Determination of Chromosome Number and Ploidy Level in Five Species of *Agave* from Mexico

José Manuel Rodríguez Domínguez[1*], Vanessa Nataly Magaña Avalos[1], Sofia Anahí Real Covarrubias[1]
[1]*Center for Research and Assistance in Technology and Design of the State of Jalisco, A.C., Mexico, Plant Biotechnology Unit; Guadalajara, Jalisco, Mexico*

Cytogenetic analysis was carried out on five species of *Agave* genus collected in Mexico. Meristematic root cells were analyzed cytogenetically using a modified steam-drop method for chromosome counting. The analysis showed that in *A. guiengola*, *A. geminiflora*, *A. attenuata*, and *A. victoriae-reginae* species, the chromosome number was 2n=60 with bimodal karyotypes composed of five pairs of large chromosomes and 25 pairs of small chromosomes. On the other hand, *A. salmiana* also showed a bimodal karyotype consisting of 30 large + 150 small chromosomes. Considering the basic number x=30, *A. salmiana* is a hexaploid species (2n=6x=180), whereas the other four species analyzed are diploid (2n=2x=60). This is the first report on the chromosome number of *A. guiengola*.
Keywords: Cytogenetic Analysis. Bimodal Karyotype. Diploid. Hexaploid.

The genus *Agave* is distributed in the tropical and subtropical parts of the world and represents a large group of succulent plants cultivated because of its commercial importance. Its distribution extends from the southern United States to Colombia, Venezuela, and the Caribbean Islands [1]. The genus has a basic chromosome number x=30 showing different degrees of ploidy, from diploid (2n=2x=60) to octoploid (2n=8x=240) species presenting asymmetric and highly conserved bimodal karyotypes, which consist of x=5 large chromosomes and x=25 small chromosomes [2-3].

Chromosomes spread without cytoplasm, and overlapping chromosomes are essential for cytogenetic studies, including chromosome counting, karyotype, and ideogram construction. In plants, creating good-quality spreads is difficult due to the rigid cell wall, which complicates chromosome separation. Several methods for obtaining good chromosome spreads have been developed, including the modified steam-drop method, one of the most effective for plant chromosome counting [4].

Received on 10 September 2024; revised 22 December 2024.
Address for correspondence: José Manuel Rodríguez Domínguez. Avenida Normalistas, nº 800, Colinas de la Normal, Guadalajara, Jalisco, México. PO Box: 44270. E-mail: mrodriguez@ciatej.mx.

J Bioeng. Tech. Health 2025;8(1):11-14
© 2025 by SENAI CIMATEC. All rights reserved.

This work aimed to determine chromosome number and ploidy level in five species of *Agave* from Mexico.

Materials and Methods

In the present investigation, ten plants (10 cm tall) of five different species of the *Agave* genus were collected from several states of Mexico on field trips over ten years: *A. guiengola* was collected from Oaxaca, *A. victoriae-reginae* from Nuevo León, *A. salmiana* from Hidalgo, *A. geminiflora*, and *A. attenuata* from Jalisco; the plants of each of the species mentioned were transported at the Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. (CIATEJ) and maintained in an *in vivo* collection in a greenhouse.

One month before cytogenetic analysis, 10 plants of each of the five species were placed in a container with a moist substrate made of peat moss vermiculite (7:3) for root production. Metaphasic chromosome preparations were performed according to the method reported by Rodríguez-Domínguez and colleagues (2017) [4]. Each slide was stained with 1% acetoorcein and coverslipped for chromosome counting.

The best ten metaphases obtained of *Agave* plants of each of the five species analyzed were photographed using a Leica DMRA2 microscope (Leica Microsystems, Wetzlar, Germany) coupled to an Evolution QEI phase-contrast camera (Media-Cybernetics, Rockville, Maryland, USA). In order to reduce noise in the images, the images were sharpened with a 9 x 9 Gaussian spatial filter.

