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Genetic Diversity Analysis of Greater Yam (*Dioscorea alata* L.) Collections Using Tuber Morphology and Simple Sequence Repeats (SSR) Markers

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The diversity of 148 greater yam (*Dioscorea alata* L.) accessions from the collections of the National Plant Genetics Resources Laboratory (NPGRL), Visayas State University (VSU), and the Institute of Crop Science - University of the Philippines Los Baños (ICROPS-UPLB) was evaluated using 54 *Dioscorea*-based SSR markers. Out of 54 SSR markers, polymorphic bands were amplified in 50 SSR markers with Polymorphic Information Content (PIC) values ranging from 0.65 to 0.89. Subsequent cluster analysis generated nine distinct clusters with a Jaccard's distance index of 0.85, implying 85% dissimilarity among the greater yam accessions. Each formed cluster from the analysis comprised a varying number of accessions: Cluster I with three, Cluster II with 24, Cluster III with 15, Cluster IV with 31, Cluster V with two, Cluster VI with four, Cluster VII with 11, Cluster VIII with 27, and Cluster IX with 31 accessions. Based on the multiscale bootstrap with 5000 times resampling, the approximately unbiased (AU) *p*-value > 0.95 was generated at Clusters II, IV, VIII, and IX with a 0.05 level of confidence. Consequently, high dissimilarity rates obtained from the molecular analysis of 148 greater yam accessions revealed high genetic diversity. Thus, eight greater yam accessions comprising PHL 33537, LA 068B, PHL 33550, PHL 31889, LA 110, LA147, TABACO, and LA 597 were potential parents for future breeding programs. Moreover, the information obtained could be essential in various greater yam improvement programs and genetic resource conservation measures.

Keywords: *Dioscorea alata* L., greater yam, genetic diversity, SSR, PIC

INTRODUCTION

Dioscorea alata L., commonly referred to as greater yam, is among the species belonging to the section Enantiophyllum of the genus *Dioscorea* L. (*Dioscoreaceae*) and is widely disseminated in the humid and semi-humid tropics (Obidiegwu et al. 2009). Greater yam originated from Southeast Asia; however, the exact origin is still debatable (Obidiegwu et al. 2009). In early times, the distribution of this species was evident in Southeast Asia, Indonesia, and the Pacific and Indian Oceans; then, during the 16th century, Portuguese and Spanish traders disseminated this species to Africa and tropical Africa (Coursey 1967). According to Egesi et al. (2002), it has a basic chromosome number of x = 10, while various polyploidy clones with 2n = 4x, 6x, and 8x were reported. Various disadvantages in the vegetatively propagated clones of *D. alata* L. were reported to lessen the capacity of various genotypes to be economically important crops. An example is the inability of the species to become sterile due to infrequent seed setting. Thus, the International Institute of Tropical Agriculture (IITA, Nigeria) and the Central Tuber Crop Research Institute (CTCRI, India) revealed that natural and artificial pollination and fertile seed production can be attained through optimized culture and management practices (Egesi et al. 2001). Nevertheless, the greater yam (*Dioscorea alata* L.) is still the oldest cultivated yam species having the largest distribution around the world. It is an essential staple food crop for millions of individuals, specifically in humid and sub-humid areas (Jyothy et al. 2017). In the Philippines, greater yam, commonly known as *ube* or *ubi*, is often used in preparing sweetened food delicacies due to its appealing purple color and distinct flavor (Lubag et al. 2008).

The production rates of greater yams continue to decline in various small-scale holders as pests and diseases are manifested during and after cultivation. This includes yam anthracnose, yam mosaic virus disease, weeds, and infestations brought by insects such as beetles and root-knot nematodes (Baquiran 2012). However, some greater yam varieties exhibit natural resistance to certain diseases that

provide promising opportunities for disease management and sustainable cultivation practices. In the Philippines, two prominent native varieties of greater yams—kinampay and kabusah—possess natural resistance to diseases like leaf spot (Martin and Delpin 1978). However, the production of greater yams in the Philippines is extremely constrained due to improper seed systems, production methods, and post-production procedures (Salda et al. 2005). Thus, proper pest and cultural management practices should be implemented to provide optimum yield. Continuous improvement of greater yam varieties is essential to enhance their resistance to pest and diseases and increase their productivity. By employing advanced breeding technologies and techniques, the genetic traits of the greater yams are improved to withstand various diseases more effectively. It ensures that cultivation of greater yam remains resilient and sustainable.

Crop improvement provides desirable characteristics that are usually based on the preference of the farmers, consumers, and breeders. This requires studies that provide information concerning the genetic relationship and genetic diversity of crops to continue the manipulation of the existing germplasm. However, just like any other crop, the genetic diversity of greater yam continues to decline; thus, the occurrence of genetic erosion in greater yam implies that there is a need to conserve and preserve the available germplasm.

The assessment of genetic relationships and genetic diversity is essential to further analyze the variations among the existing germplasms. For instance, genetic diversity studies assess the differences among individuals, groups of individuals, or populations through specific or combined methods of analysis (Mohammadi and Prasanna 2003). It can be evaluated within and between plants, and populations using morphological characterization, biochemical characterization, and molecular marker analysis (Govindaraj et al. 2015).

