

Non-homologous meiotic pairing in the A and B genomes of *Brassica*: its breeding significance in the production of variable amphidiploids

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SUMMARY

Brassica juncea Coss ($2n = 36$) is a naturally occurring allotetraploid. In the progenies of F_1 hybrids of its constituent parents *B. campestris* sub.sp. *oleifera* var. *toria* \times *B. nigra* and *B. campestris* sub.sp. *japonica* \times *B. nigra*, the majority of the plants were parthenogenetic homozygous amphidiploids like *B. juncea* whereas a few differed in morphological and physiological characteristics. The latter, presumed to have arisen parthenogenetically, were suspected to be homozygous for chromosomes changed by allosyndetic recombination. One of these derived plants with curly leaves was crossed first with one of the homozygous amphidiploids and secondly with *B. c.* sub.sp. *oleifera* var. *toria*. Sporocytes of the two hybrids were studied at meiosis. The presence of one or two quadrivalents in the first hybrid and two trivalents in the second suggested that allosyndetic recombination had occurred in the 18-chromosome hybrid. The progeny was presumed to have arisen through pseudo-diploid parthenogenesis and this in association with allosyndetic recombination may provide a means of breeding higher yielding genotypes.

1. INTRODUCTION

Brassica juncea Coss is a natural amphidiploid combining in its chromosome complement the chromosome sets of two diploid species namely the 20-chromosome *B. campestris* which provided the A genome and the 16-chromosome *B. nigra* which provided the B genome. Increased variability can be obtained in *B. juncea* by resynthesizing the amphidiploid from its highly variable diploid parental species. In this investigation, further variability arose from non-homologous chromosome recombination.

2. MATERIALS AND METHODS

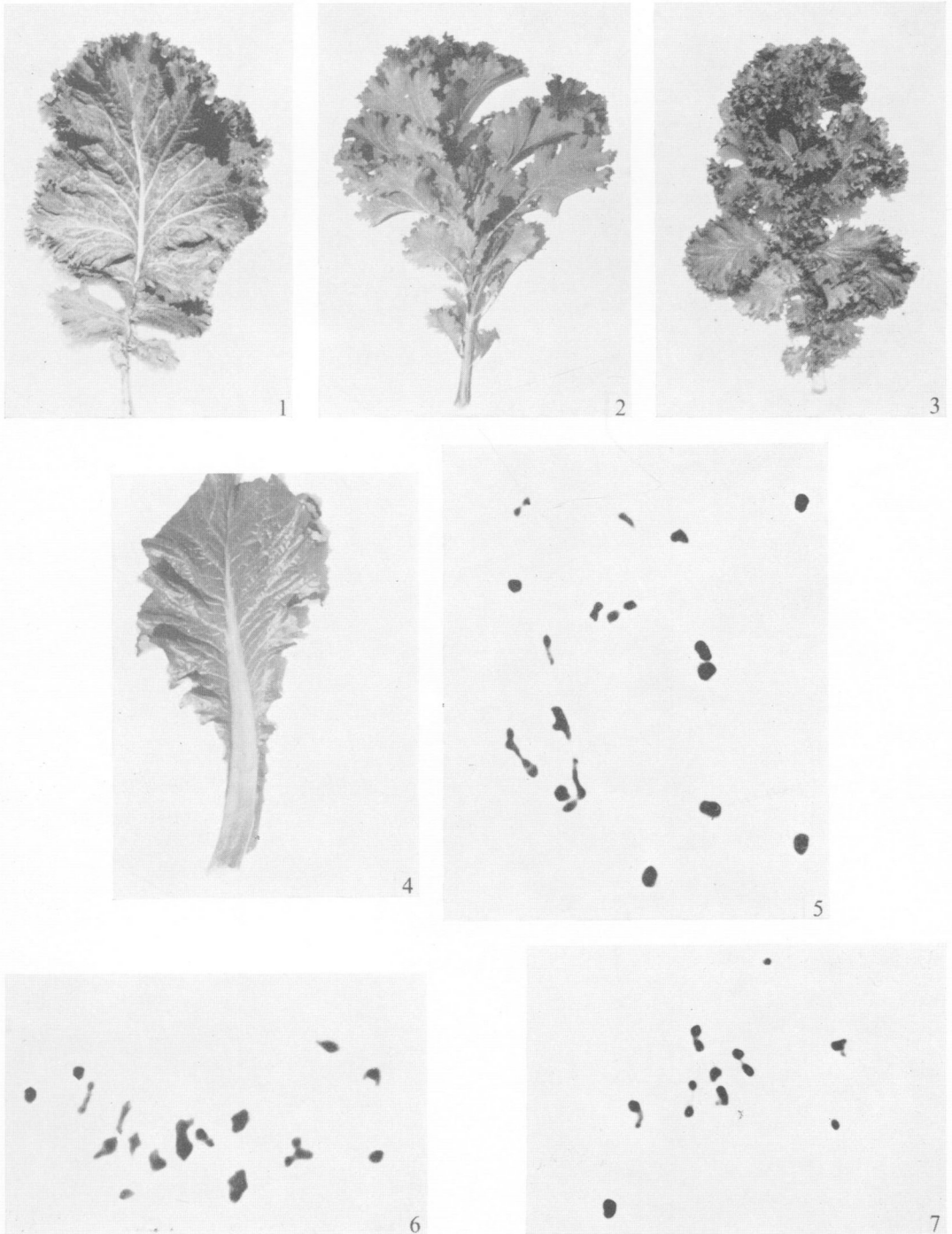
Two species, *Brassica* - *B. c.* sub.sp. *oleifera* var. *toria* and *B. c.* sub.sp. *japonica*, were crossed with *B. nigra* to give two sets of crosses termed here *torianig* and *japnig* respectively. The hybrids were allowed to out-pollinate and set some seeds which were grown and the resulting plants were screened in the following years. Meiosis was studied in acetocarmine squashes of anthers fixed in Carnoy's fluid.

