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# Diversity and Phenetic Relationship of Black Potato (*Coleus tuberosus* Benth.) in Yogyakarta Based on Morphological and Leaf Anatomical Characters

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**Abstract.** Black potato (*Coleus tuberosus* Benth.) is one of functional food. Propagation of this plant is easy to conduct and grow at various altitudes. Black potatoes are possible to widely distributed in various regions in Yogyakarta. This study aimed to examine the diversity and phenetic relationship of black potato samples based on morphological and leaf anatomical characters in Special Region of Yogyakarta as the first step in plant breeding. Samples were collected by survey method and interviewing. Samples were collected in Yogyakarta composed of tubers, stems, leaves, and flowers. Samples were characterized based on morphological and leaf anatomical characters, followed by scoring, standardization, and calculating the SI (Similarity Index) between samples (OTUs). Clustering analysis was done using UPGMA (Unweighted Pair Group Method with Arithmetic Averages) to create dendrogram of phenetic relationship. Deciding how many clusters formed in dendrogram supported by phenon line 0.7 and dominant characters supported by PCA (Principal Component Analysis). Results showed that the variation of black potatoes morphologically on stem, leaves, and tubers. Anatomically, variations were found in stomata, epidermis, xylem, and palisade parenchyma. Dendrogram based on morphological character divided into four clusters. Dominant character formed A cluster was leaf position anomaly, B cluster was adaxial leaf color, C cluster was stem erect, and D cluster was leaf rigid. Dendrogram based on leaf anatomical character divided into three clusters. A cluster formed because the similarity of palisade parenchyma layer, B cluster was neighbor cell of stomata margin shape, and C cluster was xylem arrangement.



**Keywords:** *Coleus tuberosus*, leaf anatomical, morphological, phenetic relationship

## INTRODUCTION

Black potato (*Coleus tuberosus* Benth.) is from West Africa [1]. It has been cultivated in various region of West Africa, South Asia, and Southeast Asia because its tuber was used for consumption. In Southeast Asia, the plant is grown in Sri Lanka, Malaysia, and Indonesia [2]. Black potato is one of the vegetable crops that is useful as a source of carbohydrates [3]. Carbohydrate contents per 100 g of black potatoes were 21 g while carbohydrate contents per 100 g of potatoes and sweet potatoes were 17 g and 20 g [4]. It proves that black potato has a relatively higher carbohydrate so that it can be used as carbohydrate food source. In addition, black potato is also included in one functional food.

Black potato is also useful as a medicinal plant. Based on earlier research, black potato prevented diabetes mellitus [5]. It can be used as an antioxidant to prevent cancer [5, 6], and to increase endurance and can overcome various disease [7]. Besides its benefits as a medicinal plant, black potato also has the prospect and potential to be used as flour [8]. The selection of potato flour from black potatoes can be processed into foodstuffs to make noodles, cakes, bread, cookies, and others. In addition, black potato flour has a longer shelf life than other flour. The black potato is one of the plant for future [1].

Black potato is famous less in Indonesia, only known and used by people in Java, Bali, and Madura on a limited scale, almost rarely. Its small tuber makes it less preferred to the public. The genetic diversity of these plants is low, on the other hand, these plants were distributed in many areas must be had variations at the species level due to their various environmental conditions [9, 10]. Moreover, vegetative propagation has been made it easy to do. Therefore, it is necessary to conduct research on characterization of black potato based on morphological analysis because it is easily observed [11] and anatomical for supporting characters. They will be the first step which can be used as a conservation strategy. Furthermore, by knowing the diversity of cultivated black potato germplasm, it can be utilized for the improvement of varieties. *D. alata* is divided into two cultivar groups and each became six cultivar subgroups which were useful for plant breeding [12]. The objectives of the research were to know about morphological and anatomical variations and phenetic relationship based on those characters between samples.

## MATERIALS AND METHODS

This research was conducted on from Dec 2016 to Apr 2017, begun by surveying and sampling in several subdistricts in the province of Yogyakarta (Fig. 1). Surveying in various subdistricts was aimed to get samples of black potato tubers, stems, leaves, and flowers. Interviewing local communities were also conducted to get information about its benefits for them.

