INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE

ISSN: 2067-533X Volume 3, Issue 3, July-September 2012: 199-206 www.ijcs.uaic.ro

IMPROVEMENT OF ACCESSION DISTINCTIVENESS AS AN ADDED VALUE TO THE GLOBAL WORTH OF THE YAM (DIOSCOREA SPP) GENEBANK

Gezahegn GIRMA*, Sam KORIE, Dominique DUMET, Jorge FRANCO

International Institute of Tropical Agriculture, Ibadan, P. M. B. 5320, Nigeria

Abstract

Misidentification of accessions is a common problem in genebanks. Along the years, mistakes accumulate and this is particularly true when dealing with a large number of accessions requiring annual regeneration. Human errors such as mislabeling or misreading and material mix up during planting or storage are the main causes for misidentification of accessions. The international collection of yam, maintained at IITA, has accumulated 'non true to type' accessions along the years. In the present study, 53 morphological descriptors were used to detect uniformity of individuals within accessions of the yam gene bank collection i.e. agro morphological mismatch between individual plants of the same accession. Based on a similarity matrix, individual pairs with less than 0.90 similarity coefficients, which varies in six descriptors and more, were considered as distinct and mismatched, whereas those that had similarity coefficients greater than or equal to 0.90 were considered as clones from the same parent. Overall, 20.60% of the total 3156 accessions were found not true to type i.e., misidentified individuals. The descriptive analysis shows that morphological traits like distance between lobes, upward folding of leaf along main vein, young stem color, old stem color, leaf shape, leaf density and plant vigor are the most discriminative descriptors for individual identification within accession. Some other traits were also found species specific and they may aid in distinguishing misidentifications between species.

Keywords: *mismatch identification; agro-morphological descriptors; Dioscorea spp; field bank*

Introduction

Gene banks are very important for the conservation of the diversity of species. Hence, they provide a way out in times of disaster and sustain food supplies for the coming generations. One of the most important purposes for genetic resources conservation in gene banks is offering important traits of interest for breeding in germplasm enhancement through variety development [1]. However, the actual clientele of gene bank collections is much broader and may include taxonomists, entomologists, molecular geneticists and scientists from many other disciplines [2].

Genebanks are also important in facilitating utilization, determining needs for new collections, maintaining existing collections, determining optimum regeneration methods, characterizing collections for useful agronomic traits, classifying the collections, the creation of

* Corresponding author: g.tessema@cgiar.org, Tel: +234 706 721 9077

international cooperation's that includes the exchange of methodologies and technologies to research, document, manage and utilize genetic resources [1].

The FAO Second State of The World Report on Plant Genetic Resources for Food and Agriculture reports that the total number of accessions conserved in *ex situ* collections is about 7.4 million, in over 1750 genebanks around the world [3]. About 90% of these collections is conserved as seeds in seed gene banks and the rest are maintained in field gene banks [4].

Field genebank is still the main conservation strategy and an important *ex situ* conservation method. Conservation on field can offer several advantages including the possibility of evaluation and characterization of material while being conserved, easy identification and rouging of variant genotypes compared to *in vitro* conservation, easy delivery and lower risk of losing genetic integrity [6]. Besides its importance, the regeneration of genebank accessions remains a major problem, threatening collections [5]. Moreover, the main limitations of field genebanks are that they take a great deal of space and are difficult to maintain and protect from natural disasters, susceptibility to damage from disease and insect attacks and relatively high mislabeling [6, 7]. Therefore it is important to establish a safety duplication of the living collections, by using alternate strategies of conservation like *in vitro* conservation options through tissue culture techniques. *In vitro* conservation also offers other distinct advantages. For example, the material can be maintained in a pathogen-tested state, thereby facilitating safer distribution. Furthermore, the cultures are not subjected to environmental disturbances [8, 9].

The International Institute of Tropical Agriculture (IITA) genebank maintains over 28000 accessions of major food crops of Africa, namely cowpea (*Vigna unguiculata*), cassava (*Manihot esculenta*), yam (*Dioscorea* spp.), soybean (*Glycine max*), bambara groundnut (*Vigna subterranea*), maize (*Zea mays*), and plantain and banana (*Musa* spp.) [10].

