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Ex Situ Conservation and Genetic Diversity Assessment in the Iluko Indigenous *Dioscorea* "Buga"

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ABSTRACT

The wild *Dioscorea* "buga" is an *Iluko* indigenous root crop which has high potential for food. While it is a recognized food plant, information on it is wanting. The plant's botany, ecology, ethnobotany, utilization and conservation were documented in selected sites in Ilocos Norte, Ilocos Sur, Abra and Cagayan. Germplasm materials were also assembled and conserved as living plant collections in a field genebank at MMSU, Batac City. For a more cost-effective germplasm management in the *ex situ* conservation, the sample of "buga" plants were characterized morphologically and genetic diversity was estimated using the standardized Shannon Weaver's diversity index and NTSYS-pc. The "buga" plant is distinct for the spiny anchor roots, big underground tubers and long stolons which suspend the tubers. As a wild plant, it exhibits some adaptive mechanisms enabling it to survive in the wild or cope with existing environmental conditions to which it is exposed. Linguistics, ethnobotany and indigenous knowledge systems suggest that the plant had long existed and used as an integral part of human diet in the rural areas in the Ilocos, but its use had decreased through the years. Further, conservation of the crop remains neglected. This study is so far the first conservation initiative on "buga" in the Ilocos Region. The sample of the "buga" plants is estimated to be of high genetic diversity (H'=0.79). In addition, multivariate analysis showed that each accession is unique, no duplication, and no distinct clustering of accessions coming from a particular geographic source. Experiences on the *ex situ* conservation of the plant had led to the improvement of a POT for genebank establishment and management. Assembly of accessions from areas still unrepresented in the collection is recommended. Some strategies were also recommended to improve *in situ* (on-farm) conservation of the crop, particularly on maximizing its utilization.

Keywords: Ex situ Conservation, Buga

RATIONALE

Indigenous food plants are the traditional crops grown, or gathered from the wild and consumed by the people in a given locality. These plant materials can be harnessed to provide more diversified and nutritious food on the Filipino table. These are reported to be greatly depended on during periods of famine and especially during the hunger season that precedes crop harvests (FAO, 1998) thus, making these plant forms an integral part of the daily diets of many poor rural households.

One of the Iluko indigenous plants is the wild *Dioscorea* "buga", identified taxonomically as *Dioscorea esculenta* (Lour.) Burkill *subsp. spinosa* Antonio *et al. comb. et stat. nov.* (*ined*.) (Antonio, 2008). As revealed by rural folks through a survey, the crop had been used for human food since the olden days. However, boiling and broiling were then the only known cooking methods. In an attempt to enhance the utilization of the plant, Pascua *et al.* (2004) processed buga tubers and formulated food products like *buga maja, buga halaya* and candied *buga* which were more delicious and acceptable than the standard recipes.

Although "buga" is a recognized food plant, information on it, *i.e.* botanical description, importance and method of utilization, conservation status, etc., is wanting. Further, while concerted efforts are geared on broadening the food base and increasing food production, similar attention should be concentrated on the conservation of as much biodiversity as possible. Hence, this study was conducted to assemble and conserve "buga" germplasm in a field genebank and address the above-mentioned concerns.

OBJECTIVES

Generally, the study aimed to conserve available "buga" germplasm through *ex situ* conservation method.

Specifically, it aimed to answer the following objectives:

1. To document, assess and present information on the plant's botany, ecology, ethnobotany, indigenous knowledge systems, utilization and conservation.
2. To assemble available germplasm and establish a field genebank at MMSU.
3. To develop/formulate/improve a POT for "buga" genebank establishment and management.
4. To estimate the genetic diversity of the "buga" collection.

METHODOLOGY

Documentation Activity

A survey was done in 18 sites in Ilocos Norte, Ilocos Sur, Abra and Cagayan in 2005 to 2006. A total of 180 key informants which consisted of old folks, barangay officials and other knowledgeable individuals were interviewed to provide information on the plant. Local permits to interview and collect germplasm were secured from the barangay chairmen and private land owners of the identified sites.

