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Karyotype determination of three *Tigridia* species (Asparagales, Iridaceae)

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ABSTRACT

Studies of the genus *Tigridia* Jussieu show that there is inadequate cytological information, and variable basic numbers and karyotype features have been reported. To have more accurate karyotypic information within the genus, the chromosome number, morphology of chromosomes, karyotype formula, total length of the genome (TLG), chromosomal symmetry, and asymmetry index (TF %) was determined in *Tigridia pavonia* var. *Sandra*, *Tigridia multiflora*, and *Tigridia alpestris* subsp. *obtusa*. All three species have a chromosome number of $2n = 2x = 28$, showing a bimodal karyotype consisting of four large chromosomes and 24 small ones. *Tigridia pavonia* var. *Sandra* has a karyotypic formula of 28 m with secondary constrictions on three small chromosomes; this species has a more symmetrical karyotype than the others. *T. multiflora* showed a karyotypic formula of 24 m + 4st, while *T. alpestris* subsp. *obtusa* exhibited a karyotypic formula of 22 m + 2sm + 4st. For these two species, this is the first report of the number and chromosome structure. In both, the LGT and TF% values were contrasting compared to the values found in *T. pavonia* var. *Sandra*.

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Chromosome morphology; chromosome number; karyotypic formula

Introduction

Mexico is considered the center of diversity of the genus *Tigridia* Jussieu. Forty-three species and six subspecies are currently known, of which, 41 are endemic to Mexico (Munguía-Lino et al. 2015). *Tigridia* species are potentially useful, and their size, shape, and the great variability of flower colors make these an attractive floricultural resource. Today, only *T. pavonia* (L.f) DC. is widely cultivated in Europe, Asia, and Australia, where it is marketed as a plant useful for landscaping (Vázquez-García et al. 2001). In addition, the bulb serves as food and medicinal preparations used for some diseases (González et al. 2004; Martínez et al. 2007; Botina-Galeano et al. 2008).

Despite this, there is little information on their abundance and distribution, so studies on their genetic variability are needed for efficient conservation as well as for use in breeding programs. Genetic diversity can be measured by variation in the content or size of DNA as well as the number and structure of chromosomes, because although the karyotype features are generally constant in a group of species and even a genus, often structural and/or numerical variations occur that can change the number, size, and position of the centromere on chromosomes, causing genetic variation (Levitus et al. 2010). The chromosome number is also a useful tool in systematics and plant evolution and can complement information obtained by morphological and molecular methods in which the detection of polyploidy and other highly significant genome changes are not visible (Guerra 2008).

In addition, studies of the chromosome number of a species can be uncertain to a greater or lesser degree depending on the number of analyzed plants, or due to the lack of information about their origins. Cytological studies are very few in the genera *Tigridia*: Molseed (1970) reported a chromosome number of $2n = 28$ in eight species of *Tigridia*, observing a bimodal karyotype confirmed by four large chromosomes and 24 small chromosomes, while highlighting the presence of secondary constrictions in *T. pavonia* and *T. galanthoides* Molseed. In addition, Kenton and Heywood (1984) observed a bimodal karyotype in four *Tigridia* species; however, in one of them, the authors found only two large chromosomes and 12 small chromosomes.

Accurate information about the karyotype helps to identify and classify chromosomes, and allows the detection of numerical and structural abnormalities that can subsequently be used in assisted breeding programs. The aim of this study was to determine the karyotype of three wild *Tigridia* species (*T. pavonia* var. *Sandra*, *T. multiflora* (Baker) Ravenna, and *T. alpestris* Molseed ssp. *Obtuse* Molseed) and quantify chromosomal variation among the species evaluated.

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Material and methods

Plant material

Five bulbs of each species were utilized (*T. pavonia* var. *Sandra*, *T. multiflora*, and *T. alpestris* subsp. *obtusa*). The bulbs were obtained from the Centro de Conservación de Especies Silvestres of the Centro Universitario Tenancingo, of the Universidad Autónoma del Estado de México and they were grown in pots in a substrate composed of forest soil, sand, and cow manure (1:1:1), in a rustic-type greenhouse at the Facultad de Ciencias Agrícolas of the above university.