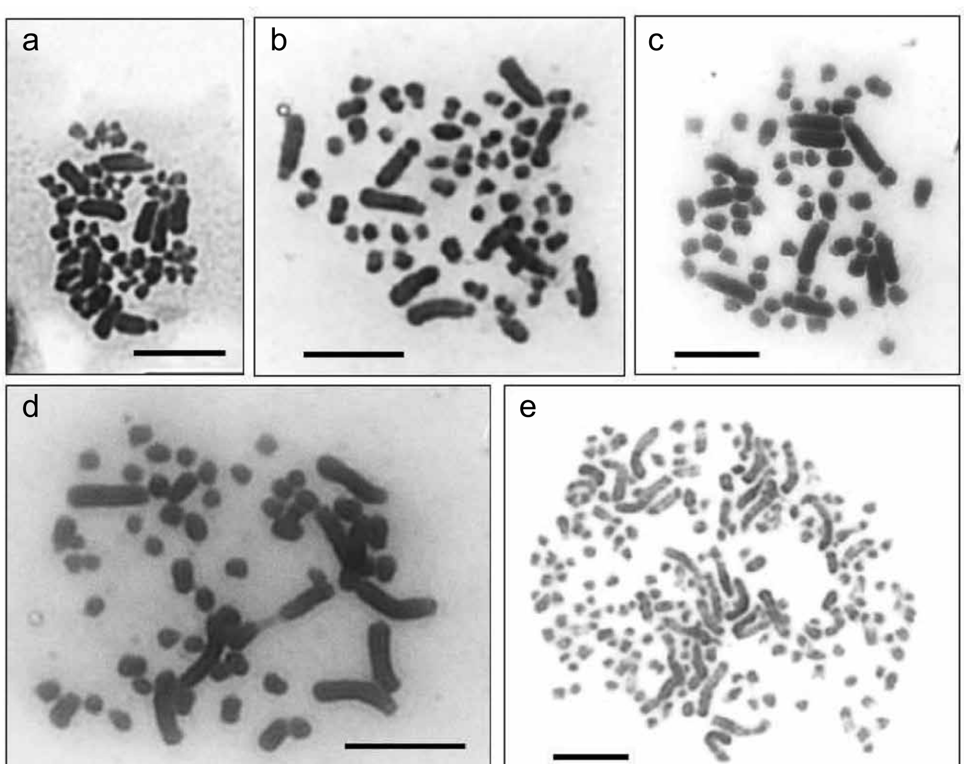
Results and Discussion

Mitotic metaphases with a good chromosome spread were obtained through the modified steam-drop method in all species analyzed. *A. guiengola*, *A. geminiflora*, *A. attenuata*, and *A. victoriae-reginae* species showed a bimodal karyotype consisting of 10 large + 50 small chromosomes (2n=60) (Figure 1a-d), on the other hand, *A. salmiana* also showed a bimodal karyotype consisting of 30 large + 150 small chromosomes (2n=180) (Figure 1e).

This is the first report on chromosome number for *A. guiengola*. Concerning *A. victoriae-reginae* and *A. geminiflora* species, the chromosome number shown in this work is consistent with that published in other studies [5-6]. On the other hand, Lingling and colleagues (2009) [7] reported that the chromosome number of *A. attenuata* ranges from 45 to 62, while our results for this species was 2n=60. Regarding *A. salmiana*, chromosome numbers 2n=120 and 2n=180 have been reported previously [8-10]. Our findings showed that the chromosome number of the analyzed plants was 2n=180. Because the genus *Agave* has a basic chromosome number x=30, the species analyzed have different ploidy levels (Table 1).

Certain chemicals can induce polyploidy; these compounds are so-called spindle poisons, which interfere with the formation of the metaphase spindle; some examples are colchicine, oryzalin, and α-bromonaphthalene among others; sometimes, these mitotic inhibitors also produce endopolyploidy in several plant species [11]. According to Ullah and colleagues (2009) [12], endopolyploidy could be achieved by at least four different mechanisms: endoreduplication, endomitosis, acytokinetic mitosis, or cell fusion; endoreduplication is the most common mode of polyploidization in plants and is estimated to occur in over 90% of angiosperms [13].

Figure 1. Chromosome number observed in mitotic metaphase of five different species of *Agave*.



a. *Agave attenuata*; b. *Agave geminiflora*; c. *Agave guiengola*; d. *Agave victoriae-reginae*; e. *Agave salmiana*. Barr=10µm.

Table 1. Somatic chromosome number and ploidy level in five species of *Agave*.

Species	Somatic Chromosome Number	Ploidy Level
Agave guiengola	2n=60	Diploid (2n=2x=60)
Agave geminiflora	2n=60	Diploid (2n=2x=60)
Agave attenuata	2n=60	Diploid (2n=2x=60)
Agave victoriae-reginae	2n=60	Diploid (2n=2x=60)
Agave salmiana	2n=180	Hexaploid (2n=6x=180)

In this study, only one species of *Agave* showed endopolyploidy. The type of endopolyploidy detected was endoreduplication.

For *A. geminiflora* (2n=60) (Figure 2a), endopolyploid cells showed 2n=120 chromosomes (Figure 2b), with a low frequency (5%); this is in agreement with Rodríguez-Domínguez and colleagues, (2020) [11] who also reported a similar frequency of endoreduplication on *Polianthes howardii* (2n=2x=60), a diploid plant belonging to the same taxonomic family: Asparagaceae.

