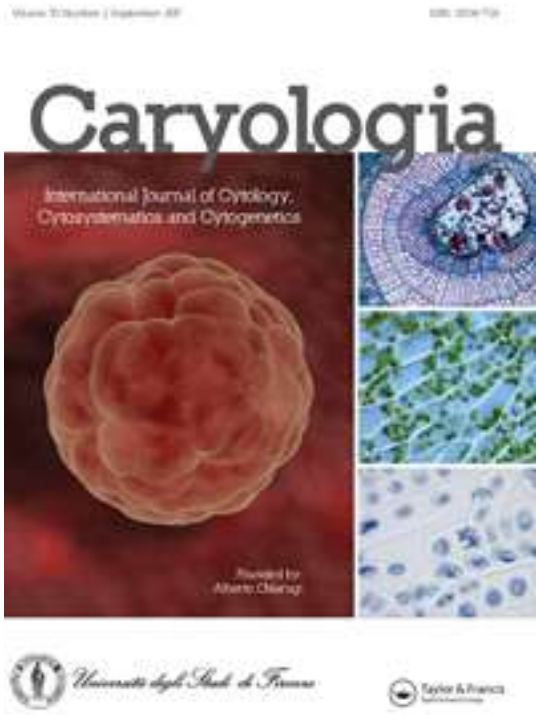
# Caryologia

International Journal of Cytology, Cytosystematics and Cytogenetics




**2016 Impact Factor** - 0.516 **5 Year Impact Factor** - 0.626

©2 016 Journal Citation Reports, Clarivate Analytics 2017®

Ranking: 157/166 (Genetics & Heredity) Ranking: 178/211 (Plant Sciences)

**Print ISSN**: 0008-7114 Online ISSN: 2165-5391 4 issues per year

**Caryologia is indexed in**: AgBiotechNet, Agroforestry Abstracts (Online), Animal Breeding Abstracts, Animal Science Database, ArticleFirst, Arts and Humanities Search, Biofuels Abstracts, Biological Abstracts (Online), Biological Abstracts (Online), BIOSIS Previews, Botanical Pesticides Abstracts, CAB Abstracts, Crop Physiology Abstracts, Crop Science Database, Current Contents, Environmental Impact, Field Crop Abstracts, Forest Products Abstracts, Forest Science Database, Forestry Abstracts, Global Health, Grasslands and Forage Abstracts (Online), Horticultural Science Database, Irrigation and Drainage Abstracts (Online), Nutrition and Food Sciences Database, Ornamental Horticulture (Online), Parasitology Database, Plant Breeding Abstracts (Online), Plant Genetics and Breeding Database, Potato Abstracts (Online), Review of Medical and Veterinary Entomology, Review of Plant Pathology (Online), Science Citation Index Expanded, Scopus, Seed Abstracts, Soil Science Database, Soils and Fertilizers (Online), Soybean Abstracts (Online), TropAg & Rural, Veterinary Science Database, Web of Science, Weed Abstracts, Zoological Record Online

Taylor & Francis
Taylor & Francis Group

Caryologia

International Journal of Cytology, Cytosystematics and Cytogenetics

ISSN: 0008-7114 (Print) 2165-5391 (Online) Journal homepage: http://www.tandfonline.com/loi/tcar20

# Karyotype analysis and physical mapping of the 5S and 45S rDNA genes in Tigridia pavonia var. Dulce (Iridaceae)

Hugo Abelardo Arroyo-Martínez, Amaury Martín Arzate-Fernández, Rodrigo Barba-González & José Luis Piña-Escutia

**To cite this article:** Hugo Abelardo Arroyo-Martínez, Amaury Martín Arzate-Fernández, Rodrigo Barba-González & José Luis Piña-Escutia (2017): Karyotype analysis and physical mapping of the 5S and 45S rDNA genes in Tigridia pavonia var. Dulce (Iridaceae), Caryologia, DOI: 10.1080/00087114.2017.1382302

**To link to this article:** http://dx.doi.org/10.1080/00087114.2017.1382302

Published online: 15 Nov 2017.

