Full Length Research Paper

SSR markers reveal diversity in Guinea yam (Dioscorea cayenensis/D. rotundata) core set


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The genetic diversity of 219 accessions of Guinea yam germplasm from Benin, Congo, Côte d’ Ivoire, Equatorial Guinea, Gabon, Ghana, Nigeria, Sierra Leone and Togo was accessed using 15 microsatellite loci. High diversity of 0.677 was found among the accessions. An allelic average of 8.06 and polymorphic information content (PIC) value of 0.65 was observed for the markers. The observed heterozygosity value of 0.563 suggests that spontaneous hybridization must have contributed to the ancestry of some of the accessions and improvement by farmers must have been far more often by selection of somatic mutants. The twenty distinct cluster groups generated by the radial phylogram shows that Dioscorea cayenensis and D. rotundata are distinct species with intermediate hybrid forms. There was no relationship between relatedness of the accessions and their geographical area of origin. This study contributes to an increased understanding of the genetic organisation of the core germplasm.

Key words: Core germplasm, Dioscorea cayenensis/D. rotundata, genetic diversity, microsatellite.

INTRODUCTION

Dioscorea cayenensis and D. rotundata (also known as Guinea yams) are the most popular and economically important yams in West and Central Africa where they are indigenous and represent the largest depository of biodiversity, as a result of centuries of large domestication, production, trade and consumption (Degras, 1993). The name Guinea yam does not only signify their intensive cultivation and great importance in the socio-cultural life of the people of this region, but also suggests similarities in various aspects of their botany and agriculture (Akoroda and Chheda, 1983). The diversity in Guinea yam provides plant breeders with the necessary options to develop, through selection and breeding, new and more productive crops that are resistant to virulent pests and diseases, and adapted to changing environments. The International Institute of Tropical Agriculture (IITA) has established a core set of Dioscorea germplasm based on morphological descriptors (Mahalakshmi et al., 2007). This core set offers a good starting point when searching for new traits (Vaughan, 1991).

The extent of genetic diversity and relationship in the established Guinea yam core set in the IITA germplasm has not been investigated using DNA based markers. Molecular marker information can help monitor the level of genetic diversity in breeding materials and assist breeders to more efficiently choose genetically diverse parents for breeding scheme. Such diversity assessment could provide a means for identifying potential gaps in the species collection and further guiding target collecting missions. Molecular markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) have been applied in yams for taxonomic, phylogenetic, diversity and mapping studies (Terauchi et al., 1992; Terauchi and Kanoma, 1994; Asemota et al., 1996; Ramser et al. 1996, 1997; Mignouna et al. 1998, 2002a, 2002b).
In this study we used SSR markers to assess the diversity of the core collection of Guinea yams held in trust by IITA.

MATERIALS AND METHODS

Plant materials

The 219 accessions of Guinea yams collected from 10 countries of West and Central Africa are listed in Table 1. These are part of the core collection of Dioscorea germplasm held in trust by the IITA genebank. The accessions were planted in 30 cm size pots filled with sterilized loamy soil and maintained in a screen-house at the IITA, Ibadan Nigeria.

DNA extraction and quantification

Genomic DNA was extracted from fresh leaf apex of young leaves using modified CTAB procedure (Mignouna et al., 1998). The quality and concentration of DNA was assessed by gel electrophoresis using 1% agarose with known concentrations of undigested lambda DNA (Sigma, St. Louis, MO, USA). Quantification of DNA was done using a spectrophotometer (Beckman Coulter DU530) at 260 nm. Extracts were diluted in water to obtain DNA concentrations of 25 ng/µl.

Polymerase chain reaction and fragment analysis

A total of fifteen SSR primer pairs were used in the study (Table 2). PCR reaction was conducted in a 20 µl volume in a 96-well microtiter plate using an automated thermal cycler (model: Peltier Thermal Cycler 200). The reaction volume contained 25 ng of template DNA, 100 µM each of dNTP, 2.5 mM MgCl₂, 0.5 µM each of fluorescently labelled forward primer and unlabelled reverse primer, 1X reaction buffer and 2 units of Taq DNA polymerase (Invitrogen). The forward primer was 5’- labeled with one of the four fluorochromes PET, 6-FAM, NED and VIC. The PCR programme consisted denaturation at 94°C for 4 min, followed by 34 cycles of 94°C for 30 s, 51 or 58°C for 1 min and 72°C for 1 min, with a final extension step at 72°C for 7 min. Capillary electrophoresis with a semi-automated system ABI PRISM 3100 Genetic Analyser was used to separate amplified PCR products. Samples for amplified product separation were prepared by adding 1 µl of diluted PCR products to 9.4 µl formamide and 0.1 µl GenSize-500 LIZ. This was dispensed in ABI 96-well plates and were denatured at 94°C for 5 min and allowed to cool down on ice.