The advances in analyzing the genetic diversity among and within the plant population pave the way for the development of marker systems that can detect the diversity on the DNA level. Among various plant populations, molecular markers or nucleotide sequences can be studied in the presence of polymorphism between the sequences (Nadeem et al. 2018). Also, changes due to deletion, duplication inversion, and/or insertion in the chromosomes can be determined using molecular markers (Govindaraj et al. 2015). According to Nadeem et al. (2018), ideal DNA markers should possess characteristics such as co-dominance, even distribution throughout the genome, high reproducibility, and detection of a higher level of polymorphism. They are categorized based on the mode of gene action that can either be co-dominant or dominant, the method of detection that includes hybridization-based molecular markers and polymerase chain reaction (PCR)- based markers, and modes of transmission such as paternal organelle inheritance, maternal organelle inheritance, bi-parental nuclear inheritance, and maternal nuclear inheritance (Nadeem et al. 2018).

The improvement of the molecular marker system leads to a more efficient and effective tool for determining genetic relatedness and variations among individuals. Simple sequence repeats (SSRs), also referred to as microsatellites, are tandem repeats ranging from 2 - 8 nucleotide units of DNA entirely present in the previously investigated genomes (Singh et al. 2016). The association of SSR markers with non-repetitive DNA is advantageous as its derived SSR markers from genomic libraries can contain either transcribed region (genic SSRs) or a non-transcribed region (genomic SSRs) (Park et al. 2009). However, SSR-based methods require a relatively high cost to develop, and technical expertise is needed especially in the production of species-specific primers and enriched libraries (Miah et al. 2013). Nevertheless, SSR markers are still widely used in various genetic diversity studies including soybean (Wang et al. 2006), maize (Pandit et al. 2016), rice (Cao et al. 2006), sweet potato (Yang et al. 2015), abaca (Boguero et al. 2016), yam (Arnau et al. 2017), and other plant species. Thus, the incorporation of SSR markers in plant breeding continues to materialize along with the improvement of various techniques. Successful genetic diversity analyses in greater yam using SSR markers have been reported including studies by Otoo et al. (2015), Arnau et al. (2017), and Olu-Olusegun et al. (2018). Aside from this, the application of species-based SSR markers extends to diverse yam species, achieving cross-amplification.

Thus, this study's objectives were to assess the genetic diversity of the existing greater yam collections available at the University of the Philippines Los Baños (UPLB) using molecular marker analysis. This particularly aimed to: 1) determine the genetic diversity and relatedness of all greater yam collections using simple sequence repeats (SSR) markers and tuber morphology, and 2) identify promising accessions of greater yam that can be used as parents for breeding or commercial production.

MATERIALS AND METHODS

Plant Materials

The 148 greater yam (*D. alata* L.) accessions were screened to analyze their molecular diversity. These included 16 greater yam accessions from ICROPS-UPLB, 71 greater yam accessions from NPGRL, and 61 greater yam accessions from VSU. Currently, these greater yam accessions are planted and maintained at the greater yam plantation and tissue culture laboratory of the National Plant Genetic Resources Laboratory (NPGRL), Institute of Plant Breeding (IPB), University of the Philippines Los Baños.

Tuber Morphological Characterization

The tuber morphological characterization was based on the available yam descriptor list formulated by the International Plant Genetic Resources (IPGRI/IITA 1997), with modifications. Based on the modified descriptor list, 22 tuber morphological characters were selected, with 21 qualitative traits and one quantitative trait. The quantitative descriptor was based on the phenotypic average of three measurements for each accession. These selected variables were used in characterizing the available greater yam tubers.

The qualitative traits included the number of tubers per hill (TPH), the relationship of tubers (RT), the presence and absence of corms (PAC), corm size (CS), tuber shape (TS), the tendency of the tuber to branch (TTB), places where the tuber branches (PTB), tuber length (TL), roots on the tuber surface (RTS), place of the roots on the tuber (PRT), cracks on tuber surface (CTS), tuber skin thickness (TST), tuber skin color (TSC), hardness of tuber (HT), skin color at the head of the tuber (SCH), fresh color at the central transverse cross-section (FCC), flesh color at the lower part of the tuber (FCL), uniformity of flesh color in cross-section (UFC), the texture of flesh (TF), flesh oxidation color (FOC), and time for flesh oxidation after cutting (TFO). On the other hand, the quantitative trait was the tuber width (TW). Each trait was characterized after harvesting the available tubers of the greater yam collections.

DNA Extraction

Leaf samples of 148 greater yam accessions maintained in the field were used in this study. The second youngest leaves in the vegetative stage or at least 20 – 30 d after emergence were collected and used as plant material. Genomic DNA was isolated using a modified Doyle and Doyle (1990) CTAB/NaCl protocol. One gram of leaf sample was frozen and pulverized using liquid nitrogen, followed by homogenization using CTAB extraction buffer, and purification of the DNA using chloroform:isoamyl alcohol. The DNA was then precipitated using isopropanol and resuspended. The obtained genomic DNA was further purified using TE buffer and RNase A. The genomic DNA extraction procedure was conducted at the Molecular Plant Breeding Laboratory, Institute of Crop Science, College of Agriculture and Food Sciences, University of the Philippines Los Baños. The concentration (ng/µL) of 2 µL isolated DNA was measured using a microplate spectrophotometer (BioTek Instruments, U.S.A). DNA samples with absorbance values (260/280) ranging from 1.8 to 2.0 were considered high-quality DNA while those with absorbance values less than 1.8 and greater than 2.0 contained impurities. Two µL of the isolated DNA was subjected to 1% agarose gel electrophoresis to assess the quality. Lastly, the DNA bands were visualized using a gel photo-documentation system from Clinx Science Instruments Co., Ltd, China, under UV light.