CARYOLOGIA: INTERNATIONAL JOURNAL OF CYTOLOGY, CYTOSYSTEMATICS AND CYTOGENETICS, 2017
https://doi.org/10.1080/00087114.2017.1382302

Taylor & Francis
Taylor & Francis Group



# Karyotype analysis and physical mapping of the 5S and 45S rDNA genes in *Tigridia pavonia* var. *Dulce* (Iridaceae)

Hugo Abelardo Arroyo-Martínez[a], Amaury Martín Arzate-Fernández[a], Rodrigo Barba-González[b] and José Luis Piña-Escutia[a]

[a]Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México, Toluca, México; [b]Unidad de Biotecnología Vegetal, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, Unidad Zapopan, Zapopan, México

**ABSTRACT**

*Tigridia pavonia* Redouté (Iridaceae) is a species with great ornamental potential of which nine different varieties are known. Among them, *Tigridia pavonia* var. *Dulce* presents the lowest seed formation. Despite this, there are no reports on cytogenetic characteristics for this variety. In the present study its karyotype was determined, and physical mapping of the 5S and 45S rDNA genes was performed by FISH. The results show that *Tigridia pavonia* var. *Dulce* exhibited a chromosomal number of $2n = 2x = 28+3B$. The karyotypic formula was 26 m + 2sm + 3B. Also, physical mapping of the 5S and 45S rDNA genes was achieved by means of the FISH technique, where the 5S rDNA gene could be mapped in eight different regions, whereas the 45S rDNA gene was observed in 10 chromosome regions, of which four corresponded to secondary constrictions. The information generated can serve as a basis for future analysis of evolutionary and/or genetic improvement in the species.

**ARTICLE HISTORY**
Received 8 June 2017
Accepted 18 September 2017

**KEYWORDS**
B-chromosome; fluorescent *in situ* hybridization; Iridaceae; ribosomal genes



## Introduction

The genus *Tigridia* (Iridaceae) includes at least 43 species and six subspecies, of which 41 are endemic to Mexico (Munguía-Lino et al. 2015). Among them, *Tigridia pavonia* Redouté, 1802 stands out for the great variability in colors and beauty of its flowers as an attractive floricultural resource. Today it is widely cultivated in Europe, Asia and Australia, where it is marketed as a gardening plant.

For this species, nine varieties are known in Mexico, which are registered in the National Catalog of Varieties of Plants (CNVV 2016).

Morphological characterization studies (Vázquez-García et al. 2001a), as well as molecular analysis (Arzate-Fernández et al. 2008; Piña-Escutia et al. 2010, 2010) performed in these varieties, have shown that genetic variation is related to the geographical origin of each variety (Piña-Escutia et al. 2010) and that this factor can exert a marked influence on flowering and fruiting, as reported in *Sisyrinchium micranthum* where cytotypes of different localities showed differences in flower opening time (Tacuatiá et al. 2012). Likewise Jordano and Godoy (2000) found that populations between two geographic limits experiment higher exchange of genes, whereas among the peripheral populations, there is a limited gene flow, and the difference of altitude of origin of the population is related to a marked difference in the phenology.

In addition, it is known that there is a relationship between flower color and fertility, with *Tigridia pavonia* var. *Sandra* (with red flowers) having a higher fertility (76.8%) compared to 38.7% of *Tigridia pavonia* var. *Angeles* (with white flowers) and 28.1% of *Tigridia pavonia* var. *Dulce* (with yellow flowers) (Vázquez-Garcia et al. 2001b).

Variation in fertility may be a result of chromosome pairing problems or presence of different chromosome structural arrangements (Kumar et al. 1987; Reed et al. 2001; Palomino et al. 2012; Alves et al. 2011) limiting seed production. In *Tigridia*, something similar has been reported by Molseed (1970) who made crosses between *T. pavonia* and related species and did not obtain seed formation. Likewise, Piña-Escutia et al. (2013) obtained a limited number of interspecific hybrids between *T. pavonia* and *Tigridia augusta* Drapiez. Due to the above, a better cytogenetic knowledge of *T. pavonia* may favor the development of hybridization programs.

In cytogenetic studies, one of the first objectives is the identification of chromosomes and the construction of the karyotype based on their morphological characteristics (Lakshmanan et al. 2015). The fluorescent *in situ* hybridization (FISH) technique facilitates the identification of specific sequences of individual chromosomes of

**CONTACT** José Luis Piña-Escutia jlpinae@uaemex.mx, joseluispina@live.com.mx

the chromosome complement (Abd El-Twab 2007; Van Laere et al. 2008; Guetat et al. 2015), allowing assessment of the types of evolutionary changes in the chromosomes of certain species, as well as the physical mapping of highly repetitive DNA sequences (Abd El-Twab 2007; Lakshmanan et al. 2015). Ribosomal genes (rDNA) have been used as probes in FISH because of their high copy number of replicate units, specific position on chromosomes and their highly conserved sequences (Gomez-Rodriguez et al. 2013; Guetat et al. 2015).