In particular, IITA has been maintaining yam germplasm since 1975, collected from West African countries. Based on the information from passport data, Togo is the largest contributor of yam germplasm, followed by Nigeria and Ghana. Currently the gene bank has 3174 yam accessions of eight different species, maintained in the field and partly duplicated in the *in vitro* genebank.

Originally, the collection was exclusively maintained in the field and standard regeneration processes involved planting several mini sets per accession (to reduce germplasm loss). Over the years it was recognized that individuals of the same accession showed different morphological characters i.e. reflecting plant mix up. There are numerous potential reasons for the mix and misidentification of accessions, the major causes being: - designation of the same name to different accessions, registration of the same cultivar under different names in different seasons, incorrect labeling on peg or test tubes, loss of label and mix up during preparation of planting material and harvest. Disparity within accessions can lead to unnecessary duplication of accession which is a waste of resources [11] and to extra complications in field germplasm management.

The incorrect labeling of accessions and misidentification is a major limitation and a serious problem for long term conservation of germplasm. In turn, it may hinder genetic improvement progresses. Moreover, as the field bank is the source for material introduction to *in vitro* conservation (including cryopreservation), any mix up at the field bank will reflect in all backup collections. The objectives of this study were therefore to assess mismatched individual plants within accessions, to develop/identify distinct morphological characteristics as a

reference for each accession and to maintain uniform individuals within accessions of the field genebank collections.

Materials and methods

Plant material

The entire international collection of yam accessions maintained at IITA was investigated. The collection includes eight different species (*D. rotundata, D. esculenta, D. cayenensis, D. alata, D. dumetorum, D. bulbifera, D. mangenotiana and D. praehensilis*). The materials were planted following standard procedures [12] as routine field bank regeneration during the 2010 main growing season at the IITA experimental plot, Ibadan (Latitude: 7°30'8"N; Longitude: 3°54'38"E), Nigeria.

Experimental layout, data collection and analysis

A total of 3156 accessions were considered in this study. Three healthy and vigorous tubers were selected for each accession. Each tuber was cut into tuber seeds of (50–250 g) as per recommendation by Dumet and Ogunsola [12] for yam field regeneration and a maximum of ten minisets per accession were used for planting. For every accession, each tuber was labeled as A, B or C (suffix to the accession number) in order to differentiate individuals belonging to the same 'maternal origin' i.e. same accession number. Accessions with small tuber size and those with only one or two tubers available were planted in nursery for careful monitoring and management. All agronomic practices were followed as per recommendation for yam production. Morphological characterization was done using 53 yam descriptors (Table 1) obtained from the IPGRI/IITA, 1997, descriptor list. GenStat for Windows software [13] was used to calculate similarities between individuals within the same accession, by using the Simple Matching coefficient. The Simple Matching coefficient is defined as the number of matching traits for two individual accessions divided by the total number of assessed morphological traits or descriptors.

Table 1. Yam morphological descriptors at different growth stages

Young stem	Mature stem	Mature leaves	Flowering	Aerial bulbils
Young stem color	Plant type	Waxiness of leaves	Sex	Absence/Presence of aerial bulbils
Absence/presence of waxiness	Vigor	Leaf Arrangement/position of leaves	Inflorescence position	Aerial tuber shape
Absence/Presence of wings	Twinning habit	Leaf density	Inflorescence type	Aerial tuber diameter
Wing color	Twining direction	Leaf type		Aerial tuber skin color
Absence/Presence of hairs	Stem height	Leaf color		Aerial tuber surface texture
Absence/Presence of spine	Stem color	Number of leaflets in compound leaf		
Absence/Presence of barky patches	Absence/Presence of waxiness	Leatheriness of leaf		
	Absence/Presence of wings	Leaf vein color (upper surface)		
	Wing color	Leaf vein color (lower surface)		
	Absence/Presence of ridges	Leaf margin color		
	Hairiness	Hairiness of upper/lower surface of leaf		
	Absence/Presence of spine	Leaf shape		
	Spine shape	Leaf apex shape		
	Absence/Presence of coalescent spines	Undulation of leaf		
		Distance between lobes		
		Upward folding of leaf		
		Downward arching of leaf		
		Widest part of leaf		
		Leaf tip length		
		Leaf tip color		
		Petiole length		
		Absence/Presence of stipule		

