

Research article

## Aberrant meiotic behavior in *Agave tequilana* Weber var. azul

Domingo Ruvalcaba-Ruiz and Benjamin Rodríguez-Garay\*

Address: División de Micropropagación y Mejoramiento Genético Vegetal, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. Av. Normalistas 800 S.H. C.P. 44270, Guadalajara, Jalisco, México

E-mail: Domingo Ruvalcaba-Ruiz - druvalcaba\_1@yahoo.es; Benjamin Rodríguez-Garay\* - brodriguez@ciatej.net.mx

\*Corresponding author

Published: 23 October 2002

Received: 11 August 2002

*BMC Plant Biology* 2002, **2**:10

Accepted: 23 October 2002

This article is available from: <http://www.biomedcentral.com/1471-2229/2/10>

© 2002 Ruvalcaba-Ruiz and Rodríguez-Garay; licensee BioMed Central Ltd. This article is published in Open Access: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article's original URL.

### Abstract

**Background:** *Agave tequilana* Weber var. azul, is the only one variety permitted by federal law in México to be used for tequila production which is the most popular contemporary alcoholic beverage made from agave and recognized worldwide. Despite the economic, genetic, and ornamental value of the plant, it has not been subjected to detailed cytogenetic research, which could lead to a better understanding of its reproduction for future genetic improvement.

The objective of this work was to study the meiotic behavior in pollen mother cells and its implications on the pollen viability in *Agave tequilana* Weber var. azul.

**Results:** The analysis of Pollen Mother Cells in anaphase I (A-I) showed 82.56% of cells with a normal anaphase and, 17.44% with an irregular anaphase. In which 5.28% corresponded to cells with side arm bridges (SAB); 3.68% cells with one bridge and one fragment; 2.58% of irregular anaphase showed cells with one or two lagging chromosomes and 2.95% showed one acentric fragment; cells with two bridges and cells with two bridges and one acentric fragment were observed in frequencies of 1.60% and 1.35% respectively. In anaphase II some cells showed bridges and fragments too. Aberrant A-I cells had many shrunken or empty pollen grains (42.00%) and 58.00 % viable pollen.

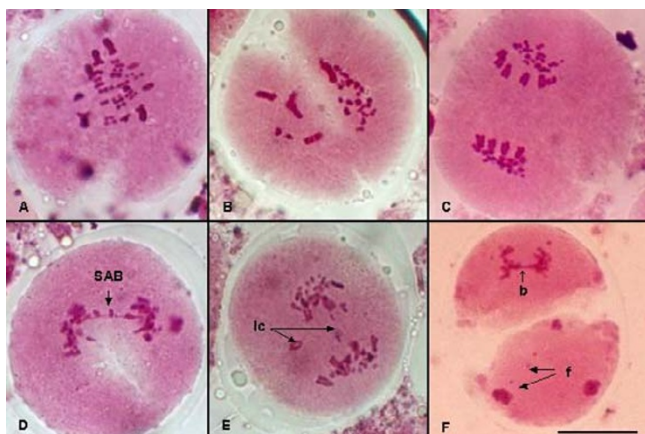
**Conclusion:** The observed meiotic irregularities suggest that structural chromosome aberrations have occurred, such as heterozygous inversions, sister chromatid exchanges, deletions and duplications which in turn are reflected in a low pollen viability.

### Background

The genus *Agave*, is distributed in the tropical and subtropical areas of the world and represents a large group of succulent plants, with 197 taxa from 136 species, and its center of origin is probably limited to Mexico [1]. The sub-genus *Agave* and particularly the sections *Rigidae* and *Sisalanae*, are cultivated because of their commercial importance for diverse purposes: a) alcoholic beverages such as tequila and mezcal; b) natural long and hard fib-

ers; and c) steroidal compounds and medicinal principles [2–4].