Documentation of some of the plants botanical and ecological data was also done on site (natural habitat). The documentation activity on the plant's ethnobotany, IKS, botany and ecology was done as a preliminary investigation establishing basic information to justify the need for the conservation of the crop, for taxonomic purposes, and serve as decision support in the various activities on germplasm establishment and management.

Assembly of Germplasm

From the survey sites, "buga" planting materials were randomly collected. Accompanying collecting descriptors were accomplished. Herbarium specimens were also prepared from natural populations to serve as voucher specimens for the taxonomic identification of the plant. The herbarium specimens were deposited at UPLB Botanical Herbarium (CAHP), and duplicates were also prepared and will be deposited at the MMSU Museum of Natural History.

Field Establishment and Management

The field genebank was established at the MMSU experimental farm in Batac City. Every year the genebank was set up at separate fields to avoid mixing or contamination of the collection. Recommended cultural management practices were followed.

Characterization and Analytical Procedures

Characterization was also done *ex situ*. Morphological characters were scored using the descriptors modified from IPGRI and IITA (1997). A total of 100 descriptors was used, which included stem, leaf, flower, aerial and underground tuber characters. Data gathering was done at 20 days after emergence (DAE) (stem and leaf), at flowering (flower), before senescence (stem and leaf), and during and after harvest (tubers).

Phenotypic diversity was estimated by the standardized Shannon Weaver's Diversity Index (H'), while genetic relationship of the collection was analyzed using the computer program NTSYS-pc.

RESULTS AND DISCUSSION

Plant Description

The "buga" plant is a vigorous left-twining vine, (Fig 1A and B), reaching about 10 m in height. It is distinct for the moderate crown of thorny anchor roots attached at the base of the stem just below the soil surface, numerous and big underground tubers and the long stolons on which the underground tubers are suspended (Fig 1C and D). The stem is cylindrical, armed with spines and hairs. Leaves are vigorous, cordate to broad cordate, pale to dark green, hairy on both sides of the lamina but denser on the under surface. The plant flowers profusely every year, approximately a month or two from sprouting. Inflorescence is a spike flower is dioecious, but only the male organ is observed. The flower is hairy epipetalous, with six petals and stamens (Fig1E), ovary poorly developed. It does not produce fruit and aerial tuber. Underground tubers are numerous, big, globose, cylindrical to oblong-oval (Fig 1F and G). Flesh color is yellowish-white or off-white.

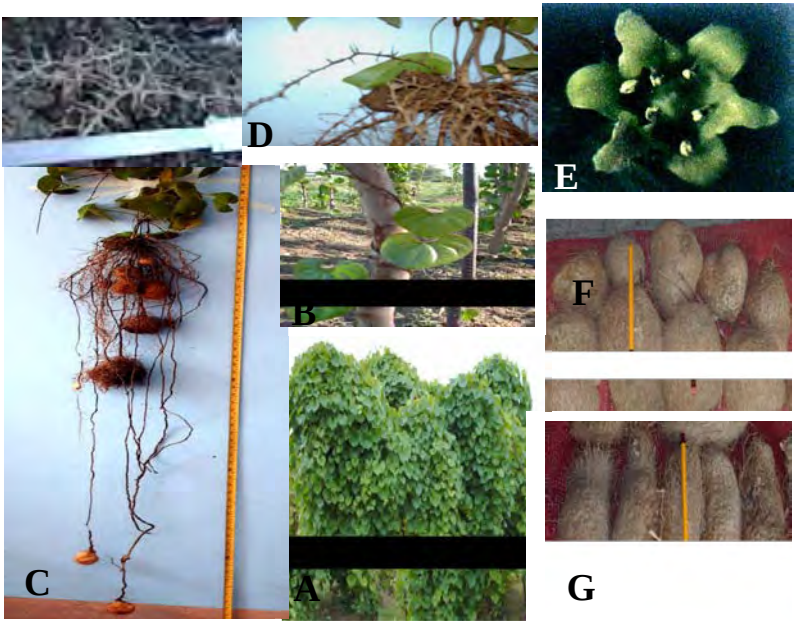

Fig. 1. "Buga" showing stem, leaf, flower, tuber and root characteristics. A. profuse leaves of mature plants, B. left-handed twining habit, C. tubers suspended on long stalks (before maturity), D. thorny anchor roots, E. epipetalous flower, and F-G. globose and cylindric tubers.

