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Research Article

Morphological Characterization and *In Vitro* Culture Optimization of Purple-Fleshed Sweet Potato (*Ipomoea batatas* L. Poiret) Accessions

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ABSTRACT

Purple-fleshed sweet potato (*Ipomoea batatas* L. Poiret) is a functional food crop known for its high anthocyanin content and nutritional value. This study aimed to characterize the morphological traits of stem and leaf organs from seven purple-fleshed sweet potato accessions and to evaluate the effectiveness of *in vitro* culture techniques for their conservation. Morphological traits were assessed using a standardized scoring system and analyzed using Principal Component Analysis (PCA) and cluster analysis. Results showed significant variation among accessions, with Ungu Lonjong appearing genetically distinct, while Ayamurasaki, KT Lampa, and Local NTT exhibited high similarity. *In vitro* culture analysis involved the sterilization and cultivation of explants on Murashige and Skoog (MS) medium and minimal growth medium. The highest sterilization success rate was observed in Ungu Lonjong (78.3%), whereas Local NTT showed the highest contamination. This study demonstrates the potential of combining morphological data and tissue culture for effective characterization, conservation, and future purple-fleshed sweet potato germplasm breeding.

Keywords: *Ipomoea batatas*, germplasm conservation, morphological characterization, tissue culture

Introduction

Sweet potato (*Ipomoea batatas* L. Poiret) is one of the most important food crops with great potential as a source of carbohydrates and essential nutrients. Among its various types, purple-fleshed sweet potato stands out due to its high anthocyanin content, reaching approximately 110.51 mg/100 g, significantly higher than other sweet potato varieties (Ginting et al., 2011). Anthocyanins are known for their health benefits, including anticancer and antibacterial properties (Rosidah, 2014), making purple-fleshed sweet potato a promising functional food. Its nutritional composition includes 22.64% starch, 0.30% reducing sugars, 0.94% fat, 0.77% protein, 70.46% water, 0.84% ash, 3.00% fiber, and 2,143 mg/100 g vitamin C (Balitkabi, 2021). Additionally, purple-fleshed sweet potato contains higher sodium and niacin levels than other varieties (Rosadi et al., 2020), and has a carbohydrate content of 57.5 g (Winayu et al., 2020).

Increased cultivation of purple-fleshed sweet potatoes can lead to greater variation and diversity in plant characteristics, in line with the species' broad genetic diversity (Prayudha et al., 2019). This may include variations in ability to produce tubers. Genetic variation can be assessed through morphological

characterization, which is key in supporting conservation and breeding programs. Such information is essential for identifying genotypes, unique traits, and population structure (Ishaq et al., 2019). Dewi et al. (2014) reported that the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD/BB Biogen)—currently the Agricultural Instrument Standardization Center for Biotechnology and Genetic Resources (BSIP Biogen)—Bogor has been routinely conducting *in vitro* conservation of various root crops, including sweet potato. *In vitro* culture is a propagation technique conducted under sterile and controlled conditions, enabling the production of healthy, pathogen-free plantlets (Ziraluo, 2021).

Sweet potato is known to have a highly heterozygous hexaploid genome, with low flower production and a high degree of incompatibility, resulting in considerable genetic diversity (Wadl et al., 2018). While this diversity supports breeding efforts, it also presents challenges in the selection process. Therefore, understanding genetic variation is essential for developing superior varieties (Hetharie et al., 2018). Morphological traits—such as leaf and tuber color, leaf shape, and growth patterns—are commonly used in genetic diversity studies (Ishaq et al., 2019; Prayudha et al., 2019). Morphological analysis and cluster analysis can help identify and eliminate duplication in germplasm collections (Hetharie et al., 2018). Several purple-fleshed sweet potato clones have been developed in Indonesia, such as Ayamurasaki, which is widely cultivated commercially in Malang and Pasuruan, Antin, a local clone bred by the Indonesian Legumes and Tuber Crops Research Institute (ILETRI/Balitkabi), and a local purple-fleshed clone from Lampung (Dewi & Sutrisno, 2014). Other local clones identified include LRE, 14(184), 5(7), 3(4), INDPFU9, LC-1, 48(46), and Biang (Prayudha et al., 2019).

