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Molecular evaluation and genetic diversity in plants of *Carludovica palmata* (Ruiz & Pavón) propagated by rhizomes and seeds

Evaluación molecular y diversidad genética en plantas de *Carludovica palmata* (Ruiz & Pavón) propagadas por rizomas y propagadas por semillas

Polyculture plantations of *C. palmata*.

Photo: L.C. Chi-Chi

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ABSTRACT

Carludovica palmata is cultivated in the northern region of Campeche (Mexico) and has agro-artisanal importance due to its soft, flexible, and durable fibers, which are used for weaving hats and other crafts. This species has two propagation mechanisms: rhizome and seed. In Mexico, the genetic diversity of the crop is unknown. The main objective of this study was to determine the genetic diversity in *C. palmate* plants with two types of propagation: rhizome and seed. Genetic diversity and differentiation were evaluated using the AFLP-V2.0 program, which estimates allele frequencies for dominant markers, assuming that populations are in Hardy-Weinberg equilibrium. The results confirmed the reproducibility of 48 loci, using Inter-Retrotransposon Amplified Polymorphism (IRAP) and Start Codon Targeted (SCoT) markers. The IRAP markers (NIKITA, SUKKULA, and 3'LTR) were polymorphic, indicating polymorphic information content (PIC). The PIC for SUKKULA was 0.28 in rhizomes and 0.24 in seed-propagated plants, while for SCoT-18 it was 0.30 in seed-propagated plants and 0.15 in rhizomes. The genetic diversity of *C. palmata* is low, as demonstrated by the Shannon-Wiener index (H'=0.986 in plants propagated from rhizomes and H'=0.981

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in plants propagated from seeds). The Nei's estimation, the expected heterozygosity (HE) was 0.2892 in rhizomes and 0.27056 in seed-propagated plants. These results indicate similar genetic diversity in both propagation groups, with no significant changes, and suggest limited genetic diversity in *C. palmata*, as well as possible crosses between parents.

Additional key words: polymorphism; molecular markers; heterozygosity; intraspecific diversity; clonal propagation.

RESUMEN

Carludovica palmata se cultiva en la región norte de Campeche (México) y tiene una importancia agro-artesanal por sus fibras suaves, flexibles y duraderas, que se utilizan para el trenzado de sombreros y otras artesanías. Esta especie tiene dos mecanismos de propagación: rizoma y semillas. En México, se desconoce la diversidad genética del cultivo. El objetivo principal de este estudio fue determinar la diversidad genética en plantas de *C. palmata* con dos tipos de propagación: rizoma y semillas. La diversidad genética y la diferenciación se evaluaron mediante el programa AFLP-V2.0, que estima frecuencias alélicas para marcadores dominantes, asumiendo que las poblaciones están en equilibrio de Hardy-Weinberg. Los resultados confirmaron la reproducibilidad de 48 loci, utilizando marcadores de polimorfismo amplificado entre retrotransposones (IRAP, *Inter-retrotransposon Amplified Polymorphism*) y marcadores dirigidos al codón de inicio (ScoT, *Start Codon Targeted*). Los marcadores IRAP (NIKITA, SUKKULA y 3'LTR) resultaron polimórficos, lo que indica contenido de información polimórfica (PIC). El PIC para SUKKULA fue de 0.28 en rizomas y 0.24 en plantas por semillas, mientras que para SCoT-18 fue de 0.30 en plantas por semillas y 0.15 en rizomas. La diversidad genética de *C. palmata* es baja, como lo demuestra el índice de Shannon-Wiener (H'=0.986 en plantas propagadas a partir de rizomas y H'=0.981 en plantas propagadas a partir de semillas). Según la estimación de Nei, la heterocigosidad esperada (HE) fue de 0.2892 en rizomas y 0.27056 en plantas a partir de semillas. Estos resultados indican una diversidad genética similar en ambos grupos de propagación, sin cambios significativos, y sugieren una diversidad genética limitada en *C. palmata*, así como posibles cruzamientos entre parentales.

Palabras clave adicionales: polimorfismo; marcadores moleculares; heterocigosidad; diversidad intraespecífica; propagación clonal.

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INTRODUCTION

The study of commercially important plants using molecular tools and omics approaches allows for the acquisition of information to answer biological questions (Bidyananda *et al*., 2024). In cultivated species, it is essential to evaluate how much genetic diversity exists in populations with different types of cultivation (Salgotra and Chauhan, 2023). Determining genetic diversity facilitates the analysis of strategies for germplasm conservation and the selection of genetic resources with tolerance to pests and diseases (Begna, 2021; Bidyananda *et al*., 2024). In crops, the selection of a few individuals or genotypes can result in low genetic diversity, because of the founder effect (Klimova *et al*., 2022; Souza *et al*., 2022). Bottlenecks in a crop can reduce population size and involve other factors, such as genetic drift, propagation system, founder effect, and cultivation conditions. All these factors increase the risk of loss of genetic diversity; therefore, populations must be sufficiently diverse and large enough to adapt to environmental changes. Otherwise, diversity would be limited (Begna, 2021; Salgotra and Chauhan, 2023). The importance of maintaining species and genotypes lies in long-term conservation and recovery. In this way, clonally propagated populations are prevented from suffering low allele diversity and, consequently, inbreeding levels (Luikart *et al*., 2021; Khoury *et al*., 2022).

Carludovica palmata L. is a monocotyledonous plant belonging to the genus Carludovica, one of the 12

genera of the family Cyclanthaceae (Leal *et al*., 2022). It is found within the subfamily Carludovicoidae, which includes four species: *C. palmata*, *C. rotundifolia*, *C. drudei*, and *C. sulcata* (Riordan *et al*., 2023). This species is commonly known as jipi palm, jipi japa, and toquilla palm (Jiménez *et al*., 2023). *C. palmata* is native to South America and is distributed throughout the Neotropics, in countries such as Guatemala, Colombia, Peru, Bolivia, Ecuador, Panama, and Venezuela. In Mexico, the introduction of the crop dates to 1866 (Perera-Hau *et al*., 2025; Méndez-Hernández *et al*., 2025), with plants originating from an unknown location in Central America. Currently, it is distributed in the states of Tabasco, Campeche, and Yucatán (Moo-Huchin *et al*., 2019). Given this, it is likely that the established populations originated from a few genotypes or related genotypes, resulting from a founder's effect. In the northern region of Campeche, the raw material used from *C. palmata* are immature leaves, which are of agro-artisanal interest due to the use of their soft, resistant, and high-quality fibers, used for weaving hats and other crafts, such as bags, brooms, carriers, umbrellas, belts, rattles, trays, and decorative products (Jiménez *et al*., 2023; Méndez-Hernández *et al*., 2025). *C. palmata* has two types of propagation: sexual, through the fusion of gametes in the formation of seeds, and asexual, through the formation of rhizomes.