Fifty-four *Dioscorea*-based SSR primer pairs that are widely distributed throughout the yam genome were used to analyze the greater yam accessions. The information and profile of the SSR primer pairs were presented in various diversity analysis studies. The final volume of the PCR mixture used for amplification was 10 µL containing 100ng/µL DNA, 1X PCR buffer, 2.5 mM MgCl2, 0.30 mM dNTPs, 0.30 µM each of forward and reverse markers, and 0.5 U/µL Taq DNA polymerase.

The PCR profile of the Dioscorea-based primers contained initial denaturation of 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, defined annealing temperature for each primer pair for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 8 min. The optimization of PCR protocol was done using thermal cycler (Bio-Rad Laboratories, Inc., Singapore) to ensure that the SSR markers would provide consistent results.

Genotyping

The products from the polymerase chain reaction were electrophoresed using 2.0% agarose gel at 100 V for 35 – 45 min. The gel was stained with GelRed™ Staining Dye and visualized under UV light using a gel photo-documentation system (Clinx Science Instruments Co., Ltd, China). Further analysis was done using the PCR products that displayed polymorphism. Polymorphic PCR products were subjected to Polyacrylamide Gel Electrophoresis (PAGE) using the ClearPAGE ™ (C.B.S. Scientific Co., Del Mar, CA) system. The PCR products were placed in the 6% polyacrylamide gel soaked in 1x TBE buffer and electrophoresed for 2.5 h at 100 V. The gels were stained with GelRed ™ Staining Dye; then, viewed under UV light with the aid of the gel photo-documentation system (Clinx Science Instruments Co., Ltd, China).

Data Analysis

The scoring of the genotypic data was based on the binary matrix where the presence (1) or absence (0) of alleles was determined after several PCR amplification procedures. Amplified bands of varying sizes were considered in each microsatellite marker using the binary matrix. The best runs that generated verified results were subjected to molecular marker scoring.

The discriminative power of SSR markers was analyzed through the values obtained in calculating the polymorphic information content (PIC). According to Ni et al. (2002), PIC is one of the key characteristics of molecular markers in assessing the capacity of the markers to differentiate within

a population. Moreover, it measures the informativeness of the molecular markers based on the amplified alleles and their frequency. The calculation of the PIC value followed the formula:

$$PIC = 1 - \sum_{i=1}^{n} Pi^2$$

where *Pi* is the frequency of the i^{th} allele over the total allele at an SSR locus; and n is the total number of alleles in that locus.

Based on the study by Botstein et al. (1980), the formulation of the PIC shows four different levels. Loci that obtained PIC > 0.50 are considered highly informative. PIC between 0.5 to 0.25 are reasonably informative, PIC < 0.25 are slightly informative, while loci with PIC near 1 are most desirable. Thus, based on their PIC value, the molecular markers' usefulness can be evaluated.

To compare the accessions and their genetic relationship, cluster analysis was done. The dissimilarity index matrix was estimated using Jaccard's distance and then clustered through the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) of R statistical analysis PC version R.4.2.2 software (R Core Team 2023).

Table 1. Total number of alleles amplified in 148 greater yam (*Dioscorea alata* L.) accessions using 50 polymorphic SSR markers.

SSR Markers	Rare Alleles	Common Alleles	Most Frequent Alleles	Number of Alleles
	(0.01 – 0.05)	(0.05 – 0.10)	(> 0.10)	
YM88	2	4	4	10
YM23	5	1	3	9
YM90	4	2	4	10
CIR60	4	1	5	10
CIR61	1	5	4	10
YM86	1	1	3	5
YM83	6	1	3	10
YM75	3	3	3	9
YM66	2	3	4	9
YM58	3	1	4	8
YM46	4	2	4	10
YM45	3	3	4	10
YM37	1	5	3	9
YM30A	3	2	5	10
YM27	2	1	3	6
YM21	1	3	6	10
YM18	3	4	3	10
YM19	3	3	4	10
YM07	2	2	6	10
YM02	6	0	4	10
YM62	3	0	4	7
YM55	2	3	5	10
YM15	1	2	5	8
H2	1	4	5	10
Da3G04	1	5	4	10
D12	3	3	4	10
D7	1	5	4	10
D9	1	3	4	8
C5	4	1	3	8
D3	0	7	3	10
D11	2	2	5	9
D4	3	1	4	8
YM33	0	6	4	10
YM52	2	5	3	10
YM09	2	1	6	9
D5	3	4	3	10
D1	3	2	4	9
D2	1	4	5	10
D6	3	5	2	10
A7	2	4	3	9
CIR17	3	2	5	10
CIR20	0	6	4	10
F1	3	4	3	10
YM13A	3	3	4	10
YM17	3	2	5	10
YM65	2	1	3	6
E11	5	1	4	10
YM10	0	5	5	10
YM12	1	5	4	10
CIR8	2	4	4	10
Total	119	147	200	466
Percentage	25.54	31.54	42.92	100%