In conserving sweet potato germplasm, *ex situ* approaches are particularly important due to the recalcitrant nature of sweet potato seeds, which cannot be stored for extended periods under low temperature and humidity conditions (Dewi et al., 2014). *In vitro* culture is an effective *ex situ* conservation method, enabling aseptic and pathogen-free plant development (Abubakar et al., 2018; Ziraluo, 2021). The initial stage of *in vitro* culture involves sterilizing the explants to remove microbial contaminants. Various sterilization methods have been evaluated, such as using 20% NaOCl (disinfecting bleach), 0.1% $HgCl_2$, and 0.1% $AgNO_3$ on patchouli leaf explants (Fitriani et al., 2019). Properly selecting initiation media is also crucial for successful *in vitro* conservation. These media serve as a platform for plant multiplication through subculture, as practiced at BB Biogen.

This study aimed to identify genetic variation in purple-fleshed sweet potato based on morphological characteristics that reflect germplasm diversity and genetic relationships among clones or accessions. Additionally, the study was evaluating the supporting and limiting factors affecting the success of *in vitro* culture analysis as an *ex situ* conservation method. The findings from morphological identification and *in vitro* culture analysis were expected to support conservation efforts and breed superior, adaptive, and nutritionally valuable purple-fleshed sweet potato varieties.

Materials and Methods

This research was conducted at the BB Biogen, Bogor, West Java, in January 2022. The plant materials used in this study consisted of seven purple-fleshed sweet potatoes accessions collected from the field by BB Biogen. These accessions included Ayamurasaki, Murasaki, KT Lampa, Local NTT, IB1530, Ungu Lonjong, and Selometir.

Equipment and Tools

Morphological characterization was carried out using measuring tools, such as rulers, calipers, and morphological descriptor guides. *In vitro* culture procedures utilized laboratory equipment, including a laminar air flow cabinet, autoclave, analytical balance, culture tubes, and other supporting tools, such as sterilization instruments and growth chambers.

Morphological Characterization

Morphological characterization was performed on the stem and leaf organs of each purple-fleshed sweet potato accession. The observed morphological traits included growth type, internode length and diameter, dominant and secondary stem color, presence of trichomes on the shoot tip, general leaf shape, leaf lobe type, number of lobes, central lobe shape, abaxial vein color, mature leaf size and color, young leaf color, and petiole length and color. Each trait was scored using a standard morphological descriptor guide, and the resulting data were used to assess genetic similarity and diversity among the accessions.

The morphological data were analyzed using Principal Component Analysis (PCA) to identify the most influential components contributing to genetic variation. PCA is a multivariate statistical technique that reduces data dimensionality and groups variables based on their contribution to variation (Hetharie et al., 2018; Janmohammadi et al., 2014). The results were visualized using scree plots, score plots, and biplots. In addition, a cluster analysis was conducted to group accessions based on morphological similarities, with the results presented in a dendrogram based on Euclidean distance (Govindaraj et al., 2015; Prayudha et al., 2019).

In Vitro Culture Analysis

In vitro culture was performed by purple-fleshed sterilizing sweet potato plant explants in the form of shoot stems and tubers. Explants were sterilized with 75% alcohol and 15% and 20% NaOCl, and were rinsed with sterile distilled water between treatments. The explants were planted in Murashige and Skoog (MS) medium. Observations were made on sterile and contaminated explants.

The culture medium used for initiation and maintenance was MS medium. For long-term conservation, a minimal growth medium supplemented with osmoregulators such as mannitol was used, as recommended by Sabda (2018). The effectiveness of *in vitro* culture was evaluated based on explant viability, contamination rate, and the duration of culture maintenance.

Results and Discussion

Morphological Characterization of Purple-fleshed Sweet Potato Leaves and Stems

According to Hetharie et al. (2018), morphological characterization combined with cluster analysis can detect and eliminate possible duplications in sweet potato accessions. In this study, morphological observations were conducted on the stem and leaf organs of each purple-fleshed sweet potato accession (Figure 1). Each morphological trait was scored to generate data on genetic similarity and relatedness among the accessions.

The analysis of genetic relationships was carried out using PCA. As Janmohammadi et al. (2014) noted, PCA is a multivariate statistical method used to explore and simplify complex datasets, enabling more precise identification of relationships among variables. In plant breeding, PCA is particularly valuable for identifying traits that contribute significantly to genetic variation (Hetharie et al., 2018).