The genus *Agave* is a semelparous perennial that produces flowers only once towards the end of its life cycle (6–8 years for *A. tequilana*). This genus has been the object of cytological investigations only after 1933, since then, chromosome counts have been made on a large number of species. The basic chromosome number is  $x = 30$  and

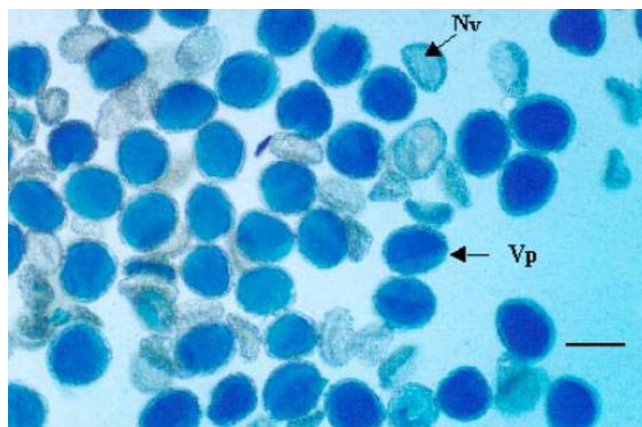


**Figure 1**  
PMC's showing meiotic regular and irregular phases in *Agave tequilana* Weber var. azul. A) M I with 30 normal bivalents. B) Aberrant M I. C) Normal A - I. D) A - I with one side arm bridge (SAB). E) Lagging chromosomes at A I (lc = lagging chromosomes). F) Dyad showing bridge and fragments at A - II (b = bridge; f = fragment). Bar for all microphotographs = 17  $\mu$ m

species ranging from diploid to hexaploid [5-7]. Cave [8] reported regular meiosis in five diploid, two tetraploid and one hexaploid species, and irregular meiosis in two polyploids, where bridges and fragments were observed at anaphase I. Brandham [9] carried out a cytological investigation in *Agave stricta*, for which he described a meiotic behavior heterozygous for a paracentric inversion, that produced a number of aberrations: one bridge and one fragment, two loops and one fragment, two bridges and two fragments, and two loops with two fragments at anaphase I.

More recently, Castorena-Sánchez *et al* [10] reported the first karyotype for *Agave tequilana* ( $2x = 60$ ), where chromosomes were classified and arm ratios, chromatin length and their variability are discussed. On the other hand, the nuclear DNA content, chromatin structure, and DNA composition were investigated in four *Agave* species including *A. tequilana* [11].

*Agave tequilana* Weber var. azul, is the only one variety permitted by federal law in México to be used for tequila production [12] which is the most popular contemporary alcoholic beverage made from agave and recognized worldwide. Despite the economic, genetic, and ornamental value of the plant, it has not been subjected to detailed cytogenetic research, which could lead to a better understanding of its reproduction for future genetic improvement.



**Figure 2**  
Pollen grains viability of *Agave tequilana* var. azul. Vp = viable pollen. Nv = non viable pollen. Bar = 0.5 mm

The objective of this work was to study the meiotic behavior in pollen mother cells and its implications on the pollen viability in *Agave tequilana* Weber var. azul.

## Results

The analysis of Pollen Mother Cells (PMC's) at metaphase I (M-I) showed 62.3% of the cells with thirty normal bivalents, (Fig. 1A), and 37.7% of PMC's presented an aberrant meiotic behavior at the same phase (Fig. 1B).

The chromosome complement of this species (and within the genus) is of a markedly bimodal persistence, having 10 large and 50 from medium to small chromosomes in the diploid. Thus, 5 large and 25 small bivalents can be seen at normal M-I (Fig. 1A).

The analysis of PMC's in anaphase I (A-I) showed 82.56% of cells with a normal anaphase (Fig. 1C) and, 17.44% with an irregular anaphase. In which 5.28% corresponded to cells with side arm bridges (SAB); 3.68% cells with one bridge and one fragment; 2.58% of irregular anaphase showed cells with one or two lagging chromosomes and 2.95% showed one acentric fragment; cells with only two bridges and cells with two bridges and one acentric fragment were observed in frequencies of 1.60% and 1.35% respectively (Fig. 1D, 1E, Table 1). Also, in anaphase II some cells showed bridges and fragments (Fig 1F). Aberrant meiotic cells produced 42% of shrunken or empty pollen grains, while the rest of normal dividing cells produced viable pollen (Fig. 2).