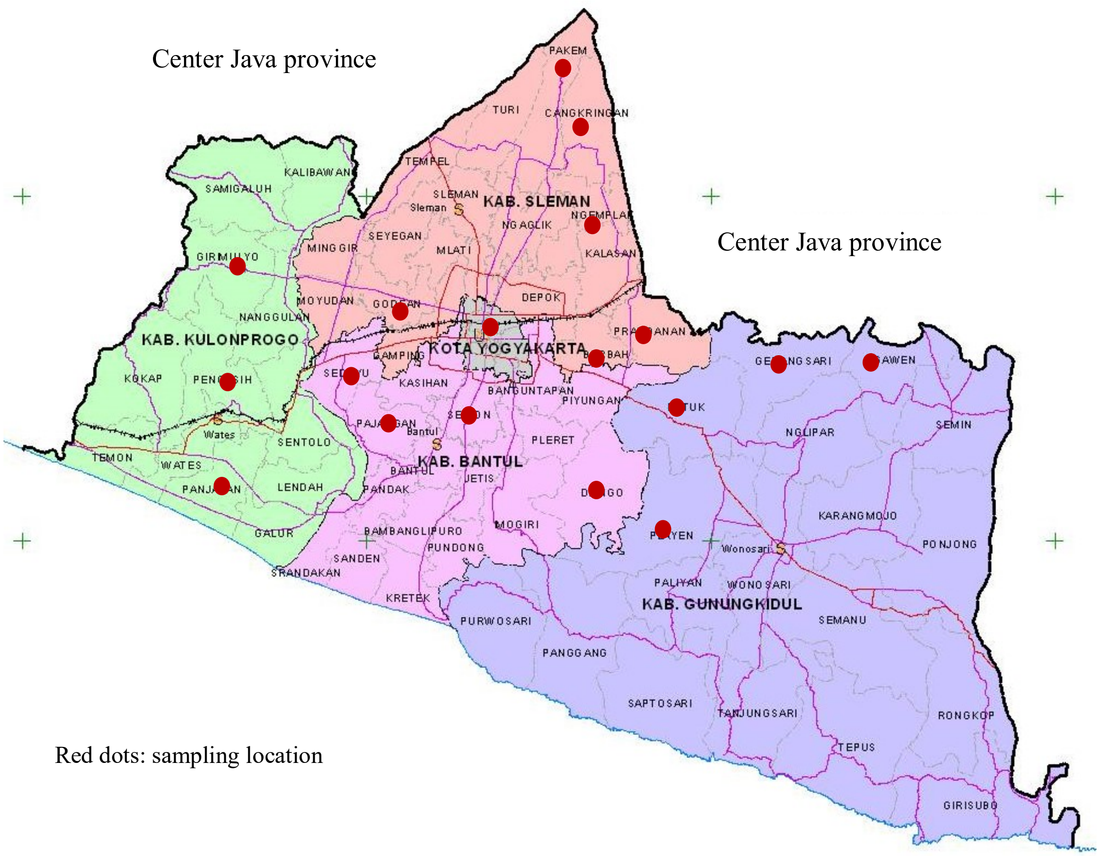



**FIGURE 1.** Map of Yogyakarta [13]

Found plants were planted and were analyzed by morphological and leaf anatomical characters in the Laboratory of Structure and Plant Development, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta.

Morphological characters consist of seven quantitative and 29 qualitative characters. Each part of the plant was characterized. Description of those characters was to get characters of each sample particularly. Scoring was for changing chosen characters into numerical data by sorting the code 0.1 for two state traits, and 0,1,2...,n for multistate traits. The scoring code can be seen in Table 1 based on *Descriptor for cultivated potato* with modification [14]. The results of scoring were standardized with Microsoft Excel to synchronize the condition of data to know present and absent characters.

**TABLE 1.** Scoring codes for chosen morphological and leaf anatomical characters.

| Characters | Scoring |
|---|---|
| Stem shape | 0 = square; 1 = square and hexagon |
| Adaxial leaf color | 0 = green; 1= light green; 2 = light dark green gradation |
| Texture of upper surface leaf | 0 = rough; 1 = almost smooth |
| Sitting leaf anomaly | 0 = nothing; 1 = exist |
| Leaf rigid | 0 = rigid; 1 = floppy |
| Stem upright | 0 = errect; 1= collapsed |
| Tuber shape | 0 = elips; 1 = elips and elongate; 2 = elips and round; 3 = elongate |
| Tuber flesh color | 0 = white; 1 = white yellowish |
| Adaxial neighbor cell stomata margin shape | 0 = straight; 1= curly |
| Adaxial surface epiderm shape | 0 = straight; 1= curly |
| Xylem structure | 0 = similar; 1 = dissimilar |
| Palisade parenchyma layer | 0 = 1 layer; 1= 2 layers |

Leaf anatomical characters were observed to support morphological characters. Leaf anatomical characters were observed through the transverse section in paraffin embedding with modification [15] and paradermal section. Paradermal section was made by soaking cutting leaves into alcohol 70 % in ± 5 d. In the paradermal section, trichomes and stomatal density were measured by the formula:

$$\frac{Number\ of\ trichomes\ /\ stomata\ in\ each\ field\ of\ view}{Area\ of\ view\ (mm^2)}$$

[16, 17].

Chosen characters which can be compared then scored. The scoring code can be seen in Table 1.

Data were analyzed descriptively and numerically. Numerical analysis was done by comparing the phenetic relationship between the data obtained from the morphological and leaf anatomical characters. The similarity index is calculated using the SSM (Simple Matching Coefficient) formula.

$$S_{SM} = \frac{a+d}{a+b+c+d} \times 100\ \%$$

[18]

Clustering analysis was done with UPGMA algorithm to create dendrogram. The dendrogram is to illustrate the similarities between samples. Dendrograms and PCA (Principal Component Analysis) were obtained through MVSP 3.1 software application.

