

## Taxonomic Significance of ISTR to Discriminate Species in *Agavaceae*

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**Abstract:** Family *Agavaceae* is endemic of American Continent. From the around 300 species recognized in this family, 217 occur in Mexico. Relevant ethnobotanic relationships among *Agavaceae* and the several native human cultures of the American Continent have been established since prehispanic times. *Agave* is one of the most important genus in that family due to its great diversity and abundance, mainly in arid and semiarid regions of Mexico. In this country, near to 15 species of *Agave* are used to elaborate alcoholic beverages. *Agave tequilana* weber var. azul is indubitable the most important of them because is the raw material to elaborate a particular worldly famous class of mescal, named tequila. *Agave salmiana*, *A. maximiliana* and *A. durangensis* are species less famous than *A. tequilana*, but they also have a high quality and level of carbohydrates and in fact, support local mescal industries. In these last species several taxonomic controversies exist concerning their specific delimitation. In this study the molecular characterization of eight species of *Agavaceae* using ISTR was performed in order to determine the significance of these markers for discriminating among specific taxa. The results suggest that these molecular markers are worthy to typify species of *Agavaceae* and detect intrapopulation variability.

**Key words:** *Agavaceae*, retrotansposons, DNA markers, ISTR

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### INTRODUCTION

*Agavaceae* is an endemic family of the American Continent, found from southern Canada to Northern Sudamerica<sup>[1]</sup>. This family reaches its highest levels of richness and diversity in Mexico, which is considered center of origin of *Agavaceae*<sup>[2]</sup>.

Citological, morphological, chemical, biogeographical<sup>[3]</sup> and molecular<sup>[4,5]</sup>, evidences, may recognize *Agavaceae* as a taxonomic family divided in two subfamilies: Yuccoideae and Agavoideae. Subfamily Yuccoideae including two upper-ovary genera: *Yucca* y *Hesperaloë* and subfamily Agavoideae including, according to García-Mendoza and Galván<sup>[1]</sup>, eight lower-ovary genera: *Agave*, *Beschorneria*, *Furcraea*, *Manfreda*, *Polianthes* and *Prochnyanthes*, but according to Rocha *et al.*<sup>[6]</sup>, besides the former ones, the genus *Hesperoyucca* is also included.

Among the 293<sup>[7]</sup> or 300 species<sup>[6]</sup> recognized in the family *Agavaceae*, 217 occur in Mexico<sup>[1]</sup>. One

hundred and fifty five species are recognized as endemic. The most rich States in *Agavaceae* are, in decrease order, Oaxaca with 52 taxa, Durango and Puebla with 43 and Sonora and Jalisco with 40<sup>[1]</sup>.

*Agave* is the biggest genus in *Agavaceae*, with around 166 species, from which 125 grow in Mexico<sup>[1,6]</sup>. The relevant ethnobotanic relationships between the elements of this genus and the ancient and present cultures of Mexico have been well documented<sup>[8,9]</sup>. Several authors have described the use of *Agave* as source of fibers, food and beverages<sup>[10]</sup>. The jesuita José de Acosta describes *Agave* as “El árbol de las maravillas, del que los nuevos o chapetones (como en Indias los llaman), suelen escribir milagros, de que da agua y vino, y aceite y vinagre, y miel, y arropo e hilo, y aguja y otras cien cosas” (Historia Natural y Moral de las Indias, 1590)<sup>[11]</sup>. In addition, *Agave* is used as natural fences to avoid the soil erosion and as cattle food<sup>[12]</sup>. However, only few reported uses yet prevail and have been transformed throughout the time.

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At the present, the relevance of *Agave* has increased mainly because of the increased demand of alcoholic beverages like tequila<sup>[13]</sup> and mescal<sup>[14]</sup> but also due to the research on potential sources of prebiotics<sup>[15]</sup>. In all the cases, the authentication of the species of *Agave* to be used is an important requirement in the quality control of the manufacturing processes. In Mexico, with exception of *Agave tequilana* Weber var. azul, species like *Agave durangensis*, which support local industries of alcoholic beverages and which with just begin an industrialization process, techniques are needed to guarantee the botanical origin of plants, according to the respective origin denomination statement. Molecular characterization is an important technique, which with plant typification and identification can be made in a relative easy and fast manner<sup>[16]</sup>.

