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Production and cytogenetics of *Brassica campestris-alboglabra* chromosome addition lines

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Abstract Four different *Brassica campestris-alboglabra* monosomic addition lines (AA + 1 chromosome from C, 2n = 21) were obtained after consecutive backcrosses between resynthesized *B. napus* (AACC, 2n = 38) and the parental *B. campestris* (AA, 2n = 20) accession. The alien chromosomes of *B. alboglabra* (CC, 2n = 18) in the addition lines were distinguished by random amplified polymorphic DNA (RAPD) marker analysis and morphology of mitotic chromosomes. Four RAPD marker synteny groups were established, which represented the four different alien chromosomes of *B. alboglabra* in the four addition lines. Three of the four addition lines were identified to harbour chromosomes 4, 8 or 9 of *B. alboglabra*. Studies on meiotic pairing in the addition lines revealed intergenomic homoeology relationships among specific chromosome arms between the A- and C-genomes. The long arm of *B. campestris* chromosome 9 was homoeologous with the long arm of *B. alboglabra* chromosome 4, while its short arm with the short arms of *B. alboglabra* chromosomes 8 and 9. Such an intergenomic homoeology relationship supports the hypothesis that *B. campestris* and *B. alboglabra* share a common ancestor but that chromosomal rearrangements have occurred during the evolution of the two species. Intergenomic introgression was observed in the progenies of the addition lines. The introgression of an entire *B. alboglabra* marker synteny group into the *B. campestris* genome implied the possible occurrence of interspecific chromosomal substitution.

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Introduction

Within the genus *Brassica*, the amphidiploid species *B. napus* L. (oilseed rape, genomes AACC, 2n = 38) is the most important oilseed crop worldwide. It is also a vegetable crop of considerable importance in some parts of China and Japan. The diploid species *B. campestris* (AA, 2n = 20) and *B. oleracea* (CC, 2n = 18) were identified as the contributors of the *B. napus* genomes following interspecific hybridization (U 1935). This early knowledge has opened up the possibility to resynthesize *B. napus* as a breeding strategy utilizing a wide range of the genetic variability of its parental diploid species (Chen and Heneen 1989). Further cytogenetic studies have revealed that *B. campestris* and *B. oleracea* together with *B. nigra* (BB, 2n = 16) represent an aneuploid series which had evolved from a common ancestor of a lower basic chromosome number. Different basic chromosome numbers such as x = 3 (Hussein and Abobakr 1976), x = 5 (Sikka 1940) and x = 6 (Catcheside 1934) have been suggested.

Modern molecular approaches to genome analysis have indicated the existence of a high frequency of duplicated chromosomal segments rather than the duplication of entire chromosomes in diploid *Brassica* species (Slocum et al. 1990; Song et al. 1991; Truco and Quiros 1994). Such findings support the idea of a *Brassica* ancestor with fewer chromosomes, and meanwhile indicate that extensive rearrangements after the initial events of chromosomal duplication had taken place in the evolution of these genomes. This evolutionary history implies that a high degree of chromosomal homoeologies prevail within as well as among these genomes. The inter- and intragenomic homoeologies in

the diploid *Brassica* species make it possible to shuttle genes among these genomes, which has been beneficial and widely exploited in practical breeding of *Brassica* crops (Prakash and Hinata 1980). On the other hand, such homoeologies pose a potential risk of undesirable interspecific gene flow in nature. In recent years, the release of transgenic oilseed rape varieties for cultivation has evoked such a risk and thus warrants timely risk assessment studies (Kerlan et al.1993; Mikkelsen et al. 1996). The transgenes resident in the transgenic *B. napus* genome may not only be backcrossed into the progenitor diploid species but also introgressed into the genomes of other related species through crossing and subsequent homoeologous recombination in the hybrids. When these aspects are considered, it is of great relevance to more elaborately address the homoeologies of the A- and C- genomes at chromosomal and molecular levels.

The dissection of genomes by generating interspecific addition lines is an effective strategy for addressing homoeology relationships among chromosomes. Research based on this strategy has been fruitful in *Brassica* due to the presence of amphidiploids and the plasticity of the diploid genomes in this genus. *B. campestris*-*oleracea*, *B. napus*-*nigra* and *Diplotaxis erucoides*-*B. nigra* chromosome addition lines have been successfully generated and used for studying chromosomal homoeologies, assigning linkage groups to chromosomes and transferring genes between species (Quiros et al 1987; Jahier et al. 1989; McGrath and Quiros 1990; This et al. 1990; Struss et al. 1991, 1992; Zhu et al. 1993).

