MORPHOLOGICAL AND CHEMICAL CHARACTERIZATION AND ASSESSMENT OF GENETIC DIVERSITY OF NATIVE YAM (*DIOSCOREA* SP.) GERMPLASM COLLECTIONS CONSERVED AT THE NPGRL

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ABSTRACT

The germplasm collections of yam at the NPGRL, IPB were characterized and evaluated to assess the diversity of the collections, facilitate selection and identify the potential germplasm for enhanced utilization, improved nutrition and sustained source of food or for livelihood. Based on the 31 accessions characterized, 17 qualitative characters exhibited high diversity index values ranging from 0.67-0.99 with texture of flesh giving the highest diversity among the characters. The sizes and dimensions of the tubers similarly obtained high diversity indices. Further evaluation of the nutraceutical properties of the 40 accessions, consisting of four species, *D. alata, D. bulbifera, D. hispida*, and *D. esculenta*, showed that *D. esculenta* tubers have the highest % crude fat while *D. alata* have the highest % total ash value, compared to other three native yam species. *D. bulbifera* and *D. hispida* on the other hand have the highest % protein per sample. *D. esculenta* and *D. bulbifera* have higher crude fiber contents compared to *D. alata*. Two *D. alata* accessions have high total phenolic and anthocyanin contents, while one *D. esculenta* collection showed high total phenolic and flavonoid contents. Highest values of antioxidant properties can be observed on PHL 4725 (tugui) and GB 53193 (ubi). Correlation of antioxidant property with anthocyanin, total phenolic and flavonoid contents formed distinct groupings that identified six (6) promising collections of yam based on functional properties. These accessions can be selected as parents for crop improvement programs.

Keywords: Yam, *Dioscorea* species, germplasm conservation, genetic diversity, nutraceutical property

INTRODUCTION

In the Philippines, yam have been associated with poor farmers and households and have been dealt with lesser priority in research, extension and government policy. For the past 10 years, the value-adding potential of yam in increasingly recognized, given the crop's new potential industrial/commercial applications (e.g. confectionery, naturally fermented food and nutrient-rich food products). However, even with the increased value-added potential of yam, volume of production decreased from 29,265 metric tons (MT) in 2007 to 15,245 MT in 2014 [1]. Recent data from the Philippine Statistics Authority [2], shows that volume of production in 2016 further decreased to 13,797 MT. One of the reasons is the highly seasonal nature of the yam crop and due to the tuber dormancy immediately after harvest which lasts for about 3-4 months**.** [3].

Yams are directly used for human consumption, as animal feed, for starch and industrial uses. The wild relatives of yam are widely used in traditional medicine. Tubers of some wild species are commercially exploited because of the steroidal sapogenins they contain, from which contraceptives, sex hormones and cortisone are manufactured [4], [5], [6].

There are about 600 species of *Dioscorea* in the world [7] but in the Philippines Merill [8] reported 15 species of Dioscorea. However, only 8 species were mentioned and these were *D. alata (ubi or greater yam), D. esculenta (tugui or lesser yam), D. hispida (nami), D. bulbifera (ubi-ubihan or air potato), D. pentaphylla (lima-lima), D. divaricata (buloy), D. loheri nd D. luzonensis*. According to Onwueme and Charles [9], among the cultivated species, *D. alata* is the most widely distributed species of yam. It originated in Southeast Asia, probably in Burma. *D. esculenta* originated in Indo-China although Papua New Guinea is considered the center of diversity of this species. *D. bulbifera* is the only species that can be found both in Asia and Africa.

Various studies have been conducted regarding the nutritional and health benefits of yams. Yam tubers are considered to be good source of energy and contains significant amount of B-complex group of vitamins and vitamin-C. Furthermore, the tuber is one of the good sources of minerals such as copper, calcium, potassium, iron, manganese, and phosphorus [10]. Lubag et al. [11] studied the antioxidant activities of purple and white cultivars of *D. alata* and was able to identify through HPLC chromatography 2 major peaks P1 and P2, to be purple anthocyanidin and phenolic in nature, respectively.

