Acta Bot. Croat. 58, 49–55, 1999

CODEN: ABCRA 25
ISSN 0365–0588

Dedicated to Prof. dr. MERCEDES WRISCHER
on the occasion of her 70th birthday.

UDC 575.11:582.683.2

CYTOGENETICAL INVESTIGATION OF CROATIAN STENOENDEMIC SPECIES *FIBIGIA TRIQUETRA* (*Brassicaceae*)

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This paper concerns the cytogenetic investigation of the Croatian endemic species *Fibigia triquetra* (DC.) Boiss. (*Brassicaceae*). The number of chromosomes in all investigated cells from three populations of *Fibigia triquetra* was 2n=2x=16. Chromosomes were small and ranged from submetacentrics to acrocentrics. One chromosome pair had a relatively small satellite. Although meiosis was not completely regular, irregularities were not very frequent. Low pollen germination rate was probably due to these irregularities. As the species is perennial, weakness of seed germination seems not to be important for its survival.

Key words: chromosomes, meiosis, pollen, seed, *Fibigia*, *Brassicaceae*

Introduction

The populations of *Fibigia triquetra* (DC.) Boiss. (*Brassicaceae*) comprise a small number of specimens present only in ten localities of South Dalmatia (KOSTOVIĆ-VRANJEŠ et al. 1994, DOMAC 1994, BALL 1996, JALAS SUOMINEN 1996). The species is strictly endemic to Croatia and to Europe (BALL 1993, 1996, JALAS and SUOMINEN 1996). It seems to be a relict from the Tertiary (TRAVIZI 1992), and it is an exceptionally valuable and protected species of Croatia.

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Fibigia triquetra has attracted the attention of many botanists since it was discovered in South Dalmatia at Klis (near Split, *locus classicus*) by Portenschlag (MAYER 1981). Different names, mentioned by MAYER (1981), have been used for this species. Due to its morphological characteristics, TRINAJSTIĆ (1980, 1983) separated *F. triquetra* from the genus *Fibigia*, and placed it into the new genus *Pevalekia* as *Pevalekia triquetra*.

In previous papers, the morphological, taxonomical and ecological characteristics (TRINAJSTIĆ 1980, 1983; MAYER 1981, TRAVIZI 1992) and clonal propagation procedure of *Fibigia triquetra* were described (PEVALEK-KOZLINA et al. 1997). It has not previously been subject to karyological investigation. The first chromosome number ($2n = 2x = 16$) of this rare endemic species from the Gata locality was reported by KOSTOVIĆ-VRANJEŠ et al. (1994). The aim of this study was a comparison of three investigated populations of *F. triquetra* based on karyological analysis.

Materials and methods

Karyological analysis was made of plant material collected in the area of Klis, Gata and Omiš in South Dalmatia (Fig.1). Root tips of germinated seeds were pretreated with colchicine, α-bromo-naphtalene or 8-hydroxyquinoline and fixed in methanol : acetic acid (3:1) for 24 h, at 4–5 °C. Feulgen staining was carried out by the conventional method with prolonged hydrolysis in 1 N HCl, for 12–15 min at 60 °C. Cells were additionally stained with 2 % aceto-carmine.

