

Assessment of genetic diversity and characterization of distinctness, uniformity, and stability of newly bred sweet potato clones

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Abstract. Mau YS, Ndiwa ASS, Arsa IGBA, Asa GV, Nana A, Londingkene JA, Hosang EY, Kotta NRE. 2022. Assessment of genetic diversity and characterization of distinctness, uniformity, and stability of newly bred sweet potato clones. *Biodiversitas* 23: 5923-5934. Genetic diversity assessment is vitally important for germplasm management and the assembly of new varieties. This study aimed to 1) assess the genetic diversity and 2) characterize the distinctness, uniformity, and stability of newly bred clones and check varieties of sweet potatoes based on morphological characters. The present study was conducted in the experimental farm of Universitas Nusa Cendana for two cycles (years) in 2021 and 2022, involving 13 newly bred (hybrid) clones and 6 control varieties. The observations were made on morphological characters of leaf, vine, and storage root, including 31 characteristics. The observed characters were given scores based on the sweet potato descriptors of IPGBR and PPVTPP. The scored morphological data were subjected to cluster analysis followed by a Principal Component Analysis to reveal the genetic diversity level. Euclidean index was used to characterize the distinctness, while the variation of the scored morphological data was used to reveal the uniformity and stability of tested genotypes. The results revealed a high genetic diversity of the studied genotypes. The cluster analysis placed the studied genotypes into four clusters; cluster I consisted of only one genotype, cluster II comprised two sub-clusters and 13 genotype members, cluster III comprised two members, and cluster IV comprised two sub-clusters and three members. The first eight principal components were responsible for about 79% of the observed variability. Euclidean distance index revealed that each of the studied genotypes is distinct from others. No off-type plant was observed; thus, each of the tested genotypes was considered uniform. Phenotypic expressions of the studied genotypes were similar over the two growing cycles; thus, each is said to be stable. The newly bred genotypes G16 and G29 genotypes that meet the distinctness, uniformity, and stability criteria based on their highest number of distinctive characters are eligible for registration as new sweet potato varieties, more specifically for their unique purplish white and purple tuber flesh characters.

Keywords: Cultivar, divergence, morphological traits, qualitative, sweet potato

INTRODUCTION

Sweet potato [*Ipomoea batatas* L. (Lam)] is grown in many parts of Indonesia. It has become the second most important tuber crop after cassava due to its high nutritional value and multiple uses as food, feed, and industrial raw materials. East Nusa Tenggara (ENT) Province is one of Indonesia's sweet potato production centers (BPS Pusat 2020). The crop has been traditionally cultivated for generations and used as a staple food besides rice and maize. Sweet potato productivity in ENT Province, however, is still low (~9 t ha⁻¹); far beyond that of the national level (~19 t ha⁻¹) (BPS Pusat 2021) and yield potential of superior varieties (>25 t ha⁻¹) (Balitkabi 2016). The use of local varieties that are mostly low yielding (Mau et al. 2013) and susceptible to sweet potato weevil/SPW (*Cylas formicarius* Fab.) (Mau et al. 2011) is among the factors contributing to low sweet potato productivity in ENT Province.

A promising approach to tackle the low sweet potato productivity is by assembling sweet potato cultivars with high yield, good resistance to the sweet potato weevil and

other desirable traits. This approach can be done through hybridizing the existing local cultivars and superior national varieties for the combination/pyramiding of desirable traits in a single clone/variety.

Breeding a sweet potato variety for desirable traits needs parental materials that harbor the traits of interest, which then can be combined in a single clone/variety through hybridization. In addition, as the sweet potato hybrids (F₁s) can be directly evaluated, selected, and registered/released as a new variety, the use of divergent parental materials is important for the maximal exploitation of high heterotic effects in hybrids. Chanda et al. (2014) stated that a breeding program employing diverse parental lines is most likely to generate superior varieties. Identification of divergent parental lines is, therefore, a crucial step to be done before the hybridization/breeding program, and this can be done by assessing germplasm genetic diversity/divergence.

The genetic diversity of sweet potatoes can be assessed using morphological and agronomical characteristics (Fongod et al. 2012; Tairo et al. 2018; Ochieng 2019). A combination of agro-morphological characters and

molecular markers has also been effectively used in dissecting the genetic diversity of sweet potatoes (Maquia et al. 2013; Koussao et al. 2014; de-Andrade et al. 2017). In addition, Mbithe et al. (2016) employed morphological characteristics to assess the genetic diversity and select dual-purpose sweet potato genotypes for food and feed.

Expression of morphological characteristics of leaf, vine/stem, and storage root are not much influenced by environmental factors; thus, they have been commonly used in assessing sweet potato diversity. Furthermore, morphological characters have also been used in the characterization of distinctness, uniformity, and stability (DUS) of plant genotypes for registration of new plant varieties and plant variety protection (PVP) of many crops (Selvi et al. 2013; Rao et al. 2013; Nehra et al. 2016).

Assessment of genetic diversity and DUS of sweet potatoes have been relying on the morphological descriptors consisting of 27 morphological characteristics of IPGBR (CIP, AVRDC, IBPGR 1991) and UPOV (2010). In Indonesia, modified descriptors of IPBGR and UPOV was recently introduced by PPVTPP (2021) to be used as a standard descriptor for the registration of new sweet potato variety. The descriptor consists of 34 morphological characteristics; 31 characteristics are compulsory, and 3 characteristics are optional.

A collection of newly bred sweet potato clones of the Agrotechnology Department of Universitas Nusa Cendana have been evaluated for important traits such as yield potential and drought tolerance (Mau et al. 2019), SPW resistance (Mau et al. 2021a) and scab disease resistance (Mau et al. 2021b). Some of these sweet potato clones meet the requirement for registration as new varieties but to be registered as a new variety, a plant genotype must have at least one character that is unique/distinct from other varieties, and the character (s) are uniform and stable for at least two growing cycles. Thus, information about the distinctness, uniformity, and stability of morphological characters/traits must be determined before a new sweet

potato variety is registered. Therefore, the objectives of the present study were 1) to assess the genetic diversity and 2) to characterize the distinctness, uniformity, and stability of the newly bred clones and control varieties of sweet potato based on morphological characteristics.