There are DNA-based tools, called molecular markers, that are functional for genetic diversity studies. These are found in the genomes of different organisms, mainly in sites that do not code proteins. Therefore, they are expected to be under natural selection; they are also known as "neutral molecular markers" (Salgotra and Chauhan, 2023; Bidyananda *et al*., 2024). Inter-Retrotransposon Amplified Polymorphism (IRAP) markers belong to Class I and are ideal for genetic variability studies, DNA identification, and genetic mapping of plants. They present fragments of 500 to 3,000 bp (Shirasu *et al*., 2000; Valizadeh *et al*., 2025). On the other hand, Arvas *et al*. (2022) report fragments of approximately 100 to 3,000 bp, using them alone and in combination withong terminal repeat (Long Terminal Repeats-LTR), for example, in rice (*Oryza sativa* L.). Other markers are those designed from conserved sequences of plant genes, such as the polymorphism directed at the translation start codon ATG, called (SCoT), which generate amplified fragments of approximately 500 to 3,000 bp (Collard and Mackill, 2009; Khodaee *et al*., 2021).

During the amplification of IRAP and SCoT molecular markers, amplicons present in the genome are generated. When they find the complement of the primer (target sequence), they present a polymorphism; in the absence of the target sequence, a mutation occurs. Therefore, these markers are considered dominant and cannot be used to evaluate the frequency of heterozygous individuals nor to perform calculations of heterozygosity, diversity, and genetic structure on their own. In studies using dominant markers, there are several options for evaluating genetic variability. One of them is related to the ability of these markers to distinguish polymorphism and their reproducibility. For this purpose, the polymorphic information content (PIC) and the Shannon-Wiener index are used, which determine allelic diversity in a population (Botstein *et al*., 1980; Serrote *et al*., 2020). Finally, genetic diversity is determined by Nei's estimator, which infers genetic diversity and is directly related to expected heterozygosity (He).

To date, no studies have assessed the genetic diversity of *C. palmata* in Mexico, nor the applicability of molecular markers in this species. This study aims to evaluate the genetic diversity of *C. palmata* using two types of dominant molecular markers—IRAP and SCoT—across two propagation systems: vegetative propagation via rhizomes and sexual propagation via seeds.

MATERIALS AND METHODS

Study species

In Mexico, *C. palmata* is characterized by having long and erect petioles ranging from 1.5 to 1.8 m in length. Its leaves have serrated edges, with between 18 and 32 teeth, each approximately 1.2 cm in length. The width of the branch ranges from 25 to 29 cm, and the plant height is around 1.9 to 2.3 m (Ortega-Haas, 2013). The reproductive biology of *C. palmata* has been studied in Campeche, based on the morphological description of inflorescences and infructescences (Bacab-Caamal, 2020). The reproductive stage occurs between October and June. The reproductive development is divided into 11 stages, from inflorescences to infructescences, with changes in the production of male and female flowers during fruit development (Bacab-Caamal, 2023).

Rhizome plants

Nine sites (App. 1; Fig. 1) were sampled for a total of 34 plants, distributed from three to eight individuals per study site (App. 2; Tab. 1). The most distant plantations correspond to young plantings of 20 to 50 years. On the other hand, the oldest plantations are from site one, with approximately 150 years of cultivation, and site five, with around 80 years of cultivation. Crop management varied among sites, including irrigation applications, use of agrochemicals, and composting systems. For subsequent analyses, three representative samples were selected by the sampling site.

Plants from lines derived from seeds

The *C. palmata* plants included in this study were adults, approximately four years old. They were obtained from seeds germinated *in vitro* conditions, adapted to a shade house, with constant irrigation and fertilization every 3 months with macro and micronutrients (Poly-feed®, 1 g L^{-1}). The plant lines derived from seeds (App. 1; Fig. 2) were 127 lines with their respective sibling lines (App. 2; Tab. 2). Only 56 plants from different lines were included in this study.

Genomic DNA extraction

Total genomic DNA extraction was performed using the modified CTAB method (cetyltrimethylammonium bromide) (Kumar and Agrawal, 2019), adapted for coconut (Edwards *et al*., 1991) (Fig 1). This method uses an analog of beta-mercaptoethanol, mono-tyacilglycerol, and 5 g of young leaf tissue. DNA quality was verified on 1% agarose gels, with EtBr 2 μL, with a running time of 30 min at 90 V. The gel was visualized on a ChemiDoc™ MP Imaging System transilluminator. DNA quality and concentration were determined using a Thermo Scientific® Nanodrop 2000 device.

PCR amplification

PCR amplification reactions were carried out according to Lapina *et al.* (2012), as described for IRAP molecular markers and, for SCoT molecular markers, based on Collard and Mackill (2009) with modifications in number of cycles proposed by Aboulila and Mansour (2017) (App. 3; Tab. 1 and 2). The PCR reaction was 25 μL and contained nuclease-free water (17.7 μL), Dream Taq Buffer with Mg 10X from Invitrogen® (2.5 μL), DNTP MIX from Invitrogen® (10 mM) (2.5 μL), primer (4 mM) (2.5 μL), DNA template (50 ng), and Dream Taq DNA polymerase from Invitrogen® (0.3 μL) (2 μL). The amplification products were run on 1.5% agarose gels, with 5 μL of amplicon, 2 μL of 6X DNA Loading Dye from Thermo Scientific®, stained with 0.8 μL of ethidium bromide from a stock of 25 mg μL^{-1} concentration, and the running time was 70 min at 75 V.

Figure 1. Plant propagated from *C. palmata* seed, grown in a shade house nomenclature (S12.3), the box shows the section collected for genomic DNA extraction.

Analysis of molecular markers

The presence (1) and absence (0) of clear bands were recorded visually. Each band of different molecular weights was considered a locus. For the IRAP (NIKITA, 3'LTR, and SUKKULA) and SCoT (SCoT-3, SCoT-5, and SCoT-18), a presence-absence matrix was constructed.

