Theor Appl Genet (2000) 101:538–546 © Springer-Verlag 2000

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Construction of synteny groups of *Brassica alboglabra* by RAPD markers and detection of chromosome aberrations and distorted transmission under the genetic background of *B. campestris*

Received: 26 February 1999 / Accepted: 30 December 1999

Abstract Interspecific hybrids were produced by crosses between the inbred lines of *B. campestris* and *B. alboglabra*, and were backcrossed twice to *B. campestris*. Genetical constitutions of the BC_2 plants were analyzed by RAPD (random amplified polymorphic DNA), flow cytometry and cytological observations. By using 140 arbitrary primers, a total of 137 polymorphic bands were obtained and 125 were found to be specific to *B. alboglabra*. Based on the presence and absence of the specific RAPD markers of *B. alboglabra*, 13 synteny groups were constructed. The number of markers in each synteny group was found to be different and varied from 2 to 28. This reflects the difference in the degree of genetic variability among the *B. alboglabra* chromosomes from those of *B. campestris*. Losses or gains of RAPD markers were observed frequently in most of the synteny groups, which indicated the occurrence of chromosome translocations and/or deletions in the chromosomes of *B. alboglabra*. In a population of 40 BC_2 plants, chromosome transmission rates were analyzed by using the RAPD markers in each synteny group. Most of the chromosomes of the synteny groups were transmitted with rates of 0.37–0.68. An extremely high transmission rate, 0.98, was however observed in one of the synteny groups. Inheritance data of the synteny groups revealed relationships among themselves. The plants lacking the RAPD markers of two synteny groups tended to lose others belonging to the rest of the synteny groups, indicating the effects of these groups on the transmission of *B. alboglabra* chromosomes to the *B. campestris* background.

Communicated by V. Glimelius

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Key words *Brassica campestris* · *Brassica alboglabra* · Chromosome addition · RAPD markers · Intergenomic recombination

Introduction

The genus *Brassica* has abundant variations among and within its species, which are widely cultivated throughout the world. This genus is composed of three diploid species, *B. campestris* L. (AA, 2n=20), *B. oleracea* L. (CC, 2n=18), and *B. nigra* (L.) Koch (BB, 2n=16), and three amphidiploids, *B. napus* L. (AACC, 2n=38), *B. juncea* (L.) Czern. (AABB, 2n=36) and *B. carinata* Braun (BBCC, 2n=34). The amphidiploid species originated from the hybridization between pairs of the three diploid species (U 1935). Cytological analyses, such as chromosome pairing and chiasma formation, in haploid plants revealed that the diploid species had diverged from a common ancestor (Röbbelen 1960; Armstrong and Keller 1981; Attia and Röbbelen 1986a, b), which suggests the possibility of the occurrence of chromosomal recombination in the hybrids between the *Brassica* species.

Attempts at the identification of *Brassica* chromosomes have been made in recent years (Wang et al. 1989; Nishibayashi 1992; Olin-Fatih and Heneen 1992; Fukui et al. 1998). Each chromosome was characterized and classified based on its morphology and its banding pattern. Nevertheless, cytogenetic analysis of each of the discriminated chromosomes is still difficult because of limited information on each of the chromosomes and the technical difficulties encountered by the smallness of their size.

Genetic markers are one of the most-effective methods for chromosome analysis. Isozymes and RFLP markers were effectively used for the identification of the chromosome addition lines of *B. campestris-oleracea* (Quiros et al. 1987; McGrath and Quiros 1990; McGrath et al. 1990; Hu and Quiros 1991) and *B. napus-nigra* (Chèvre et al. 1991). Chen et al. (1990) examined the in-

heritance of isozyme markers in the progenies of a trigenomic hybrid between *B. napus* and *B. campestris*. Isozyme and morphological features, like flower color and seed color, were used as markers and the detected specific chromosomes were compared to the known chromosome number in *B. campestris-B. alboglabra* addition lines (Chen et al. 1992; Cheng et al. 1994). These studies gave an insight for the intergenomic homology in *Brassica* by revealing intergenomic recombination (Quiros et al. 1987; Chen et al. 1992), chromosome substitution and gene duplication (McGrath et al. 1990), as well as deletions in alien chromosomes (Hu and Quiros 1991).

For detailed analysis of the chromosomes, many markers are required to cover each of them as well as to cover the whole genome. In this respect, RAPD could be one of the effective genetic markers (Williams et al. 1990). Quiros et al. (1991) found genome-specific RAPD markers in the A, B and C genomes of *Brassica* species. By using RAPD markers, the inheritance of *B. napus* genes was examined in a cross of *B. juncea* and *B. napus* (Frello et al. 1995), and the identification of *B. nigra* chromosomes added to *B. napus* and *B. oleracea* has also been reported (Struss et al. 1992; Chèvre et al. 1997). Recently, Chen et al. (1997) produced four types of *B. campestris-B. alboglabra* chromosome addition lines and constructed four synteny groups with RAPD markers; using this approach intergenomic introgression was shown in the progenies of the addition lines.

