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Karyotypes and meiotic behavior of chromosomes in two male sterile strains of *Brassica campestris* L.

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Male sterility was examined in backcross families of the hybrid *Diplotaxis muralis* × *B. campestris* (*mur*-cytoplasm). Segregation of sterile and fertile plants did not fit to any of the Mendelian ratios. Since an extra chromosome is known to occur in sterile plants having a similar combination of *mur*-cytoplasm and *B. napus* nucleus, the mitotic and meiotic chromosomes were analysed. Similarly, the chromosomes were examined in another male sterility system, in backcross families of *B. napus* × *B. campestris* (*pol*-cytoplasm). The normal chromosome number (2n=20) was detected in all the sterile as well as the fertile plants. After improvements of the staining technique, all the chromosomes were identified in some cells. Ten bivalents were formed in meiosis and the segregation of chromosomes appeared to be normal. The visibility of satellites was examined at diakinesis. Variation among plants was observed but no correlation with male sterility was found.

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A system that facilitates cross pollination is a necessity for hybrid seed production. Cytoplasmic male sterility (CMS) has proved to be the most suitable method in the field scale. Hence, different CMS systems based on native or alien cytoplasms have been tested in cultivated *Brassica* species (e.g., ERICKSON et al. 1986; KEMBLE et al. 1986). One of these is *mur*-cytoplasm originating from the related wild species *Diplotaxis muralis*. HINATA and KONNO (1979) reported a CMS system with *mur*-cytoplasm and nuclear genome from *B. campestris* (2n=20). On the other hand, when FAN et al. (1985) crossed *D. muralis* as female parent and *B. napus* (2n=38), they observed in the sixth backcross families to *B. napus* that all the male sterile plants had an extra chromosome, while the fertile plants had the normal chromosome complement of *B. napus*. They concluded that the presence of the extra chromosome obviously derived from *D. muralis* is the sole cause of male sterility in these families. In the present study, we have examined backcross families bearing *mur*-cytoplasm and nuclear genome from *B. campestris*. The proportion of male sterile plants varied from 0 to 100 % in different families, and no Mendelian ratios could be confirmed. In another male sterility system, *pol*-cytoplasm (from *B. napus* cv. 'Polima') with *B. campestris* genome, the segregation of sterile and fertile plants was more regular, one or two dominant genes restoring the fertility (SOVERO unpubl.). Karyotypes and meiosis of sterile and fertile plants have been analysed in both systems.

Another aim of the present work was to improve the methods used for karyotyping the somatic chromosomes of *Brassica* species. The chromosomes are small, but size differences have been observed already by CATCHESIDE (1934), ALAM (1936) and RICHHARIA (1937). The most detailed descriptions of the chromosomes are the pachytene maps of RÖBBELEN (1960) and VENKATESWARLU and KAMALA (1971). The basic chromosome number in *Brassica* species is thought to be six on the basis of the secondarily paired groups of bivalents in meiosis (CATCHESIDE 1937). Hence, *B. campestris* should be regarded as a partially polyploid species. According to RÖBBELEN (1960) six basic chromosome types could be recognized. The formula AABCDDEFFF indicates that chromosome type A is tetrasomic (satellited chromsomes 1 and 2), B disomic (chromosome 3), C disomic (the tiny chromosome 4), D tetrasomic (chromosomes 5 and 6), E disomic (chromosome 7) and F hexasomic (chromosomes 8, 9 and 10).

In the present contribution, the name *B. campestris* L. is used as in most works concerning the breed-

ing of this species, although according to OOST (1985) the more correct name is *B. rapa* L. on the basis of the nomenclature rules.

Material and methods

The male sterile strains with *mur*-cytoplasm originated from a cross between *Diplotaxis muralis* and an Indian toria type cultivar 'TL-15' of *B. campestris*. Sixth backcross families to 'TL-15' were used. The plants with *pol*-cytoplasm were, similarly, the sixth backcross families of the hybrid between *B. napus* and *B. campestris* cv. 'Tobin'. In addition, spring turnip rape cv. 'Ante' was used for testing the cytological methods.