Mizuochi et al. (2007) mentions that 45S rDNA genes are highly repetitive units (18S, 5.8S and 26S rDNA), while the 5S rDNA genes, which are also highly repetitive, are independent of the region of the 45S rDNA gene in eukaryotes. In addition, both the location and number of rDNA sites vary in plants between closely related species, as well as at intraspecific level (Raskina et al. 2008), whereby such sequences have been used in numerous phylogenetic and plant evolutionary studies (Mizuochi et al. 2007; Raskina et al. 2008; Van Laere et al. 2008; Lakshmanan et al. 2015).

To date, cytological studies performed in *T. pavonia* are very scarce. Only aspects referring to chromosome number and bimodality of the chromosome complement have been reported, with a chromosomal number of $2n = 28$ in *T. pavonia* var. *Sandra*, the only formal variety studied so far (Arroyo-Martínez et al. 2017). In addition, characteristics such as the position of the secondary constrictions vary among varieties (Molseed 1970; Kenton and Heywood 1984). Therefore, the objective of the present work was to determine the karyotype and to perform the physical map of the 45S and 5S rDNA genes in *Tigridia pavonia* var. *Dulce*.

## Material and methods

### Plant material

Twenty bulbs of *Tigridia pavonia* var. *Dulce* were used. The bulbs were obtained from the Wild Species Conservation Center of the Centro Universitario Tenancingo, of the Universidad Autónoma del Estado de México and were planted in pots containing a substrate composed of mountain soil, sand and cattle manure (ratio 1:1:1), in a greenhouse of the Facultad de Ciencias Agrícolas of the above university.

### Mitotic chromosome preparations

Chromosome slides preparation was done according to the methodology proposed by Barba-Gonzalez et al. (2005) and Palomino et al. (2015) with a few changes, using five metaphase cells of five plants of *T. pavonia* var. *Dulce*. The root meristems were placed in a solution of 8-hydroxyquinoline 0.002 M for 6 h at 4 °C under dark conditions. Subsequently, they were fixed in Farmer's solution for 24 h, and hydrolyzed with HCl (1 N) for 8 min at 60 °C. Chromosomes were stained with Schiff reagent for 1 h; and subsequently treated with a mixture of enzymes to a final concentration of 1% (cellulase, pectolyase, cytohelicase) in citrate buffer pH 4.5 for 2 h at 37 °C. After enzymatic digestion, the meristem was placed in the slide and a drop of acetoorcein (1%) was added. After placing the coverslips, the tissue was disrupted and the cells were left in a single plane by the squash method, the preparations were made permanent by the method of liquid nitrogen. The preparations were analyzed using an Olympus BX43 microscope (Olympus, Tokyo, Japan) equipped with a Leica MC170 HD camera (Leica Microsystems, Jurong East, Singapore).

### Analysis of mitotic chromosomes and karyotype determination

Measurement of the chromosome arms was performed utilizing LAZE V.4 software (Leica Microsystems, Switzerland, https://www.leica-microsystems.com). The parameters determined were: chromosome morphology, the total genome length in μm (TGL), and asymmetry index (TF%).

The classification of chromosome morphology was performed following the methodology proposed by Levan et al. (1964). Chromosomal homology was established according to the similarities in length and centromeric position. The idiograms were elaborated according to the mean values of the short arm and long arm in each pair of chromosomes and were grouped in metacentric (m), submetacentric (sm), subtelocentric (st) and telocentric (t) chromosomes. The asymmetry index (TF%) was obtained as reported by Sinha and Roy (1979).

### Physical mapping of the 45S and 5S rDNA genes

The physical mapping of the 45S and 5S rDNA genes was done according to the methodology proposed by Barba-Gonzalez et al. (2005) with some changes, for the slide preparation the root meristems were placed in a solution of 8-hydroxyquinoline 0.002 M for 6 h at 4 °C under dark conditions. Subsequently, they were fixed in Farmer's solution for 24 h, and incubated in an enzyme digestion mixture containing 0.2% (w/v) Y23 pectolyase, 0.2% (w/v) cellulase, and cytohelicase 0.2% (w/v) in citrate buffer 10 mmol $l^{-1}$ (pH 4.5), at 37 °C for about 2 h. Cell squash was performed in a drop of 50% acetic acid and frozen in liquid nitrogen, the coverslips were removed with a razor blade and immediately dehydrated in 96% ethanol and air dried.

Two different probes were used for the FISH technique: clones pTa71 and pTa794, which contain the EcoRI fragments of 45S and 5S ribosomal DNA, respectively, from wheat (Gerlach and Bedbrook 1979; Gerlach and Dyer 1980), and which were isolated with the High Pure Plasmid Isolation kit (Roche Diagnostics GmbH, Mannheim, Germany) and labelled with biotin-16-dUTP (Biotin-Nick Translation Mix) and Dig-11-dUTP

(Digoxigenin-Nick Translation Mix) respectively according to the manufacturer's instructions.