In the present paper, we report the inheritance of C genome-specific RAPD markers in the progeny obtained by backcrossing *B. campestris-B. alboglabra* hybrids with *B. campestris*. Intergenomic chromosomal exchange, or deletions and intergenomic chromosomal relationships, were revealed through the construction of synteny groups and transmission of the RAPD markers.

Materials and methods

Plant materials

Inbred lines of Pak-Choi (*B. campestris* L. ssp. *chinensis*, 2n=20, AA), nos. 7-17, 7-23 and 7-39, and Chinese kale (*B. alboglabra* Bailey, a form of *B. oleracea*, 2n=18, CC), nos. 7-25 and 7-33, were used as the parents. These lines were produced by selfing at least three times to remove their heterozygosity. Two species were crossed reciprocally and the ovaries were excised and cultured in vitro after Inomata (1976). One of the hybrids obtained from the cross combination 7–17×7–33 was selected for further analysis. Stems of the hybrid plant were treated with 0.5% colchicine for chromosome doubling, and amphidiploid (AACC) shoots were obtained. The flowers on amphidiploid shoots were pollinated with Pak-Choi, 7–17, to produce the BC_1 (AAC) plants. The AAC plant was crossed again as a female with Pak-Choi, 7–17, in two seasons, spring and autumn of 1997. The BC_2 plants obtained (AA+various numbers of C-genome chromosomes) were used for RAPD analysis.

RAPD analysis

DNAs were extracted from young leaves according to Dellaporta et al. (1983) with minor modifications. DNA samples were amplified by PCR (Williams et al. 1990) using 140 arbitrary primers of Oligo 10-mer Kits (Operon Technologies Inc.). PCR was conducted in a 15-µl reaction buffer with 0.4 units of the Tth DNA polymerase (Toyobo Inc.), 200 µM each of dATP, dCTP, dGTP and dTTP, 0.8 µM of a single primer and 1.0 ng of genomic DNA, in a 0.6-ml microfuge tube. The reaction solutions were overlaid with a drop of mineral oil.

Amplifications were performed in a Thermal Cycler (MJ Research Inc.), programmed for three cycles at 94°C for 2 min, 40°C for 2 min and 72°C for 2 min, 40 cycles at 94°C for 0.5 min, 40°C for 0.5 min and 72°C for 2 min, and one cycle at 94°C for 0.5 min, 40°C for 0.5 min and 72°C for 10 min. Amplified DNA products were separated in 2% agarose gels and stained with ethidium bromide and photographed under ultraviolet illumination.

Flow cytometric analysis

The relative DNA contents of the 40 BC_2 plants were determined by flow cytometry. A Partec PA cytometer, equipped with a mercury lamp, with the filter combinations KG1, BG38, UG1, TK420 and GG435, and a dichroic mirror TK420, was used. About 25 mm^2 of young leaves of the BC_2 plants, along with that of Pak-Choi 7–17 as the standard, were chopped with a razor blade in buffer (solution A of plant high-resolution DNA kit type P, Partec GmbH-Munster). After incubating at room temperature for 5 min, 1 ml of staining solution containing 10 mM Tris, 50 mM sodium citrate, 2 mM $MgCl_2$, 1% (w/v) DABCO, 1%(w/v)PVP, 0.1%(v/v) Triton X-100 and 2 mg/l of DAPI, pH 7.5, was added to the cell solution, which was then filtered through a 40-µm nylon mesh. After 5 min, the solutions were used for measurement.

Cytology

Root tips, 7–10 mm long, were collected from the BC_2 plants and pre-treated with 2 mM of 8-hydroxyquinoline solution at 4°C in the dark for 4 h, and then fixed and stored in ethanol: acetic acid (3:1). After rinsing in water, the root tips were digested with enzyme solution (2% Cellulase Onozuka R-10, Yakult Pharmaceutical Industry Co., Ltd., Japan, and 1% Pectolyase Y-23, Seishin Pharmaceutical Co., Ltd., Japan) at 37°C for 3 h. After rinsing again in water they were placed on a glass slide and tapped softly with a needle. A small drop of the fixative was applied to the root-tip cells which were then dried in air. The chromosomes were stained with aceto-carmine and observed under a microscope. For examination of the meiosis, young flower buds were fixed in the same fixative solution. The anthers were squashed in aceto–carmine solution and reduction divisions of pollen mother cells were observed under a microscope.

Results

Production of backcross progeny

Seven combinations of crosses were carried out between the three lines of *B. campestris* and two lines of *B. alboglabra* (Table 1). The efficiency of the crosses varied from 0 to 1.18. The highest efficiency, 1.18, was observed in the cross 7–17×7–33. No hybrid was formed in cases where *B. alboglabra* was used as the female parent.