RESULTS AND DISCUSSION

PCR Amplification

Fifty-four SSR markers were used to screen the diversity of 148 *D. alata* L. collections. The SSR markers used were all *Dioscorea*-based markers obtained from various publications. Of the screened SSR markers, 19 were from *D. alata* L., 2 were from *D. abyssinica*, 2 were from *D. praehensilis*, and 31 were from *D. cayenensis* (Table 2). All SSR markers amplified bands on the screened greater yam collections with annealing temperatures ranging from 50°C to 62°C. However, among the screened SSR markers, only 50 SSR markers exhibited polymorphism, while the remaining four SSR markers produced monomorphic bands. Despite the absence of polymorphism in the four SSR markers, the number of polymorphic SSR markers is relatively high compared to the study of Arnau et al. (2017) where 24 microsatellite markers determined the genetic diversity of 384 accessions of *D. alata* from different geographical regions including South Pacific, Asia, Africa, and the Caribbean. Also, considering the capacity of the selected SSR markers, the observed amplification proved that the screened SSR markers from the *Dioscorea* species exhibited transferability among the 148 *D. alata* L. accessions. This was supported by the study of Tostain et al. (2006) which concludes that there is consistent transferability among the SSR markers from various Dioscorea species in agreement with their botanical classification. Thus, the rate of cross-species amplification increases for species that are found in the same botanical family of the developed SSR markers.

Allelic Frequency

The 50 polymorphic markers amplified reproducible fragments with a total of 466 alleles and an average of 9.32 alleles per locus (Table 1). Among the analyzed markers, YM86 detected the lowest number of alleles observed with five alleles in total, followed by YM65 (6 alleles), YM27 (6 alleles), YM62 (7 alleles), C5 (8 alleles), YM58 (8 alleles), Da1F08 (8 alleles), YM15 (8 alleles), and D9 (8 alleles). The size of amplified alleles of the analyzed markers ranged from 100 to 470 bp. The lowest amplified allele size was observed in locus Dpr3F12, while the highest amplified size was observed in the alleles of YM15. Based on the classification of allele frequency adapted by Korsa et al. (2022), 25.54% of the detected alleles exhibited rare alleles with frequencies between 0.01 and 0.05, common alleles with frequencies ranging from 0.05 to 0.1 accounted for 31.54% of the detected alleles (147 alleles), while the remaining percentage (42.92%) was observed to have the most frequent alleles with frequencies ranging from 0.11 to 0.50.

Polymorphic Information Content

The calculated PIC values of the SSR markers obtained in this study ranged from 0.65 to 0.89 with an average PIC value of 0.83 (Table 2). Loci YM86 obtained the lowest PIC value while YM10 and YM21 recorded the highest PIC values with 0.8944 and 0.8940, respectively. On average, the obtained PIC values of the SSR markers used in this study indicate a high level of information. The result corroborates with the PIC values reported by Korsa et al. (2022) and Otoo et al. (2015). However, the observed PIC values in the study are higher than the result of Girma et al. (2015). Different results were also observed in the *D. alata* L. reported by Siqueira et al. (2011) with an average PIC value of 0.65, Olu-Olusegun et al. (2018) with a mean PIC value of 0.63, and an average PIC value of 0.66 from the study of Siqueira et al. (2012). In comparison, the mean PIC value observed in this study is relatively higher than in the previous studies. The findings thus demonstrate that the SSR markers used in this study were highly informative and had discriminatory power to identify genetic variation among the examined greater yam accessions. Also, the observed information reveals that the markers were useful in the phylogenetic relationship and genetic diversity studies.

Cluster Analysis

The clustering analysis formed nine distinct clusters (Cluster I, II, III, IV, V, VI, VII, VIII, IX) at a Jaccard's distance of 0.85 indicating that 85% of the generated clusters were observed to exhibit dissimilarities (Fig. 1). This observation signifies a notable dissimilarity between the accessions within 1 cluster and those belonging to the other cluster. Consequently, members within the same cluster exhibited a higher degree of relatedness compared to those from different clusters, emphasizing the distinct genetic relationships present among the various accession groups.

Cluster I grouped 3 greater yam accessions—1 from the VSU (LA 075) and 2 from the NPGRL collections (PHL 31887 and PHL 33537). It was observed that Cluster I was divided into two sub-clusters at a 0.82 dissimilarity index. The accessions PHL 31887 and PHL 33537 were grouped together due to the similarities they exhibited in some important tuber morphological characteristics such as irregular tuber shape, unbranched tuber, and < 1-mm tuber skin thickness. Cluster II grouped 24 greater yam accessions (15 from VSU and 9 from NPGRL collections). It was observed that five greater yam accessions (PHL 9599, PHL 5012, LA 108B, PHL 33488, and PHL 4640) formed distinct clusters and were considered dissimilar to the other remaining 14 greater yam accessions. These accessions exhibited unbranched tuber, absence of corm and tuber cracks, < 1-mm tuber skin thickness, completely separate and distant tubers, hard tuber, uniform flesh color, and yellow flesh oxidation color. On the contrary, the lowest rate of dissimilarity was observed in two VSU yam collections—LA 068B and LA 067. Among the 15 greater yam accessions in Cluster III, 12 accessions were from the NPGRL,

Table 2. The description of 50 polymorphic Dioscorea-based SSR markers and their polymorphic information content (PIC) amplified across the 148 greater yam (*Dioscorea alata* L.) accessions.