Hybridization with the probes was performed by incubating the slides in RNAse (100 µg ml$^{-1}$) for 1 h, and pepsin (5 µg ml$^{-1}$) for 10 min, both at 37 °C, and then in paraformaldehyde (4%) for 10 min at room temperature; then dehydrated with 70, 90 and 100% ethanol for 3 min each, and air dried. Hybridization continued using a mixture consisting of 20 × SSC, 50% formamide, 10% sodium dextran sulfate, 10% SDS, and 25–50 ng ml$^{-1}$ of each probe. The DNA was denatured by heating the hybridization mixture to 70 °C for 10 min and placing it on ice for at least 10 min. For each slide, 80 µl of the hybridization mixture was used. The slides were denatured at 80 °C for 10 min and hybridized overnight at 37 °C in a humid chamber, then the slides were washed at room temperature in 2× SSC for 15 min and in 0.1× SSC at 42 °C for 30 min. Chromosomes were counterstained with 1 µl ml$^{-1}$ of DAPI (4′diamidino-2-phenylindole), and a drop of Vectashield antifade (Vector Laboratories, Burlingame, CA, USA) was added before examining the slides under a Leica DMRA2 microscope (Leica Microsystems, Wetzlar, Germany) equipped with epifluorescent lighting and coupled to an Evolution QEi camera (Media-Cybernetics, Rockville, USA).

## Results

In the present study, *Tigridia pavonia* var. *Dulce* exhibited a chromosome number of $2n = 2x = 28 + 3B$ with the presence of three putative B chromosomes (Figure 1). In spite of the three additional chromosomes, this variety retains its bimodal karyotype, with the presence of four large and 24 small chromosomes. In this way *T. pavonia* var. *Dulce* presented a karyotypic formula of 26 m + 2sm + 3B showing a total genome length of 124.94 µm and an asymmetry index of 44.29, whereas the length of large chromosomes was 11.46–7.77 µm, and small chromosomes had a length range of 4.77–2.11 µm. The putative B chromosomes had a length range of 1.75–1.40 µm, being markedly smaller than any normal chromosome.

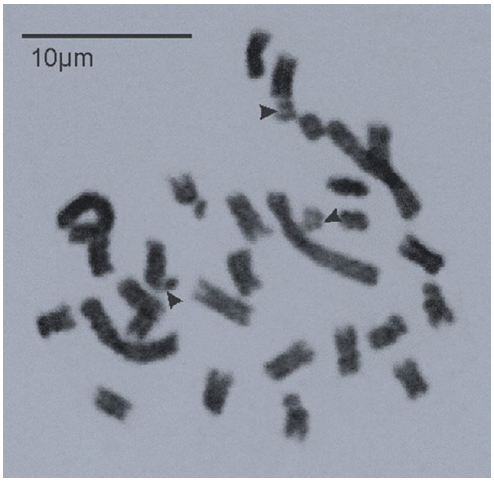



**Figure 1.** *Tigridia pavonia* var. *Dulce* mitotic metaphase chromosomes. $2n = 2x = 28 + 3B$. Arrow heads indicate putative B chromosomes.

The amplification of the 5S (red) and 45S (green) genes of rDNA for *T. pavonia* var. *Dulce* (Figure 2) was achieved for the first time. The 5S rDNA gene amplified in eight regions, whereas the 45s rDNA gene amplified in 10 regions of different chromosomes respectively.

The data obtained by FISH were integrated into the idiograms, indicating the number and position of the 5S and 45S rDNA loci (Figure 3).

