Cluster analysis of *dioscorea* spp. based on amilum and tuber morphology

Siti Maqfiroh, Jumari, Murningsih

1,2,3Department of Biology, Faculty of Science and Mathematics, Diponegoro University
Jl. Prof. Soedharto, SH, Tembalang, Semarang 50275, Indonesia
E-mail: smaqfiroh@gmail.com

Abstract. *Dioscorea* Spp. is a group of plants that having great potential to be developed, but its presence are increasingly marginalised. Semarang district and Boyolali-Indonesia have variants of *Dioscorea* Spp. that has not been characterized. The purpose of this research to clustering and know the similarity range of *Dioscorea* Spp. from Semarang district and Boyolali-Indonesia based on starch type and tuber morphology. Ten variants of *Dioscorea* Spp. from Semarang district and Boyolali-Indonesia are characterized its starch and tuber, than arranged using cluster analysis with Past ver. 9.0 programs. The result of cluster analysis showed that 10 variants were found clustered into two main groups. First group consist of ‘gembili wulung’, ‘tomboreso’, ‘gembili brol’, ‘gembili tropong’ and ‘gembili pulung’, while second group are ‘uwi legi’, ‘uwi ungu’ and ‘uwi bangkulit’. Varieties from Semarang district and Boyolali have different morphological forms, but still have close kinship relationship.

1. Introduction
Central Java is one of the areas that became center of genetic diversity resources (Rustini, 2003). According to Jumari (2017), rural areas in Central Java have various types of sweet potato that need to be preserved. Semarang district and Boyolali-Indonesia are two areas that still have variants of *Dioscorea* Spp. because existence is preserved by society. *Dioscorea* Spp. is one of species that must be preserved its existence because it has great potential, especially in the field of food.

Semarang district is a hilly area with a slope of 15-14% (Bappeda Semarang, 2014). The area of Boyolali, especially in the Simo area is a mountainous area located at an altitude of 432 masl (BPS Simo, 2014). A small proportion of people in these two regions cultivate *Dioscorea* Spp. as a side crop, so the diversity of *Dioscorea* Spp. in these areas can still be found. According Jumari (2017), there are five types of member *Dioscorea* Spp. located in 12 districts and some rural areas in central Java. Some species can be encountered in Semarang district and Boyolali-Indonesia areas. So far, Dioscorea Spp. from Semarang and Boyolali not yet characterized definitively, so it is necessary to carry out the group characterization activities to find out how much potential that *Dioscorea* Spp. can be develop.
Differences in the characteristics of a plant not only occur in plants originating from areas where production centers are different, but also from the same region (Hambali, 1994). The diversity occurring causing a classified plant/ taxonomy still shows the diversity among populations (Sofro, 1994). The equations and the existence of a plant morphology can be used to know how far closer kindship relationship (Suskendriyati et al, 2000). Characterization activities based on morphological markers are important because their existence can be preliminary data to complement the diversity of a plant (Trimanto, 2012).

2. Material and Methods
*Dioscorea* Spp. sampling activity was conducted from April-August 2017. The location of the accession took place in the village of Jabungan, Mluweh, and Simo. Characterization of tuber morphology tubers was carried out in Ecology and Systematic Laboratory, Observation of starch was done in the laboratory of Basic Biology Department of Biology, Faculty of Science and Mathematics, Diponegoro University. Tuber sample from Jabungan, Mluweh, and Simo has identified to morphological identification based on their stem and leaf using guidelines from IRETA (1998) and then characterized using Yam’s Descriptor from IPGRI (1997). The characteristic of the tubers observed are the number of tubers per hill, tuber spacing, shape, size, and colours. Photomicrograph 4x (4x10) magnification used for characterization of amylum type. Similarity analysis was calculated using similarity index then cluster analysis was done to determine Dendogram of *Dioscorea* Spp. similarity distance. Before determining the distance of similarity, scaled data was processed. Scoring is done by giving score 0 if not fulfilled and score 1 if fulfilled.