Several marker systems are based on retrotransposons<sup>[17, 18]</sup>, these markers are a ubiquitous and ancient particular class of transposable elements of plant genome<sup>[19]</sup>, replicating through a cycle of successive transcription, reverse transcription and integration into genome<sup>[20]</sup>. Inverse Sequence Tagged Repeats (ISTR) is a molecular marker based on retrotransposons, using encoded reverse transcriptase sequences bounded by *copia-like* elements. ISTR has showed to be a worthy molecular tool to detect intrapopulation variability in economically important cultivars of coffee, soya, *Agave tequilana*, *Agave fourcroydes* and *Agave cocui*<sup>[21-26]</sup>. In this study the molecular characterization of eight species of *Agavaceae* (*Furcraea* sp. (L.) Haw, *Agave americana* L., *Agave guadalajarana* Trelease, *Agave maximiliana* (Berger) Gentry, *Agave salmiana* Otto, *Agave desmettiana* Jacobi, *Agave angustifolia* Haw, *Agave tequilana* Weber and *Agave durangensis*) was performed, using ISTR, in order to determine the significance of these markers to typify and discriminate among these species of *Agavaceae* and detect intrapopulation variability.

## MATERIALS AND METHODS

**Plant material:** Leaves of *Furcraea* sp., *A. americana*, *A. desmettiana*, *A. maximiliana*, *A. salmiana*, *A. tequilana* and *A. angustifolia* were obtained from four different individuals of each species, growing in the *Agavetum* of Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara (UdG), México. These seven all taxa were identified by Ana Lilia Viguera, botanist of UdG. Leaves of *Agave durangensis* were also collected from

four plants growing in a natural population in Sierra de Registro, Durango, México (23°59.4' 38'' N, 104°22.5' 13'' W, 1928 m), reference samples were deposited in Herbarium CIIDIR.

**DNA extraction:** Total DNA of each sample was prepared from leaves using the CTAB procedure of Keb-Llanes *et al.*<sup>[27]</sup>.

**ISTR analysis:** Retrotransposon fragments were amplified using the following primer pairs F9/B6 (d5'[TTA CCT CCT CCA TCT CGT AG]3'/d3' [GGT TTC ACT TTG TCC TTA G]5'), and FI/B6 (d5'[GCA CTC CAC CAA GAA TAC C]3'/d3' [GGT TTC ACT TTG TCC TTA G]5'). PCR amplifications were performed according to Osorio *et al.*<sup>[23]</sup>, on 2.5 ng of DNA in 20 µL reaction volumes containing 0.25 units of Taq DNA polymerase (Promega®), 3 mM MgCl<sub>2</sub>, 0.3 µM each primer, 0.25 mM each dNTP and the buffer supplied with the enzyme. Cycling conditions were 3 min at 95°C followed by 40 cycles of 30 sec at 95°C and annealing at 45°C 1 min and extension at 72°C 2 min.

Amplification fragments were analyzed by denaturing polyacrilamide gels 6%. Samples were electrophoresed at 200W constant power and stained with silver salts, according to Sanguinetti *et al.*<sup>[28]</sup>.

**Data analysis:** The amplification profile of each sample was made up of all fragments resolved in their respective electropherogram. Each fragment was treated as a single molecular character. A binary (presence-absence) matrix formed by all individual samples (32 fragments vs. 32 individuals) was analyzed using the unweighted paired group of arithmetic averages method (UPGMA). Similarity coefficients were estimated using the program NTSYS 2.11.

## RESULTS AND DISCUSSION

In order to demonstrate the reproducibility and consistency of ISTR results in the eight species of *Agavaceae* analyzed, DNA of the same leaves was extracted and amplified with the same primer combination by duplicate in different days. The ISTR profiles were reproducible (Fig. 1).

Thirty two fragments (or loci) of the reverse transcriptase domain were amplified from the eight taxa of *Agavaceae* analyzed (Fig. 1). These results represent a strong contrast with most of the reports on ISTR markers indicating that a large number of loci are detected in a single ISTR analysis<sup>[24, 29, 30]</sup>, particularly with those of reported for *Agave fourcroydes*, in which

from 94-111 loci were detected<sup>[29]</sup>. Our results also represent a contrast with the 43-135 loci detected with AFLPs for eight species of *Agave*<sup>[31]</sup> and with the 93-262 loci founded also with AFLPs for several varieties of *Agave tequilana* Weber<sup>[16]</sup>, but are according to the results of Torres-Morán et al.<sup>[26]</sup> on different plantations of *Agave tequilana* Weber var. azul, who reported profiles composed by 35 amplification fragments, using the same ISTR pairs of primers, and with the 24-48 loci detected for eight species of *Agave* by Infante et al.<sup>[31]</sup>.

Only one amplified fragment was common to the eight taxa of *Agavaceae* analyzed. This could represent a family marker, as long as it would be found in more species of *Agavaceae* treated with the same pair of primers.