Estimators of genetic diversity

The polymorphic information content (PIC) (Botstein *et al*., 1980) indicates the discriminatory power of a molecular marker in relation to others and its

efficiency in determining polymorphic loci (Serrote *et al*., 2020; Zhang *et al*., 2021). The formula for calculating the PIC is as follows:

$$PIC = 1 - \sum_{Pi^2} - \sum(2Pi^2Pj^2)$$

Where, *Pi* is the frequency of the *i*-th allele. The criteria for classifying the PIC are: i) PIC>0.5: highly informative (highly polymorphic locus), ii) 0.25<PIC≤0.5: reasonably informative, and iii) PIC≤0.25: low informative (Zhang *et al*., 2021).

Allelic diversity was determined using the Shannon-Wiener index, which is frequently used to measure genetic diversity (Sun and Ren, 2021). Genetic diversity was estimated using the AFLP-SURV 1.0 program (Vekemans, 2002). Allelic frequencies for each matrix were obtained using a Bayesian method with a non-uniform prior frequency. With the estimators, the percentage of polymorphic loci (PPL) and expected heterozygosity (He) were calculated, where H' represents the Shannon-Wiener Index and NB with the number of bands/loci.

RESULTS

Reproducibility of IRAP markers in *C. palmata*

The marker profiles were confirmed in both groups of plants, with band sizes ranging from 550 to 3,000 bp, and three loci (700; 1,300 and 1,500 bp) were monomorphic in both groups. The IRAP molecular markers that were functional and reproducible for the *C. palmata* samples, demonstrating that the markers 3'LTR, NIKITA, and SUKKULA are suitable for identifying polymorphism (Fig. 2; Tab. 1; Fig. 3). On the other hand, the LTR-6149 and 5'LTR-2 markers were not functional for our studies, as nonspecificity was observed with both molecular markers (App. 1; Fig. 3A-B), and thus they were discarded for genetic diversity studies.

Reproducibility of SCoT markers in *C. palmata*

The profiles of the SCoT molecular markers were validated and evaluated in both propagation groups,

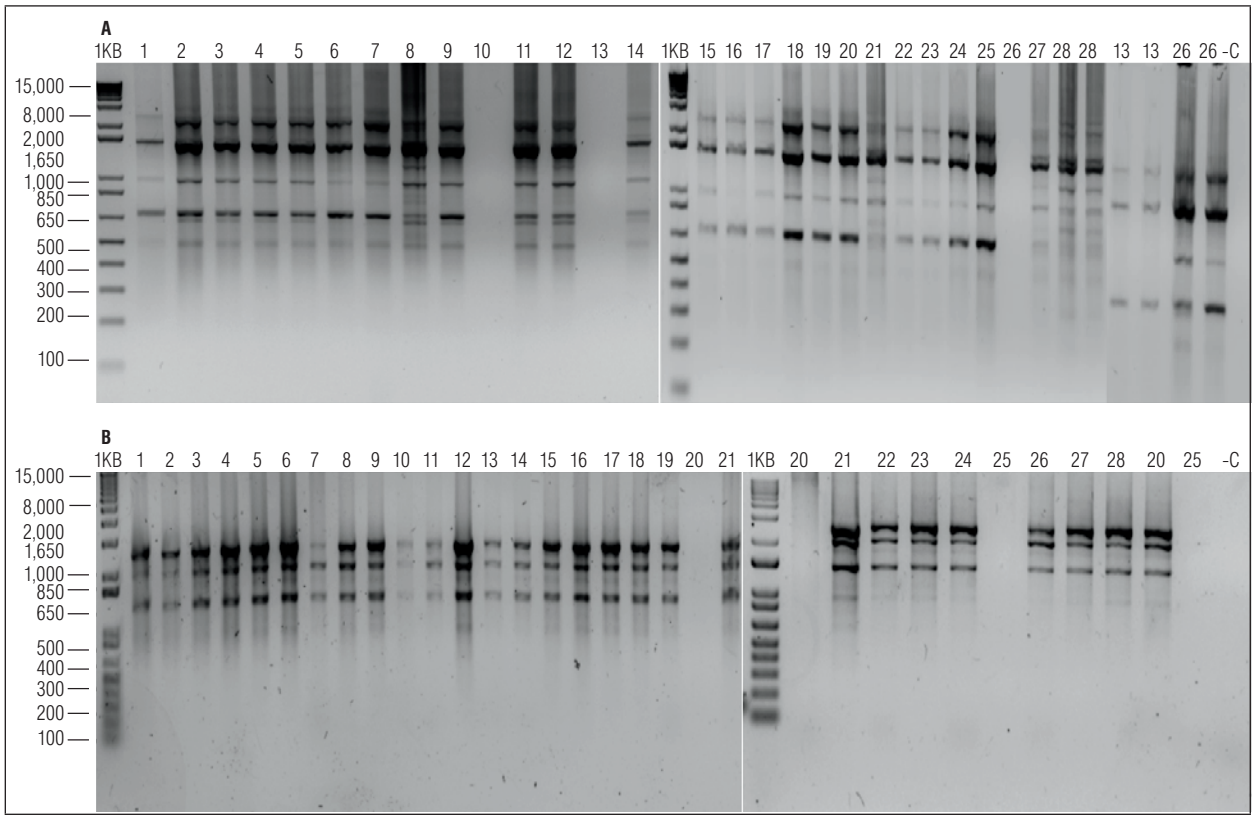

Figure 2. Identification of polymorphism in *C. palmata* plants propagated from seeds. A, 3'LTR molecular marker. B, NIKITA molecular marker. Conditions 1.5% agarose with a run time of 75 V for 70 min. MPM: 1 KB Plus Ladder Invitrogen.

generating bands ranging from 400 to 3,000 bp. The SCoT-3 molecular marker generated seven loci in plants propagated from seeds, while SCoT-5 generated six loci (Fig. 4A-B; Tab. 1). SCoT-3 generated five loci and SCoT-5 generated six loci in plants propagated by rhizomes, while SCoT-18 generated nine loci (700; 1,300; and 1,500 bp), indicating polymorphism (Fig. 5A-B). Only three markers (SCoT-10, SCoT-20, and SCoT-36) were not functional (App. 1; Fig. 4).

Table 1. Description of LTR-based molecular markers in *C. palmata*.

