Plant Genetic Resources: Characterization and Utilization

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Genetic purity of yam (*Dioscorea* spp.) multiplied through different seed multiplication techniques based on DArT SNP markers

Norbert G. Maroya[1], Paterne A. Agre[1] and Balogun Morufat[1,2]

[1]International Institute of Tropical Agriculture (IITA), PMB 5320, Oyo Road, Ibadan 200001, Oyo State, Nigeria and [2]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria

Research Article

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Author for correspondence:
Paterne A. Agre, E-mail: p.agre@cgiar.org

Abstract

Obtaining high-quality planting material for cultivation is a persisting challenge for yam (*Dioscorea* spp.) production in Africa. Efforts to provide a solution to this challenge have led to varying seed multiplication techniques but whose efficiency in maintaining the genetic purity of yam genotypes during the rapid multiplication process is yet unknown. Three improved varieties Swaswa, Kpamyo and Asiedu were multiplied through tissue culture, aeroponics system, field condition and vine cutting techniques. Leaf samples were collected at every stage of multiplication in the different techniques as well as the original mother plant for DNA fingerprinting. From a total of 16,922 SNP markers, an average heterozygosity of 0.091 was obtained with minor allele frequency of 0.119, and polymorphic information content of 0.166. The transition to transversion ratio was 62:38%. Hierarchical clustering of the genotypes and technologies discriminated the multiplied materials into three clusters with the first cluster consisting of only the variety Asiedu multiplied through aeroponics, vine and tubers collected from vine cutting and grown from the field. The second cluster consisted predominantly of the variety Kpamyo, with a little admixture from Asiedu. The third cluster consisted of only Swaswa. The different seed multiplication methods showed great potentials in conserving the genetic purity of genotypes used. Therefore, the use of these seed multiplication techniques could offer a lasting solution to the low multiplication ratio of yam without compromising the genetic integrity and offers a great opportunity for establishing a formal yam seed system.

Introduction

Yam is a staple crop in many countries of the tropics where it provides daily dietary calories to over 300 million people (Darkwa *et al*., 2020; Agre *et al*., 2021). In West Africa, yam is a major source of income and has high cultural value. It belongs to the genus *Dioscorea* (family: *Dioscoreaceae*). It has over 600 species of which about six are economically important staple species. These include *D. rotundata* (Poir.) (white Guinea yam), *D. cayenensis* (Lam.) (yellow Guinea yam), *D. esculenta* (Lour) (Asiatic yam or lesser yam), *D. alata* L. (water yam or greater yam), *D. bulbifera* L. (aerial yam) and *D. dumetorum* (Pax) (trifoliate yam or bitter yam) (Mondo *et al*., 2021). Among the major constraints associated with yam production, the high cost and limited availability of quality planting materials are considered as most critical. Planting materials account for about 50% of the production cost and farmers must set aside 30% of their harvest for the next planting season (Okoli and Akoroda, 1995). The need to develop and use technologies that will hasten the production of clean seed yam and reduce the breeding cycle for the development of new varieties is primordial. At the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, different methods of seed multiplication have been developed. Among these are the single node vine cuttings (SNVC) technique, the conventional tissue culture (CTC) technique, the aeroponics system (AS) and the temporary immersion bioreactor system (TIBS). Of the different seed multiplication methods developed, CTC, AS, HS and TIBS are well known to be more appropriate in limiting the disease propagation. However, these techniques are more expensive and not accessible by a common farmer. Concerning the SNVC, this technique is less expensive and been adopted by many farmers but has tendency of increasing the disease incidence.

The existing yam multiplication techniques developed within the last two decades have proven to be highly effective and have offered a promising solution to overcome the challenge of low multiplication ratio in yam, and thus, suitable for the multiplication of clean elite yam genotypes to produce clean seed yams in large quantities sufficient for cultivation by farmers. However, propagation and preservation of elite yam varieties, selected for their superior characteristics, require a high degree of genetic uniformity amongst the regenerated and the

mother plants. Therefore, there is a need to assess the degree of genetic uniformity of outcomes from these various multiplication methods. The objective of this study is to ascertain the true to type value of the different varieties maintained in different seed multiplication systems. As hypothesis tested, the different seed multiplications have effects on the genetic constitution of the mother plant.

