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THE GIEMSA C-BANDED KARYOTYPE OF DIPLOID SYMPHYTUM OFFICINALE (BORAGINACEAE)

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SUMMARY

The twelve pairs of chromosomes of the diploid cytotype of *Symphytum officinale* L. (2n = 24) can be distinguished individually with the use of their Feulgen-Giemsa banding pattern in combination with the relative length of the chromosomes and the position of the centromeres. The karyotypes of plants from France, Hungary and The Netherlands do not differ significantly, except for the number of satellites.

1. INTRODUCTION

For *Symphytum officinale* L. the chromosome numbers 2n = 24, 40, 48 and 56 have been reported (GADELLA & KLIPHUIS 1967, 1978). In addition all the chromosome numbers between 2n = 40 and 2n = 48 occur occasionally in nature due to hybridization of the two tetraploid cytotypes (GADELLA 1972, GADELLA & KLIPHUIS 1971). The diploid cytotype is morphologically indistinguishable from the tetraploid plants with the chromosome number 2n = 48, except for the colour of the flowers which is always white in the diploid plants whereas it is white or purple in the tetraploid plants (l.c.). The tetraploid cytotypes are fairly common and widely distributed throughout Europe, but the diploid plants have only been found in a small number of scattered populations in western, central and eastern Europe. They have been reported from Britain, France, Germany, Hungary, Italy and The Netherlands (GADELLA & KLIPHUIS 1978, GADELLA et al. 1983).

Morphologically the diploid plants of *S. officinale* are rather uniform, though plants from different parts of Europe may differ somewhat in the length of the flowering stems, the indumentum and the shape of the corolla (KLIPHUIS, pers. comm.). In the present study we have tried to establish whether the twelve pairs of chromosomes of the diploid cytotype can be distinguished with the use of the Giemsa banding technique and in addition whether the karyotypes of plants from different origins are identical or not. Since differential staining of chromosomes has proved to be very useful in the study of polyploid complexes (VOSA

1976, GERLACH 1977, KENTON 1978, JEWEL 1979, VAN RAAMSDONK 1984) these results will be used in further studies to elucidate the origin of the tetraploid cytotypes of *S. officinale*.

2. MATERIAL AND METHODS

The plants of *S. officinale* were collected in nature and transplanted to the experimental garden of the State University of Utrecht. The plants were grown in pots in the temperate greenhouse. Voucher specimens of the plants studied have been deposited in the Botanical Institute of Utrecht (U).

Prior to fixation the plants were placed in the cold overnight (c. 0 °C). Root-tips of 0.5–1.5 cm were fixed in a mixture of ethanol (96%) and acetic acid (3:1). They were stained according to the Feulgen-Giemsa staining procedure described by GOSTEV & ASKER (1979), but the roots were macerated in a mixture of equal parts of pectinase (4% in glycerol) and cellulase (1% in Gurr buffer pH 4.0) at 27 °C for 1 hour. The slides were stained in 2% Giemsa (Merck) for 5 minutes, and after drying to the air for about 1 hour mounted in Gurr's DePex.

Of each plant 5 metaphase plates were drawn with the use of a camera lucida (magnification about 9000 times). All measurements were made in the drawings.

3. RESULTS AND DISCUSSION

The karyotypes were studied of six diploid plants of *S. officinale* (2n = 24) from Hungary, France and The Netherlands (two plants from each locality, *table 1*). In these plants, the combined Feulgen-Giemsa method revealed the presence of bands of heterochromatin in all the chromosomes. However, the bands varied in size, position and intensity.

The chromosomes of the diploid cytotype of *S. officinale* are very small (*fig. 1*). The mean length of the longest chromosome is 2.4 microns (SD 0.1 micron) and of the smallest chromosome 1.1 micron (SD 0.1 micron). The chromosomes are acrocentric; the size of the long arm of the chromosomes is two to four times as long as the short arm. With the use of a combination of the banding pattern and the relative length of the chromosomes and the position of the centromeres it is possible to distinguish the twelve pairs of chromosomes of the diploid cytotype of *S. officinale*.

Table 1. Collection number and origin of the diploid plants of *Symphytum officinale* L. (2n = 24).

Coll. no.	Origin
11314	Hungary: prov. Heves; Lőrinci, ca. 10 km north of Hatvan.
11754	Hungary: prov. Pest; Dabas, ca. 37 km south-east of Budapest in the direction of Kecskemét.
25559, 25580	France: dep. Isère; Le Fayet ca. 4 km south-west of Pontcharra.
28255, 28257	Netherlands: prov. Utrecht; Vic. of IJsselstein.

