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CYTOGENETICAL STUDIES ON *BRASSICA NIGRA* x *B. OLERACEA* HYBRIDS

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ABSTRACT

Chromosomal associations were studied in three F_1 hybrids from the cross *Brassica nigra* x *B. oleracea* var. *alboglabra*. Differences were observed in the extent of chromosome pairing in the three hybrids. Trivalents were observed at a high frequency, indicating allosyndetic pairing and a higher possibility of gene transfer between B and C genomes.

Key words: *Brassica nigra*, *B. oleracea*, *B. carinata*, allosyndesis, amphihaploid.

Cytogenetic investigations of the cultivated *Brassica* species have shown that there are three monogenomic diploid species and three digenomic allotetraploid species [1]. The diploid species are *B. campestris* L. (2n = 20, AA), *B. oleracea* L. (2n = 18, CC), and *B. nigra* (L.) Koch (2n = 16, BB). It has been established that in nature, crosses between pairs of these three elementary diploid species followed by chromosome doubling have given rise to the three amphidiploid species, namely, *B. napus* L. (2n = 38, AACC), *B. juncea* (L.) Czern. (2n = 36, AABB), and *B. carinata* A. Braun (2n= 34, BBCC).

Interspecific hybridization among the diploid *Brassica* species has received considerable attention both to establish chromosome relationships [2–5] and for exploitation in crop improvement [6–9]. These studies have shown that variable degree of chromosome pairing occurs in the hybrids (amphihaploids) between the three diploid species. The AC amphihaploids show consistently high amount of pairing compared to AB and BC hybrids. This implies that *B. campestris* is closer to *B. oleracea* than either is to *B. nigra*. Several authors have reported the cytology of AC hybrids [4, 10], while studies on BC hybrids have been very limited. Even these deal only with *B. oleracea* x *B. nigra* hybrids [3]. The present paper examines the chromosome pairing in three hybrids from the reciprocal cross *B. nigra* x *B. oleracea*.

MATERIALS AND METHODS

In a programme on the synthesis of *Brassica carinata* [11], three hybrids (1–3) were obtained from the cross *B. nigra* (IC 257) x *B. oleracea* var. *alboglabra*. The first hybrid

(Hybrid 1) was obtained by hand-pollination, and other two (Hybrids 2, 3) through ovary culture. Chromosome pairing was studied in these three hybrids.

Flower buds were fixed in 6:3:1 (alcohol:chloroform:acetic acid) mixture containing ferric chloride as a mordant, and the anthers were squashed in 1% acetocarmine. Chromosomal configurations were scored at metaphase I. Stages up to pollen formation were also observed for any abnormalities.

RESULTS

The three amphihaploid F_1 hybrids between *B. nigra* and *B. oleracea* were analysed for meiotic chromosome behaviour. Each of the hybrids had 17 chromosomes as expected. Univalents occurred with a high frequency in all the three hybrids. While 7–8 univalents were most frequently present in Hybrid 1, Hybrids 2 and 3 showed predominance of 11 univalents (Table 1).

Bivalents were present in all the three hybrids. At least one bivalent was present in all the PMCs, with three bivalents per PMC occurring most frequently. The number of bivalents ranged from 1 to 5. The average number of bivalents per PMC was lowest in Hybrid 2 (Table 2).

In addition to bivalents, trivalents were also found in all the three hybrids. The frequency of trivalents in Hybrid 1 was more than five times higher than that in Hybrids 2 and 3 (Table 2). Correspondingly, the frequency of univalents was low in Hybrid 1 compared to that in Hybrids 2 and 3. While only 4–8 univalents were present in 72.5% PMCs of hybrid 1, more than 8 univalents were observed in 90 and 75% PMCs of Hybrids 2 and 3, respectively.

Table 1. Chromosome associations at PMC metaphase I of three hybrids from the cross *B. nigra* x *B. oleracea* var. *alboglabra* (values in parentheses indicate percentages)

Chromosome association	Hybrid 1	Hybrid 2	Hybrid 3
III + 5 II + 4 I	2 (5)	1	1 (2.5)
1 III + 4 II + 6 I	7 (17.5)	—	—
1 III + 3 II + 8 I	12 (30)	2 (5)	4 (10)
1 III + 1 II + 12 I	5 (12.5)	2 (5)	1 (2.5)
5 II + 7 I	8 (20)	—	5 (12.5)
4 II + 9 I	2 (5)	6 (15)	7 (17.5)
3 II + 11 I	4 (10)	26 (65)	12 (30)
2 II + 13 I	—	3 (7.5)	6 (15)
1 II + 15 I	—	1 (2.5)	4 (10)
Total PMCs	40	40	40