Molecular markers	Tm	Characteristic	Candidates
NIKITA	58	Monomorphic	*
SUKKULA	66	Polymorphic	Best marker (DNP)
5'LTR1	63	Does not amplify	X
5'LTR2	58	Does not amplify	X
3'LTR	69	Polymorphic	*
LTR 6149	61	Does not amplify	X
LTR 6150	62	Does not amplify	X

* Monomorphic molecular marker, x did not amplify in samples.

Discriminatory power of molecular markers

In plants propagated by seed and by rhizome, the SUKKULA molecular marker generated seven polymorphic loci, while SCoT-18 generated nine polymorphic loci in both genetic groups (Tab. 3). Based on the polymorphic content index (PIC) and the Shannon Wiener index, the loci demonstrated low intraspecific genetic diversity, with PIC values >0.28 in plants propagated by rhizomes with the SUKKULA marker and PIC>0.30 in plants propagated by seeds with the SCoT-18, resulting in reasonably informative values. Therefore, it is suggested that SUKKULA and SCoT-18 could be useful for genetic diversity assessments of *C. palmata* in future studies.

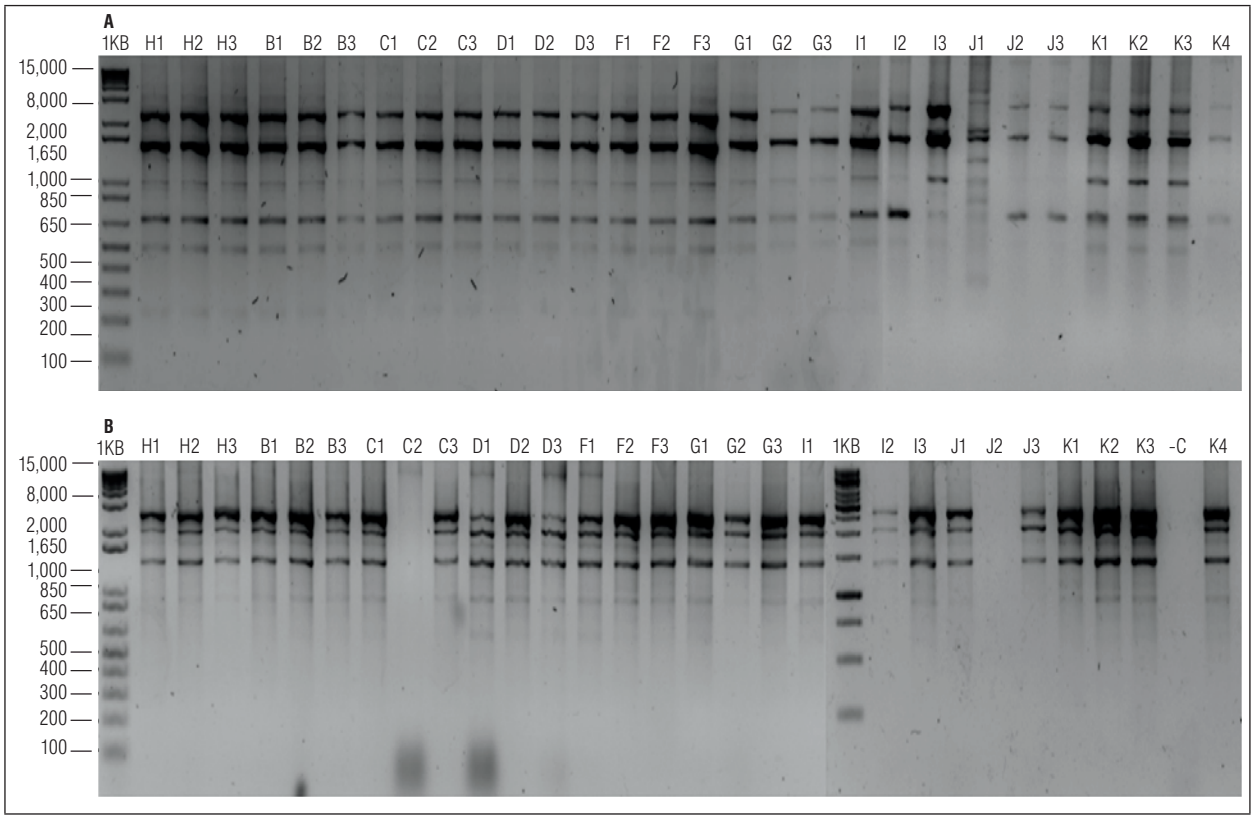

Figure 3. Identification of polymorphism in plants propagated by rhizomes of *C. palmata*. Lanes (HI-K4). A, 3'LTR molecular marker. B, NIKITA molecular marker. Conditions 1.5% agarose with a run time of 75 V for 70 min. MPM: 1 KB Plus Ladder Invitrogen.

Similarly, SCoT-3 demonstrated a discriminatory power with PIC>0.21 in rhizomes and the 3'LTR with PIC>0.25 in plants from seeds (Tab. 3), making them reasonably informative markers for genetic diversity studies in *C. palmata*.