3. Results and discussions
Jabungan and Mluweh villages located in Semarang are located at an altitude 123 amsl and 103 amsl. Simo village is located in Boyolali with an altitude of 216 amsl. Simo has the highest humidity (70.1%) compared to Jabungan and Mluweh. This means that mean that water vapor in the ground is still high because this area is close to the water flow. Soil moisture in Jabungan and Mluweh is about 25%-37.5%. Soil in the Jabungan area is more humid than Mluweh because in Jabungan there are many shaded. Mapping in Jabungan makes the intensity of light that coming into this area decreased. Jabungan and Mluweh have neutral soil, while Simo has acid soil (PH 5.5). But, these three areas have environmental conditions that suitable to the cultivation of *Dioscorea* Spp. In accordance with Abdillah (2015), the environmental requirements to the cultivation of *Dioscorea* Spp. in the form of lowland to the mainland that is at an altitude of 800 amsl, optimal soil moisture is 40% and soil’s PH around 5.5 to 6.5. Although Simo has a moist soil, some members of *Dioscorea* Spp. still survive. This is because *Dioscorea* Spp. able to survive in a critical environment (poor nutrients). According to Zalindau (2015), Dioscorea is able to grow in places such as moor, rich field and lea.

The result showed that in Jabungan area there are 4 variants *Dioscorea* Spp. (namely gembili brol, uwi legi, uwi bangkulit and tomboreso), from Mluweh there is 1 variant that is gembili wulung and from Simo got 5 variant (namely gembili brol, gembili tropong, gembili pulung, uwi bangkulit and uwi ungu). Based on leaf and stem characteristics, the ten sample variants obtained were placed on three types of Dioscorea Spp. members. There are *D. alata, D. esculenta* and *D. pentaphylla*. Samples from Semarang and Boyolali have various characteristics. One of the factors that influence the diversity of morphological characteristic is the environment. Tuber characterization results are shown in Table 1.
Table 1. Tuber Characteristic *Dioscorea* Spp. From Semarang and Boyolali-Indonesia

| No | Local Name               | Shape                | Skin Color | Length | Color after oxidation | Fresh tuber color | Oxidation duration | Relationship between tubers | There are Root spines |
|----|--------------------------|----------------------|------------|--------|-----------------------|-------------------|---------------------|--------------------------|----------------------|
| 1  | Uwi legi                 | Round-elongated      | Dark brown | 27 cm  | Brown                 | White             | < 1 minute          | United                   | Yes                  |
| 2  | Uwi bangkulit Semarang   | flattened            | Light brown| 26 cm  | White                 | White             | < 1 minute          | United                   | No                   |
| 3  | Gembili wulung           | rounded              | Purple     | 6 cm   | Purple-yellowish      | White-purple      | > 1 minute          | Separate                 | Yes                  |
| 4  | Gembili brol Semarang    | Cylindrical          | Light brown| 18 cm  | Purple-yellowish      | White             | < 1 minute          | Separate                 | Yes                  |
| 5  | Tomboreso                | Rounded              | Light brown| 21 cm  | Purple-yellowish      | White             | > 1 minute          | United                   | No                   |
| 6  | Uwi ungu                 | Cylindrical-oblong   | Dark brown | 28 cm  | Purple-yellowish      | White-purple      | < 1 minute          | United                   | No                   |
| 7  | Uwi bangkulit Boyolali   | Round-elongated      | Dark brown | 30 cm  | Orange-yellowish      | Orange            | < 1 minute          | United                   | No                   |
| 8  | Gembili tropong          | Cylindrical          | Light brown| 18 cm  | Brown                 | Yellowish         | > 1 minute          | Separate                 | No                   |
| 9  | Gembili brol Boyolali    | Cylindrical          | Light brown| 5 cm   | Brown                 | White             | < 1 minute          | Separate                 | No                   |
| 10 | Gembili pulung           | Cylindrical          | Dark brown | 4.5 cm | Brown                 | White             | < 1 minute          | Separate                 | No                   |