The amphidiploid (AACC) from 7–17×7–33 was crossed as a female with 7–17 (*B. campestris*) (Table 2). The BC_1 seeds were produced with high frequency by using the AACC plant as female. On the other hand, only one seed was obtained from 294 flowers pollinated by AC plants, and which seems to be derived from an unre-

Table 1 Production of interspecific hybrids from crosses between three lines of *B. campestris* (Pak-Choi) and two lines of *B. alboglabra* (Chinese kale) by ovary culture

Item	*B. campestris*×*B. alboglabra*					*B. alboglabra*×*B. campestris*	
	7–17×7–33	7–23×7–25	7–23×7–33	7–39×7–25	7–39×7–33	7–25×7–17	7–33×7–23
No. of ovaries cultured	11	45	52	62	60	5	35
No. of plants obtained	13	3	11	1	1	0	0
Efficiency of ovary culture[a]	1.18	0.07	0.21	0.02	0.02	0	0

[a] No. of plants obtained/no. of ovaries cultured

Table 2 Progenies of the backcrosses of inter-specific hybrids (AACC and AC) with *B. campestris* (AA)

Cross	No. of floweres pollinated	No. of siliques formed	No. of seeds obtained
BC_1			
AACC×*B. camp.*	24	–[a]	51
AC×*B. camp.*	294	–	1
BC_2			
AAC×*B. camp.* (Spring)	246	96	30 (0.122)[b]
AAC×*B. camp.* (Autumn)	403	208	374 (0.928)
BC_3			
No. 5×*B. camp.*	90	81	0
No. 6×*B. camp.*	93	81	0
No. 7×*B. camp.*	43	2	0

[a] Not conducted
[b] No. seeds obtained/no. flowers pollinated

duced gamete. To obtain BC_2 plants, the BC_1 was again crossed with 7–17, in spring and autumn. The efficiency in the latter was higher than in the former. The difference in the efficiency between the two seasons is considered to be due to environmental variations between them. Three plants of BC_2, nos. 5, 6 and 7, were selected and used for further backcrosses. However, although many siliques were formed, no seeds were found in them. The effect of the self-incompatibility gene in the A genome might have suppressed the production of the BC_3 plants.

Construction of synteny groups

A total of 140 primers were used to examine the inheritance of *B. alboglabra* specific markers. In the BC_2 plants, 137 polymorphic bands were observed among which 125 bands were confirmed to be specific to *B. alboglabra* (Fig. 1).

Synteny relationships among the markers were revealed by analyzing the presence and absence of the bands in ten BC_2 plants (nos. 1–10). A group of RAPD loci showing similar banding patterns were assumed to be in a same synteny group. Initially, the number of synteny groups was too large and each of the groups had a small number of markers. In order to make the number of synteny groups close to the expected haploid number of *B. alboglabra* (n=9), one miss-matching marker was ignored. With this manipulation, 13 synteny groups were constructed. Eight markers were found to be ungrouped and five markers, D15-650, D20-660, K17-610, K01-540 and D02-880, were excluded from the construction of the synteny groups, as their patterns were dissimilar compared with other markers. The excluded markers could, however, be joined with more than two synteny groups if one miss-matching was ignored. The synteny groups formed are presented in Fig. 1, numbered from 1 to 13. The largest synteny group, no. 1, included 28 RAPD markers, comprising 20% of all the markers. The smallest groups, nos. 11, 12 and 13, each consisted of only two markers (1.5%).