Cornago et al [12] compared the phenolic contents and antioxidant activity of *D. alata* (purple) and *D. esculenta*, tugui. Significant correlation was observed between total phenolic content and DPPH radical scavenging activity (R=-0.7664, p0.05), for both purple yam and tugui. Significant correlation between total phenolic content and iron-chelating capacity was observed only for the tugui varieties (R= -0.9859, $p<0.05$). Tongco et al. [13] studied the nutritional content of *D. divaricata* or buloy, a culturally important species for the Aytas of Morong, Bataan, Philippines. Their results showed that compared with other yams, buloy had relatively higher crude protein and ash content. Thus, the shift from buloy to rice compromises additional nutritional benefits such as dietary fiber, calcium and ash.

Germplasm collections of yam exist at the National Plant Genetic Resources Laboratory, Institute of Plant Breeding, PhilRootcrops Center in Visayas State University and at the Northern Philippines Rootcrops Research and Training Center. But these collections are not fully utilized to make improvements in pest resistance, productivity and quality needed to promote the use of the crop. The extent of diversity of the collection can be assessed through characterization and evaluation, thus facilitating selection and identification of potential germplasm for crop improvement programs identified for their special traits for improved nutrition and resilience to climate change.

Experimental

Characterization and preliminary evaluation of root crops, medicinal and herbal plants Characterization descriptors' lists for yam was used to determine the morphological features of the germplasm collections. Vegetative, root and inflorescence characters were gathered. The data obtained from characterization were used to assess the diversity of collection using the standardized Shannon Weaver Diversity Index (H') where it was categorized as low (0-0.33), medium (0.34-0.66) and high (H'>0.67). The data obtained will be used to select promising accessions for direct utilization and as potential parents for crop improvement. The formula is as follows:

$$H' = -\sum pi \frac{\log2\ pi}{\log\ (n,2)}$$

where: n = number of classes for a character

pi = proportion of entries in the ith class

Identification and screening for phytoactive compounds of nutraceuticals

1. Nutritional evaluation and analysis included chemical properties in terms of protein, ash, fats, crude fiber, dry matter and nitrogen free extract (NFE).

a. Protein -The Kjeldahl method of determining total N was used.

b. Ash - 100 mg of well-mixed powdered sample was ignited at 550°C in a muffle furnace until sample particles turned to light gray ash. The sample was cooled to room temperature in a desiccator and weighed.

c. Fats -A 1.0g of freshly dried sample was defatted in a Soxhlet fat extractor using anhydrous hexane as solvent. The sample was removed from the fat extractor and air-dried under the hood until hexane-free. The sample was heated for 1 hour at 105°C, desiccated for 1 hour, and weighed.

d. Crude fiber -To a 1.0g moisture- and fat-free sample, 0.5 g asbestos and 2 drops of octanol was added, weighed in 500 ml Erlenmeyer flask and boiled for exactly 30 min. with constant shaking. The sample was filtered using filtering cloth, washed with boiling water until residue was acid-free, and 200 ml boiling H_2SO_4 solution was added to the sample in a pre-heated digestion apparatus. The residue was transferred back to the flask quantitatively, the cloth was washed with 200 ml boiling NAOH solution and similarly digested for 30 min. The residue was washed until alkali-free, filtered through suction in a gooch crucible by washing the residue with distilled water, rinsed with about 15 ml of 95% ethanol, removed from suction, dried in the oven at 105°C overnight, cooled in a desiccator and weighed. The sample was ignited in the muffle furnace at 550°C for 1 hour or longer, cooled in a desiccator and weighed. The total weight loss is the crude fiber.

e. Dry Matter -A well-mixed finely macerated fresh sample weighing 15 – 20 g was put in 1.5 g aluminum foil dish (previously heated at 130°C to constant weight) then placed inside the air-oven at 45°C for 2 days. The samples was removed after the required number of days and cooled in the desiccator. Weight of the dried sample and moisture dish was recorded.

