DNA extraction from roots of xoconostle

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Abstract

High contents of polysaccharides present in cactus of the genus *Opuntia* make it difficult to obtain optimal quality DNA for genotyping analysis. In this work, DNA was extracted from the young roots of xoconostle (*Opuntia matudae* Scheinvar). The quality of the DNA was assessed by random amplification with PCR using RAPD. The extraction method will help to solve technical problems that are commonly reported by different researchers for this group of plants, and it will lead to more efficient handling of samples for DNA analysis. The use of small samples of young roots as an alternative to cladodes, which are normally used for genetic or genomic analysis, was appropriate because high concentrations of DNA with optimum quality were obtained, and this DNA contained low concentrations of the polysaccharides characteristic of this group of cacti. The amplified fragments obtained from the different plants were clear and reproducible.

Key Words: Contaminating, polysaccharides, PCR.

Introduction

DNA analysis is now a very useful tool that has been used in different fields of biology. Genotyping studies can complement the characterization and differentiation of organisms, especially those with similar morphological characteristics (Scheinvar, 2004; Reyes-Agüero *et al*., 2005). Such studies have also been used extensively to identify both taxonomic and phylogenetic relationships (Wang *et al*., 1998). With these tools, it has been possible to categorize many species, especially microorganisms (Morton and Redecker, 2001), more accurately and even to propose new phylogenetic lines, e. g. the reunification of the Cactaceae family (Griffith and Portert, 2009). In particular, molecular markers have contributed to molecular systematics and have advanced new and fresh answers to old problems (Nobel, 2002; Gonzalez *et al*., 2004). The molecular markers are a tool in molecular genetics that provides estimates of genetic variation and differences at the inter- and intra-specific levels among individuals that make up a population. In addition, they have a neutral effect with respect to the environment, and are generally not affected by the phenological stage of the organism (Griffith, 2003). Genetic

markers can be found abundantly in the genome, and they provide valuable information (Peterson and Richardson, 1996; Luna-Paez *et al*., 2004).

So, the taxonomic study of cacti, besides using morphological and biochemical descriptors, has turned now to DNA analysis, by using different techniques. However, the presence of high concentrations of polysaccharides (De la Cruz *et al*., 1997; Schmitt *et al*., 2001; Mondragón-Jacobo, 2003) and polyphenols, which are isolated simultaneously with the DNA, hinders the analysis. This contamination is difficult to remove by existing methodologies, and it can drastically alter the analysis of molecular markers (Cota-Sánchez *et al*., 2006).

This paper describes the use of young roots of the xoconostle cactus for DNA extraction and analysis. This organ has not been used for this purpose in cacti. It contains the same genetic information as the cladodes but has a lower concentration of polysaccharides. This protocol provides a simple alternative for obtaining and purifying DNA from cacti and *O. matudae* (xoconostle), thereby helping to solve the problems related to the presence of secondary metabolites.

Materials and methods

For this reserach, we collected xoconostle (*O. matudae*) cladodes from two municipalities: Chapantongo, which is located at 20°17' LN 99°24' LW, at an altitude of 2,145 m, where the xoconostle site contains wild and small cultivated plots arranged in a grove; and Alfajayucan, which is geographically located in the west, within the Valle del Mezquital at 20°24' LN 99°21' LW and at an altitude of 1,880 m, where the xoconostle is found in the wild and in cultivated backyards. Both municipalities are within the state of Hidalgo and have a semi-temperate, semi-cold, humid climate, with temperatures averaging between 16 and 17 °C and an average annual rainfall of approximately 540 mm (Hidalgo Statistical Yearbook, 2000) (Table 1). The cladodes healed at room temperature and were planted in a sterile mixture of clay and river sand (1:1). A black plastic bag containing 4 kg of soil (Radix-1500) was used for each of the cladodes collected. The cladodes were subsequently placed in the open field and rinsed every 4 days, at field capacity. The young roots were harvested after 30 days of establishment, snipped, rinsed with tap water to eliminate all of the waste from the substrate, and immediately processed for DNA extraction.