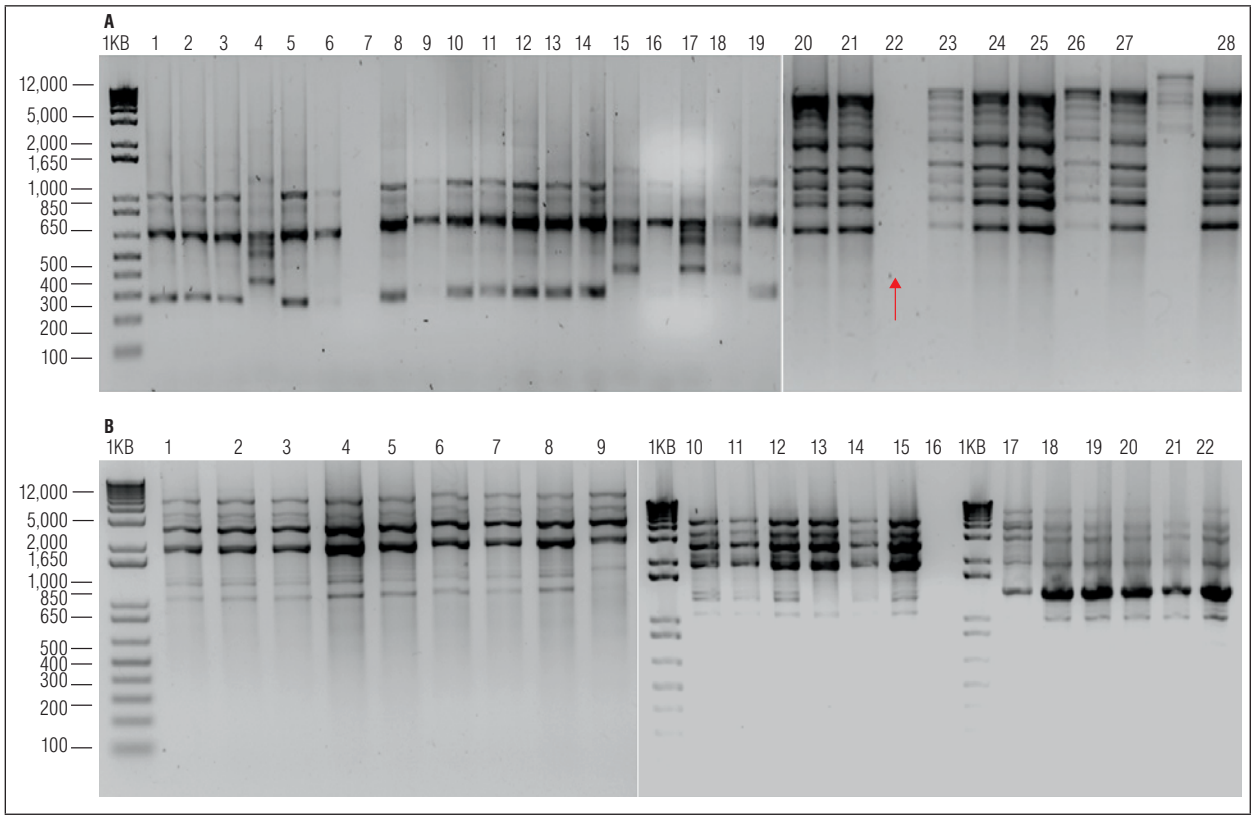

Figure 4. Identification of polymorphism in *C. palmata* plants propagated from seeds. A, SCoT-3 molecular marker. B, SCoT-5 molecular marker. Conditions 1.5% agarose with a run time of 75 V for 70 min. MPM: 1 KB Plus Ladder Invitrogen.

Table 2. Description of the SCoT molecular marker in relation to reproducibility for *C. palmata*.

Molecular marker	Tm	Characteristic	Candidates
SCoT-5	50	Monomorphic	*
SCoT-25	67	Polymorphic	Candidate
SCoT-10	56	Does not amplify	X
SCoT-20	67	Does not amplify	X
SCoT-36	56	Does not amplify	X
SCoT-4	50	Polymorphic	*
SCoT-6	56	Monomorphic	*
SCoT-1	50	Monomorphic	*
SCoT-2	56	Monomorphic	*
SCoT-3	56	Monomorphic	Candidate
SCoT-17	61	Monomorphic	Candidate
SCoT-18	67	Polymorphic	Best marker candidate
SCoT-7	56	Monomorphic	*
SCoT-8	50	Monomorphic	*
SCoT-9	50	Monomorphic	*
SCoT-24	56	Monomorphic	
SCoT-13	61	Monomorphic	Candidate
SCoT-14	67	Monomorphic	Candidate
SCoT-12	61	Monomorphic	Candidate
SCoT-16	56	Monomorphic	Candidate
SCoT-15	67	Monomorphic	Candidate

* Monomorphic molecular marker, x did not amplify in samples.

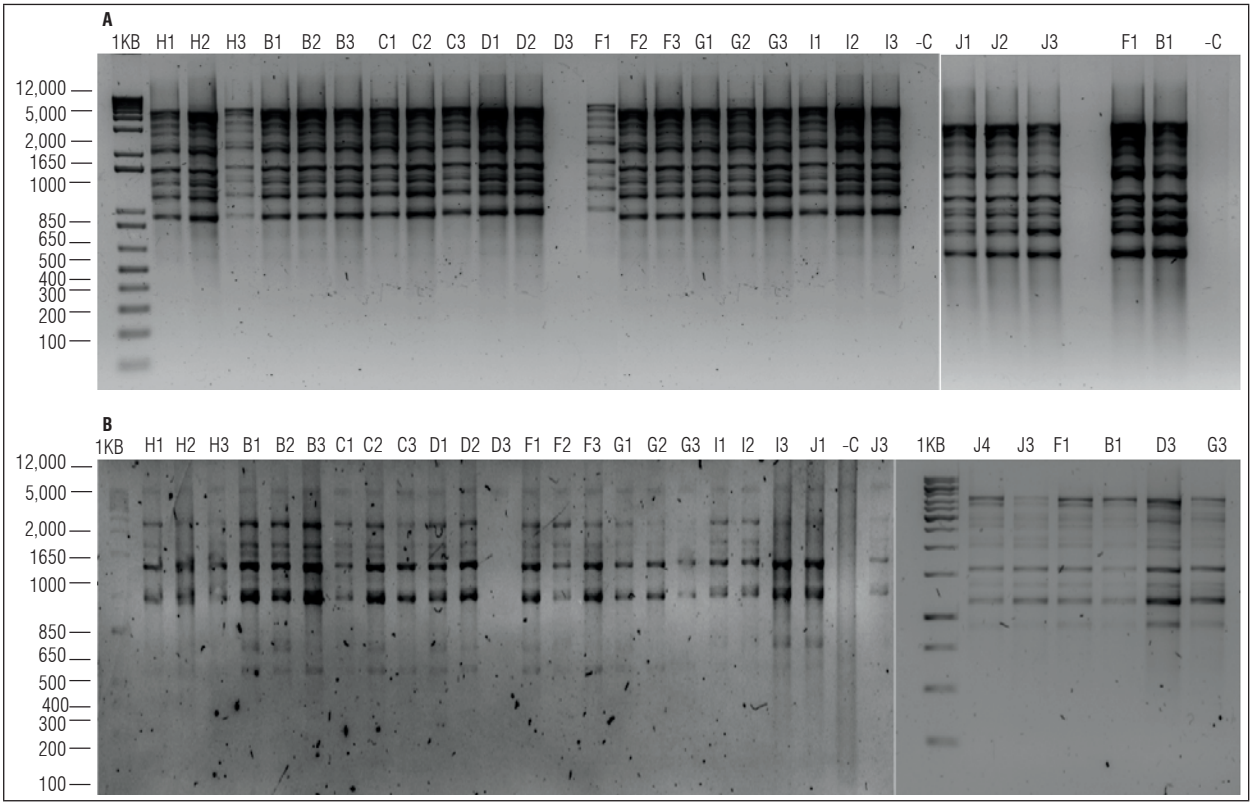

Figure 5. Identification of polymorphism in plants propagated by rhizomes of *C. palmata*. A, SCoT-3 molecular marker. B, SCoT-5 molecular marker. Conditions 1.5% agarose with a run time of 75 V for 70 min. MPM: 1 KB Plus Ladder Invitrogen.

