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Comparison of four DNA extraction methods on various tissues and types of *Sechium edule* (Jacq.) Sw.

Comparación de cuatro métodos de extracción de ADN en diferentes tejidos y tipos de *Sechium edule* (Jacq.) Sw.

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Abstract. *Sechium edule* is an important crop in Southern Mexico and Central America, and México was the main producer, with 53% of the world production in 2012. This species presents high levels of morphological variation that have resulted in different types of varieties not clearly defined. However, morphological characteristics have not been sufficient to classify *S. edule* varieties, and it has been difficult to study the species genetic diversity. Because of it, there is a need to develop methods for studying the genetic diversity in *S. edule*. In this regard, the first step is to find a suitable DNA extraction method. For this reason, in this study four DNA extraction methods (two laboratory protocols, and two commercial kits) were compared for their efficiencies in yield and quality of DNA obtained from four different tissues of four *S. edule* types. Clear effects of extraction methods, types and tissues of *S. edule* were observed on DNA yield and quality.

Keywords: *Sechium edule*; Chayote; DNA quality; DNA yield.

Resumen. *Sechium edule* es una planta importante en el sur de México y América Central, y México, con el 53% de la producción en 2012, es el principal productor del mundo. Esta especie presenta altos niveles de variación que han resultado en diferentes tipos o variedades, no claramente definidos. Las características morfológicas no han sido suficientes para clasificar las variedades de *S. edule* y ha sido difícil el estudio de la diversidad genética de la especie. Debido a esto, existe la necesidad de desarrollar métodos apropiados para el estudio de la diversidad genética del chayote. En este aspecto, el primer paso es contar con un método confiable para extracción de ADN. Por esta razón, en este estudio se comparó la eficiencia de cuatro métodos de extracción de ADN (dos protocolos de laboratorio y dos paquetes comerciales). Se probaron cuatro variedades y cuatro tejidos diferentes de *S. edule*. Se observaron efectos claros en la cantidad y calidad de ADN extraido, tanto entre los métodos de extracción, como entre los tipos y tejidos de *S. edule*.

Palabras clave: *Sechium edule*; Chayote; Calidad de ADN, Cantidad de ADN.

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INTRODUCTION

Chayote [*Sechium edule* (Jacq.) Sw] is a Mesoamerican cucurbitaceae. Mexico and Guatemala are most likely the centers of origin for this crop (Newstrom, 1991). *Sechium edule* is extensively cultivated in Mesoamerica. In the opinion of the Mexican Business Web, Mexico was in 2012 the main producer of this crop species with 53% of the world production. In this country, chayote has a social, economic, cultural and environmental importance, which must be comprehensively addressed. In Mexico, the states of Veracruz, Chiapas and Oaxaca show the highest diversity levels for fruit characteristics (e.g., size, form, color, flavor, skin type or presence of spines). Likewise, different leaf forms, coloring, leaf vein structure, vines and flowers have been reported (Cadena et al., 2008). However, it has been difficult to develop genetic improvement programs, since it is not clear how much of the variation is either genetic or environmental. Morphological and anatomical variations have been studied in leaves and fruits of *S. edule* (Cadena et al., 2008). However, there are no records of molecular studies that could give information about the genetic composition of the species. A number of techniques for DNA extraction have been published in different crops. Nevertheless, there is not a DNA extraction method reported for *S. edule*.

Molecular techniques have facilitated the study of plant diversity and provide tools and opportunities for plant breeding and conservation. However, it is essential to develop a DNA extraction method that can be applied to the isolation of suitable DNA for molecular applications (Varshney & Tuberosa, 2007). Therefore, it is necessary to get a proper extraction method for DNA amplification in PCR and sequencing to study *S. edule* diversity and examine breeding material to select genotypes.

The objective of this study was to search for a suitable DNA extraction method. Because of this, four different methods were compared, including two laboratory protocols and two commercial kits, on four tissues and four *S. edule* types.

MATERIALS AND METHODS

This investigation is part of a project on *S. edule* genetic diversity and was carried out in plants from the central region of the Veracruz State, Mexico, in two municipalities (Ixtaczoquitlan and Huatusco), at an altitudinal gradient of 900-1120 m.a.s.l. Experimental material was collected in four commercial orchards and in the CRUO (Centro Regional Universitario Oriente - Universidad Autónoma de Chapingo) germplasm collection.

