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Genetic diversity of Philippine sweet potato (*Ipomoea batatas* L. Lam) germplasm using morphological and molecular markers

Lalusin, A. G.*, Heredia, A. C., Batalon-Gaviña, L. J. S. and Iranista, E. J. A.

Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines at Los Baños, Philippines.

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Abstract The genetic and phenotypic diversity of sweet potato (*Ipomoea batatas* L. Lam*)* germplasm collected from Luzon, Philippines, was assessed through integrated morphological and molecular approaches. A total of 264 accessions, including released varieties, advanced lines, and hybrids, were characterized. Morphological evaluation revealed distinct variability in vegetative and storage root traits. Leaf morphology ranged from triangular to semi-elliptical with pigmentation varying from green to purple, while lobe number reflected high phenotypic plasticity. Storage roots exhibited diverse shapes, from elliptic to oblong, and variable pigmentation (orange to purple), indicative of carotenoid and anthocyanin accumulation. Environmental conditions, particularly excessive soil moisture, influenced trait expression by reducing pigmentation intensity and inducing structural deformities such as grooves and constrictions. Phenotypic diversity analysis using the Shannon Diversity Index revealed high variability in vine pubescence and skin pigmentation ($H' \geq 0.67$) but limited diversity in mature leaf color and flesh pigmentation ($H' \leq 0.33$). Cluster analysis (UPGMA) grouped the genotypes into 10 clusters, reflecting both genetic variation and environmental effects. Complementary molecular characterization using 54 SSR primers, of which 33 were polymorphic, confirmed substantial allelic diversity. The mean polymorphic information content (PIC) was 0.909, demonstrating strong discriminatory power. SSR-based clustering grouped 156 genotypes into 12 clusters at a Jaccard similarity coefficient of 0.89, providing higher resolution than morphological analysis and delineating regional populations and hybrid groups. The integration of phenotypic and molecular data highlights the extensive diversity within Philippine sweetpotato germplasm. While morphological traits were environmentally influenced, SSR markers effectively resolved genetic relationships and population structure. This combined framework provided a robust basis for germplasm management and utilization. The findings underscored the importance of conserving diverse alleles and exploiting them for breeding programs focused on pigmentation, root quality, stress resilience, and yield improvement, thereby supporting sustainable sweet potato production in the Philippines.

Keywords: Sweet potato, Genetic diversity, Germplasm collection, Morphological, Molecular markers

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***Corresponding Author:** Lalusin, A. G.; **Email:** aglalusin@up.edu.ph

Introduction

Sweet potato (*Ipomoea batatas* L. Lam.) is an indispensable crop globally, particularly in developing countries, where it accounts for over 95% of production. It ranks among the top five crops worldwide due to its adaptability, high yield potential, and nutritional value. Its resilience to a variety of stresses, including high temperatures, drought, pests, and diseases, further solidifies its role in ensuring food security. Sweet potato thrives across diverse geographical regions and has a short production cycle, making it a preferred crop in over 100 developing nations. Notably, sweet potato provides more edible energy per hectare per day than major staples like rice, wheat, and cassava, demonstrating its efficiency in addressing global food demands (Grüneberg *et al*., 2009).

Nutritionally, sweet potato is superior to many staple foods. It is an important source of beta-carotene, especially in orange-fleshed varieties (OFSP), which can be converted into Vitamin A. This attribute makes OFSP a powerful tool for addressing vitamin A deficiency, a condition that disproportionately affects children and lactating mothers in developing regions (Chandrasekara and Kumar, 2016). Vitamin A deficiency can lead to blindness and increased vulnerability to infectious diseases. A single medium-sized sweetpotato can provide the recommended daily allowance of Vitamin A, offering an accessible solution to this public health challenge. In addition to OFSP, purple-fleshed sweet potato (PFSP) varieties are also gaining attention due to their high anthocyanin content. Anthocyanins are potent antioxidants with potential anti-cancer, anti-diabetic, and anti-inflammatory effects (Ayeleso *et al*., 2016). These pigments, primarily peonidins and cyanidins, have been found in complex forms in PFSP, contributing to their stability and health benefits (Khoo *et al*., 2017). Advanced analytical techniques like mass spectrometry have identified up to 22 unique anthocyanins in purple sweet potatoes, providing valuable insights for breeding programs aimed at maximizing their health potential (Terahara *et al.,* 2004; Tian *et al*., 2005). Beta-carotene, responsible for the orange coloration in sweet potato, is also a significant dietary component. It acts as an antioxidant and can be converted into two molecules of Vitamin A. Its health benefits include reducing the risk of cancer, cataracts, and cardiovascular diseases (Grüne *et al.,* 2010). While beta-carotene is also present in dark green leafy vegetables, its intake from sweet potato has proven to be an efficient means of combating vitamin A deficiency in populations with limited dietary diversity.

Consumer preferences for sweet potato have shifted over time, moving from white-fleshed varieties, which are higher in starch, to orange- and purple-fleshed varieties, which are richer in nutrients like beta-carotene and anthocyanins. This shift reflects an increasing awareness of the crop’s nutritional benefits. Sweet potato is not only an essential food source but also holds promise as a functional food for improving human health.

Despite its significance, sweet potato production faces numerous challenges. One of the most critical threats is the Sweet Potato Virus Disease Complex (SPVD), which results from the interaction of the Sweet Potato Feathery Mottle Virus (SPFMV) and the Sweet Potato Chlorotic Stunt Virus (SPCSV). SPVD causes severe symptoms such as stunting, leaf curling, and chlorosis, leading to yield losses of up to 80% (Karyeija *et al.,* 2000; Gutiérrez *et al.,* 2003). SPFMV, the most widespread sweet potato virus, is aphid-transmitted and often asymptomatic in single infections. However, when combined with SPCSV, transmitted by whiteflies, the effects are devastating (Kreuze, 2002; Sim *et al.,* 2000). In the Philippines, SPVD, locally known as "kulot," is a major constraint to sweet potato farming.

Efforts to manage SPVD include the use of virus-free planting materials, sanitation practices, and breeding resistant varieties. However, controlling the insect vectors that transmit these viruses is often economically unfeasible for smallholder farmers. Furthermore, SPFMV can persist between cropping cycles in infected cuttings, making it difficult to eliminate. Such viral diseases continue to pose significant constraints on sweetpotato production, with an estimated 10-60% incidence observed in fields globally (Prasanth and Hegde, 2008).

Conserving sweet potato germplasm also presents challenges. Field genebanks, which are critical for maintaining genetic diversity, are expensive to maintain and vulnerable to environmental stresses, pest infestations, and cross-contamination. Genetic losses are exacerbated by small plot sizes and virus infections, particularly during dry periods (Huaman, 1999). Addressing these challenges requires the use of both morphological and molecular tools to evaluate and preserve genetic diversity effectively.

The diversity of sweet potato germplasm is a vital resource for breeding programs aimed at enhancing productivity and resilience. Morphological markers assess traits like root size, shape, flesh color, and foliage characteristics, which are essential for meeting farmer and consumer preferences (Laurie *et al.,* 2012). For instance, the orange and purple flesh colors are directly associated with high levels of beta-carotene and anthocyanins, respectively. Molecular markers such as Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs) complement morphological evaluations by revealing genetic variation at the DNA level. These markers enable researchers to identify traits associated with disease resistance, stress tolerance, and nutritional quality (Zhang *et al.,* 2000; Vaeasey *et al.,* 2008). Combining morphological and molecular data provides a comprehensive understanding of sweetpotato diversity, which is essential for selecting parent genotypes for breeding.

The integration of morphological and molecular approaches has already led to significant advancements in sweet potato improvement. For example, genetic diversity analyses have identified genotypes resistant to SPVD and other diseases, as well as varieties with enhanced nutritional profiles (Yada *et al*., 2010). Moreover, the high genetic variability observed in sweet potato populations highlights its potential for developing climate-resilient varieties. This is particularly important in regions like Southeast Asia and sub-Saharan Africa, which are centers of sweet potato diversity and home to many smallholder farmers who rely on the crop for their livelihoods.