SSR primer	Origin	Expected Size (BP)	Primer sequences (5' - 3')	Annealing Temperature (°C)	PIC	References
Dpr3F04 (D11)	Dpr	115–127	F: AGACTCTTGCTCATGT R: GCCTTGTTACTTTATTC	51	0.855	Tostain et al. 2006
Dpr3F12 (D12)	Dpr	162–171	F: TCCCCATAGAAACAAAGT R: TCAAGCAAGAGAAGGTG	51	0.860	
Dab2C05 (D5)	Dab	168–198	F: CCCATGCTTGTAGTTGT R: TGCTCACCTCTTTACTTG	51	0.872	
Dab2C12 (D6)	Dab	179	F: AGGCATCTTGGGAAA R: CGAACGATCCAATAAAA	51	0.819	
Dab2D08 (D7)	Dab	335	F: ACAAGAGAACCGACATAGT R: GATTTGCTTTGAGTCCTT	51	0.867	
A7	Da	267	F: GCCCCACCTTAATTTCAT R: GGAATGAGATGGGACGAGAA	53	0.844	Siqueira et al. 2012
C5	Da	145–180	F: AACCAATTACCCTTTGTCATGG R: GCCTTGCAAGCAATTTTGA	58	0.752	Siqueira et al. 2011
E11	Da	240–280	F: ATGGTGTTCTCCCATGCTTC R: ACCAAAAATCAGGCTTGTGC	55	0.839	
F1	Da	130–180	F: ATGGCTCAAGAGCACACG R: GGGCCTCATAAACATGCAAT	58	0.822	
H2	Da	160–250	F: AAACCAAACAGGCAAAGCAT R: TGCCCTGCTTGTAAGATTGA	58	0.883	
D9	Da	160–320	F: CTCACTAGCGTGGGGAAGAC R: TGAAAGAAAGAAGCGCAAGG	55	0.809	Otoo et al., 2015
YM13 A	Da	200–275	F: TTCCCTAATTGTTCCTCTTGTTG R: GTCCTCGTTTTCCCTCTGTGT	55	0.847	
YM30 A	Da	200–390	F: GGTCCTCTTCTATCCCAACAA R: CACGTATTAACTCCATCTATCCAA	55	0.853	
YM02	Dca	263	F: TAGATTTCGCTTTTCCACTAGC R: CCTAATCATCATCATCGTCATC	58/57	0.749	Tamiru et al. 2015
YM07	Dca	203	F: AGCATTGGGTCC TTTCATCCC R: ACAATTCACACAAAGCATGGC	59	0.882	
YM09	Dca	193	F: AGGAACATTCCCACTCAGTTATG R: ATTGGGCAAGTGTGGTGTG	59	0.867	
YM10	Dca	348	F: ACCCAAAATATTCTCCCCATTATAC R: TTGACACTCATCTTATATTGCTC	57	0.894	
YM12	Dca	215	F: TGAGCATTCTTGTTTTGCCG R: CTTTCAGGGCGTGCATGG	58/60	0.881	
YM15	Dca	485	F: CCATCTCCTCCCTTATCTACAC R: GGGATTGAAGTTCC AGAGACTA	57	0.830	
YM17	Dca	181	F: TCCCTCAATTAAAGCATAGCCTC R: AGCCACCAAACATCTTGCTC	59/60	0.856	
YM18	Dca	266	F: GACATTGGGGATCTCTTATCAT R: TAGCAGCAGTAACGTTAAGGAA	57	0.846	
YM19	Dca	219	F: ACGGAAGCAGCAAGAGGAG R: GTGTCATCAGCATCTGGGC	59/60	0.852	
YM21	Dca	340	F: AATGATGCATCTGAGGATAGTG R: GATGCTATTACGACAACCTTGA	57	0.894	
YM23	Dca	200	F: TTAAGACTTGCAGGGTTAAAGG R: GTGGCTAGTTTTTGTAGCTGGT	58	0.698	
YM27	Dca	231	F: TCCAGCTCTTTAGCACAGG R: AGGAGCATAGGCAACAAGC	58/59	0.719	
YM33	Dca	199	F: ACC ATGGGATGAAGGGAAGG R: ACC ATGGGATGAAGGGAAGG	60	0.884	

Cont...Table 2. The description of 50 polymorphic Dioscorea-based SSR markers and their polymorphic information content (PIC) amplified across the 148 greater yam (*Dioscorea alata* L.) accessions.