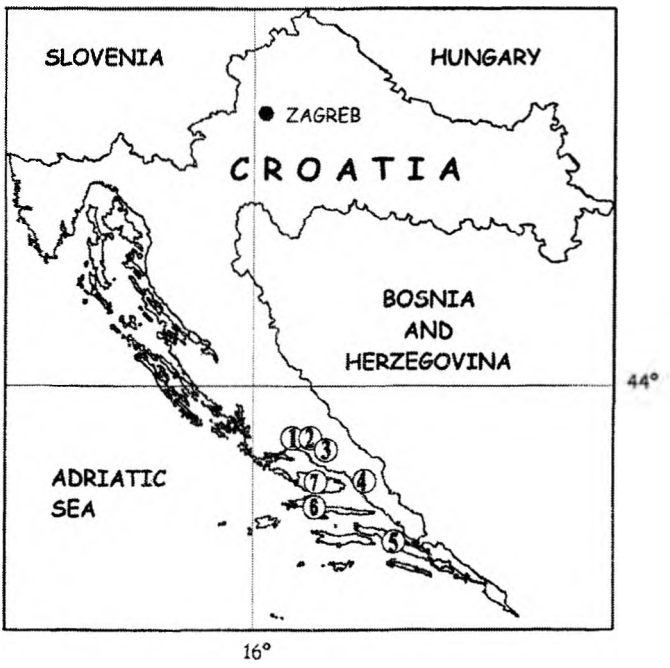

Fig.1. Distribution range of the Croatian endemic species *Fibigia triquetra*: 1– Klis, 2 – Gata, 3 – Omiš, 4 – Biokovo, 5 – Pelješac, 6 – Hvar, 7 – Brač.

The best results were obtained with 0,002 M 8-hydroxyquinoline (for 3–4 h, at 18–20 °C). The length of the long and short arm of the metaphase chromosomes was measured. The ratio between the arms, the total length of the chromosome and the centromeric index were calculated. An average value was estimated on the metaphase plates of four different plants from each population. The chromosomes were classified according to the system suggested by LEVAN et al. (1964).

Analyses of meiotic chromosomes were performed on flower buds fixed in absolute ethanol : chloroform : glacial acetic acid (6:3:1). For studying pollen mother cells, anthers were squashed in 2 % aceto-carmine. Process of meiosis was analysed on buds of ten different plants from each population.

Pollen viability and germinability were investigated by a germination test on an artificial germinating medium according to SHARMA and SHARMA (1972). After 6 hours germination the diameter of 1000 pollen grains was determined. Seed germination percentage was studied and monitored from the fifth to the twentieth day. Seeds were germinated in Petri dishes on wet filter paper.

Voucher specimens of all investigated plants are kept in the Herbarium of the Department of Biology, Faculty of Science, University of Split.

Results

The chromosome number of plants from all three localities investigated (Klis, Gata, Omiš: Fig. 1) was 2n = 2x = 16 chromosomes (Fig. 2). The chromosomes were small, with decreasing values inside the set. Their size ranged from 1.0 to 1.9 μm. The chromosomes were submetacentrics to acrocentrics, and one pair had a satellite on the shorter arm. The total karyotype size was 24.7 μm for locality Klis, 23.5 μm for Gata and for Omiš 22.9 μm.

The process of meiosis in pollen mother cells was also investigated. Seven "ring-shaped" bivalents with terminal chiasmata and a rod-like bivalent were observed from diakinesis to metaphase. In a few cases an irregular segregation of some chromosomes that were not included in metaphase I and anaphase I was

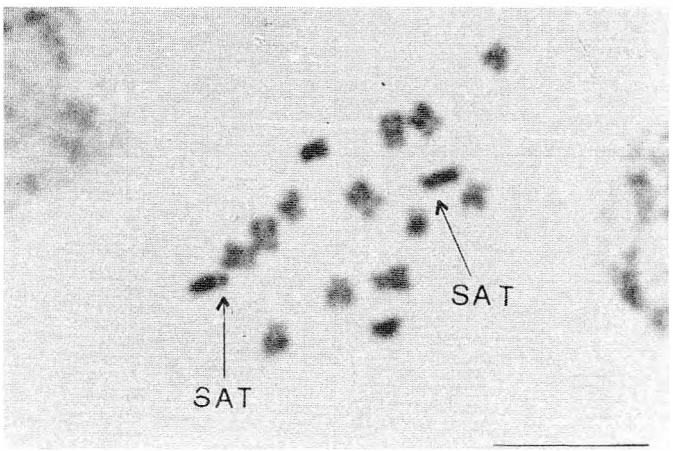

Fig. 2. Mitotic chromosomes of *Fibigia triquetra*. Bar 10 μm.

noticed (Fig. 3). Also, an anaphase I bridge formation and a laggard chromosome in metaphase II were found. The second meiotic division and cytokinesis proceeded synchronously to form tetrads, which later developed into pollen. Irregularities in the process of meiosis were not frequent.