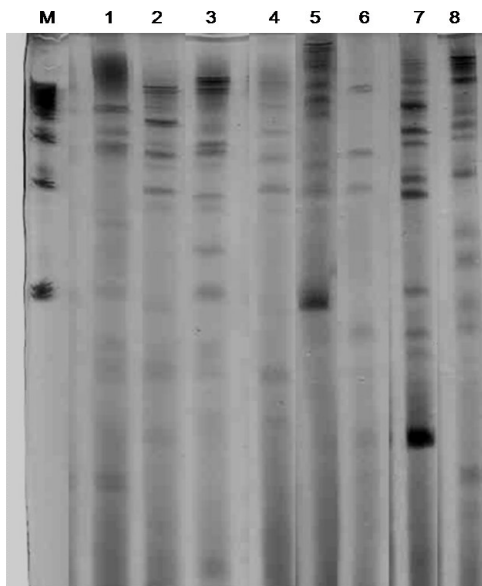


Fig. 1: Typical banding pattern founded using ISTR for eight species of *Agave*. M = 200 pb leader, 1 = *A. angustifolia*, 2 = *A. maximiliana*, 3 = *A. americana*, 4 = *A. desmettiana*, 5 = *A. salmiana*, 6 = *Furcraea* sp., 7 = *A. tequilana* and 8 = *A. durangensis*

According to Fig. 1, the number of amplified fragments per species was variable, six fragments were found in every individual of *Furcraea* sp., 16 were found in *A. salmiana*, 14-17 in *A. tequilana*, seven in *A. desmetiana*, 6-12 in *A. americana*, 7-10 in *A. maximiliana*, 8-9 in *A. angustifolia* and 13-14 in *A. durangensis*. Despite the observed variability in *A. tequilana*, *A. americana*, *A. maximiliana* and *A. durangensis*, each analyzed taxon displays a unique ISTR amplification profile.

The UPGMA analysis clearly distinguishes two groups (Fig. 2). One group was formed by *A. angustifolia*, *A. maximiliana*, *A. desmetiana* and *A. americana* (group I) and other group formed by *A. salmiana*, *A. durangensis*, *A. tequilana* and *Furcraea* sp. (group II).

ISTR markers display a close relationship between *Furcraea* sp. and the group II of *Agave*, although clearly separated (Fig. 2). Contrary to that stated on morphological basis by Gentry<sup>[8]</sup>, *A. angustifolia* and *A. tequilana*, two species belonging to the same group Rigidae, are grouped separately in this analysis.

The UPGMA analysis indicates that each of these eight species of *Agavaceae* can be distinguished from the others by a unique ISTR amplification profile. Although it was affirmed that markers revealing a great number of loci are more efficient in revealing polymorphisms<sup>[16]</sup> the number of fragments obtained from ISTR analysis is enough to reveal intrapopulation variability. Our results suggest that ISTR markers could be used in taxonomic analysis to delimitate and discriminate among species of *Agavaceae*.

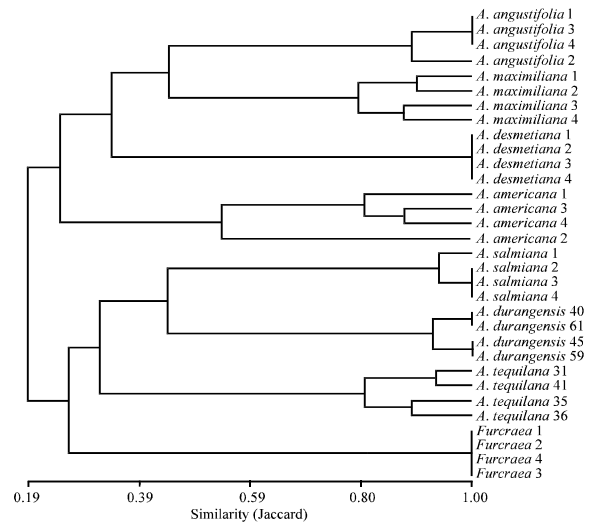


Fig. 2: Results of clustering depicting similarity among four individuals for eight *Agavaceae* species

ISTR amplification profiles confirm specific taxonomic separation made on a morphological basis. The eight species of *Agavaceae* are clearly distinguished from each other (Fig. 2).

ISTR profiles displayed variability in *A. angustifolia*, *A. maximiliana*, *A. americana*, *A. salmiana*, *A. durangensis* and *A. tequilana* (Fig. 2). This suggests that these markers could be worthy to detect intrapopulation variability in those species.

Previously, it has been reported genetic variability detected by ISTR among asexually propagated plants of *A. fourcroydes*<sup>[32]</sup> and in micropropagated plants of *A. tequilana*<sup>[25]</sup> despite the expecting fact that those plants should be genetically identical.

### CONCLUSION

ISTR markers are well distributed in the eight taxa of *Agavaceae* analyzed in this study, in a such a way that it is possible distinguish species-specific profiles. The complex pattern of retrotransposon polymorphism enables the discrimination among these species from the patterns of shared and unique bands. These profiles can be considered a valuable molecular marker at specific level in *Agavaceae*. Although more population studies on the distribution of ISTR markers among more species of this family are needed, these results suggest that ISTR profiles could be specific taxonomic markers in *Agavaceae*.

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