Data analyses

Observed allelic data were binned into discrete units and SSR fragment sizes were called using Genemapper v. 3.7 software (Figure 1). The fragment sizes in base pairs for each genotype across SSR markers were converted to binary data where alleles were transformed into presence (1) or absence (0) of an SSR band. Missing data accounted for less than 5% (that is, marker x genotype) of the entire data set. The genetic diversity parameters (Table 2) such as number of alleles per locus, percent of polymorphic loci, observed heterozygosity and gene diversity were estimated with FSTAT v. 2.9.3 software (Goudet, 2002). The tree structure (Figure 2) of the genetic diversity was constructed using DARwin 5.0 software.

RESULTS AND DISCUSSION

A total of 121 alleles were amplified with 15 SSR loci analyzed in 219 accessions, with the number of alleles observed per locus varying from 6 to 9 alleles (Table 2). The observed heterozygosity of 0.563 on average, varied from 0.276 (Dpr3F12) to 0.750 (Dab2D06). A total gene diversity of 0.677 was observed according to Nei diversity indices (Nei, 1973) for the accessions. Polymorphism was observed in all fifteen microsatellite loci analysed (Table 2). Polymorphic information content (PIC) ranged from 0.37 (Da1A01) to 0.80 (Dpr3D06). Average PIC value was 0.65. The UPGMA-derived radial phylogram constructed for the studied accessions provides an overview of the diversity structure (Figure 2) resulting into twenty distinct clusters groups. Accessions from different countries were fairly represented within each cluster.

In our study genetic diversity was detected in accessions with an average of 8.06 alleles per locus. Gene diversity of 0.677 on average was also found. The results demonstrate a genetic polymorphism in the studied germplasm from Benin, Congo, Côte d’Ivoire, Equatorial Guinea, Gabon, Ghana, Nigeria, Sierra Leone and Togo and high potential for genetic improvement. These findings suggest that morphological descriptors earlier used to develop this core set were discriminatory enough to capture as much diversity. Polymorphism was observed at all fifteen microsatellite loci analysed (Table 2). Polymorphic information content (PIC) ranged from 0.37 (Da1A01) to 0.80 (Dpr3D06) with an average value of 0.65. Tostain et al. (2007) also found SSR markers as discriminatory enough in diversity studies of yam. The 0.563 value for observed heterozygosity in this vegetative propagated crop is expected due to the fact that yams are dioecious and implies that spontaneous hybridization must have contributed to the ancestry of some of the accessions and improvement by farmers must have been far more often by selection of somatic mutants. Accessions from different countries were grouped together in the thirteen clusters (Figure 2). There was no relationship between relatedness of the accessions and their geographical area of collection. This could be due to the fact that cultivars must have been distributed over great distances as clones in the course of human migration. D. cayenensis accessions clustered specifically in group 11 and 13 with overlapping mixtures with D. rotundata in group 10. This trend seems to uphold the view that Guinea yams are two distinct but related species and perhaps share a common secondary gene pool as proposed by Akoroda and Chheda (1983). Mignouna et al. (1998) showed that the varietal groups of D. cayenensis were genetically distant from those of D. rotundata. The overlapping D. cayenensis and D. rotundata in cluster group 10 could be regarded as hybrid derivatives as a result of natural hybridization. This view
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### Table 2. Primer sequences (forward/reserve) used in the SSR analyses and their respective size annealing temperature ($T_a$), number of alleles per locus ($A$), observed heterozygosity ($H_{obs}$) and polymorphic information content (PIC).

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is in agreement with Hamon and Touré (1990) who recognized some intermediate accessions. Taxonomic relationship between *D. cayenensis* and *D. rotundata* (Guinea yams) has often been a subject of controversy and speculation. The existences of numerous vernacular names specifying a given cultivar (Dansi et al., 1999; Mignouna et al., 1998), and the existence of many intermediate forms (Akoroda and Chheda, 1983; Hamon and Touré, 1990; Mignouna et al., 2002c), have complicated their exact classification.

Our study contributes to an increased knowledge of the taxonomic classification and genetic arrangement of core set of Guinea yams.

**ACKNOWLEDGEMENTS**

This study would not have been possible without the support of Generation Challenge Program (GCP) in Project Number 3d of sub-programme 1. The technical assistance of Athanson Blessing, Hammed Adeola and Olayunti Aina of the Central Biotechnology Laboratory (CBL),
International Institute of Tropical Agriculture (IITA), Ibadan is acknowledged.

REFERENCES

Mignouna HD, Mank RA, Ellis THN, Van den Bosch N, Asiedu R, Ng...