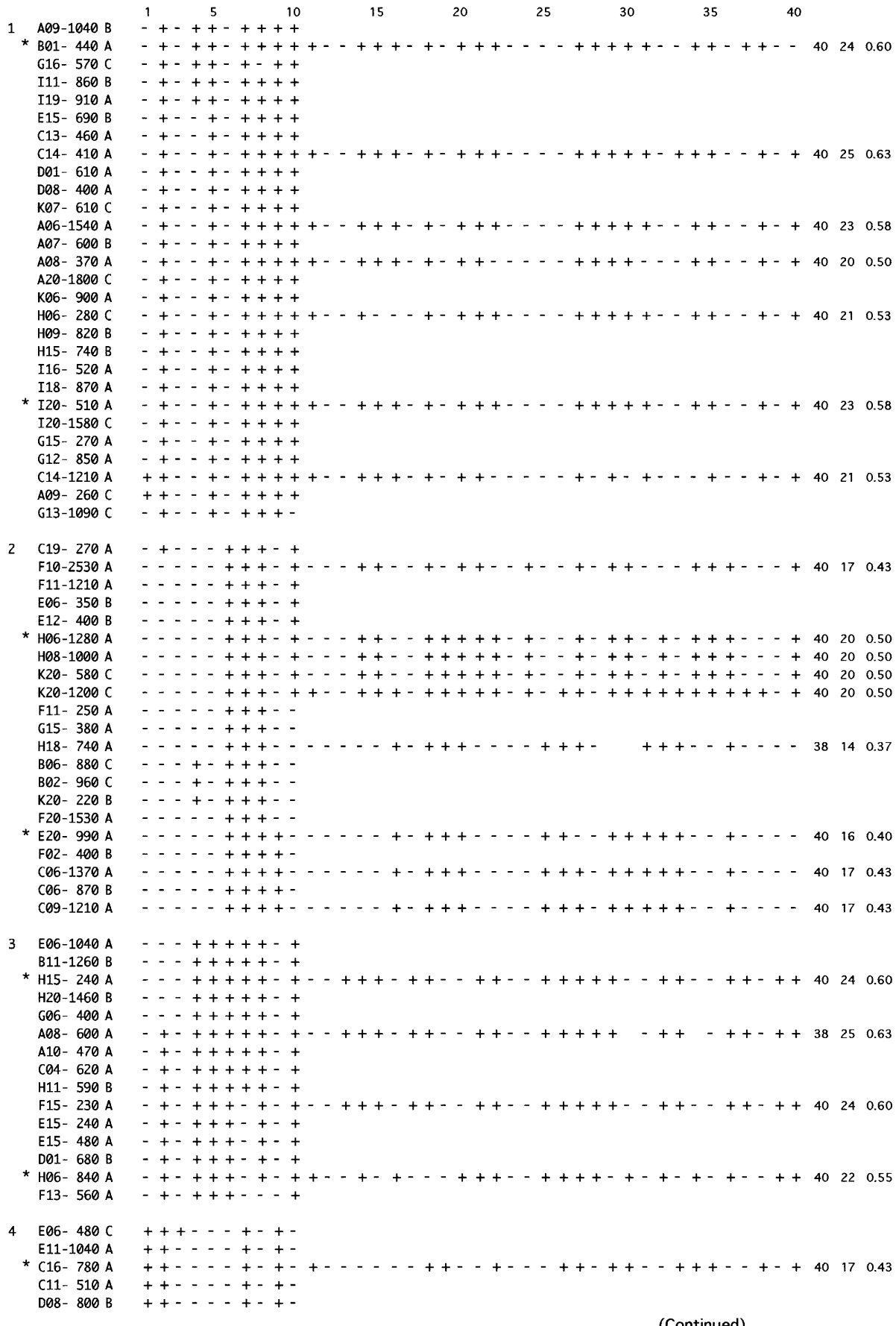
Chromosomal translocations and/or deletions in BC_2 plants

Some *B. alboglabra* markers were found deleted in the constructed synteny groups. For example, in synteny group no. 1, five markers, A09-1040, B01-440, C16-570, I11-860 and I19-910, were present in plant no. 4, while the other 23 markers were absent. This kind of event was observed in nine synteny groups, except for nos. 5, 8, 11 and 12, and in all of the plants except no. 6. In groups 1, 2 and 6, these deletion events were observed four times in the ten plants. These phenomena are considered as a reflection of chromosomal abnormalities, such as translocations and/or deletions, which occurred during meiosis of the BC_1 plant.

DNA contents and chromosome numbers of the BC_2 plants

The DNA contents of the BC_2 plants were estimated with a flow cytometer and the DNA differences among

Fig. 1 Synteny groups of *B. alboglabra* revealed by RAPD markers from BC_2 plants of *B. campestris-B. alboglabra*. Presence and absence of the markers is designated with + and –, respectively. The nos. of the synteny groups are shown at the left end, followed by the marker names and evaluation of the markers, *A* very fine, *B* fine, *C* not so fine. The number of plants observed and those having the markers are shown in the 3rd and 2nd columns from the right, respectively, and the chromosome transmission rates are shown at the right end column. The markers used for analysis of relationships among chromosomes are indicated by *asterisks* ▶



(Continued)

