Molecular Characterization of Colombian Yam Germoplasm by "Selective Amplification of Microsatellite Polymorphic Loci" (SAMPL)

Caracterización molecular del germoplasma de ñame colombiano utilizando "Selective Amplification of Microsatellite Polymorphic Loci" (SAMPL) en condiciones radioactivas

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ABSTRACT
In Colombia, yam (*Dioscorea* spp.) has been a basic and traditional crop for small-and medium-scale farmers. It has been an important source of family employment in the Colombian Atlantic Coast for the past several centuries. However, yam is the most fragile crop regarding market issues and disease susceptibility (e.g. to anthracnose and viruses). At present, the crop is under progressive recovery from a previous drastic reduction in area and production, and apparently allows high profits. Is imperative for the future of the crop, that the rural farmers are supplied with selected varieties, thereby increasing their sustainability and economic yield. However, such varieties are not available, but have to be bred. Breeding of yam in turn is difficult, and presupposes knowledge about the genetic diversity in the breeding germplasm, which is not known. For these reasons, we characterized the Colombian yam varieties used in the fields as well as wild relatives with a molecular marker technique: SAMPL (Selective Amplification of Microsatellite Polymorphic Loci). Positions of 160 unequivocally scorable bands (of which 56 were monomorphic, 35% of total, with an average of 2.8 polymorphic bands per assay) were transformed into a binary character matrix and analyzed. The resulting similarity matrix was transformed into a dendrogram with the UPGMA algorithm. The diversity of the collections with this technique was 0.0471 with a mean similarity of 0.9529.

Key Words: *Dioscorea* spp, microsatellites, DNA

RESUMEN
En Colombia el ñame (*Dioscorea* spp.) ha sido un cultivo básico tradicional a pequeña y mediana escala de cultivadores de la Costa Atlántica para abastecer el consumo propio y el mercado local, convirtiéndose en una gran fuente de empleo durante muchos años, sin embargo el ñame es uno de los cultivos más susceptibles a enfermedades (vg. la antracnosis y virus). En la actualidad el cultivo ha ido recuperándose progresivamente de una drástica reducción en área, como en producción, permitiendo aparentes resultados benéficos para los productores. Es imprescindible para el futuro del cultivo, que los productores cuenten con variedades seleccionadas para incrementar la sostenibilidad y rentabilidad del cultivo. Sin embargo esas variedades no están disponibles en la naturaleza, sino que tienen que ser mejoradas. El mejoramiento del ñame es difícil y presupone un conocimiento de la diversidad genética del germoplasma de *Dioscorea* en Colombia, la cual no se conoce todavía. Por estas razones nosotros caracterizamos las variedades de ñame colombiano utilizadas en campo, así como las silvestres con una técnica de biología molecular: *Selective Amplification Microsatellite Polimorphic Loci* (SAMPL). Se detectaron 160 bandas identificables, (de las cuales 56 fueron monomórficas, 35% del total, con un rango de 2.8 bandas polimórficas por análisis). Con la información anterior se construyó una matriz binaria que fue transformada en un dendograma utilizando el algoritmo UPGMA. La diversidad de la colección determinada con esta técnica fue de 0.0471 con una media de similaridad de 0.9529.

Palabras clave: *Dioscorea* spp, microsatélites, DNA

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INTRODUCTION

Yam (*Dioscorea spp*) belongs to the family Dioscoreaceae, order Dioscoreales. It is monocotyledonous and constitutes an important staple food crop for millions of people in the humid and subhumid tropics. Tubers provide an important food resource for people from these regions. In Colombia, yam (*Dioscorea* spp.) has been a basic and traditional crop for small- and medium-scale farmers from the Atlantic Coast (Córdoba, Sucre, Cesar, Atlántico and Bolívar departments). Formerly it was a self-supporting crop for local markets only, but more recently became an export product. In 1989, production has been drastically affected by a massive infection of *Colletotrichum gloeosporioides*, the causative agent of the foliar disease anthracnose (Álvarez, 1991). In subsequent years, smallholders dramatically reduced the acreage for yams, replacing it with other crops such as cassava and plantains. Knowledge of germplasm diversity and relationships among elite materials has a significant impact on the improvement of crop plants generally (e.g. Witcombe, et ál., 2001), and of yam in particular for example the works done in Asian and Africans *Dioscorea bulbifera,* Guinea yam *Dioscorea rotundata - D.cayenensis* (Ramser, et ál., 1996, 1997 respectively) and Jamaican yam germplams (Asemota, et ál., 1996), were, using different molecular techniques was possible detected genomic variation for phylogeny and taxonomy studies as well as obtained information about de genome of these collection. Efficient breeding of yam in Colombia can only be achieved if its genetic diversity is known and promising varieties can be selected. This, however, is not the case. For these reasons, we characterized a comprehensive collection of the presently grown Colombian yam varieties with a molecular marker techniques: selective amplification of microsatellite polymorphic loci (SAMPL; Witsenboer, et ál. 1997).

