

## Karyotype studies in *Pulsatilla zimmermannii*

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MÁRTONFIOVÁ, L., Karyotype studies in *Pulsatilla zimmermannii*. *Biologia, Bratislava*, 59: 61–64, 2003; ISSN 0006-3088.

Karyology of *Pulsatilla zimmermannii* SOÓ, a pannonian endemic, was studied. The chromosome number recorded,  $2n = 16$ , represents the first karyological statement for this species. The karyotype was studied in detail: according to the position of centromere, the largest 5 chromosome pairs are metacentric, followed by two submetacentric and then one subtelocentric chromosome pair. Comparing one Slovak with two Hungarian populations, no difference between their karyotypes was found. The comparison with further available data on *Pulsatilla* chromosomes, especially the presence of satellites are discussed.

Key words: chromosome number, satellites, endangered species, Slovakia, Hungary.

### Introduction

The genus *Pulsatilla* MILL. comprises about 30 species distributed in the temperate zone of northern hemisphere (SKALICKÝ, 1988). Two chromosome numbers are known in the genus, diploid  $2n = 16$  and tetraploid  $2n = 32$  (BOLKHOVSKIKH et al., 1969; GOLDBLATT, 1981, 1984, 1985, 1988; GOLDBLATT & JOHNSON, 1990, 1994, 2000). Chromosome number and karyotype characteristics of *Pulsatilla zimmermannii* SOÓ, a pannonian endemic, has not been reported yet.

*Pulsatilla zimmermannii* SOÓ is distributed mainly in northern and central Hungary. The northern limit of its distribution area lies in eastern Slovakia (VAGENKNECHT & ČEROVSKÝ, 1999). In Hungary it is given from Zempléni-hegység Mts and Bükk Mts (RUŠČANČINOVÁ & MRAZOVÁ, 2001), as a collin – montane species, calciphilous, growing in warm places in light gravelly soils rich in nutrients, in grass fields with forests and colline pasture-lands (SOÓ, 1966). In Slovakia it is given from dry pastures on not calciferous soils from lowland degree (FUTÁK, 1982). In

Slovakia *P. zimmermannii* SOÓ was recently confirmed in 3 localities – Tarbucka hill near village Velký Kamenec, nature reserve Poniklecová lúčka (pasque flower meadow) between the villages of Malý Horeš and Svätušé and in the locality near the village Ladmovce (MRAZOVÁ & RUŠČANČINOVÁ, 2001). In Slovakia *P. zimmermannii* occurs in the same or very close localities with diploid ( $2n = 16$ ) *Pulsatilla pratensis* subsp. *flavescens* (HOLUB) HAZSL. Both the sympatric species are influenced by introgressive hybridization, and the various types connecting two parental taxa occur (VAGENKNECHT & ČEROVSKÝ, 1999). *P. zimmermannii* is endangered and protected by the law in both Hungary and Slovakia.

### Material and methods

Plant material was collected in two localities in Hungary, the localities Gomboshegy and Pancérhegy, both in Zempléni-hegység Mts, and in the locality Tarbucka near Velký Kamenec village in Slovakia. Since in both Hungary and Slovakia the species belongs to the protected ones (in Slovakia, according to the IUCN categorization it is included in the category CR – Crit-

Table 1. Average and relative chromosome length and centromeric index of particular chromosome pairs in *Pulsatilla zimmermannii* SOO from the localities Gomboshegy and Pancérhegy.

| Chromosome pair | Chromosome length ( $\mu\text{m}$ ) | Relative chromosome length | Centromeric index | Chromosome designation |
|-----------------|-------------------------------------|----------------------------|-------------------|------------------------|
| I               | 10.08                               | 0.160                      | 47.2              | m                      |
| II              | 9.92                                | 0.157                      | 48.5              | m                      |
| III             | 9.28                                | 0.147                      | 47.8              | m                      |
| IV              | 8.16                                | 0.130                      | 48.3              | m                      |
| V               | 7.52                                | 0.120                      | 43.1              | m                      |
| VI (SAT)        | 6.24 (0.5)                          | 0.099                      | 28.2              | sm                     |
| VII             | 6.24                                | 0.099                      | 27.0              | sm                     |
| VIII            | 5.36                                | 0.085                      | 18.8              | st                     |

ically endangered), we were allowed to take only one plant (locality Tarbucka) or two plants from each locality (Gomboshegy, Pancérhegy) for research purposes. Herbarium specimens are deposited in KO herbarium (Botanical Garden of P. J. Šafárik University in Košice, Slovakia).

For the karyotype analysis, root tips of potted plants were used. The adult plants did not grow well in culture, the first new roots suitable for analyses appeared only in autumn. The root tips were pre-treated with 0.25% aqueous solution of colchicine for 3 h, fixed in acetic ethanol (1:3), hydrolyzed for 5 minutes in 1N HCl at 60°C. The meristems were squashed using cellophane technique (MURIN, 1960) and stained in 10% Giemsa stain solution in Sørensen phosphate buffer. The slides were washed in distilled water, dried and observed in a drop of immersion oil. Chromosome number was calculated first in about 10 metaphases from each locality, then the best metaphase plates were selected for calculation of chromosome characteristics (two from the locality Gomboshegy, four from the locality Pancérhegy and two from the locality Tarbucka). The selected metaphases were photographed, the chromosomes measured. For the chromosome identification and comparison, the absolute chromosome length, relative chromosome length (the ratio of the length of particular chromosome to the sum of lengths of all chromosomes in one chromosome set) and centromeric index (ratio of the length of shorter arm to the length of chromosome) were used. The classification of chromosomes is according to LEVAN et al. (1964).

## Results

All the plants studied were diploid,  $2n = 16$ .