Fig. 1 Part 1

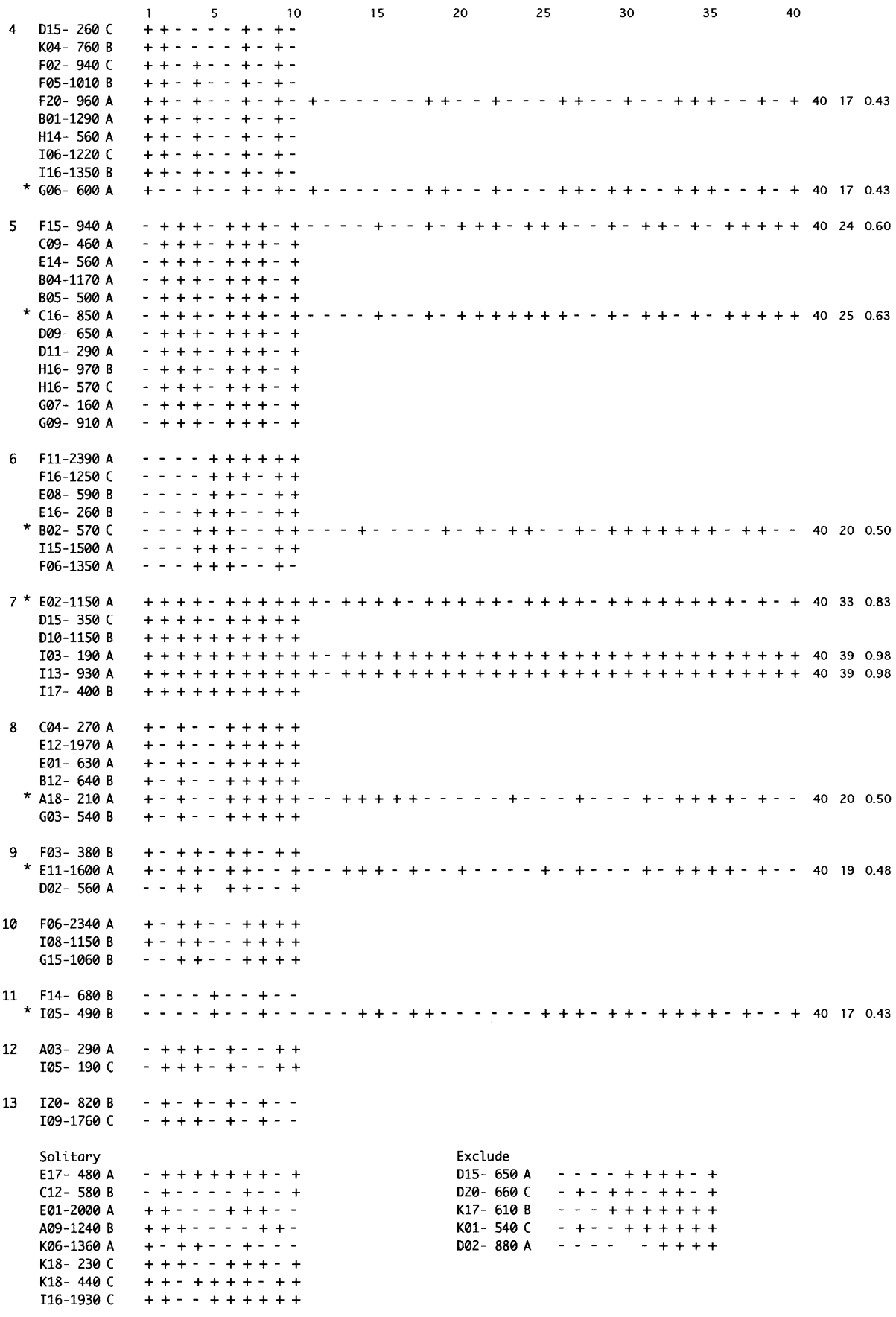

1 5 10 15 20 25 30 35 40
4 D15- 260 C + + - - - - + - + -
K04- 760 B + + - - - - + - + -
F02- 940 C + + - + - - + - + -
F05-1010 B + + - + - - + - + -
F20- 960 A + + - + - - + - + - + - - - - - - - + + - - + - - - + + - - + - + 40 17 0.43
B01-1290 A + + - + - - + - + -
H14- 560 A + + - + - - + - + -
I06-1220 C + + - + - - + - + -
I16-1350 B + + - + - - + - + -
* G06- 600 A + - - + - - + - + - + - - - - - - - + + - - + - - - + + - - + - + 40 17 0.43
5 F15- 940 A - + + + - + + + - + - - - - + - - + - + + + - + + + - - + - + + - + - + + + + + 40 24 0.60
C09- 460 A - + + + - + + + - +
E14- 560 A - + + + - + + + - +
B04-1170 A - + + + - + + + - +
B05- 500 A - + + + - + + + - +
* C16- 850 A - + + + - + + + - + - - - - + - - + - + + + + + + + - - + - + + - + - + + + + + 40 25 0.63
D09- 650 A - + + + - + + + - +
D11- 290 A - + + + - + + + - +
H16- 970 B - + + + - + + + - +
H16- 570 C - + + + - + + + - +
G07- 160 A - + + + - + + + - +
G09- 910 A - + + + - + + + - +
6 F11-2390 A - - - - + + + + + +
F16-1250 C - - - - + + + - + +
E08- 590 B - - - - + + - - + +
E16- 260 B - - - + + + - - + +
* B02- 570 C - - - + + + - - + + - - - + - - - - + - + - + + - - + - + + + + + + + + - + + - - 40 20 0.50
I15-1500 A - - - + + + - - + +
F06-1350 A - - - + + + - - + -
7 * E02-1150 A + + + + - + + + + + + - + + + + - + + + + + - + + + + - + + + + + + + + + - + - + 40 33 0.83
D15- 350 C + + + + - + + + + +
D10-1150 B + + + + + + + + + +
I03- 190 A + + + + + + + + + + + - + 40 39 0.98
I13- 930 A + + + + + + + + + + + - + 40 39 0.98
I17- 400 B + + + + + + + + + +
8 C04- 270 A + - + - - + + + + +
E12-1970 A + - + - - + + + + +
E01- 630 A + - + - - + + + + +
B12- 640 B + - + - - + + + + +
* A18- 210 A + - + - - + + + + + - - + + + + - - - - - + - - - + - - - + - + + + + - + - - 40 20 0.50
G03- 540 B + - + - - + + + + +
9 F03- 380 B + - + + - + + - + +
* E11-1600 A + - + + - + + - - + - - + + + - + - - + - - - - + - + - - - + - + + + + - + - - 40 19 0.48
D02- 560 A - - + + + + - - +
10 F06-2340 A + - + + - - + + + +
I08-1150 B + - + + - - + + + +
G15-1060 B - - + + - - + + + +
11 F14- 680 B - - - - + - - + - -
* I05- 490 B - - - - + - - + - - - - - + + - + + - - - - - + + + - + + - + + + + - + - - + 40 17 0.43
12 A03- 290 A - + + + - + - - + +
I05- 190 C - + + + - + - - + +
13 I20- 820 B - + - + - + - + - -
I09-1760 C - + + + - + - + - -
Solitary
E17- 480 A - + + + + + + + - +
C12- 580 B - + - - - - + - - +
E01-2000 A + + - - - + + + - -
A09-1240 B + + + - - - - + + -
K06-1360 A + - + + - - + - - -
K18- 230 C + + + - - + + + - +
K18- 440 C + + - + + + + - + +
I16-1930 C + + - - + + + + + +
Exclude
D15- 650 A - - - - + + + + - +
D20- 660 C - + - + + - + + - +
K17- 610 B - - - + + + + + + +
K01- 540 C - + - - + + + + + +
D02- 880 A - - - - - + + + +