Calculation:

$$\%\ \text{Moisture} = \frac{[\text{wt. sample + dish before drying}] - [\text{wt. sample + dish after drying}]}{\text{weight of sample}} \times 100$$

f. Nitrogen Free Extract

Is calculated using the formula below:

% NFE=100 - %moisture - %Protein - % Fat - % Crude fiber - % ash

2. Nutraceutical Value and Metabolites

a. Antioxidant activity - Antioxidant activity was evaluated using the DPPH method. DPPH is 2,2 diphenyl-1-picryl hydrazyl, a stable free radical. A negative control and different concentrations of BHA (butylatedhydroxyanisole) as positive control was included in the analysis. BHA is a synthetic antioxidant. Absorbance will then be measured at 517 nm. The percentage of DPPH scavenging activity is expressed by [1-(test sample absorbance /blank sample absorbance)] x 100. The result is compared to the standard. High % Scavenging activity means high antioxidant capacity.

b. Total Phenolic Content - Total phenolics was determined colorimetrically using Folin-Ciocalteau reagent using the method described by Veliogluet al. with slight modifications. Absorbance will be measured at 725 nm using a Cecil UV Visible spectrophotometer. Total phenolics will be quantified by calibration curve obtained from measuring the absorbance of known concentrations of catechin standard (25-150 μg/mL of 50% methanol). The concentrations are expressed as g of catechin equivalents per gram of dry weight.

c. Total Flavonoid Content - The total flavonoid concentration was measured using a colorimetric assay developed by Zhishen et al (1999). Absorbance of the mixtures upon the development of pink color were determined at 510 nm relative to a prepared blank. The total flavonoid contents of the samples are expressed in milligrams gallic acid equivalent per gram sample.

RESULTS AND DISCUSSION

Characterization and preliminary evaluation of yam

Morphological characterization using the NPGRL descriptors list for yam was used to describe the diversity of 31accessions. Four species are represented in the germplasm collection of yam characterized and evaluated. The data obtained from characterization were used to assess the diversity of collections using the standardized Shannon-Weaver Diversity Index.

Forty two (42) qualitative characters were analyzed for diversity (Table 1). 17 qualitative characters out of 42 exhibited high diversity indices ranging from 0.67-0.99 with texture of flesh giving the highest diversity among the characters and the dominant trait being the smooth texture of flesh. This is then followed by leaf density (0.94) and hardness of tubers (0.90). Fourteen (14) morphological characters obtained medium diversity index values while eleven (11) characters obtained low index values. The qualitative characters leaf length, leaf width, tuber length, tuber width, and tuber weight all obtained high diversity index values (Table 2). Diversity on tuber length shows that GB 60555 is the longest tuber with slight difference to GB 60118 and GB 60994 is the smallest. While in tuber width GB 61163 has the widest tuber and GB 60994 being the smallest tubers for both length and width. Tuber length and width is directly related to its shapes.

Table 1. 17 qualitative characters with high Shannon-Weaver diversity index (H'). (n=31)

Qualitative character	Dominant trait	Diversity Index	Diversity
Texture of flesh	Smooth	0.99	High
Leaf density	Intermediate	0.94	High
Hardness of tubers	Easy	0.90	High
Stem height (m)	> 10 m	0.88	High
Time for flesh oxidation after cutting	> 2 min	0.87	High
Vigor	Intermediate	0.78	High
Flesh oxidation color	Purple	0.78	High
Uniformity of flesh color in cross-section	Yes	0.77	High
Place of roots on tuber surface	Entire tuber	0.76	High
Leaf color	Dark green	0.76	High
Leaf vein color (upper surface)	Green	0.74	High
Leaf shape	Sagittate long	0.71	High
Petiole color	All green with purple base	0.71	High
Flesh color at central transverse cross-section	White	0.70	High
Relationship of tubers	Completely separate and distant	0.67	High
Number of tubules per hill	One	0.67	High
Tuber shape	Irregular (not uniform)	0.67	High

Table 2. Shannon-Weaver diversity index (H') of quantitative characters in yam accessions (n=31)

Quantitative characters	Minimum	Maximum	Mean	Diversity Index	Diversity
Leaf length (cm)	8.96	17.6	13.21	0.78	High
Leaf width (cm)	5.3	11.8	8.46	0.78	High
Tuber length (cm)	2.98	35.15	15.94	0.73	High
Tuber width (cm)	2.68	14.58	8.65	0.85	High
Tuber weight (kg)	0.3	9.8	2.91	0.79	High

Evaluation of Yam

A total of 40 accessions of four *Dioscorea* species were evaluated for percent moisture, crude fat, protein, ash, crude fiber and nitrogen free extractives (NFE). 23 *D. alata* (ubi), 13 *D. esculenta* (tugui) and 2 accessions each of *D. bulbifera (ubi patatas)* and *D. hispida* (nami) were included in the laboratory analysis.