Descriptive analysis of morphological traits was conducted using SAS software [14], to determine the most descriminant descriptors for the identification of individual variation within accession. The extent of a variable distribution in terms of spread can be used to determine its ability to discriminate individual differences within accessions in the data matrix. Spread is often measured by the range and variance. So, the ranges and variances were calculated for all

the traits and then sorted in descending order. The more spread a trait the more discriminating information it contains. A Gower distance matrix was also computed for comparison among individuals and to observe the distance within and between species, by using SAS software. For calculating the Gower distance, ordinal data were ranked and then the Manhattan distance was calculated. For nominal and binary data simple matching distance was calculated.

Results

The computed similarity matrix was used to compare variations within accessions. Individual accessions in a pair greater than or equal to 0.90 in similarities with less than six descriptors difference were considered as individuals originating from the same clone. Table 2 shows individuals TDr1952A and TDr1952B had a 1.00 similarity value, while both shared only 0.78 similarity value with TDr1952C. That implied that TDr1952C was different from the other two individuals within that accession. Similar comparisons were done for individuals within all accessions. Overall, 20.60% of the total collection was found as misidentified (Table 3). A greater proportion of disparity (28.20 %) was observed in *D. rotundata* (2140 accessions) followed by (16.40 %) in *D. cayenensis*. A lower level of misidentification was observed in *D. bulbifera, D. dumetorum and D. alata* with non matching proportions of 4.34, 3.81 and 3.77% respectively. Morphological descriptors could not reveal any discrepancy among 20 accessions of *D. esculenta*.

Table 2. List of accessions with similarity matrix showing variation of individuals within the same accession (less than 0.90 similarity shows distinct individual within accession)

1951A	1.00										
1951B	**0.87**	1.00									
1951C	1.00	0.87	1.00								
1952A	0.76	0.67	0.76	1.00							
1952B	0.76	0.67	0.76	1.00	1.00						
1952C	0.80	0.89	0.80	**0.78**	**0.78**	1.00					
1953A	0.78	0.80	0.78	0.65	0.65	0.74	1.00				
1953B	0.78	0.80	0.78	0.65	0.65	0.74	1.00	1.00			
1953C	0.78	0.80	0.78	0.65	0.65	0.74	1.00	1.00	1.00		
1954A	0.72	0.65	0.72	0.85	0.85	0.76	0.72	0.72	0.72	1.00	
1954B	0.69	0.63	0.69	0.93	0.93	0.74	0.70	0.70	0.70	0.93	1.00
1954C	0.72	0.65	0.72	0.85	0.85	0.76	0.72	0.72	0.72	1.00	0.93
	1951A	1951B	1951C	1952A	1952B	1952C	1953A	1953B	1953C	1954A	1954B

Table 3. List of species, number of accessions, percentage, total number and percent mismatch

Species	Number of accessions	Percentage (%)	Total number of mismatch	Percent mismatch (%)
D. rotundata	2140	67.42	604	28.22
D. esculenta	20	0.63	0	0.00
D. cayenensis	61	1.92	10	16.40
D. dumetorum	53	1.67	2	3.77
D. alata	813	25.61	31	3.81
D. bulbifera	69	2.17	3	4.35
Total	**3156**	**100**	**650**	**20.60**

The registration of two different species with the same accession number, accession name different from passport on field data and loss of accession number, either on passport, or in field data were some other misidentification problems and causes where management actions were indispensable.

The descriminant analysis showed that all descriptors were not equally important, in regard to the identification of an individual variation within an accession (Table 4). Some

descriptors were found more spread in terms of range and variance, which was directly related to the capacity of the descriptors to discriminate individual variation within accession. Among 53 different yam descriptors used in this study, the distance between lobes with a variance of 5.68 was found to be the most descriminant descriptor, followed by upward folding of leaf along main vein, leaf shape, old stem color, young stem color, leaf density and vigor, in descending order.