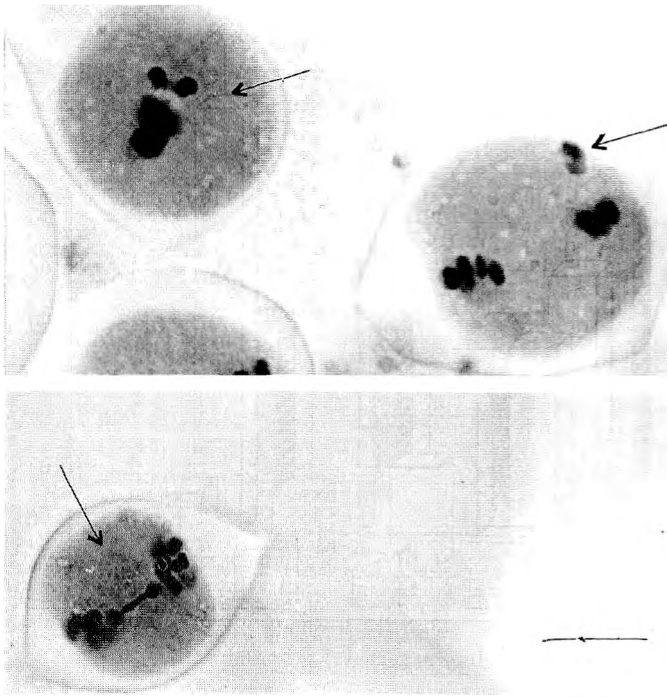

Fig. 3. Meiotic chromosomes with irregularities in meiotic division of *Fibigia triquetra*. Bar 10 μm.

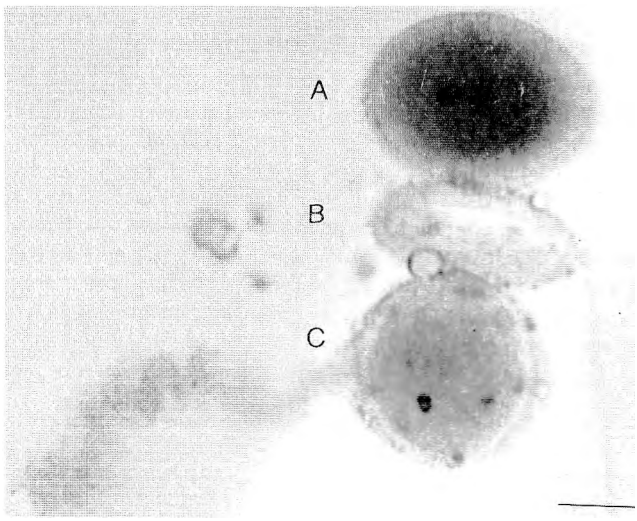

Fig. 4. Pollen grains of *Fibigia triquetra*. A: mature pollen grain; B: empty pollen rain; C: germinating pollen grain. Bar 10 μm

The average diameter of pollen ranged from 27.9 to 34.5 μm. The analysis of germination showed that only 20.88 % grains germinated regularly, 35.61 % of them germinated irregularly and 43.54 % did not germinate at all (Fig. 4). Comparison of average pollen diameter and the percentage of germination showed that grains with a larger diameter germinated better. The best germination was observed with pollen grains of 32.3 μm (Fig. 5).

Seeds of *Fibigia triquetra* germinated from the fifth to the twentieth day (Fig. 6). The best germination percentage was between the seventh and the eleventh day. The average germination percentage was 76.75 %.

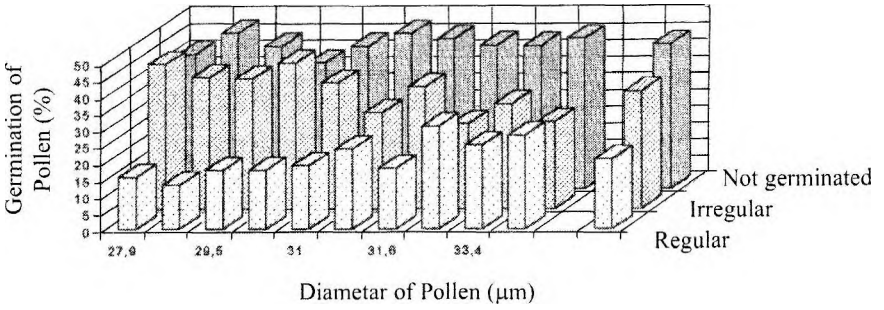

Fig. 5. Pollen grain germination of *Fibigia triquetra*.