We produced resynthesized *B. napus* lines through interspecific hybridization between selected *B. campestris* and *B. alboglabra* (a form of *B. oleracea*) materials which have desirable agronomic or marker traits (Chen et al. 1988, 1989, 1990). Such resynthesized *B. napus* lines with well-defined genomic constitutions are particularly useful in the genetic analysis of agronomic and marker traits. *B. campestris*-*alboglabra* monosomic addition lines (AA + 1 chromosome from C, 2n = 21) were generated by consecutive backcrossing of the resynthesized *B. napus* to the parental *B. campestris* (Chen et al. 1992; Cheng et al. 1994a; Cheng 1996). Using these addition lines, we have been able to identify the chromosomes with genes controlling important agronomic traits such as erucic acid content and seed colour in the *B. alboglabra* genome (Cheng et al. 1995a; Chen et al. 1996; Cheng 1996). Meiotic pairing in the addition lines revealed intergenomic homoeology relationships between specific chromosomes (Cheng et al. 1994b; Cheng 1996). Moreover, random amplified polymorphic DNA (RAPD) markers linked with the genes for erucic acid and seed colour have been established (Jørgensen et al. 1996; Chen et al. 1996). In the present paper, we report on the production and cytogenetics of three new *B. campestris*-*alboglabra* chromosome addition lines.

Materials and methods

Plant material

Resynthesized *B. napus* line No. 7406 is an amphidiploid between *B. campestris* var 'yellow sarson K-151' and *B. alboglabra* accession No. 4003 (Chen et al. 1988, 1989). 'K-151' is yellow-flowered and yellow-seeded, whereas No. 4003 is white-flowered and black-seeded. The dominant white flower and black seed traits of *B. alboglabra* are two useful markers. Addition of the *B. alboglabra* chromosomes with the gene loci W_c and Bl_c for white flower and black seed to K-151 background could be ascertained by their effect on the phenotype (Chen et al. 1992; Cheng et al. 1994a; Chen et al. 1996). No. 7406 was backcrossed to 'K-151' to produce the AAC (2n = 29) hybrid. The AAC hybrid was further backcrossed to develop the BC_1 aneuploid progeny (AA + variable numbers of chromosomes from C). In the BC_1 progeny, one *B. campestris*-*alboglabra* monosomic addition plant, No. 8 (AA + 1 from C, 2n = 21), was identified by chromosome counting. Plant No. 8 was yellow-flowered and yellow-seeded, indicating that the chromosome of *B. alboglabra* in this plant did not carry the genes for white flower or black seed traits. Another BC_1 aneuploid plant, No. 36, had 2n = 23 chromosomes. Plant No. 36 was white-flowered and produced yellow seeds, indicating that one of the three *B. alboglabra* chromosomes in this plant carried the gene locus W_c for white flower colour. The BC_1 plants No. 8 and No. 36 were backcrossed, as female parents, again to 'K-151' to develop the BC_2 progeny. *B. campestris*-*alboglabra* monosomic and double-monosomic addition plants (AA + 2 different chromosomes from C, 2n = 22) in the BC_2 progeny were identified by cytogenetic and RAPD marker analyses. Flower colour of the individual BC_2 plants derived from plant No. 36 was scored.

Cytology and RAPD marker analysis

The chromosome number of each plant was ascertained by studying more than 20 pollen mother cells (PMCs) at metaphase I/anaphase I. For meiotic pairing in the monosomic and double-monosomic addition lines, a minimum of 50 PMCs were analysed at diakinesis for each addition line. The methods of meiotic and mitotic analyses have been reported by Chen et al. (1992) and Cheng et al. (1995a), respectively.

RAPD marker analysis was carried out according to the protocols of Jørgensen et al. (1996). The RAPD fragments originating from *B. alboglabra* were identified in No. 7406 by comparing the amphidiploid with its parents 'K-151' and No. 4003 (Jørgensen et al. 1996; Chen et al. 1996). These RAPD fragments are useful markers for the chromosomes of *B. alboglabra*. Genetic markers and gene loci, when located on the same chromosome of *B. alboglabra*, will co-segregate in the BC_1/BC_2 aneuploid progeny. Deviation from the co-segregation pattern can arise if inter- or intragenomic homoeologous recombinations or chromosomal deletions due to breakage occur.

Results

Chromosome numbers of the BC_2 plants

Chromosome numbers of 9 BC_2 plants derived from the BC_1 plant No. 8 were 2n = 20 or 21 (4 and 5 plants, respectively). For 21 BC_2 plants derived from the BC_1 aneuploid plant No. 36, the chromosome numbers

were either 2n = 20, 21 or 22 (4, 11 and 6 plants, respectively).