Color, form, size and spines of the fruit were the characteristics used for selection of four cultivated chayote types, that were identified by their popular name as smooth green (Co), cambray (Ca), black (Ne) and spiny black (Es). According to Avendaño et al. (2010), these types correspond to *S. edule* var. *virens levis*, *S. edule* var. *albus minor*, *S. edule* var. *nigrum xalapensis* and *S. edule* var. *nigrum spinosum*.

Completely expanded leaf blades of each chayote type were collected at random from five plants in each orchard. In addition, five fruits were collected from each plant. In total, five replicates of each *S. edule* type, leaf blades and three fruit tissues (mesophyll, epicarp and seed embryo) were compared using four DNA isolation methods: CTAB (Zhow et al., 1999) and Dellaporta (Dellaporta et al., 1983) laboratory methods, and MoBio (MoBio, 2009) and Wizard (Promega, 2010) commercial kits.

DNA quality was analyzed considering DNA fragmentation and 260/280 absorbance. The analysis of DNA fragmentation was performed on ethidium bromide-stained 1% agarose gel electrophoresis. Absorbance and DNA quantity were determined in an Eppendorf Biophotometer. DNA amplification methods are useful for various biotechnology applications. It is often unclear whether unsuccessful amplification using the polymerase chain reaction (PCR) is the result of poor quality template, the presence of inhibitors, or a combination of both factors. Therefore, the DNA obtained was tested for amplification by PCR using a RAPD primer (5' TGCGCCCTTC 3').

The factorial variation of 260:280 absorbance ratio and DNA quantity were evaluated, and statistically analyzed with a factorial variance analysis and a Tukey test, using the SAS program.

RESULTS

The DNA fragmentation analysis performed using 1% agarose gel electrophoresis showed banding patterns corresponding to high molecular weight in almost all samples. Some DNA samples showed degradation (smearing), indicating different structural integrity.

Dellaporta method showed no DNA degradation but smeared bands in the bottom of the gel, suggesting the presence of contamination. This was probably due to the presence of proteins, also indicated by a 260:280 ratio lower than 1.8. In order to examine the DNA quality, it was amplified by PCR, using a RAPD primer (5' TGCGCCCTTC 3'), and clear and well defined bands were observed.

Wizard method showed little fragmentation and a few smeared bands pointing to the presence of contamination. The best DNA quality was observed on leaves, with almost no fragmentation and no contamination evidence on agarose gels. The presence of contaminating substances in the fruit tissues also suggested that the leaf would be the best source of DNA in *S. edule*.

In the four types of used tissues, CTAB and MoBio methods showed fragmentation and contamination bands in the agarose gels, denoting lower DNA quality.

All methods produced small quantities of DNA (Table 1), but sufficient to be analyzed, following amplification by the polymerase chain reaction. Even when Dellaporta method

Table 1. DNA concentration (ng/µL) obtained using four extraction methods (Dellaporta, Wizard, CTAB, MoBio), four *Sechium edule* varieties referred to as Ca (cambray), Co (smooth green), Ne (black), Es (spiny black), and four plant tissues referred to as Le (leaf), Me (mesophyll), Ep (epicarp) and Em (embryo).
Tabla 1. Concentración de ADN (ng/µL) obtenido con cuatro métodos de extracción (Dellaporta, Wizard, CTAB, MoBio), cuatro variedades de *Sechium edule* referidas como Ca (cambray), Co (verde liso), Ne (negro), Es (negro espinudo), y cuatro tejidos de la planta referidos como Le (hoja), Me (mesófilo), Ep (epicarpio) y Em (embrión).