Fig. 1 Part 2

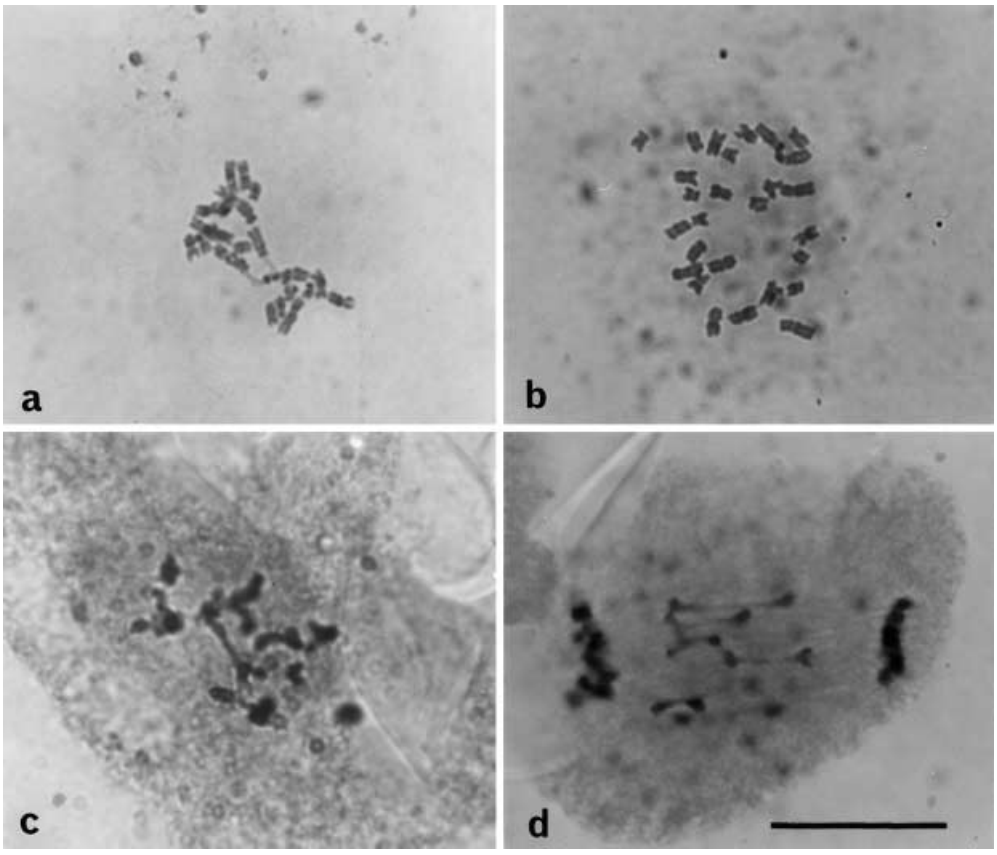


Fig. 2 a–d The somatic chromosomes of plants no. 5 (2n=23) (**a**) and no. 8 (2n=27) (**b**) in the BC_2 plants. Reduction division of pollen mother cells at metaphase-I (**c**) and anaphase-I (**d**) in the BC_1 plant. The scale bar indicates 10 µm

Table 3 Genetic components of the ten BC_2 plants deduced by RAPDs, DNA content and chromosome number

Item	Plant no. (BC_2)									
	1	2	3	4	5	6	7	8	9	10
No. of synteny groups revealed[a]	6	8	8	11	5	9	10	10	9	10
Excess of DNA content[b]	22.54	67.78	–[c]	–	37.08	67.01	77.41	75.54	52.02	–
No. of chromosomes observed	22	27	–	27	23	26	28	27	27	–

[a] The groups having at least one of the markers are included
[b] Relative % to A genome
[c] Not tested

the plants were presented as percentages of the recurrent parent. The DNA contents of the ten BC_2 plants considered for synteny group construction are given in Table 3. From the 40 BC_2 plants measured, the values were shown to have a wide range (1.1–90.7%) (data not shown). The chromosome numbers varied from 22 to 28 in the ten plants, which were composed of the recurrent parent and various numbers of *B. alboglabra* chromosomes (Fig. 2a and b). The number of synteny groups was correlated with the excess of DNA content, r=0.81 (Fig. 3), and also with the chromosome number.