Analysis of mitotic chromosomes

For the observation of mitotic chromosomes and karyotype analysis, the methodology proposed by Barba-González et al. (2005) and Palomino et al. (2015) was used with some changes: 15 cells were observed in metaphase from five plants per species. The root meristems were placed in a solution of 8-hydroxyquinoline 0.002 M for 6 h at 4°C in the dark. Subsequently, they were fixed in Farmer solution for 24 h and hydrolyzed with HCl (1 N) for 8 min at 60 °C. The chromosomes were stained with Schiff reagent for 1 h; subsequently, they were treated with an enzyme mixture at a final concentration of 1% (cellulase, pectolyase, citohelicase) in citrate buffer pH 4.5 for 2 h at 37 °C; once the enzymatic digestion was completed, the meristem was placed on slide in a drop of aceto-orcein (1%). After placing the coverslip, the tissue was disaggregated and the cells were left in a single plane by the squash method; the chromosome preparations were made permanent by the liquid nitrogen method. The preparations were analyzed using an Olympus BX43 microscope (Olympus, Tokyo, Japan) equipped with a Leica MC170 HD camera (Leica Microsystems, Singapore).

Karyotype analysis

The chromosome arm measurements were performed utilizing LAZE V.4 software (Leica Microsystems, Switzerland, http://www.leica-microsystems.com) and based on this the chromosome morphology, total length of the genome in µm (TLG), and asymmetry index (TF %) were determined. The classification of chromosome morphology was carried out following the methodology proposed by Levan et al. (1964). Chromosome homology was established according to the similarities in lengths and centromeric positions. Idiograms were prepared according to the average values of the short and long arm on each chromosome pair and were grouped into metacentric (m), submetacentric (sm), subtelocentric (st), and telocentric (t) chromosomes. The asymmetry index (TF %) was obtained as reported by Sinha and Roy (1979).

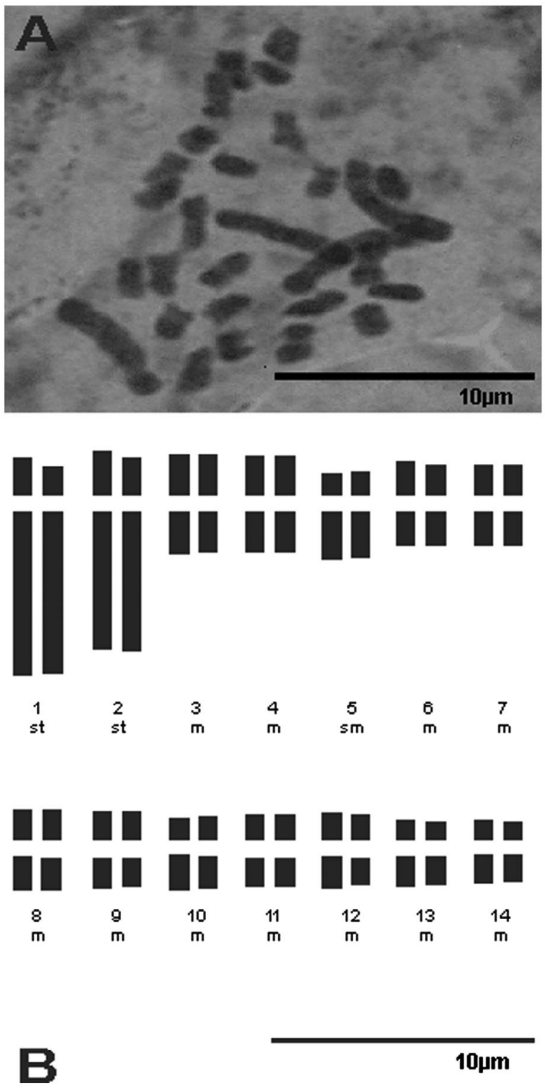

Figure 1. *Tigridia alpestris* subsp. *obtusa* diploid $2n = 2x = 28$. (A) Mitotic cell with metaphase chromosomes. (B) Idiogram with a karyotype of 22 m + 2sm + 4st.

Statistical analysis

The differences of LGT and TF% between the species studied were evaluated by an ANOVA test with the software STATGRAPHICS Centurion XVI. (Statpoint Technologies,Virginia, USA, http://www.statgraphics17.com). The least significant difference test ($p \leq 0.05$) was performed to corroborate the differences between the values evaluated.

Results and discussion

The karyotype is the result of many forces acting in the genome in the structural, organizational, and functional levels (Guerra 2008), so the information about the morphology and karyotypic structure of an organism is essential (Baltisberger and Widmer 2009).

