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Genetic variability in mother plants and rhizome offshoots of *Agave* híbrido 11648

Variabilidad genética en plantas madre y vástagos de rizoma de *Agave* híbrido 11648

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ABSTRACT

Agave hybrid 11648 is the most widely cultivated agave for fiber production worldwide and reproduces, mainly, by clonal rhizome shoots. To determine the influence of asexual propagation on the reduction of the genetic diversity of hybrid 11648, this study evaluated the genetic variability between mother plants and their clonal offspring using six primer combinations. 586 AFLP markers were generated, with 40 % polymorphism and significant genetic differentiation (Φ_{ST} = 0.455, p = 0.0001). The Shannon indices (I = 0.226) and expected heterozygosity (He = 0.153) show low genetic diversity. Knowledge of genetic variability is essential to develop strategies that favor generating more disease-resistant plants and help mitigate the negative effects of low genetic diversity in the long term.

KEY WORDS: AFLP, *Agave*, fiber, genetic variability.

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RESUMEN

El *Agave* híbrido 11648 es el agave que más se cultiva a nivel mundial para la producción de fibra y este se reproduce, principalmente, por vástagos clonales de rizoma. Para determinar la influencia de la propagación asexual en la reducción de la diversidad genética del híbrido 11648, este estudio evaluó la variabilidad genética entre plantas madre y sus vástagos clonales utilizando seis combinaciones de cebadores. Se generaron 586 marcadores AFLP, con un 40 % de polimorfismo y una diferenciación genética significativa (Φ_{ST} = 0.455, p = 0.0001). Los índices de Shannon (I = 0.226) y heterocigosidad esperada (He = 0.153) muestran una baja diversidad genética. El conocimiento de la variabilidad genética es fundamental para desarrollar estrategias que favorezcan la generación de plantas más resistentes a enfermedades y ayuden a mitigar los efectos negativos de la baja diversidad genética a largo plazo.

PALABRAS CLAVE: AFLP, *Agave*, fibra, variabilidad genética.

Introduction

The hybrid *Agave* 11648 (H.11648) was obtained during the first half of the twentieth century by George W. Lock in Tanzania, Africa, by crossing between *Agave angustifolia*×*Agave amaniensis* Trel & Nowel and later backcrossing with *A. amaniensis* (Zhang *et al*., 2013; Bautista-Montes *et al*., 2022). It is characterized by the absence of marginal spines and higher leaf fiber content compared to *Agave fourcroydes* Lem. and *Agave sisalana* Perr. (Zhang *et al*., 2013); according to Hartemink & Kekem (1994), H.11648 can be harvested earlier and reach a yield of up to 3 t ha^{-1} in contrast to *A. sisalana*, which produces about 1.5 t ha^{-1} of fiber using the whole leaf. Consequently, the main *Agave* crop worldwide for obtaining fiber is H.11648 (Jin *et al*., 2020).

Plants of the genus *Agave* can be propagated both sexually, through seeds, and asexually through clonal rhizome stems or inflorescence bulbils (Abraham-Juárez *et al*., 2015; Figueredo-Urbina *et al*., 2017; Sánchez *et al*., 2020). Although agave populations that propagate clonally through rhizomes are genetically homogeneous (Infante *et al*., 2003), their asexual propagation has resulted in a loss of crops diversity, increasing their vulnerability to various pathogens (Rivera-Lugo *et al*., 2018; Trejo *et al*., 2018; Klimova *et al*., 2023; Figueredo-Urbina *et al*., 2024). Although H.11648 can reproduce by seed, it is propagated mainly by clonal rhizome stems. Asexual propagation in species such as *Agave tequilana* (Trejo *et al*., 2018; Chávez-Sánchez *et al*., 2022), *Agave mapisaga* (Figueredo-Urbina *et al*., 2021), and *Agave fourcroydes* (Infante *et al*., 2003), has been the subject of study due to its implications for genetic variability.