Dellaporta		Wizard		CTAB		MoBio	
Treatment	DNA (ng/µL)	Treatment	DNA (ng/µL)	Treatment	DNA (ng/µL)	Treatment	DNA (ng/µL)
CaLe	1304.22	CaLe	884.46	CaLe	336.74	CaLe	265.66
CaMe	666.06	CaMe	274.58	CaMe	218.18	CaMe	221.76
CaEp	699.98	CaEp	416.04	CaEp	333.96	CaEp	229.6
CaEm	1094.52	CaEm	290.46	CaEm	193.38	CaEm	231.3
CoLe	2049.89	CoLe	1063.6	CoLe	331.52	CoLe	167.5
CoMe	754.54	CoMe	134.86	CoMe	438.88	CoMe	293.2
CoEp	685.7	CoEp	251.06	CoEp	347.08	CoEp	580.58
CoEm	829.28	CoEm	372.46	CoEm	378.12	CoEm	171.64
NeLe	830.48	NeLe	1842.58	NeLe	319.96	NeLe	231.02
NeMe	417.36	NeMe	143.4	NeMe	301.12	NeMe	146.78
NeEp	318.12	NeEp	435.5	NeEp	236.78	NeEp	242.98
NeEm	258.9	NeEm	232.52	NeEm	376.98	NeEm	118.92
EsLe	1013.72	EsLe	1088	EsLe	233	EsLe	415.08
EsMe	230.22	EsMe	115.74	EsMe	187	EsMe	113.26
EsEp	882.94	EsEp	259.32	EsEp	277.72	EsEp	78.04
EsEm	1047.96	EsEm	199.3	EsEm	357.42	EsEm	388.46

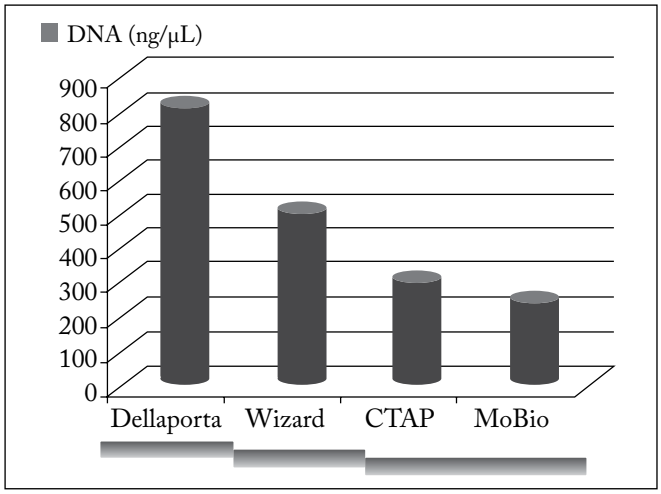

Fig. 1. Average DNA yield in ng/µL, obtained from the combination of four chayote types (smooth green, cambray, black, spiny black) and four types of tissues (leaf blade, mesophyll, epicarp and seed embryo) with four DNA extraction methods. Rods show Tukey test differences.
Fig. 1. Concentración promedio en ng/µL de ADN obtenido con cuatro diferentes métodos de extracción combinado con cuatro tipos de chayote (verde liso, cambray, negro y negro espinudo) y cuatro tejidos (lámina foliar, mesófilo, epicarpio y embrión). Las barras indican las diferencias de la prueba de Tukey.

showed the highest DNA yield, PCR products were observed in all four methods used on the four study tissue types. However, more clear bands were observed with Dellaporta and Wizard than with CTAB and MoBio methods.

For each *S. edule* type and tissue, the extraction method clearly determined the DNA yield and quality. Results were combined for each extraction method including types and tissues. According to a variance analysis and application of a Tukey test, the highest quantity and the best quality were observed with the Dellaporta DNA extraction method (Fig. 1). In addition, DNA obtained from leaf blades showed concentrations ranging from 2049 to 830 (ng/µL); the highest value was for the smooth green chayote variety (Table 1). The second best option was the Wizard Kit using leaf blades of the smooth green variety.

CONCLUSIONS

This study revealed that the DNA extraction method affects the DNA yield and quality in different types and tissues of *S. edule*. Dellaporta was the best method, the leaf the best tissue and the smooth green chayote the best variety for this kind of analysis. There is no doubt that there is much to be done, specially to test methods that render better quality

for molecular analysis. However, the results of this study open the way for evaluating *S. edule* genetic diversity, and initiate formal programs for the genetic improvement of this species.

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