Table 4. List of most descriminant morphological traits with respective variance, minimum and maximum score, range and total number of individuals (N)

Trait	Variance	Range	Minimum	Maximum	N
Distance between lobes	5.68	9	0	9	8785
Upward folding of leaf along main vein	3.99	6	1	7	8785
Leaf shape	3.22	8	1	9	8785
Old stem color	2.44	4	1	5	8785
Young stem color	2.27	4	1	5	8785
Leaf density	2.08	5	2	7	8785
Vigor	1.29	4	3	7	8785

Similarly, some descriptors were found to be species specific (Table 5). Even though, no misidentification was observed between species in this study, descriptor specificity is an important criterion for the discrimination among species. In addition, sex and inflorescence type in flowering, the presence or absence of aerial bulbils and the type of tubers were important to distinguish individual variation within accession and among species.

The distance matrix for group comparison revealed a lower Gower distance within species (GD≤0.3), ranging from 99.3% of the cases in *D. rotundata* to 100% within all other species. On the other hand, a higher Gower distance (GD>0.3) was also found between species. Similarly, histogram generated through Gower distance was found to explain bimodal distribution (Figure1), with the maximum on the left showing a lower frequency than the one on the right.

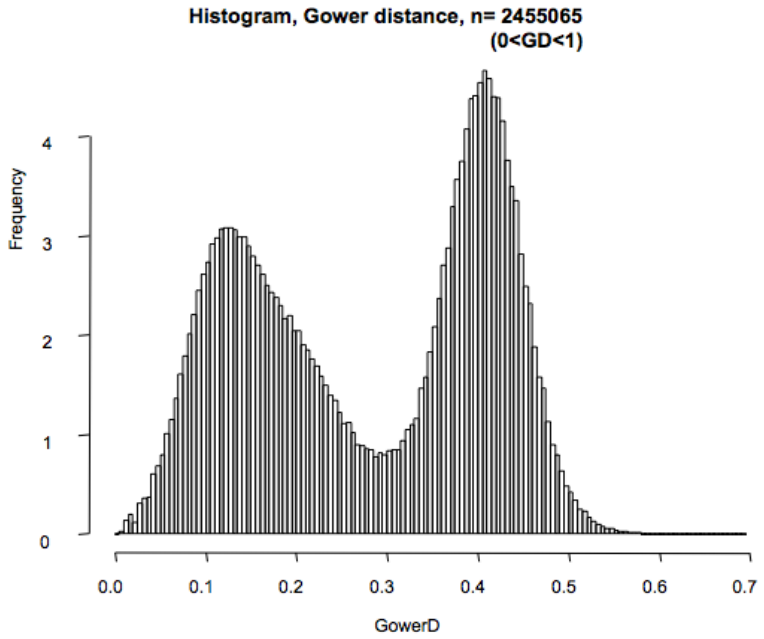

Fig.1. Histogram showing bimodal distribution for Gower distances

Discussion

Morphological descriptors are basic tools for improving germplasm management in general and particularly in duplicate identification [11, 15], the development of core collections [16] and the identification of mislabeling [17]. Similarly, a significant number of mismatches were identified in this study using morphological descriptors (Table 3). The high proportion of mismatch is possibly due to mislabeling, mix up of materials, loss of label and other human errors.

Mislabeling was noted to be one of the common problems in clonally propagated crops such as potato [18] and enset [19]. Likewise, mislabeling has been reported in some other crop genebanks, ranging from 12.30% in cacao [20] and reaching, in some cases, up to 30% in cocoa [21, 22]. The 20.60% mismatch found in this study (Table 3) confirms the existence of more misidentification and human errors in yam germplasm field regeneration. Due attention during harvesting and mini set preparation for planting, closer follow up on field label and rewriting of lost or fade label(s), proof reading before taking any actions and bar coding each accession for identification can help reduce misidentification between and within accessions. Moreover, this and some other management actions can help achieve the objective of any genebank, which is to maintain the genetic integrity of accessions as much as possible. According to D. Spooner et al. [1] the loss of genetic integrity from variable accessions will always occur and what the best genebanks can do is to reduce it as much as possible.