# RESULTS AND DISCUSSION

## The Source Sample

The source samples of this research were ten samples from Bantul, Kulon Progo, Gunungkidul district. It can be seen in Table 2.

**TABLE 2.** Samples of black potato found on explorations.

| No. | Village and district | No. sample | Subdistrict |
|---|---|---|---|
| 1. | Triwidadi, Pajangan | KH1 | Bantul |
| 2. | Sendangsari, Pajangan | KH2 | Bantul |
| 3. | Sidomulyo, Pengasih | KH3 | Kulon Progo |
| 4. | Kenteng, Patuk | KH4 | Gunungkidul |
| 5. | Ngalang, Gedangsari | KH5 | Gunungkidul |
| 6. | Bugel, Panjatan | KH6 | Kulon Progo |
| 7. | Bugel, Panjatan | KH7 | Kulon Progo |
| 8. | Beji, Ngawen | KH8 | Gunungkidul |
| 9. | Argodadi, Sedayu | KH9 | Bantul |
| 10. | Beji, Ngawen | KH10 | Gunungkidul |

## Morphological Variations

### *Tubers*

Tuber shapes are elliptic and elongate to round, tuber length is around 0.8 cm to 4.4 cm, and the diameter is around 0.14 cm to 1.64 cm. Tuber colors have variation from light brown until dark brown according to their age (Fig. 2). Tuber flesh color is white to yellowish white. The tubers have a strong scent, surrounded by a few root fibers and a coarse leather texture, and the tubers are not gummy.

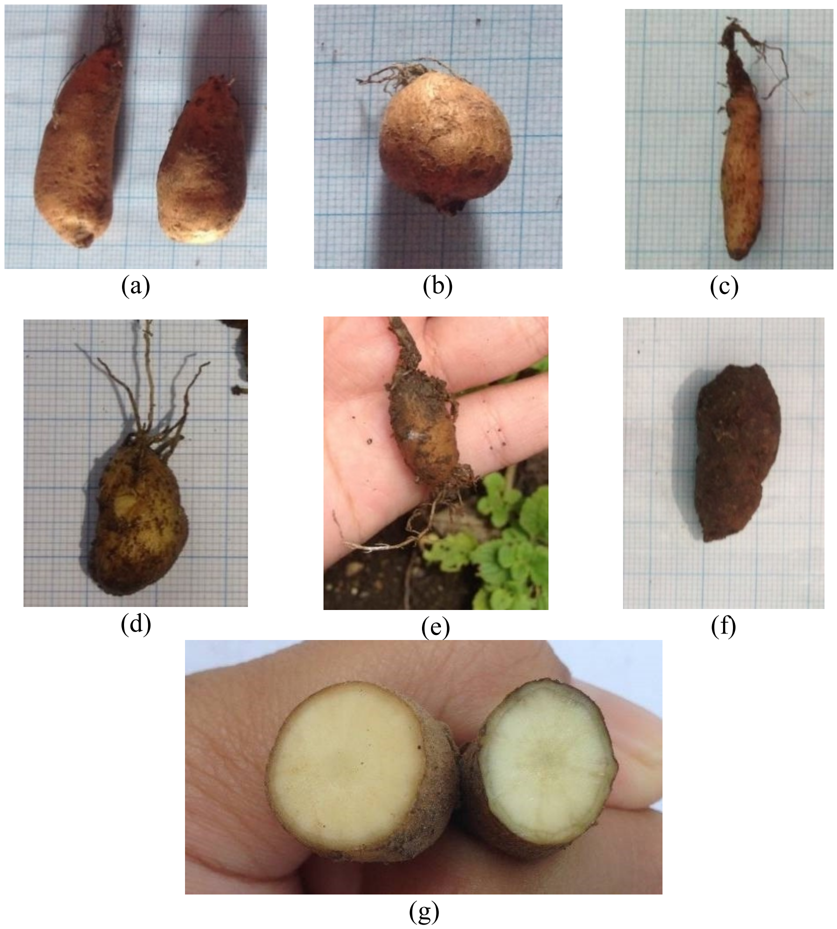



**FIGURE 2.** Morphologies of black potatoes's tubers from various sub-district in Yogyakarta: (a) elliptic, (b) round, (c) elongate, (d) light brown color, (e) brown color, (f) dark brown color, (g) flesh tuber colors, right: white, left: white yellowish.

Harvesting of black potato's tubers is seasonal. According to [19], the black potato is an annual herbaceous plant and the tuber usually can be harvested approximately 3 months after the planting period. The period of tuber production is when the generative phase of the plant has begun so that the tubers can be harvested when the plant canopy has dried up [20, 21].