Materials and methods

Plant materials

Three varieties namely Swaswa (TDa9801176), Kpamyo (TDr9519177) and Asiedu (TDr8902665) were grown through different seed yam multiplication techniques at IITA Ibadan, Nigeria. The variety Swaswa is a *D. alata* released clone while Kpamyo and Asiedu are *D. rotundata* released genotypes. These three varieties were released in Nigeria, and multiplied through different techniques before dissemination into Nigerian yam production systems. Tubers developed from the AS were then planted into the field while vines were collected from the aerial parts. The vines collected from the AS were planted in mini plastic bag to allow the tuber formation. At each stage of the tuber multiplication, four samples were collected for each genotype (Fig. 1).

Leaf sampling and DNA extraction

For each seed yam multiplication system (aeroponics, vines cutting and field), 2 g of young leaves were collected in ziploc bag and kept in ice. The mother plants (TDa9801176 and TDr9519177) which have not gone through any form of seed multiplication system were sampled as well and served as control. The collected leaves were kept at −80°C before lyophilization. DNA was extracted using the CTAB procedure with slight modification (Dellaporta *et al.*, 1983). DNA quality and concentration were evaluated through 1% agarose and Nanodrop, respectively.

Genotypic data assessment

The DArTSeqTM protocol for genome complexity reduction through digestion of genomic DNA and ligation of barcoded adapters was followed as described by Xia *et al.* (2005). Single read sequencing runs for 94 bases were performed to sequence libraries. Polymorphism identification and calling, and generation of quality control parameters for selection of polymorphic markers were performed in the secondary pipeline in KDCompute plug-in platform using DArTSoft14. The genomic representation of the sample and the generated SNP markers were aligned to the white yam reference genome v2 (Sugihara *et al.*, 2020).

Analysis of molecular data

Hapmap file received from the DArT platform was converted into a variant call format (VCF) (Danecek *et al.*, 2011). A total of 22,140 SNP markers were identified from the raw data. After filtering for minor allele frequencies (MAF) (0.01), 10% missing and depth (>5), 16,922 SNP markers were retained.

Summary statistics including MAF, heterozygosity for both SNP markers and genotypes was assessed using VCFtools and Plink (Purcell *et al.*, 2007).

A pairwise dissimilarity genetic distance matrix was calculated using the identity by state (IBS) method implemented using plink. A Ward's minimum variance hierarchical cluster dendrogram was then built from the IBS using the analyses of phylogenetics and evolution (ape) package implemented in R (Paradis *et al.*, 2004). The genetic distance was then studied to identify possible genetic variation among the yam maintained and multiplied through different seed multiplication techniques.

Results

Summary statistics

A total of 16,922 DArT SNP-based markers were used to sequence the yam varieties multiplied through various techniques.