MATERIALS AND METHODS

Research site

The study was conducted on the experimental farm of Universitas Nusa Cendana, located at Kupang Tengah Sub-district, Kupang District, East Nusa Tenggara Province, Indonesia. The study site is 68 m a.s.l, 10° 09'02" South latitude and 123° 42' 02" East longitude. The study was conducted for two growing cycles, from March to August 2021 (cycle 1) and March to August 2022 (cycle 2). The soil type of the research site is Grumosol (USDA), with a sandy clay texture.

Plant materials

Nineteen sweet potato genotypes were evaluated in this study. The studied sweet potato genotypes comprised 13 hybrids (newly bred) clones, four national reference/checks varieties, and two local cultivars. The national control varieties and local cultivars were used as comparable varieties. The control varieties (Antin 3, Beta 3, Papua Salossa, and Pating 1) were provided by BALITKABI (Balai Penelitian Kacang-kacangan dan Umbi-umbian/Indonesian Legume and Tuber Crop Research Institute), Malang, East Java, while the hybrid clones and local varieties were obtained from the collection of Plant Breeding Section, Agrotechnology Department, University of Nusa Cendana. The plant materials were collected as vine cuttings from the Archipelagic Dryland Field Laboratory of Universitas Nusa Cendana. A list of the studied sweet potato genotypes is presented in Table 1.

Table 1. List of 19 sweet potato genotypes used in the present study

Genotype/code	Crosses/origin/source
G1	NPL/PSOL.16//JPV-01, Universitas Nusa Cendana collection
G4	NPL/PSOL.16//JPV-04, Universitas Nusa Cendana collection
G5	NPL/PSOL.16//KDL.11-05, Universitas Nusa Cendana collection
G6	NPL/PSOL.16//KDL.11-06, Universitas Nusa Cendana collection
G9	JPV/KDL.11-02, Universitas Nusa Cendana collection
G11	NPL/PSOL.16//JPV-01, Universitas Nusa Cendana collection
G15	CIL/JPV-01, Universitas Nusa Cendana collection
G16	NPL//CIL/JPV-01, Universitas Nusa Cendana collection
G18	JPV/NPL-02, Universitas Nusa Cendana collection
G21	JPV/KDL.11, Universitas Nusa Cendana collection
G23	KDL/V1-CIL.01, Universitas Nusa Cendana collection
G24	KDL/NPL.02, Universitas Nusa Cendana collection
G29	CIL/ATN 3, Universitas Nusa Cendana collection
JPV-01	Local Cultivar, West Timor, ENT Province, Indonesia
Loel Molo	Local Cultivar, West Timor, ENT Province, Indonesia
Antin 3	Indonesian released/control variety, Balitkabi
Beta 3	Indonesian released/control variety, Balitkabi
Papua Salossa	Indonesian released/control variety, Balitkabi
Pating 1	Indonesian released/control variety, Balitkabi

Note: G: genotype; denoted to newly bred/hybrid clones

Research design

The research employed a Randomized Block Design in each cycle. The treatments assigned were 19 sweet potato genotypes, 13 newly bred/hybrid clones, and 6 national and local varieties/cultivars as control. Each genotype/treatment was four replicates in each growing cycle (year), with 76 plots per cycle or 152 plots in two cycles.

Field preparation and planting

The experimental field was plowed at 30 cm depth, and 3 m x 1.5 m (4.5 m²) plots were prepared for each experimental unit. Spacing between blocks was 100 cm, while within-plot spacing was 50 cm. A basal compound fertilizer (NPK: 16:16:16) at a rate of 300 kg ha⁻¹ (135 g plot⁻¹) was applied at sowing. The planting materials used were shoots of 30-40 cm in length or about 4 - 6 nodes each. The shoots were obtained from each sweet potato genotype of two months old. Plant spacing was 50 cm within the row; one cutting was planted in one planting hole, with five plants per plot. A standard sweet potato cultivation technique was applied throughout the two growing cycles (PPPTP 2012).

Observation

Observations were made on morphological characters of sweet potato descriptors (CIP, AVRDC, IBPGR 1991; PPVTPP 2021). The descriptors consisted of 31 characters, 26 visually observed (VG) and 5 measured (MS) characters, as described in Table 2. The shoot and stem characters were observed 100 days after planting, while the tuber morphological characters were observed at harvest (140 days after planting). The observation was done on five plants per plot/replication, or 20 plants per cycle.

Data analysis

Visually observed characters of each plant/plant's organs were directly scored based on their phenotypic expression/classes. Meanwhile, the measured characters were first averaged over all observed plants/plant organs per plot, and then, the means were scored for phenotypic classes, as presented in Table 2. As the phenotypic classes of the observed and measured characters of the studied genotypes were similar between the two growing cycles (2021 and 2022), the data of the two trials/cycles were pooled for analysis. Cluster analysis employing scored morphological data based Euclidean distance and Un-Weighted Pair Group Method with Arithmetic mean (UPGMA) was performed to assess the genetic diversity of the studied genotypes. Principal Component Analysis was also carried out to reveal the characters mostly contributing to the observed variation. The distinctness between genotypes was assessed based on the genetic distance (Euclidean index) employing the pooled morphological characters over the two growing cycles. At the same time, the uniformity was determined based on the presence or absence of off-type plants. Finally, the stability was determined descriptively based on the level of variation of the scored morphological characters over the two growing cycles. All the statistical analysis was performed using PAST (Hammer et al. 2001) version 4.03.

RESULTS AND DISCUSSION

Morphological characteristics

Observed variables included leaf, shoot/stem, and tuber morphological characteristics. The observed morphological characters are presented in Table 3. This table show variation among the sweet potato genotypes on each observed morphological character. Among the 31 observed characters, 12 characters (38.71%) were dimorphic, and the rest 19 characters (61.29%) were polymorphic (≥ 3 expressed scores/phenotypic classes) for all genotypes. None of the characters was monomorphic for all the genotypes. The dimorphic characters included 10 characters, i.e., 6 (vine secondary color), 7 (anthocyanin coloration on the tip of the vines), 8 (anthocyanin coloration on internode), 9 (anthocyanin coloration on the node), 12 (presence of lobules on the leaf), 17 (main color of mature leaf upper surface), 18 (anthocyanin coloration on leaf upper surface vein), 19 (anthocyanin coloration on leaf margins), 20 (main color of mature leaf lower surface), 21 (anthocyanin coloration and distribution on abaxial leaf vein), 30 (storage root flesh secondary color), and 31 (distribution of storage root flesh secondary color). Meanwhile, the rest 21 characters were polymorphic (three or more expression levels/scores).