### *Stems*

Black potato has rectangular shaped succulent stem-like members of Lamiaceae in general and the stem has glandular hairs that contain essential oils [22]. Square shaped stems were found mostly when did the sampling, but there were anomalies in the leaf position because of a hexagonally shaped stem (Fig. 3b). The height of black potato reaches 23 cm with the diameter of stem is around 0.216 cm to 0.374 cm. The stem has rough surface texture because it is filled with many trichomes. Black potato usually has an erect growth (Fig. 3c) and there are some that creeping (Fig. 3d).

The stem color is various, that is green with a purple color in the corner, green, green on top of the stem and purple on the bottom of the stem which almost touched the ground, and a mixture of green and purple, determined by the age of the plant. When young, the stem is bright green, while growing older, the edge of the stem turned into purple and eventually, it turned purple on the entire surface of the lower stem nearest the ground.

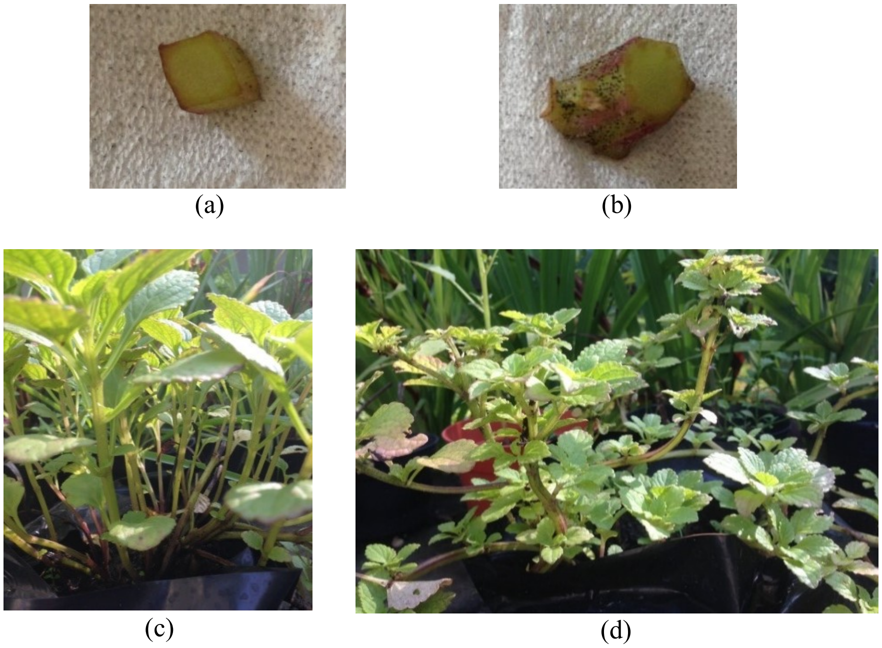



**FIGURE 3.** Morphologies of black potatoes' stems from various sub-district in Yogyakarta: (a) rectangular shaped, (b) hexagonal shaped, (c) upright growth, (d) fall growth

*Leaves*

Black potato leaf has a single leaf type with opposite position, without leaf sheath [23]. The leaf shape is ovate with the pointed tip, attenuate base, crenate margin (Fig. 4a), the color of the abaxial surface of leaf is light green (Fig. 4b), and leaf petiole color is green. The ratio of leaf length and width is about 1.5 to 2:1, leaf with pinnate venation. Leaf surface texture is rough depends on trichome density. The anomalies were found in KH 5 and KH 9, there were three single leaves in each node (verticillate) (Fig. 4d). Those variations were likely due to genetic factors. The phenotype is controlled by a gene arrangement that correlates with environmental factors causing by mutation [24].



