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Research Paper

Analysis of diversity and determination of duplicates among twenty one *Dioscorea* accessions through morphological and molecular characterization

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Abstract: Several accessions of *Dioscorea*, a tuber crop of the family Dioscoreaceae, have been conserved under field and *in-vitro* conditions at the Plant Genetic Resources Center, Sri Lanka. Therefore, identification of duplicates in the collection is necessary as they cause a considerable wastage of input during the conservation process and leads to confusion when using these accessions in plant variety improvement programs. In this study, 21 *Dioscorea* accessions belonging to *Dioscorea alata, D. esculenta, D. bulbifera* and *D. pentaphylla* were characterized by 75 morphological characters and 15 simple sequence repeat (SSR) markers. The morphological characters were distributed as; 31 leaf, 21 stem, five flower and 18 tuber characters. The dendrogram showed that there are four main clusters without duplication. Accession 107 recorded as *D. pentaphylla* was well separated from other accessions while four accessions (21, 36, 113 and 92) were clustered into non-related clusters with their species. The SSR analysis was conducted on 20 *Dioscorea* accessions (except for the accession 107) through Power Marker. Polymorphism was detected among all *Dioscorea* accessions with 2 to 5 alleles per marker and a genetic distance ranging from 0.1333 (among accessions 101, 102, 103 and 109) to 0.7529 (between accessions 83 and 133). The phenogram resulted in three major clusters, which is almost in agreement with the existing classification. A disagreement was observed with respect to the accession 36 recorded as *D. alata* and 127 recorded as *D. bulbifera* as both were clustering with *D. esculenta.* Duplicates, accession 113 and 101, were classified with *D. bulbifera* and accession 62, 26 were identified with *D. esculenta*, respectively.

Keywords: *Dioscorea* accessions, morphological analysis, SSR (simple sequence repeats)

Introduction

Root and tuber crops are vital for providing food for over two billion people, especially in the rural regions of Africa, Asia and the Caribbean. The most prominent root and tuber crops grown in the world are potato (*Solanum tuberosum* L.), cassava (*Manihot esculenta* Crantz), sweet potato [*Ipomoea batatas* (L.) Lam.], yams (*Dioscorea* spp.) and aroids (*Colocasia* spp.) (Sangakkara and Emmanuel, 2014). Those are efficient solar energy transferors, a good source of starch (Senanayake *et al.*, 2011) while the productivity is 2.4 Mg dry matter ha^{-1} with 182 MJ of energy ha^{-1} day^{-1} when compared to

1.9 Mg dry matter ha^{-1} and 151 MJ of energy $ha^{-1}day^{-1}$ produced by rice (Sangakkara and Emmanuel, 2014).

Dioscorea is a genus consisting of 630 identified species including *D. alata*, *D. esculenta*, *D. bulbifera*, *D. fenterfila*, *D. rotundata*, *D. trifida*, *D. composita*, *D. spicata* and *D. tomentosa*. Nearly 40 *Dioscorea* varieties are grown in Sri Lanka (Senanayake *et al*., 2011) with many of *D. alata* and few *D. esculenta* and *D. bulbifera* varieties being edible. Because of many benefits such as being a cash crop and a food crop with health benefits, conservation of the *Dioscorea* has become important. There are about 85 *Dioscorea* accessions conserved under *in vitro* and field conditions at the Plant Genetic Resources Center (PGRC), Sri Lanka. Conservation is a high input process and having duplicates cause considerable wastage of inputs. Accession details derived mostly from ordinary community such as from farmers without scientific background are having a low credibility and therefore, incorrect identification is a barrier to deploy those accessions for further use. In addition, *Dioscorea* having high diversity with many beneficial characters are not thoroughly analyzed. Therefore, morphological and molecular characterization of *Dioscorea* accessions is a timely requirement. In this study, 21 *Dioscorea* accessions were characterized up to the species level with the objectives of identifying duplicates and the genetic diversity through morphological and molecular characterization.

Materials and Methods

Selection of site and plant material:

The study was conducted in the biotechnology division and an open field of the Plant Genetic Resources Center (PGRC) at Gannoruwa, Peradeniya in Sri Lanka. Twenty one *Dioscorea* accessions including four accessions of *D. esculenta* (accession 111, 83, 21 and 133), five of *D. bulbifera* (accession 127, 102, 103, 101 and 109), 11 of *D. alata* (accession 113, 92, 36, 30, 53, 65, 135, 128, 74, 26, 62) and one of *D. pentaphylla* (accession 107) were selected as shown (Table 1).