Polymorphic characters that were expressed in more than three categories/scores included length of the main shoot (2), pubescence of tips of the vines (10), the shape of leaf blade (13), depth of lobules (14), number of lobules (15), central lobule format (16), anthocyanin coloration and distribution on abaxial leaf vein (21), distribution of anthocyanin on petioles (24), the pattern of anthocyanin distribution on petioles (25), shape of storage root (26), the predominant color of storage root bark (28), and storage root flesh predominant color (29).

Among the 31 morphological characters observed, six characters are categorized as grouping characteristics. Grouping characteristics are the characters whose documented states of expression can be used, either individually or in combination with other such characters. The grouping characters are used to (i) select common knowledge varieties to be excluded from the trial used for assessment of distinctness, and (ii) to organize the growing trial so that similar varieties are grouped (UPOV 2010). The agreed grouping characters include; (i) plant growth habit, (ii) stem anthocyanin coloration of tips, (iii) presence of leaf blade lobules, (iv) shape of storage root, (v) storage root main color of bark, and (vi) storage root flesh main color. The observed grouping characters of the tested sweet potato genotypes are presented in Table 4.

Most of the sweet potato genotypes (57.89%) exhibited a semi-erect plant type, while the rest of the genotypes exhibited erect (21.05%) and spreading (21.05%) plant type. Anthocyanin pigmentation on the tips of the vines was present in 9 genotypes (47.37%), while the rest of the genotypes (10 genotypes, 52.63%) showed no anthocyanin pigmentation on the vine tips. Lobules on the leaf were present in the majority (68.42%) of tested genotypes, while six genotypes (G4, G5, G6, G21, G23, and Beta 3, 31.58%) produced no lobules on the leaf. The shape of the storage

root fell into five categories, i.e., round and elliptical (G1, G21, G23, and Beta 3, 21.05%), elliptical (G4, G9, G15, G29, Loel Molo, and Papua Salossa, 31.58%), oblong and long (Pating 1, 5.26%), long and elliptical (G6, G16, G24, JPV-01, and Antin 3, 26.32%) and long irregular or curved (G5, G11, and G18, 17.79) (Table 4).

Table 2. Morphological characteristics/descriptors observed in the present study

Characteristics	Expression and score (Phenotypic class)
Plant type (VG)	3-erect; 5-semi compact; 7-spreading; 9-extremely spreading
Length of the main vines (MS)	3-short (< 75 cm); 5-medium (75-150 cm); 7-long (151-250 cm); 9-very long (> 250 cm)
Shoot internode diameter (MS)	1-very thin (< 43 mm); 3-thin (4-6 mm); 5-medium (7-9 mm); 7-thick (10-12 mm); 9-very thick (>12 mm)
Shoot internode length (MS)	1-very short (< 3 cm); 3-short (3-5 cm); 5-intermediate (6-9 cm); 7-long (10-12 cm); 9-very long (> 12 cm)
Vine predominant color (VG)	1-green; 2-red; 3-purple; 4-deep purple
Vine secondary color (VG)	0-absent; 1-present
Anthocyanin coloration on the tip of the vines (VG)	0-absent; 1-present
Anthocyanin coloration on internode (VG)	0-absent; 1-present
Anthocyanin coloration on the node (VG)	0-absent; 1-present
Pubescence of tips of the vines (VG)	0-absent; 1-sparse; 2-moderate; 3-heavy, 4-very heavy
Upper part color of the immature leaf (VG)	1-greenish yellow; 2-light green; 3-medium green; 4-dark green; 5-light purple; 5-medium purple; 7-purplish brown; 8-light brown; 9-dark brown
Presence of lobules on the leaf (VG)	0-absent; 1-present
Shape of leaf blade (only for varieties with no lobules)c(VG)	1-cordate; 2-broad triangular; 3-narrow triangular; 4-reniform; 5-circular
Depth of lobules (VG)	1-very slight; 3-slight; 5-moderate; 7-deep; 9-very deep
Number of lobules (VG)	1-1 lobule; 3-3 lobules; 5-5 lobules; 7-7 lobules; 9-9 lobules
Central lobule format (VG)	1-toothed; 2-triangular; 3-semicircular; 4-semi elliptic; 5-elliptic; 6-lanceolate; 7-oblong lanceolate; 8-linear broad; 9-linear narrow
The main color of mature leaf upper surface (VG)	1-greenish yellow; 2-green; 3-greyish green
Anthocyanin coloration on leaf upper surface vein (VG)	0-absent; 1-present
Anthocyanin coloration on leaf margins (VG)	0-absent; 1-present
The main color of mature leaf lower surface (VG)	1-greenish yellow; 2-green; 3-greyish green
anthocyanin coloration and distribution on abaxial leaf vein (VG)	3-small; 5-medium; 7-large
Petiole length (MS)	1-very short (1-2 cm); 2-short (2.1-3.0 cm); 3-intermediate (3.1-5.0 cm); 4-long (5.1-7.0 cm); 5-very long (> 7 cm)
Presence of anthocyanin on petioles (VG)	0-absent; 1-present
Distribution of anthocyanin on petioles (VG)	1-only at basal; 2-only at apex; 3-basal and apex; 4-more than half of the petiole; 5-fully distributed on petiole
The pattern of anthocyanin distribution on petioles (VG)	1-spot; 2-line; 3-spot and line; 4-thorough
The shape of the storage root (VG)	1-oblate; 2-round; 3-round and elliptical; 4-elliptical; 5-oval; 6-oboval; 7-oblong; 8-oblong and long; 9-long and elliptical; 10-long irregular or curved
Storage root cortex thickness (MS)	1-very thin (< 1 mm); 3-thin (1 mm); 5-intermediate (2-3 mm); 7-thick (>3-4 mm); 9-(very thick (> 4 mm)
The predominant color of storage root bark (VG)	1-white; 2-cream; 3-yellow; 4-orange; 5-brownish orange; 6-pink; 7-red; 8-purplish red; 9-dark purple
Storage root flesh predominant color (VG)	1-white; 2-cream; 3-dark cream; 4-pale yellow; 5-yellow; 6-pale orange; 7-orange; 8-dark orange; 9-purple
Storage root flesh secondary color (VG)	0-absent; 1-white; 2-cream; 3-yellow; 4-orange; 5-pink; 6-purplish red; 7-purple; 8-dark purple
Distribution of storage root flesh secondary color (VG)	1-absent; 2- narrow ring in the cortex; 3- broad, narrow ring in the cortex; 4- scattered spots; 5- narrow ring in flesh; 6- broad ring in flesh; 7- ring and other areas in the flesh; 8- in longitudinal section; 9- covering most of the flesh; 10- covering all flesh; 11- scattered spots and flush