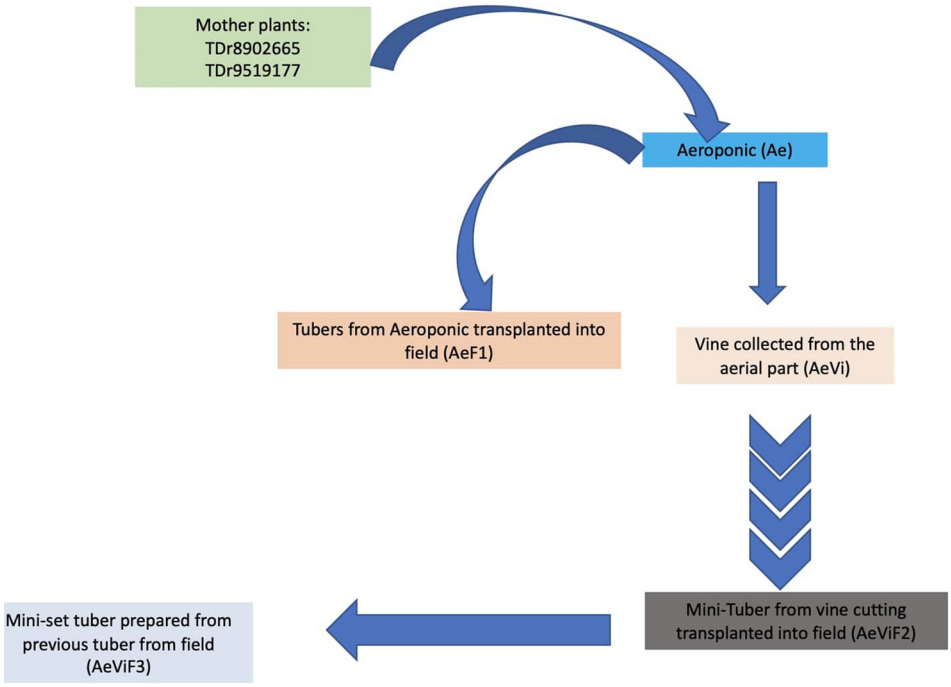

Fig. 1. Flowchart showing the different seed multiplication from which yam leaves were collected for DNA fingerprinting.

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MAF varied from 0.011 to 0.51, with an average of 0.119 and the lowest MAF (0.099) was observed on chromosome 7. The observed heterozygosity (Ho) ranged from 0.076 on chromosome 7 to 0.106 on chromosome 12, with an average value of 0.091. About the polymorphic information content, an average value of 0.166 was obtained across chromosomes (Table 1).

Of the 16,922 SNP markers used in this study, the transition and transversion SNPs were estimated. Transition (Ts) SNPs (62.22%, 10,531 SNPs) were more abundant than transversion SNPs (37.78%, 6391 SNPs) (Fig. 2).

Identification of possible somaclonal variation-based genetic distance

Hierarchical clustering analysis based on the IBS pairwise genetic matrix revealed the presence of three genetic groups (Fig. 3). Group 1/red colour consisted of only the variety Asiedu multiplied through the aeroponics, vine cuttings and tubers collected from vine cutting and grown in the field. In this cluster, a very low genetic distance was observed among accessions of this group and can be considered as duplicates irrespective of the multiplication technique.

Group 2 (blue) was made of a mixture of Asiedu and Kpamyo and the two mother plants. In this group, dominated by Kpamyo, Asiedu tuber generated from the aeroponic system, and transplanted into the field showed high genetic correlation with all Kpamyo originated from the different seed multiplication system

Table 1. Summary statistics of gene diversity (observed Ho and expected heterozygosity He), minor allele frequency (MAF) and polymorphic information content (PIC) across the 20 yam chromosomes

SN	Chromosome	Ho	He	MAF	PIC
1	chrom1	0.086	0.188	0.116	0.164
2	chrom2	0.089	0.199	0.124	0.172
3	chrom3	0.098	0.204	0.131	0.175
4	chrom4	0.083	0.189	0.117	0.165
5	chrom5	0.088	0.187	0.114	0.164
6	chrom6	0.095	0.191	0.118	0.166
7	chrom7	0.076	0.165	0.099	0.146
8	chrom8	0.092	0.202	0.128	0.174
9	chrom9	0.089	0.193	0.121	0.167
10	chrom10	0.085	0.199	0.125	0.172
11	chrom11	0.100	0.189	0.118	0.164
12	chrom12	0.106	0.207	0.131	0.178
13	chrom13	0.093	0.182	0.111	0.159
14	chrom14	0.087	0.178	0.109	0.157
15	chrom15	0.091	0.185	0.113	0.162
16	chrom16	0.090	0.199	0.125	0.172
17	chrom17	0.097	0.200	0.127	0.173
18	chrom18	0.096	0.189	0.116	0.165
19	chrom19	0.091	0.182	0.111	0.159
20	chrom20	0.085	0.186	0.115	0.162
	Average	**0.091**	**0.191**	**0.119**	**0.166**