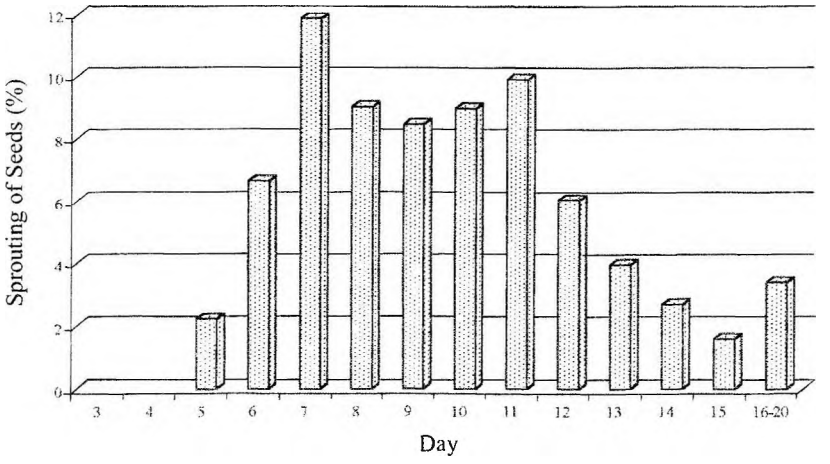

Fig. 6. Seed germination of *Fibigia triquetra*.

Discussion

Cytogenetic investigations of three populations of *Fibigia triquetra*, a species endemic to Croatia, are reported for the first time. It has been established, by classical chromosome staining technique, that plants from all three localities studied (Klis, Gata, Omiš) had 2n = 2x = 16. The basic number of chromosomes 2n = 2x = 16 is common to numerous species of the *Brassicaceae* family. For *Fibigia clypeata* (typical species of genus *Fibigia*) the number of chromosomes was 2n = 2x = 16 in the case of plants collected in Italy, Albany, Greece, Turkey and Bulgaria (ANČEV 1981; BALTISBERGER 1987, 1991). The chromosome number of *Fibigia eriocarpa* was 2n = 14 and 2n = 16 (DARLINGTON and JANAKI AMAL 1945). The basic number of chromosomes of *Fibigia triquetra* (2n = 2x = 16) corresponded to the chromosome number of species in the genus *Fibigia*.

The mitotic chromosomes in *Brassicacea* are characterised by their small size, which makes it difficult to identify homologues and to distinguish between different pairs in the complement (OLIN-FATIH and HENEEN 1992). The same authors noticed that *Brassica* metaphase chromosomes were condensed after the treatment with colchicine or α-bromo-naphtalene, so it was very difficult to see the pairs in the complement. In our investigations, the root tips of *Fibigia triquetra* were pretreated with colchicine, α-bromo-naphtalene or 8-hydroxyquinoline. The best results were obtained with 8-hydroxyquinoline, and in this sample metaphase chromosomes were measured.

The chromosomes of *Fibigia triquetra* from all three studied localities were small submetacentrics to acrocentrics, and one pair had a small satellite on the shorter arm. Analysis of chromosome size showed that there was no difference in morphology or in the length of karyograms in the three investigated localities. It was not possible to compare the form and the size of *Fibigia triquetra* chromosomes with those of other species in the genus *Fibigia*, because there were not enough data in the available literature.

The process of meiosis in pollen mother cells of the plants from all three localities studied (Klis, Gata, Omiš) was not completely regular. In a few cases an irregular segregation of some chromosomes was observed, as well as the formation of anaphase I bridges. The second meiotic division and cytokinesis proceeded synchronously and the irregularities in the process of meiosis were not frequent. The weak pollen germination (20.92 %) was probably a consequence of some irregularities in the process of meiosis.

Investigations of *Fibigia triquetra* seed germination showed that 76.75 % of seeds germinated. Since this endemic species is a perennial, the relative reduction in seed germination is not important for its survival. Contrariwise, a decrease of seed germination is a very important for consideration in the survival of biennial plants like *Fibigia clypeat* and *Fibigia eriocarpa*.

Acknowledgement

This work was financially supported by the Ministry of Science and Technology, Republic of Croatia (Projects No. 119–112 and 119–116).

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