The "buga" plant is growing in the wild, climbing among bushes and trees. It thrives in marginal areas, border fields, or hills and forests from low to high elevations. It is highly adaptable to different soil types and conditions. Based on the collecting descriptors, it could thrive in sandy, clay, loam or silt; or in stony, reddish brown or black soil. Since it is left growing in the wild, it could thrive even without care and management practices like land tillage, irrigation and fertilizer and chemical application. Rainwater, the more or less humic soil in its habitat and the natural trellis provided by the trees and bushes are the only provisions for its growth and development. It is hardy, not susceptible to pests and diseases. The crop however needs soil with good drainage since water logging was observed to be the most serious abiotic factor threatening the crop.

Some of the plant's adaptive mechanisms to survive in the wild include: 1) the production of numerous and big tubers, 2) synchronization of leaf emergence and stem elongation, 3) presence of long petioles and big leaf blades, 4) thorny anchor roots and spiny stems, and 5) presence of long stolons.

Evidences of the Plant's Importance and Existence in the Ilocos

"Buga" is an important root crop in the Ilocos owing to its high potential for human food. "Buga" tuber is a cheap source of carbohydrates. Identified as

a subspecies of *D. esculenta,* it may contain 26 to 36 g of starch and sugar per 100 g edible portion (Onwueme in Flach and Rumawas, 1996). It could also possibly contain similar components like its cultivated counterpart, which include fat, 0.1 – 0.3 g; Vit. A, 0.017 mg; Vit. B1, 0.08 mg; Vit. B2, 0.02 mg and Vit. C, 20.3 mg.
Linguistics, ethnobotany and indigenous knowledge point to the perceived importance and existence of the crop in the Ilocos early in time (Table 1).

The plant's long existence and importance in the Ilocos is evidenced by: a) its popularity among Ilocanos as indicated by the presence of local or vernacular names assigned to it and coined terms referring to or associated with it, b) developed procedures and ways on the management, preparation, cooking and utilization of the tubers, and c) developed indigenous knowledge on harvesting and postharvest.

Conservation Status

Despite the proven potential of the crop for human food, buga's utilization had decreased through the years. Only a few families now in Ilocos Norte still use it as human food – and only as an emergency food during the lean months. It has now become more popular as boiled feedstuff for swine raised on a backyard scale.

The reduced utilization of the crop for food is attributed to the following reasons: 1) The areas where the plant used to grow are gone or converted to other land uses. 2) Digging the tubers is very laborious (and constrained by the meshwork of spines at the base of the plant. 3) The plant has negatively-selected characters, 4) Other plants which are readily available and easily prepared have taken the place of "buga". 5) Food preferences of the people have been altered, and have shifted to instant and fast foods.

Table 1. Linguistics, ethonobotany and IKS on "buga".

INFORMATION	ILOCOS NORTE	ILOCOS SUR	ABRA	CAGAYAN
A. Linguistics	"buga"	"buga"	"buga"	"amakay" (according to secondary source)
Common name		"dolian"		
Coined terms associated with the crop	*Nagares* (crunchy-like even after cooking) *Kumudisir/kimmudisir* (hard to cook/soften) *Panagilebben* (storage technique by burying tubers in the soil)			

