CHARACTERIZATION OF THE BRASSICA ALBOGLABRA GENOME

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INTRODUCTION

The Brassica genus includes many agronomically important species such as B. napus, B. campestris and B. oleracea. While B. napus and B. campestris are the two most important oleiferous Brassica species, B. oleracea is widely cultivated as a vegetable crop.

Through interspecific hybridization, U (1935) elegantly demonstrated the Brassica species interrelationships which he illustrated in the well-known U-triangle. This knowledge has been a solid foundation in connection with practical breeding as well as academic research dealing with Brassica crops. For example, resynthesis of B. napus (genomes: AACC) through interspecific hybridization between B. campestris (AA) and B. oleracea (CC) has been widely adopted as an effective strategy for the purpose of gene transfer (e.g. Chen et al. 1988b), genome mapping and evolutionary studies (Hosaka et al. 1990).

Brassica alboglabra is a form of B. oleracea, which has been also utilized as the C-genome contributor to resynthesized B. napus (Chen et al. 1988b). The white flower and erucic acid content of B. alboglabra are each controlled by a single locus, W_c and E_c, respectively (Chen et al. 1988a; Chen and Heneen 1989), and thus can be used as ideal genetic markers. Isozyme loci specific for the B. alboglabra genome have been also identified and are used as additional markers (Chen et al. 1989; 1990). Furthermore, resynthesized B. napus has been successively backcrossed to the parental B. campestris to generate an aneuploid progeny, i.e. B. campestris with various numbers of B. alboglabra chromosomes. Taking advantage of the known genetic markers and the available aneuploid progeny, we are making efforts to characterize the B. alboglabra genome. The present report is concerned with the recent progress of our work.

MATERIALS AND METHODS

Plant Materials

Two resynthesized B. napus lines, No2305 and No7406, were used (Chen et al. 1988a; 1989). The C-genome contributor for both No2305 and No7406 was the B. alboglabra line No4003 which was high-erucic, white-flowered and black-seeded (black seed coat with black hilum). The A-genome of No2305 was contributed by the B. campestris line Sv85-38301 which was zero-erucic, yellow-flowered and yellow-seeded (yellow seed

coat with black hilum). No7406 haboured the A-genome of B. campestris var. yellow sarson line K-151 which was high-erucic, yellow-flowered and yellow-seeded (yellow seed coat with light-coloured hilum). The black-seeded character of No2305 and No7406 was thus controlled by the B. alboglabra genome although the inheritance pattern of this character was not known in the present material.

No2305 and No7406 were backcrossed to their respective parental B. campestris lines. The resulting F1 trigenomic hybrids (2n=29, AAC) were either selfed or successively backcrossed to the B. campestris lines to produce an aneuploid progeny. Aneuploid seeds derived from selfing and backcrossing were pooled. These aneuploid seeds were assayed for erucic acid content and their resulting plants were further studied for isozymes, flower colour and seed colour (including both seed coat colour and hilum colour).

Assay for Erucic Acid Content and Isozymes

Aneuploid seeds were analysed for erucic acid contents by the so-called half-seed technique in order to be able to raise plants from these seeds. The methods for assaying isozymes were described in our previous reports (Chen et al. 1989; 1990). In the present study, it has been possible to score, in the aneuploid plants, the presence or loss of the following B. alboglabra specific isozyme loci: Gpi-2C^a (glucosephosphate isomerase), Lap-1C^c (leucine aminopeptidase), 6Pgd-1Cb (6-phospho-gluconate dehydrogenase) and Sdh-1C^a (shikimate dehydrogenase) (for more details, see Chen et al. 1990).

Cytological Methods

For mitotic chromosome counting, root tips were taken from about 3 to 4 week-old plants raised in pots in the greenhouse. The root tips were put on wet filter paper in a petri dish on ice for about 8 hours, and then fixed in Farmer's solution (3 parts of absolute ethanol: 1 part of glacial acetic acid, v:v). Preparations were made according to the Feulgen squash method (Darlington and La Cour 1960). For meiotic analyses, flower buds were also fixed in Farmer's solution supplemented with some ferric chloride as a mordant, and then stored in 70% ethanol. The flower buds were stained in Snow's carmine in water bath at 60°C for 3 hours. Squashing was done in Hoyer's medium to obtain permanent slides.

RESULTS

Transmission Frequencies of the Loci or Characters Specific for the B. alboglabra Genome

Various B. alboglabra genome specific loci or characters were transmitted at extremely different frequencies; and also strongly influenced by the B. campestris background in the trigenomic hybrids (Table 1). In the aneuploid progeny derived from No2305, the highest transmission frequency was observed for the erucic locus (E_c) at 53%, while a very low transmission frequency (29%) was recorded for the isozyme

Table 1. Transmission frequencies of various B. alboglabra genome specific loci or characters in the aneuploid progeny of the trigenomic Brassica hybrids (2n=29, AAC) derived from two resynthesized B. napus lines, N02305 and No7406.

Locus or character	No2305				No7406			
	No. of plants (N)	Presence (A)	Absence (B)	A/N in %	No. of plants (N)	Presence (A)	Absence (B)	A/N in %
E_c	121	65	56	53.7	-	-	-	-
W_c	110	56	54	50.9	90	88	2	97.8
Gpi-2C^a	115	58	57	50.4	93	73	20	78.5
Lap-1C^c	116	60	56	51.7	110	106	4	96.4
6pgd-1Cb	116	61	55	52.6	98	86	12	87.8
Sdh-1C^a	109	32	77	29.4	98	70	28	71.4
Seed colour(1)	39	8	31	20.5	35	34	1	97.1

(1) In the case of seed colour, presence is for black seed; absence for yellow seed.

locus Sdh-1C^c. The strong influence of B. campestris background was indicated e.g. by the transmission frequencies for the locus controlling white flower in the two trigenomic hybrids, being at 51% and 98%. The transmission of black-seeded character, however, showed a complex pattern. Compared with the other loci, this character was transmitted at a very high frequency in the trigenomic hybrid involving No7406 but at a very low frequency in the other hybrid involving No2305.