Seeds were germinated on moist filter paper in Petri dishes. Mitoses could be examined after two or three days, but usually the seedlings were transferred into paper pots filled with garden soil and cultivated for a week to obtain enough material from individual plants. The same plants were used for the examinations of meiosis.

Root tips were pretreated according to NEWELL et al. (1984), first in iced water for 3 h (0–4°C), thereafter in 2mM 8-hydroxyquinoline solution for 4 h at 18°C, fixed in absolute ethanol—glacial acetic acid (3:1) for 24 h, and stored in 70 % ethanol at 4°C. Various other pretreatments have been tested. After the following pretreatment, longer and classifiable late prophase chromosomes were observed: 2mM 8-hydroxyquinoline for 3 h at 18°C followed by treatment in enzyme solution (1 % pectinase, 2 % cellulase) for 90 min. Material was fixed and stored as above. Young flowers were fixed in 6:3:1 solution of absolute ethanol, chloroform and acetic acid, and stored in the fixative in refrigerator until used.

All the preparations were stained according to the Feulgen-Giemsa double staining method (PURO and NOKKALA 1977). The material was transferred from 70 % ethanol (overnight) through 50 % (30 min) and 30 % (5 min) ethanol into water, followed by a treatment in 1N HCl for 20 min at room temperature and hydrolysis in 1N HCl at 60 °C for 8 min, stained with dilute Schiff's reagent (200 ml boiling water poured on 1g basic fuchsin, filtered and added 0.8 g $K_2S_2O_5$ and 8 ml 1N HCl) for 45 min, and rinsed in water. The material was dissected in 45 % acetic acid. Cover slip was placed on the material, pressed gently and removed after dry ice treatment. Slides were rinsed in absolute ethanol for 5 min, in glacial acetic acid for 25 sec, air-dried and stored overnight or longer at 40°C. Then, the slides were rinsed in Sørensen's phosphate buffer pH 6.8 for 5 min, stained with 4 % Giemsa in the same buffer for 15 min, rinsed in water, air-dried, and mounted in Entellan.

Table 1. Number of chromosomes in root tip cells in different strains of *B. campestris*

Strain	No. of plants	No. of cells	2n
cv. 'Tobin'	5	9	20
cv. 'TL-15'	6	8	20
BC6of *B. napus* × 'Tobin'	4	16	20
BC6of *D. muralis* × 'TL-15'	9	33	20

Results

Somatic chromosomes

Karyotypes were examined from root tip cells of cv. 'Tobin', toria type cv. 'TL-15', and the two backcross families showing male sterility. In all of them, the chromosome number 2n=20 was observed (Table 1). Karyotype analysis in *Brassica* species is complicated, besides the small size of the chromosomes, by varying expression of the nucleolus-organizing regions in chromosomes 1 and 2, and also by somatic association of chromosomes. As shown in Fig. 2 the euchromatic parts of different chromosomes are closely associated at their telomeric regions in prophase cells. Classification of the chromosomes is very difficult at this stage, but associations have been observed between similar and very dissimilar chromosomes. Usually, the associations are absent in fully condensed chromosomes, but occasionally two metaphase chromosomes lie in close proximity (Fig. 3).