RAPD marker analysis of the BC_2 plants

The 9 progeny plants derived from plant No. 8 were analysed with RAPD markers specific for *B. alboglabra* (Fig. 1a). Fifteen markers co-segregated in the 5 progeny plants with 2n = 21, thereby defining these plants as the monosomic addition line with the *B. alboglabra* chromosome-carrying synteny group I (Table 1). However, 1 of these plants had some RAPD markers specific for synteny group IV, indicating that at least part of the C-chromosome carrying this synteny group is present in this addition line. The 4 diploid plants (2n = 20) lacked the extra chromosome and did not exhibit any of the 15 markers of synteny group I or any of those markers of synteny group IV found in 1 plant with 2n = 21.

Segregation analysis of the RAPD markers and the flower colour in the BC_2 progeny plants derived from the BC_1 aneuploid plant No. 36 established three marker synteny groups II, III and IV (Table 1, Fig. 1b, c), which represented the three different *B. alboglabra* chromosomes occurring in these plants. In addition to presenting the chromosome numbers, Table 2 also gives the genomic constitutions of the 21 BC_2 progeny plants, as deduced from the RAPD marker analysis. Among the 4 plants with 2n = 20, 1 plant did not have any markers of the synteny groups; 2 plants displayed genetic introgression by exhibiting variable numbers of the markers; and 1 plant exhibited introgression of the entire synteny group IV, indicating the possible occurrence of interspecific chromosomal substitution. Among the 11 plants with 2n = 21 chromosomes, 2 did not contain any markers, indicating that they were most likely AA trisomics. Among the other 9 plants with 2n = 21, three types of monosomic addition lines were defined: 2 plants with synteny group II, 2 plants with synteny group III and 4 plants with synteny group IV. The remaining plant with 2n = 21 had all the markers of both synteny groups II and IV, implying the presence of two *B. alboglabra* chromosomes and meanwhile absence of one *B. campestris* chromosome. One of the 2 plants with synteny group III also displayed introgression of 6 RAPD markers belonging to synteny group IV into the background A-genome. Six plants with 2n = 22 were defined as representing three types of double-monosomic addition lines: 1 plant with synteny groups II and III, 4 plants with II and IV and 1 plant with III and IV. In the double-monosomic addition line with synteny groups II and III, 4 markers (A09-705, A15-650, H06-650 and H12-950) were missing from synteny group III. Also in 1 of the 4 plants with synteny groups II and IV, 2 markers (C18-1450 and H06-1650) were missing from synteny group IV. In both cases, this could be a result of intergenomic homoeologous recombination or chromosomal deletion.

Identification of *B. alboglabra* chromosomes in the addition lines

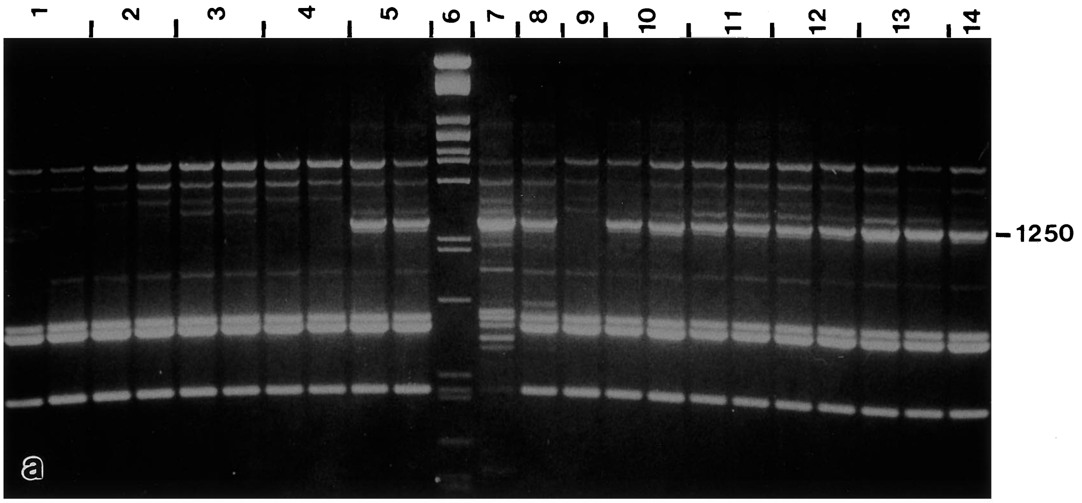
In the addition line with marker synteny group I (Table 1), the alien chromosome of *B. alboglabra* was distinguished from the background *B. campestris* complement by having a large size and a subterminal centromere (Fig. 2). On the basis of measurements made on five prometaphase cells with 2n = 21, the alien chromosome was 4.4 μm long and had an arm ratio of 3.14 ± 0.09. With reference to the karyotype of *B. alboglabra* (Cheng et al. 1995a), the alien chromosome was revealed to be chromosome 8 (C8).