Table 1. Details of the selected *Dioscorea* accessions

Accession no:	Common Name	Species
111	*Kukulala*	*Dioscorea esculenta*
083	*Kukulala*	*D. esculenta*
021	*Kukulala*	*D. esculenta*
133	*Nartala*	*D. esculenta*
127	*Rassawelli*	*D. bulbifera*
102	*Rassawelli*	*D. bulbifera*
103	*Rassawelli*	*D. bulbifera*
101	*Rassawelli*	*D. bulbifera*
109	*Mothakawelli*	*D. bulbifera*
113	*Raja ala*	D. alata
092	*Raja ala*	D. alata
036	*Raja ala*	D. alata
030	*Kiriala*	D. alata
053	*Kiriala*	D. alata
065	*Higurala*	D. alata
135	*Higurala*	D. alata
128	*Weliala*	D. alata
074	*Welala*	D. alata
026	*Paniala*	D. alata
062	*Rathabala*	D. alata
107	*Katuala*	*D. pentaphylla*

Morphological characterization:
Diversity analysis was conducted based on 75 morphological characters and 15 SSR markers. Morphological data were collected based on the PGRC descriptor of yam (IPGRI/IITA, 1997) from the 20th day after emergence of shoots to end of the life cycle considering 31 leaf characters, 21 stem characters, 5 floral characters and 18 tuber characters and analyzed via Minitab 17.

Molecular characterization: The DNA was extracted from young *Dioscorea* leaves according to Mignouna *et al.* (2009). Fifteen SSR primers designed for microsatellites regions of *Dioscorea* genome were selected (Table 2) for the study.

Table 2. Details of the selected SSR primers

Primer Name	Forward Primer Sequence	Reverse Primer Sequence	Molecular weight (bp)
DA1A01	TATAATCGGCCAGAGG	TGTTGGAAGCATAGAGAA	212 - 225
Dab2D08	ACAAGAGAACCGACATAGT	GATTTGCTTTGAGTCCTT	124 - 368
Dab2E07	TTCCCTAATTGTTCCTCTTGTTG	GTCCTCGTTTTCCCTCTGTGT	129 - 173
Dab2C05	CCCATGCTTGTAGTTGT	TGCTCACCTCTTTACTTG	168 - 198
Da1C12	GCCTTTGTGCGTATCT	AATCGGCTACACTCATCT	140 - 160
YM13	TTCCCTAATTGTTCCTCTTGTTG	GTCCTCGTTTCCCTCTGTGT	175 - 250
Da1F08	AATGCTTCGTAATCCAAC	CTATAAGGAATTGGTGCC	166 - 179
MDaCIR17	GATTAGGTTGGACTTTGCAT	ACCACTGCACTCAACAGC	228 - 236
H_2	AAACCAAACAGGCAAAGCAT	TGCCCTGCTTGAAGATTGA	160 - 250
Dpr3B12	CATCAATCTTTCTCTGCTT	CCATCACACAATCCATC	129 - 141
C_5	AACCAATTACCCTTTGTCATGG	GCCTTGCAAGCAATTTTGA	145 - 180
MDaCIR20	TGCCTTAATCTGCTGACAC	CATGGTGCTCCGATTCTA	172 - 206
F_1	ATGGCTCAAGAGCACACG	GGGCCTCATAAACATGCAAT	145 - 205
H_{12}	TTGTAATTGGGTGTTGTATTTGC	CGGCCAAAACATTTTCTGAT	140 -160
MDaCIR55	CTCCCATCTCATGGAACA	ACGTTGTGAGCAAACACA	

(Source: Otoo *et al*., 2015; Arnau *et al*., 2017; Siqueira *et al*., 2012; Tostain *et al*., 2006)

The polymerase Chain Reaction (PCR) was done for 20 *Dioscorea* accessions (whole set PCR) for each 15 primers according to PCR Program shown in Table 3. The polyacrylamide gel images obtained from PCR products were manually scored for the presence or absence of alleles for each SSR marker and analyzed using Power Marker version 3.25.

Table 3. Composition of the PCR Mixture (Promega® USA)

Chemical	Volume per PCR tube (µl)	Volume per 21 PCR tubes (µl)
Nuclease free water	7.03	147.63
5x PCR buffer	3.00	63.0
$MgCl_2$ (25 mM)	1.02	21.42
Deoxyribose nucleotides (dNTPs) (10 mM)	0.15	3.15
Primer (Forward)	0.30	6.3
Primer (Reverse)	0.30	6.3
Taq Polymerase(5 U/µl)	0.20	4.2
DNA (25 ng/µl)	3.00	-
Total	15.0	252.0

Results and Discussion

The dendrogram of the morphological analysis revealed four clusters at 50% similarity level without duplicates (Figure 2). Accession 107 (*Katuala* cultivar) recorded as *D. pentaphylla* was well separated from the other accessions while four accessions (21, 36, 113 and 92) were clustered into non-related clusters with their species.