Ultimately, sweet potato remains a cornerstone of food security, particularly in developing regions. Its high nutritional value, adaptability, and genetic diversity make it a critical resource for addressing global challenges such as malnutrition, climate change, and sustainable agriculture. By leveraging both morphological and molecular tools, researchers and policymakers can ensure that sweetpotato continues to play a central role in improving human health and livelihoods worldwide (Grüneberg *et al*., 2009; Low *et al.,* 2007; Islam, 2006). The study aimed to analyze and characterize the extent of genetic diversity of Philippine sweetpotato germplasm collections specifically in Luzon, using morphological and molecular markers.

Materials and methods

Sweetpotato germplasm collection and characterization

The success of any breeding program is dependent on the understanding of distribution of genetic diversity present in a gene pool. A total of 264 sweet potato germplasm collection consisting of 63 NSIC registered varieties, 45 advanced lines, 97 collected from Mindoro, Bicol, Region 4A (CALABAZON), 6 from NPGRL and 53 new hybrids were planted and evaluated in pots (Figure 1) and in the field. These accessions were characterized using the Revised Protocols for Sweet potato Characterization in the Philippines developed by NPGRL, CIP, and PhilRoots. A total of sixteen morphological traits for shoots and leaves such as leaf color and shape, color and pigmentation of vine and petiole, and root morphology were used to assess the sweetpotato collection.

DNA isolation

Genomic DNA of the 264 sweet potato accessions were isolated using a modified Doyle and Doyle (1987) protocol. One gram of leaf tissue was ground with liquid nitrogen and PVP, then mixed with CTAB extraction buffer and incubated at 65°C for an hour. The aqueous phase was treated with chloroform alcohol and centrifuged. The aqueous layer was mixed with NaCl and PEG solution, incubated at -4°C, and centrifuged again. DNA was precipitated with isopropanol, incubated overnight at -20°C, collected, washed with ethanol, and air-dried. The DNA pellet was resuspended in TE buffer with RNAse A and incubated at 37°C. DNA quality was assessed using a Biotek Epoch™ UV-VIS spectrophotometer and agarose gel electrophoresis and visualized under UV light with a Clinx GenoSens 1510 system.

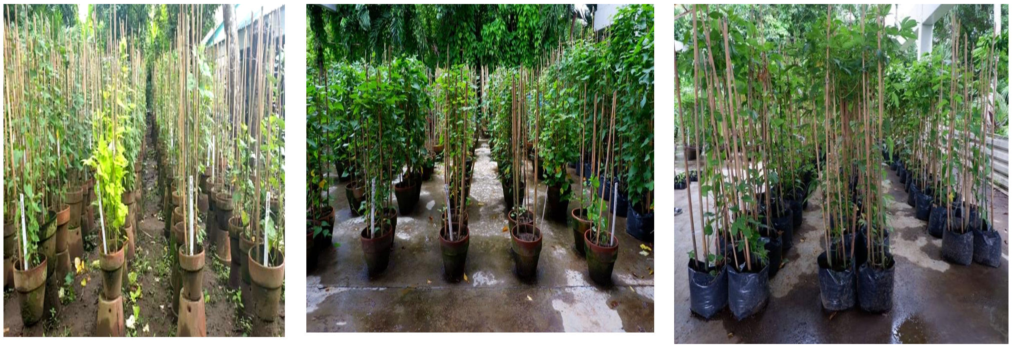


Figure 1. Sweetpotato germplasm collection maintained in clay and plastic pots

Primer selection and polymerase chain reaction

Microsatellites, or simple sequence repeats (SSRs), are molecular markers used in genetic map construction, marker-assisted selection (MAS), and population analysis. These markers, consisting of one to six nucleotide repeats, are common throughout eukaryotic genomes. In this study, 54 SSR markers (Table 1) showing high polymorphism were selected from the studies of Meng *et al.* (2018), Amoanimaa-Dede *et al.* (2020), and Naidoo *et al.* (2022). DNA templates were normalized to a concentration of 60 ng/µl for screening. Each marker was optimized which based on source literature, with annealing temperatures adjusted during optimization. The PCR profile included an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 45-64°C for 30 seconds, and extension at 72°C for 60 seconds, with a final extension at 72°C for 5 minutes. Amplified products were resolved using 6% polyacrylamide gel electrophoresis (PAGE) with Biotium GelRed™ staining. The PAGE gel consisted of 89.2 ml dH2O, 8 ml tris buffer, 12 ml 29:1 acrylamide, 120 µl 10% ammonium persulfate (APS), and 80 µl tetramethylethylenediamine (TEMED). The gel was visualized under a UV trans-illuminator using the GenoSens 1510 Gel documentation analysis system.

Table 1. List of selected SSR markers for molecular characterization of sweetpotato (*Ipomoea batatas*) genotypes

Primer	**Repeat motif**	**Forward sequence**	**Reverse sequence**	**Size (bp)**	**Chromosome**	**Allele . No**	**Ta (°C) literature**	**Ta (°C) Optimized**	**Literature cited**
IBM 2193	(AT)16	TGCATGTTTGGATGTTACAGG	CAATTACCGGAAAATTTTGGTC	248	Chr 15	4	55	-	Amoanimaa-Dede *et al.,* 2020
IBM 222	(AT)18	GTTCGCAGTTCCAAATTGCT	GCATCAACAACAAAAACAAAACA	254	Chr 7	7	55	-	Amoanimaa-Dede *et al.,* 2020
IBM 244	(A)24	GATCCCTCGAGGTGTGAAAG	AGGATCATGCTTCACCAACC	250	Chr 8	7	55	55	Amoanimaa-Dede *et al*., 2020
IBM 210	(A)25	CCTGTCCACTGGTCTAAGGC	GCGGTCTTCATCTTCTCTGG	278	Chr 7	10	55	55	Amoanimaa-Dede et al., 2020
IBM 442	(T)33	AATCTGTCAGGGAGTGGTGG	AAATGCAACCCAAACAAAGC	236	Chr 10	2	55	55	Amoanimaa-Dede *et al.,* 2020
IBM 203	(T)36	CCATATCAATAGGCCGTGCT	CGAACCTCAGTGAAGACGAA	231	Chr 7	7	55	-	Amoanimaa-Dede *et al*., 2020
IBM 182	(A)78	GCCTTTGCTTTTCCTCTCCT	CCGGAAACCAGCTAATCAAA	262	Chr 6	9	55	55	Amoanimaa-Dede *et al.,* 2020
IBM 1895	(CT)12	AATTCAATGTGGGGTCTTGC	GCTTGATCTAACTCGGTGGC	236	Chr 12	2	55	55	Amoanimaa-Dede *et al.,* 2020
IBM 335	(A)25	CTTGAACAACACCTCAGGCA	CGAGAGGAATCAGAGCCAAC	199	Chr 9	2	55	-	Amoanimaa-Dede *et al*., 2020
IBM 346	(ATT)13	ATGCCCACATCATCATCATC	GAATCACATATTTGCCCCTGA	254	Chr 9	2	55	-	Amoanimaa-Dede *et al*., 2020
IBM 56	(TA)11	GGCCTTAGTCTTCGAAACACAT	CGTTTGGTCTTCTGGGGTTA	236	Chr 2	4	55	55	Amoanimaa-Dede *et al., 2020*
IBM 228	(TGA)8	CTCTCTTTCTTCCTTTGCCG	GGTAGAGAAGGGAGGAGAAAGG	259	Chr 7	4	55	-	*Amoanimaa-Dede et al., 2020*