Table 1. Location of sampling sites of plants of *O. matudae* Scheinvar in the state of Hidalgo, Mexico.

Municipality	Weather	Altitude (m)	p.p. (mm)	Temp. (°C)	LN	LW	Sup. (km^2)
Chapantongo	Temperate Cold	2,145	545	16	20°17’45’’	99°25’45’’	198.10
Alfajayucan	Temperate Humid	1,880	510	17	20°24’00’’	99°21’00’’	467.70

Source: Anuario Estadístico Hidalgo, 2000.

DNA extraction

The extraction was based on the modified method described by Dellaporta *et al*. (1983). Fresh young root tissue (0.15 g) was used. The adhering substrate was removed by washing with running water. Disinfection was carried out with a solution of 5 % $NaClO_4$. The excess of chemical was removed with sterile distilled water. The tissue was crushed by using sterile mortars with liquid nitrogen. The pulverized tissue was placed in Eppendorf tubes containing 700 µL of extraction buffer (100 mM Tris-HCl, pH 8.0; 50 mM EDTA; 500 mM Na Cl; 10 mM β-mercaptoethanol; 4 % CTAB, previously heated to 65 °C. To each sample, 4 µL of beta-mercaptoethanol was added followed by mixing. The mixture was incubated at 65 °C for 10 minutes. Subsequently, 210 µL of 5 M potassium acetate was added to the mix, and the samples were mixed carefully by inversion and then incubated for 60 minutes on ice. Next, the samples were centrifuged for 15 minutes at 2040 x *g*, and the supernatant was transferred to another Eppendorf tube, to which was added one volume of isopropanol that had been precooled to 4 °C. The tubes were incubated at 20 °C for 60 minutes (although it is desirable to incubate overnight). The samples were centrifuged for 15 minutes at 2040 x*g*, and then the excess isopropanol was removed by inverting

the tubes on absorbent paper for 5 minutes. The DNA was resuspended in 100 µL of sterile distilled water. RNA was removed by the addition of 4 µL of RNase (10 mg / mL) and incubation for one hour at 37 °C. Next, 210 µL of ammonium acetate at a concentration of 3 M (or potassium acetate at 5 M) and two volumes of absolute ethanol were added. The samples were incubated at 20 °C for 15 minutes and centrifuged at 5000 x*g* for 10 minutes. The supernatant was removed, and the pellets were washed with 500 µL of 70 % ethanol. The samples were again centrifuged at 2040 x *g* for 10 minutes, and the 70 % ethanol was removed by inverting the tubes. The DNA pellets were finally resuspended in 50 µL of TE, and the DNA was stored in solution at 4 °C.

Quantification and quality

The DNA concentration was quantified with a spectrophotometer (Perkin Elmer Lambda Bio UV / VIS) and assessed for quality in 0.8 % agarose gels, using 5 µL of sample DNA solution and 3.0 µL of loading buffer (0.25 % bromophenol blue, 0.25 % xylene cyanol, 0.30 % glycerol and 0.20 % water).

Evaluation by PCR of DNA functionality

The evaluation of the functionality of the extracted DNA was carried out with the RAPD (*R*andom *A*mplified *P*olymorphic *D*NA) PCR technique in a final reaction volume of 25 µL. The reaction mixture contained 100 ng DNA, 1.5 U of *Taq* polymerase, 1X *Taq* buffer, 2 mM $MgCl_2$, 200 µM dNTPs, 20 pm Primer K-04 from the Roth Company and sterile distilled water. The reaction tubes were covered with a drop of sterile mineral oil. The amplification was performed in a thermocycler (model 2720 Thermal Cycle, Applied Biosystems) with the following program: an initial denaturation cycle of 95 °C for 1 min, 35 cycles [94 °C, 30 sec; 40 °C, 30 sec; 72 °C, 1.5 min] and a final extension step at 72 °C for 2.5 min. The amplification products were separated in polyacrylamide gels (29:1) at 230 volts and stained with a solution of 2 % silver nitrate (Sambrook *et al*., 1989). RAPD analyses were performed with various oligonucleotides of the K series to choose the banding and to provide greater definition (Table 2).

