RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
49 (1) 1-8, 2017

VARIABILITY AND INTRASPESIFIC CLASSIFICATION OF GEMBILI (*Dioscorea esculenta* (Lour.) Burk.) BASED ON MORPHOLOGICAL CHARACTERS

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SUMMARY

Gembili species of *Dioscorea esculenta* (Lour.) Burk. a climber plant producing tubers, belongs to *Dioscoreaceae*. This plant species has morphological variation on its vegetative organs (i.e. stems, leaves, and tubers). Thirty accessions of gembili were collected from Java, Sumatera, Kalimantan, and Celebes (Sulawesi) of Indonesia. Tuber samples were collected using survey methods and cultivated as a living collection. Based on IPGRI plant descriptors for *Dioscorea* tubers, stems, leaves, flowers, and fruits were scored and analyzed using UPGMA method. The results show that the level of diversity of gembili is quite high, ranging from 0.64-1.00 similarity values. Those accessions were divided into 2 groups: the first cultivar group has dense thorns on roots, oblong to cylindrical tuber shapes with white to yellowish-white tuber flesh. Those characters are similar to *D. esculenta* (Lour.) Burk. var. *spinosa* (Lour.) Burk. whereas the second group showed rare thorn roots, irregular tuber shapes, and violet tuber flesh color, and the characters are similar to *D. esculenta* (Lour.) Burk. var. *fasciculata* (Lour.) Burk. This result revealed that the variability of *D. esculenta* in Indonesia is high.

Key words: *Dioscorea esculenta*, morphology, variation, intraspecific classification

Key findings: This research detected morphological variation within *Dioscorea esculenta* for classification purposes into certain groups. Breeders can utilize morphological variation data to create new cultivated varieties in accordance with the public interest.

Manuscript received: April 23, 2016; Decision on manuscript: July 24, 2016; Manuscript accepted: August 28, 2016.

Communicating Editor: Bertrand Collard

INTRODUCTION

Species of *Dioscorea esculenta* (Lour.) Burk. is the tuber crop of Dioscoreaceae family and its belongs to the Opsophyton section (Onweume & Ganga, 1996; Chair et al., 2005). The plants have local names such as Lesser yam, Chinese yam, Asiatic yam, or sweet yam (English), and in Indonesia they have several local names, e.g. *gembili* (Java), and *ubi opa'* (Central Sulawesi) (Heyne, 1987; Purnomo, 2010). This species has geographical distribution in Nigeria, China, and Mexico, and this species also has its center of diversity in China (Zannou *et al.*, 2009).

Morphologically *D. esculenta* was differentiated from the other species of *Dioscorea* based on its sinister voluble, spiny, bulbil stem, and with spines on the root above

the tuber (Backer and Bakhuizen van den Brink, 1968; Sastrapradja and Rifai, 1989; Purnomo *et al.*, 2012). Anatomical analysis indicates that trichome with T-shape on stems and leaves are also specific characters of *D. esculenta* (Onweume & Ganga, 1986; Purnomo, 2013).

Based on morphological and molecular characters, genetic variability of *D. esculenta* in Africa has already been investigated, and it has the second highest genetic variability after *D. alata* L. (Zannou *et al.*, 2009; Tamiru *et al.*, 2007; Norman *et al.*, 2011). However, genetic variability of *D. esculenta* in Asia based on morphological characters was studied because these crops are planted frequently in the dry areas around village fences. Hence, these may be relics of cultivation of edible tubers found in these areas (Backer and Bakhuizen v.d. Brink, 1968; Heyne, 1987).