Based on the analysis, eight principal components (PCs) were obtained, each with varying eigenvalues and proportions of variance (Table 1). The highest proportion of variance was explained by PC1 (35.5%), followed by PC2 (29.2%), PC3 (16.5%), and PC4

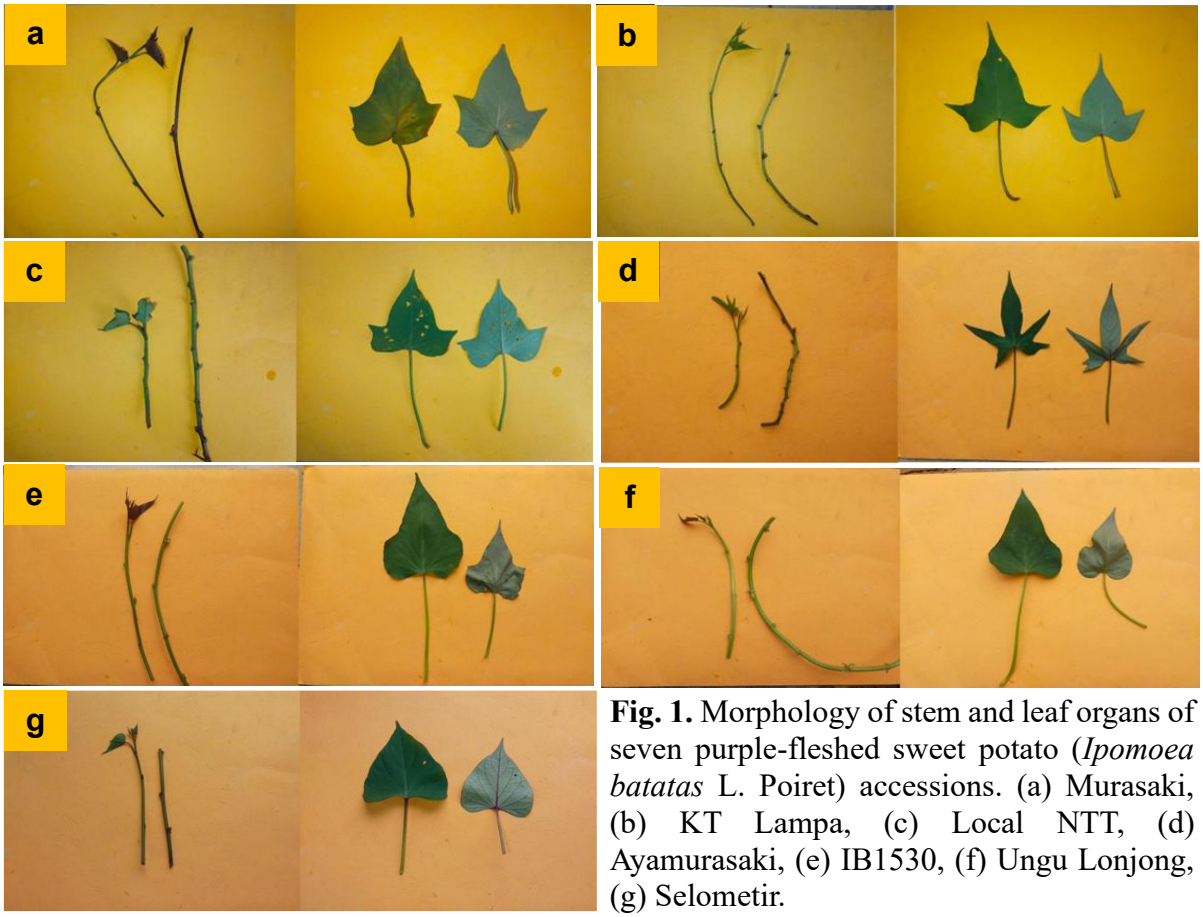

Fig. 1. Morphology of stem and leaf organs of seven purple-fleshed sweet potato (*Ipomoea batatas* L. Poiret) accessions. (a) Murasaki, (b) KT Lampa, (c) Local NTT, (d) Ayamurasaki, (e) IB1530, (f) Ungu Lonjong, (g) Selometir.

(11.1%) (Figure 2). The remaining components contributed lower percentages (Table 2). Thus, PC1 and PC2 were selected for further analysis using score plots and biplots, as they account for the most significant variation in the dataset. These components reflect the most influential traits differentiating the accessions and provide valuable insights into genetic variation within the population (Evgenidis et al., 2011).

