

Table 4. Identification Key for Tobacco Cultivars in Central Java and Yogyakarta

Macromorphology	Micromorphology
1. A. Leaves Petiolate .... 4 B. Leaves Sessile... 2	1. A. Stomatal Length <40 µm........ 'Mantili','Garut' B. Stomatal Length >40 µm 2
2. A. Sessile, Apex Acute .... 'Mantili' B. Sessile, Apex Attenuate 3	2. A. Stomatal Width <20 µm .... 'Jawa','Siluk' B. Stomatal Width >20 µm.......... 3
3. A. Attenuate, Lighter Shade........................... 'Garut' B. Attenuate, Darker Shade............................ 'Gober Boyolali'	3. A. Stomatal Density<40 µm² ...... 'Manila','Gober Boyolali' B. Stomatal Density <40 µm²...... 'Virginia','Uler Magetan'
4. A. Low Trichomes... 5 B. Moderate Trichomes................................... 6	
5. A. Low Trichomes, Lighter Shade................. 'Virginia' B. Moderate Trichomes, Darker Shade .... 'Manila'	
6. A. Lamina Ovate .... 'Uler Magetan' B. Lamina Oblong... 7	
7. A. Oblong, Lighter Shade .... 'Siluk' B. oblong, Darker Shade.................................. 'Jawa'	

Glandular trichomes on leaves could produce certain secretaries for defence and metabolic aid processes (Maryani et al., 2009). In contrast with non-glandular trichomes that are common to plant for defence agents (Fajri, 2013). This variations makes the trichomes one of the characters used commonly in plant systematics. The variations of these characters are then arranged as identification key, to find out the distinctive character of each cultivars. A dendrogram then supports the identification key to determine its relationship (Table 4).

Based on dendrograms cultivars samples have a similarity index ranging from 0.50 to 1.00 (Figure 5). Some cultivars have a similarity index close to 100% although planted in two different regions. 'Manila' from Central Java is closely related to 'Virginia' and 'Java' from Yogyakarta. The close relationship between cultivars grown in two different regions is caused by similar environmental conditions around the plants. The dendrograms show the existence of two clusters A and B based on the presence of petiole, and the density range of stomata. Cluster A is a cultivar with petiolated leaves, and cluster B is the sessile leaves.

The cluster A has stomatal density >40 ind/mm², and cluster B has stomatal density <40 ind/mm². In micromorphologys' dendrogam the similarities bertween cultivars are 100%. This means that cultivars in each subclaster have high similar characters, thus its relationship are very close. Both dendrograms show that the sample will have a closer relationship in one cluster, especially for cultivars that grow in one region such as 'Java'-'Siluk'. A lower similarity index means closer relationships with the sample such as 'Java'-'Manila'. The morphological characters used can also be used to group and know their relationship. The data can be used as a reference to choose iductess with high variation, that when crossed, could produce offspring with better quality for cultivation.
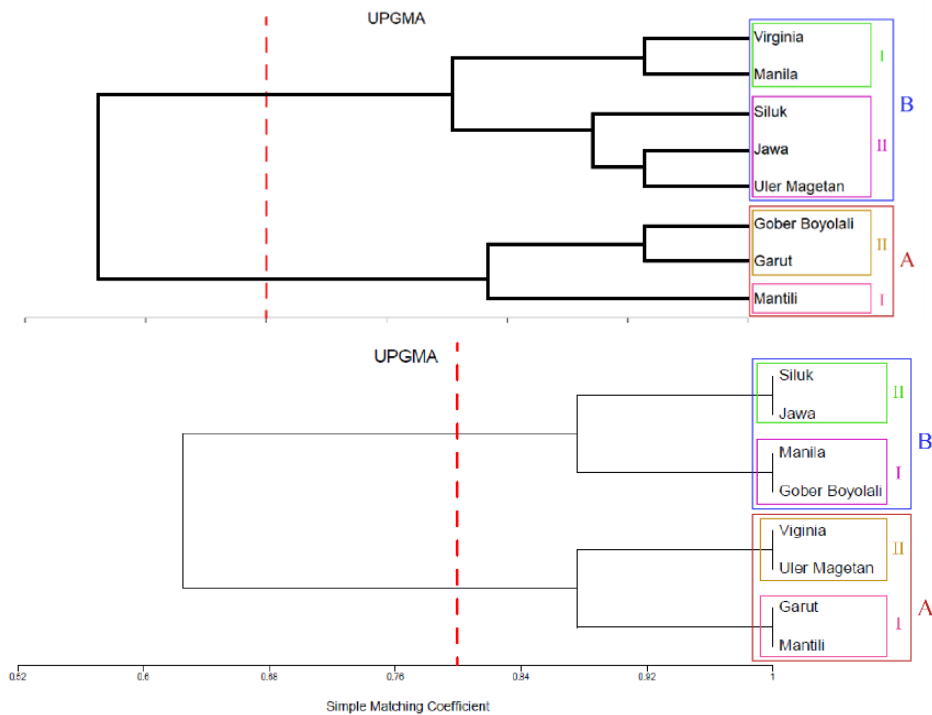

Figure 5. Phenetic relationship dendrogram of tobacco cultivars in Central Java and Yogyakarta based on morphological characters. a=macromorphology, b=micromorphology

Figure 6. A) Petiolate leaves, B) Sessile leaves

CONCLUSION

Tobacco cultivars that can be found include 5 cultivars in Central Java namely 'Mantili', 'Uler Magetan', 'Garut', 'Scrooge Boyolali', 'Manila', and 3 cultivars in Yogyakarta namely 'Siluk', 'Java', and 'Virginia'. Variations of morphological characters are used to construct dendrogram. Dendrograms form two clusters (A and B) with the similarity index of each cluster above 0.80. Cultivars with close relationships are 'Siluk'-'Java', and far related are 'Java'-'Manila'.

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