Note: Based on sweet potato descriptor (CIP, AVRDC, IBPGR 1991; PPVTPP 2021). VG = Visual assessment by observing a single plant or a group of plants or parts of plants, MS = Measurement, by measuring a number of individual plants or parts of plants

Table 3. Expression of morphological characters of 19 newly bred clones and comparable/check varieties of sweet potato

Genotype	Morphological descriptors*																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
G1	7	7	9	3	1	0	0	0	0	2	2	1	1	3	5	4	2	0	0	2	0	5	0	0	0	3	7	2	2	0	1	
G4	7	7	9	5	1	1	0	0	1	1	2	0	0	0	0	0	2	1	1	2	5	5	1	3	3	4	7	2	2	0	1	
G5	7	7	9	5	1	1	0	1	1	1	2	0	0	0	0	0	2	0	0	2	7	5	1	3	3	10	5	8	6	0	1	
G6	5	5	9	5	1	1	0	1	1	1	5	0	0	0	0	0	2	0	0	2	5	5	1	4	3	9	5	6	6	0	1	
G9	7	7	9	5	1	0	0	0	0	0	1	2	1	2	5	5	4	2	0	0	2	0	5	1	2	1	4	7	6	6	0	1
G11	7	7	7	5	1	0	0	0	0	0	2	1	3	5	5	4	2	0	0	2	3	5	0	0	0	10	5	2	2	0	1	
G15	3	3	9	3	1	1	1	1	1	1	2	1	3	7	5	4	2	0	0	2	3	5	0	0	0	4	5	8	9	0	1	
G16	5	5	7	5	1	1	1	0	0	2	2	1	2	3	3	2	2	1	1	2	3	5	1	0	0	9	9	2	9	1	11	
G18	5	5	7	7	2	1	1	1	1	1	2	1	3	7	5	5	2	1	1	2	9	5	1	3	3	10	7	7	6	0	1	
G21	5	5	7	5	1	0	0	1	1	4	2	0	0	0	0	0	2	1	0	2	3	3	1	2	3	3	5	8	4	0	1	
G23	5	5	9	7	1	0	0	1	1	4	2	0	0	0	0	0	2	1	1	2	3	4	1	2	3	3	7	8	4	0	1	
G24	9	9	9	5	1	1	0	1	1	1	2	1	3	5	5	6	2	1	1	3	3	5	1	4	1	9	9	9	9	0	1	
G29	5	5	3	3	1	1	1	1	0	2	5	1	3	9	5	7	3	1	1	3	0	5	0	0	0	4	7	9	9	0	1	
JPV-01	5	5	7	3	1	1	1	0	1	4	2	1	2	1	3	2	2	0	0	2	0	5	0	1	1	9	7	9	9	0	1	
Loel Molo	7	7	9	5	1	0	0	0	0	2	2	1	2	2	3	2	2	0	0	2	3	5	1	2	1	4	7	2	5	0	1	
Beta 3	5	5	7	5	1	1	1	1	1	2	2	0	0	0	0	0	2	0	1	2	3	5	1	3	3	3	7	6	7	0	1	
Antin 3	5	5	7	3	3	1	1	1	1	1	5	1	2	3	4	4	2	1	1	3	7	5	1	3	2	9	7	8	9	0	1	
P. Sallosa	5	5	7	3	1	1	1	1	1	1	4	1	3	5	5	4	2	1	1	2	3	5	1	4	3	4	7	2	5	0	1	
Pating 1	7	7	9	5	1	1	1	1	1	0	5	1	2	2	3	1	2	1	1	2	5	4	1	3	3	8	7	1	1	0	1	

Note: Based on phenotypic classes of sweet potato descriptors (CIP, AVRDC, IBPGR 1991; PPVTPP 2021) as described in Table 2. Each genotype data point was obtained from the mean score of four replicates over two growing cycles

Table 4. Distinct morphological profiles for 19 sweet potato genotypes based on grouping characters

Grouping characters	Expression	Genotypes	Percentage (%)
Growth habit/plant type (characteristic 1)	Erect (3)	G15, G18, Papua Salossa	21.05
	Semi-erect (5)	G4, G5, G6, G9, G16, G21, G23, G29, JPV-01, Antin 3, Beta 3	57.89
	Spreading (7)	G1, G11, G24, Loel Molo	21.05
	Extremely spreading (9)	-	-
Anthocyanin coloration on the tip of the vines (characteristic 7)	Absent (0)	G1, G4, G5, G6, G9, G11, G21, G23, G24, Loel Molo	52.63
	Present (1)	G15, G16, G18, JPV-01, G29, Antin 3, Beta 3, Papua Salossa, Pating 1	47.37
Presence of lobules on the leaf (characteristic 12)	Absent (0)	G4, G5, G6, G21, G23, Beta 3	31.58
	Present (1)	G1, G9, G11, G15, G18, G24, G29, JPV-01, Loel Molo, Antin 3, Beta 3, Papua Salossa, Pating 1	68.42
The shape of the storage root (characteristic 26)	Oblate (1)	-	-
	Round (2)	-	-
	Round and elliptical (3)	G1, G21, G23, Beta 3	21.05
	Elliptical (4)	G4, G9, G15, G29, Loel Molo, Papua Salossa	31.58
	Oval (5)	-	-
	Oboval (6)	-	-
	Oblong (7)	-	-
	Oblong and long (8)	Pating 1	5.26
	Long and elliptical (9)	G6, G16, G24, JPV-01, Antin 3	26.32
	Long irregular or curved (10)	G5, G11, G18	17.79
The predominant color of root bark (characteristic 28)	White (1)	Pating 1	5.26
	Cream (2)	G1, G4, G11, G16, Loel Molo, Papua Salossa	31.58
	Yellow (3)	-	-
	Orange (4)	-	-
	Brownish orange (5)	-	-
	Pink (6)	G6, G9, Beta 3	15.79
	Red (7)	G18	5.26
	Purplish red (8)	G5, G15, G21, G23	21.05
	Dark purple (9)	G24, G29, JPV-01, Antin 3	21.05
	The predominant color of the storage root flesh (characteristic 29)	White (1)	Pating 1
Cream (2)		G1, G4, G11	15.79
Dark cream (3)		-	-
Pale yellow (4)		G21, G23	10.53
Yellow (5)		Loel Molo, Papua Salossa	10.53
Pale orange (6)		G5, G6, G9, G18	21.05
Orange (7)		Beta 3	5.26
Dark orange (8)		-	-
Purple (9)		G15, G16, G24, G29, JPV-01, Antin 3	31.58