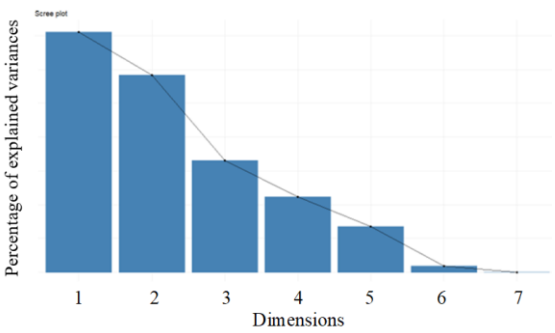

Fig. 2. Scree plot from the Principal Component Analysis (PCA) of the morphological traits of purple-fleshed sweet potato accessions.

The score plot and biplot from the PCA offer a visual representation of the distribution and genetic relationships among the purple-fleshed sweet potato accessions (Figures 3 and 4). According to Hetharie et al. (2018), accessions within the same quadrant indicate close genetic relationships, whereas accessions in quadrants separated by a 90° angle suggest distant relationships. Figure 4 shows that the Selometir accession did not cluster within the four main quadrants, indicating a relatively distant relationship from the other six accessions. In contrast, Ayamurasaki, KT Lampa, and Local NTT exhibited close genetic similarities. Murasaki was also found to be closely related to IB1530. Meanwhile, Ungu Lonjong stood apart and showed no notable similarity to the other accessions. These relationships reflect similarities in traits such as general leaf shape, central lobe shape, number of lobes, dominant stem color, and lobe type.

In addition to PCA, cluster analysis was performed to group accessions based

Table 1. Morphological traits of stem and leaf traits of purple-fleshed sweet potato accessions.

No.	Accession	Twining	Plant type	Internode length	Internode diameter	Dominant stem color	Secondary stem color	Shoot tip hair	General leaf shape	Leaf lobe type	Number of lobes	Central lobe shape	Abaxial vein color	Mature leaf size	Mature leaf color	Young leaf color	Petiole length	Petiole color
1	Murasaki	3	7	7	3	6	2	3	6	5	5	4	3	5	2	9	1	6
2	KT Lampa	0	3	3	1	4	5	0	6	5	5	4	3	5	2	2	1	3
3	Local NTT	0	3	1	3	3	6	3	6	3	5	2	2	5	2	3	3	1
4	Ayamurasaki	0	3	3	1	3	6	0	6	7	5	6	3	5	2	5	1	3
5	IB01530	5	5	5	3	3	6	0	5	3	3	2	2	5	2	9	3	1
6	Ungu Lonjong	3	5	3	1	0	0	4	1	3	1	2	2	5	2	6	3	1
7	Selometir	0	3	3	1	3	6	0	3	1	1	1	8	3	2	2	1	3

Twining: 0 = untwisted, 3 = slightly twisted, 5 = moderately twisted. Plant type: 3 = upright, 5 = semi-compact, 7 = creeping. Internode length: 1 = very short, 3 = short, 5 = medium, 7 = long. Internode diameter: 1 = very thin, 3 = thin. Dominant stem color: 0 = green, few purple spots, 3 = green, few purple spots, 4 = green, many purple spots, 6 = dominant purple. Secondary stem color: 0 = none, 2 = green top, 5 = purple top, 6 = purple internode. Shoot tip hair: 0 = none, 3 = few, 4 = medium. General leaf shape: 1 = rounded, 3 = heart-shaped, 5 = lanceolate, 6 = lobed. Leaf lobe type: 1 = very small, 3 = small, 5 = medium, 7 = deep. Central lobe shape: 1 = tooth, 2 = triangular, 4 = semi-elliptical, 6 = lanceolate. Abaxial vein color: 2 = green, 3 = purple spots on lower part, 8 = leaf vein mostly/totally purple. Mature leaf size: 3 = small, 5 = medium. Mature leaf color: 2 = green. Young leaf color: 2 = green, 3 = green, purple leaf margin, 5 = green, purple leaf veins on upper surface, 6 = slightly purple, 9 = purple on both surfaces. Petiole length: 1 = very short, 3 = short. Petiole color: 1 = green, 3 = green with purple near leaf, 6 = green with purple stripes.

on morphological similarities. As stated by Prayudha et al. (2019), cluster analysis is effective in classifying individuals based on shared characteristics. The resulting dendrogram (Figure 5) used Euclidean distance to quantify genetic distances among the accessions (Govindaraj et al., 2015). A Euclidean distance greater than one suggests broad genotypic diversity (Nair et al., 2017), as also confirmed in the findings of Prayudha et al. (2019), where most accessions did not exhibit close genetic relationships.