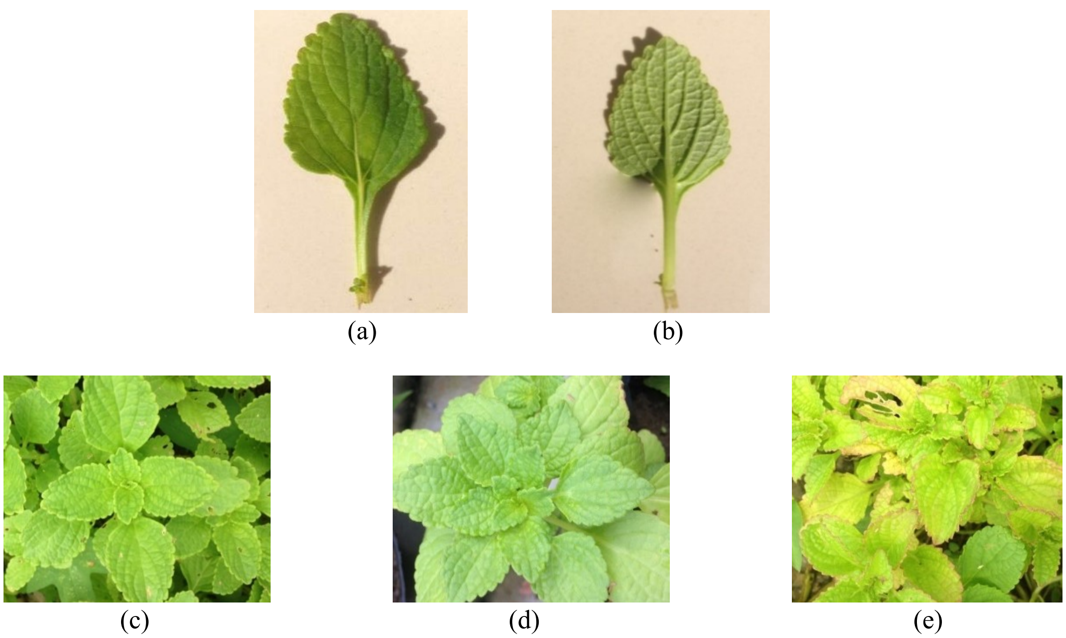



**FIGURE 4.** Leaf morphology of black potatoes from various sub-district in Yogyakarta: (a) adaxial leaf surface, (b) abaxial leaf surface, (c) normal leaf position (verticillate with three leaves), light and dark green gradation, (d) anomaly leaf position and green color, (e) light color

Flower characteristics contribute excellent clues to identification [25] because flowers structure was constant. Not all flowers could be found in all samples, but there were only a few already flowered samples, so the flower characters could not be used for scoring. Flowers characters were only for descriptive data because the variation was not found. The arrangement of raceme (Fig. 5a) each node can be up to five to eight flowers. Corolla composed of five petals. Three anterior petals as anterior lip and two posterior lip with a purple color (Fig. 5b).

(a) (b)

**FIGURE 5.** Flowers morphology of black potatoes from various sub-district in Special Region of Yogyakarta: (a) wreath, (b) appearance flowers from above

## Phenetic Relationship Between Samples of Black Potato Based on Morphological Characters

The dendrogram indicates samples relationship of black potato based on morphological characters from Yogyakarta. A number indicates the similarity index between OTUs. The similarity was determined using Ssm (Simple Matching Similarity. Figure 7 shows that the black potato in Yogyakarta produces a similarity index 0.48 to 1. Inter-sample relationships that have high similarity indexes indicate that the samples have close genetic relationship. Samples with genetic closeness, allegedly from close-knit elders, whereas those with high genetic distances are thought to have originated from a distant relative to another elder [26].

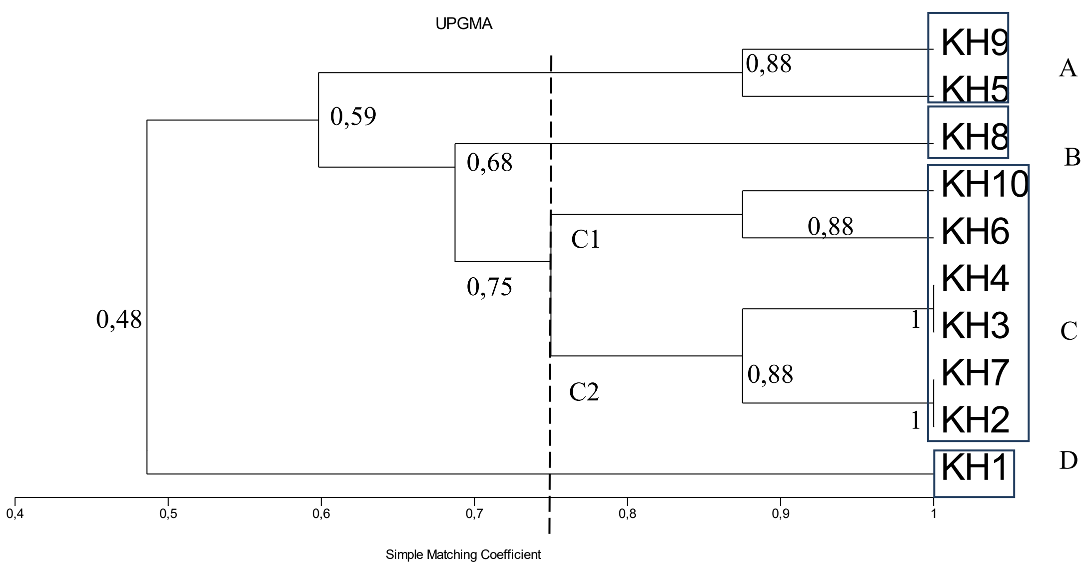



**FIGURE 6.** Dendrogram of phenetic relationship between sample of black potato plant in Yogyakarta based on the morphological character using Simple Matching Similarity (Ssm)

Group A, i.e., KH9 and KH5 have a high similarity index that is 0.88. According to Singh [27], the similarity index > 80 % presumes that the samples have high similarity. This means KH9 and KH5 have very closely related. KH6, KH7 and KH8, KH10 have many similarity index, although both come from the same region with relatively similar environmental conditions. Two samples in the same area may not necessarily have the same similarity or within the same group [28, 29]. While KH8 and KH10 are from Beji Village, Gunungkidul with similar environmental conditions but, soil pH is much different. KH10 lives on the acid pH 4.2 and KH8 at pH 6. Although both are from the same region, their different environments lead to genetic diversity [30].