Note: Each data point was obtained from the average of four replicates over two growing cycles

Similar to the storage root shape, the predominant color of storage root bark also fell into five categories, i.e., white (5.26%), cream (31.58%), pink (15.79%), red (5.26%), purplish red (21.05%) and dark purple (21.05%). Only Pating 1 and Beta 3 have, respectively, a white and orange storage root flesh color, while three genotypes (G1, G4, and G11, 15.79%) have cream root flesh color, two genotypes (G21 and G23, 10.53%) have pale yellow. Two genotypes (Loel Molo and Papua Salossa, 10.53%) have yellow root flesh color. Pale orange storage root flesh color was observed in 21.05% of tested genotypes (G5, G6, G9, G18), while the purple storage root flesh color was demonstrated in 31.58% of the tested genotypes, i.e., G15, G16, G24, G29, JPV-01, Antin 3. Samples of images of grouping characters of the tested sweet potato genotypes are presented in Figure 1. Data in Table 4 and Figure 1 implies that the tested sweet potato

genotypes exhibited a distinctive and high variability in the six grouping morphological characteristics of sweet potato.

Genetic diversity based on morphological characters

All 31 observed morphological character scores were dimorphic and polymorphic; thus, they were used to assess the genetic diversity of the sweet potato genotypes. An Un-Weighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis employing Euclidean distance resulted in a dendrogram, as shown in Figure 2. The dendrogram shows that four main clusters were formed at a truncation point of 12.0 (Clusters I, II, III, IV). Cluster I is a stand-alone cluster comprised of only G16. Cluster II was sub-classified into two sub-clusters, each consisting of 7 and 6 members. Cluster III comprised only two members, while cluster IV had three genotype members.

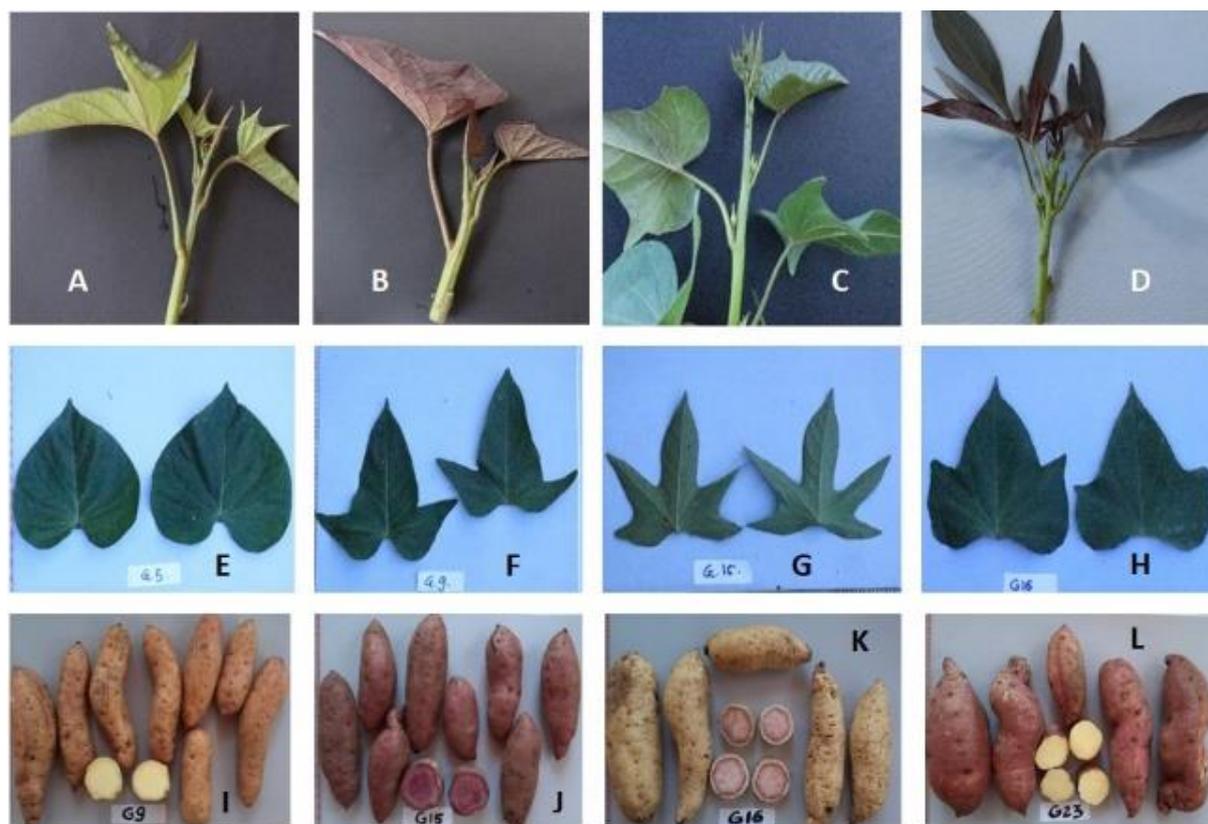


Figure 1. Samples of images of grouping morphological characters of tested sweet potato genotypes: anthocyanin coloration on the tips of the vines (A: Papua Salossa, B: Antin 3, C: G16, D: G29), the presence of lobules of the leaf (E: G5, F: G9, G: G15, H: G16), and the storage root shape, root bark predominant color and root flesh predominant color (I: G9, J: G18, K: G16, L: G23)

Principal Component Analysis (PCA) revealed 18 independent components responsible for the total (100%) observed variation. About 79% of the variation in the data set was contributed by the first 6 components with eigenvalues greater than 1.5. The first six components include component 1 (24.94%), component 2 (17.95%), component 3 (12.26%), component 4 (10.30%), component 5 (7.74%), and component 6 (5.77). Biplots of PCA (PC1-PC2, PC1-PC4) involving the 31 morphological characters of the tested sweet potato genotypes are presented in Figure 3.