Table 2. Amylum Characteristics *Dioscorea* Spp. From Semarang and Boyolali-Indonesia

| No  | Local Name               | Amylum Shape | Hilum Position | Amylum length (µm) | Amylum wide (µm) | Amylum Type |
|-----|--------------------------|--------------|----------------|-------------------|------------------|-------------|
| 1   | Uwi legi                 | Oval         | Eccentric      | 422.5             | 275.5            | Monoadelph  |
| 2   | Uwi bangkulit Semarang   | Oval         | Eccentric      | 432.5             | 263.5            | Monoadelph  |
| 3   | Gembili wulung           | Circle       | Concentric     | 5.9               | 4.9              | Monoadelph  |
| 4   | Gembili brol Semarang    | Circle       | Concentric     | 8.7               | 7                | Monoadelph  |
| 5   | Tomboreso                | Oval         | Eccentric      | 422.5             | 275.5            | Monoadelph  |
| 6   | Uwi ungu                 | Oval         | Eccentric      | 427.5             | 340              | Monoadelph  |
| 7   | Uwi bangkulit Boyolali   | Triangle     | Eccentric      | 370               | 299.5            | Monoadelph  |
| 8   | Gembili tropong          | Circle       | Concentric     | 8.1               | 5.7              | Monoadelph  |
| 9   | Gembili brol Boyolali    | Circle       | Concentric     | 13.4              | 11.4             | Monoadelph  |
| 10  | Gembili pulung           | Circle       | Concentric     | 15.1              | 11.9             | Monoadelph  |

Based on Table 1, varieties originating from different regions have different morphological characteristics. Ten varieties have tuber types and annual growth. Tubers can only be harvested during the dry season, because during the rainy season the unprofitable tubers will experience the growth of shoots and other vegetative organs. Different morphological characteristics are evident in groups *D. esculenta* and *D. alata*. *D. esculenta* has cylindrical tuber, has 2-5 or >5 tubers per hill, and is not branched. *D. alata* and *D. pentaphylla* have round tuber shape, one form per hill with size large than *D. esculenta*. Differences in shape and surface of the tubers are influenced by different temperatures. Lower temperature made the tubers elongate and pale. Crack on the surface of the bulbs is affected by the hottest light intensity in the area. The temperature intake as a result the growth of the ball rose normally. This is in accordance with the Nonnecke (1989) statement that is if during tuber growth experienced high temperature stress the resulting tuber will be abnormal due to the growth of new
tubers from the performed bulbs called secondary growth (tuber fractures, tuber lengthening and tuber series).

Tubers pulps have a variety of colors. *D. esculenta* white to yellowish white, *D. alata* and *D. pentaphylla* have the color of white, orange and purplish white. Variations in the color of pulps are influenced by the amount of anthocyanin. Highly low anthocyanin content is influenced by environmental conditions. Both of light factors, microorganisms and nutrients are in the soil. According to Damanhuri (2005), differences in anthocyanin levels are caused by season and environmental conditions. The accumulation of anthocyanins is caused by environmental factors such as light, temperature, nitrogen source, pathogen attack and some substances such as cytokines, GA and ethylene. Tubers that produce much sap, will more quickly experience oxidation. The most rapidly oxidized and decayed variant is the gembili brol of Semarang.

Based on the results of the characterization of starch in Table 2, the type of starch *D. esculenta* is monoadelph, circular and the location of hilum in the center (concentris). Groups *D. alata* and *D. pentaphylla* have a monoadelph starch type, eccentric hilum and oval-shaped amylum and triangle. Circular, oval and triangular shapes are the common form of starch in the tuber groups. The shape of the starch is a special character that can be used to identify the type of starch from tuber plants. The circle is a typical form ‘gembili’ and oval shape is a hallmark of ‘uwi’ and ‘tomboreso’ starch. According to Richana (2004), granular form is characteristic of each starch that can be used to identify the types of starch.

Tuber characteristic morphology can used to determining the kinship relation resemblance variant based on the similarity. Result of cluster analys clumped many variant become a group at the certain distance based on their characteristic similarity. The higer similar between variant it will increase similarity presentage and closer the distance kinship. Based on analysis of 30 character of tuber and starch, get the result in Table 3 and Figure 1.