Conclusion

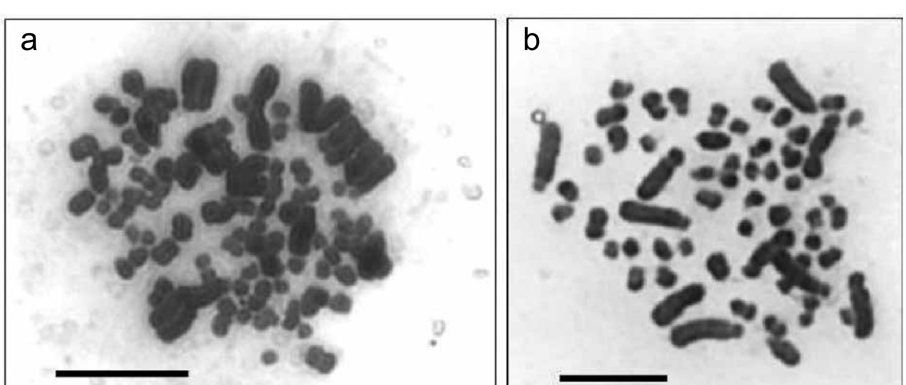
Based on the chromosome number obtained and considering the basic number x=30 for the *Agave* genus, *Agave salmiana* is identified as a hexaploid species (2n=2x=180), whereas *A. guiengola*, *A. geminiflora*, *A. attenuata*, and *A. victoriae-reginae* are diploid species (2n=2x=60). Notably, this study presents the first report on the chromosome number of *A. guiengola*.

One of the main challenges in counting chromosomes in polyploid species is obtaining preparations with well-spread metaphase chromosomes. However, this issue can be effectively addressed using the methodology described by one of our team members [4], which enabled the successful preparation of high-quality metaphase spreads in the hexaploid species *A. salmiana*. At CIATEJ, we continue our research to determine chromosome numbers in additional *Agave* species. The findings of this study provide valuable insights that will support future breeding programs within the *Agave* genus.

Acknowledgments

The authors express their gratitude to Project PlanTECC for funding this research.

Figure 2. Normal and endopolyploid cells in *Agave geminiflora*.



a. Endoreduplication with 4n appearance where the chromosomes are close to their homologous pair (2n=120); b. Normal cell (2n=60). Barr=10µm.

References

1. García-Mendoza A. Distribution of *Agave* (Agavaceae) in Mexico. Cactus and Succulent Journal 2002;74(4):177-187.
2. Thiede J. *Agave agavaceae*. In: Eggli U, Nyffeler R. (eds) Monocotyledons. Illustrated Handbook of Succulent Plants, Springer, Berlin, Heidelberg, p. 21-311, 2020.
3. Robert ML et al. Wild and agronomically important *Agave* species (Asparagaceae) show proportional increases in chromosome number, genome size, and genetic markers with increasing ploidy. Botanical Journal of the Linnean Society 2008;158(2):215-222.
4. Rodríguez-Domínguez JM et al. An improved technique for obtaining well- spread metaphases from plants with numerous large chromosomes. Biotechnic & Histochemistry 2017;92(3):159-166.
5. Bhattacharyya GN. Chromosomes in different species of *Agave*. Journal of Cytology and Genetics 1968;3:1-6.
6. González-Gutiérrez AG et al. Aspectos relevantes del desarrollo sexual en el género *Agave* y otras asparagáceas. Los *Agave*s y sus Derivados: Tendencias Científicas, Uso Sostenible y Patrimonio 2023:21-49, 2023.
7. Lingling LV et al. Determination of chromosomal ploidy in *Agave* ssp. African Journal of Biotechnology 2009;8(20):5248-5252.
8. Vignoli L. Cariologia del genere *Agave*. Lavori del Reale Istituto Botanico di Palermo 1936;7:176-217.
9. Granick EB. A karyosystematic study of the genus *Agave*. American Journal of Botany 1944;31(5):283-298.
10. García-Mensoza A. Los *Agave*s de México. Ciencias 2007;87:14-23.
11. Rodríguez-Domínguez JM et al. Detection of endopolyploid cells in root tips from two geophytic species pretreated with α-bromonaphthalene. Acta Horticulturae 2020;1288:117-122.
12. Ullah Z et al. Cip/Kip cyclin-dependent protein kinase inhibitors and the road to polyploidy. Cell Division 2009;4(10).
13. D'Amato F. Role of polyploidy in reproductive organs and tissues. Embryology of Angiosperms, B.M. Johri, ed. (New York: Springer-Verlag), p. 519-566, 1984.