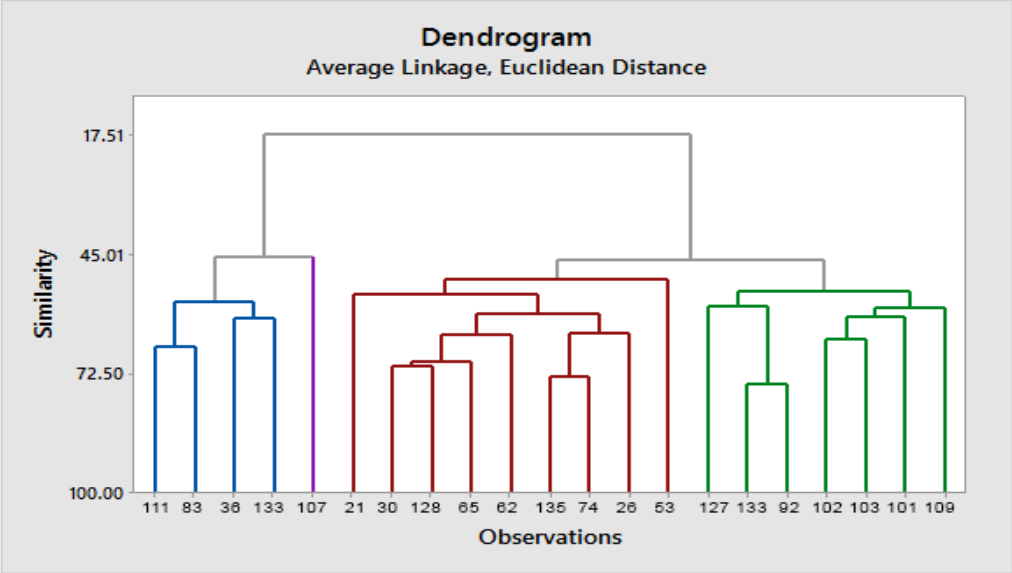

Figure 2. Dendrogram of the morphological analysis

Molecular analysis done for 20 accessions (except accession 107) revealed that there is polymorphism among all 20 *Dioscorea* accessions with 2 to 5 alleles per marker, with a genetic distance ranging from 0.1333 (among accessions 101, 102, 103 and 109) to 0.7529 (between accessions 83 and 133). The *phenogram* resulted in three major clusters (Figure 3). These three clusters were in agreement with the existing classification demarcating them to the above three species. However, there was a mismatch with respect to the accession 36, which was recorded as *D. alata* and 127 recorded as *D. bulbifera* as both clustered with *D. esculenta.* Two sets of duplicates (accessions: 113, and 101, 62 and 26) were identified by molecular characterization.

Both morphological and molecular characterization showed a high diversity among 21 *Dioscorea* accessions. In morphological characterization, some accessions were clustered away from main cluster of their species (accessions 113, 92 and 21) however, it was not so in the molecular characterization. Duplicates could be identified only by molecular characterization, which can be expected to provide more accurate clustering than the morphological characterization as morphology can be influenced by environmental factors (Carovic stanko *et al*., 2011). The genetic distance was minimum among the accessions belonging to the same species and maximum among different species. According to the cluster analysis, misidentifications such as with accession 36, which was recorded as *D. alata* in the PGRC records, was resolved and identified as *D. esculenta* according to both morphological and molecular clustering. Accordingly, identification of duplicates have helped minimizing high input requirement in conservation and to resolve issues with improper distribution of accessions from PGRC in future. The study also confirmed the highest divergence of *D. pentaphylla* (accession 107) and its unique species status.

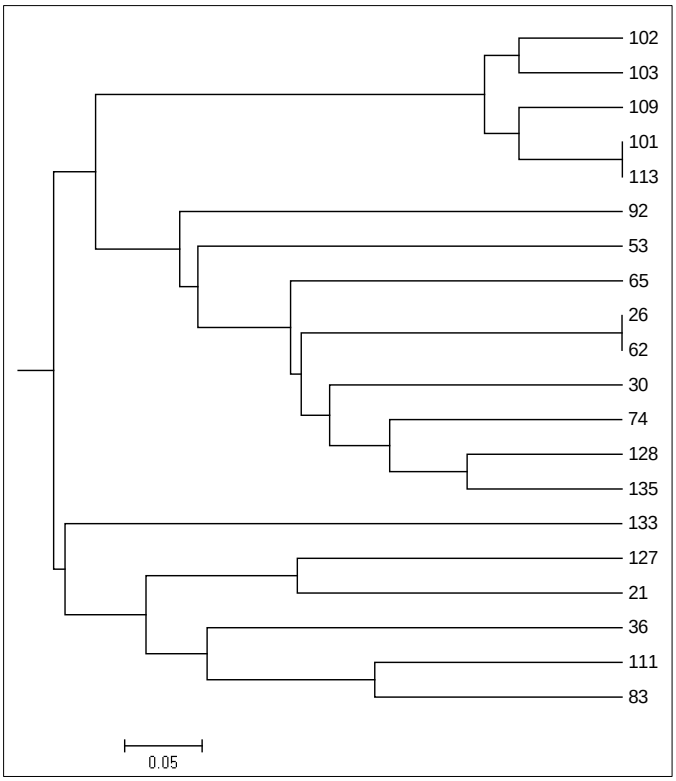

Figure 3. *Phenogram* of the molecular analysis

Conclusion

A wide diversity could be identified among 21 *Dioscorea* accessions. There is a disparity between morphological and molecular characterization. Molecular characterization provides more precise results than the morphological characterization. Accession 36 (recorded as *D. alata*) and accession 113 (a dubious accession) can be recorded under species *D. esculenta*. Accessions 113 and 101, and 62 and 26 are the two sets of duplicates.

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