Table 2. Frequency of univalents, bivalents and trivalents in three hybrids of *Brassica nigra* x *B. oleracea* var. *alboglabra*

Hybrid No.	PMCs with various associations		
	univalents	bivalents	trivalents
Hybrid 1	8.10	3.47	0.65
Hybrid 2	10.85	2.92	0.10
Hybrid 3	10.40	3.70	0.12

The maximum pairing observed in any of the hybrids was 1 III + 5 II + 4 I (Fig. 1:1). In Hybrid 1, the chromosomal associations ranged from 3 II + 11 I to 1 III + 5 II + 4 I. The chromosomal association with the highest frequency (30% PMCs) was 1 III + 3 II + 8 I. In Hybrid 2, the chromosomal associations ranged from 1 II + 15 I to 1 III + 3 II + 8 I. The highest frequency of chromosomal association (65% PMCs) was 3 II + 11 I (Fig. 1:2). In Hybrid 3, the range of pairing was 1 II + 15 I to 1 III + 5 II + 4 I. As in Hybrid 2, the most common configuration was 3 II + 11 I (30% PMCs).

Bivalent and trivalent associations persisted through late anaphase I (Fig. 1:3) in all the three hybrids. Sometimes well defined metaphase plates could be recognised but not all the chromosomes were arranged in the equatorial region. In other cells, the chromosomes lay scattered from one pole to the other, making distinction between metaphase and anaphase difficult (Fig. 1:4). Distribution of chromosomes at anaphase was random.

In all the hybrids, abnormalities of the spindle apparatus occured occasionally. Tripolar spindles with laggards in the equatorial region were observed in some PMCs (Fig. 1:5). All the PMCs did not divide synchronously, as dyads were observed among tetrads in the ratio of 2 dyads : 26 tetrads (Fig. 1:6). The pollen of the three hybrids was highly sterile (0.005% fertile pollen grains).

DISCUSSION

On the basis of mitotic chromosome morphology [12] and pachytene analysis [13], the three elementary diploid species of *Brassica* themselves have been proposed to be derived from a common ancestor with x=6 chromosomes. Thus, in interspecific hybrids betwen the diploid species both autosyndetic and allosyndetic pairing is expected. Genome B forms a maximum of two bivalents autosyndetically [14] and genome C a maximum of one trivalent and one bivalent autosyndetically [15]. Thus, theoretically, a maximum allosyndetic pairing of 1 III + 3 II or 4 II is possible in BC hybrid. Mizushima [16] observed a maximum pairing of 4 II in BC hybrids and suggested that they were allosyndetic, since they appeared 10 times more frequently than the autopairs in haploid [16].

In the present investigation, maximum pairing of 1 III + 5 II + 4 I was observed in the *B. nigra* x *B. oleracea* hybrids, suggesting that both auto- and allosyndetic pairings occur. Trivalents or higher associations have not been previously reported in the *B. nigra* x *B. oleracea* hybrids [15]. However, in the reciprocal cross between *B. oleracea* x *B. nigra*, trivalents were observed in 3% PMCs [3]. The occurrence of a high frequency of trivalents at least in Hybrid 1 indicates that they are of allosyndetic origin since the C genome by itself forms a trivalent at a very low frequency [15]. Such a high pairing between B and C genomes has not been reported before. Attia and Robbelen [3] observed that more than 13 out of the 17 chromosomes were left unpaired in the *B. oleracea* x *B. nigra* hybrids [3]. Whether reciprocal crosses have any bearing on the degree of chromosome pairing needs to be evaluated.