IBM 126	(CA)12	GCGAAAATGTCACCGAGTTT	GCTCTTTTCTCATCGCACCT	191	Chr 4	7	55	55	Amoanimaa-Dede *et al.*, 2020
IBM 422	(A)25	TTGTTCTTGCCCAATTTGCT	AAACAATCAGCCCACACACA	211	Chr 9	2	55	55	Amoanimaa-Dede *et al.*, 2020
IBM 89	(T)26(T)30	CTTAGCGCTTCATGGGAGAC	GGCATAATCAGCTCAATTCCA	259	Chr 3	4	55	55	Amoanimaa-Dede et *al*., 2020
IBM 127	(TAA)8	TTCATCCTGCAAACACATGC	TTAACGCCAACCCAACTTTC	266	Chr 4	6	55	55	Amoanimaa-Dede *et al.*, 2020
IBM 265	(T)27	AAACTTAGGTGATCCCAATCC	AACATAGTTGGTTCGTCGCC	209	Chr 8	13	55	-	Amoanimaa-Dede *et al.*, 2020
IBM 185	(T)52	TACGTTGTCTTCCCTTCCCA	TTGGAATTACATCAACCCCC	224	Chr 6	8	55	-	Amoanimaa-Dede *et al.*, 2020
IBM 2166	(TA)15	TGGGTTGAGGTTGAGGAAAC	CTTCTAAAACCATCGCCCAA	208	Chr 15	3	55	55	Amoanimaa-Dede *et al.*, 2020
IBM 59	(AT)12	ATCCAATGACGCTAGTTCGG	CCAAAAACACAGCCATCAGA	175	Chr 2	3	55	-	Amoanimaa-Dede *et al.*, 2020
IBM 124	(T)26(T)36	TCTTGAAGGGGTAAGGCAAA	CATAAAATTTTGCTCCACATGC	234	Chr 4	4	55	55	Amoanimaa-Dede *et al.*, 2020
IBM 2060	(CAA)10(ATA)16	AAGAAATCTTTTTGGAATGCGA	ACCGTACAACGACGGTTCAT	255	Chr 14	4	55	55	Amoanimaa-Dede *et al.*, 2020
IBM 1745	(ATAC)6(AT)16	TGTGTTTGGTTCAACAAGGAA	ACGAGTTGGGTATGAATCGG	244	Chr 11	7	55	55	Amoanimaa-Dede *et al.*, 2020
IBM 2209	(TTG)8	ATGGTTTTGTGGGCAAAAGT	ACGCTCTCTTCATGCCAAGT	138	Chr 15	1	55	55	Amoanimaa-Dede *et al.*, 2020
IBM 2010	(TAT)14(TGTTAT)6	TTAATAAAAGTTTGCGCGGG	ATGCAGATCCCTGATTTTGG	208	Chr 13	7	55	55	Amoanimaa-Dede *et al.*, 2020

IBM 255	(TAT)16	AAATTTATTTAGATTTTGGATACGGA	ATTGTTACCATGCACAGGCA	234	Chr 8	5	55	55	Amoanimaa-Dede *et al.,* 2020
IBM 211	(A)23(A)21	GACACTGAATTGATCTCCCGA	TCGGTTGTTGTTGTTGTTTTT	207	Chr 7	10	55	55	Amoanimaa-Dede *et al.,* 2020
IBM 760	(T)25	GCCAGAATTTTCTGTCAACCA	AAAAGAACGTGGGGAAGGAA	180	Chr 10	1	55	55	Amoanimaa-Dede *et al.,* 2020
IBM 1174	(A)25	TGCAACATGCCATAAATGCT	CCTAAAGCTTTCCCGTTTTG	267	Chr 10	2	55	55	Amoanimaa-Dede *et al.,* 2020
IBM 106	(T)87	TTGGGGAAGGCTTTTAGGTT	TTGTGATCCTTTCTCAGTTAAGGT	280	Chr 3	10	55	-	Amoanimaa-Dede *et al.,* 2020
IBM 52	(TTA)11	GCACTTAGCCACCCCTATCA	AAACAAAATTGTGGGAGAGCA	171	Chr 2	10	55	-	Amoanimaa-Dede *et al.,* 2020
IBM 261	(TAA)9(TAT)8(ATC)7	TGCATTTAAAAACTCCGTAATACA	GAATGAATGCAATTCTAAAAACCC	218	Chr 8	9	55	-	Amoanimaa-Dede *et al.,* 2020
IBM 27	(A)24	GGTTTGAATTTGGAGTGAACATC	TGAGTTGTGACGTGTGAGCA	217	Chr 1	1	55	55	Amoanimaa-Dede *et al.,* 2020
IBM 2082	(AT)16	TATCTACCCAACCGACCTGC	CCGTTAGATCTGAACACGTGAA	238	Chr 14	1	55	55	Amoanimaa-Dede *et al.,* 2020
IBM 241	(T)25	CTGCACACATGCAACACAAC	TCAGTATCACAAAGCTCCACAA	189	Chr 8	10	55	-	Amoanimaa-Dede *et al.,* 2020
IBM 1923	(ATT)9	CAACCAAACCCCCTAATGTG	ACATGGTTTCAGAGGGACCA	211	Chr 13	4	55	-	Amoanimaa-Dede *et al.,* 2020
IBM 1733	(TA)19	TGATTTTGGATGTTATTTCATCATTT	TCTTGGCTTAAGTTATCGGCA	270	Chr 11	6	55	55	Amoanimaa-Dede *et al.,* 2020
IBM 119	(A)24	GGAAACGTTAGTACAAGTTGACACA	TCGCACATTATTAAAAACGGTCT	273	Chr 4	4	55	-	Amoanimaa-Dede *et al.,* 2020

IBM 1895	(CT)12	AATTCAATGTGGGGTCTTGC	GCTTGATCTAACTCGGTGGC	236	Chr 12	2	55	55	Amoanimaa-Dede *et al.*, 2020
IBM 291	(A)30	CCAAGCAAGCACACAACTTT	GCACGCTGTGCTTAAAATGA	277	Chr 9	7	55	-	Amoanimaa-Dede *et al.*, 2020
IBM 53	(A)45	CCAAACACCCACATAGACACC	AAGCACACTGATGTGCCACT	239	Chr 2	10	55	-	Amoanimaa-Dede *et al.*, 2020
IBM 582	(ATT)9	AAGGTTATGATGGCCGACTG	AAAAACTCCGTTCCCATCAA	157	Chr 10	7	55	-	Amoanimaa-Dede *et al.*, 2020
IBM 296	(A)25	ATAAGAAGAGAGCGGGTCGG	TGCACTTTGAATGCACAACA	220	Chr 9	5	55	-	Amoanimaa-Dede et al., 2020
IBM 1984	(AT)23	TGACATGTGCCGATACTCTAAAA	GCAAAACACTTCTTCATGGG	250	Chr 13	3	55	-	Amoanimaa-Dede *et al.*, 2020
IBM 32	(T)29	TCCACATAAGGGAGATGAGGA	TGTGGAGGGGAGAGAGTGTT	252	Chr 1	10	55	-	Amoanimaa-Dede *et al.*, 2020
SPGS1	(GGC)5	CTCGCTCACGATTGATGATG	CGGAGTGGTAGGGCTAAACC	100-900	-	41	59	-	Meng *et al.* 2018
SPGS2	(AAG)5	AGACTGGACTCCCAGAAGCA	CAAGCAGTCAGAAGTCGACAA	100-900	-	18	56.8	-	Meng *et al.* 2018
SPES1	(GTCTG)5	AATGCCAACCAAAGCCATAG	CGATGACAAAGCAGCTGAAG	100-900	-	35	57.9	-	Meng *et al.* 2018
SPES2	(AGAAG)6	TCGGAACGGAGATAGATTGG	AAGCAAGAAAAAGAAGTGAAGGAA	100-900	-	19	59	-	Meng *et al.* 2018
SPES3	(CAGCCC)3	ATGACATCCCAAGGAGCATC	GAGGTTGAGGGCGTATCTGA	100-900	-	28	57.4	-	Meng *et al.* 2018
IB-286	(CT)12	AGC CAC TCC AAC AGC ACA TA	GGT TTC CCA ATC AGC AAT TC	90–122			50	55	Naidoo *et al.* 2022
IB-316	(CT)3C(CT)8	CAA ACG CAC AAC GCT GTC	CGC GTC CCG CTT ATT TAA C	150			54	55	Naidoo *et al.* 2022
IBSSR 17	(AG)6N(AG)17	ACG TGC AGA CTT AGC CAC AC	AGG AAG CCA GAT GTT CAG ATG	201–245			56	55	Naidoo *et al.* 2022
IBSSR 18	(GA)7(AG)5(GA)4	GAT CTT GAA TTA GCC CAC	AGA TGG ATG ACC GTA TGC	90–110			58	55	Naidoo *et al.* 2022