The *B. campestris*-*alboglabra* monosomic addition line with the synteny group II (Table 1) harboured the same alien *B. alboglabra* chromosome as found in an addition line obtained earlier (Chen et al. 1992). The alien chromosome in this addition line carried the gene for white flower colour and was identified as chromosome 4 (C4) (Cheng et al. 1995a).

The alien *B. alboglabra* chromosome in the addition line with synteny group III (Table 1) was the most readily identifiable since it was satellited. The alien chromosome had a small satellite, whereas the nucleolar pair (A9) of the background *B. campestris* 'K-151' carried large satellites (Fig. 3). Obviously, the *B. alboglabra* chromosome in this addition line is the satellited chromosome 9 (C9), according to the karyotype designations given by Cheng et al. (1995a). The *B. alboglabra* chromosome with synteny group IV remains to be identified.

Meiotic pairing of the addition lines

In PMCs at diakinesis, the nucleolar chromosomes of *B. campestris* (A9) are morphologically easy to distinguish from the other bivalents by having a large size and a darkly stained distal part (Cheng et al. 1994b). As revealed by diakinesis analysis, the alien chromosome C8 in the monosomic addition line stayed as a univalent in 88% of the PMCs (Table 3). When the alien chromosome C8 paired with the chromosomes of *B. campestris*, it paired with the nucleolar chromosomes (A9) by