Table 3 presents the results of the proximate analysis in the harvested tubers of 40 germplasm accessions of *Dioscorea* species. The functional properties were also determined which included antioxidant property, total phenolics, anthocyanin content and flavonoid content of the corms. Among the four (4) species of native yams analyzed, *D. esculenta* tubers have the highest percent value in crude fat (2.42-2.79). *D. alata* have the highest percent total ash value (1.01-1.35), compared to other three native yam species which means high mineral contents. *D. bulbifera* and *D. hispida* on the other hand have the highest percentile value in protein per sample (2.28-2.56). *D. esculenta* and *D. bulbifera* have higher crude fiber contents compared to *D. alata* and *D. hispida*. Percent NFE indicates the presence of some compounds like lignins, tannins and soluble carbohydrates and hemicelluloses. *D. alata* have higher range of values of %NFE compared to *D. esculenta. D. bulbifera* also contain high % NFE considering that only two accessions were included in the analysis.

Table 3. Results proximate analysis of yam germplasm accessions

	% Moisture	% Crude Fat	% Protein	% Total Ash	% Crude Fiber	% NFE
D. esculenta						
PHL 4725	69.97	2.76	0.84	0.79	0.80	24.84
PHL 4737	75.33	2.52	0.91	0.76	0.77	19.72
PHL 4742	66.2	2.66	0.96	0.84	0.85	28.51
PHL 4750	78.5	2.75	1.00	0.82	0.78	16.16
PHL 4758	65.91	2.65	0.99	0.84	0.85	28.76
PHL 4766	80.01	2.79	0.98	0.75	0.77	14.71
PHL 4768	63.71	2.76	1.00	0.76	0.81	30.96
PHL 4775	79.58	2.63	1.00	0.79	0.83	15.17
PHL 4776	64.75	2.52	1.00	0.75	0.85	30.15
PHL 4783	60.15	2.47	1.00	0.82	0.83	34.73
PHL 9557	60.68	2.71	0.98	0.77	0.80	34.06
PHL 9683	71.96	2.66	0.98	0.83	0.77	22.81
PHL 9684	67.34	2.74	0.96	0.87	0.85	27.25
D. alata						
GB 51346	67.74	0.21	1.89	1.04	0.67	28.46
GB 51347	65.00	0.2	1.83	1.01	0.7	31.26
GB 51966	66.04	0.2	2.14	1.03	0.64	29.95
GB 52211	77.03	0.2	1.91	1.23	0.75	18.88
GB 52212	62.70	0.17	1.91	1.12	0.72	33.38
GB 53078	73.80	0.18	1.79	1.10	0.69	22.44
GB 53191	65.20	0.21	1.41	1.11	0.75	31.32
GB 53193	71.98	0.22	1.89	1.16	0.71	24.04
GB 54334	78.41	0.19	1.7	1.24	0.73	17.74
PHL 3496	64.32	0.18	1.83	1.11	0.67	31.89
PHL 3507	70.48	0.19	1.74	1.33	0.66	25.59
PHL 3524	69.15	0.21	1.79	1.31	0.69	26.86
PHL 4614	70.96	0.22	1.58	1.24	0.74	25.26
PHL 4641	72.85	0.21	1.52	1.22	0.65	23.55
PHL 4970	69.45	0.21	1.91	1.35	0.72	26.37
PHL 5012	68.21	0.22	1.87	1.02	0.66	28.02
PHL 8282	71.39	0.23	1.91	1.11	0.67	24.70
PHL 9599	64.27	0.22	1.47	1.02	0.67	32.34
PHL 9624	72.40	0.23	2.08	1.04	0.71	23.55
PHL 9629	75.52	0.23	1.97	1.24	0.59	20.44
PHL 9635	65.19	0.21	1.89	1.01	0.73	30.97

PHL 9641	68.16	0.22	1.97	1.10	0.67	27.88
PHL 9642	76.35	0.22	1.85	1.03	0.73	19.82
D. bulbifera						
GB 55647	63.8	0.11	2.53	0.79	0.85	31.93
GB 58487	63.35	0.13	2.56	0.8	0.86	32.31
D. hispida						
GB 51000	72.44	0.29	2.28	0.62	0.64	23.72
GB 51004	71	0.31	2.39	0.68	0.68	24.94

Presented in Table 4 are the functional properties of the different yam species. While Figures 1, 2 and 3 present the correlation of the antioxidant capacity with anthocyanin content, total phenolics, and flavonoid content. Ubi accessions GB 53191 and PHL 5012 both have high total phenolic and anthocyanin contents, while PHL 4750 (tugui) has high total phenolic and flavonoid contents. Highest values of antioxidant properties can be observed on PHL 4725 (tugui) and GB 53193 (ubi) with 94.98 % and 92.69 % scavenging activity, respectively. The results of this study supports the work done by Cornago et al..