Field evaluation and characterization of collected sweetpotato germplasm

Sweet potato collections initially planted in clay and plastic pots in the screenhouse were planted in the field for multiplication to produce enough planting materials. Accessions with sufficient planting materials underwent field evaluation for root characteristics and yield using a randomized complete block design (RCBD) with three replicates (Figure 2). Each genotype was planted in two 3-meter plots per row per replicate, with 11 cuttings per row and 0.3-meter spacing between hills. Rows and blocks were spaced 1 meter apart. The initial trial was conducted in the dry season, starting in November 2020, and harvested in March 2021, at 105 days after planting. During harvest, storage roots were collected and categorized into marketable and unmarketable yield. Both tuber number and weight were recorded for each genotype replication. Morphological traits such as skin and flesh color were characterized, and roots were analyzed for dry matter, starch content, anthocyanin content, and beta-carotene content at the Analytical Service Laboratory of the Institute of Plant Breeding.

In the subsequent wet season preliminary trial, the same procedures were followed, with planting in May 2021 and harvesting in September 2021. Promising genotypes from these trials underwent a comprehensive general yield trial using an RCBD with four replicates. Each replicate consisted of two 6-meter plots per row, with 21 plants/cuttings spaced 0.3 meters apart. Rows and blocks were again spaced 1 meter apart.

Figure 2. Field planting and harvesting for root characteristics evaluation of sweet potato germplasm collection

Results

Morphological characterization of sweet potato genotypes

Morphological characterization for foliage and vine was conducted using sixteen descriptors based on the Revised Protocol for Sweet potato

Characterization in the Philippines. Traits such as leaf color and shape, vine and petiole color pigmentation, and number and type of lobes were noted and scored. It showed various leaf phenotypic variations across sweet potato genotypes collected in different areas of Luzon, Philippines (Figure 3). Some of the predominant phenotypic traits observed from leaves across genotypes were the triangular leaf outline and semi-elliptical central lobes. Leaf color variations were mostly green or purple with veins that can also be green or strongly pigmented with anthocyanin. There are also genotypes with both green mature leaves and purple young leaves. Some genotypes even display a variation of leaf shape and lobe number on the same plant.

On the other hand, root morphological assessment was carried out after harvesting. The presence of purple and orange color pigmentation in the root flesh denotes high anthocyanin and carotenoid biosynthesis. Hence, flesh color was scored through their cross sections (Figure 4). Most of the purple-fleshed genotypes were characterized as intermediate purple to strongly pigmented with anthocyanin and a few individuals were recorded with pale purple color. Pale to dark orange root flesh color were also recorded among orange genotypes. Interestingly, similar to the check variety, genotypes that showed high pigmentation of purple and orange color during dry season were observed to have lighter flesh color or less pigmentation during the wet season. This might be associated with some environmental factors such as excess amount of moisture during wet season considering that color intensity depends on environmental condition. For accurate quantification of dry matter, starch, anthocyanin, and beta-carotene content, storage roots of each genotype per replicate were sent to Analytical Service Laboratory of Institute of Plant Breeding. Moreover, storage root shape, size, and skin color were also noted. Most genotypes have red purple to dark purple skin while shape varies from elliptic, round elliptic, ovate, obovate, and oblong (Figure 5). Most genotypes showed normal root surface, but some showed some defects such as horizontal constriction, longitudinal grooves, and vein-like skin.

Shannon Diversity Index (H’) was calculated for each morphological character to assess phenotypic diversity across accessions (Table 2). The Shannon Diversity Index of the morphological traits ranged from 0.28 (distribution of secondary flesh color) to 0.94 (vine tip pubescence). It indicated a high diversity among sweetpotato genotypes based on vine tip pubescence. Out of all the morphological characteristics assessed, high variability was observed in 6 traits ($H' \geq 0.67$), intermediate variability was observed in 7 traits ($0.34 \leq H' \leq 0.66$), and low variability was observed in 3 traits ($0.01 \leq H' \leq 0.33$). Predominant vine color, secondary vine color, immature leaf color, petiole pigmentation, and intensity of predominant skin color were the traits that obtained high variability (H’ ≥ 0.67) among the tested accessions. The traits that obtained intermediate variability were general outline of the leaf, leaf lobe number, shape of central leaf lobe, leaf lobe type, abaxial leaf vein pigmentation, predominant skin color, and secondary flesh color. Lastly, the morphological characteristics that were considered to have low variability (0.01 ≤ H’ ≤ 0.33) were mature leaf color, predominant flesh color, and distribution of secondary flesh color. The H’ value of these 3 traits indicated low diversity among sweetpotato genoty1es in terms of the aforementioned properties.

Table 2. Modified Standardized Shannon Diversity Index (H’) of sweetpotato foliage and root morphological characteristics

SWEETPOTATO MORPHOLOGICAL CHARACTERISTICS	H’
FOLIAGE	
Predominant vine color (PVC)	0.82
Secondary vine color (SVC)	0.84
Vine tip pubescence (VTP)	0.94
General outline of the leaf (GOL)	0.47
Leaf lobe type (LLT)	0.64
Leaf lobe number (LLN)	0.61
Shape of central leaf lobe (SCLL)	0.51
Abaxial leaf vein pigmentation (ALVP)	0.66
Mature leaf color (MLC)	0.31
Immature leaf color (ILC)	0.82
Petiole pigmentation (PP)	0.85
ROOT	
Predominant skin color (PSC)	0.65
Intensity of predominant skin color (IPSC)	0.85
Predominant flesh color (PFC)	0.29
Secondary flesh color (SFC)	0.36
Distribution of secondary flesh color (DSFC)	0.28

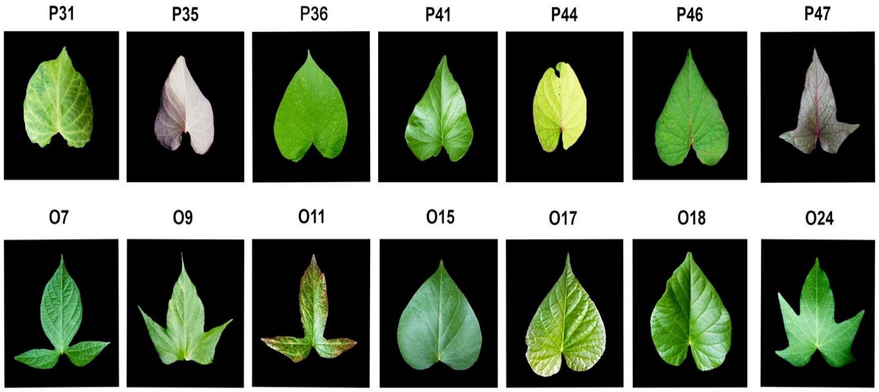


Figure 3. Variations in the leaf phenotypic characteristics of sweetpotato germplasm collections collected in different parts of the Philippines