Table 3. Comparison of genetic diversity estimation in *C. palmata* with dominant markers using the Polymorphic Content Index and Shannon Wiener Index.

Molecular marker	Plants propagated by rhizome			Plants propagated by seeds		
	NB	PIC	H'	NB	PIC	H'
SUKKULA	7	0.28	0.984	7	0.24	0.9838
3'LTR	5	0.17	0.980	5	0.25	0.9802
NIKITA	5	0.13	0.980	4	0.15	0.9808
SCoT-3	5	0.21	0.987	7	0.20	0.9868
SCoT-5	6	0.13	0.989	6	0.11	0.9859
SCoT-18	9	0.15	0.988	9	0.30	0.9880

PIC: Polymorphic content index, *H': Shannon Wiener index. *NB: number of bands/loci.

Genetic diversity

Genetic diversity in *C. palmata* was similar for the Shannon Wiener index, both in plants propagated by rhizome (H' = 0.986) and in those propagated by seed (H' = 0.981) (App. 2), showing no significant differences between the two genetic groups when using the IRAP and SCoT molecular markers (Tab. 4).

Nei's genetic diversity

The Nei's genetic variation generated was He=0.27505 in the seed group and He=0.28149 in plants propagated from rhizomes, demonstrating that plants from rhizomes exhibit greater genetic diversity (Tab. 4; FIS=0.25). Additionally, using an FIS=0.5, the expected heterozygosity was higher in

Table 4. Genetic diversity estimators in *C. palmata* (Lynch and Milligan, 1994), when FIS=0.25 and FIS=0.5, using 22 IRAP loci (SUKKULA, NIKITA, 3'LTR) with the AFLP-SURV 1.0 program (Vekemans, 2002) in the two genetic groups.

IRAP markers	Groups	Number of individuals	NLocP	Locus per group	PLP	He	S.E. (Hj)
FIS 0.25							
SUKKULA, NIKITA, 3'LTR	Seeds	28	22	15	68.2	0.27505	0.03831
	Rhizomes	28	22	17	77.3	0.28149	0.03568
FIS 0.5							
SUKKULA, NIKITA, 3'LTR	Seeds	28	22	15	68.2	0.25352	0.03810
	Rhizomes	28	22	18	81.8	0.24527	0.03132

NLocP: number of polymorphic loci at 5%. Evaluates allelic frequencies (0.05-0.95).
PLP: percentage of polymorphic loci at 5%.
He: expected heterozygosity, in proportion to Hardy-Weinberg genotypic data indicating genetic diversity.
S.E. (Hj): standard error of genetic diversity.
FIS (0.25 and 0.5, if H&W assumed) 1,000 bootstrap.

plants propagated by seeds (He=0.25352) and lower in plants by rhizome (He=0.24527). The percentage of polymorphic loci (PLP) was 81.8% in plants propagated by rhizome, compared to 68.2% in plants from seeds with an FIS=0.5. Therefore, using the IRAP molecular markers, no significant differences in genetic diversity were observed between the two genetic groups.

Polymorphic loci with SCoT molecular markers

The behavior of the SCoT marker system in relation to the genetic groups was identified. With FIS=0.25 and FIS=0.5 (Tab. 5), using 26 polymorphic loci of the SCoT markers, heterozygosity and inbreeding were determined through the SCoT-3, SCoT-5, and SCoT-18 markers. These results demonstrated that the seed group exhibited a higher number of polymorphic loci with the SCoT markers, indicating that these markers are robust and informative for genetic diversity studies.

Nei's genetic diversity with SCoT molecular markers

Genetic diversity was higher in the group of plants from rhizomes (He=0.29560) compared to the seed propagation group (He=0.26788), suggesting that rhizome plants are more heterozygous. Additionally, assuming an FIS=0.5 with the SCoT molecular markers, the results demonstrated greater heterozygosity in plants from rhizomes (He=0.25861) compared to plants from seeds (He=0.23249). These differences indicate the presence of heterozygous individuals in the northern region, although genetic diversity is similar in both genetic groups without significant differences (Tab. 5).

Genetic diversity in plants from rhizomes

Genetic differentiation was confirmed through the SUKKULA molecular marker, by sampling 24 individuals of *C. palmata* within the northern region. The results show different polymorphic loci of approximately 700; 1,000; and 1,300 bp (Fig. 6), demonstrating interspecific genetic variation in plants from rhizomes of the site with older plants (site where *C. palmata* was first cultivated over 150 years ago). The evaluation of polymorphism in samples H1, H3, and H4-H5 identified null alleles due to the absence of bands, which could be induced by somatic mutations or by the constant cultivation of rhizomes in the region. These data suggest that there is greater genetic diversity in plants propagated by rhizomes, indicating a possible introduction of elite genotypes in the sampled sites.

Table 5. Genetic diversity estimators (Lynch and Milligan, 1994), when FIS=0.25 and FIS=0.5, in *Carludovica palmata*, using (26) SCoT loci (SCoT-3, SCoT-5, SCoT-18) With the AFLP-SURV 1.0 program (Vekemans, 2002) in the two genetic groups.

ScoT markers	Groups	Number of individuals	NLocP	Locus per group	PLP	He	S.E. (Hj)
FIS=0.25							
SCoT-3, SCoT-5, SCoT-18	Seeds	28	26	19	73.1	0.26788	0.03003
	Rhizomes	28	26	18	69.2	0.29560	0.03665
FIS=0.5							
SCoT-3, SCoT-5, SCoT-18	Seeds	28	26	19	73.1	0.23249	0.02966
	Rhizomes	28	26	18	69.2	0.25861	0.03455

NLocP: number of polymorphic loci at 5%. Evaluates allelic frequencies (0.05-0.95).
PLP: Percentage of polymorphic loci at 5%.
He: Expected heterozygosity, in proportion to Hardy-Weinberg genotypic data indicating genetic diversity.
S.E. (Hj): Standard error of genetic diversity.
FIS (0.25 and FIS=0.5, if H&W assumed) 1,000 bootstrap.