short arm association in 6% of the PMCs, or with another chromosome by long arm association in 6% of the PMCs (Table 3). The alien chromosome C9 in the monosomic addition line stayed as a univalent (Fig. 4a) in 44% of the PMCs, or paired with the nucleolar chromosomes (A9) of *B. campestris* by short arm association (Fig. 4b) in 46% of the PMCs (Table 3). In the remaining 10% of the PMCs, the long arm of the alien chromosome C9 paired with an arm of *B. campestris* chromosomes A1 (Fig. 4c), which were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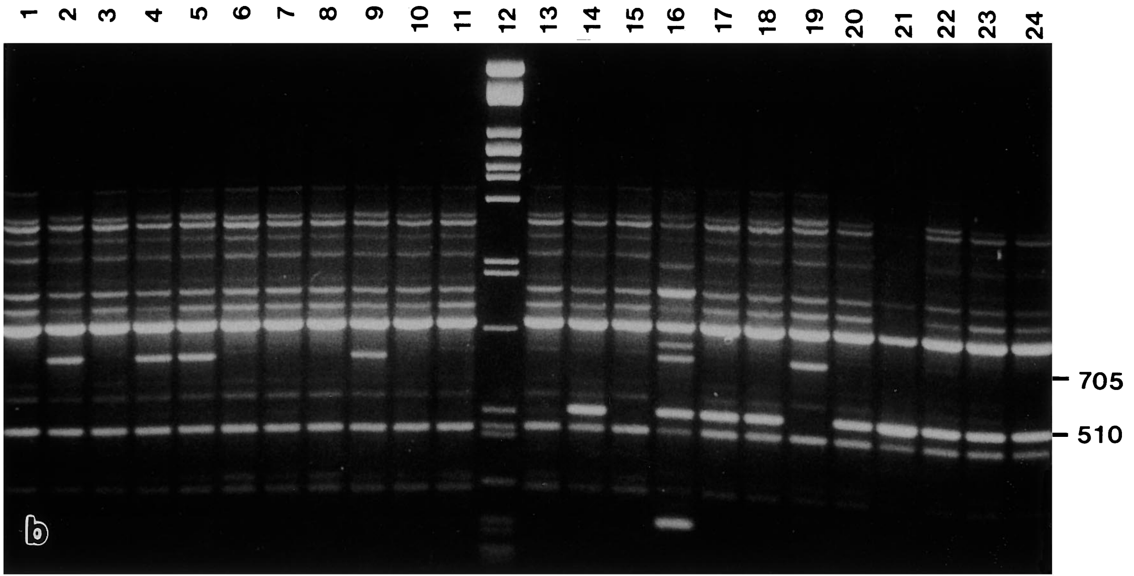
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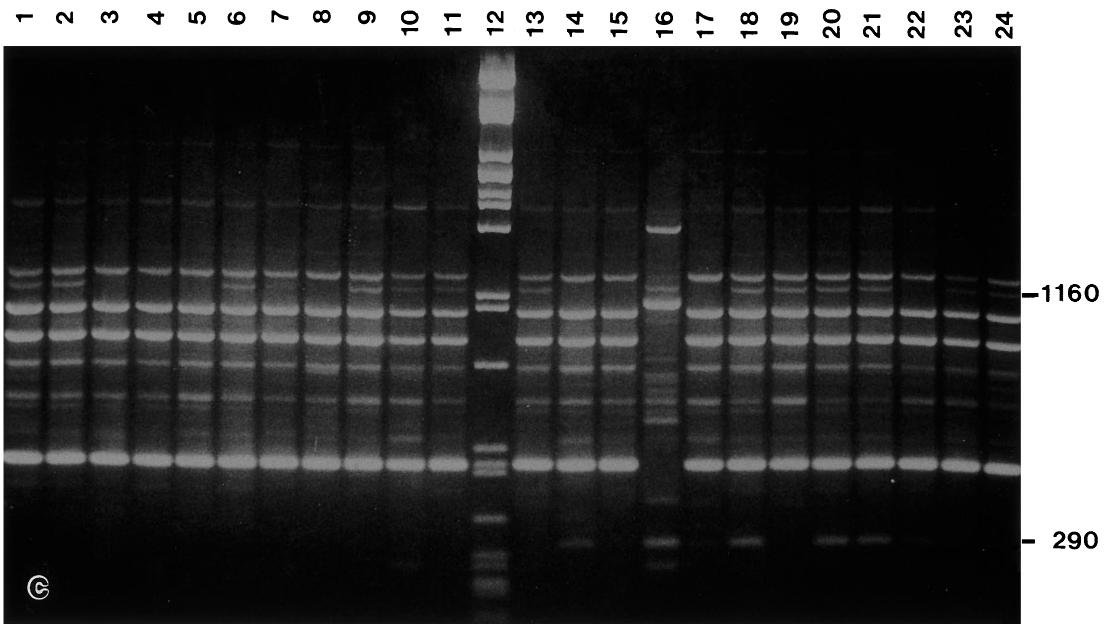
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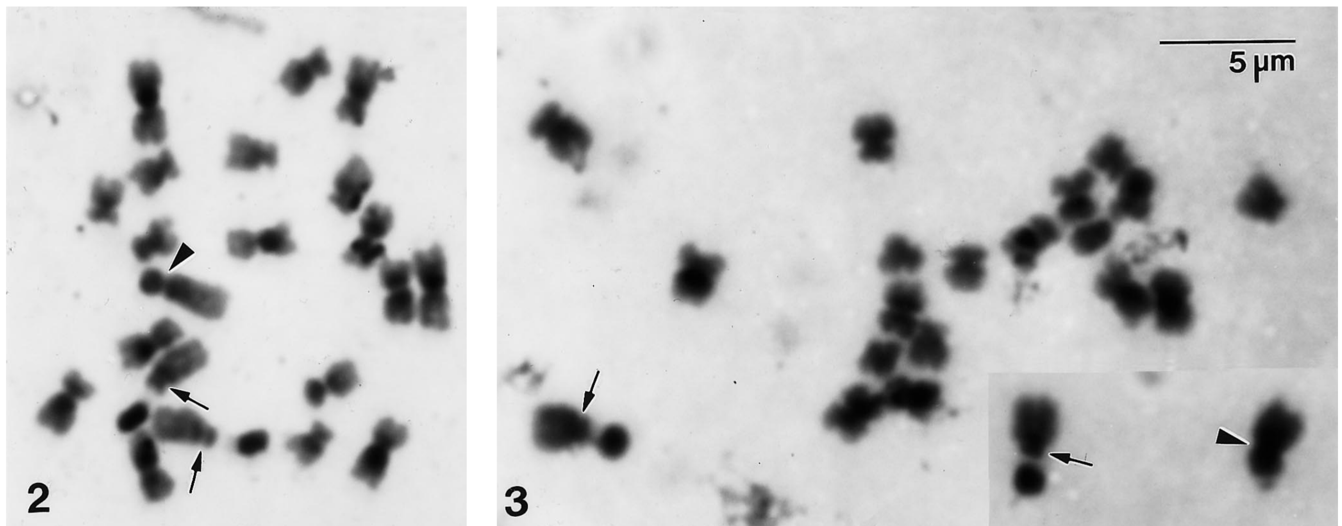

Figs. 2, 3 Mitotic chromosomes of *B. campestris-alboglabra* monosomic addition lines (AA + 1 chromosome from C, 2n = 21). The *arrows* indicate the nucleolar pair (A9) of the A-genome. The *arrowheads* indicate the alien C-genome chromosomes C8 at late prometaphase (**Fig. 2**) and C9 at metaphase (**Fig. 3**)

easily identifiable by their median pericentric heterochromatin and large size (Cheng et al. 1995a; Heneen et al. 1995). In the monosomic addition line with synteny group IV (Table 1), the alien chromosome stayed as a univalent in 59 PMCs (82%) or paired with *B. campestris* chromosomes to form a trivalent in 13 PMCs (18%).