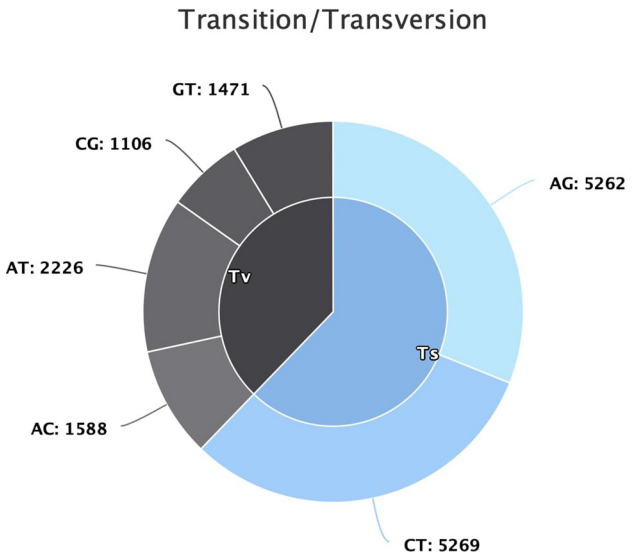

Fig. 2. Distribution of transition and transversion of 16,922 SNP markers across chromosomes. Tv, transversions; Ts, transitions; A, adenine; T, thymine; G, guanine; C, cytosine. Chart developed using SNIPLAY web-based software.

and could be the result of the mixture during planting or likely Asiedu was mistakenly planted as Kpamyo. The third group was made of *D. alata* clone Swaswa, which was used as a control to assess the performance of the markers to distinguish among yam species.

Principal component analysis (PCA) revealed the presence of a well-defined group based on the varieties and species. It appeared that *D. alata* formed a distinct group while the *D. rotundata* clones also formed a group. In the group formed by *D. rotundata*, three separate sub-groups could be identified. The first is a cluster of the plant materials from the variety Asiedu alone, and the second sub-group consists of predominantly materials from the variety Kpamyo mixed with those from Asiedu, which could have resulted from an error at planting or mislabelling in the field. The third sub-group, however, consists of materials from the variety Kpamyo and two parents used as control (Fig. 4).

Discussion

Gene diversity is one of the vital parameters used to assess the level of variation within a population. The low level of observed heterozygosity in the study compared to the expected heterozygosity indicates that the genetic purity of the genotypes has not been affected by the various seed multiplication methods employed in this study. Thus, the use of any of the seed multiplication techniques described in this study can guarantee the large-scale production of clean and high-quality seeds for farmers and generate seeds that can facilitate activities of yam breeding programmes (Maroya *et al.*, 2014a, 2014b).

Despite the interesting outcomes from the various multiplication methods tested, not all the methods are farmer-friendly, i.e. not easily practiced directly by farmers (Maroya *et al.*, 2017). This is due to one or a combination of the following factors: the complexity of the technique, the high cost associated with the technique and high dependency on technical know-how. The aeroponic system and the TIBS have been developed with the primary target of priming and sustaining the formal seed yam system for pre-basic and basic seed production (Balogun *et al.*, 2014; Maroya *et al.*, 2014a; Aighewi *et al.*, 2015). These systems, however, could also be very