Tubers of *D. esculenta* have been used traditionally by Indonesian people, especially in Java, for a long time. They boiled and consumed cooking tubers of *D. esculenta* directly, and in traditional markets there are always boiled tubers of *D. esculenta* moreover. Gembili in West Papua is used as the staple food after talas (Achmady and Schneider, 1995). Tubers of *D. esculenta* is an ingredient for the manufacture of various types of food (Maneenoon *et al*., 2008). Harvest tuber of *D. esculenta* contains starch of 758.65 ± 8.83 mg/g, non-reduced sugar of 68.28 ± 0.85 mg/g, and reduced sugar of 8.67 ± 0.41 mg/g, and in the dormant periods, tubers show the presence of sucrose, glucose, fructose, and maltose (Otoo *et al*., 2009**)**. Tuber starch of *D. esculenta* is similar with batatas tuber (*Ipomoea batatas*) both physically and in chemistry (Jayakodi *et al.*, 2007). Clones from specific cultigens were already made for *D. esculenta* with high sugar content (Malaurie *et al*., 1993). Wheat and lesser yam composite flour at the ratio of 80:20 w/w could be used for the production of bread that is comparable to those made with sole wheat bread flour in Nigeria (Ukpabi, 2010). Preliminary phytochemical screening confirmed the presence of saponins, disgenin, sistosterol, stigmasterol, cardiac glycosides, fat and starch. The extract exhibited significant inhibition of the carrageenan-induced oedema that was dose-dependent with a good initial effect in 1 h and 2 h at doses of 100 mg/kg and 150 mg/kg, respectively. *D. esculenta* tuber methanol extract supports the folkloric use for management of inflammation (Olayemi & Ajaeyeoba, 2007). Native yam starch (*D. esculenta*) and its carboxymethyl yam starch (CMS) derivative can be used as tablet disintegrates formulation (Nattapulwat *et al*., 2008).

The objectives of this study are to determine the genetic variability and to classify *D. esculenta* into cultivar groups or intraspecific classification. This study also has objectives to select the best cultigens from nature and to create a specific cultivar, which is suitable for human consumption.

MATERIALS AND METHODS

Plant materials of *D. esculenta* were collected from Java, Sumatera, Kalimantan, and Sulawesi of Indonesia, covering representatives of local cultivars. Samples were collected using survey method based on the center of *D. esculenta* cultivation. Samples of tubers, stems, leaves, flowers, and fruits are made as dried specimens. The tubers were planted as a living collection. Morphological characterization was based on International Plant Genetic Resources Institute (IPGRI) (Annonymous, 1997) for *D. esculenta* with some modifications (Table 1). Scoring and coding were done by using binary and multistate techniques. Jaccard similarity coefficient was calculated as a measure of similarity among accessions as Operational Taxonomic Unit (OTU). Qualitative morphological data were analyzed descriptively to determine the specific character among accessions. The Unweighted Pair-Group Method Using Arithmetic Average (UPGMA) clustering technique was used to construct dendrogram illustrating taxonomic affinity of accessions. Data analysis was performed using Numerical Taxonomy and Multivariate Analysis System (NTSYSpc2.1) (Rohlf, 2000).

Table 1. Scoring and coding characters based on descriptor of *Dioscorea* (IPGRI) of *D. esculenta* accessions.

Morphological characters	Scoring and coding
Stem twist	0 = Dexter, 1 = sinister
Thorns in leaf base	0= absence, 1= presence
Color of stem	0= green, 1= purplish green, 2= purple
Color of thorns	0= green, 1= purplish green, 2= purple
Lignification of young stem	0= presence, 1= absence
Thorns on stem surface	0= absence, 1= presence
Stem cross section	0= rounded, 1= angular
Thorn on leaf petiole	0= absence, 1= presence
Petiole size	0 $\leq$ 5 cm, 1= 6-9 cm, 2$\geq$ 9 cm
Petiole shape	0= cylindrical, 1=plat, 2= cylindrical with clave
Color of petiole	0= green, 1= purplish green, 2= purple
Leaf shape	0= ovate, 1= cordate, 3= sagitate
Leaf base	0= emarginated, 1= imbricate
Leaf tip	0= acute, 1= acuminate
Leaf margin	0= integer, 1= serrate, 2= crenate
Number of lateral nerves	0= 3, 1= 5, 2$\geq$ 5
Venation arching	0= densely, 1= rare
Color of Staminate flower rachis	0= green, 1= purplish green, 2= purple
Color of sepal and petal	0= green, 1= reddish green
Color of anthers	0= light brown, 1= dark brown
Color of pistilate flower rachis	0= green, 1= reddish green
Color of fruit wing	0= green, 1= reddish green
Thorns root above the tuber	0= short densely, 1= long rarely
Sharpness of thorns root	0= sharp, 1= obtuse
Tuber shape	0= single oblong, 1= irregular, 2= cylindrical
Tuber surface	0= densely rooted, 1= rarely rooted, 3= absence
Color of tuber skin	0= dark brown, 1= light brown, 2= yellow
Color of tuber flesh	0= white, 1= yellowish white, 2= violet
Texture of tuber flesh	0= soft, 1= sandy, 2= hard
Tuber sap	0= presence, 1= absence