The dendrogram revealed two major clusters. Cluster I consisted of only one accession, Ungu Lonjong, indicating it had the most distinct morphology. This finding aligns with PCA results, which also placed Ungu Lonjong apart from the others. Cluster II included the remaining six accessions, which were further subdivided into two sub-clusters. Sub-cluster II.1 comprised Selometir, Local NTT, KT Lampa, and Ayamurasaki, with Local NTT, KT Lampa, and Ayamurasaki showing particularly close relationships. Selometir, while part of the group, showed more

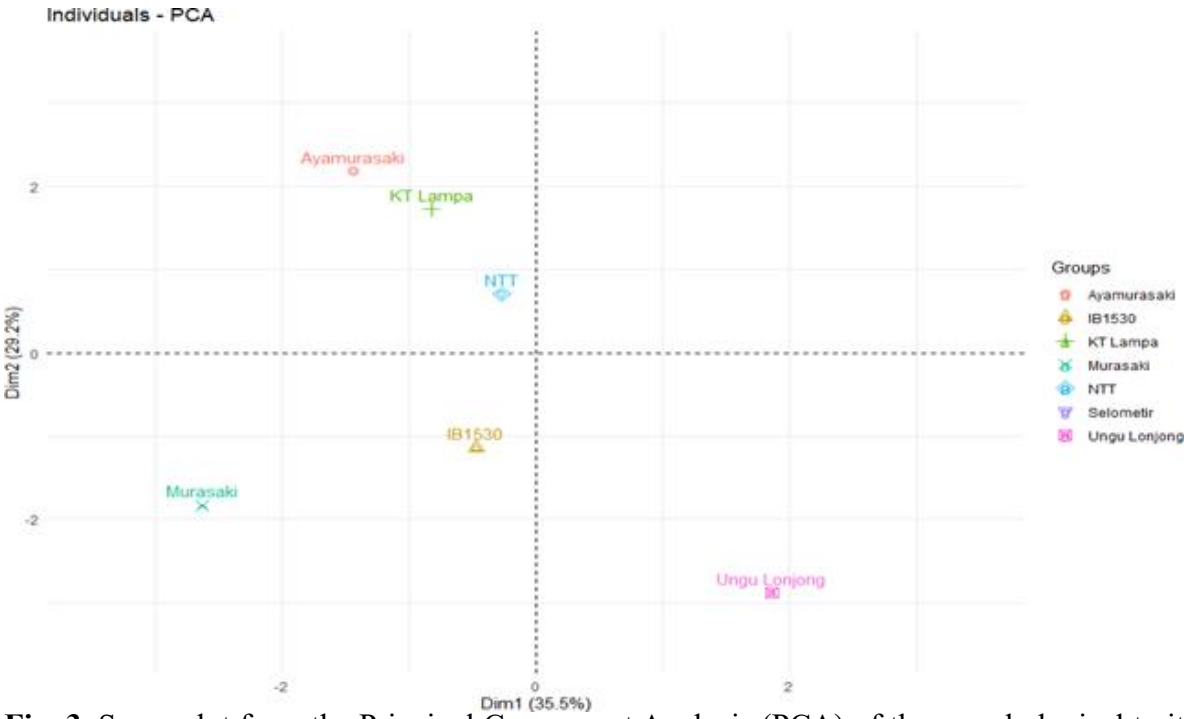

Fig. 3. Score plot from the Principal Component Analysis (PCA) of the morphological traits of purple-fleshed sweet potato accessions.

distant similarity. Sub-cluster II.2 included Murasaki and IB1530, indicating a very close genetic resemblance in leaf and stem morphology.

Table 2. Summary of the Principal Component Analysis (PCA) of the morphological traits of purple-fleshed sweet potato accessions.