The principal component analysis showed that five morphological characters with positive loading factors (0.30-0.32) are responsible for maximum variability in principal component 1 (PC1), which explains 24.94% of the total observed variability. These five characteristics include the presence of lobules on the leaf (12), the shape of the leaf blade (13), the depth of lobules (14), the number of lobules (15), and the central lobule format (16) (Figure 3A). The morphological characters with the highest and positive loadings (0.25 - 0.31) responsible for variability in principal component 2 (PC2) included 6 (vine secondary color (6), anthocyanin coloration on internode (8), anthocyanin coloration on the node (9), anthocyanin coloration on leaf margins (19), anthocyanin coloration and distribution on abaxial leaf vein (21) (Figure 3A). Meanwhile, the characters that were mostly responsible for variability in

principal component 3 (PC3) included plant type (1) and length of the main vine (2), each with loading factors of 0.39, and the presence of anthocyanin on petioles (23), the shape of storage root (16) and storage root cortex thickness (27) with loading factors, respectively, 0.22, 0.22, and 0.24. In the principal component 4 (PC4), the highest and positive loadings were contributed by characteristics number 30 (storage root flesh secondary color) and 31 (Distribution of storage root flesh secondary color); both have similar loading factors of 0.51. The morphological characteristics mostly responsible for variability in component 5 (PC5) included the main color of the mature leaf upper surface (loading factor 0.31), anthocyanin coloration on leaf upper surface vein (0.34), anthocyanin coloration on leaf margins (0.24), main color of mature leaf lower surface (0.27) and root cortex thickness (0.24). In comparison, those in principal component 6 (PC6) were the main color of the mature leaf lower surface (0.24), the predominant color of root bark (0.349), and the root flesh predominant color (0.47).

Biplot of PC1 versus PC4 (Figure 3B) clearly shows that G16 is clustered separately and apart from other genotypes., and vertical lines show the characteristics responsible for this with positive loading factors, i.e., characters number 30 (storage root flesh secondary color) and 31 (distribution of storage root flesh secondary color). These two characteristics are unique for G16, as shown in Table 3,

which also supports the grouping of the genotype in Figure 2. Figure 3B places G15 and G29 in one quadrant, supporting the clustering of these two genotypes in one cluster (III), as shown in Figure 2.

Distinctness, uniformity, and stability of tested sweet potato genotypes

Distinctness

According to UPOV (2010), assessing sweet potato's distinctness needs to consider two important points, i.e., 1). consistent differences, and 2). clear differences. One way to ensure that a characteristic's difference is sufficiently consistent is to examine the characters in at least two independent growing cycles. Meanwhile, the clear difference between two varieties in character depends on, particularly, the type of expression of a character being examined, i.e., qualitative, pseudo-qualitative, and quantitative.

In the present study, the distinctness of the tested sweet potato genotypes was assessed based on an analysis of the genotypes' dissimilarity (genetic distance) employing the pooled morphological characteristic (Table 3). The dissimilarity/divergence level of each genotype pair was assessed based on the Euclidean distance index, as presented in Table 5. This table demonstrates that the genetic distance index of the genotype pairs, except the self-pair genotype, ranged from 3.7 - 19.2, indicating that each of the tested genotypes was distinct from other genotypes for at least one morphological character. The lowest Euclidean index was observed on the G21-G23 pair (3.7), followed by G6-G5 (5.2) at the second place, Beta 3-G21 (5.5), and Beta 3-G23 (5.5) at the third place. Meanwhile, the highest Euclidean distance is shown by the genotype pairs G29-G4 (19.2), G29-G5 (18.6), and G29-Pating 1 (18.3).

The genetic distance index in Table 4 demonstrated that each genotype evaluated in the present study is distinct from other genotypes for at least five morphological characters, as shown by the genotype pair G21 and G23 (Euclidean index of 3.7). Furthermore, the number of distinctive characters between genotype pair increases with the increase

in the Euclidean index, with the highest number of distinctive characters being 19 morphological characters, as shown by the genotype pair G29-G24 with a Euclidean index of 19.2.

Data in Table 5 also shows that genotype pairs involving G16 have high Euclidean indices, ranging from 13.0 to 16.9, indicating that G16 is highly distinct from other studied genotypes, with the lowest distance in G16-Loel Mollo pair with 15 distinctive characteristics. The highest distance is in the G16-G29 pair with 17 distinctive characters (Table 3). The distinctness of G16 from other genotypes, as shown in Table 3 and Table 4, is in line with the cluster analysis results, which placed G16 separately, as shown in the dendrogram (Figure 2).

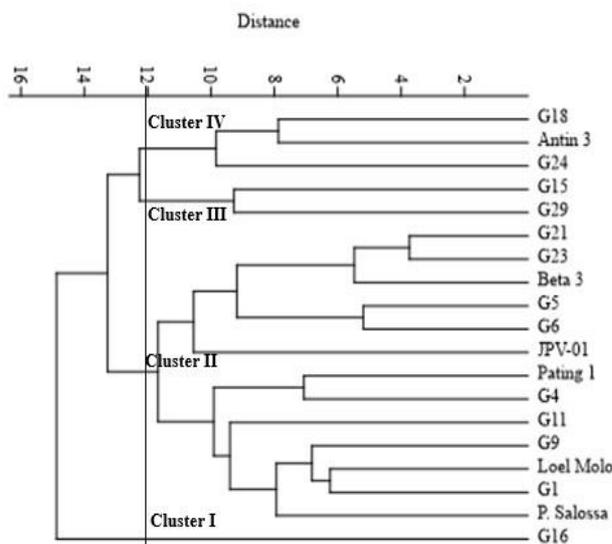


Figure 2. UPGMA Dendrogram, based on Euclidean distance coefficient, of 19 sweet potato genotypes generated using 31 morphological characters of leaf, vine/stem, and storage root. Each genotype scored data point was obtained from the average of four replicates over two growing cycles