After a combined pretreatment with iced water and 8-hydroxyquinoline (Fig. 1), cells were observed where all the chromosomes could be classified on the basis of the pachytene map of RÖBBELEN (1960). Chromosome 1 has a larger and darker satellite than chromosome 2. However, the non-satellited homologues are usually difficult to distinguish from the rest of the chromosomes. Chromosome 3 is submetacentric and the largest of the whole complement in *B. campestris*. On the contrary, chromosome 4 is the smallest one, although sometimes the size difference between that and the next smallest chromosomes 10 and 9 is inadequate

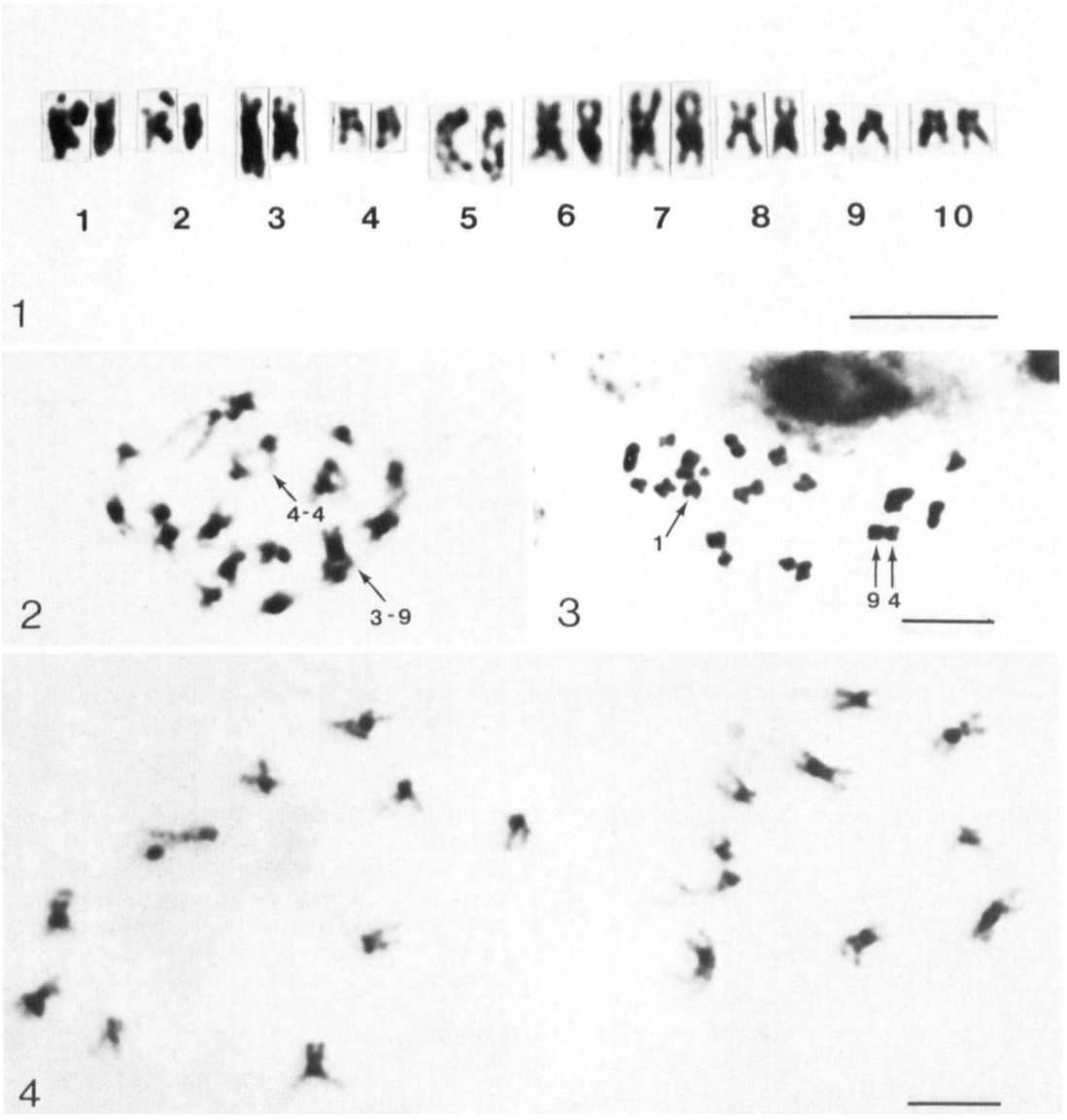

Fig. 1–4. Somatic chromosomes of *B. campestris*. **Fig. 1.** Karyotype of BC 6 of *B. napus* × *B. campestris* cv. 'Tobin' after cold and 8-hydroxyquinoline treatment. **Fig. 2.** Prophase chromosomes of cv. 'Tobin' showing associations of similar (4–4) and dissimilar chromosomes (3–9). **Fig. 3.** Metaphase chromosomes of BC 6 of *Diplotaxis muralis* × *B. campestris* cv. 