## Discussion

Martínez et al. (2010) reported that polyploidy was important in the early diversification of the Iridaceae family, and polyploid cultivars are common. For example, in *Crocus* the presence of more than a basic chromosome number has resulted in what appear to be aneuploid cultivars (Ramanna et al. 2012), which has also been observed in *Xiphium* (Mill.) Spach species (Martínez et al. 2010). In the present study cytogenetic analysis showed a diploid chromosome number of $2n = 28 + 3B$ for *T. pavonia* var. *Dulce*, in agreement with Arroyo-Martínez et al. (2017) who reported a chromosomal number of $2n = 28$ for *T. pavonia* var. *Sandra*; and with Molseed (1970) and Kenton and Heywood (1984) who reported the same chromosomal number in *Tigridia pavonia*, although in their studies the variety was not given. Likewise, parameters like TGL and TF% varied between *T. pavonia* var. *Dulce* and those reported for var. *Sandra* (Arroyo-Martínez et al. 2017), observing that as the TGL increase (101.45 µm in var. *Sandra*), the TF% decrease (47.276 in var. *Sandra*) in var. *Dulce*. The increase in LGT could be due to the presence of supernumerary chromosomes, or because, where B chromosomes do occur, they increase the nuclear DNA values (Jones et al. 2008). On the other hand the change in the TF% could be because the formation of the B chromosomes (depending on their origin) can involve a series of changes to the original chromosome set, as explained by Houben and Carchilan (2012). All of this could suggests that each of the nine varieties of *T. pavonia* may have unique structural chromosome changes, as reported by Alves et al. (2011) in *Alophia drummondii* Foster 1945 and Gettner (2005) in *Bellevalia saviczii* Woron 1927. These authors observed that plants of the same species from different localities showed variation in chromosome number and that B chromosomes were only present in some of them.

It is often reported that most B chromosomes do not confer any advantage (Houben and Carchilan 2012; Banaei-Moghaddam et al. 2013), but recent studies indicated that the presence of B chromosomes might increase pollen stability and seed production,

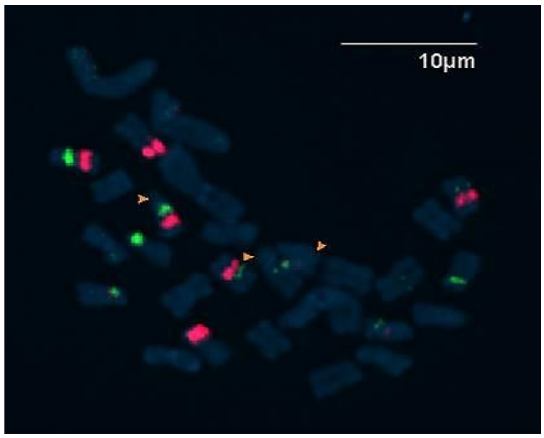



**Figure 2.** Fluorescent *in situ* hybridization of the 5S genes (red fluorescence) and 45S rDNA (green fluorescence) in *Tigridia pavonia* var. *Dulce*. Chromosomes were counterstained with DAPI. Arrow heads indicate putative B chromosomes.

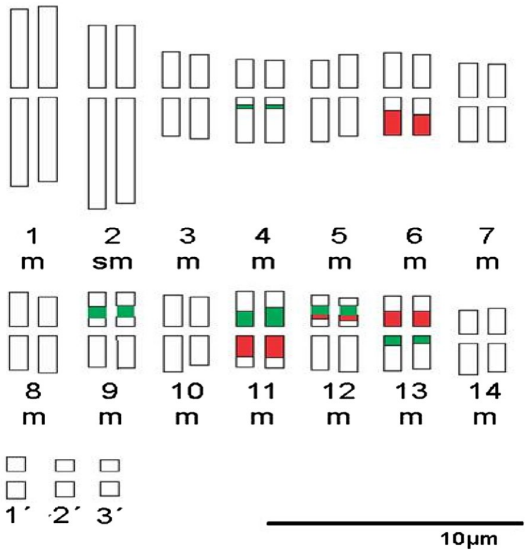



**Figure 3.** Idiogram of *Tigridia pavonia* var. *Dulce* with a karyotype formula of 26 m + 2 sm + 3 B. The red marks indicate the 5S rDNA gene and the green marks indicate the 45S rDNA gene.

as in *Acanthophyllum laxiusculum* (Ghaffari and Bidmeshkipoor 2002). In fact, it has been reported that their presence may affect the synthesis of essential oils (Tomson and Thoppil 2005), as well as the fertility of the organism (Jones et al. 2008). Our results seem to agree with the latter idea because the three putative B chromosomes were observed in var. *Dulce*, which is one of the varieties with less fruit formation and lower seed production, although additional studies are necessary in the other varieties to confirm this.

Navrátilová et al. (2003) mention that the karyotype of various species is established on the basis of chromosome size, centromeric position and banding pattern, which in many species is limited by the morphological similarity of the chromosomes. One way to overcome such limitations is the use of specific DNA sequences in the chromosomes through FISH, as this enables specific probe patterns to be obtained for discrimination of similar chromosomes, as was observed in the present study where the amplification of the 5S and 45S rDNA allowed the identification of homologous chromosomes. Similar results were found by Gomez-Rodriguez et al. (2013) who analyzed three species of *Agave* and observed that, despite the difference in chromosome number assignment, those chromosomes that had the same position as the 18S and 5S rDNA genes were homologous; concluding that the length, intensity and position of the amplified probe are important characteristics that can help the identification of homologous chromosomes.