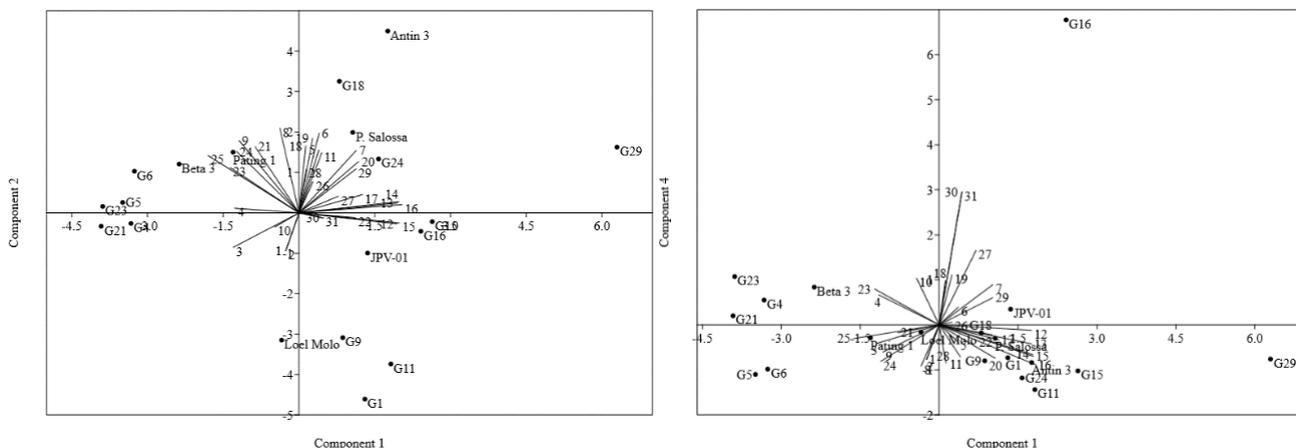


Figure 3. Scatter plots showing distribution and morphological characters mostly responsible for the observed variability of 19 sweet potato genotypes in PC1 versus PC2 (above), and PC1 versus PC4 (below). Numbers 1 - 31 are the sweet potato morphological characters/descriptors, as presented in Table 2

Table 5. Genetic distance matrix (Euclidean index) of 19 sweet potato genotypes based on 31 morphological characteristics

	G1	G4	G5	G6	G9	G11	G15	G16	G18	G21	G23	G24	G29	JPV-01	Loel Molo	Beta 3	Antin 3	P. Salossa	Pating 1	
G1	0.0																			
G4	10.3	0.0																		
G5	15.3	9.9	0.0																	
G6	14.1	9.1	5.2	0.0																
G9	7.0	11.7	13.1	12.4	0.0															
G11	9.1	12.2	13.1	12.8	9.6	0.0														
G15	12.5	15.9	14.7	13.8	8.8	13.0	0.0													
G16	15.1	15.6	15.6	14.7	14.1	14.0	15.0	0.0												
G18	15.7	14.9	11.7	12.4	12.3	11.2	11.7	15.2	0.0											
G21	12.1	8.8	9.8	8.9	11.2	14.5	13.1	16.3	15.0	0.0										
G23	12.2	8.4	9.9	9.1	10.9	14.8	13.5	16.1	14.7	3.7	0.0									
G24	14.0	15.6	12.4	13.5	8.9	12.5	12.2	15.3	10.3	15.3	14.8	0.0								
G29	14.4	19.2	18.6	17.6	10.7	14.8	9.3	16.9	14.2	16.2	16.9	12.6	0.0							
JPV-01	13.0	14.3	10.9	10.4	9.8	13.0	10.4	13.5	13.5	10.6	11.0	10.7	12.5	0.0						
Loel Molo	6.2	6.7	11.1	10.0	6.6	9.0	11.7	13.0	12.9	9.5	9.2	12.0	14.9	11.2	0.0					
Beta 3	11.9	7.9	9.4	7.9	10.2	14.4	12.2	14.5	14.2	5.5	5.5	13.9	15.5	9.8	8.1	0.0				
Antin 3	14.8	13.9	9.7	9.2	11.4	12.6	10.2	14.0	7.9	12.8	13.1	9.3	12.6	9.5	11.7	11.3	0.0			
P. Salossa	8.7	10.6	13.9	11.7	7.8	9.9	10.2	14.0	11.2	12.1	12.4	12.2	12.1	12.4	7.3	10.4	10.3	0.0		
Pating 1	10.5	7.1	10.9	9.4	11.6	8.7	15.6	14.9	12.4	12.3	12.1	14.2	18.3	14.5	7.9	11.7	12.4	8.9	0.0	

Note: Higher Euclidean distance index indicates a higher dissimilarity level, and vice versa. A lower Euclidean distance index indicates a lower dissimilarity level. Each genotype's data used in the analysis was the average of four replicates over two growing cycles

Uniformity

Morphological data used in the present study were obtained from trials in two growing cycles (2021 and 2022). As the observed phenotypical expressions of the individual morphological character of each genotype were similar over the two growing cycles, the data were then pooled as an average in Table 3.

UPOV (2010; 2019) states that a population standard of 1% and an acceptable probability level of at least 95% should be applied for uniformity assessment. Thus, in the case of a sample size of 50 plants, two off-types are allowed, and only one off-type is allowed for a population of about 25 - 30 plants. No off-type is allowed for a population size of 20 plants or less.

The observed morphological data in the present study were recorded from 4 replications/plots of 5 plants per plot, with 20 plants observed per genotype in each growing cycle and 40 plants per genotype for two growing cycles. There were no off-type characters of each genotype during the two growing cycles. Thus, by the OPOV (2010; 2019) standard, each tested genotype is considered uniform in its phenotypic expression of morphological characteristics over the two growing cycles.