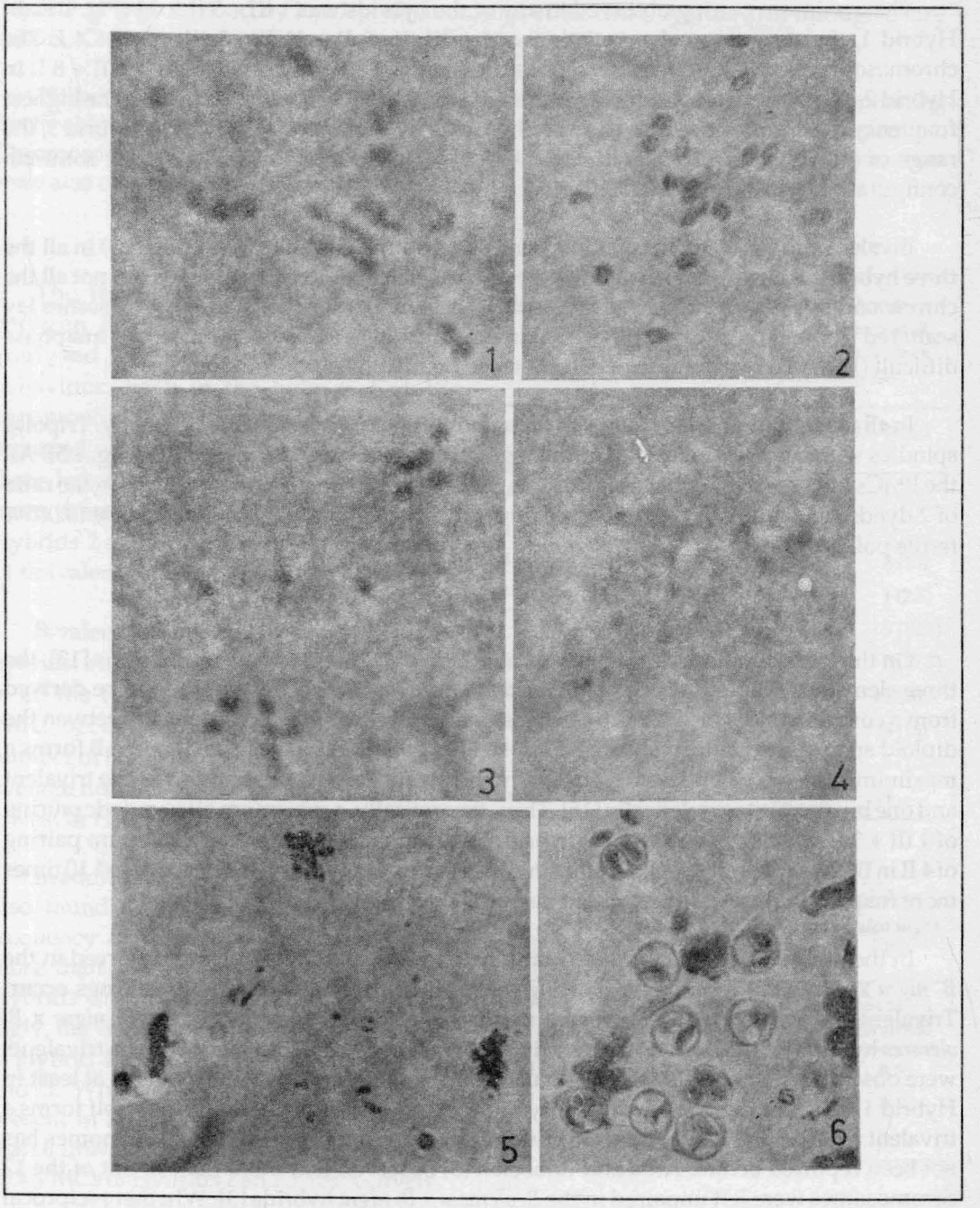

Fig. 1. Meiosis in *B. nigra* x *B. oleracea* hybrids: 1) 1 III + 5 II + 4 I at metaphase I, x 4000. 2) 3 II + 11 I. Two larger bivalents terminalized, x 4000. 3) 1 III + 3 II + 8 I at meta-anaphase I, x 4000. 4) 2 II + 12 I at meta-anaphase I, x 2000. 5) Late anaphase II showing abnormal tripolar spindles and laggards x 450. 6) Dyad among tetrads, x 400.

The three hybrids in the present study had the same cytoplasm of *B. nigra*, therefore, the differences in chromosome pairing can be assumed to be genetic and not influenced by the cytoplasm. That genetic differences exist in the control of meiotic pairing is indicated by the variation in the extent of pairing in the three hybrids studied. Both the parents *B. nigra* and *B. oleracea* var. *alboglabra* are self-incompatible species and genetic differences in the plants used could have also contributed to the observed differences in chromosome pairing. Our results are in agreement with earlier observations on variation in pairing in different amphihaploid plants raised from a single cross [3].

If chromosome pairing is an index of gene transfer, the greater homology between B and C genomes noted in the present study indicates a higher possibility of transferring genes between these genomes and to the amphidiploid species as well.

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