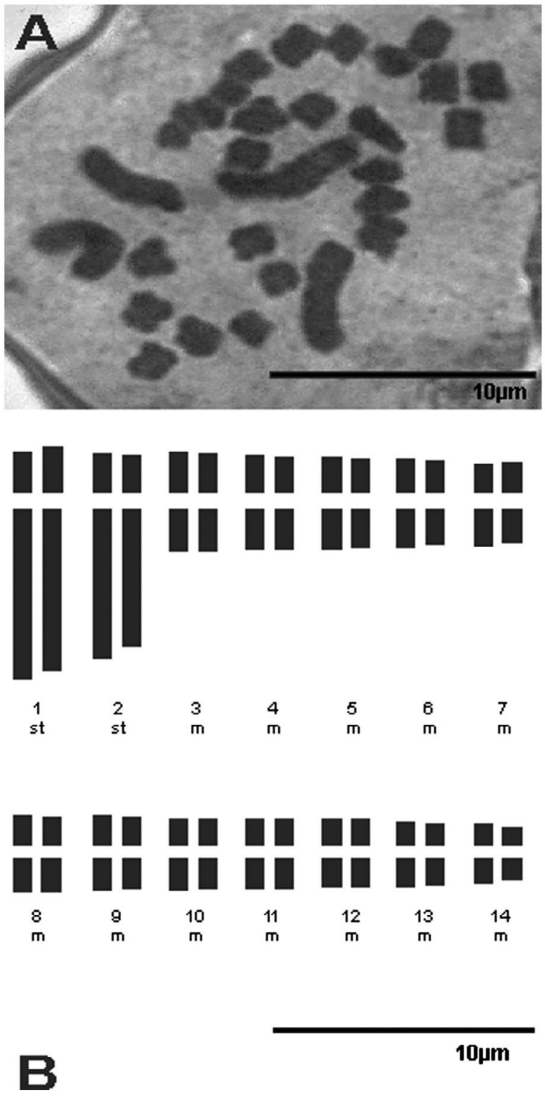

Figure 2. *Tigridia multiflora* diploid $2n = 2x = 28$. (A) Mitotic cell with metaphase chromosomes. (B) Idiogram with a karyotype of 24 m + 4st.

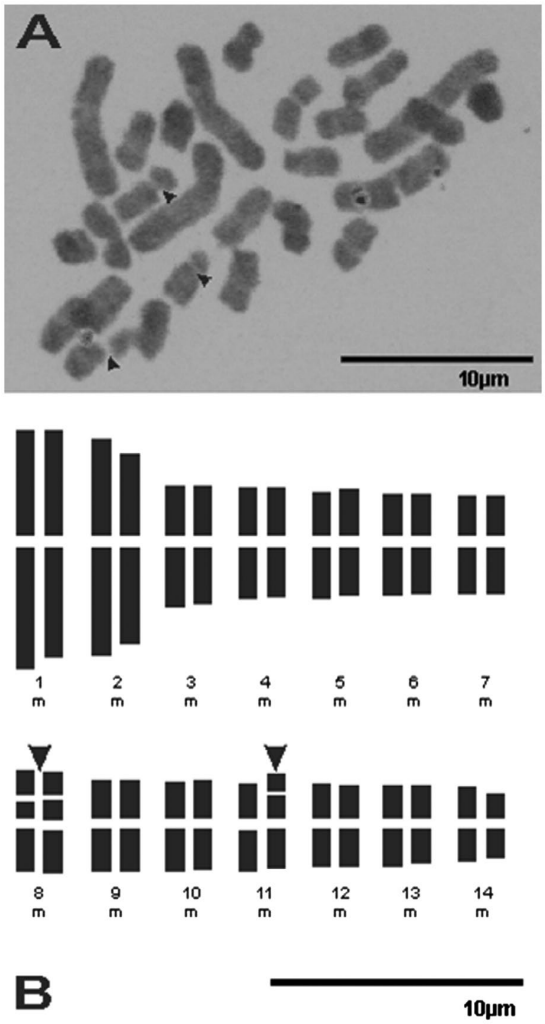

Figure 3. *Tigridia pavonia* var. *Sandra* diploid $2n = 2x = 28$; arrows indicate secondary constrictions in the respective chromosome. (A) Mitotic cell with metaphase chromosomes. (B) Idiogram with a karyotype of 28 m.