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Studies carried out on *A. fourcroydes* and *A. tequilana* indicate that the analysis of genetic variability allows the identification of elite materials that can be selected to reproduce plants with superior agronomic characteristics, such as greater genetic resistance to diseases or higher performance in fiber production (Infante *et al*., 2003; Chávez-Sánchez *et al*., 2022). Since the use of molecular markers has proven useful for the identification of individuals with desirable characteristics in genetic improvement programs, the present work aimed to determine the genetic variability in the mother plants and their clonal rhizome offshoots of the *hybrid Agave* 11648 using molecular markers of amplified fragment length polymorphism (AFLP).

Material and Methods

Plant material

Leaves were collected from six H.11648 mother plants (designated P2 to P6) and five clonal rhizome offshoots from each. Samples were also collected from a potted mother plant (P1) and two of its clonal offspring as a control group. In total, 39 samples were analyzed, corresponding to the sum of seven mother plants and 32 clonal offspring, collected at the Yucatan Scientific Research Center, A.C., located in Mérida, Yucatán. To ensure the homogeneity of the samples, healthy plants with an approximate height of 2 m for mother plants and 0.6 m for their offspring were chosen; The control group reported a height of 0.8 m for the mother plant and 0.3 m for its offshoots.

DNA extraction was performed from a 1 cm segment of the leaf base, according to the method of Echevarría-Machado *et al.* (2005), with slight adaptations. First, the tissue was ground in liquid nitrogen, and a 0.5 g sample was taken to which polyvinylpyrrolidone (Sigma-Aldrich®) was added. Next, the extraction buffer β-mercaptoethanol (100 µL, 100mM) and SDS (100 µL, 20 % Sigma-Aldrich®) were incorporated. Samples were incubated at 65°C for 15 min in a Thermo Scientific® AquaBath™ incubator and then allowed to stand at room temperature for 12 h. After incubation, 500 µL of 5M potassium acetate (Sigma-Aldrich®) was added, and the tubes were mixed by inversion and then incubated for 20 min in ice. The tubes were then centrifuged at 13,000 rpm for 20 min. The supernatant was transferred to a 2 mL tube, and 300 µL of silica (Sigma-Aldrich®) was added. Samples were inversion mixed for 5 min and centrifuged at 12,000 rpm for 6 min. The supernatant was discarded, and the pellet was allowed to dry for one hour before being re-suspended in 50 µL of sterile water (PISA®). The purity evaluation and DNA quantification were performed as described by Ballesteros-Rodríguez *et al*. (2022).

AFLP Development

AFLP analyses followed the protocol Vos *et al*. (1995) described, with slight modifications proposed by Ballesteros-Rodríguez *et al*. (2022). In a preliminary evaluation of five plants, six pairs of EcoRI and MseI primers were selected based on the number of amplified bands. The EcoRI primers used, which were fluorescently labeled, were AAC/CAT, ACG/CAT, ACT/CAT, AAC/CAC, ACG/CTA, and ACT/CAC. The amplified bands were detected with a Beckman Coulter CEQ

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8800 DNA sequencer®.

Data analysis

Data analysis was performed as described by Ballesteros-Rodríguez *et al*. (2022), using the GeneMarker® v1.75 software and the UPGMA grouping method of the NTSYSpc® software. Bootstrap values were calculated from 1000 trees re-sampled using FreeTree software (Felsenstein, 1985).

The binary matrix generated with 586 AFLP markers was used to perform a molecular analysis of variance (AMOVA) using GenAlEx 6.5 software (Peakall & Smouse, 2012). The AMOVA provided metrics such as the percentage of polymorphic loci, the Shannon information index (I), and the expected heterozygosity (He). The metrics were calculated assuming each locus represents a pair of alleles and considering the presence and absence of the AFLP fragments in each band.

Results and Discussion

Six primer combinations in selective amplification generated 586 AFLP markers, an average of 98 markers per combination. AMOVA determined that 55 % of molecular variation occurred between mother plants and offshoots and 45 % within each mother plant and its offshoots. These results indicate significant genetic differentiation between the mother plants and their offspring, with a value of Φ_{ST} = 0.455 (p = 0.0001), suggesting that the offspring present a different genetic variability than the parent plants (Table 1).