In addition to sorting out and identifying causes of mismatch, it is important to implement barcoding techniques in field genebanks as a management option to avoid misidentification. Otherwise, the process may be both time-consuming and more error introducing if done manually. Hence, accession barcoding, by clearly showing the name of the crop and the accession number on a barcode sticker, is very important for direct data capture and further improvement of yam germplasm management.

The six morphological descriptors (Table 4) determined through descriminant analysis and identified to have high capacity to distinguish individual variation within accession is important, because it is not always possible to use all descriptors. Moreover, using all morphological descriptors every year is not practicable due to its high cost and time implication, especially for large collection. The species specific descriptors identified are also important for the identification of any mix up among species. Since morphological characteristics are often affected by environmental factors that in turn may influence the reliability of the collected information, developing and implementing molecular tools to support morphological descriptors is an important topic, which needs to be taken into consideration.

Table 5. List of important descriptors for identification of different yam species

Descriptors	***Species***					
	D. rotundata	***D. esculenta***	***D. cayenensis***	***D. dumetorum***	***D. alata***	***D. bulbifera***
Twinning direction	anticlockwise	clockwise	anticlockwise	clockwise	anticlockwise	clockwise
Presence of wing on stem	Absent	Absent	Absent	Absent	Present	Absent
Ridges	Absent	Absent	Absent	Absent	Present	Absent
Aerial bulbils	Absent	Absent	Absent	Absent	Present in few and in small size	Present
Barky patches	Present	Absent	Absent	Absent	Absent	Absent
Stem shape	Round	Round	Round	Round	Polygonal	Round
Leaf type	Simple	Simple	Simple	Compound	Simple	Simple
Hairy leaves	Absent	Absent	Absent	Present	Absent	Absent

The appropriate management of materials in any field bank is a fundamental issue for maintenance, the quality of accessions and the reliability of further regeneration techniques (*in*

vitro and cryopreservation). Hence, identification and proper action in germplasm management problems, the creation and proper use of passport data and the characterization of *ex situ* collections, should be given priority in any genebank germplasm management.

Conclusions

In conclusion, significant number of accessions in the collection was found with mixed individuals /mismatched. This is attributed to Human errors such as mislabeling, loss and fade of label(s), material mix during regeneration process and other field genebank management problems. Understanding these causes are therefore essential for proper action and maintaining the germplasm integrity. Some morphological descriptors were with high capacity to distinguish mismatch. These descriptors are therefore very important for quick assessment of individual uniformity as implementing all descriptors may not be always practical due to time and cost implication.

References

[1] D. Spooner, R. Treuren, M.C. Vicente, *Molecular markers for genebank management*, **IPGRI Technical Bulletin,** No. 10, International Plant Genetic Resources Institute, Rome, 2005.

[2] J.M.M. Engels, L. Visser L, *A guide to effective management of germplasm collections*, **IPGRI Handbook for Genebanks,** No. 6, International Plant Genetic Resources Institute, Rome, 2003.

[3] * * *, **The Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture**, Food and Agriculture Organization of the United Nations, Rome, 2010.

[4] B. Koo, P.G. Pardey, B.D. Wright, *The economic costs of conserving genetic resources at the CGIAR centers*, **Agricultural Economics**, **29**, 2003, pp. 287-297.

[5] * * *, **The State of the World's Plant Genetic Resources for Food and Agriculture**, Food and Agriculture Organization of the United Nations, Rome, 1998.

[6] M.E. Dulloo, D. Hunter, T. Borelli, *Ex Situ and In Situ Conservation of Agricultural Biodiversity: Major Advances and Research Needs*, **Notulae Botanicae Horti Agrobotanici Cluj-Napoca**, **38**(2), 2010, pp. 123-135.