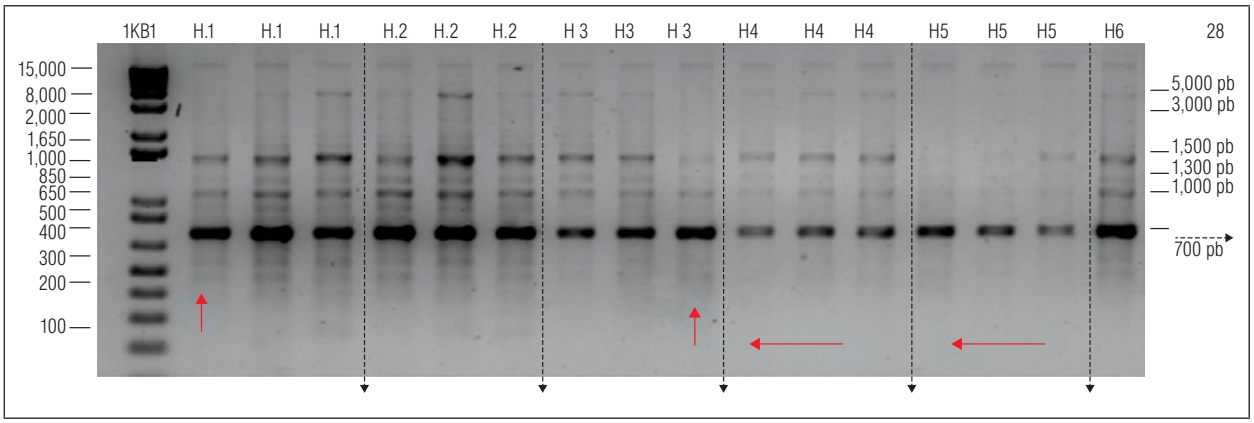

Figure 6. Genetic variation in plants propagated by rhizome within sites for stady, with the SUKKULA molecular marker. Conditions: 75 V, 70 min in 1.5% agarose. Molecular weight marker 1 KB Plus Ladder Invitrogen, ethidium bromide 0.8 µL, and 2 µL 6X DNA Loading Dye, Thermo Scientific. (H1-H6): groups of sampled individuals. H1: samples from hacienda 1. H6: samples from hacienda 6. Red arrows indicate polymorphism.

DISCUSSION

Cultivation of *C. palmata* in Mexico

In the northern region of the state of Campeche, *C. palmata* is primarily cultivated through vegetative propagation (rhizome). Farmers remove the inflorescences and infructescences to obtain the heart (immature leaves), an activity that limits the sexual reproduction of the species. Salgotra and Chauhan (2023) mention that during vegetative propagation, there are losses of some components of genetic diversity, increases in somatic mutations, the generation of genetic bottlenecks, increased inbreeding, low gene flow, and greater susceptibility to pathogen attacks.

In a study with the species *Nymphoides peltata* (Menyanthaceae), Barrett (2015) demonstrated that during exclusively clonal propagation, there are alterations during cross-pollination, leading to problems in the fertilization of ovules. Therefore, seed reproduction is affected. Begna (2021) also points out that during exclusively clonal propagation, there is a loss of genetic diversity and important alleles, in addition to being prone to inbreeding events.

Genetic diversity with dominant molecular markers

The seven Inter-Retrotransposon Amplified Polymorphism (IRAP) markers described for barley (Shirasu

et al., 2000) three were reproducible in *C. palmata*: SUKKULA, NIKITA, and 3'LTR. SUKKULA generated a greater number of polymorphic loci, which were reasonably informative (PIC<0.25). There are 36 molecular markers described for rice (Collard and Mackill, 2009). In the study with *C. palmata*, 34 molecular markers presented monomorphic bands, and three were reproducible: SCoT-3, SCoT-5, and SCoT-18. Only SCoT-18 generated polymorphic loci that were reasonably informative (PIC<0.25, Tab. 2), most generated monomorphism for both propagation groups. These results suggest good reproducibility of the markers in generating monomorphisms, making them potential candidates for genetic stability studies, as they are well conserved in generating monomorphic loci.

Botstein *et al*. (1980) mention that the polymorphism information content (PIC) is the result of the total number of loci in relation to the fraction of loci (the discriminatory power of the molecular marker), as well as the efficiency and robustness of the molecular marker used. In our work, like a study with *Crocus sativus*, IRAP markers were evaluated (Alsayied *et al*., 2015). We obtained plants with polymorphism using the SUKKULA marker and monomorphic bands with NIKITA. In *C. sativus*, different geographical sites and collections did not show significant differences in the generation of polymorphic loci, obtaining monomorphism in their molecular analyses.

The reproducibility of the SUKKULA marker demonstrated a PIC of 0.28 in plants from rhizomes and 0.30 in plants propagated by seeds with the SCoT-18 marker, indicating that for each genetic group, the marker functions for the determination of polymorphism (Tab. 3; Fig. 2A-B). Quantitatively, the degree of polymorphism is measured with heterozygosity (Nei and Roychoudhury, 1974). In both propagation groups, the polymorphic content index was greater than 2.5, demonstrating that the markers used are functional for *C. palmata*.

On the other hand, Serrote *et al*. (2020) in their classification of PIC values, mention that a PIC value between 0 and 0.10 indicates low reproducibility of a dominant marker, while a PIC greater than 2.5 indicates high reproducibility of the molecular marker. Our results, validated with IRAP and SCoT markers, demonstrated that they are suitable for the analysis of genetic variation in *C. palmata*, being reasonably informative and reproducible.

In studies with *C. sativus*, like our work, SUKKULA was the most polymorphic. While it did not show specific loci for *Crocus*, it did generate polymorphic and monomorphic bands in the clonal propagation groups of 500; 1,000; 1,500; and 3,000 bp. In *Crocus*, bands were generated from 100 bp to approximately 4 kb, in addition to the absence of bands (null alleles), which were taken as accessions with different polymorphic loci.