Stability

The stability parameter of the observed characters of each genotype was assessed based on the character's expression during the two growing cycles. UPOV (2010; 2019) highlighted that, for many types of plant variety, when a variety has been shown to be uniform, it could also be considered stable. The present study results demonstrated that each tested genotype's morphological characters were expressed in similar phenotypic levels (scores) during the two growing cycles, indicating their uniformity. Thus, the study results revealed that the phenotypic expressions of all morphological characters of tested genotypes were stable.

Discussion

Characterization and assessment of agro-morphological diversity and relationships among sweet potato varieties are important for the conservation of germplasm, and the development of new superior varieties through breeding programs (Laurie et al. 2004; Norman et al. 2014). Cluster analysis and principal component analysis (PCA) are the statistical methods most frequently used for the assessment of the genetic diversity of crops (Chanda et al. 2014; Joshi et al. 2015; Mau et al. 2017; Ochieng 2019; Seid et al. 2021). In the present study, cluster analysis placed the 19 sweet potato genotypes into four main clusters, each comprised of one to 13 genotype members, indicating high genetic variability among the genotypes. The dendrogram shows that G16 was clustered in Cluster I on its own, presumably due to its uniqueness in character number 30 (storage root flesh secondary color) and character number 31 (distribution of storage root flesh secondary color (Table 3). Meanwhile, the grouping of G15 and G29 in one cluster (Figure 2) is presumably due mainly to their unique leaf blade shape (character number 19) (Table 3), as also evident in Figure 3B, where G15 and G29 were placed in one quadrant and apart from other genotypes. Furthermore, the three

genotypes having anthocyanin pigmentation on the vine tips and purple storage flesh color were classified into one cluster (IV), indicating the usefulness and effectiveness of characteristics number 7 (anthocyanin coloration on the tip of the shoots) and number 29 (the predominant color of the storage root flesh) as grouping characters of sweet potato (UPOV 2010).

The clustering of these genotypes into several clusters and sub-clusters indicates that morphological characters used in this study effectively reveal the genetic diversity of sweet potatoes. The effectiveness of morphological characters as discriminators of sweet potatoes has been reported in previous works (Elameen et al. 2011; Fongod et al. 2012; Mbithe et al. 2016; de-Andrade et al. 2017; Tairo et al. 2018; Ochieng 2019).

The plant variety protection (PVP) system currently relies on the plant morphological description (Yu and Chung 2021). A plant variety candidate must meet the DUS criteria to be eligible for PVP. The DUS assessment determines whether a new variety is unique, uniform, and stable in its phenotypic expression.

The study results in Table 5 show that the Euclidean index matrix of pair-wise combinations of the studied genotype pairs had a Euclidean index ranging from 3.7 - 19.2, indicating that each genotype in the genotype pair is different from its counterpart for at least one morphological character. The genotype pair G21-G23, for instance, has an index of 3.7. Data in Table 3 show that both genotypes are distinct from each other in five morphological characteristics such as vine internode diameter (3), vine internode length (4), anthocyanin coloration on leaf margins (19), petiole length (22) and root cortex thickness (27). Meanwhile, the genotype pair with the highest Euclidean index (19.2), i.e., G29-G24, are distinct in 19 out of 31 observed morphological characteristics (Table 3). This finding highlights that the studied genotypes are distinct for at least five morphological characters and, at most, 19 characters. As with the distinctness criterion, the study results also reveal that all the tested genotypes are uniform and stable, as shown by the absence of off-type plants and the homogeneous phenotypic expressions of the morphological characters over the two growing cycles (UPOV 2010, 2019).

The use of morphological descriptors for the assessment of genetic diversity and characterization of DUS criteria has been reported in inbred maize lines (Selvi et al. 2013), moringa (Angadi and Jagadeesha 2018), pearl millet inbreds (Nehra et al. 2016), and farmer rice variety (Rao et al. 2013). The present study results proved the effectiveness of morphological characters for assessing genetic diversity and identifying DUS in sweet potato germplasm (Tairo et al. 2018; Ochieng 2019).

The present study results revealed that each of the newly bred sweet potato clones has unique, stable and uniform characters, and thus, by regulation (PPVTPP 2021), each of them is eligible for registration as a new variety. However, registration of a new variety must also consider the potency of the variety to be released as a superior variety. Only crop genotypes that have superior traits will only be considered for release as superior varieties. Although all tested sweet

potato clones are eligible for registration as new varieties, not all of them possess superior traits as compared to the existing sweet potato varieties in Indonesia. Thus, we decided that only a few clones with unique and superior traits would be registered as new varieties; and these include G16 and G29.

The genotypes G16 and G29 have, respectively, purplish-white and purple tuber flesh characters, which are distinct from other clones. The purple-fleshed character is considered a superior trait as the sweet potato varieties with this character is only few in Indonesia (Balitkabi 2016). In addition, purple-fleshed sweet potatoes have attained increasing public interest in recent years due to their beneficial attributes related to human health (Ginting et al. 2020) due to their high anthocyanin content. Anthocyanin compounds possess medicinal properties such as antioxidant which can prevent vascular diseases such as hypertension and heart attack, lower the risk of cancer, protect against type 2 diabetes, hepato-protective, antimicrobial, and anti-inflammatory (Lee et al. 2012; Pojer et al. 2013; Xu et al. 2015; Chen et al. 2016).

In addition to the unique tuber morphological characters, the genotypes G16 and G29 also exhibited high mean tuber yield (> 25 t ha⁻¹) and also high anthocyanin content (Mau et al. 2022), which are comparable to the Indonesian-released purple-fleshed varieties Antin 1, Antin 2 and Antin 3 (Balitkabi 2016; Ginting et al. 2020; Indriani et al. 2020). Considering the above mentioned unique and superior traits, it is reasonable to consider the sweet potato genotypes G16 and G29 to be registered as a new variety, which may latter be further evaluated for release as superior sweet potato varieties.

In conclusion, the present study results revealed high genetic diversity among newly bred and the control varieties of sweet potato genotypes included in the study. Genetic distance analysis demonstrated that each of the tested genotypes is distinct from the other for at least five of the 31 observed morphological characters. Each of the studied genotypes is uniform and stable over the two growing cycles. The newly bred clones are distinct from the control varieties and uniform and stable; and the most distinctive genotypes G16 and G29, which have unique and superior purplish white and purple tuber flesh characteristics can be further processed for registration as new varieties and plant variety protection.

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