SSR primer	Origin	Expected size (BP)	Primer sequences (5' - 3')	Annealing temperature (°C)	PIC	References
YM37	Dca	216	F: CCTTACCACCGGACTCCTC R: TGCAGCAATACACC GGAAC	59/60	0.786	
YM45	Dca	235	F: GCTGTTTTTGGGATTTAGTAGG R: GCAACTAGATATGCTTGGACAC	57	0.863	
YM46	Dca	250	F: ACTTGACATCCAGAAGGTGC R: GCTGTAACTTATCAAGGGTAGC	57/59	0.850	
YM52	Dca	384	F: TGCATAGAGTGAGAGCTTAAGAG R: ATGGGTAGTTGAGCAAGAGAAT	56/57	0.874	
YM55	Dca	380	F: TCTTCCGAGATATACACATCCA R: ACAGTGACAATGAGAAGGAACA	57	0.877	
YM58	Dca	199	F: TCTGGGGCACTGTCCTTTC R: CCACATGGACTGGAATAGCTC	59/60	0.780	
YM62	Dca	195	F: GAGCTCTCCTCTAGACCTTCAC R: CCAACGGCCTAGATTGCTC	59/60	0.737	Tamiru et al. 2015
YM65	Dca	183	F: ACAAATGCACGCTCTGAAGG R: GGGCAGTAGAATTTGGTGCG	60	0.672	
YM66	Dca	246	F: ATATTGACTGACCACCAGATCA R: GAAGAGTCTTGGATTTCTACCA	55/57	0.842	
YM75	Dca	350	F: TCGCTCAACCTAATCCTCTATT R: TCAAACCAGCCAAAACATC	57	0.816	
YM83	Dca	239	F: TCGGAATTCAACTGTGATGGC R: AGCACACCATTCACACATAGG	59/60	0.773	
YM86	Dca	176	F: GCGCAAAGCTGTATCTGGC R: CTGTCCCATTAGGGCACTTG	59/61	0.652	
YM88	Dca	257	F: CTTTATCCATTAGAGCTTTGGG R: GTGAAATAACCTAGCAAATCCC	57	0.873	
YM90	Dca	304	F: GTGTGTGGATGGAGTTTCAAT R: GAATACCCCCAACAGATGTAAT	57	0.836	
MDaCIR17	Da	228–236	F: GATTAGGTTGGACTTTGCAT R: ACCACTGCACTCAACAGC		0.859	Arnau et. al. 2017
MDaCIR20	Da	172–206	F: TGCCTTAATCTGCTGACAC R: CATGGTGCTCCGATTCTA		0.889	
MDaCIR8	Da		F: ACAGCAGCAAAATAACTG R: TCTTTGCAGGAGAAGAGG		0.880	Nemorin et al. 2013
MDaCIR60	Da		F: CAAAGACCAGGGAATGTG R: AGAATGCAGAGCATGGTG		0.814	
MDaCIR61	Da		F: CTAACCCCTCCAAAGCTG R: GGGCATTCACGTCTTTAT		0.877	
Da1A01 (D1)	Da	212–236	F: TATAATCGGCCAGAGG R: TGTTGGAAGCATAGAGAA	51	0.811	Tostain et al. 2006
Da1C12 (D2)	Da	160–162	F: GCCTTTGTGCGTATCT R: AATCGGCTACACTCATCT	51	0.824	
Da1D08 (D3)	Da	320–358	F: GATGCTATGAACACAACTAA R: TTTGACAGTGAGAATGGA	51	0.888	
Da1F08 (D4)	Da	159–173	F: AATGCTTCGTAATCCAAC R: CTATAAGGAATTGGTGCC	51	0.788	
Da3G04	Da	318–341	F: CACGGCTTGACCTATC R: TTATTCAGGGCTGGTG R: GATTTGCTTTGAGTCCTT	51	0.877	

Note: *Dioscorea alata* (Da), *Dioscorea abyssinica* (Dab), *Dioscorea praehensilis* (Dpr), *Dioscorea cayenensis* (Dca).