Physical mapping of four loci of the 45S rDNA gene observed in the secondary constrictions (chromosome pairs 9 and 13) is noteworthy because it coincides with Abd El-Twab (2007), Van Laere et al. (2008) and Lakshmanan et al. (2015) who indicate that 45S rDNA gene is associated with the nucleolar organizer region (NOR) and is usually positioned in the secondary constriction of satellite chromosomes. It is important to highlight the large number of regions localized with both genes as well as those regions of the 45S rDNA gene that were located on different regions to the secondary constrictions, which according to Lakshmanan et al. (2015) may be due to a polyploidization event in which the number of regions of such genes is generally doubled and can be detected by the FISH technique.

Jones and Houben (2003) mentioned that some B chromosomes could be composed by ribosomal DNA. In this regard, Xie et al. (2014) observed in hybrids of *Lilium* that one B chromosome of the analyzed chromosome complements was completely labeled by the 5S rDNA probe, whereas in other hybrid the B chromosome was only partially marked by the probe 45S. In contrast, in our study, none of the B chromosomes were labeled by the probes 5S or 45S evaluated, which may indicate that this type of ribosomal gene does not intervene in the origin of the B chromosomes in the analyzed plants, as pointed out by Jones et al. (2008).

## Conclusions

The karyotype of *Tigridia pavonia* var. *Dulce* was determined, with a chromosome number of $2n = 2x = 28 + 3B$, with the presence of three putative B chromosomes, showing a karyotype formula of 26 m + 2sm + 3B. Likewise, we could visualize the 5S and 45S rDNA genes by means of FISH, where the 5S rDNA gene is located in eight different chromosomes (four homologous pairs), whereas the 45S rDNA gene was observed in 10 chromosomes (five homologous pairs), of which four corresponded to secondary constrictions.

Using the FISH technique, homologous chromosomes were identified and it is suggested that *T. pavonia*

var. *Dulce* is a diploid species and, due to a possible process of ancestral polyploidization, formed its characteristic bimodal karyotype. The information generated may serve as a basis for future evolutionary analyzes, and/or breeding programs of the species.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This work was supported by Consejo Nacional de Ciencia y Tecnología [grant number 243266]. Scholarship granted to Arroyo-Martinez H.A. (616509).

## ORCID

*Hugo Abelardo Arroyo-Martínez* http://orcid.org/0000-0001-8475-0666
*Amaury Martín Arzate-Fernández* http://orcid.org/0000-0001-8603-0099
*Rodrigo Barba-González* http://orcid.org/0000-0001-9336-090X
*José Luis Piña-Escutia* http://orcid.org/0000-0001-9678-9939

## References

Abd El-Twab MH. 2007. Physical mapping of the 45S rDNA on the chromosomes of *Triticum turgidum* and *T. aestivum* using fluorescence *in situ* hybridization for chromosome ancestors. Arab Journal of Biotechnology. 10(1):69–80.

Alves FLI, Lima ASA, Felix PL. 2011. Chromosome characterization and variability in some Iridaceae from Northeastern Brazil. Genet Mol Biol. 34(2):259–267. doi:10.1590/S1415-47572011000200016.

Arroyo-Martínez HA, Arzate-Fernández AM, Barba-González Piña-Escutia JL. 2017. Karyotype determination of three *Tigridia* species (Asparagales, Iridaceae). Caryologia. 11:27. doi:10.1080/00087114.2017.1321312.

Arzate-Fernández AM, Hoyos-Basurto A, Vázquez-García LM, Gutiérrez-Martínez MG. 2008. Isozyme characterization of nine botanical varieties of *Tigridia pavonia* (L.f.) DC. Agrociencia. 42(5):519–528. doi:https://www.scielo.org.mx/pdf/agro/v42n5/v42n5a4.pdf.

Banaei-Moghaddam AM, Meier K, Karimi-Ashtiyani R, Houben A. 2013. Formation and expression of pseudogenes on the B chromosome of rye. Plant Cell. 25(7):2536–2544. doi:10.1105/tpc.113.111856.

Barba-Gonzalez R, Ramanna MS, Visser RGF, Van Tuyl JM. 2005. Intergenomic recombination in $F_1$ lily hybrids (Lilium) and its significance for genetic variation in the BC1 progenies as revealed by GISH and FISH. Genome. 48(5):884–894. doi:10.1139/g05-057.

CNVV. 2016. Catálogo Nacional de Variedades Vegetales. México: Secretaría de Agricultura Ganadería, Desarrollo Rural, Pesca y Alimentación; p. 38.