In our work, like *C. sativus*, we obtained plants with polymorphism using the SUKKULA marker, which presented a polymorphic locus at the 600 bp position in plants propagated by rhizomes. Another molecular marker was NIKITA, which in *C. sativus* and different geographical sites did not show significant differences in the generation of polymorphic loci, obtaining monomorphism in their molecular analyses. In comparison with our analyses using the NIKITA marker, we obtained monomorphism, like *C. sativus*, in both propagation groups, and there were no significant differences (Tab. 4 and 5).

In their analyses, Sathapondecha *et al*. (2021) validated SCoT-18 as the best marker compared to RAPD, being reproducible for different monocotyledonous species, such as rice, oil palm, and onion, suggesting that SCoT-18 is a suitable polymorphic marker. In our work with *C. palmata*, 34 SCoT molecular markers were standardized, generating monomorphic bands ranging from 100 to 3,000 bp. Only SCoT-18 presented a greater number of polymorphic loci in plants from seeds. On the other hand, SCoT-10, SCoT-20, and SCoT-36 did not amplify any of the DNA samples in *C. palmata*, indicating that these markers are not functional for the species (App. 1; Fig. 4). The best polymorphic molecular marker is SCoT-18.

In their studies, Saboori *et al*. (2019) estimated genetic diversity and structure in date palm cultivars, an economically important palm in Eastern Iran, where the genetic structure was unknown. They used different genetic diversity parameters and demonstrated that two cultivars were the most diverse: 'Mazafati' showed a Shannon index of I=0.348, a heterozygosity of He=0.233, and a polymorphism percentage of %P=67.86; the other most diverse cultivar was Kalooteh with I=0.311, He=0.206, and %P=62.5, demonstrating moderate levels of genetic diversity with the different loci of the SCoT markers.

In our analyses, different genetic diversity estimators were also used, such as the Shannon index (I or H'),

Nei (Ne), expected heterozygosity (HE), and percentage of polymorphic loci (PPL), for the two sites and with the two marker systems. The results with the SUKKULA marker in rhizome plants showed NA=9, while in plants from seeds NA=7. Genetic diversity (He or Hj) was greater in the rhizome plant group (HJ=0.2892), with a percentage of polymorphic loci (PPL=75%), while in plants propagated by seeds (HJ=0.27056) and PPl=75% (Tab. 4). No significant differences were found between both groups, although greater heterozygosity was obtained in the rhizome group. For both markers, genetic diversity was similar without significant changes between the groups and in relation to the markers used.

Genetic structure in *C. palmata*

Shannon-Weiner index in C. palmata

The total allelic diversity of the propagation groups was determined using the Shannon-Weiner index. This index is used in the biological field to measure total diversity and genetic diversity (Sun and Ren, 2021). The results with the Shannon-Weiner index were H'=0.986 in plants propagated by rhizomes, and H'=0.981 in plants from seeds. These results suggest that there is low genetic diversity in propagation systems. Although the Shannon index (H') can vary in dominance, distribution, abundance, and genetic composition (uniformity) and species richness (Omayio *et al*., 2019; Kunakh *et al*., 2023), in *C. palmata*, in both genetic groups, genetic diversity was low. The scenario of factors affecting the species is broad, including limitations in sexual reproduction (limitation in seed production), as well as its exclusively clonal propagation and possible crossings between related parents.

Nei's genetic diversity in C. palmata

The heterozygosity of a marker indicates the probability that an individual will be heterozygous at a marker site position, depending on allelic frequency. When the value is equal to 1, it represents high allelic frequency, and it is calculated using Nei's formulation (Nei and Li, 1979). The level of genetic diversity of Nei in *C. palmata* was performed using the AFLP V2 software, and genetic diversity, genetic structure, and possible related scenarios among individuals were calculated using Wright's F statistic (Wright, 1977). This statistic, based on the inbreeding coefficient of a population (FIT), uses the FIS estimator, which determines the inbreeding of matings in a population and is related to heterozygosity (Pérez-Pereira *et al*., 2022).

In *C. palmata*, the inbreeding coefficient (FIS=0.25) demonstrated genetic diversity for both groups, resulting in greater heterozygosity in plants from rhizomes (He=0.2892) than in plants from seeds (He=0.2756). This scenario demonstrated that mating types may exist between siblings, and that genetic diversity is similar without significant differences. These results indicate that there is inbreeding within the northern region, and that genetic diversity is limited, possibly due to the founder's event more than 150 years ago. Additionally, it was demonstrated that *C. palmata* is experiencing crossings between parents.

Perspectives on genetic diversity studies

In the northern region of Campeche (Mexico), genetic diversity is limited, and there is crossing between parents in the region. Several factors are involved, such as the domestication bottleneck that the species suffered, followed by a founder effect during its introduction to the northern region of Campeche approximately 150 years ago. Exclusively clonal propagation and the limitation in the production of infructescences, since farmers attribute the premature decline of the plant to the maturation of reproductive structures, could worsen over the years. This risks the species losing the ability to produce fertile seeds definitively and increasing crossings between parents, leading to inbreeding events. Similarly, exclusive propagation by rhizomes (suckers) would seriously affect cultivation. Another inconvenience is the lack of studies on pathogens that affect the species in Mexico, which are important to consider in future research works.

To address these problems, the following alternatives would be considered: selection of genotypes efficient in rapid propagation (rhizomes) and fiber quality; selection of genotypes with excellent production of immature leaves for germplasm banks; establishment of seed germination systems for elite clonal lines; selection of genotypes resistant to drought and diseases; validation of new genetic markers that allow differentiation of clonal lines in the field; conducting other genetic diversity studies in the region to understand the risk the crop is in, as it is currently propagated exclusively by clonal means; study of inbreeding events in the species; and introduction of new genotypes of *C. palmata* in the northern region.

CONCLUSIONS

The genetic diversity studies of *C. palmata* in the northern region of Mexico demonstrated low genetic diversity using IRAP and SCoT molecular markers. The use of these dominant markers demonstrated their functionality and that they are reasonably reproducible for the determination of genetic diversity in *C. palmata*.

The genetic diversity of *C. palmata* in plants propagated by rhizomes demonstrated the introduction of a few selected genotypes (founder effect); therefore, genetic diversity is limited. Expected heterozygosity in *C. palmata* was similar in plants propagated by rhizomes and in plants propagated by seeds, without significant changes due to a narrow genetic base. In the northern region, *C. palmata* is experiencing crossings between parents (inbreeding events).

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