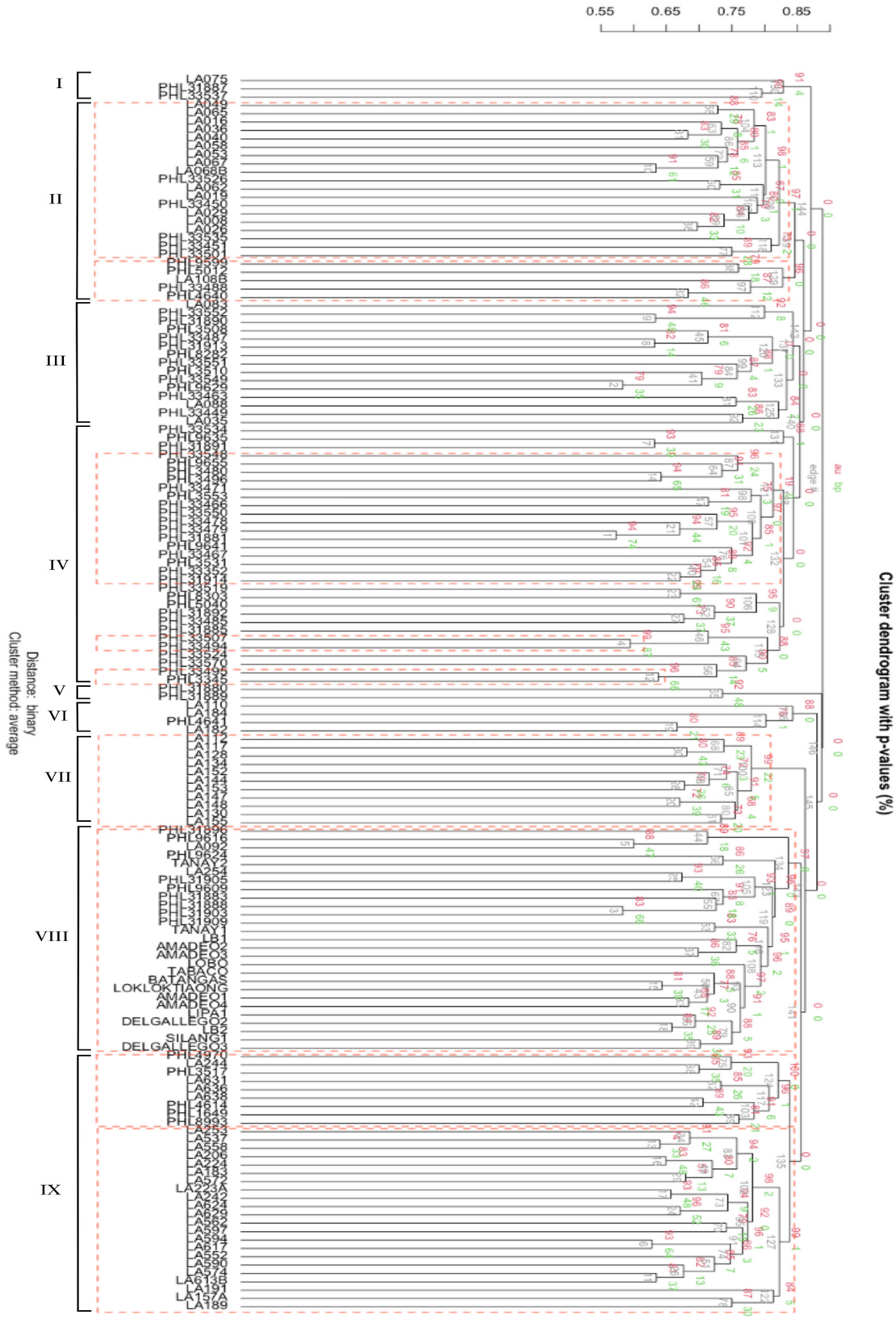

Fig. 1. Cluster dendrogram of 148 greater yam (*Dioscorea alata* L.) accessions with AU/BP *p*-values (%) and bootstrapping of 5000 based on Jaccard dissimilarity index.

and the three remaining accessions (LA 083, LA 088, and LA 035) were from the VSU collections. Of the 15 greater yam accessions in Cluster III, PHL 9629 and PHL 33549 exhibited the lowest dissimilarity rate. For instance, the characteristics of PHL 9629 and PHL 33549 differed in the number of tubers they produced, shape of tuber, tuber width, presence and absence of cracks, hardness of tuber, tuber skin and flesh color, texture of flesh, and time of tuber oxidation.

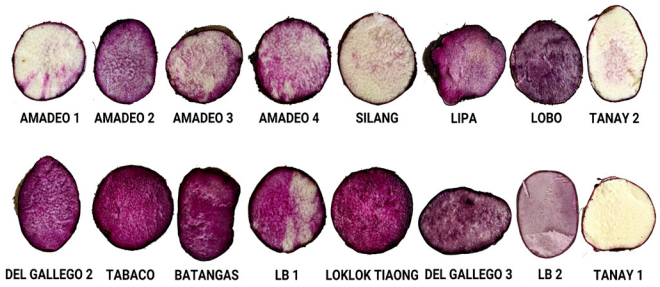

Fig. 2. Tuber flesh of 16 ICROPS-UPLB greater yam (*Dioscorea alata* L.) accessions.

Most of the greater yam accessions (31) from the NPGRL greater yam collections were grouped in Cluster IV. Among the greater yam accessions, the lowest dissimilarity rate was derived from the comparison between 2 NPGRL greater yam collections (PHL 33479 and PHL 31881) from Cluster IV. The variation between PHL 33479 and PHL 31881 was observed in 9 out of 22 tuber morphological characteristics that were examined. On the other hand, Cluster V generated clustering of 2 NPGRL greater yam accessions (PHL 31880 and PHL 31889) with 75% dissimilarity. Cluster VI was formed with 4 accessions, with one accession from NPGRL greater yam collections (PHL 4641) and with the remaining 3 accessions from VSU collections. In this cluster, the NPGRL greater yam collection had a 69% dissimilarity rate with LA 182, a VSU collection. The cluster analysis also generated clustering (Cluster VII) that comprised different VSU greater yam collections. Among the VSU greater yam collections in this cluster, LA 147 and LA 148 exhibited the lowest dissimilarity rate. Cluster VIII grouped 27 accessions: 9 NPGRL greater yam collections, 2 VSU greater yam collections, and 16 ICROPS-UPLB greater yam collections. In the same cluster, it was observed that among the 16 ICROPS-UPLB greater yam collections, Tanay 2 greater yam accession clustered differently. However, all ICROPS-UPLB greater yam accessions exhibited a dark maroon tuber skin color. Nevertheless, it was observed that Tanay 2 exhibited white with purple flesh, while the other ICROPS-UPLB greater yam accessions had varying flesh colors—white, white with purple, purple with white, and purple (Fig. 2). At a 0.83 Jaccard's distance, three accessions (PHL 31896, PHL 9616, and LA 092) formed distinctly within the cluster, encompassing variations from the 24 yam accessions. The last cluster formed 31 greater yam accessions. Majority of the greater yam accessions in Cluster IX were from VSU greater yam collections, totaling 26 accessions, while the remaining 5 accessions were from the NPGRL greater yam collection. Cluster IX was further divided into two groups at a distance of 0.83, implying that the greater yam accessions differ at a rate of 83%.