### *Plants from Gomboshegy and Pancérhegy*

The four plants examined exhibited no differences in karyotype. Their particular chromosome pairs are characterised in Table 1.

The first five chromosome pairs are metacentric (m), the pairs VI and VII are submetacentric, the pair VIII subtelo-centric. The satellites detected



Fig. 1. Idiogram of *Pulsatilla zimmermannii* SOO from the localities Gomboshegy and Pancérhegy.

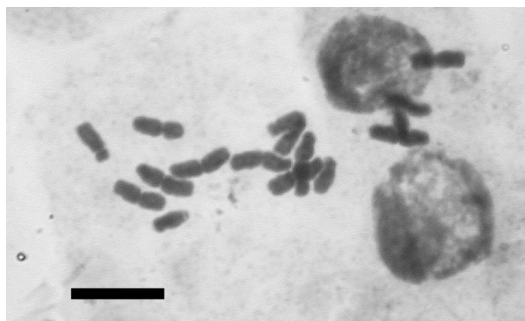


Fig. 2. Metaphase plate of *P. zimmermannii* SOO from the locality Gomboshegy. Scale bar = 10  $\mu\text{m}$ .

on the pair VI were well-visible. The idiogram is given in Fig. 1, a microphotograph of metaphase plate is given in Fig. 2.

### *Plants from Tarbucka*

In the locality Tarbucka *P. zimmermannii* grows

Table 2. Average and relative chromosome length and centromeric index of particular chromosome pairs in *Pulsatilla zimmermannii* SOÓ from the locality Tarbucka.

| Chromosome pair | Chromosome length ( $\mu\text{m}$ ) | Relative chromosome length | Centromeric index | Chromosome designation |
|-----------------|-------------------------------------|----------------------------|-------------------|------------------------|
| I               | 9.92                                | 0.170                      | 46.9              | m                      |
| II              | 9.04                                | 0.155                      | 48.5              | m                      |
| III             | 8.56                                | 0.147                      | 46.7              | m                      |
| IV              | 7.84                                | 0.135                      | 46.9              | m                      |
| V               | 6.72                                | 0.116                      | 40.8              | m                      |
| VI (SAT)        | 5.68 (0.6)                          | 0.098                      | 26.3              | sm                     |
| VII             | 5.60                                | 0.096                      | 25.0              | sm                     |
| VIII            | 4.80                                | 0.083                      | 21.7              | st                     |

together with *P. pratensis* subsp. *flavescens* (HOLUB) HAZSL. Therefore, in this locality most of the plants are hybrids and introgressants with the characters closer to one of the parental taxons. For the analyses, one plant which resembled *P. zimmermannii* the most, was chosen. The absolute and relative chromosome length and centromeric index are given in Table 2. Despite the fact that the plant analysed was an introgressant, the comparison with the karyotype of the plants from Gomboshegy and Pancérhegy showed almost no differences. Comparing the both karyotypes, there is a difference in length of particular chromosome pairs, which is probably due to different degree of contraction in the metaphases selected. The differences in relative chromosome length are, however, negligible. Very slight differences in centromeric index (when compared with *P. zimmermannii* from Pancérhegy and Gomboshegy) are probably due to low number (only two) of metaphase plates suitable for calculation of chromosome characteristics from the locality Tarbucka.

## Discussion

In the literature no data on chromosome numbers and karyotype of *P. zimmermannii* SOÓ were found.

When the karyotype of *P. zimmermannii* is compared with karyotypes of several *Pulsatilla* species published by BAUMBERGER (1971), no obvious differences, as far as haploid chromosome sets are concerned, were present. The comparison of the karyotype of *P. zimmermannii* with the karyotype of *Pulsatilla patens* (L.) MILLER (MIHOKOVÁ & MIKOLÁŠ, 1995) showed the same fact. Only the question of satellites remains unclear. In the karyotype of *P. zimmermannii* one chromosome pair bears well visible satellites. MI-

HOKOVÁ & MIKOLÁŠ (1995) stated that no satellites were well-visible in *P. patens*. In the paper of BAUMBERGER (1971) the question of satellites remains also open. However, in one photograph of the karyotype of *Pulsatilla bungeana* C. A. MEYER which was published there no satellited chromosomes were visible.

The comparison, as far as satellites presence/absence is concerned, with closely related taxon *P. pratensis* subsp. *flavescens* (HOLUB) HAZSL. is desirable. However, we were not allowed to take any plant of this taxon from Hungary because of endangerment of the species (the locality Bodrogköz, Bodrogkeresztúr, where the plants are not influenced by introgressive hybridization, is concerned), for research purposes. In addition, I found no available literature data on this topic. However, it is necessary to mention that the plant from Tarbucka, studied in this work, was influenced by introgressive hybridization with *P. pratensis* subsp. *flavescens* (HOLUB) HAZSL and possessed the same karyotype characteristics, including the position and size of satellites, as the plants of *P. zimmermannii* from Hungary.

The similarity of the karyotypes of various *Pulsatilla* species are probably one of the main reasons, why various species of *Pulsatilla* (even from various sections) hybridize easily.

## Acknowledgements

The author is also thankful to K. MIČIETA, J. ŠTĚPANKOVÁ, P. MRÁZ, A. KRAHULCOVÁ for the help with some literature sources, V. MRÁZOVÁ, A. ŠIMKOVÁ for bringing the plants from the localities, P. MARTONFI for some technical help and critical reading of the paper and an anonymous reviewer for valuable comments. This study was carried with financial support of the grant VTP 324/2000 of the Ministry of Education of the Slovak Republic.

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Received Dec. 5, 2002

Accepted Oct. 19, 2003