In the three types of double-monosomic addition lines, the two alien chromosomes of the C-genome either stayed as two univalents or paired with the chromosomes of the A-genome. Multivalent formation in the double-monosomic addition line with C4 and C9 further confirmed the homoeology relationships as inferred by analysis of the monosomic addition lines for

Table 1 Four marker synteny groups representing four different chromosomes of *B. alboglabra*

I	II	III	IV
A04-1050	A09-510	A09-705	A11-400
A13-520	C10-290	A11-960	B05-1100
B05-470	C12-1250	A15-650	C07-1400
B05-900	C14-600	B05-500	C09-1900
C06-1250	G11-600	C07-900	C10-1160
C09-2800	G17-520	C18-1650	C14-2400
G03-525	G17-1090	G06-1050	C18-1450
G06-1600	H06-1160	G09-850	H06-1650
G09-1200	H15-570	G11-350	
G11-950	W_c[a]	G17-950	
H06-540		H06-650	
H11-730		H12-950	
H12-970			
H15-750			
H15-1750			

[a] The gene locus for the dominant white flower colour of *B. alboglabra*

these chromosomes (Cheng et al. 1994b; Cheng 1996; the present study). The quadrivalent in Fig. 5 indicated that a long arm and a short arm of the *B. campestris* nucleolar chromosomes (A9) paired with the long arm of the alien chromosome C4 and the short arm of the alien chromosome C9, respectively. This pairing pattern was further observed as part of a hexavalent in another PMC, in which two additional A1 chromosomes were associated with the long arm of chromosome C9 (not shown).

←

Fig. 1a–c RAPD analyses. **a** Nine offspring plants from plant No. 8 tested by primer C06. Two separate reactions were made for each plant. Four of these offspring plants has 2n = 20 and lacked the C-chromosome carrying synteny group I (*lanes 1–4*), and the other 5 plants had 2n = 21 and harboured the C-chromosome (*5, 10–13*). These two groups could be identified by the absence or presence of the C–genome marker C06–1250. *Lane 6* size marker, (*7*) *Brassica alboglabra* No. 4003, (*8*) resynthesized *B. napus* No. 7406, (*9*) *B. campestris* K–151, (14) mother plant No. 8, **b** Twenty-one offspring plants from plant No. 36 tested by primer A09 (*lanes 1–11, 13, 14, 17–24*). Markers A09–510 (*lanes 14, 17, 18, 20–24*) and A09–705 (*2, 4, 5, 9, 19*) represent synteny groups II and III, respectively. *Lane 12* size marker, (*15*) *B. campestris* 'K-151' (*16*) resynthesized *B. napus* No. 7406. **c** Offspring plants from plant No. 36 tested by primer C10 (numbering as in **b**). Markers C10–1160 (*lanes1, 2, 6, 9–11, 13, 18–21, 23, 24*) and C10–290 [*14, 17, 18, 20–24* (*17* and *22–24* faint in this gel but distinct in another gel)] represent synteny groups IV and II, respectively

Discussion

Four *B. campestris-alboglabra* chromosome addition lines have been developed in the present material. The addition line with marker synteny group II comprising the gene locus W_c for white flower colour (Table 2),

Table 2 Chromosome numbers, genomic constitutions and marker synteny groups of the 21 BC_2 progeny plants derived from the BC_1 aneuploid plant No. 36 (2n = 23). Details on the intergenomic recombination or chromosomal deletion in the progeny plants are given in the text

Genomic constitution and marker synteny group	Number of plants
2n = 20:	4
Diploid AA	1
Introgression line of AA:	
with some markers of synteny group III	1
with some markers of synteny groups III and IV	1
Substitution line of AA:	
with the entire synteny group IV	1
2n = 21:	11
AA trisomic	2
Monosomic addition line:	
with synteny group II	2
III	2
IV	4
Monosomic addition and substitution line:	
with synteny groups II and IV	1
2n = 22:	6
Double-monosomic addition line:	
with synteny groups II and III	1
II and IV	4
III and IV	1

harboured chromosome 4 (C4) of *B. alboglabra* (Cheng et al. 1995a). The alien *B. alboglabra* chromosomes in the remaining three addition lines with synteny groups I, III and IV were distinguished from one another by their different marker synteny groups or morphology. As a result, synteny groups I and III have been assigned to chromosomes 8 and 9 (C8 and C9) of *B. alboglabra*. Segregation analyses of RAPD markers in the aneuploid progeny derived from the AAC (2n = 29) hybrid and *B. campestris-alboglabra* addition lines have established a total of nine synteny groups, which presumably represent the nine different chromosomes of the *B. alboglabra* complement (the present study, Chen et al. 1996; Jørgensen et al. 1996, unpublished). These synteny groups will greatly assist in developing the whole set of the nine different *B. campestris-alboglabra* addition lines.