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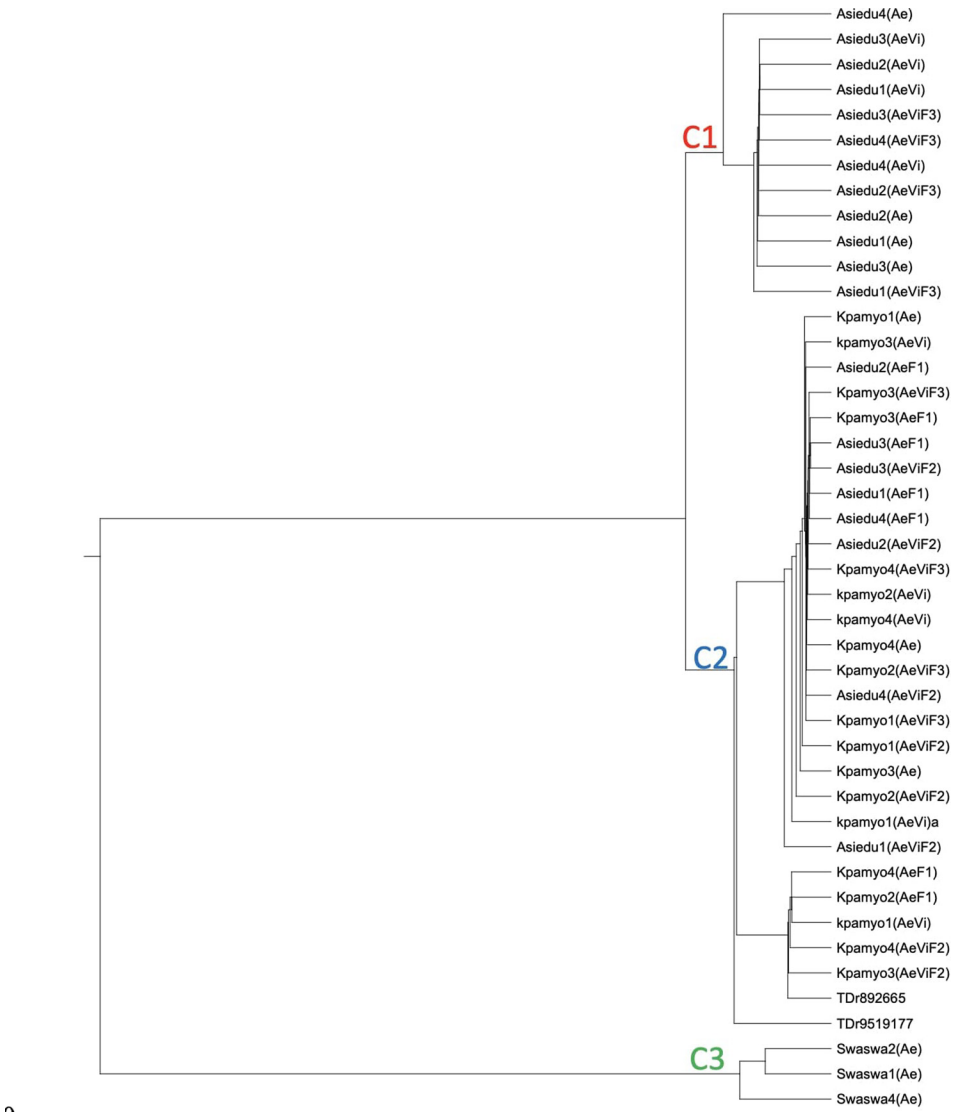

Fig. 3. Hierarchical clustering showing the genetic relationship among the sequenced genetic material.

useful in yam breeding programmes for developing large quantities of high-quality seed yam for various multi-location trials. This will help reduce the lengthy time spent on varietal development. The success of the aeroponics and the TIBS in achieving the targets for which they were developed has been observed at IITA (Balogun *et al.*, 2014; Maroya *et al.*, 2014a).

The tissue culture system even though not farmer-friendly, due to the dependency on a controlled laboratory environment unsusceptible to changing weather conditions, has been very helpful in generating clean, high-quality and uniform plants from otherwise unclean mother plants (Yam and Arditt, 2009). The system has also been identified to be key in creating genetic variants due to somaclonal variations in yam (Abil *et al.*, 2012; Vandenbroucke *et al.*, 2016; Gabriel *et al.*, 2021) and other crops that are not amenable to improvement in the same manner as sexually propagated plants due to polyploidy, flowering difficulty and self-incompatibility (Ahloowalia, 1998; Vandenbroucke *et al.*, 2016; Delgado-Paredes *et al.*, 2017). In this study, and through the genetic distance analysis, very low values obtained among accessions under different multiplication techniques provide evidence that genetic purity has been preserved rather than compromised even though plants have been through *in vitro* propagation to ensure cleanliness and purity. However, the slight mixture observed in the second cluster where Asiedu tubers from the AS and Kpamyo from other multiplication methods were genetically similar could be attributable to the mixture during planting where Asiedu might have been mistakenly planted as Kpamyo or an error from field labelling. The observed mixture in this context is a result of poor field management which has resulted in wrong clones' labelling. To address this, bulk segregation for DNA fingerprinting should be conducted to address such mixture in large yam seed production. PCA and the clustering analysis revealed high similarity in the result and thus one of the methods can be used to ascertain the genetic variability in such study.