Table 2. Sequence of the oligonucleotides of the K series used in RAPD (Luna-Paez *et al*., 2004).

Oligonucleotide	Sequence
K-01	CATTCGAGCC
K-04	CCGCCCAAAC
K-07	AGCGAGCAAG
K-12	TGGCCCTCAC
K-17	CCCAGCTGTG

Results

The quality of DNA obtained from young roots was satisfactory to conduce the PCR amplification. The results obtained for DNA extraction from young roots were positive. The amount of DNA from 0.15 g of young root tissue averaged 4.0 µg / µL in *O matudae*. The visual assessment of DNA in agarose gels was acceptable compared with that of the cladodes, which was assessed simultaneously using the same methodology and compliance with the guidelines of the protocol (Figure 1).

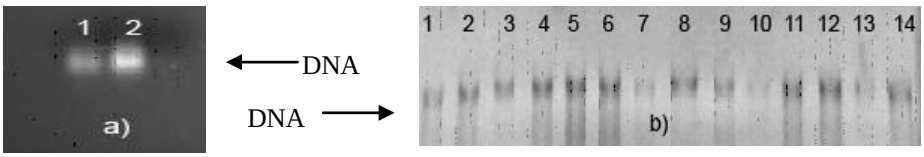

Figure 1. The high molecular weight DNA extracted from xoconostle samples was separated in 0.8% agarose gels. The gel (a) compares the quality of DNA extracted from cladodes (1) with that from roots (2). The gel (b) shows the quality of DNA extracted from the roots of different individual plants.

Even when DNA obtained from cladodes or roots was observed as a defined band (Figure 1a and 2a), the functionality of the DNA from the two structures differed even when amplified with the same primers by PCR. This is because the polysaccharides associated with DNA may interfere with the recognition of potential binding sites for the PCR primers used for amplification (Mondragón-Jacobo, 2000; Mondragón-Jacobo, 2003).

However, the DNA from the roots was virtually free of these substances, and when assessed by RAPD, a technique that is commonly used for genotyping analysis, the obtained amplified products produced sharp and reproducible bands. The separation of the fragments of one of the repeats is shown in an acrylamide gel, which reveals polymorphisms among the different specimens of O. *matudae* (Figure 2). The xoconostle, similar to the nopales, have ecotypes that vary morphologically with the medium. Variants of *O. matudae* differ mainly in the color, size and number of fruits, the color of spines and the number of areolas in the rows, which makes differentiation very subjective if no fruit is present (Reyes-Agüero *et al*., 2005).

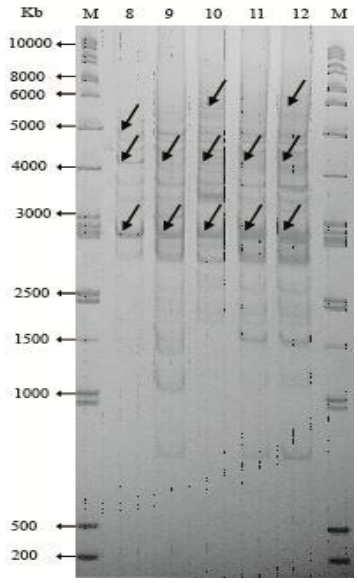

Figure 2. Polyacrylamide gel (29:1 to 8 %) with RAPD products amplified with the K-04 primer. Different samples of DNA extracted from young roots show similarities and differences.

Conclusions

Purification of DNA from the young roots of cacti is useful for PCR because the quality of the starting material is optimized, which is one of the most important requirements for molecular analysis. The use of 4 % CTAB was ideal for the extraction of the genetic material because this product binds tightly to DNA, removes proteins efficiently, prevents oxidation, prevents the degradation of DNA, and helps to eliminate the high concentrations of polysaccharides that are present in most tissues of the cacti. The DNA obtained from the roots allowed successful amplification in RAPD reactions. The reproducible and clear separation of the amplified fragments in polyacrylamide gels resulted in high quality results, and therefore young roots should be considered as an excellent source of DNA for the molecular analysis of cacti.

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