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Efficiency of RAPD, ISSR, AFLP and ISTR markers for the detection of polymorphisms and genetic relationships in camote de cerro (*Dioscorea* spp.)

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ABSTRACT

Background: At present, species known as camote de cerro (*Dioscorea* spp.) are found only in the wilderness in Mexico, but their populations are extremely depleted because they are indiscriminately collected, it is urgent to evaluate the conservation status of these plants in order to design conservation genetics programs. In this study, genetic diversity parameters along with cluster analysis based on Jaccard's coefficient were estimated with the objective to assess the efficiency of Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR), Amplified Fragment Length Polymorphism (AFLP) and Inverse Sequence Tagged Repeat (ISTR) molecular DNA markers in the *Dioscorea* genus.
Results: The polymorphic information contents were quite similar for all markers (≈0.48). Genetic variation of *Dioscorea* spp., in terms of average heterozygosity was lower with ISTR (0.36), and higher when other markers were used (RAPD = 0.43; ISSR = 0.45 and AFLP = 0.47).
Conclusion: This indicates an important level of genetic differences despite the fact that the plant is asexually propagated. Based on the diversity statistics, any marker tested in present work can be recommended for use in large-scale genetic studies of populations. However, the low correlations among different molecular marker systems show the importance of the complementarity of the information that is generated by different markers for genetic studies involving estimation of polymorphism and relationships.

1. Introduction

The genus *Dioscorea* (family Dioscoreaceae) comprises multiple species that grow in Africa, Asia, South America and the Caribbean Islands [1]. Some of these species are polyploid, producing tubers, which allow their vegetative propagation. The genus *Dioscorea* has been reported as producing diosgenin, a secondary metabolite which is very important in pharmaceutical industry because it is used as raw material for the semi synthesis of steroidal drugs and also as a complement to traditional medicine in the treatment of various diseases [2]. The tuber is used as food because of its contents of carbohydrates, vitamins (especially vitamin C), minerals and proteins [3,4]. This genus includes 600 species, of which 65 to 70 were reported for Mexico [5]. In western Mexico this plant, commonly known as camote de cerro, cannot be found cultivated but occurs in wild form [6]. It is distributed in the mountainous areas with evergreen and subperennifoliar forests. From ancient times these tubers were harvested, cooked and consumed as food by people of the region. Because this plant is collected only, the extensive acceptance of its tuber is threatening the genetic diversity of many local populations. Therefore, studies designed to the estimation of the genetic diversity of camote de cerro, can be very important in supporting breeding and conservation strategies of this genetic resource, in assessing its potential as field crop and to determine the origin and relationships among different forms and species. There are many molecular techniques that can help to generate information and assess the polymorphism among individuals and populations. In those cases in which institutions of developing countries do not have access to next generation sequencing technologies, the most popular molecular markers can be quickly and easily utilized to begin to assess genetic diversity of plant genetic resources. Molecular tools have been proved

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useful for the correct identification of taxa, species and specific genotypes [7,8,9]. DNA fingerprinting techniques have been utilized to characterize the diversity of genetic collections. Molecular markers can detect specific locations at DNA level that differ among cultivars or improved species, and they can be selected for many purposes and technical facilities. Examples of markers are: RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeat); AFLP (Amplified Fragment Length Polymorphism) and ISTR (Inverse Sequence Tagged Repeat). Even if the markers are randomly determined, they have different properties, and require different DNA quantities and qualities. The four molecular markers listed above have the ability to detect different parts of the genome, they have a dominant inheritance and used together they can be more informative. RAPD is used to amplify a specific sequence of the genome. Williams et al. [10] utilizes short synthetic oligonucleotides (10 bases long) of random sequences as primers to amplify nanogram amounts of total genomic DNA under low annealing temperatures by PCR. The ISSR [11] is a type of genetic marker that allows us to get the levels of variation in microsatellite regions that are scattered in various genomes, particularly nuclear. These regions consist of tandem repeats of simple patterns as (CT)n or (AC)n repeating sequences located between nuclear eukaryotic genome. Repeated motifs, also called SSRs (simple sequence repeats) may be penta-, tetra-, tri-and dinucleotides. The biallelic markers are dominant AFLP [12]. They can detect single nucleotide simultaneous variations in unknown regions of the genome in which a mutation can be found frequently in functional genes indeterminate. ISTR is a retrotransposon based marker which has the ability to characterize wild species and genetic relationships at an individual level [13,14]. It profits from the abundant repeats that are characteristic of plants with large genomes [15,16]. The acquisition of knowledge about the polymorphisms that can be detected in the wild *Dioscorea* genus with molecular markers is essential for the implementation of conservation programs, as well as for domestication and breeding.