RESULTS AND DISCUSSION

Morphological variability

The roots of *D. esculenta* are adventitious roots; many lateral roots grow from stem base, while long slender and sharp spines grow on the root branch near the top of the tuber. The spine by peoples in Java is called as *duri kemarung* (Figure 1. k,l). *D. esculenta* thorns used to defend against predators such as hedgehogs, wild boar, and the others (Purnomo, 2010).

Tubers of *D. esculenta* are divided into oblong and cylindrical tuber shapes (Figure 1. h,i,j,k). Each tuber has a papyrus tuber skin yellow to brown in color. Tuber flesh has 3 color variations (i.e. white, yellowish white and violet (Figure 1. j,l,m).

Stems of *D. esculenta* are a sinister twist, have a pair of spines on the leaf base, a cylindrical cross section, and a ligneous stem. Stems of *D. esculenta* with green color always produce white or yellowish white tuber flesh color and stems with purple color produce violet tuber color (Figure 1. a,b).

D. esculenta has a single leaf with spread leaf position, petiole as long as leaf blade, cordate leaf blade, acuminate apex, and integer margin, and leaf base is split into 2 lobes. Venation is palmate type, 5-7 nerves and prominent. Leaves venation of *D. esculenta* is green and or violet color (Figure 1. c,d), which is correlated to the tuber flesh color.

Specific characters on stem and leaves of *D. esculenta* are trichomes shape, dichotomous branching trichomes with single

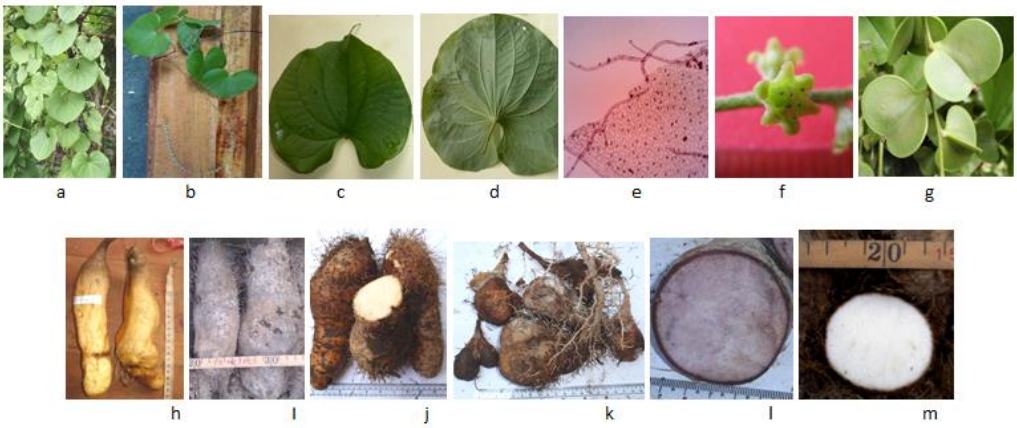

Figure 1. Morphological characters of *D. esculenta*: a. green stem type, b. Purple stem type, c. lobes of leaf base, d. imbricate leaf base, e. T-shapes trichome on leaf surface, f. staminate flower with 6 perianths and 6 stamens, g. pistilate flower with 3 wings, h. tuber shape of *gembili pelus*, i. tuber shape of *gembili biasa*, j. tuber of gembili jewot with dense root on the surface and yellowish-white tuber flesh, k & l. tuber of *gembili ungu* (*senggani*) with dominance thorns root above the tuber, with violet tuber flesh color, m. white tuber flesh color of *gembili pelus*.

cells at the base of the tip covered with 2 branches shaped similar to the letter T (i.e. T-shaped hairs) (Figure 1. e).