Table 1. Karyotype analysis in three Tigridia species.

Species	Chromosome number	Karyotypic formula	Chromosome size range (µm)	Secondary constriction	Total genome lengthµm	Asymmetry indexTF%
T. pavonia var. *Sandra*	$2n = 28$	28 m	1.99 – 8.24	15 la, 16la and 22la	101.45[a]	47.276[a]
T. multiflora	$2n = 28$	24 m + 4 st	1.234 – 6.924		74.647[b]	38.136[b]
T. alpestris subsp. *obtusa*	$2n = 28$	22 m + 2 sm + 4 st	1.408 – 6.549		72.205[b]	36.153[c]

Abbreviations: m, metacentric; sm, submetacentric; st, subtelocentric; la, large arm.
LSD ($p \leq 0.05$).
Values followed by different letters are significantly different.

In the present study, the three analyzed species have a chromosome number of $2n = 2x = 28$ which coincides with that reported by Molseed (1970) and Kenton and Heywood (1984). It is important to mention that for *T. alpestris* subsp. *obtusa* and *T. multiflora*, a bimodal karyotype consisting of four large chromosomes and 24 small chromosomes is reported for the first time, and this is similar to that found in *T. pavonia* var. *Sandra*.

Gianfranco et al. (2008) mention that the karyotype of a species is usually subject to small variations and two similar species may be different due to a number of chromosomal rearrangements correlated with the phylogenetic distance between them. Accordingly, in this study, it was observed that despite the similarity in the numbers and bimodal karyotypes in *Tigridia* species analyzed, each species presented morphological characteristics and different karyotypic formulae:

Tigridia alpestris subsp. *obtusa* (Figure 1) exhibited a karyotypic formula of 22 metacentric, two submetacentric, and four subtelocentric (22 m + 2sm + 4st) chromosomes.

Tigridia multiflora (Figure 2) presented a karyotype formula with 24 metacentric and four subtelocentric chromosomes (24 m + 4st).

As for *T. pavonia* var. *Sandra* (Figure 3), this species presented a karyotype consisting of 28 metacentric, showing the presence of secondary constrictions in chromosomes 15, 16, and 22. Brown and Bertke (1979) mention that often the secondary constrictions are in at least two chromosomes (homologous chromosome pair); therefore the presence of such structures can be used for the identification of specific chromosomes. By contrast in this study, the observed constrictions were located on different chromosomes to those reported by Molseed (1970), who reports them in only two of the large chromosomes, and those reported by Kenton and Heywood (1984), who reported them in the chromosomes 22, 23, and 24. It is important to mention that in their research, the three previous studies did not indicate the variety used, suggesting that each of the nine botanical varieties of *T. pavonia* may have unique structural chromosome changes; additional cytogenetic studies are required to confirm this.

It can be seen that there are notable differences in the karyotype formulae of the evaluated species, and a considerable decrease in TLG and TF % (Table 1). Schubert (2007) indicates that eukaryotic chromosomes may differ in number, size, shape, and DNA composition, and that one of the mechanisms of variation is the loss of dispensable segments, which could explain the difference of TLG between the species evaluated in our study.

Levitzky (1931) and Stebbins (1950) also mention that the karyotypes with a higher degree of symmetry are more primitive than asymmetric karyotypes. Martinez et al. (2000) observed that the genetic divergence found in different populations of *Echeandia nana* was the result of geographic isolation, which favored speciation processes. It is possible that the symmetry and TLG values found in the present study of *T. pavonia* var. *Sandra* are responsible for the higher distribution of this species (Munguia-Lino et al. 2015), and this species could be considered more primitive than *T. alpestris* subsp. *obtusa* and *T. multiflora* which, by evolutionary processes, may have lost their adaptive capacities, and consequently have narrower distributions.

Conclusions

It was found that the chromosome number of *T. pavonia* var. *Sandra T. multiflora*, and *T. alpestris* subsp. *obtusa* is $2n = 2x = 28$. It was also observed that the evaluated species retain a bimodal karyotype formed by four large chromosomes and 24 small ones, establishing that the karyotypic formula for *T. pavonia* var. *Sandra* was 28 m, presenting secondary constrictions on chromosomes 15, 16, and 22; for *T. multiflora* it was 24 m + 4st; and finally *T. alpestris* subsp. *obtusa* was 22 m + 2sm + 4st.

Disclosure statement

No potential conflict of interest was reported by the authors.

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