[7] N.K. Rao, *Plant genetic resources: Advancing conservation and use through biotechnology*, **African Journal of Biotechnology**, **3**(2), 2004, pp. 136-145.

[8] R.A. Shibli, M.A. Shatnawi, W.S. Subaih, M.M. Ajlouni, *In Vitro conservation and cryopreservation of plant Genetic resources*, **World Journal of Agricultural Sciences, 2**(4), 2006, pp. 372-382.

[9] L.A. Withers, F. Engelmann, *In vitro conservation of plant genetic resources*, **Biotechnology in Agriculture** (Editor Altman A), Marcel Dekker Inc., New York, 1997, pp 57-88.

[10] * * *, **Descriptors for Yam (Dioscorea spp.),** International Institute of Tropical Agriculture, Ibadan, Nigeria/International Plant Genetic Resource Institute (IPGRI/IITA), Rome, 1997.

[11] T.J.L. Hintum, H. Kntipffer, *Duplication within and between germplasm collections. I. Identifying duplication on the basis of passport data*, **Genetic Resources and Crop Evolution, 42**, 1995, pp. 127-133.

[12] D. Dumet, D. Ogunsola, *Regeneration guidelines: yams*, **Crop Specific Regeneration Guidelines** (Editors: M.E. Dulloo, I. Thormann, M.A. Jorge, J. Hanson), CGIAR System-wide Genetic Resource Programme, Rome, 2008.

[13] * * *, **GenStat for Windows software** (Discovery Edition 3), VSN International, Hemel Hempstead, UK, 2007.
[14] A.F. Chiorato, S.M. Carbonell, L. Dias, R.R. Moura, M.B. Chiavegato, C.A. Colombo, *Identification of common bean (Phaseolus vulgaris) duplicates using agro morphological and molecular data*, **Genetics and Molecular Biology, 29**(1), 2006, pp. 105-111.
[15] * * *, **The SAS System for Windows, Version 9.2,** SAS Institute Inc., Cary, NC, USA, 2007.
[16] V. Mahalakshmi, N. Obidiegwu, J.J. Ng, D. Ogunsola, M. Lawson, R. Ortiz, *Development of a West African yam Dioscorea spp. core collection*, **Genetic Resources and Crop Evolution**, **54**(8), 2007, pp. 1817-1825.
[17] E.S. Johnson, A. Mora, R.J. Schnell, *Field Guide efficacy in the identification of reallocated clonally propagated accessions of cacao (Theobroma cacao L.)*, **Genetic Resources and Crop Evolution, 54**(6), 2007, pp. 1301-1313.
[18] Z. Huamán, R. Ortiz, R. Gómez, *Selecting a Solanum tuberosum subsp. andigena core collection using morphological, geographical, disease and pest descriptors*. **American Journal of Potato Research, 77**, 2000, pp. 183-190.
[19] A. Negash, A. Tsegaye, R. Treuren, B. Visser, *AFLP analysis of enset clonal diversity in south and southwestern Ethiopia for conservation*, **Crop Science**, **42**, 2002, pp. 1105-1111.
[20] B.M. Irish, R. Goenaga, D. Zhang, S.R. Apeng, J.S. Brown, J.C. Motamayo, *Microsatellite Fingerprinting of the USDA-ARS Tropical Agriculture Research Station Cacao (Theobroma cacao L.) Germplasm Collection*, **Crop Science, 50**, 2010, pp. 656-667.
[21] Y. Christopher, V. Mooleedhar, F. Bekele, F. Hosein, *Verification of accessions in the ICG, T using botanical descriptors and RAPD analysis*, **Annual Report for 1998,** Cocoa Research Unit, University of the West Indies, St. Augustine, Trinidad, 1999, pp. 15-18.
[22] C.J. Turnbull, D.R. Butler, N.C. Cryer, D. Zhang, C. Lanaud, A.J. Daymond, C.S. Ford, M.J. Wilkinson, P. Hadley, *Tackling mislabeling in cocoa germplasm collections*, **Ingenic Newsletter, 9**, 2004, pp. 8–11.

Received: March, 19, 2012
Accepted: July, 28, 2012