Components	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Standard deviation	2.1486	1.9473	1.4648	1.2035	0.93723	0.34507	3.57E-16
Proportion of variance	0.3551	0.2917	0.1651	0.1114	0.06757	0.00916	0
Cumulative proportion	0.3551	0.6468	0.8119	0.9233	0.99084	1	1

These characterization results are essential for plant breeding, particularly in classifying and selecting superior accessions. They help differentiate between accessions and serve as a reference for identifying promising genetic materials for varietal development (Prayudha et al., 2019). The observed genetic diversity among purple-fleshed sweet potato accessions highlights the potential to produce high-quality varieties through targeted breeding programs.

The results of explant sterilization from five purple-fleshed sweet potato accessions showed that 41.7% of the explants were successfully sterilized, 55.9% were contaminated, and 2.4% were dead (Table 3). The Local NTT accession had the highest contamination rate, whereas the Ungu Lonjong accession had the highest sterilization success. One factor contributing to contamination was the presence of numerous fine hairs (trichomes) on the stem, which can harbor microorganisms. This was particularly evident in the Local NTT accession, which had a high density of stem trichomes.

The Murashige and Skoog (MS) medium was optimal for initiating and maintaining *in vitro* cultures of purple-fleshed sweet potato. This medium maintained cultures in good condition for 4 to 9 months (Sabda, 2018). Table 4 showed that, for long-term storage, minimal growth media based on MS supplemented with

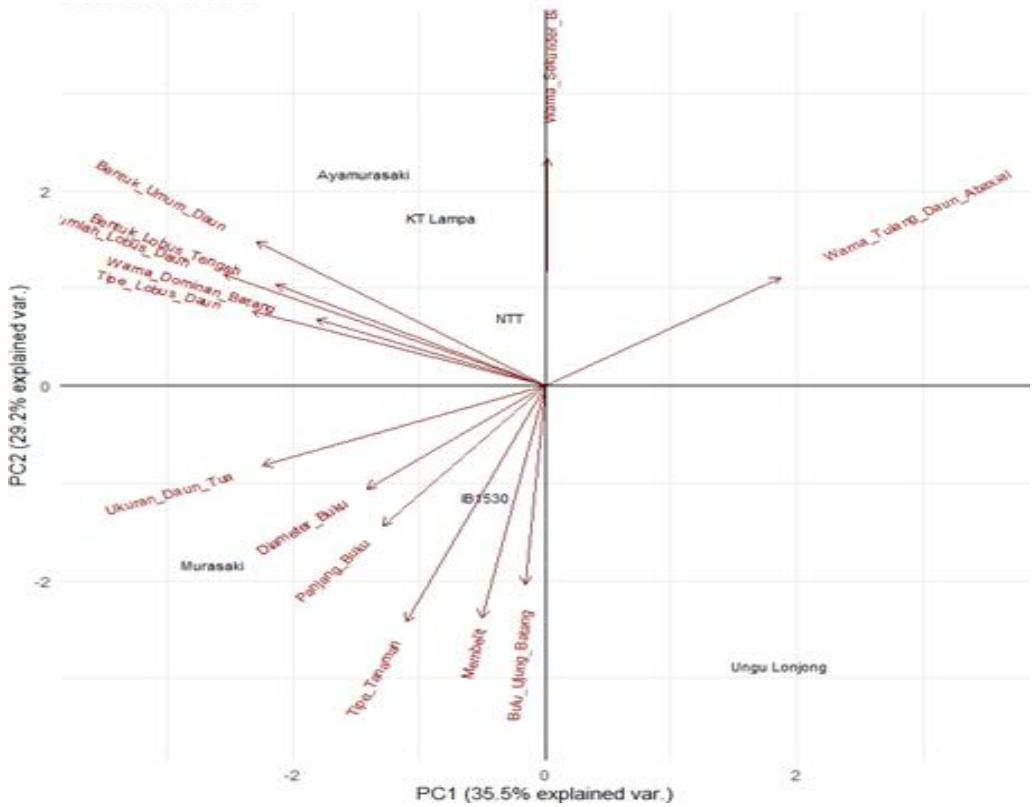

Fig. 4. Biplot from the Principal Component Analysis (PCA) of the morphological traits of purple-fleshed sweet potato accessions.

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osmoregulators such as mannitol were used to slow plantlet growth while maintaining viability. Conditions of the five tested sweet potato accessions during *in vitro* cultures were shown in Figure 6.