Meiotic studies on the amphihaploid (AC), digenomic hybrid (AAC) and resynthesized amphidiploid *B. napus* (AACC) have only revealed that chromosome pairing generally occurs between and within the A- and C-genomes (Attia and Röbbelen 1986; Attia et al. 1987; Heneen et al. 1995). By taking advantage of the *B. campestris-alboglabra* addition lines, we are able to further address homoeology relationships among particular chromosomes of these two genomes, thus providing a deeper insight into the evolution of the genomes. Meiotic studies on the addition lines revealed that chromosomes C4, C8 and C9 of *B. alboglabra* all had more than one pairing partner in the A-genome (Cheng et al. 1994b; Cheng 1996; the present study). For instance, the short arm of C9 paired with the short arm of A9, whereas the long arm of C9 paired with an arm of A1 rather than the long arm of A9. Another striking point is that these three chromosomes of the C-genome had a common pairing partner, i.e. the nucleolar chromosomes (A9) of the A-genome. More specifically, the long arm of A9 is homoeologous with the long arm of C4, while the short arm of A9 is homoeologous with the short arms of both C8 and C9 (Cheng et al. 1994b; Cheng 1996; the present study). The intergenomic homoeology among these chromosomes is thus only segmental rather than along entire chromosomes. A combination of the long arm of C4 with the short arm of C9 would make up a *B. alboglabra* chromosome which is homoeologous with A9 of *B. campestris* along both arms. These results suggest that *B. campestris* and *B. alboglabra*/*B. oleracea* have evolved from a common ancestor but that extensive chromosomal rearrangements must have taken place during the evolution of the two species. Molecular marker mapping in *Brassica* has revealed conservation of extensive linkage blocks and only occasionally entire linkage groups between the A- and C-genomes (Slocum et al. 1990; Song et al. 1991; Parkin et al. 1995). One linkage group of *B. campestris* was found to be split up on two different synteny groups of *B. oleracea* (Quiros et al. 1994).

Intragenomic homoeology was not observed in the three *B. campestris-alboglabra* double-monosomic addition lines. However, intragenomic homoeology

Table 3 Meiotic behaviour of the alien *B. alboglabra* chromosomes at diakinesis in two *B. campestris-alboglabra* monosomic addition lines (2n = 21)

Addition line	Number of PMCs observed	Staying as a univalent (10 II + 1I)		Pairing with chromosomes of *B. campestris* (9 II + 1 III)			
		PMCs	%	With the nucleolar chromosome (A9) through the short arms		With another chromosome through the long arms	
				PMCs	%	PMCs	%
C8	80	70	88	5	6	5	6
C9	108	47	44	50	46	11	10