MATERIALS AND METHODS

Plant material

Different yam cultivars were collected from fields at different locations at the Caribbean Colombian Coast (Durangoand Padilla, 1998). The samples from Africa were generously donated by the International Institute of Tropical Agriculture (IITA Ibadan, Nigeria). *Dioscorea sansibarensis* and *D. vittata* were provided by in the yam collection of Frankfurt University (table 1).

DNA isolation

DNA was isoled according to the procedure of Asemota (1991) with some modifications. For example, dithiotreithol (DDT) was employed for the reduction of quinones instead of β-mercaptoethanol.

SAMPL analysis

The SAMPL technique makes use of compound microsatellite primers to reveal genetic polymorphisms (Morgante and Vogel, 1994). It combines the high multiplex ratio typical for amplification fragment length polymorphisms (AFLPs; Vos et ál. 1995) with the high level of variability typical for SSRs. The protocol was based on the procedures reported by Morgante and Vogel (1994) and Witsenboer et ál. (1997). Sequences of primers and adapters are given in Table 2. All PCR steps were performed in a Perkin Elmer 9700 thermocycler. Initial restriction enzyme digestions were performed in final volumes of 25 µL containing 200 ng of DNA template, 10 units of *Eco*RI (MBI Fermentas), 33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, and 0.1 mg/mL BSA. After three hours at 37 ºC, 10 µL of a ligation mix containing 5.5 ng of *Eco*R1 adapter, 1 mM ATP, 33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA and 2 U T4 DNA ligase (MBI Fermentas) were added. After overnight incubation at 37 °C, ligation-restriction reactions were diluted 1:4 with bi-distilled sterile water. Pre-amplifications were performed in 20 µL reactions containing 5 µL of diluted ligation products, 75 mM Tris-HCl (pH 9), 20 mM $(NH_4)_2SO_4$, 0.01% Tween-20, 1.5 mM $MgCl_2$, 200 µM of dNTPs, 0.5 µM *Eco*RI adapter-specific primer, and 0.5 U *Taq* polymerase (Biotherm, Gene Craft). The PCR program consisted of an initial denaturation step at 94 °C (120 s), 34 cycles at 94 °C (30 s), 50 °C (30 s), and 72 °C (60 s). After pre-amplification, 3 µL aliquots were separated in 1% agarose gels and stained with ethidium bromide. The remaining samples were diluted 1: 9 and stored at –20 ºC until use. Compound microsatellite primers (see table 2) were end-labelled with [γ^{32}P]-

Table 1. Origin of plant material

Species	c.v	Origin	Species	c.v	Origin
D.alata	9805003	Córdoba	***D.alata***	Da11	Africa (IITA)
	9503004	Sucre		Da12	África (IITA)
	9503005	Sucre		Da13	África (IITA)
	9503006	Sucre		Da14	África (IITA)
	9503007	Córdoba		Da15	África (IITA)
	9503008	Córdoba		Da16	África (IITA)
	9504009	Córdoba		Da17	África (IITA)
	9506016	Córdoba		Da18	África (IITA)
	9506019	Córdoba		Da19	África (IITA)
	9506020	Córdoba	***D.rotundata***	9603041	Córdoba
	9506021	Córdoba		9605060	Sucre
	9502022	Córdoba		9804075	Córdoba
	9506024	Bolívar		9804078	Córdoba
	9506028	Atlántico		9804080	Córdoba
	9602034	Córdoba		9804084	Córdoba
	9602035	Córdoba		9804085	Córdoba
	9603037	Córdoba		9404087	Córdoba
	9603040	Córdoba		9804092	Córdoba
	9603042	Córdoba		9903093	Córdoba
	9603043	Córdoba		9903094	Córdoba
	9701043	Córdoba		Dr22	África (IITA)
	9605044	Córdoba		Dr24	África (IITA)
	9605045	Bolívar		Dr30	África (IITA)
	9605047	Bolívar		Dr32	África (IITA)
	9605049	Bolívar		Dr33	África (IITA)
	9605052	Magdalena		Dr34	África (IITA)
	9605053	Magdalena		Dr35	África (IITA)
	9605054	Magdalena		Dr36	África (IITA)
	9505059	Sucre		Dr37	África (IITA)
	9605061	Bolívar		Dr38	África (IITA)
	9605063	Córdoba		Dr39	África (IITA)
	9605064	Antioquia		Car 1	Córdoba
	9605065	Córdoba	***D.trífida***	9805001	Córdoba
	9505066	Antioquia	***D.bulbífera***	9507030	Córdoba
	9605070	Córdoba	***D.vittata***		Frankfurt University collection
	9701071	Córdoba	***D.sansibarensis***		Frankfurt University collection