The objectives of this study were to evaluate the value of RAPD, ISSR, AFLP and ISTR marker systems for their ability to distinguish *Dioscorea* populations and their efficiency to estimate genetic diversity parameters, including number of fragments, unique profiles and polymorphic levels per assay unit.

2. Materials and methods

2.1. Plant material

Fresh leaf tissue was collected from 24 asexually propagated wild individuals from nine locations in various regions of state of Jalisco in Mexico (Fig. 1). Tubers of each individual were planted in a nursery shade mesh at Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA), Universidad de Guadalajara, Jalisco, Mexico (20°45′N, 103°31′ W; 1650 msnm). Materials were placed in plastic containers 60 × 40 × 27 cm with substrate to promote budding. The plants were maintained with fertilizer formula 20–10–20 until the branches were sufficiently developed to collect leaves for DNA extraction. Names and codes for vegetal materials are shown in Table 1.

2.2. DNA extraction

DNA was isolated from fresh leaves. Two protocols, reported by Keb-Llanes et al. [17] and Cota-Sánchez et al. [18], respectively, were assessed in order to determine the ability to eliminate the excess content of carbohydrates, phenolic compounds, and proteins present in the leaves, which may cause inhibition on *Taq* polymerase action during PCR [3]. The DNA extracted was assessed for quality, using electrophoretic and spectrophotometric methods, and stored at -20°C until processing. The yield and quality obtained measuring OD_{260} and OD_{280} (OD = Optical Density) with both methods were compared by Analysis of Variance (ANOVA).

2.3. Analysis of molecular markers

In this study, the informativeness and efficiency of the molecular markers RAPD, ISSR, AFLP and ISTR were compared. All markers used are based on random sequences and involve different levels of technical difficulty. Different numbers of primer sequences were used, as reported in Table 2.

Conditions for PCR were specific for each marker. RAPD and ISSR analyses were carried out in a 25 μL reaction container for RAPD: 2 ng

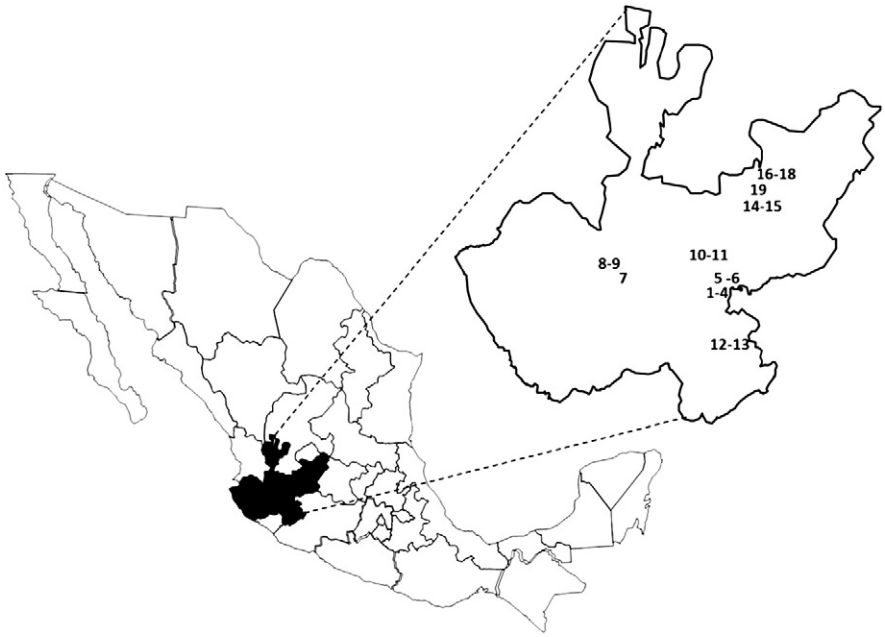

Fig. 1. Locations of the State of Jalisco, Mexico, where the number of 1 to 4 belong to Chapala, 5–6 San Antonio, 7 Cocula, 8–9 Ahualulco, 10–11 Ixtlahuacan de los Membrillos, 12–13 La Manzanilla de la Paz, 14–15 Acatic, 16–18 Ixtlahuacan del Rio and 19 San Gabriel.