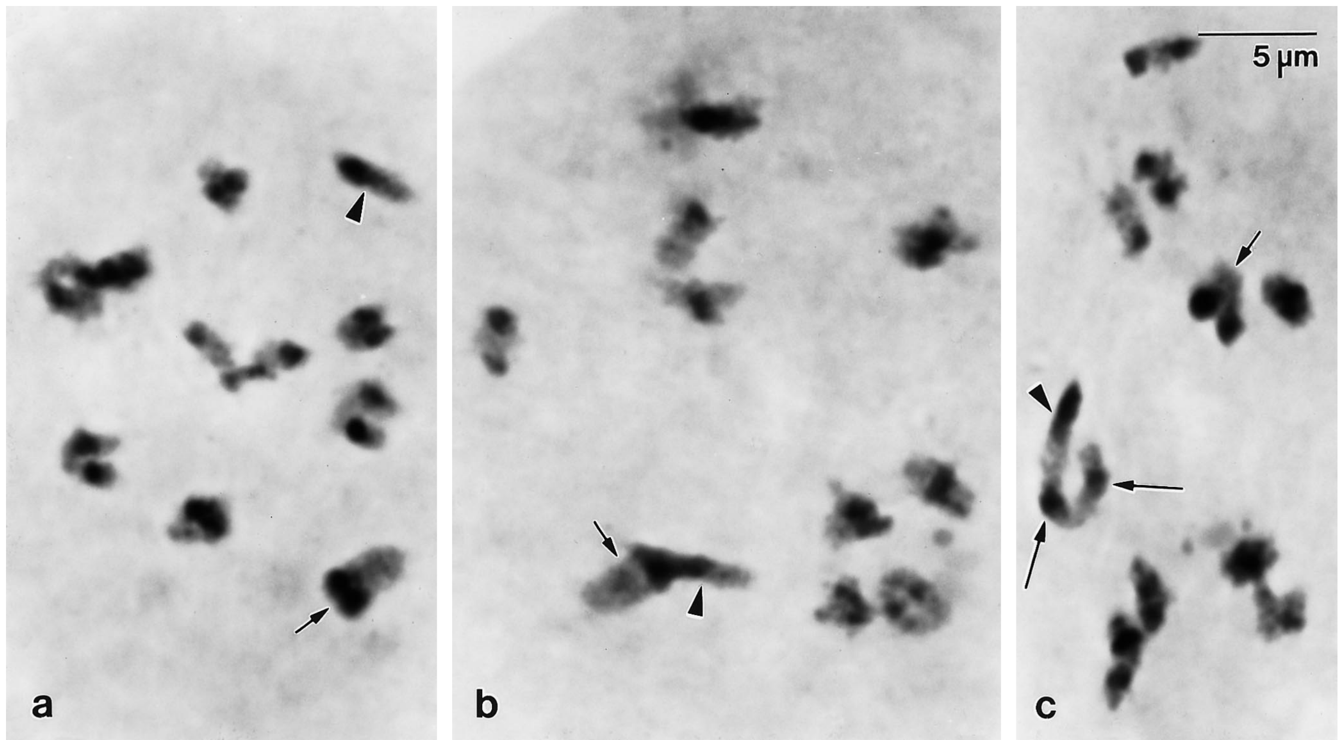

Fig. 4a–c Diakinesis chromosomes in PMCs of the *B. campestris-alboglabra* monosomic addition line with the alien chromosome C9. **a** 10 II + 1 I (*arrowhead*). The *arrow* indicates the nucleolar bivalent (A9) of the A-genome. **b** 1 III + 9 II. The trivalent is a result of association between the short arm of C9 (*arrowhead*) and short arms of the nucleolar chromosomes A9 (*arrow*). **c** 1 III + 9 II. The trivalent is a result of pairing between the long arm of C9 (*arrowhead*) and an arm of *B. campestris* chromosomes A1 (*long arrows*), both of which have median pericentric heterochromatin and a large size. The nucleolar pair (A9) is indicated by a *short arrow*

between the short arms of chromosomes 8 and 9 (C8 and C9) of *B. alboglabra* was revealed by the presence of rRNA gene loci (Cheng et al. 1995b). Lack of pairing between these two chromosomes of the C-genome in the double-monosomic addition line might be due to a low degree of intragenomic homoeology or a greater intergenomic homoeology leading to a preferential pairing between the chromosomes of the A and C genomes.

Homoeologous pairing in the addition lines led to the occurrence of intergenomic introgression, as revealed by RAPD marker analysis. In an extreme case, introgression of the entire synteny group D into the *B. campestris* genome was observed, implying the possible occurrence of interspecific chromosomal substitution. Intergenomic gene and marker introgression and chromosomal substitution between the A- and C-genomes of *Brassica* have been reported (Quiros et al. 1987; McGrath et al. 1990; Chen et al. 1992; Jørgensen et al. 1996). These results point to the possible application

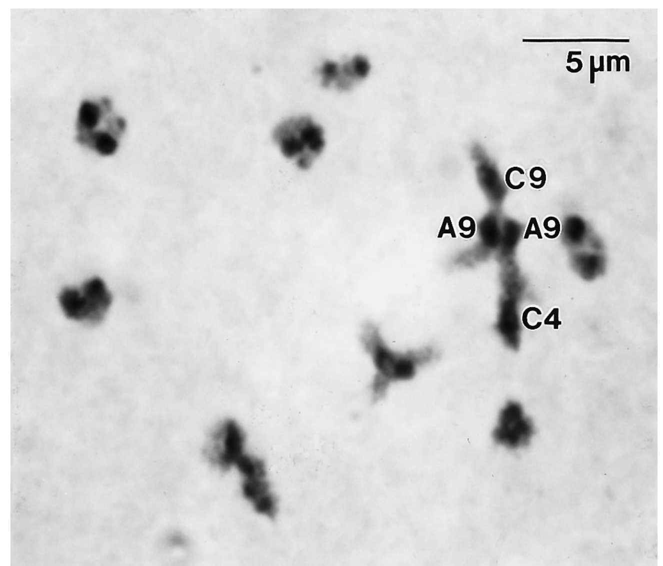

Fig. 5 Diakinesis chromosomes in a PMC of the *B. campestris-alboglabra* double-monosomic addition line (AA + 2 different chromosomes from C, 2n = 22) with alien chromosomes C4 and C9. 1 IV + 9 II. The quadrivalent demonstrates that a long arm of the nucleolar chromosomes A9 is paired with the long arm of C4 while a short arm of A9 is paired with the short arm of C9

in crop improvement but meanwhile indicate a potential risk of interspecific gene flow which should be considered when releasing transgenic oilseed rape varieties for cultivation.

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