The percentage of polymorphism observed varied in the mother plants analyzed and their offshoots, being 28 % for P1, 39 % for P2, 41 % for P3, 44 % for P4, 36 % for P5, 45 % for P6 and 44 % for P7. With 40 % as the general mean of polymorphism, it can be inferred that there is genetic variability between the mother plants and their offspring, and although they share a common genetic basis, certain changes in the genetic profile of the offspring are observed. In *A. tequilana*, genetic variability in materials propagated asexually through rhizomes has been documented by AFLP analysis (Torres-Morán *et al*., 2005; 2013; Chávez-Sánchez *et al*., 2022). Abraham-Juárez *et al*. (2009) reported 75 % polymorphism in clonal offshoots, while inflorescence bulbils showed polymorphism of 86 % and seed-derived plants of 90 %.

The Shannon information index (I = 0.226) and the expected heterozygosity value (He = 0.153) obtained in this study reflect a relatively low genetic diversity among the mother plants and their offspring. Both values suggest that although genetic variability exists, diversity is limited due to asexual reproduction, as both mother plants and offspring share a similar genetic basis.

In previous studies on *A. tequilana* Weber var. Azul, Ruiz-Mondragón *et al*. (2022), when analyzing 68 mature plants in the state of Jalisco, obtained a value of He = 0.120, similar to what is reported in this research. With five plants in Guanajuato employing AFLP, Rivera-Lugo *et al*.

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(2018) reported a value of He = 0.205, while Vargas-Ponce *et al*. (2009), using ISSRs in 22 plants collected in Tequila, Jalisco, obtained a value of He = 0.118. Trejo *et al*. (2018), using microsatellites in 23 plants from cultivated fields in Tequila, Jalisco, reported He = 0 because all individuals presented the same genotype. However, in populations with less intensive management, they observed greater genetic diversity, for example, the varieties 'Sigüin' (He = 0.409) and 'Chato' (He = 0.435) presented higher levels of expected heterozygosity. Similarly, Figueredo-Urbina *et al*. (2021), in the analysis of 19 traditional varieties of pulque agave (varieties of *A. americana, A. salmiana*, and *A. mapisaga*), obtained He values between 0.204 and 0.721, which reflects a remarkable genetic diversity.

The genetic similarity index among the 39 individuals ranged from 0.59 to 0.94. In P1 and its offshoots, the index ranged between 0.82 and 0.91; in P2, between 0.83 and 0.92; in P3, from 0.77 to 0.91; in P4, from 0.78 to 0.93; in P5, from 0.83 to 0.93; in P6, from 0.75 to 0.86; and in P7, from 0.77 to 0.84. These results are similar to those reported in *A. tequilana*, where the coefficient of similarity between the mother plants and their offspring ranged from 0.770 to 0.890 (Torres-Morán *et al*., 2010).

The dendrogram generated showed two main groups: one composed of P7 and its clonal offspring and another subdivided into six subgroups (Figure 1). A remarkable similarity is observed between the mother plants and their offspring, consistent with the Torres-Morán *et al.* (2010) findings in *A. tequilana.* However, differences in the pattern of bands between the mother plants and the shoots and between the shoots themselves are evident. This variability is essential for agave improvement, as it allows the selection of individuals with desirable characteristics in cloned populations. Selection and *in vitro* culture could facilitate obtaining elite materials with improved genetic disease resistance (Chávez-Sánchez *et al*., 2022).

In farmed agaves, genetic variability is variable and depends on factors such as species, intensity of use, management practices, and degree of domestication. In *A. tequilana*, clonal propagation has been widely promoted, resulting in a decrease in genetic variability. In contrast, genetic variability in wild *Agave* populations tends to be high, in part due to the intervention of pollinators such as nectarivorous bats, which play a crucial role in the conservation of genetic diversity (Figueredo-Urbina *et al*., 2021; Ruiz-Mondragón *et al*., 2022). It should be highlighted that the contrast between the results of this research and those of other studies is complex due to the differences in the molecular methodologies and sampling designs used.