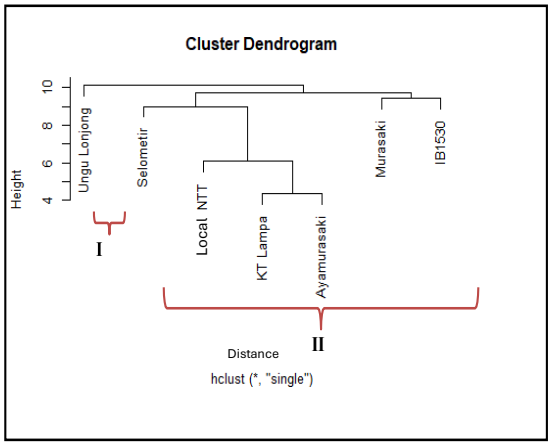

Fig. 5. Cluster dendrogram of seven purple-fleshed sweet potato accessions based on leaf and stem morphological traits.

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The MS medium was optimal for initiating and maintaining *in vitro* cultures of purple-fleshed sweet potato. This medium maintained cultures in good condition for 4 to 9 months (Sabda, 2018). Table 4 showed that, for long-term storage, minimal growth media based on MS supplemented with osmoregulators such as mannitol were used to slow plantlet growth while maintaining viability. Conditions of the five tested sweet potato accessions during *in vitro* cultures were shown in Figure 6.

Table 3. Sterilization results of explants from five purple-fleshed sweet potato accessions.

Accession	Sterile (%)	Contaminated (%)	Dead (%)
KT Lampa	45	53	2
IB1530	40	55	5
Local NTT	13	87	0
Murasaki	32	63	5
Ungu Lonjong	78.3	21.7	0
Average	41.7	55.9	2.4

Table 4. Optimization of storage duration for five purple-fleshed sweet potato accessions.

Accession	MS medium (months)	Minimal growth medium (months)
KT Lampa	4–6	18
IB1530	3–5	17
Local NTT	4.5–6.5	20
Murasaki	4–6	15
Ungu Lonjong	3–5	14

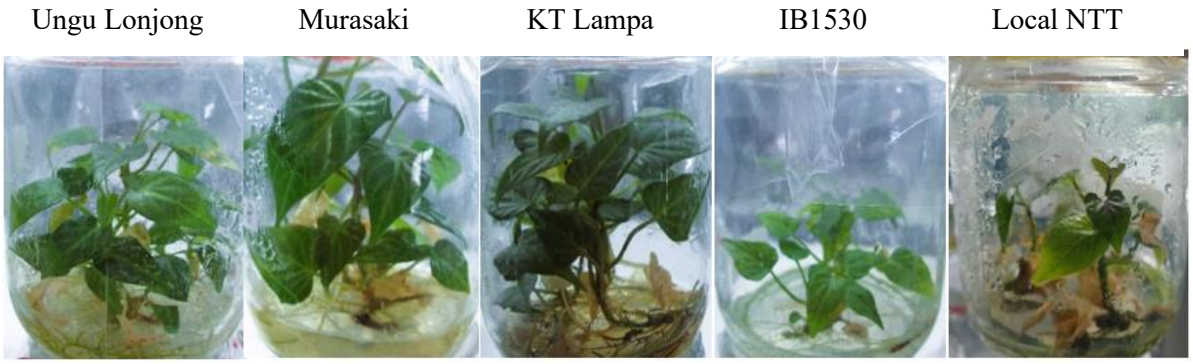

Fig. 6. Appearance of five purple-fleshed sweet potato accessions under *in vitro* conditions at different ages (3, 4, 4, 2, and 4 months, respectively).

Conclusion

Morphological variation analysis of purple-fleshed sweet potato revealed that all seven accessions exhibited diverse characteristics. Ungu Lonjong showed the most distinct morphology compared to the other six accessions. Local NTT, KT Lampa, and Ayamurasaki were found to be closely related, as were Murasaki and IB1530. The closer the genetic distance between accessions, the greater their morphological similarity. In the *in vitro* culture optimization, 41.7% of explants were successfully sterilized, 55.9% were contaminated, and 2.4% were dead. The Local NTT accession had the highest contamination rate, while Ungu Lonjong had the highest sterilization success rate.

Conflict of Interest

All authors have no conflicts of interest to disclose.

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