

# CYTOGENETICAL INVESTIGATIONS OF FIVE POPULATIONS OF THE SPECIES *Muscari comosum* (L.) MILL. (*Alliaceae*) FROM DALMATIA

VESNA KOSTOVIĆ-VRANJEŠ<sup>1</sup> & DRAŽENA PAPEŠ<sup>2</sup>

<sup>1</sup>Faculty of Science, Department of Biology, University of Split, Teslina 12/III,  
21000 Split, Croatia

<sup>2</sup>Faculty of Science, Department of Biology, University of Zagreb, Rooseveltov  
trg 6, 10000 Zagreb, Croatia

Kostović-Vranješ, V. & Papeš, D.: Cytogenetical investigations of five populations of the species *Muscari comosum* (L.) Mill. (*Alliaceae*) from Dalmatia, *Nat. Croat.*, Vol. 8, No. 2., 131–136, 1999, Zagreb.

The Mediterranean populations of the species *Muscari comosum* (L.) Mill. are well investigated, and results of these investigations have shown that it is most frequently diploid with  $2n=18$ , and rarely  $2n=19$ ,  $19+1B$ ,  $27$  or  $36$ . A literature search showed that the populations from Dalmatia mentioned have not been karyologically explored. In this work we present cytogenetic investigations of the species *Muscari comosum* (L.) Mill. collected from five localities in central Dalmatia.

**Key words:** Chromosomes, meiosis, pollen germinability, *Muscari comosum* (L.) Mill., *Alliaceae*

Kostović-Vranješ, V. & Papeš, D.: Citogenetička istraživanja pet populacija vrste *Muscari comosum* (L.) Mill. (*Alliaceae*) s područja Dalmacije, *Nat. Croat.*, Vol. 8, No. 2., 131–136, 1999, Zagreb.

Mediterranske populacije vrste *Muscari comosum* (L.) Mill. vrlo dobro su istražene. Rezultati njihovih istraživanja pokazuju da su većinom diploidi s  $2n=18$ , a rjeđe  $2n=19$ ,  $19+1B$ ,  $27$  ili  $36$ . Pregledom literaturnih podataka ustanovljeno je da navedena vrsta s područja Dalmacije nije kariološki analizirana. U ovom radu navedeni su rezultati citogenetičkih analiza biljaka vrste *Muscari comosum* (L.) Mill. sakupljenih na pet lokaliteta središnje Dalmacije.

**Key words:** Kromosomi, mejoza, klijavost peluda, *Muscari comosum* (L.) Mill., *Alliaceae*

## INTRODUCTION

Results of cytogenetic investigations of the species *Muscari comosum* (L.) Mill. (*Alliaceae*) have shown that it is most frequently diploid with  $2n=18$  (RUIZ REJON *et*

*al.*, 1985, 1986; VALDES & DIAZ LIFANTE, 1992; LOZANO *et al.*, 1990), and rarely  $2n=19, 19+1B, 27$  or  $36$  (BENTZER, 1972; RUIZ REJON *et al.*, 1981, 1986; VALDES & DIAZ LIFANTE, 1992).

The karyotype is asymmetric with one pair of large telocentric chromosomes, one pair of intermediate sized subtelocentric chromosomes and seven pairs of small metacentric chromosomes (RUIZ REJON *et al.*, 1986).

According to the literature, populations from Dalmatia have not been karyologically explored. Cytogenetic investigations of the species *Muscari comosum* (L.) Mill. collected from five localities from central Dalmatia are presented in this work.

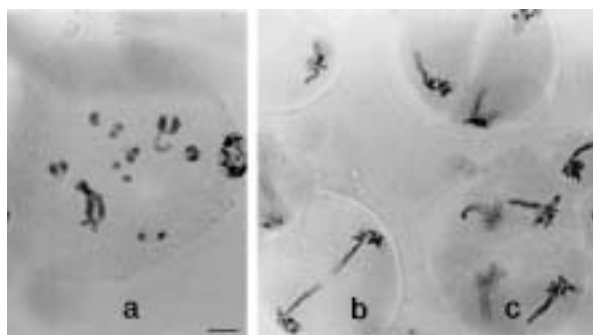
## MATERIALS AND METHODS

Karyological investigations were made on plant material collected from five localities in central Dalmatia: the Marjan hill near Split, the Sv. Križ hill near Mravinci, Klis, Rupotina and Soldun on the island of Čiovo (Fig. 1).