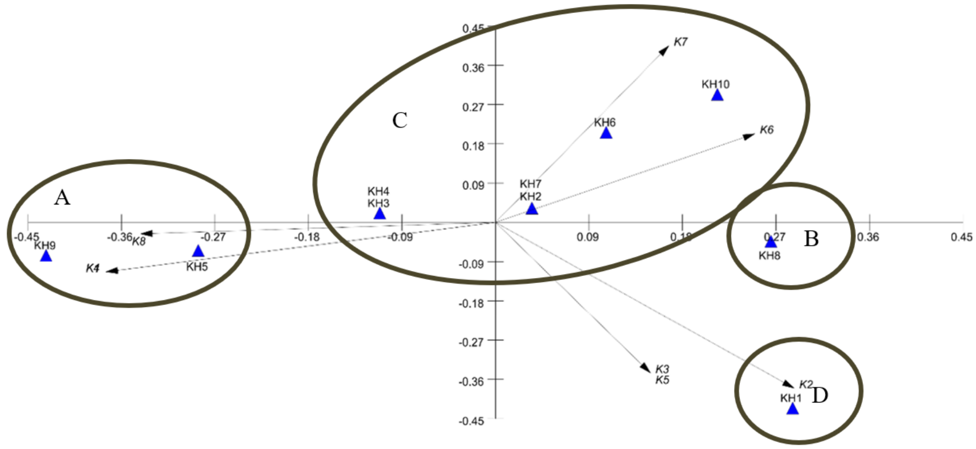



**FIGURE 7**. Scatter plot of the main component analysis of the black potato on morphological characters from several subdistricts in Yogykarta

The black potato relationship is divided into two major groups in the dendrogram on a low index of similarity, indicating that the black potato samples in Yogyakarta have variation. Variations can be found at the species level [31]. A low SI value indicates that the samples of the black potatoes are distantly related [32]. Variations can occur due to growth habits in response to environmental factors. Plasticity of these plants affects morphological characters [33].

## Leaf Anatomical Variations

The epidermal tissue of a layer is in the abaxial and adaxial parts with irregular shapes; some are elongated and rounded. Mean epidermal thickness ± 21.2 µm in each sample. In leaf organs there are epidermal derivatives, those are stomata and trichomes.

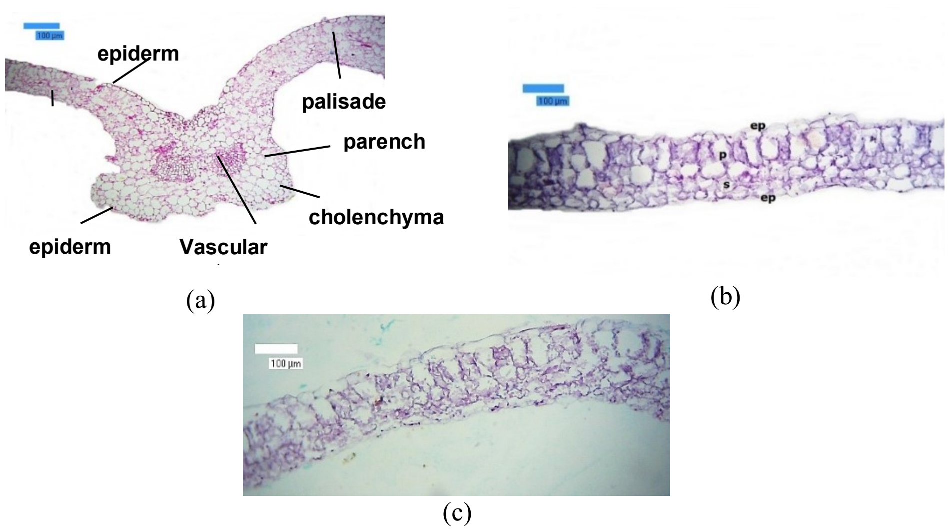
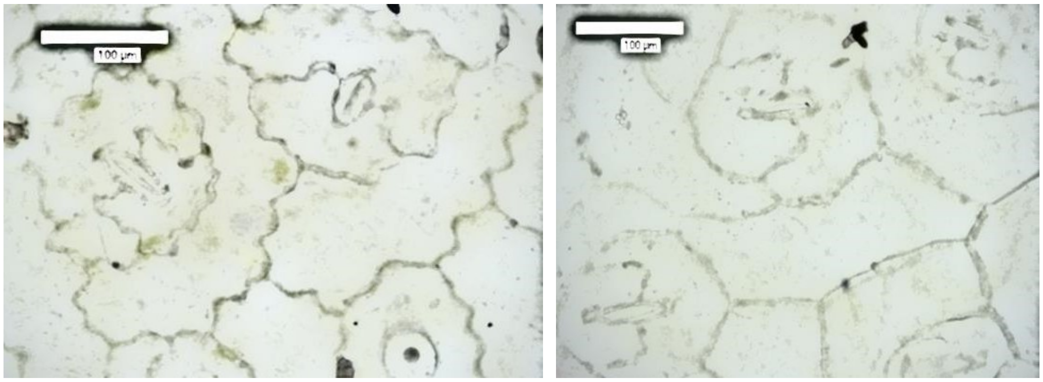