Three distinct clusters were shown when the functional properties are correlated with the antioxidant activity of the collections with anthocyanin, total phenolics and flavonoid contents. Correlation of antioxidant property with anthocyanin content shows the first group has low antioxidant and anthocyanin contents, the second group has high antioxidant capacity but with low anthocyanin content and the third group possess medium to high antioxidant and anthocyanin contents. The third group consists of 23 accessions and these are potential or promising accessions that can be used for crop improvement programs. Same trends were observed for correlation with total phenolics and flavonoid contents. PHL 4750 (*D. esculenta*), PHL 4758 (*D. esculenta)*, PHL 5012 (*D. alata*) and GB 53191 (*D. alata*) are grouped (Group III) as the collections with high antioxidant and total phenolic contents. The correlation of antioxidant capacity with flavonoid content shows two accessions distinctly separated and these are GB 53191 and PHL 5012 both with high antioxidant and flavonoid contents.

Based on the results of the laboratory analysis, six (6) promising collections of yam can be selected based on functional properties and these are PHL 5012, PHL 4750, PHL 4725, PHL 4758, GB 53191 and GB 53193.

Table 4. Functional components analysis results for yam germplasm accessions

PHL/GB No.	Antioxidant Capacity	Total Phenolics	Flavonoid Content	Anthocyanin Content
	% Scavenging activity	mg GAE/ 100 g	mg QE/100g	mg PGAE/ 100g
D. esculenta				
PHL 4725	**94.98**	200.00	132.84	11.70
PHL 4737	87.67	238.10	132.84	18.27
PHL 4742	73.97	150.60	90.67	18.15
PHL 4750	83.11	**401.19**	**434.38**	16.14
PHL 4758	68.95	162.50	122.30	15.96
PHL 4766	84.02	217.86	153.93	16.93
PHL 4768	78.08	127.38	115.98	9.75
PHL 4775	79.91	198.21	101.21	15.35
PHL 4776	73.52	169.05	90.67	13.95
PHL 4783	79.00	186.31	160.26	17.00
PHL 9557	76.26	120.83	80.13	14.62
PHL 9683	76.26	203.57	143.39	16.20
PHL 9684	81.74	192.86	164.47	16.02
D. alata				
GB 51346	78.08	164.88	196.10	68.71
GB 51347	89.95	236.31	326.84	66.52
GB 51966	89.95	244.70	373.23	51.41
GB 52211	91.78	211.31	215.08	57.75
GB 52212	58.90	117.86	175.02	38.74
GB 53078	61.19	268.45	322.62	66.52
GB 53191	70.32	**513.10**	**782.30**	74.32
GB 53193	**92.69**	176.79	151.82	68.47
GB 54334	57.53	393.45	379.55	**83.09**
PHL 3496	61.19	111.9	162.37	58.72
PHL 3507	59.36	205.95	347.93	68.71
PHL 3524	60.27	183.93	242.49	62.62
PHL 4614	67.12	190.48	113.87	55.56
PHL 4641	64.38	200.00	326.84	48.49
PHL 4970	68.49	151.79	122.30	45.32
PHL 5012	58.90	**395.24**	**560.90**	**77.97**
PHL 8282	69.86	108.33	166.58	60.92
PHL 9599	60.73	157.74	358.47	66.76

PHL 9624	60.27	236.9	261.47	59.94
PHL 9629	54.79	200.00	265.69	63.84
PHL 9635	68.49	189.88	307.86	64.08
PHL 9641		204.17	234.06	56.04
PHL 9642		203.57	134.95	64.81
D. bulbifera				
GB 55647		252.98	337.38	17.79
GB 58487		244.64	189.78	16.2
D. hispida				
GB 51000		177.38	80.13	15.53
GB 51004		136.31	69.59	13.22

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