Staminate flowers in panicle, with 6 stamens and anthers with 2 cells. Pistilate flower in spike, with wing ovary, 3 cells, and ovule parietal-lateralis position. Fruit with 3 dominance wing permanence (Figure 1. f,g).

Intraspecific Classification

Accessions of D. esculenta

Accessions of *D. esculenta* is determined based on differences in the shape and color of tubers, and in this study area 18 accessions were found. Accession information is presented in Table 2.

Table 2 shows that there are 6 local names based on tuber shapes and colors, namely gembili *gembili biasa, gembili jewot, gembili ungu, gembili pelus, gembili welut,* and *gembili alus. Gembili biasa* and *jewot* are distinguished by the dense roots on the tuber surface, with a dark brown tuber skin color. *Gembili pelus, welut,* and *alus* have the rare rooted on the tuber surface with light brown to yellow tuber skin color (Figure 1).

Cluster analysis

A dendrogram (Figure 2) was created based on morphological similarity index (Jaccard formula) and UPGMA cluster analysis (Sokal and Sneath, 1963), with NTSYSpc 2.1 software (Rohlf, 2000). Furthermore, for scoring and coding 30 morphological characters were compared (Table 1). The dominant characters that affect the dendrogram are the color of stem, petiole, thorns, tuber skin, and tuber flesh.

The dendrogram (Figure 2) shows that the accession of *D. esculenta* is divided into 2 clusters A and B. Morphological variability was shown by the similarity among the accessions (OTUs) that were tested. Clusters A and B split the similarity index value of 0.64 and cluster A.a consists of all accessions with similarity index value of 0.82, while cluster A.b was grouped on the similarity index value of 0.84. The accessions with morphological similarity index value in cluster A.a. is very high (i.e. between accessions 2-9-19, 13-18-21-25). This data indicates that they were vegetatively propagated (clones) through tuber of *D. esculenta* (Tamiru *et al.*, 2007; Zannou *et al.*, 2009; Ukpabi, 2010). Similarly, in cluster A.b., all accessions clustered based on morphological similarity index value were above 0.91.

Table 2. Accession number, local name, location, and tuber character.