**FIGURE 8.** Appearance of transversal and longitudinal section of black potato leaves: (a) costae, (b) lamina with two palisade layers, (c) lamina with one palisade layer. ep: epiderm, p: palisade, s: spons




(a) (b)

**FIGURE 9.** Stomata adaxial with neighbor cell shape: (a) curly, (b) straight

The palisade tissue is tightly arranged, shaped like a barrel, and consists of two layers of cells, except in KH1 there is only one layer of palisade cell. Spongy tissue seen there are (one to two) layers of the cells. The thickness of the mesophyll (palisade and sponge) averaged ± 178.23 µm in each sample. Leaves are dorsiventral because the ventral part differs from the dorsal part and the palisade parenchymal layer is found only on the upper side of the leaf [35].

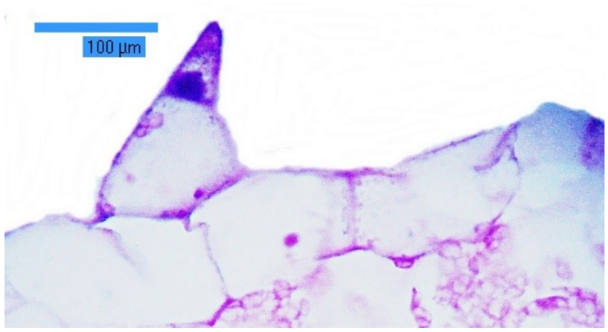
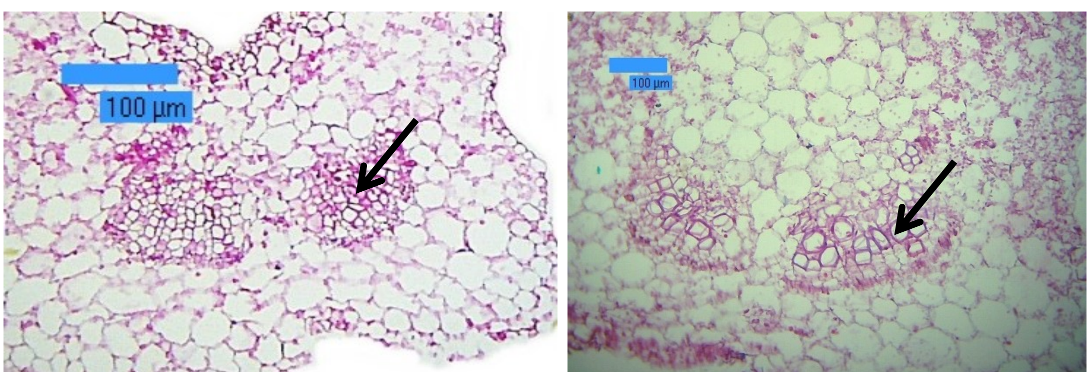



**FIGURE 10**. Trichome on epidermis leaf

The vascular is surrounded by sclerenchyma tissue as a protector. The average vascular thickness is ± 124.6 µm in each sample. Opened collateral vascular type [34]. Variations in the properties of the xylem arrangement in the mother of the black potato bone are seen as the arrangement of xylem cells appear to be more similar in Fig. 11 (a) than in the arrangement of xylem in Fig. 11 (b).




(a) (b)

**FIGURE 11.** Xylem arrangement on vascular system in costae (a) similar, (b) disimilar

## Phenetic Relationship Between Samples of Black Potato Based on Leaf Anatomical Characters

The dendrogram results (Fig. 12) describe the division of three large clusters formed with the help of the 80 % phenon line and supported by PCA (Fig. 13). Group A is formed due to the similarity of the two palisade layer characters. Group A is divided into two clusters, namely A1 and A2, which are separated by different characters. The A1 cluster, KH9, has the edge of the neighboring stomata adaxial cells and the edges of the epidermis are straight, and the arrangement of xylem cells is the same

Group B is formed due to the similarity of the four qualitative anatomical characters, i.e., the shape of the neighboring edge of the adaxial stromata, the epidermal edge of the cell, the arrangement of xylem cells and the number of layers of palisade since the cluster B similarity index reached the perfect number 1. Group B and group C united on the similarity index 0.67 because of the similarity of the neoplasmic cell shape of the adaxial stomata and the epidermal edge of the epidermis.

Group C is divided into 2 clusters, i.e., C1 and C2 are united on a similarity index of 0.75. Both clusters are separated due to differences in the number of palisade layers. Cluster C2 has one palisade layer. The three major groups (Groups A, B, and C) united at a low similarity index of 0.34. This indicates that the variety of black potato based on anatomical characters is very diverse. Genetic and environmental factors also influence the variation of anatomical characters [31, 36].