Chromosome transmission and relationship among the *B. alboglabra* chromosomes in the BC_2 plants

Thirty two RAPD markers covering most of the synteny groups were selected for further analyses. The transmission rates were calculated using 40 plants and are presented in the right column of Fig. 1. The transmission rates of most of the RAPD markers were in the range of 0.38 to 0.68. However, extremely high transmission rates, 0.83, 0.98 and 0.98, were observed in E02-1150, I03-190 and I13-930, respectively. These three RAPD markers with extremely higher transmission rates belonged to synteny group no. 7. This fact indicates that whole or a part of a chromosome represented by synteny group no. 7 tended to be transmitted with high frequency in the backcross process.

The inheritance data of RAPD markers in each synteny group were also applied to reveal the relationships among these groups. When a certain synteny group was examined, the difference between the average numbers of the rest of the synteny groups per plant, in terms of the presence and absence of the synteny group, was tested by using the markers (Table 4). Significant differ-

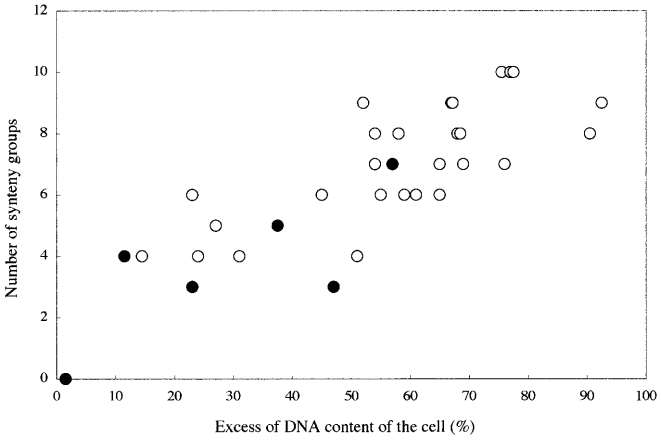


Fig. 3 Relationship between the excess of DNA contents of the cells and the number of synteny groups revealed by RAPD markers in the BC_2 progenies. The plants lacking a part of synteny group no. 7 are shown with *black circles*

Table 4 Average number of synteny groups in the BC_2 progenies classified in terms of the presence and absence of the RAPD bands in each synteny group

Synteny group no.	RAPD band	Present	Absent	*t*-value
1	B01-440	5.71	4.81	1.621
	I20-510	5.74	4.82	1.676
2	H06-1280	6.00	4.65	2.736**
	E20-990	5.50	5.21	0.531
3	H15-240	5.42	4.94	0.807
	H06-840	5.23	5.22	0.009
4	C16-780	5.71	5.39	0.534
	G06-600	5.82	5.30	0.887
5	C16-850	5.36	5.33	0.043
6	B02-570	5.75	5.20	0.967
7	E02-1150	5.45	2.86	3.906***
8	A18-210	5.65	5.30	0.601
9	E11-1600	5.84	5.19	1.159
11	I05-490	6.12	5.13	1.828

, * Significant at 1% and 0.1% levels, respectively

ences were observed in the markers of synteny group no. 2 and no. 7. In addition to the number of synteny groups, a significant relationship was also confirmed between the DNA content and the number of markers in group no. 7 (t=2.07, P<0.05) (not tested for no. 2). The plants not includes in no. 7 tended to lose other synteny groups and their DNA contents (Fig. 3). Therefore, it seems certain that all or part of the chromosomes corresponding to synteny groups no. 2 and no. 7 are responsible for the transmission of the other C-genome chromosomes.

Discussion

The effectiveness of ovary culture for obtaining hybrids between *B. campestris* and *B. alboglabra* differed both among the lines used and between the reciprocals. Olsson (1960) reported that the genotype and physiological state of the maternal parent play important roles in hybridization. In the present study, the difference of hybridization rates among the material lines of *B. campestris* might be partly due to genotypic differences among the lines. The higher rates of hybrid productivity of the crosses using *B. campestris* as female are consistent with the normal tendency, as reported by Namai et al. (1980).

In the present study, the rate of polymorphic bands per primer was 0.98, which is higher than with an intraspecific cross of *B. campestris*, where the rate was reported to be 0.60 (Nozaki et al. 1997). In contrast to the intraspecific cross, a backcross population was used in the present study, and the highest number of polymorphic bands came from one of the parents. The difference between the rates reflects the genetic difference between the parental lines involved in the crosses. Therefore, effective marker-assisted genetic analysis can be achieved by wide crosses.