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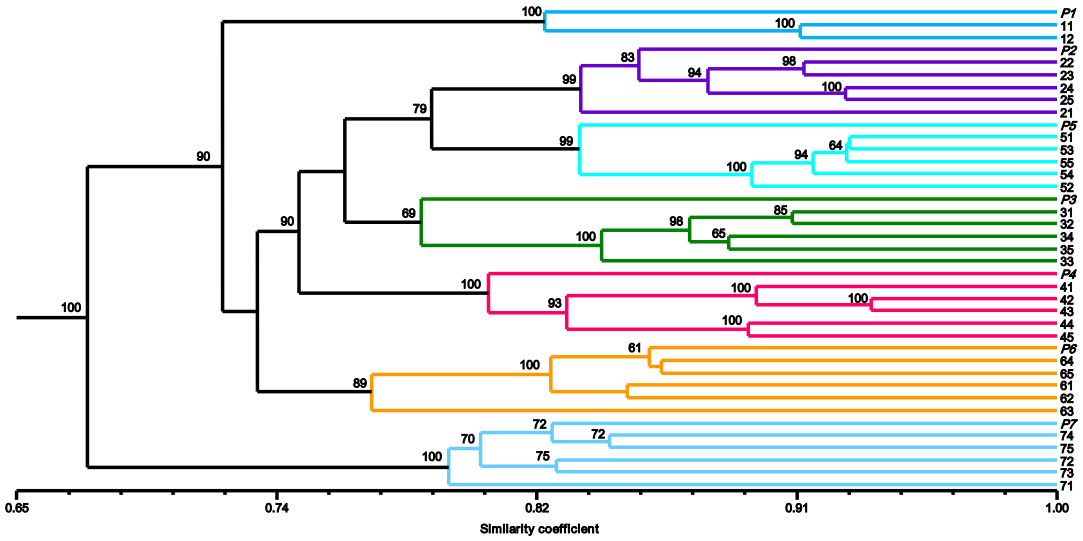

Figure 1. Dendrogram showing the genetic relationships between mother plants and their clonal rhizome offspring.

The numbers indicate the percentage of *bootstrap* repeats obtained at each node from 1000 re-sampled trees. The labels correspond to the following identifications: P1 indicates the mother plant; 11 and 12 are clonal rhizome offshoots derived from P1; P2 is another mother plant, and 21, 22, 23, 24, 25 are clonal rhizome offshoots derived from P2, and so on.

Source: Authors' elaboration based on AFLP results

Table 1. Analysis of molecular variance between mother plants and clonal rhizome offshoots of hybrid *Agave* 11648 by AFLP.

	df	SCD	MSD	Variance	Φ_{ST}	*P-value*
Between mother plants and offshoots	6	1873.526	312.254	46.350	0.455	0.0001
Within each mother plant and its offspring	32	1777.500	55.547	55.547		
Total	38	3651.026				

df: degrees of freedom, SCD: Sum of squares of differences, MSD: Mean of quadratic deviations. P values were derived from a random permutation test with 10,000 permutations. Source: Own elaboration

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Conclusions

This study shows that although asexual reproduction is the main route of propagation, the genetic profile of clonal offspring does not completely homogenize. Polymorphism, expected heterozygosity, and genetic diversity are relatively low, which could be attributed to clonal propagation. However, proper management of this genetic variability through selection could facilitate the genetic improvement of clonal populations.

It is important to highlight that the low genetic diversity observed also carries risks, such as a reduced ability to adapt to environmental changes and increased vulnerability to disease. Therefore, knowledge of genetic variability is essential to generate strategies that favor flowering and the production of viable seeds. These strategies could, in turn, contribute to creating more disease-resistant plants and mitigate the adverse effects of low genetic diversity in the long term.

Authors' contribution

Conceptualization of Work, LFST; methodology development, EBR, LFST; software management, EBR; experimental validation, EBR, LFST; analysis of results, data management, writing and preparation of the manuscript, EBR; writing, revision, and editing, EBR, LFST.

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Conflict of interest

The authors declare no conflict of interest.

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