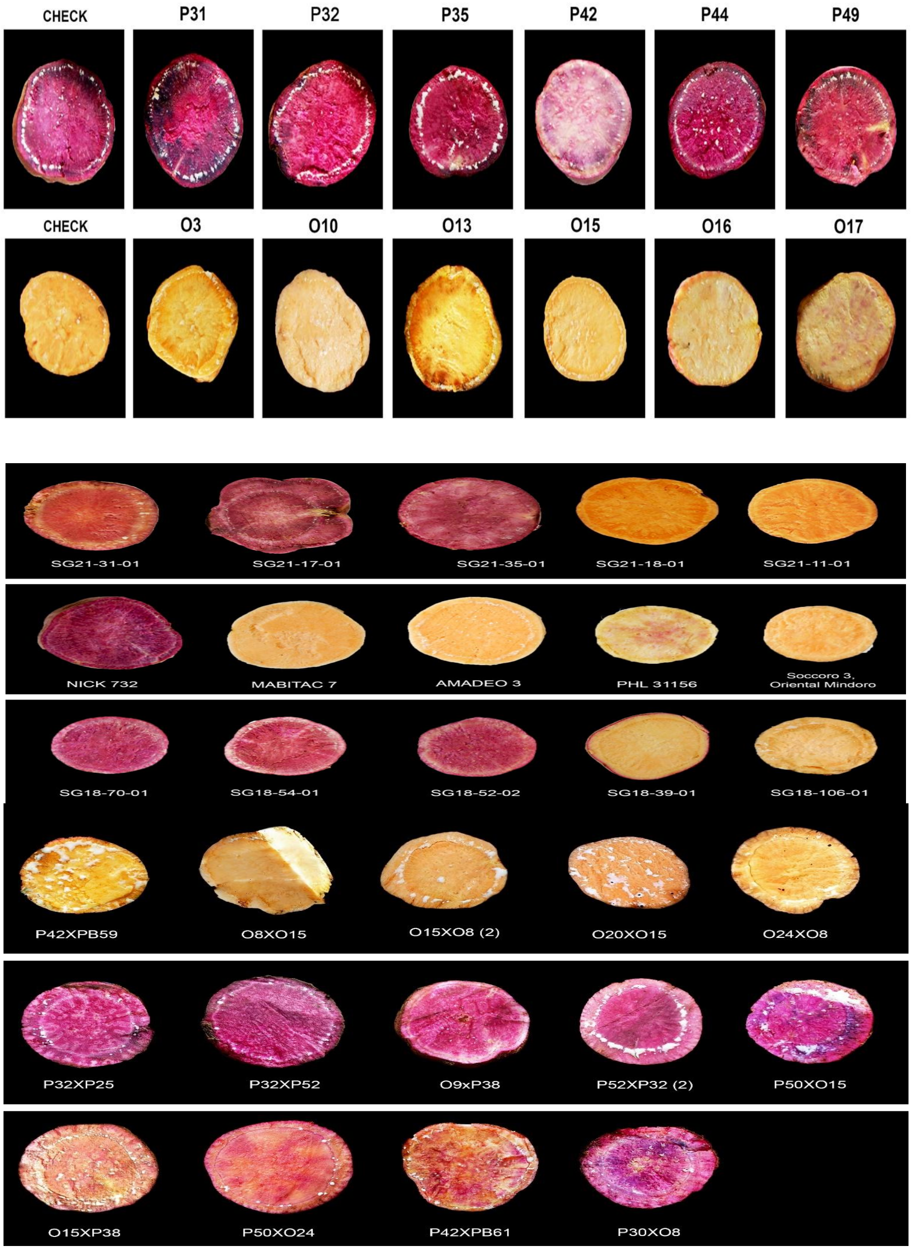


Figure 4. Variations in the root flesh color of the different sweetpotato germplasm collection from Luzon, Philippines

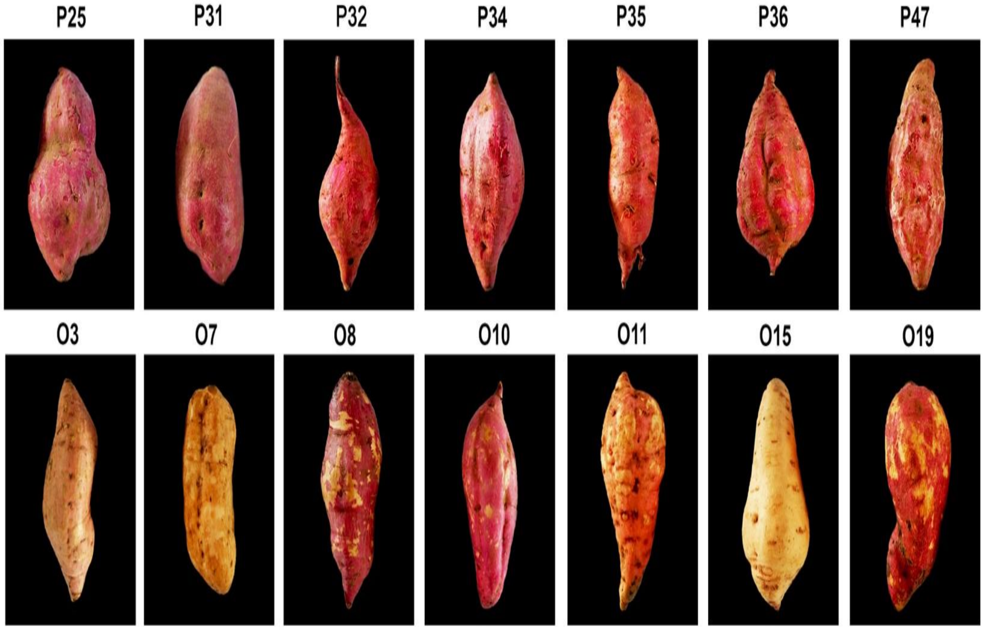


Figure 5. Variations in the root shape and root color of the sweetpotato germplasm collected in different parts of Luzon, Philippines