* **c.v :** cultivar number or name of clone
Note: IITA, International Institute for Tropical Agriculture, Ibadan, Nigeria

ATP and polynucleotide kinase (MBI Fermentas) using standard procedures (Sambrook, et ál. 2000). Selective amplification was performed in 10 µL reactions containing 2 µL of diluted pre-amplified fragments, 0.5 µM labelled microsatellite primer, 5 µM *Eco*RI adapter-specific primer, 400 µM of dNTPs, 75 mM Tris-HCl (pH 9), 20 mM $(NH_4)_2SO_4$, 0.01% Tween-20, 1.5 mM $MgCl_2$, and 0.5 U *Taq* polymerase (Biotherm, Gene Craft). The PCR program consisted of an initial denaturation step at 94 °C (120 s), 11 cycles at 94 °C (30 s), 65 °C (30 s) [with a touch-down of -0.7 °C each cycle], and 72 °C (60 s), followed by 25 cycles at 94 °C (30 s), 56 °C (30 s), and 72 °C (60 s). Final extension was at 72 °C (120 s). Products were separated on 4% sequencing gels and autoradiographed (Sambrook, et ál., 2000). The compound microsatellite primers used for the selective amplification were designed and provided by Dr. Peter Winter (unpublished data). Additionally, a newly designed adapter-primer ("*Eco RI short"*) was employed in combination with TA-rich selective primers (Palacios, et ál. 2002).

Data analysis

Unequivocally scorable SAMPL bands were transformed into a binary character matrix, with 1 for the presence, and 0 for the absence of a band at a particular position. Pairwise distances were directly computed with the STATISTICA sofware package (1984-1999 Stat Soft. Inc) using the percent disagreement. The final dendrogram was constructed using UPGMA cluster algorithm (Dhillon and Ishiki, 1999).

RESULTS AND DISCUSSIONS

Genomic DNAs from 36 Colombian and 9 African *D. alata* accessions, 12 Colombian and 11 African *D. rotundata* accessions, one accession each of *D. trífida*, *D. bulbífera, D. vittata* and *D. sansibarensis*, respectively, were analyzed by SAMPL with a set of 5 compound microsatellite primers and a modified *EcoRI* adapter primer (Palacios, et ál., 2002) (table 2). Distinct polymorphic

Table 2. Primers sequences

SAMPL		
	Adapters	**Sequence 5' - 3'**
Eco R1 1		CTCGTAGACTGCGTACC
		AATTGGTACGCAGTC
	Eco RI primer*	CCATGCGTCAG
Compound microsatellites primers		
P-SAMPL-34		TAATAATAACAACA
P-SAMPL-35		TATATATATAGAAGA
P-SAMPL-36		GAAGAAGAATATAT
P-SAMPL-37		GAAGAAGAACACAC
P-SAMPL-38		GAAGAAGAAGTGTG