No	Accession number	Local name	Location	Tuber Characters (tuber shape, surface root, color of tuber skin, color of tuber flesh)
1	01/TTBIO	Gembili jewot	Karebet, Sendangsari, Yogyakarta	Oblong (cylindrical), dense, dark brown, yellowish-white
2	02/TTBIO	Gembili jewot	Karebet, Sendangsari, Yogyakarta	Oblong (cylindrical), dense, dark brown, yellowish-white
3	03/TTBIO	Gembili pelus	Karebet, Sendangsari, Yogyakarta	Oblong (cylindrical), rare, light brown to yellow, white
4	04/TTBIO	Gembili ungu	Karebet, Sendangsari, Yogyakarta	Irregular obovate, densely root, dark brown, white ring with violet centre
5	05/TTBIO	Gembili jewot	Purworejo, Central Java	Oblong (cylindrical), dense, dark brown, yellowish-white
6	06/TTBIO	Gembili jewot	Pajangan, Bantul, Yogyakarta	Oblong (cylindrical), dense, dark brown, yellowish-white
7	07/TTBIO	Gembili ungu	Gedang sari, Gunung Kidul, Yogyakarta	Irregular obovate, densely root, dark brown, white ring with violet centre
8	08/TTBIO	Gembili pelus	Nanggulan, Kulon Progo, Yogyakarta	Oblong (cylindrical), rarely root, light brown to yellow, white
9	09/TTBIO	Gembili biasa	Cawas, Klaten, Central Java	Oblong (cylindrical), dense, dark brown, yellowish-white
10	10/TTBIO	Gembili biasa	Karang Mojo, Gunung Kidul, Yogyakarta	Oblong (cylindrical), dense, dark brown, yellowish-white
11	11/TTBIO	Gembili jewot	Kasihan, Bantul, Yogyakarta	Oblong (cylindrical), dense, dark brown, yellowish-white
12	12/TTBIO	Gembili jewot	Pandak, Bantul, Yogyakarta	Oblong (cylindrical), dense, dark brown, yellowish-white
13	13/TTBIO	Gembili jewot	Purworejo, Central Java	Oblong (cylindrical), dense, dark brown, yellowish-white
14	14/TTBIO	Gembili welut	Klaten, Central Java	Oblong (cylindrical), rarely root, light brown to yellow, white
15	15/TTBIO	Gembili biasa	Moyudan, Sleman, Yogyakarta	Oblong (cylindrical), dense, dark brown, yellowish-white
16	16/TTBIO	Gembili alus	Banjarnegara, Central Java	Oblong (cylindrical), rarely root, light brown to yellow, white
17	17/TTBIO	Gembili biasa	Sendang Sari, Bantul, Yogyakarta	Oblong (cylindrical), dense, dark brown, yellowish
18	18/TTBIO	Gembili jewot	Galur, Kulon Progo, Yogyakarta	Oblong (cylindrical), dense, dark brown, yellowish
19	19/TTBIO	Gembili	Tanah Bumbu, South Kalimantan	Oblong (cylindrical), dense, dark brown, yellowish
20	20/TTBIO	Gembili	Tanah Bumbu, South Kalimantan	Oblong (cylindrical), dense, dark brown, yellowish
21	21/TTBIO	Gembili	Batu Licin, South Kalimantan	Oblong (cylindrical), dense, dark brown, yellowish
22	22/TTBIO	Gembili	Banjar Baru, South Kalimantan	Oblong (cylindrical), dense, dark brown, yellowish
23	23/TTBIO	Gembili	Mojopahit, Lampung , South Sumatera	Oblong (cylindrical), dense, dark brown, yellowish
24	24/TTBIO	Gembii	Tanggamus, Lampung , South Sumatera	Oblong (cylindrical), dense, dark brown, yellowish
25	25/TTBIO	Gembili	Tanggamus, Lampung, South Sumatera	Oblong (cylindrical), dense, dark brown, yellowish
26	26/TTBIO	Ubi Opak	Luwuk, Central Sulawesi	Oblong (cylindrical), dense, dark brown, yellowish
27	27/TTBIO	Ubi Opak	Toili, Central Sulawesi	Irregular obovate, densely root, dark brown, white ring with violet centre
28	28/TTBIO	Ubi Opak	Banggai Island, Central Sulawesi	Oblong (cylindrical), dense, dark brown, yellowish
29	29/TTBIO	Ubi Opak	Luwuk, Central Sulawesi	Oblong (cylindrical), dense, dark brown, yellowish
30	30/TTBIO	Ubi Opak	Banggai Island, Central Sulawesi	Oblong (cylindrical), dense, dark brown, yellowish