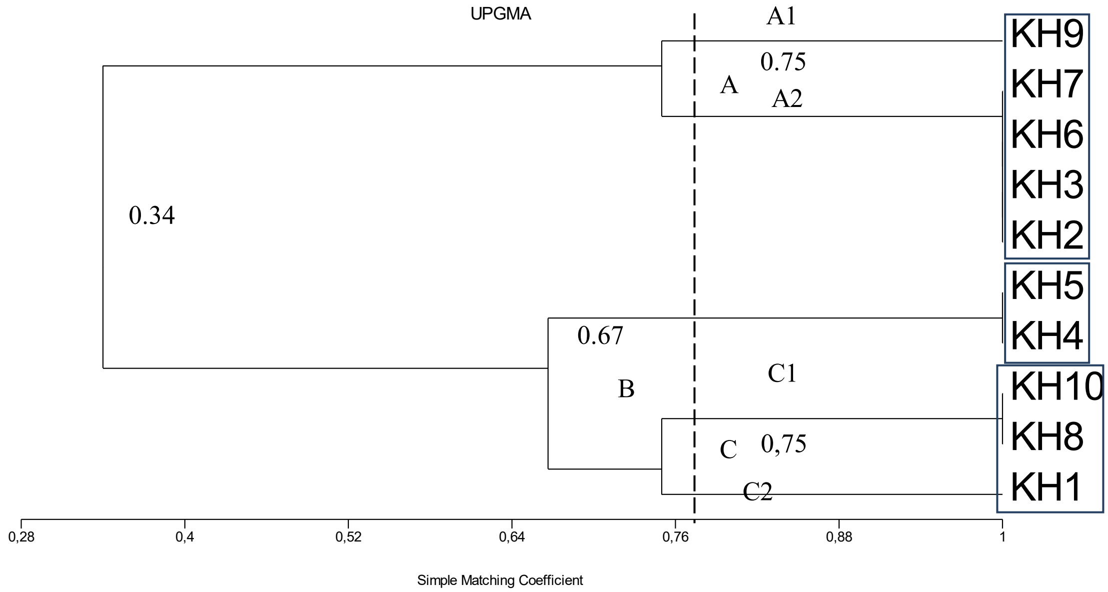



**FIGURE 12.** Dendrogram of phenetic relationship between sample of black potato plant in Yogyakarta based on leaf anatomical character using Simple Matching Similarity (Ssm)

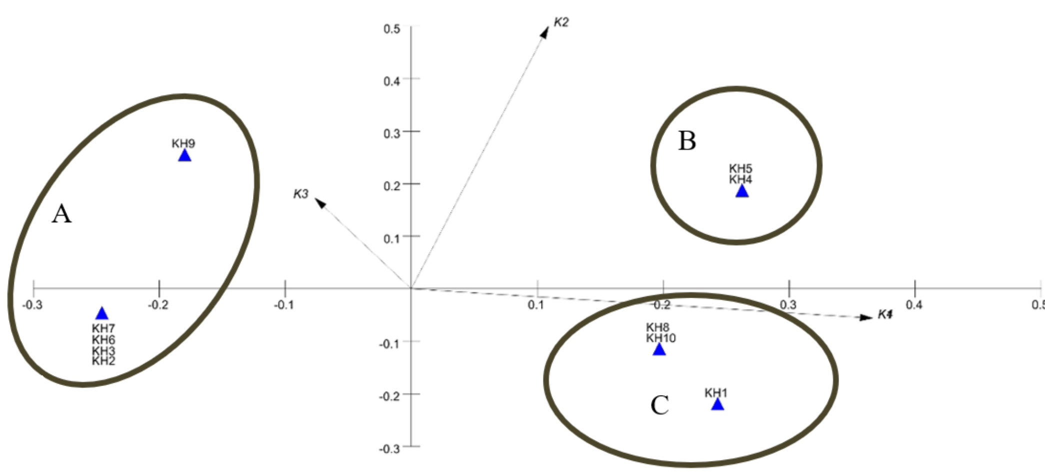



**FIGURE 13**. Scatter plot of the main component analysis of the black potato on leaf anatomical characters from several subdistricts in Yogyakarta

## CONCLUSIONS

Based on this research, it can be concluded that morphologically, black potatoes in Special Region of Yogyakarta are various in these characters: stem shape, adaxial leaf color, adaxial leaf surface texture, leaf position, leaf rigid, plant upright, tuber shape, and flesh tuber color. Leaf anatomically, black potatoes in Special Region of Yogyakarta are various in these characters: neighbor cell shape of adaxial stomata, epidermis edge shape, xylem arrangement on costae, and palisade layers amount. The relationship of the black potato in Yogyakarta based on morphological and leaf anatomical characters is a distant relative. While the phenetic relationship of the black potato in Yogyakarta based on a combination of both morphological and anatomical characters of the leaves is closely related.

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