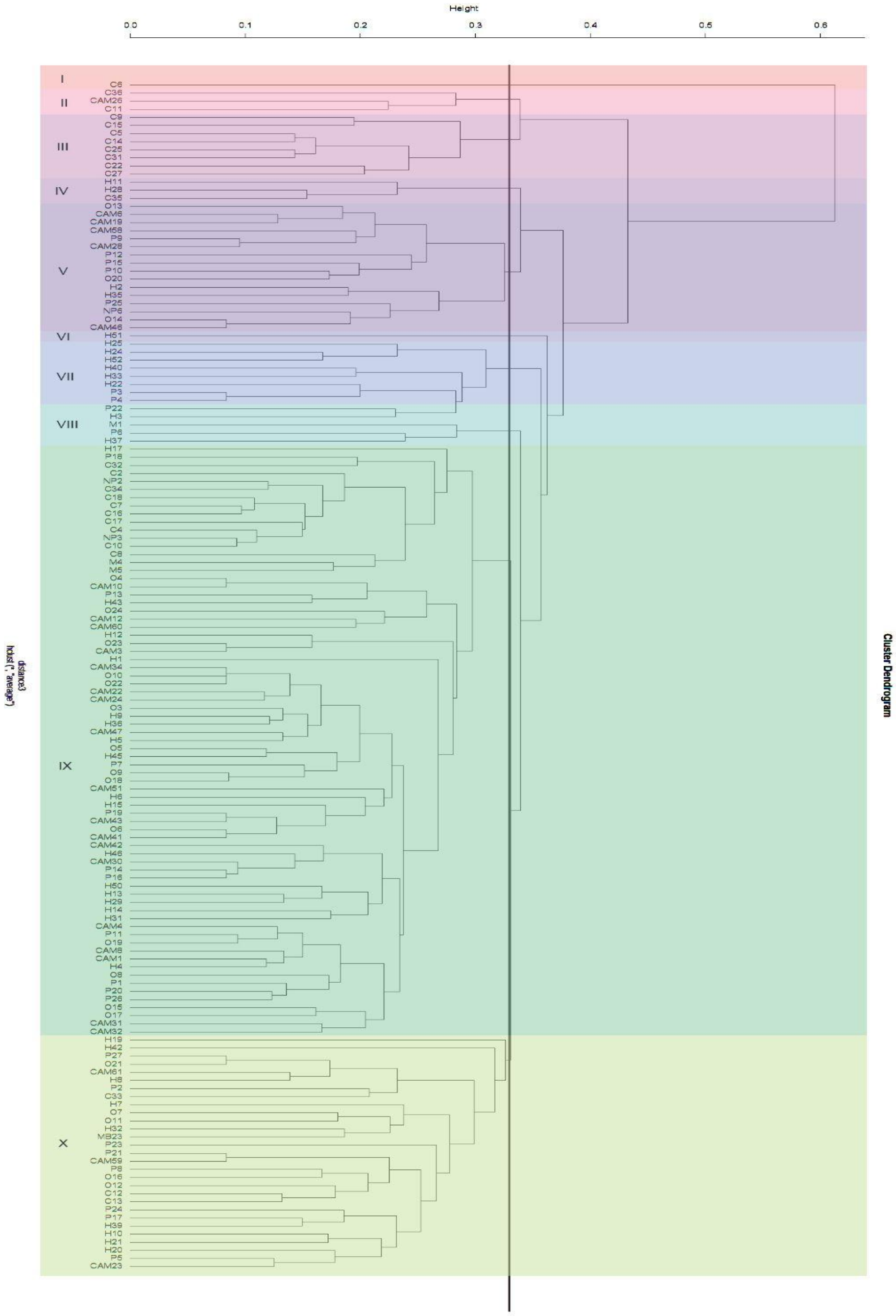


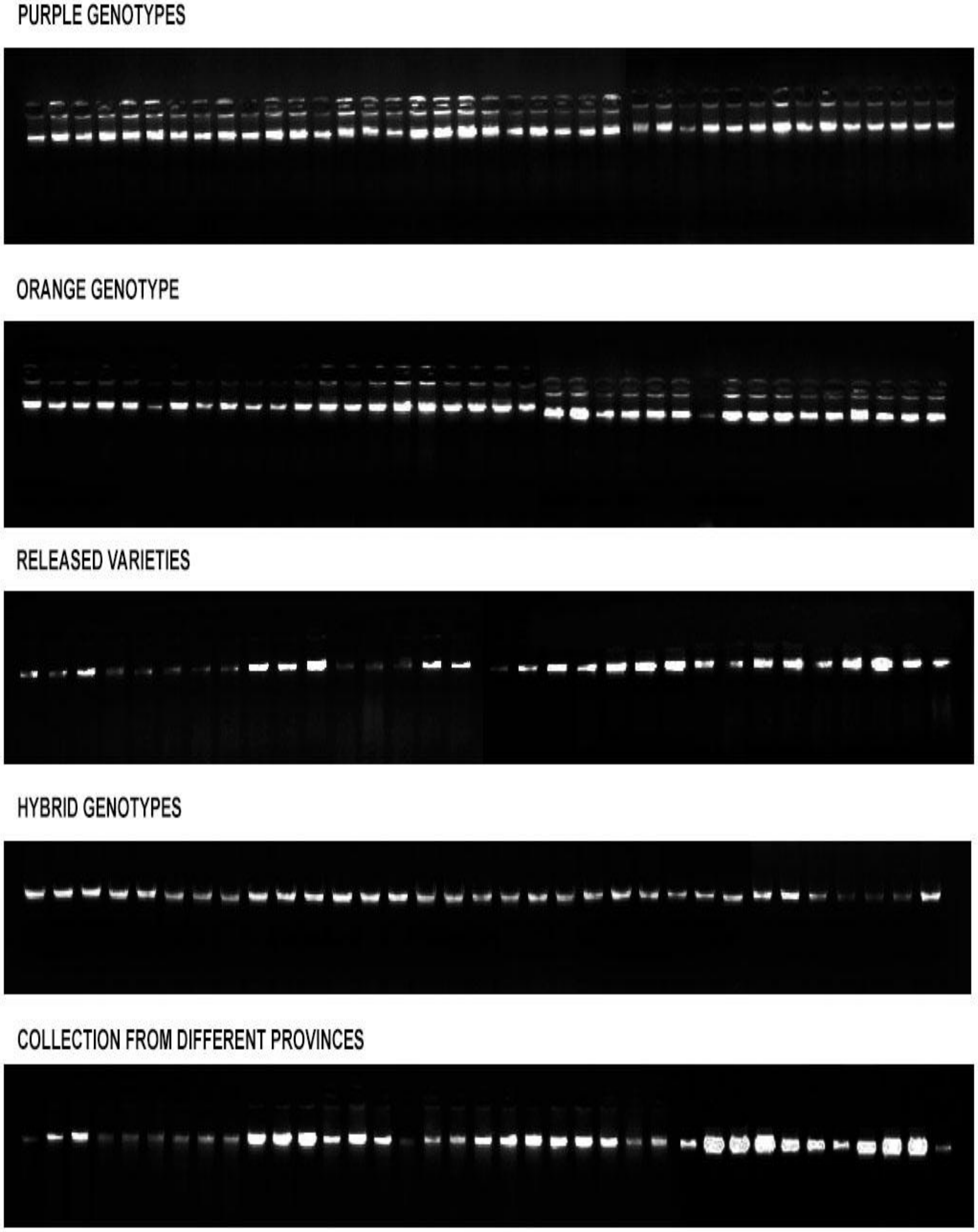
Figure 6. Dendrogram of sweet potato genotypes based on morphological characteristics generated using R studio showing 10 clusters at 0.34 coefficient of dissimilarity

Cluster analysis from morphological data was generated which based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) using R studio (Figure 6). From 264 accessions collected, only 147 accessions were fully characterized in the field, and they were grouped into 10 major clusters at 0.34 dissimilarity coefficient. Cluster I was comprised of 1 accession (C6), cluster II was comprised of 3 accessions (predominantly accessions from CALABARZON), cluster III was comprised of 8 accessions (all accessions from CALABARZON), cluster IV was comprised of 3 accessions (predominantly hybrids), cluster V was comprised of 16 accessions (hybrid genotypes and their parents), cluster VI was comprised of a single hybrid accession, cluster VII was comprised of 10 accessions (all hybrids), cluster VIII was comprised of 3 accessions (predominantly hybrids), cluster IX was comprised of 73 accessions (predominantly hybrids and their parents), and cluster X was comprised of 29 accessions (predominantly hybrids).

Molecular characterization of sweetpotato genotypes

Genomic DNA was isolated using the modified Doyle and Doyle (1987) protocol. Intact bands were generated using 1% AGE gel, indicating absence of degradation (Figure 7). Moreover, quality and quantity assessment through spectrophotometry showed an A_{260}/A_{280} ratio reading of 1.8-2.0 indicating good quality DNA. Normalization of template concentration was done by dilution of DNA to a final concentration of 60ng for all samples. Moreover, primer selection and synthesis were done to carry out the molecular characterization of sweetpotato genotypes.

DNA amplification was carried out across genotypes. Thirty-three of the 54 screened markers produced band polymorphism. Amplicons were visualized through polyacrylamide gel electrophoresis and analyzed using GelAnalyzer software based on binary scoring. Unique banding patterns were generated by SSR markers (Figure 8). Polymorphic information content (PIC) was calculated for each marker to assess the genetic variation (Table 3). Highest PIC was obtained from IBM 296 with 0.964 while the lowest value was from IBM 119 with 0.744. The high average overall PIC of 0.909 indicated polymorphism among genotypes. Furthermore, it also indicated that the used markers were discriminatory and highly informative.


PURPLE GENOTYPES

ORANGE GENOTYPE

RELEASED VARIETIES

HYBRID GENOTYPES

COLLECTION FROM DIFFERENT PROVINCES

Figure 7. Genomic DNA of sweet potato representative genotypes resolved in 1% agarose gel

R Studio software was used to run molecular binary data for the dendrogram establishment. Unweighted pair group method with arithmetic mean (UPGMA) was used as clustering method. The dendrogram of 156 sweet potato accessions grouped into twelve major clusters at approximately 0.89 Jaccard similarity coefficient is shown in Figure 9. Cluster VI generated the largest number of genotypes with 37 accessions (all hybrids), followed by cluster IX with 30 accessions (all parental genotypes), cluster VII with 28 accessions (all accessions from CALABARZON area), cluster III with 25 accessions (predominantly purple genotypes), cluster V with 17 accessions (all orange genotypes), cluster XII with 6 accessions (all orange genotypes), cluster X with 5 accessions (predominantly purple), cluster IV with 3 accessions (all orange genotypes), cluster VIII with 2 accessions (both orange genotypes), and finally, clusters XI, I, and II with 1 accession each. The relatively high resolution of the phylogenetic tree generated from SSR markers implies a more effective discriminatory ability for trait characterization as compared to identification on the basis of morphological characteristics.