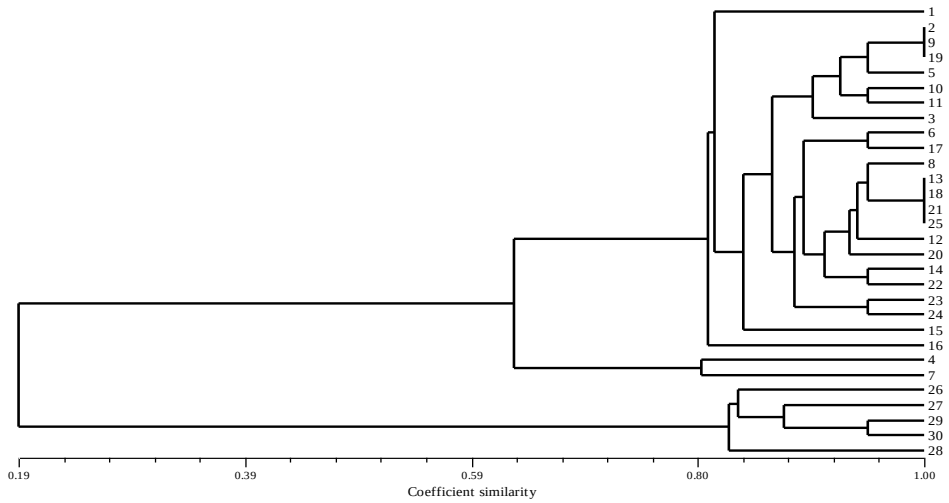

Figure 2. Dendrogram showing phenetic relationships between accession and intraspecific classification of gembili (*Dioscorea esculenta* L) based on morphological characters. The right number is the OTUs (Tables 1 and 2).

Cluster A.a is the accession of *D. esculenta* that has a white to yellowish white tuber flesh color, consisting of 16 accessions. This cluster was divided based on the tuber skin and tuber flesh color. Cluster A.a was composed of accessions 1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 and 25 which were characterized by oblong (cylindrical) tuber shape, densely rooted on the tuber surface, with dark brown tuber skin and yellowish-white tuber flesh. People in Yogyakarta and the surrounding areas called it *gembili biasa* or *gembili jewot*. There are many roots on the tuber surface like dense hairs. According to Onweume and Ganga (1996) and Burkill (1954), this group accessions is similar to the characteristics of *D. esculenta* (Lour.) Burk. var. *spinosa* (Roxb.) Prain & Burk. While Heyne (1987) refers to this variety as *huwi butul* or *huwi landak*.

Cluster A.b, which was composed of accessions 4 and 7, has specific morphological characters including an oblong (cylindrical) tuber shape rarely rooted on the tuber surface, with light brown to yellow tuber skin and white tuber flesh. The second cluster is composed of *gembili pelus*, *gembili welut*, or *gembili alus* group. The characters resemble *D. esculenta* (Lour.) Burk. var. *fasciculata* (Roxb.) Prain & Burk., which only has a few thorns on the roots (Onweume and Ganga, 1996). Other characters that are specific are tuber skin color, which light brown to yellow with a smooth surface. This variety includes *gembili bulu*, which has long roots with spread spines, and tuber flesh, which has no fibers (Heyne, 1987).

Cluster B is composed of 5 accessions of *D. esculenta*, namely 26, 27, 28, 29 and 30. This cluster has an irregular tuber shape and is white with a violet ring tuber flesh and the local name is *gembili ungu*; the population of its morphological variant in Indonesia is very rare. *Gembili ungu* is used by people for traditional medicine, especially digestive complaints. The third group has a different population abundance such as *gembili jewot* with the greatest abundance, then *gembili pelus*, and the least abundant is *gembili ungu.*

Gembili ungu is used for drugs due to its containing diosgenin derivatives or because of the presence of antosian in the tuber (Olayemi and Ajaeyeoba, 2007). Native lesser yam starch (*D. esculenta*) and its carboxymethyl yam starch (CMS) derivative can also be used as constituent materials in the manufacture of tablets (Nattapulwat *et al.*, 2008).

CONCLUSION

Based on the data and discussion, it can be concluded that the population of *D. esculenta* in Indonesia has a high genetic variability based on morphological characters. The range of values of the morphological similarity index is 0.64-1.0 0. Morphological variation of *D. esculenta* is shown by the density of root thorns, root density at the surface of the tuber, tuber skin color, and tubers flesh color. Based on tuber shape, tuber flesh color, density of root thorns, and the density of the root tuber surface, *D. esculenta* has been divided into 3 groups of accessions, which are *gembili jewot* (*D. esculenta* (Lour.) Burk. var. *spinosa* (Roxb.) Prain & Burk.), *gembili pelus* (*D. esculenta* (Lour.) Burk. var. *fasciculata* (Roxb.) Prain & Burk.), and *gembili ungu.*

ACKNOWLEDGMENT

We would like to thank the Indonesian Managing Higher Education for Relevancy and Efficiency (I-MHERE) Project, Faculty of Biology, Gadjah Mada University, Indonesia, for supporting us financially through Research Grant No: UGM/BI/1157/I/ 05/04.

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