Gerlach WL, Bedbrook JR. 1979. Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res. 7(7):1869–1885.

Gerlach WL, Dyer TA. 1980. Sequence organization of the repeating units in the nucleus of wheat which contain 5S rRNA genes. Nucleic Acids Res. 8(21):4851–4865.

Gettner ME. 2005. Variable karyotype with B-chromosomes in *Bellevalia saviczii* (Liliaceae). Genetica. 124(2-3):223–234.

Ghaffari SM, Bidmeshkipoor A. 2002. Presence and behaviour of B-chromosomes in *Acanthophyllum laxiusculum* (*Caryophyllaceae*). Genetica. 115(3):319–323. doi:10.1023/A:1020676119886.

Gomez-Rodriguez VM, Rodriguez-Garay B, Palomino G, Martínez J, Barba-Gonzalez R. 2013. Physical mapping of 5S and 18S ribosomal DNA in three species of *Agave* (Asparagales, Asparagaceae). CompCytogen. 7(3):191–203. doi:10.3897/CompCytogen.v7i3.5337.

Guetat A, Rosato M, Rosello JA, Boussaid M. 2015. Karyotype analysis in *Allium roseum* L. (Alliaceae) using fluorescent in situ hybridization of rDNA sites and conventional stainings. Turk J Bot. 39:796–807. doi:https://journals.tubitak.gov.tr/botany/issues/bot-15-39-5/bot-39-5-6-1408-50.pdf.

Houben A, Carchilan M. 2012. Plant B chromosomes: what makes them different? In: Bass HW, Birchler JA, editors. Plant cytogenetics plant genetics and genomics: crops and models, vol 4. Springer, New York, NY, p. 59–77. doi:10.1007/978-0-387-70869-0_1.

Jones N, Houben A. 2003. B chromosomes in plants: escapees from the A chromosome genome? Trends Plant Sci. 8(9):417–423. doi:10.1016/S1360-1385(03)00187-0.

Jones RN, Viegas W, Houben A. 2008. A Century of B Chromosomes in Plants: So What? Ann Bot. 101(6):767–775. doi:10.1093/aob/mcm167.

Jordano P, Godoy JA. 2000. RAPD variation and population genetic structure in *Prunus mahaleb* (Rosaceae), an animal-dispersed tree. Molecular Ecol. 9(9):1293–1305. doi:10.1046/j.1365-294x.2000.01009.x.

Kenton A, Heywood CA. 1984. Cytological studies in South American Iridaceae. Plant Syst Evol. 146(1-2):87–104. doi:10.1007/BF00984056.

Kumar OA, Ramesh CP, Raja Rao KG. 1987. Cytogenetic studies of the $F_1$ hybrids of *Capsicum annuum* with *C. chinense* and *C. baccatum*. Theor Appl Genet. 74(2):242–246. doi:10.1007/BF00289975.

Lakshmanan PS, Van Laere K, Eeckhaut T, Van Huylenbroeck J, Van Bockstaele E, Khrustaleva L. 2015. Karyotype analysis and visualization of 45S rRNA genes using fluorescence *in situ* hybridization in aroids (Araceae). CompCytogen. 9(2):145–160. doi:10.3897/CompCytogen.v9i2.4366.

Levan A, Fredga K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. Hereditas. 52:201–220. doi:10.1111/j.1601-5223.1964.tb01953.x.

Martínez J, Vargas P, Luceño M, Cuadrado A. 2010. Evolution of *Iris* subgenus *Xiphium* based on chromosome numbers, FISH of nrDNA (5S, 45S) and *trnL–trnF* sequence analysis. Plant Syst Evol. 289(3-4):223–235. doi:10.1007/s00606-010-0345-7.

Mizuochi H, Marasek A, Okazaki E. 2007. Molecular cloning of *Tulipa fosteriana* rDNA and subsequent FISH analysis yields cytogenetic organization of 5S rDNA and 45S rDNA in *T. gesneriana* and *T. fosteriana*. Euphytica. 155(1-2):235–248. doi:10.1007/s10681-006-9325-y.

Molseed E (1970) The genus *Tigridia* (Iridaceae) of Mexico and Central America. Berkeley, CA: University of California Press; p. 126.

Munguía-Lino G, Vargas-Amado G, Vázquez-García LM, Rodríguez A. 2015. Riqueza y distribución geográfica de la tribu *Tigridieae* (Iridaceae) en Norteamérica. Rev Mex De Biodivers. 86(1):80–98. doi:10.7550/rmb.44083.