Table 3. Index Similarity *Dioscorea* Spp. From Semarang and Boyolali-Indonesia

|          | USM2 | USM4 | GSM1 | GSM2 | TSM | UBY2 | UBY4 | GBY1 | GBY2 | GBY3 |
|----------|------|------|------|------|-----|------|------|------|------|------|
| USM2     | 1    |      |      |      |     |      |      |      |      |      |
| USM4     | 0.35 | 1    |      |      |     |      |      |      |      |      |
| GSM1     | 0.25 | 0.26 | 1    |      |     |      |      |      |      |      |
| GSM2     | 0.41 | 0.27 | 0.47 | 1    |     |      |      |      |      |      |
| TSM      | 0.5  | 0.35 | 0.56 | 0.41 | 1   |      |      |      |      |      |
| UBY2     | 0.5  | 0.21 | 0.19 | 0.2  | 0.41| 1    |      |      |      |      |
| UBY4     | 0.41 | 0.21 | 0.25 | 0.14 | 0.26| 0.41 | 1    |      |      |      |
| GBY1     | 0.5  | 0.15 | 0.38 | 0.41 | 0.33| 0.33 | 0.41 | 1    |      |      |
| GBY2     | 0.5  | 0.15 | 0.38 | 0.41 | 0.33| 0.33 | 0.41 | 1    | 1    |      |
| GBY3     | 0.5  | 0.15 | 0.38 | 0.41 | 0.33| 0.33 | 0.41 | 1    | 1    | 1    |

Noted: USM2 (‘Uwi’ Legi), USM4 (‘Bangkulit_Semarang’), GSM1 (‘Gembili Wulung’), GSM2 (‘Brol Semarang’), TSM (‘Tomboreso’), UBY2 (‘Uwi Ungu Boyolali’), UBY4 (‘Uwi bangkulit Boyolali’), GBY1 (‘Gembili Tropong’), GBY2 (‘Gembili Brol Boyolali’) and GBY3 (‘Gembili Pulung’).

Based on Table 3, shows that variants that have similarities will be indicated by number one (1). While, variants that do not have any similarities will be indicated by number zero (0). ‘Gembili Tropong’, ‘Gembili Brol Boyolali’ and ‘Gembili Pulung’ have a close relationship. They have a similar characteristics based on their tuber morphologi and amylum type.
Based on Fig. 1, it shown that at the value of similarity 40, ten variants Dioscorea Spp. from Semarang and Boyolali-Indonesia clustering to be two clusters. The first cluster consist of ‘Gembili wulung’, ‘Tomboreso’, ‘Brol Semarang’, ‘Uwi Ungu’, ‘Uwi legi’, ‘Gembili Tropong’, ‘Gembili Brol Boyolali’, ‘Gembili Pulung’ and ‘Uwi Bangkulit Boyolali’. 2\textsuperscript{nd} cluster consist of ‘Uwi Bangkulit Semarang’. We know that ‘Uwi Bangkulit Semarang’ has a different characteristic from the other variant. It’s tuber length, wide and not have branches. At the value of similarity 1.0 ‘Gembili tropong’, ‘Gembili Brol boyolali’ and ‘Gembili Pulung’ are same. They have same tuber, from their size, color, and amyylum type.

According to Trimanto (2012), there are variations on the uwi caused of environmental factors. The environmental conditions that cause stress for long periods allow the plant to undergo a genetic change.

4. Conclusion

Semarang and Boyolali area there are three types of members Dioscorea spp. namely D. alata, D. esculenta and D. pentaphylla. D. alata has big tuber, flattened, one per hill has branch, variant colors, and oval or triangle amyylum. D. esculenta has small tuber, oval, ±5 tubers per hill, variant color, without branch and circle amyylum. D. pentaphylla has round tuber, branch, big tuber, white tuber and oval amyylum. From this research we know that variants ‘gembili pulung’, ‘gembili brol’, and ‘gembili tropong’ have same characteristics, so its have near relationship. And we know that ‘tomboreso’ near to ‘gembili’ than ‘uwi’.

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