For chromosomal analysis the root-tips of bulbs collected in the field and grown in the laboratory, were pretreated with 0,002 % colchicine for 3–4 h, and fixed in Carnoy's solution for 24 h, at 4–5 °C. Feulgen staining was carried out by the conventional procedure with additional staining by carmine acetic acid. An idiogram was made from an average of ten somatic metaphases.

Analyses of meiotic chromosomes were performed on flower buds fixed in a mixture of absolute ethanol : chloroform : glacial acetic acid (6:3:1 v/v). For the study of pollen mother cells (PMCs), anthers were squashed in 2% aceto-carmine.

Pollen viability and germinability were examined by a germination test on an artificial germinating medium ( $H_3BO_3$ ,  $Ca(NO_3)_2 \times 4H_2O$ ,  $MgSO_4 \times 7H_2O$ ,  $KNO_3$ , sucrose, water) according to SHARMA & SHARMA (1972). The diameters of 1500 pollen grains from some localities was also determined.



**Fig. 1.** Investigated localities of the species *Muscari comosum* (L.) Mill. from central Dalmatia: 1 – Marjan Hill near Split, 2 – Sv. Križ Hill near Mravinci, 3 – Klis, 4 – Rupotina, 5 – Soldun on the island of Čiovo.

## RESULTS AND DISCUSSION

The cells of all plants from five investigated localities (Marjan Hill, Sv. Križ Hill, Klis, Rupotina and Soldun) had  $2n=2x=18$  chromosomes (Fig. 2). Chromosome size ranged from 2  $\mu$ m to 12  $\mu$ m. According to Levan's classification (LEVAN *et al.*, 1964), the karyotype is asymmetric with one pair of large telocentric chromosomes (average value 11.78  $\mu$ m), one pair of intermediate size subtelocentric chromosomes (average value 7.6  $\mu$ m) and seven pairs of small metacentric chromosomes (from 2 to 4.6  $\mu$ m). The karyogram formula is  $2t + 2st + 14m$  (Fig. 3). Chromosome measurements showed that there was no difference in morphology of karyograms from the five localities investigated. There was also no difference in the profiles of single karyograms. The results obtained were in agreement with results of RUIZ REJON *et al.* (1995).

The process of meiosis studied in pollen mother cells (PMCs) was regular. In metaphase I, 9 bivalents were easily counted. Chiasmata were missing in the interstitial region of the long pair of chromosomes. The second pair of chromosomes formed »ring-shaped« bivalents with terminal chiasmata. The seven pairs of small metacentric chromosomes formed bivalents with one chiasma. (Fig. 4). Both in anaphase I and anaphase II the long arms of the largest chromosome were the latest to separate, which gave the false impression of anaphase bridges (Fig. 4). The meiotic second division and cytokinesis proceeded synchronously to form tetrads, which developed later into pollen.

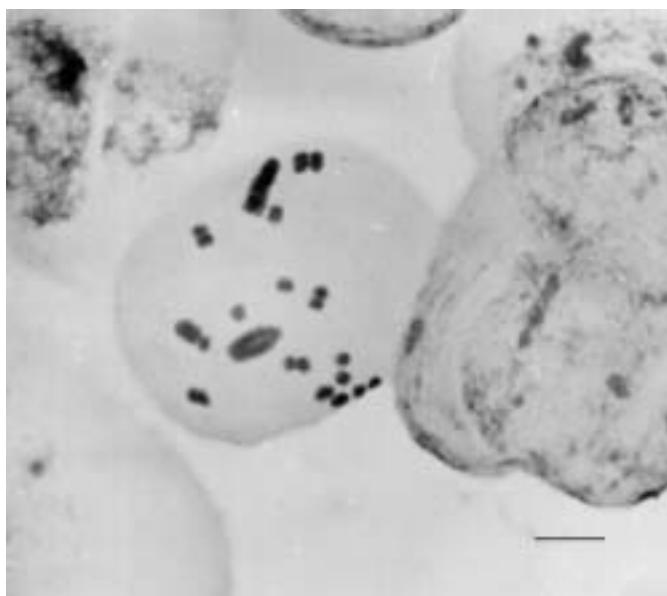


Fig. 2. Mitotic chromosomes of the species *Muscari comosum* (L.) Mill. ( $2n=2x=18$ ).  
Bar 10  $\mu$ m.