Navrátilová A, Neumann P, Macas J. 2003. Karyotype analysis of four *Vicia* species using *in situ* hybridization with repetitive sequences. Ann Bot. 91(7):921–926. doi:10.1093/aob/mcg099.

Palomino G, Martínez J, Barba-González, Méndez I, Rodríguez-Garay B (2012) Mexican geophytes III. Cytotypes and meiotic behavior in Mexican populations of species of *Echeandia* (anthericaceae). Floriculture and Ornamental Biotechnology 6 (1): 140–152.

Palomino G, Martínez J, Méndez I, Cepeda-Cornejo V, Barba-González R, Rodríguez-Garay B. 2015. Nuclear genome size and cytotype analysis in *Agave parviflora* Torr. subsp. *flexiflora* Gentry (Asparagales, Asparagaceae). Caryologia. 68(3):159–168. doi:10.1080/00087114.2015.1032575.

Piña-Escutia JL, Vences-Contreras C, Gutiérrez-Martínez MG, Vázquez-García LM, Arzate-Fernández AM. 2010. Morphological and molecular characterization of nine botanical varieties of *Tigridia pavonia* (L.f.) DC. Agrociencia. 44(2):147–158. doi:https://www.scielo.org.mx/pdf/agro/v44n2/v44n2a3.pdf.

Piña-Escutia JL, Vázquez-García LM, Arzate-Fernández AM. 2010. Variety discrimination of *Tigridia pavonia* (L.f.) DC. assesed by different length RAPD primers. Electron J Biotechnol. 13(4):10–11. doi:10.2225/vol13-issue4-fulltext-7.

Piña-Escutia JL, Vázquez-García LM, Arzate-Fernández AM. 2013. Interspecific hybridization between *Tigridia pavonia* and *T. augusta* through ovary slice culture. Genet Mol Res. 12(1):15–22. doi:10.4238/2013.January.16.4.

Ramanna MS, Marasek-Ciolakowska A, Xie S, Khan N, Arens P, Van Tuyl JP. 2012. The significance of polyploidy for bulbous ornamentals: a molecular cytogenetics assessment. Floriculture Ornamental Biotech. 6(1):116–121.

Raskina O, Barber JC, Nevo E, Belyayev A. 2008. Repetitive DNA and chromosomal rearrangements: speciation-related events in plant genomes. Cytogenet Genome Res. 120(3-4):351–357. doi:10.1159/000121084.

Reed SM, Riedel GL, Pooler MR (2001) Verification and establishment of *Hydrangea macrophylla* "Kardinal" x *H. paniculata* "Brussels Lace" interspecific hybrids. Plant Breeding 19 (2): 85–88.

Sinha SSN, Roy H. 1979. Cytological studies in the genus Phaseolus I. Mitotic analysis in fourteen species. Cytologia. 44:191–199.

Tacuatiá LO, Souza-Chies TT, Am Flores, Eggers L, Siljak-Yakovlev S, Kaltchuk-Santos E. 2012. Cytogenetic and molecular characterization of morphologically variable *Sisyrinchium micranthum* (Iridaceae) in southern Brazil. Bot J Linnean Soc. 169(2):350–364. doi:10.1111/j.1095-8339.2012.01229.x.

Tomson M, Thoppil JE. 2005. Influence of B-chromosome on essential oil content and composition in *Salvia coccinea* Buc'hoz ex Etl. (Lamiaceae). Caryologia. 58(3):246–248. doi:10.1080/00087114.2005.10589458.

Van Laere K, Van Huylenbroeck J, Van Bockstaele E. 2008. Karyotype analysis and physical mapping of 45S rRNA genes in *Hydrangea* species by fluorescence *in situ* hybridization. Plant Breeding. 127(3):301–307. doi:10.1111/j.1439-0523.2007.01456.x.

Vázquez-Garcia LM, Przybyla AA, De la Cruz TE, Torres Navarro H, Rodriguez G. 2001b. Morphological description of nine botanical varieties of *Tigridia pavonia* (L. F.) Ker. Gawl J Appl Bot. 75:14–19.

Vázquez-García LM, Norman MTH, Corona RMC (2001a) Oceloxochitl Tigridia pavonia (L. f.) DC. Colección: Ciencias Naturales y Exactas, Serie: Ciencias Agrícolas. México: Universidad Autónoma del Estado de México ; p. 69.

Xie S, Marasek-Ciolakowska A, Ramanna MS, Arens P, Visser RJF, van Tuyl JM. 2014. Characterization of B chromosomes in *Lilium* hybrids through GISH and FISH. Plant Syst Evol. 300(8):1771–1777. doi:10.1007/s00606-014-1004-1.