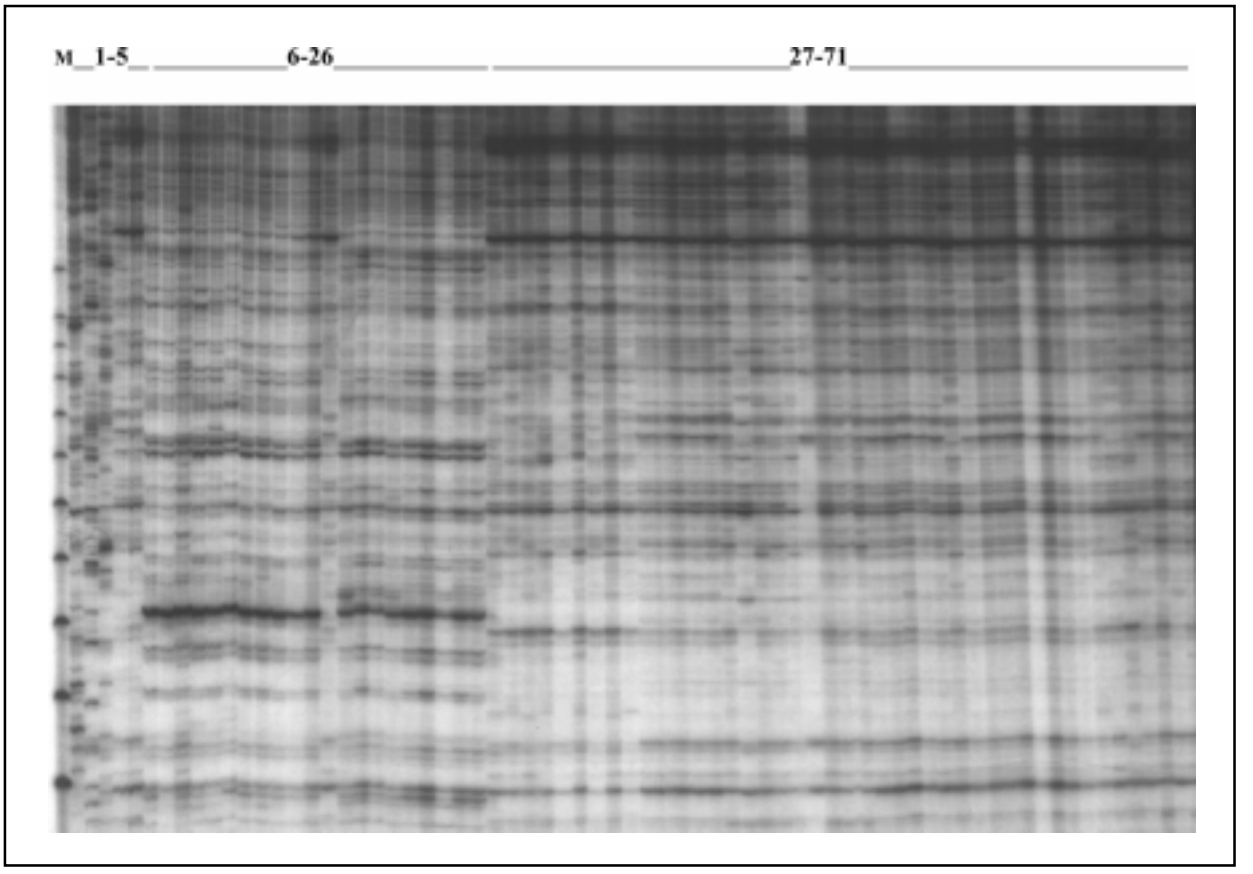

Figure 1. SAMPL analysis of the whole *D. alata* and *D. rotundata* collections using SAMPL primer38. M: Molecular weigh marker 25pb, MBI Fermentas, 1- 5: *D. trífida, D. bulbifera, D. vittata, D. sansibarensis*. Car1, 6 - 26: *D. rotundata,* 26 - 71: *D. alata*.

banding patterns were obtained with all primers, as exemplified by a subset of samples and *SAMPL* primer *38* (figure.1). Positions of 160 unequivocally scorable bands (of which 56 were monomorphic, 35% of total, with an average of 2.8 polymorphic bands per assay) were transformed into a binary character matrix (1 for presence, 0 for absence) and analyzed. The resulting similarity matrix was transformed into a dendrogram with the UPGMA algorithm. The diversity of the collections with this technique was 0.0471 with a mean similarity of 0.9529.

The obtained SAMPL banding patterns clearly reflect the lack of genetic diversity observed within and between sampling sites. Basically the clusters observed obey to species separation. African accessions are found in close similarity with Colombian individuals, since the way of propagation applied by the farmers is in principle clonal, some accessions are still identical with the African "very likely" ancesters (Coursey and Martin, 1970; Coursey, 1976a, 1976b). On the other hand, somaclonal variation, which is the only source of variability, shows also to be rather low. Within the same species, form hundred-sixty scored characters, only four polymorphic bands were observed among individuals form separated clusters. The same type of results were observed with DAF markers (Bustamante, et ál., 2003) were was evident the low genetic variability. Is important to develop varieties carrying as many different genes for resistance as possible, in order to provide stable resistance against a broad spectrum of the pathogens. Therefore, is imperative to extend the

yam germplasms in Colombia by plant breeding or introducing new varieties.

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