## Discussion

Our cytogenetic studies confirmed that *Agave tequilana* Weber var. azul is a diploid species with  $2n = 60$  chromosomes, and thirty bivalents from pollen mother cells confirm the basic chromosome number of  $x = 30$  [10].

**Table 1: Normal and irregular anaphase I (A-I) of *Agave tequilana* Weber var. azul**

PMC's	Total 814	%100.00
Regular	672	85.56
Cells with one or two lagging chromosomes	21	2.58
Cells with one bridge (SAB)	43	5.28
Cells with one bridge and fragment	30	3.68
Cells with two bridges (SAB)	13	1.60
Cells with two bridges and fragment	11	1.35
Cells with acentric fragment	24	2.95

The presence of bridges with or without fragment reflected structural changes such as heterozygous inversions, chromatid exchanges, deletions and duplications [14–16]. PMC's in A II with the presence of bridges and segment in each of the two cells of a dyad (Fig. 1F), could be an evidence of heterozygous inversions with 3 crossover points within the inversion loop [17], where similar aberrations were reported for *Agave stricta* [9].

The observed subchromatic or side arm bridges (SAB) could be originated from an abnormal function at the places where the chiasmata are formed [9,14]. In this study it could be observed that SAB involved long chromosomes as it was previously described for *Agave stricta* (Fig. 1D) [9]. Abnormal disjunction in some chromosomes, showed irregular anaphase I (2.58%) with one or two lagging chromosomes (Fig. 1D). In this study acentric fragments were also recorded (2.95%) as it was observed in *Gibasis schiedeana* where acentric fragments probably resulted from sister chromatid reunion [18].

It could be observed that an asynchronous movement of chromosomes was evident at the beginning of the rainy season. The occurrence of desynchronization in the movement of chromosomes and the advance of the meiotic process produced aberrant metaphase I behavior as it was observed in the 37.7% of the total of PMC's studied. For example, dyads were formed when the homologous chromosomes (bivalents) remained coupled (Fig. 1B). This aberrant meiotic behavior could also have an influence in the low viability of pollen.

On the other hand, Piven *et al* [19] working with henequen (*A. fourcroydes* Lem.) showed meiotic and post-meiotic alterations in the formation of the male gametophyte, which could be due to its polyploid nature ( $5x = 150$ ), and these alterations might be responsible for the high sterility of pollen grains of this species (66.4%), on the contrary, *A. tequilana* is a diploid with  $2n = 60$ , how-

ever, it had a high percentage (42.00%) of nonviable pollen grains.

### Conclusions

The observed meiotic irregularities suggest that structural chromosome aberrations have occurred, such as heterozygous inversions, sister chromatid exchanges, deletions and duplications which in turn are reflected in a low pollen viability.

### Methods

Inflorescences from 7 plants were collected from commercial plantations (Santa Fe, Jalisco, México). Fresh anthers from young buds ( $\pm 1.8$  cm) were selected, and squashed in 1% aceto-orceine, and 320 pollen mother cells (PMC's) at metaphase I (M – I) and 814 PMC's at anaphase I (A – I) were analyzed.

Pollen viability was estimated by staining a sample of 1,583 pollen grains with 1% blue aniline in lactophenol [13] and the percentage of viable stained grains was recorded. The best cells for chromosome analysis and pollen samples were photographed using an Olympus BH2 microscope coupled with an automatic photograph camera.

### Authors' contributions

DR-R participated with the laboratory methods and chromosome analysis. BR-G was involved in the design and coordination, and drafted the manuscript.

### Acknowledgements

The authors wish to thank Tequilas del Señor, S. A. de C. V. for providing the biological materials from their agave plantations and to Dr. Fernando Santacruz-Ruvalcaba for his advise and assistance in field work. D. Ruvalcaba-Ruiz is a graduate student at Posgrado en Procesos Biotecnológicos. U. de G. – CIATEJ, A. C., and financially supported by CONACYT-México.