With the C genome-specific bands, 13 synteny groups were constructed, which are higher than the expected number of nine. In synteny group no. 2, the ten BC_2 plants showed almost common banding patterns for the 21 markers. However, two types of banding patterns of the nine markers composed of the upper five and lower four in Fig. 1 were clearly shown in the additional 30 plants. This implies that group 2 might be composed of two different synteny groups. By the inclusion of additional RAPD markers specific to the C genome, the number of synteny groups could be brought to expectation.

A wide range in the number of RAPD markers was observed among the synteny groups. Since these RAPD markers were detected to be specific to the C genome, the number in each synteny group reflects the degree of genetic difference of the chromosomes of the C genome from those of the A genome. Even if the chromosome sizes are different, the present results revealed large differences in the number of markers among the C-genome chromosomes. From chromosome pairing, Röbbelen (1960) pointed out that *Brassica* species were derived from one ancestral species. The present result also indicated the possibility that the chromosomes represented by the synteny groups having a relatively large number of C genome-specific markers, e.g. nos. 1 and 2, differentiated rapidly during species differentiation.

Simultaneous loss and gain of clusters of markers were observed with high frequency in some of the synteny groups. These phenomena could result from translocations and/or deletions of the chromosomes. There are two possibilities concerning the fate of the C-genome chromosomes at meiotic division in the BC_1 plants. First, a fragment of the C-genome chromosomes might be incorporated into an A-genome chromosome after intergenomic chromosome pairing. When the recombinant A-genome chromosome was transmitted to the BC_2 generation and the donor C-genome fragment was lost, only the markers of the inherited fragment would be expressed. Multivalent formation in the BC_1 plants was observed at MI which was assumed to be between the

A- and C-genome chromosomes (Fig. 2c). The second possibility is the loss of the C-genome chromosomes after intragenomic chromosome pairing due to partial homology. Pairing between intragenomic chromosomes was reported in *B. campestris* by using haploids (Armstrong and Keller 1981). In the present report, the observed pairing and bridge formation in the lagging chromosomes seemed to belong to the C genome in the BC_1 plants (Fig. 2d).

The occurrence of intergenomic recombination or introgression between the A and C genomes were revealed by using isozyme and RAPD markers by Quiros et al. (1987) and Chen et al. (1997), respectively. Attia and Röbbelen (1986b) reported that synthetic amphidiploids of AACC showed multivalents at meiotic pairing. On the other hand, the chromosome deficiency in the B-genome chromosomes did not occur in the *B. oleracea*– *B. nigra* and *B. napus*– *B. nigra* chromosome addition lines (Chèvre et al. 1997). These reports imply that, in contrast to the chromosome pairing between the A and C genomes, B genome chromosomes did not pair with those of the C or A genome. Actually, chromosome pairing occurred more frequently in amphihaploids of *B. napus*, AC, than that of *B. carinata*, BC (Attia and Röbbelen 1986a). From a phylogenetic study, *B. nigra* must have resulted from a different lineage to those of *B. campestris* and *B. oleracea*, the latter two having a close relationship (Warwick and Black 1991; Pradhan et al. 1992). Thus, it might be concluded that chromosomal recombination had occurred between the A and C genomes due to the phylogenic relationship between *B. campestris* and *B. alboglabra*.

The theoretical transmission rate of a univalent chromosome from the BC_1 to BC_2 generation is 0.5, under conditions of random distribution, no elimination and no selection. On the other hand, the transmission rates of the additional alien chromosomes reported in the cases of *B. oleracea*– *B. nigra* and *B. napus*– *B. nigra* were 0.14–0.24 in the female (Chèvre et al. 1997), and in *B. napus*– *B. campestris* 0.30–0.38 (McGrath and Quiros 1990). These reports showed tendency for a low transmission rate. By contrast, the present data is almost close to the theoretical value, except in the case of synteny group 7. The relatively high rates are probably due to the absence of chromosome elimination and selection against the C-genome chromosomes.

Considering the fact that the markers in synteny group no. 7 showed extremely high transmission rates of 0.83–0.98, gametes or embryos which do not have the chromosome represented by this group might be subject to some kind of selection pressure. When the chromosomes represented by a part of synteny group no. 2 or no. 7 were fully or partially deleted, the plants showed a significant tendency to lose other C-genome chromosomes. From this result, it can be assumed that the gametes not having the gene(s) on the chromosome shown by synteny group no. 7 could not participate in the fertilization process, or else after fertilization the zygotes not having the gene(s) would have died. In addition, it appears certain that the genetic factor(s) on the chromosomes corresponding to synteny groups no. 2 and no. 7 are responsible for the transmission of the other C-genome chromosomes. There is the possibility for the presence of such kinds of chromosomes in other *Brassica* species which would play an important role in maintaining amphidiploidization after crosses between the diploid species.

Acknowledgements The authors are grateful to Dr. Khwaja G. Hossain for his critical reading of the manuscript. This work was supported in part by a Grant-in-Aid for Scientific Research, No. 10306001, to M.M. from the Ministry of Education, Science and Culture, Japan.

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