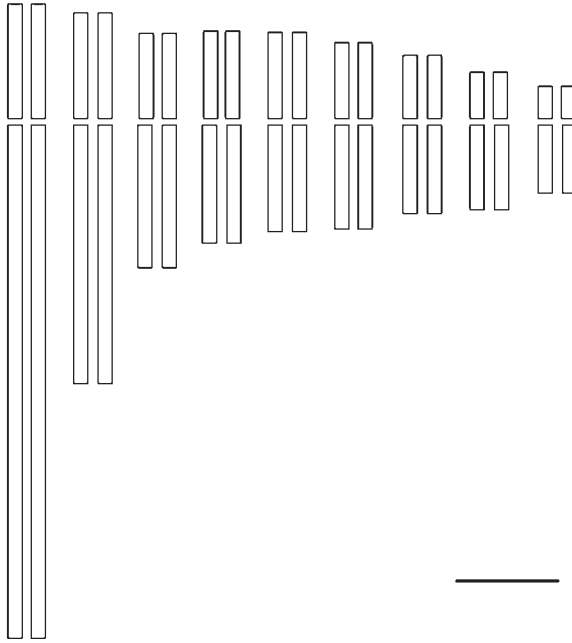


FIG. 3. The idiogram of the species *Muscari comosum* (L.) Mill. ( $2n=2x=18$ ).  
Bar 10  $\mu$ m.

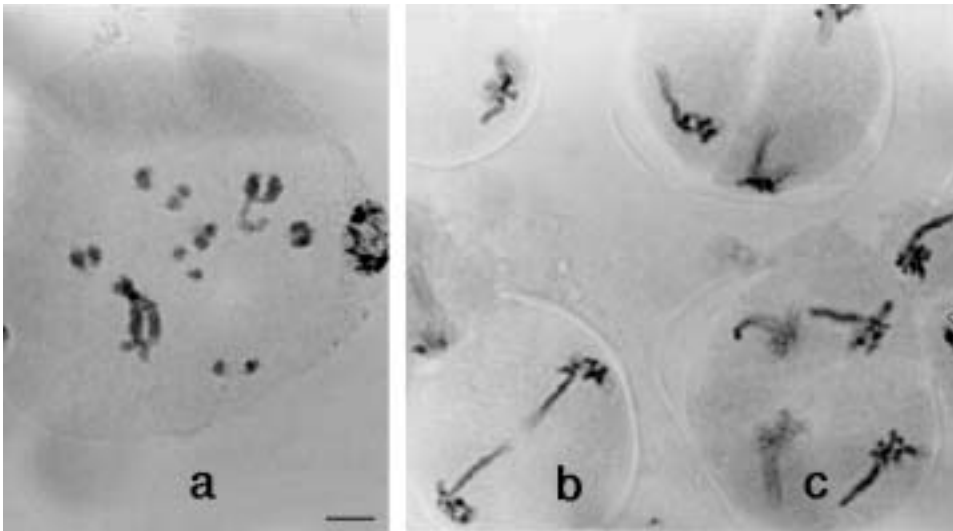


Fig. 4. Meiotic chromosomes of the species *Muscari comosum* (L.) Mill.:  
a – bivalents, b – long arm of the longest bivalent protrudes from the mass of  
other chromosomes, c – anaphase II. Bar 10  $\mu$ m.

The diameter of pollen was from 25.3  $\mu$ m to 57.5  $\mu$ m, while the mean value was 45.1  $\mu$ m. By analysing the results of sprouting from the five localities investigated we discovered that 46.52% grains germinated regularly, 17.63% germinated irregularly and 34.63% did not germinate at all (Fig. 5). Comparing the percentage of pollen sprouting from different localities, it was determined that pollen from the locality Rupotina germinated the best, while grains from other localities showed similar values of germination.