B. Ethnobotany Popularity as staple food (substitute of rice)	Boiled, broiled, or cooked as guinatan, halaya or as *inabraw* or *dinengdeng* (Iluko vegetable viand) mixed with malunggay (*Moringa oleifera)* leaves, squash (*Cucurbita moschata*) flowers or other leafy vegetables		Respondents not familiar with the plant
	Preparation of the tubers: • Wash throuroughly and drain • Remove upper sett - this part cannot be cooked easily (nagares) • Peel up to the rootlet base - to eliminate the itchy or biting taste		No developed practices
C.Indigenous knowledge Harvesting and Postharvest practices	Dig tubers at one time (Jan- Feb) With bruised tubers: Scrape off wound, cure with woodash or sandy soil Airdry; strictly no washing *Panagilebben* – bury tubers in dry shaded area near abodes for accessibility	Dig sackfuls of tubers at one time (Oct); Leave tubers in the open inside hut *(abong*) or barn *(kamarin,kamalig)* Mark hills before plant senesces, Go back to dig when needed	No developed practices due to unfamiliarity of the plant Sprayed with herbicide since considered as weed in corn farms

Further, conservation of the plant remains neglected and genetic erosion is possible in the next years if the plant remains in its natural habitat. In fact, nobody from the key informants has said that he/she sees the crop being domesticated like the "kamangeg" *(D. luzonensis)*, another wild *Dioscorea.* The above-listed factors are contributory to the non-conservation of said plant.

Ex Situ Conservation of "Buga"

With the anticipated genetic erosion of "buga" if left out in its natural habitat, "buga" germplasm were assembled and conserved *ex situ*. This study is so far the first conservation initiative in this part of the region. The "buga" collection together with their collection numbers, respective sources and accession (GB) numbers are presented in Table 2.

Table 2. The "buga" collection and their respective sources.

COLLECTION NUMBER	SOURCE	GENEBANK NUMBER
MAA-01	Sitio Gusod, Brgy. Parang Sarrat, Ilocos	GB 012
MAA-02	Norte	GB 013
MAA-03	Sitio Imingan, Brgy. Monggoc Pidigan,	GB 014
MAA-04	Abra	GB 015
	Sitio Barbarangay, Brgy.Alinaya Pidigan,	
MAA-05	Abra	GB 016
MAA-06	Sitio Paypayao, Brgy. Tangadan San	GB 017
MAA-07	Quintin, Abra	GB 018
MAA-08	Brgy. Kakaldingan, Narvacan, Ilocos Sur	GB 019
	Brgy. Baresbes, Dingras, Ilocos Norte	
MAA-09	Brgy. Buyon, Bacarra, Ilocos Norte	GB 020
MAA-10	Sitio Malampa, Brgy. Nambaran Bacarra,	GB 021
	Ilocos Norte	
MAA-11	Bangui, Ilocos Norte	GB 022
MAA-12	Sitio Marabanos, Brgy.Cabisuculan	GB 023
MAA-13	Vintar, Ilocos Norte	GB 024
	Brgy. Talugtug, Solsona, Ilocos Norte	
MAA-14	Sitio Upay, Brgy. Oaig, Paoay, Ilocos	GB 025
MAA-15	Norte	GB 026
MAA-16	Sitio Allangigan, Brgy. Payao, Batac City,	GB 027
MAA-17	Ilocos Norte	GB 028
MAA-18	Brgy. Calaoagan Dackel, Gattaran,	GB 029
	Cagayan	
	Palagao Sur, Gattaran, Cagayan	
	Brgy. Baraoidan, Gattaran, Cagayan	
	Sitio Dolores, Brgy. Mocag, Baggao,	
	Cagayan	
	Brgy. Mocag, Baggao, Cagayan	

The three-year experiences on the *ex situ* conservation of the crop had led to the formulation of a POT for genebank establishment and management for "buga" (Table 3). This is an improvement of the recommended practices for yam but modified and improved to suit the growth habit and other characters of "buga". The "buga" genebank POT include recommended practices for site selection, land and planting material preparation, planting, weeding, hilling-up, trellising, harvesting, and postharvest handling.

Table 3. Genebank establishment and management practices for "buga".