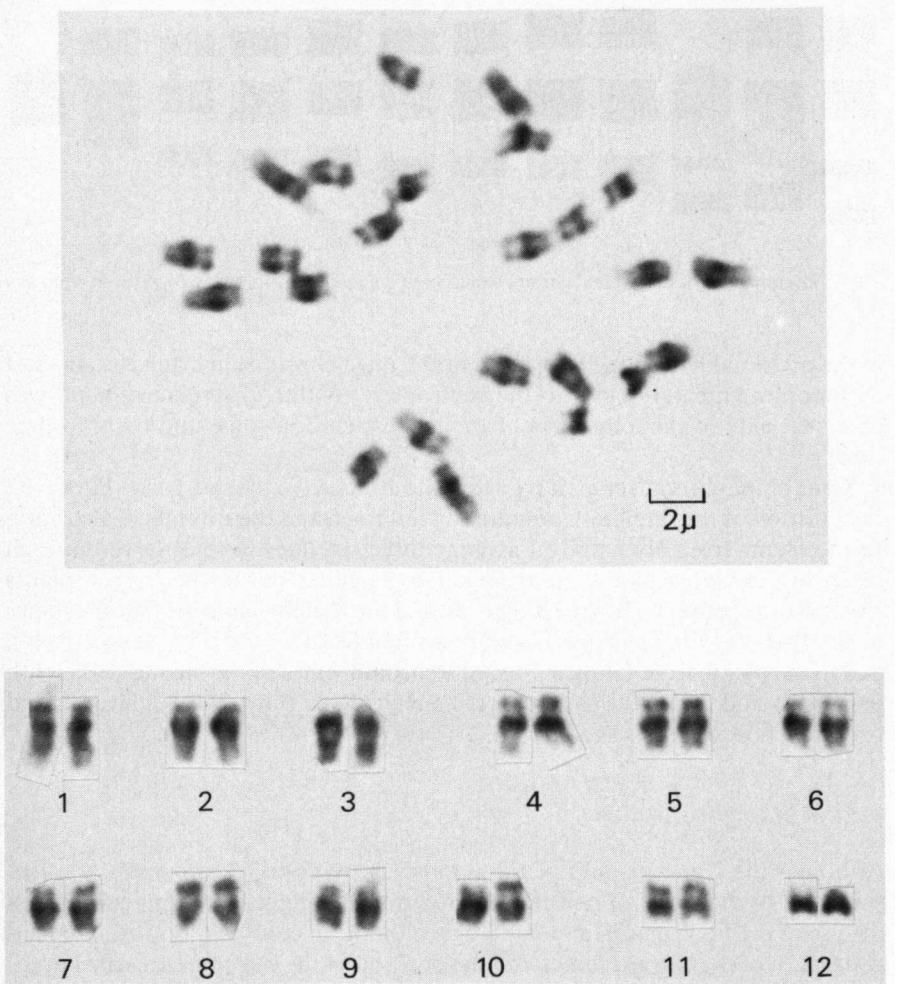

Fig. 1. The Giemsa C-banded karyotype of a diploid plant of *Symphytum officinale* L. from Le Fayet (France).

The karyotypes of the six plants of *S. officinale* from France, Hungary and The Netherlands do not differ significantly. The idiogram of the haploid karyotype (*fig. 2*) consists of three long chromosomes (2–2.4 micron), two very short chromosomes (1.1–1.3 micron) and seven chromosomes of intermediate length (1.5–2 micron). The chromosomes have a heterochromatic terminal band in the short arm, except for chromosome three and the smallest chromosome in both of which the short arm is Giemsa negative. The long arms of all the chromosomes have a distinct heterochromatic band in the proximal half near the centromere and a narrow, usually faint terminal band, except for the two smallest chromosomes in which the long arms are completely heterochromatic. The distinct band

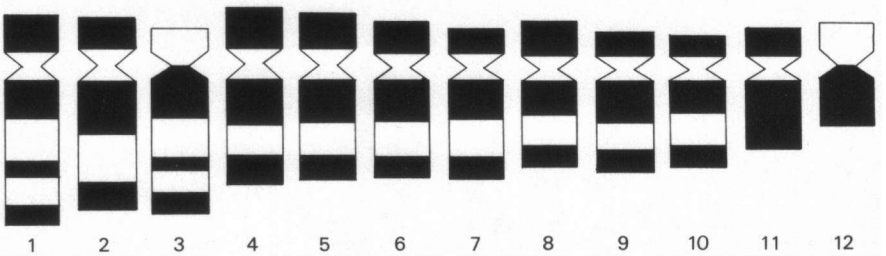

Fig. 2. The idiogram of the haploid chromosome complement of the diploid cytotype of *Symphytum officinale* L.

in the proximal half of the long arms of the third chromosome and the smallest chromosome are very close to the centromere. A third, intercalary band was observed only in the long arms of the longest chromosome and chromosome three.

Some of the chromosomes have a satellite attached to the short arm. However, the position of the satellites is apparently not fixed and their numbers also vary. In the plants from Hungary on average three satellites were observed in each plate, in the Dutch plants on average two satellites but in the French plants less than one per plate. In the Hungarian and the Dutch plants the chromosomes of the first and fifth pair usually bore a satellite (70% and 55%, respectively), and in the plants from Hungary also the chromosomes of the second and eighth pair often had a satellite. The chromosomes of the third, tenth, eleventh and twelfth pair never had a satellite.

4. CONCLUSIONS

Although the chromosomes of the diploid cytotypes of *S. officinale* are very small the twelve pairs of chromosomes can be distinguished individually with the use of the Feulgen-Giemsa banding technique in combination with the relative length of the chromosomes and the position of the centromeres. The karyotypes of the six plants from France, Hungary and The Netherlands did not differ significantly, except for the number of satellites which was much higher in the Hungarian plants than in the others. The results indicate that the Feulgen-Giemsa banding technique can be used in further studies about the origin of the karyotypes of the tetraploid cytotypes of *S. officinale* (2n = 40 and 48).

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