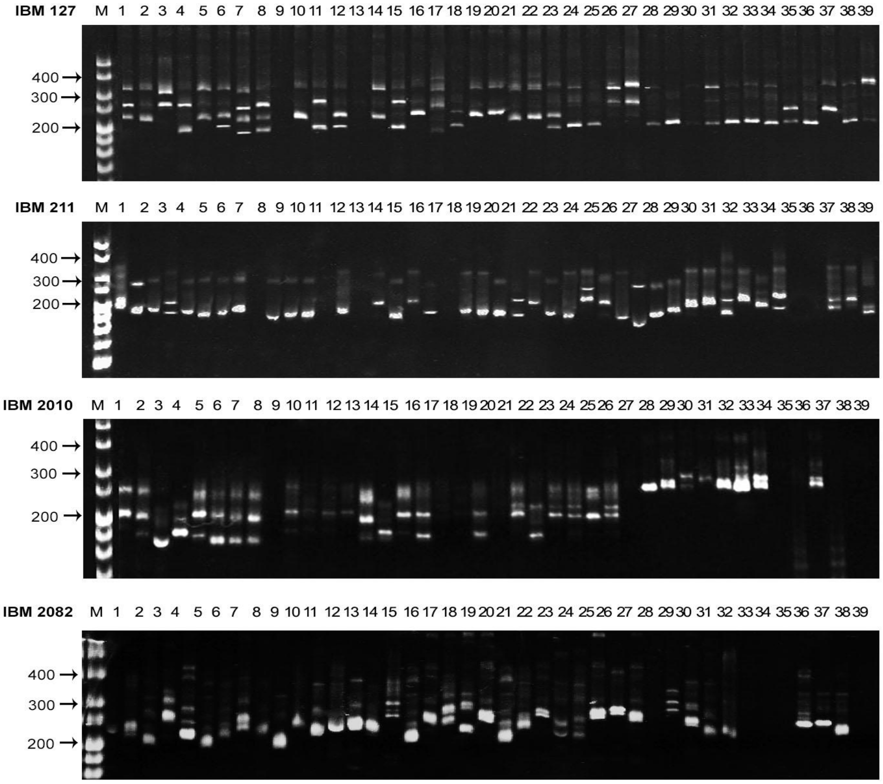


Figure 8. PAGE gel (6%) depicting the banding patterns of sweet potato genotypes generated by markers IBM 127, IBM 211, IBM 2010, and IBM 2082 with 25 base pairs ladder

Table 3. SSR markers with high PIC values screened across sweet potato genotypes collected in different parts of Luzon, Philippines

Marker	Allele number	Max Allele frequency	Min Allele frequency	Total P^2	PIC
SSR18	8	0.336	0.004	0.255	0.745
SSR17	54	0.147	0.002	0.050	0.950
IB316	12	0.252	0.004	0.158	0.842
IBM1923	22	0.118	0.004	0.069	0.931
IBM1745	28	0.159	0.005	0.065	0.935
IBM241	22	0.150	0.005	0.074	0.926
IBM222	24	0.098	0.006	0.059	0.941
IBM124	20	0.146	0.007	0.081	0.919
IBM261	50	0.101	0.003	0.045	0.955
IBM265	30	0.142	0.004	0.071	0.929
IBM185	41	0.082	0.004	0.040	0.960
IBM52	17	0.272	0.004	0.152	0.848
IBM53	25	0.135	0.003	0.081	0.918
IBM126	34	0.143	0.003	0.080	0.920
IBM119	7	0.323	0.006	0.256	0.744
IBM32	26	0.137	0.005	0.098	0.902
IBM422	20	0.143	0.004	0.086	0.914
IBM182	24	0.147	0.004	0.077	0.923
IBM1174	19	0.155	0.010	0.093	0.907
IBM296	43	0.071	0.006	0.036	0.964
IBM2010	29	0.128	0.003	0.072	0.928
IBM2060	48	0.075	0.003	0.039	0.961
IBM2209	11	0.278	0.006	0.173	0.827
IBM244	44	0.071	0.005	0.039	0.961
IBM442	13	0.191	0.007	0.120	0.880
IBM89	22	0.135	0.004	0.076	0.924
IBM56	36	0.112	0.004	0.058	0.942
IBM2082	38	0.155	0.004	0.056	0.944
IBM27	23	0.150	0.003	0.098	0.902
IBM127	36	0.085	0.004	0.045	0.955
IBM1895	21	0.172	0.003	0.083	0.917
IBM59	31	0.149	0.003	0.079	0.921
IBM106	17	0.181	0.009	0.124	0.876