'TL-15' after cold and 8-hq treatment. 1 = satellited chromosome. Two chromosomes lie close to each other (9–4). **Fig. 4.** Late prophase chromosomes of cv. 'Ante' after 8-hq and pectinase-cellulase treatment. (Bars equal 5 μm).

for classifications. Chromosome 5 is of medium size and easily identifiable as an extreme acrocentric. On chromosomes 6 and 7 the centric heterochromatin is conspicuously asymmetrical, the long arm bearing the larger block. Chromosome 7 is a little larger and more metacentric than chromosome 6. Of the rest of the chromosomes, number 8 is metacentric, and of the acrocentric ones chromosome 9 is larger than 10. According to RÖBBELEN (1960) the centric heterochromatin is asymmetri-

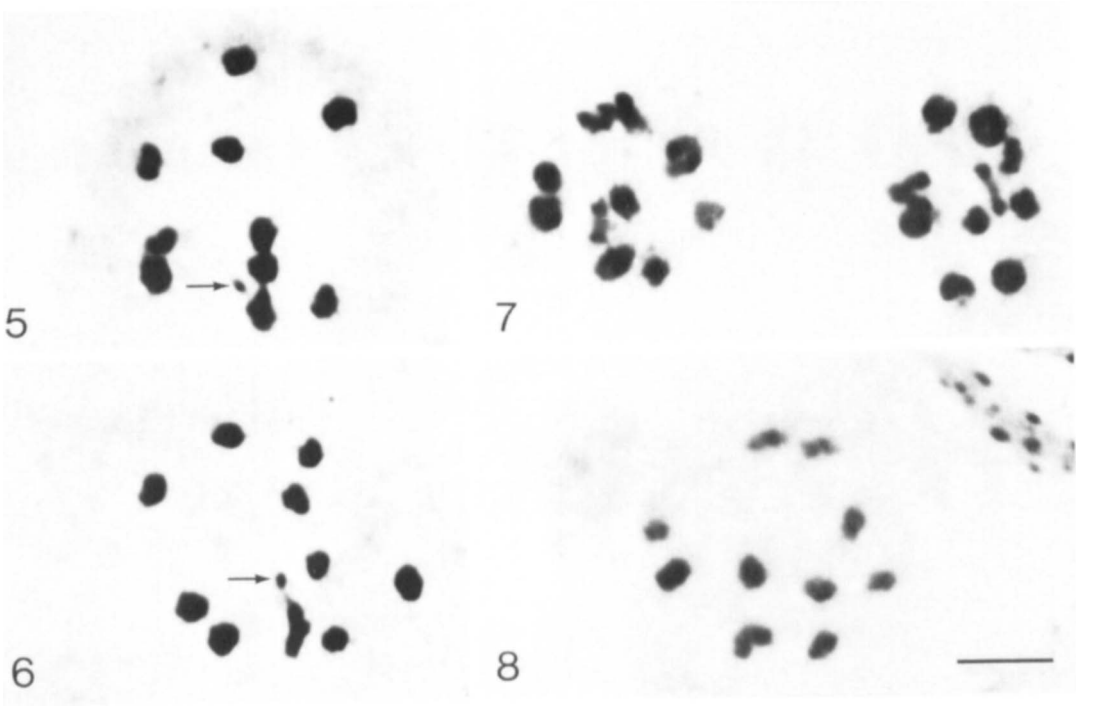

Fig. 5–8. Diakinesis of *B. campestris*. Ten bivalents with a satellite visible (**Fig. 5 and 6**) and invisible (**Fig. 7**) of BC 6 of *B. napus* × *B. campestris*, and BC 6 of *D. muralis* × *B. campestris* (**Fig. 8**). (Bar equals 5μm).