MANAGEMENT PRACTICE	DESCRIPTION
Site selection	• Select an area with good drainage and porous soil. • Transfer site every year. • Do not plant in sacks.
Land and Planting Material Preparation	• For level plain, plow and harrow the field twice. • For sloping/hilly area, follow zero tillage. • Pre-sprout tubers. Water them once a week. • Transplant when most of the tubers have produced sprouts.
Planting	• Plant at a distance of 1.0 m between hills, 1.5 m between rows and at 10 cm depth. • Maintain five hills per accession.
Weeding, Hilling-up and Trellising	• Weed as the need arises. Use hand tractor *cum* weeder while the stake set-up still allows; otherwise use a grass cutter or manual weeding. • Hill-up not later than one month after planting using a hand tractor. • Put trellis before the vines started crawling on the ground. • Use sturdy poles (bamboo or wood), 1.0 to 1.5 m high.
Harvesting	• Harvest annually, when 80% of the foliage had turned yellow or dried up. • Harvest manually by carefully digging the tubers using a fork shovel to minimize bruises. • Group tubers according to accession, place in fine net bags, label properly.
Post-harvest	• Treat bruised tubers with wood ash, sandy soil or fine sand. • Store in wire racks in a shaded area until needed. Label properly and instigate measures to avoid tuber mixing. • Inspect tubers regularly, discarding rotten or decaying ones. Segregate but don't discard shriveled tubers.

Phenotypic Diversity of Wild "Buga"

The sample of the Iluko indigenous "buga" germplasm used in this study was estimated to be of high diversity (H'=0.79) based on the standardized Shannon-Weaver diversity index. The quantitative (H'=0.89) and qualitative (H'=0.75) descriptors both amounted to high diversity and contributed to this observed high variability.

Flower descriptors were most variable with an average of H'=0.92. Following in variability were the underground tubers and leaf descriptors (H'=0.80), and the least variability was observed in the stem descriptors (H'=0.74).

Genetic Relationship of "Buga"

Multivariate analysis of morphological data showed that each accession is unique and distinct since no two or more individuals appear one and the same genotype (Fig 2). Such result also suggests no duplication in the collection. At a distance of 0.72, the "buga" cluster is divided into 4 subgroups, each group of which has some unique features. Fig. 2 also showed no distinct clustering of accessions coming from a particular geographic source. This indicates that even when originally grown in different geographic locations, there is little variability in these individuals. The crop is vegetatively-grown, hence sexual reproduction (outcrossing) cannot introduce greater variability in the yam taxa. Most probably, it is only by somatic mutation that variation can be achieved in them.

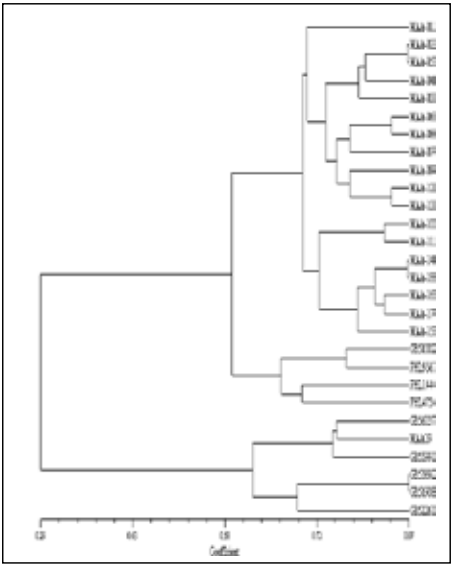

Fig. 2. Dendrogram for qualitative characters produced by SAHN clustering using simple matching coefficient and UPGMA method.

CONCLUSIONS AND RECOMMENDATIONS

Results showed that the plant is an imprtant root crop in the Ilocos, both as human food and swine feedstuff. However, utilization of the crop had decreased through the years due to its negatively-selected characters. Conservation of the plant also remains neglected. A POT for "buga" genebank establishment and management was formulated and recommended.

The following component technologies are recommended for enhanced utilization and conservation of the crop: improved cultural management for increased yield,

a) selection of accessions with good quality traits like less spiny roots and better yield, and

b) provision of technical assistance on product formulation and value adding, and marketing assistance for formulated food products.

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