Locus/Chromosome Relationships

When two loci are carried by the same C-genome chromosome, they will show simultaneous presence or loss in the offspring from the trigenomic hybrid (2n=29, AAC) if their linkage is not broken up by recombination following autosyndesis or allosyndesis. If the two loci are located on separate C-genome chromosomes, a concurrent presence or loss of these chromosomes would lead to the same result, which is, however, unlikely to occur in a large sample (Chen et al. 1990). The simultaneous presence or loss of the three C-genome loci coding for erucic acid content (E_c), white flower (W_c) and the faster band of leucine aminopeptidase (Lap-1C^c) was observed in a large sample comprising 110 aneuploid plants derived from No2305. Therefore, it was concluded that these three loci were located on the same B. alboglabra chromosome. This conclusion was further confirmed by the development of a B. campestris-alboglabra monosomic addition line showing simultaneous presence of the three loci.

In the aneuploid progeny derived from No7406, the high erucic acid content controlled by the B. campestris (K-151) genome made it unrealistic to record the presence or loss of the B. alboglabra erucic locus. However, the simultaneous presence or loss of the other two loci, W_c and Lap-1C^c, was observed, thereby providing additional evidence for a chromosomal linkage between them. In addition, it was found that one plant produced yellow seeds with black hilum, indicating the presence of the B. alboglabra chromosome(s) that code for black hilum.

In the progeny originating from both No2305 and No7406, there was no evidence of chromosomal linkage between the B. alboglabra loci, Gpi-2C^a, 6Pgd-1Cb and Sdh-1C^a.

Development of Alien Chromosome Addition Lines

Three B. campestris-alboglabra alien monosomic chromosome addition lines have been developed in the aneuploid progeny involving No2305. It is possible to distinguish these three addition lines from one another. One addition line haboured the C-genome chromosome carrying the three loci, E_c, W_c and Lap-1C^c. The other addition line showed the presence of the two loci, Gpi-2C^a and Sdh-1C^a, although there was no evidence for chromosomal linkage between the two loci. The third addition line remained unmarked. Chromosome configurations in the pollen mother cells (PMCs) at diakinesis or the first metaphase of meiosis were 10 bivalents and 1 univalent, or 9 bivalents and 1

trivalent at various frequencies in the three monosomic addition lines (Table 2).

Table 2. Chromosome configurations in the pollen mother cells (PMCs) at diakinesis or the first metaphase of meiosis in the three B. campestris-alboglabra monosomic addition lines.

Addition line	No.of PMCs	Chromosome configurations			
		(10 II + 1 I)	in %	(9 II + 1 III)	in %
$E_c/W_c/Lap\text{-}1C^c$	76	67	88	9	12
$Gpi\text{-}2C^a$ or $Sdh\text{-}1C^a$	83	76	92	7	8
Unmarked	42	33	79	9	21

DISCUSSION

Transmission of various B. alboglabra specific loci reflected the transmission of the chromosomes carrying these loci. The transmission frequency for a locus would thus be determined by the biological importance and possibly the physical size of that chromosome carrying the locus. Large chromosomes might not be lost as easily as small ones. It is reasonable to assume that two genetically divergent B. campestris lines such as Sv85-38301 and K-151 may have, to a different degree, homology or homoeology to the B. alboglabra genome, thereby leading to the different transmission frequencies of the same C-genome locus in their background.

Though situated on the same chromosome, the three loci, E_c, W_c and $Lap\text{-}1C^c$ showed a little difference in their transmission frequencies. This was due to the inability to score the three characters on each aneuploid plant. For example, one seed might have been analysed for erucic acid content, but the resulting plant failed to grow into blooming phase, thus not permitting flower colour determination.

In the process of developing addition lines, genetic recombination following autosyndesis or allosyndesis can break up chromosomal linkage between genes. Great caution should be taken as to whether the alien chromosome is intact or recombined in such addition lines. In this respect, it is very helpful to study the joint segregation of various C-genome specific loci in a large sample of the aneuploid progeny derived from the trigenomic hybrids (2n=29, AAC).

In the aneuploid progeny derived from No7406, the observation of a plant bearing yellow seeds with black hilum indicated that the seed coat colour and hilum colour are regulated by two separate genetic systems that code for seed pigmentation in the B. alboglabra genome. Furthermore, the gene(s) controlling black hilum are likely to be located on different chromosome(s) compared to the gene(s) controlling black-seeded character. However, this conclusion is open to

verification since the small sample size can not exclude such a phenotype being a result of breaking-up of linkage between the genes controlling these two characters.

CONCLUSIONS

(1) In the aneuploid progeny derived from trigenomic Brassica hybrids (2n=29, AAC), various B. alboglabra (CC) specific loci or characters were transmitted at extremely different frequencies, which were also strongly influenced by the B. campestris (AA) background.

(2) In the B. alboglabra genome, the same chromosome carried the three loci coding for erucic acid content, white flower and the faster band of leucine aminopeptidase.

(3) Three monosomic addition lines have been developed, which habour three different B. alboglabra chromosomes.

(4) The seed colour and hilum colour could be regulated by two separate genetic systems responsible for seed pigmentation in the B. alboglabra genome.

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