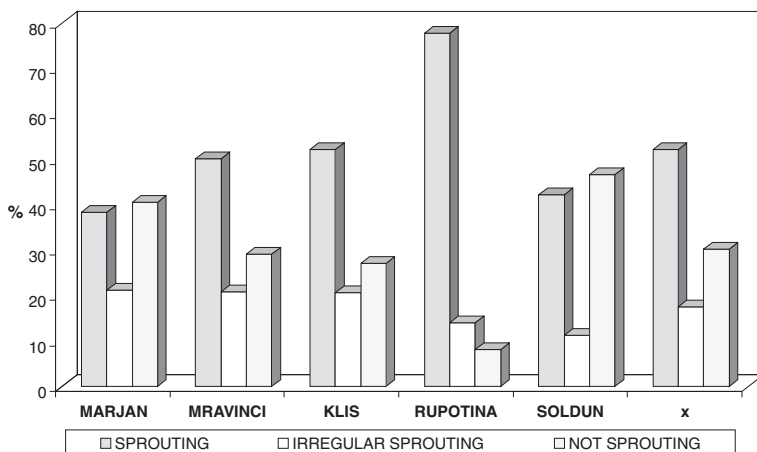


Fig. 5. Sprouting of pollen grains (%) of the species *Muscari comosum* (L.) Mill. from five localities: Marjan, Mravinci, Klis, Rupotine, Soldun, x – average value.

## ACKNOWLEDGMENT

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

Received March 22, 1999

## REFERENCES

- BENTZER, B., 1972: Variation in the chromosome complement of *Leopoldia comosa* (L.) Parl. (Liliaceae). *Botaniska Notiser*, **125**, 406–418.
- BENTZER, B., 1973: Taxonomy, variation and evolution in of *Leopoldia comosa* (L.) Parl. (Liliaceae) in the southern and central Aegean. *Botaniska Notiser*, **126**, 69–132.
- LEVAN, A., FREDGA, K. & SANDBERG, A. A., 1964: Nomenclature for centromeric position of chromosomes. *Hereditas* **52**, 201–219.
- LOZANO, R., JAMILENA, M., RUIZ REJON, C. & RUIZ REJON, M., 1990: Characterization of the chromatin of some liliaceous after digestion with restriction endonucleases and sequential Giemsa, fluorochrome and silver staining. *Heredity* **64**, 185–195.

- RUIZ REJON, C., LOZANO, R. & RUIZ REJON, M., 1986: Numerous cromosomicos flora Espanola. Numeros 479-484. *Lagascalia* **10**, 292-297.
- RUIZ REJON, M., LOZANO, R. & RUIZ REJON, C., 1981: Numerous cromosomicos flora Espanola. Numeros 240-244. *Lagascalia* **10**, 247-252.
- RUIZ REJON, M., PASCUAL, L., RUIZ REJON, C., VALDES, B. & OLIVER, J. L., 1981: A new species of *Muscari* subgenus *Leopoldia* from Iberian Peninsula. *Biochemical Systematics and Ecology*, **13**, 239-250.
- SHARMA, A. K. & SHARMA, A., 1972: *Chromosome techniques theory and practice* (2. ed.) Butterworth & Co (Publishers) LTD. London, University Park Press Baltimore, 312.
- VALDEZ, B. & DIAZ LIFANTE, Z., 1992: Karyology and reproductive biology of *Muscari matiricensis* M. Ruiz Rejon *et al.* (Liliaceae). *Botanical Journal of the Linnean Society*, **109**, 125-130.

## SAŽETAK

### Citogenetička istraživanja pet populacija vrste *Muscari comosum* (L.) Mill. (*Alliaceae*) s područja Dalmacije

V. Kostović-Vranješ & D. Papeš

Mediterranske populacije vrste *Muscari comosum* (L.) Mill. vrlo dobro su istražene. Rezultati njihovih istraživanja pokazuju da su biljke većinom diploidi s  $2n=18$ , a rjeđe je naveden broj kromosoma  $2n=19$ ,  $19+1B$ , 27 ili 36. Pregledom literaturnih podataka ustanovljeno je da navedena vrsta s područja Dalmacije nije kariološki analizirana. Stoga je i rađena citogenetička analiza biljaka vrste *Muscari comosum* (L.) Mill. sakupljenih na pet lokaliteta središnje Dalmacije. Primjenom klasičnih tehnika za analizu kromosoma pronađeno je da biljke u svim analiziranim stanicama imaju diploidan broj kromosoma  $2n=18$ . Prosječna dužina kromosoma je 2-12  $\mu$ m. Kariotip je asimetričan, s jednim parom velikih telocentričnih kromosoma, jednim parom subtlocentričnih kromosoma srednje veličine i sedam parova malih metacentričnih kromosoma. U mejozi nisu utvrđene nepravilnosti. Kljavost peludnih zrna razlikovala se na pojedinim lokalitetima (36-77%). Analiza kljavosti pokazuje da 46.52% peludnih zrna sa svih lokaliteta pravilno proklija.