Across the West Africa, farmers have adopted the vine cutting method for rapid seed multiplication (Aighewi *et al.*, 2015). The system has been an alternative to the use of tubers in seed yam production. However, farmers need more training in selecting clean plant/free from disease symptoms, time for vine cutting and adequate media to be used for optimum production (Aighewi *et al.*, 2015). To achieve this, we are suggesting the training of farmers (plant selection and age for obtaining vines) through an open day for showcasing the advantage of the vine cutting technique. The vine cutting method like others in this study has the potential to efficiently increase the multiplication ratio of yam (Acha *et al.*, 2004; Kikuno *et al.*, 2007; Agele *et al.*, 2010).

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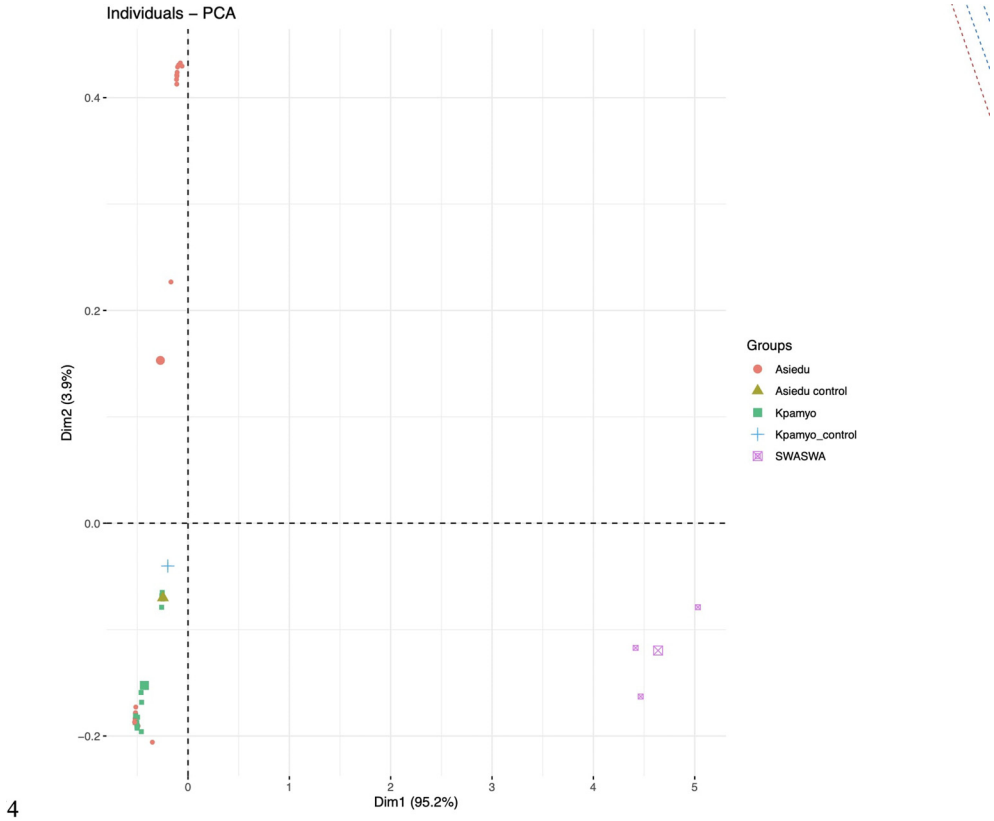

Fig. 4. Principal component analysis of DArT SNP-based markers.

Conclusion

The different seed multiplication methods used in the study have not shown any discrepancy concerning genetic purity within genotype from one yam seed multiplication to another. Bulk segregation DNA fingerprinting is highly recommended to maintain clean seed and to produce true-to-type varieties from generation to generation. Alongside this method, proper phenotyping using key traits for easy varietal identification should be deployed and applied during the seed multiplication. Barcoding should be deployed as well for field monitoring and varietal tracking.

Data. The VCF file used in this study for the analysis is available upon request from the corresponding author.

Author contributions. N. G. M. and B. M. designed the research. P. A. A. analysed the data and wrote the manuscript. N. G. M. and B. M. reviewed and edited the manuscript.

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Conflict of interest. The authors declare that the research was conducted in the absence of any potential conflict of interest.

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