Table 2. Visibility of the satellite at diakinesis in a BC 6 family of *B. napus* × cv. 'Tobin'

	No. of plants	A satellite visible/ Total no. of cells	%
Fertile plants	4	4/246	1.6
Partly sterile plants	10	1/86	1.2

cally located in the two last mentioned chromosomes, the long arm of chromosome 9 having the larger block while on chromosome 10 it is located in the short arm. This difference could not be confirmed with the present technique.

Since classifiable chromosomes were only infrequently observed after the pretreatment described above, several other treatments have been tested. The most promising method seems to be a pectinase-cellulase digestion before fixation combined with 8-hydroxyquinoline (Fig. 4). The late prophase chromosomes appear now more swollen with clear constrictions and sharp boundaries of heterochromatic regions. However, the results were rather variable, and improvements of the method are still needed.

No constant chromosomal differences between the strains could be found, perhaps because of the small number of cells with classifiable chromosomes.

Meiosis

Usually, ten bivalents were observed in all the plants examined. Occasionally two or three bivalents were very close to each other. However, it is very difficult to examine the real positions of the bivalents in squashed material, although the preparations were made with very gentle pressing. Hence no comparisons with the "secondary pairing" described by Catchesidi (1937) have been made. Segregation at anaphase II was normal in nearly all the cells of both male sterile strains examined.

In one nearly totally male sterile plant with *pol*-cytoplasm (BC 6 of *B. napus* × 'Tobin') the satellite was still visible at diakinesis in a large number of cells, when it was invisible in all cells at the same stage in a fertile sister plant. In order to examine if

the expression of the nucleolus-organizing region is different in male sterile and fertile plants, the visibility of the satellite was examined in another family of the same cross. As indicated in Table 2 and Fig. 5–8, the satellite was visible only in a few cells, and no correlation with the male sterility could be established. The visibility of the satellite seems to be rather variable, depending on the strain and the staining method.

Discussion

No extra chromosomes were discovered in the male sterile plants bearing cytoplasm from *D. muralis* and nuclear genome from *B. campestris*. In a similar combination between *D. muralis* cytoplasm and *B. napus* genome (FAN et al. 1985), male sterility was always combined with the presence of an extra chromosome. Hence, the systems producing male sterility with *mur*-cytoplasm in these two *Brassica* species seem to differ, e.g., in the restorer genes of the genomes examined. Meiosis appeared to be normal, as it was in the other male sterile strain having *pol*-cytoplasm.

The somatic chromosomes were identified on the basis of the pachytene map of RÖBBELEN (1960). However, there were certain differences in some chromosomes. The satellited chromosomes 1 and 2 appeared more metacentric than in pachytene chromosomes, and chromosome 5 more acrocentric. There is no way to confirm or disprove the hypothesis of six chromosome types (CATCHESIDE 1937; RÖBBELEN 1960) on the basis of the somatic chromosomes. While chromosomes 1 and 2 (supposed tetrasomic type A) appeared to be morphologically similar, chromosomes 5 and 6 (tetrasomic type D) were very dissimilar, and chromosome 8 differs clearly from 9 and 10 (hexasomic type F). ARMSTRONG and KELLER (1981) found, in haploids of *B. campestris,* the expected maximum of meiotic chromosome pairing, two bivalents and a trivalent in the same cell, but the size of chromosomes involved in the trivalent does not fit to the F chromosomes. The final determination of the partial homology of the chromosomes can be made only with the aid of more efficient molecular methods.

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