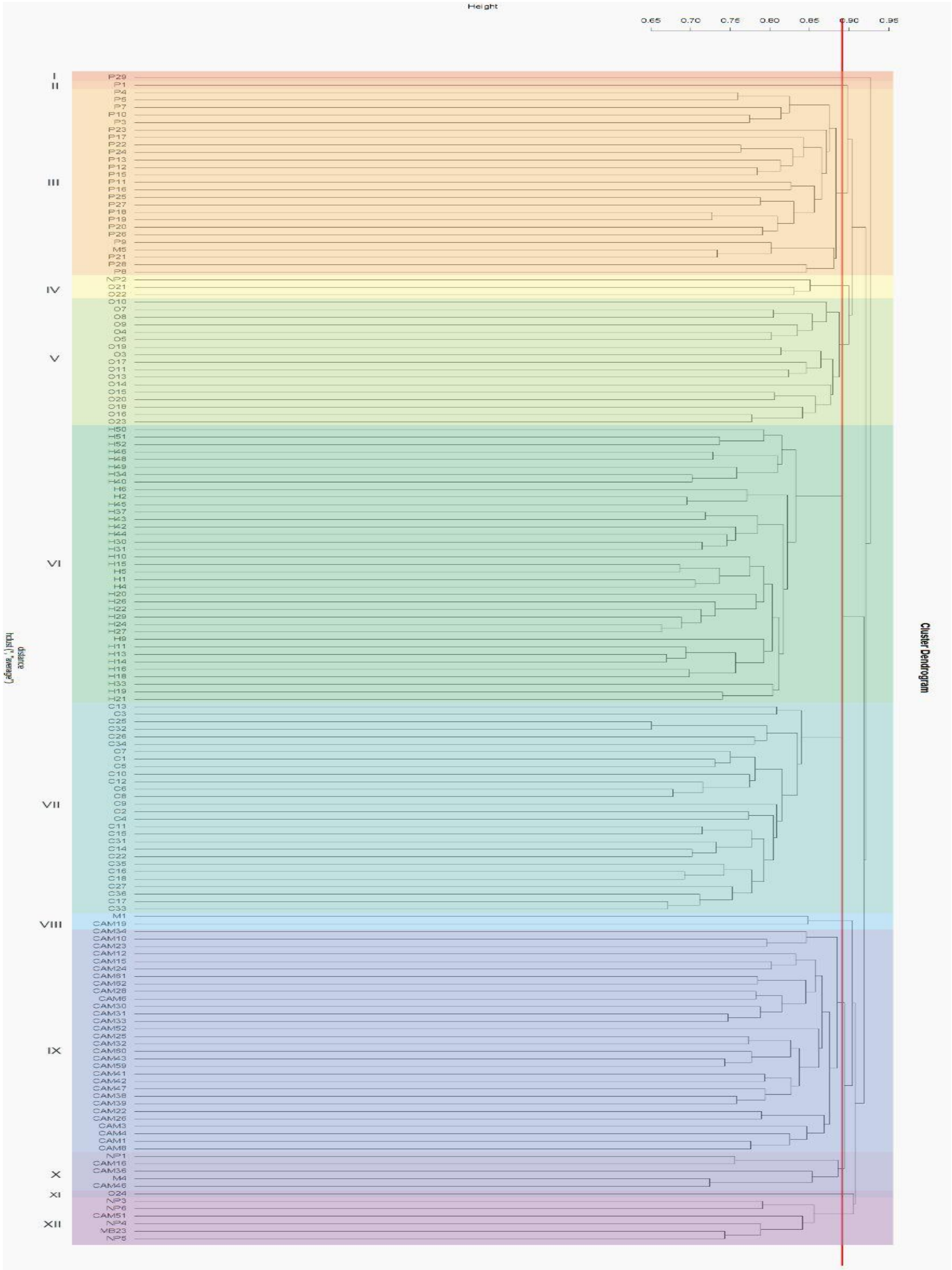


Figure 9. Dendrogram of sweet potato genotypes based on binary scoring generated using R studio showing twelve clusters at approximately 0.89 Jaccard similarity coefficient

Discussion

An in-depth investigation into the morphological and genetic diversity of sweet potato genotypes, shedding light on how phenotypic variations, root characteristics, and genetic factors interact was elucidated in this study. By integrating morphological assessment with molecular analysis, it provided a comprehensive understanding of diversity patterns and the influence of environmental factors on phenotypic expression. The large-scale evaluation of sweet potato germplasm for genetic diversity is vital for exploiting the valuable alleles present in diverse collection and varieties of this crop. However, the number of sweetpotato accessions with clear genetic diversity is still limited to date (Yang *et al.,* 2015; David *et al.,* 2018; Meng *et al.,* 2018).

The study observed notable leaf and root morphological variations across the 147 genotypes. Leaf traits were generally consistent, with triangular outlines, semi-elliptical lobes, and a range of pigmentation from green to purple. However, some plants exhibited heterogeneity in leaf shape and lobe number, indicating a degree of phenotypic plasticity. Root characteristics also varied, with pigmentation ranging from purple to orange, indicative of anthocyanin and carotenoid biosynthesis. Interestingly, pigmentation intensity diminished in the wet season, likely due to excessive moisture disrupting the phenotypic expression of these traits. The shape of the sweetpotato roots varied from elliptic to oblong, and skin pigmentation ranged from red purple to dark-purple. Environmental factors, such as soil conditions and moisture saturation, were noted to influence defects in root morphology, such as grooves and constrictions.

Morphological traits were evaluated for diversity using the Shannon Diversity Index. The traits with high variability (H' ≥ 0.67) included vine tip pubescence and skin pigmentation, while traits like mature leaf and flesh colors showed lower variability (H' ≤ 0.33), suggesting less phenotypic variation. The phenotypic data were analyzed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), resulting in the grouping of the 147 genotypes into 10 clusters. These clusters reflected both genetic and environmental factors, with some regions (e.g., CALABARZON) and hybrids having prominent representation. Cluster sizes varied, from single accession groups to larger clusters of 73 genotypes. A study on the morphological diversity of *Ipomoea batatas* in Nigeria highlighted significant variation in tuber shape, color, and yield among accessions (Egesi *et al*., 2003). Research on morphological diversity has shown that traits such as root color, tuber weight, and leaf shape are often reliable indicators for differentiating between varieties, which can be valuable for crop improvement or conservation purposes (Hauser *et al.,* 2013).

Moreover, genetic diversity of 264 sweet potato accessions were also analyzed using 54 SSR primer pairs of which 33 were polymorphic. These markers had a high Polymorphic Information Content (PIC), averaging 0.909, indicating their high discriminatory power for genetic analysis. The PIC value and gene diversity analyses revealed that these sweet potato accessions have richer alleles and gene diversity. These results are consisted with those of Yang *et al.* (2015), possibly because of the self-incompatibility of sweet potato (Yang *et al.,* 2017). The molecular data also revealed a distinct genetic structure, with 156 genotypes grouped into 12 clusters at a Jaccard similarity coefficient of 0.89. Notably, Cluster VI contained 37 hybrids, and other clusters represented regional or parental genotypes. The genetic analysis demonstrated greater resolution than the morphological data, revealing a more detailed understanding of the genetic diversity among the sweet potato genotypes. SSR markers are widely used to assess the genetic diversity in crops because of their advantages of co-dominance, high polymorphism, reliability and reproducibility (Huang *et al.* 2014; Xiao *et al.* 2021; Ngailo *et al.* 2016). In a study by Rojas *et al.* (2015), SSR markers were found to offer a high level of resolution in distinguishing between sweet potato varieties from various regions, emphasizing the genetic variability both within and between populations. Another study by Mahuku *et al.* (2002) demonstrated the utility of SSR markers in identifying sweet potato cultivars with unique genetic signatures, which could be beneficial for breeding programs focusing on disease resistance or quality traits.

This study emphasizes the substantial phenotypic and genetic diversity present in sweet potato genotypes collected in Luzon, Philippines. While environmental factors like moisture significantly influence morphological traits such as pigmentation, molecular analysis using SSR markers revealed a high degree of genetic variability. This work contributes valuable insights that can enhance breeding programs aimed at improving traits such as pigmentation, root quality, and environmental adaptation in sweet potato. The combination of morphological and molecular approaches offers a comprehensive framework for future research and breeding efforts in sweet potato cultivation. Analyzing the diversity of sweet potato through morpho-agronomic traits and Simple Sequence Repeat (SSR) markers involves evaluating both observable physical traits and genetic markers to better understand the variation within and between sweet potato accessions or populations. By integrating both morphological and molecular markers like SSRs, researchers can obtain a more holistic understanding of sweet potato diversity. Morphological traits shed light on phenotypic variation, while SSR markers provide a more precise genetic view. This combination is essential for selecting desirable traits for breeding programs or conserving genetic resources.

A study combining both morphological and SSR markers found considerable genetic diversity within and among different *Ipomoea batatas* populations in Sub-Saharan Africa, suggesting a rich reservoir of untapped genetic resources for breeding and improvement (Olayemi *et al.,* 2020). Additionally, research on sweet potato populations in Central America revealed that while morphological markers detected some diversity, SSR markers identified much greater variation, particularly in regions with high cultivation pressure (Hernandez *et al.,* 2016). Using both morphological traits and SSR markers offers a comprehensive approach to studying sweet potato diversity. Morphological markers facilitate low-cost, straightforward assessments, while SSR markers deliver high-resolution genetic data. Together, they provide valuable insights into both genetic and phenotypic variation, which can inform breeding strategies, conservation efforts, and the development of new cultivars with improved traits. This combined approach is particularly useful in areas with extensive sweet potato cultivation, where genetic conservation and crop improvement are vital for ensuring food security and agricultural sustainability

Sweet potato ranks among the top five crops in the world in terms of production, with over 95% of the total output being produced in developing countries. Its high yield, nutritional value, adaptability, and resistance to various stresses make it a superior staple compared to other crops. It is increasingly popular, producing more edible energy per hectare per day than wheat, rice, or cassava. Despite its benefits, maintaining sweet potato collections in the field presents challenges. The maintenance of field genebanks is costly, and crops are exposed to diseases, pests, and environmental stresses. The germplasm collection consists of 264 accessions, including released varieties, advanced lines, and new hybrid genotypes. Morphological assessment of foliage and roots revealed high diversity among sweet potato genotypes. Traits such as leaf color, vine pigmentation, and root flesh color were analyzed. The presence of purple and orange pigmentation indicates high anthocyanin and carotenoid content. Molecular characterization using SSR markers showed high polymorphism, indicating significant genetic variation. These genotypes hold a promise for enhancing sweet potato production and supporting food security efforts.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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