### References

1. Gentry HS: **Agaves of Continental North America.** Tucson, The University of Arizona Press 1982

2. Blunden G, Carabot C, Jewers K: **Steroidal sapogenins from leaves of some species of *Agave* and *Fureraea*.** *Phytochemistry* 1980, **19**:2489-2490
3. Banerjee S, Sharma AK: **Structural differences of chromosomes in diploid *Agave*.** *Cytologia* 1988, **53**:415-420
4. Cedeño GM: **Tequila production.** *Crit Rev Biotechnol* 1995, **15**:1-11
5. McKelvey SD, Sax K: **Taxonomic and cytological relationships of *Yucca* and *Agave*.** *J Arnold Arbor Harv Univ* 1933, **14**:76-81
6. Whitaker TV: **Chromosome constitution in certain monocotyledons.** *J Arnold Arbor Harv Univ* 1934, **15**:135-143
7. Darlington CD, Wylie AP: **Chromosome atlas of flowering plants.** London, George Allen and Unwin Ltd 1955
8. Cave MS: **Cytological observations on some genera of the *Agavaceae*.** *Madroño* 1964, **17**:163-169
9. Brandham PE: **Inversion heterozygosity and sub-chromatid exchanges in *Agave stricta*.** *Chromosoma* 1969, **26**:270-286
10. Castorena-Sánchez R, Escobedo M, Quiroz A: **New cytotaxonomical determinants recognized in six taxa of *Agave* in the sections *Rigidae* and *Sisalanae*.** *Can J Bot* 1991, **69**:1257-1264
11. Cavallini A, Natali L, Cionini G, Castoreña-Sánchez I: **Cytophotometric and biochemical analyses of DNA in pentaploid and diploid *Agave* species.** *Genome* 1996, **39**:266-271
12. Diario Oficial de la Federación: **Norma Oficial Mexicana NOM-006-SCFI-1993. Bebidas Alcohólicas-Tequila-Especificaciones.** Secretaría de Comercio y Fomento Industrial México, D.F. 1993, 48-52
13. Hauser EJP, Morrison JH: **The cytochemical reduction of nitro tetrazolium as an index of pollen viability.** *Am J Bot* 1964, **51**:748-752
14. Brandham PE: **Chromosome behaviour in the *Aloineae*. III Correlations between spontaneous chromatid and sub-chromatid aberrations.** *Chromosoma* 1970, **31**:1-17
15. Kenton A: **Chromosome evolution in the *Gibaris linearis* alliance (*Commelinaceae*). I. The Robertsonian differentiation of *G. venustula* and *G. speciosa*.** *Chromosoma* 1981, **84**:291-304
16. Palomino Gq: **Vázquez: Cytogenetic studies in mexican populations of species *Crotalaria L.* (*Leguminosae-Papiloideae*).** *Cytologia* 1991, **56**:343-351
17. Schulz-Schaeffer J: **Cytogenetics: Plants, Animals, Humans.** New York, Springer-Verlag 1980
18. Martínez J, Palomino G: **Evidence of heterozygous chromosome interchange and chromatid exchange in an autotetraploid cytotype of *Gibasis schiedeana* (*Tradescantieae-Commelinaceae*).** *Cytologia* 1997, **61**:215-223
19. Piven NM, Barredo-Pool FA, Borges-Argáez IC, Herrera-Alamillo MA, Mayo-Mosqueda A, Herrera-Herrera JL, Robert ML: **Reproductive biology of henequen (*Agave fourcroydes*) and its wild ancestor *Agave angustifolia* (*Agavaceae*). I Gametophyte development.** *Am J Bot* 2001, **88**:1966-1976

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMedcentral will be the most significant development for disseminating the results of biomedical research in our lifetime."

Paul Nurse, Director-General, Imperial Cancer Research Fund

Publish with **BMC** and your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours - you keep the copyright

Submit your manuscript here:

<http://www.biomedcentral.com/manuscript/>



**BioMedcentral.com**

[editorial@biomedcentral.com](mailto:editorial@biomedcentral.com)