The significance of the clustering was determined using the calculated *p*-values obtained from the multiscale bootstrap with 5000 times resampling. According to De Croos and Pálsson (2012), clusters with AU *p*-value > 0.95 are considered as significant at 0.05 level of significance as the hypothesis that 'the cluster does not exist' was rejected. Based on the hierarchical clustering, nine groups were generated with AU *p*-value > 0.95 (Fig. 1). Among the 9 significant groups, 1 group was observed in Cluster VIII, 2 groups from Clusters IX and II, and 4 groups from Cluster IV. The AU *p*-value obtained in the hierarchical clustering ranged from 1.0 to 0.96. Cluster II had 2 significant groups with AU *p*-value = 0.97 and AU *p*-value = 0.96 at a distance of 0.84, while only 1 group was obtained in Cluster VIII with AU *p*-value = 0.97 at a distance of 0.83. In Cluster IX, the further division of two groups within the cluster showed both significances with an AU *p*-value of 1.00 and an AU *p*-value of 0.96 grouped at a distance of 0.83. Lastly, Cluster IV generated most of the significant groupings having an AU *p*-value of 0.96 at 0.76 distance index, AU *p*-value of 0.97 at 0.81 distance index, AU *p*-value of 0.99 at 0.61 distance index, and AU *p*-value of 0.96 at 0.65 distance index. The significance observed in the AU *p*-value of the clustering proved that the nine groups observed in Clusters II, IV, VIII, and IX are more likely to exist compared to the other clustering.

The high Jaccard distance value inferred that the 148 greater yam accessions screened using 50 polymorphic SSR markers exhibit high genetic diversity. It was corroborated in the study of Siqueira et al. (2013) where 12 SSR markers revealed high genetic variation among the collected greater yam accessions (72 local varieties and 17 commercial accessions) from the four distinct regions of Brazil. On the other hand, various articles reported high genetic diversity despite considering lower genetic coefficients while clustering the population. Wu et al. (2019) clustered the greater yam population of China into two groups at a genetic coefficient of 0.62 and concluded that the greater yam population exhibited broad genetic variation. In terms of reproduction, greater yam has a high chance of cross-pollination that causes significant modification in the genetic makeup of the populations (Mulualem et al. 2018). Also, greater yam, an asexually propagated crop, often maintains a high level of heterozygosity that accounts for the high genetic diversity of greater yam (Olu-Olusegun et al. 2018). Aside from the cultural, social, and economic aspects, agricultural techniques might also contribute to the variation of greater yams (Siqueira et al. 2013).

CONCLUSION

The genomic DNA of all 148 yam accessions (71 from the National Plant Genetics Resources Laboratory [NPGRL], 61 from the Visayas State University [VSU], and 16 from and the Institute of Crop Science-University of the Philippines Los Baños [ICROPS-UPLB] greater yam collections) screened using 54 *Dioscorea*-based simple sequence repeat (SSR) markers amplified 50 markers polymorphic bands. The informativeness of the markers resulted in Polymorphic Information Content (PIC) values ranging from 0.65 to 0.89, with an average of 0.83. Thus, the SSR markers had high discriminatory power in identifying variation among the greater yam accessions. The gathered binary profile data was then analyzed using R statistical analysis which clustered the greater yam accessions at a 0.85 Jaccard's distance. The cluster analysis formed nine distinct groups: Cluster I (3 greater yam accessions), Cluster II (24 greater yam accessions), Cluster III (15 greater yam accessions), Cluster IV (31 greater yam accessions), Cluster V (2 greater yam accessions), Cluster VI (5 greater yam accessions), Cluster VII (11 greater yam accessions), Cluster VIII (27 greater yam accessions), and Cluster IX (31 greater yam accessions). The AU *p*-values generated nine significant (AU *p*-values > 0.95) clustering at Clusters II, IV, VIII, and IX which implies that the clusters exist at a 0.05 level of confidence. Considering the tuber morphology and clustering observed in the molecular diversity analysis of 148 greater yam accessions, eight greater yam accessions are recommended in the selection of promising parents for future breeding programs. The recommended accessions are PHL 33537, LA 068B, PHL 33550, PHL 31889, LA 110, LA147, TABACO, and LA 597. Among the eight greater yam accessions, white tuber flesh was observed in LA 110 and LA 068B, while white with purple flesh was observed in PHL 31889 and PHL 33550, and purple tuber flesh was observed in LA147, TABACO, PHL 33537, and LA 597. Overall, the results of this study revealed a high genetic diversity among the greater yam collections from NPGRL, VSU, and ICROPS-UPLB. This information from the genetic diversity analysis could be valuable in improving greater yam breeding programs and in contributing to the proper conservation and management of greater yam genetic resources.

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