3. RESULTS AND DISCUSSION

The F_1 hybrids were generally sterile and had usually 18 univalents in P.M.C.s. Bivalents were seen occasionally being more frequent in *japnig* than in *torianig* hybrids. The few seeds that set under open pollination produced three types of plants: 1. Parthenogenetic homozygous amphidiploids ($2n = 36$). These were the commonest plants. 2. A few plants with $2n = 36$ but differing from 1 in certain characters (Table 1), probably due to segmental interchanges between chromosomes following bivalent formation in F_1 hybrids. Tentatively these may be called parthenogenetic homozygous non-homologous recombinants. 3. Aneuploids – one plant with $2n = 20 + 1$ was noticed. Plants of classes 1 and 2 bred true for five generations. All the derived amphidiploids of these two classes with normal and deviant phenotypes behaved cytologically alike and had fairly disturbed meiosis in the A_1 generation. Besides the normal pairing pattern of 18 bivalents, there was a high frequency of univalents ranging from 4 to 8. Surprisingly no multivalent association was observed. In the subsequent generations, i.e. A_2 and A_3 , the pairing behaviour improved gradually as the cells displayed a diminished number of univalents. Afterwards the meiotic process attained a marked degree of stabilization, so much so that in A_5 invariably 18 bivalents were regularly seen, with only occasional univalents.

An experiment was carried out to test whether plants of group 2 were homozygous for allosyndetic recombination. One line (curley leaf) was crossed with a plant of group 1 and meiosis was studied in the hybrids. Most P.M.C.s showed one or two quadrivalents (Table 2, Fig. 5) as would be expected if chromosome interchanges had occurred in F_1 hybrids of the class 2 amphidiploids. The curley leaf line was also crossed with its parent *B. c.* sub.sp. *oleifera* var. *toria*. At meiosis this hybrid showed a number of trivalents, the maximum being two but no quadrivalent was observed (Table 3, Fig. 6.)

18-chromosome F_1 hybrids with the A and B genomes of *Brassica* have been reported earlier to form 3-7 bivalents (U, Mizushima & Saito, 1937; Ramanujam & Srinivasachar, 1943 and Olsson, 1960). In the present investigation, however, a maximum of 8 bivalents occurred at first metaphase (Fig. 7). Röbbelen (1960), Prakash (1969) and Nwankiti (1970) have shown that the basic chromosome number in *Brassica* is six. The genetical constitution of A genome is AABCDDDEFFF and that of B genome as ABCDDEFFF, consequently the maximum number of bivalents due to autosyndesis should be five. Therefore, in cells with eight bivalents at least three were possibly allosyndetic. Olsson (1960) concluded that one out of six bivalents in his material was allosyndetic. Apparently there may be homoeologous pairing between some chromosomes of the two genomes. It may be noted that chiasma formation and recombination between non-homologous chromosomes leads to occurrence of changes that must be recognized as translocation relative to original chromosome structure (Riley & Kempanna, 1963). The occurrence of quadrivalents in the F_1 hybrid between homozygous allosyndetic recombinants (class 2 with $2n = 36$) and homozygous amphidiploids (class 1 with $2n = 36$)



Figs. 1-4. Morphological variations in radical leaves of allosyndetic recombinants.

Fig. 5. Metaphase I in the F_1 of allosyndetic recombinant \times normal amphidiploid of *B. juncea* 2 IV + 14 II.

Fig. 6. Metaphase I in the F_1 of allosyndetic recombinant \times *B. campestris* 2 III + 8 II + 6 I.

Fig. 7. Metaphase I in the F_1 hybrid of *B. c.* sub.sp. *oleifera* var. *toria* \times *B. nigra* 8 II + 2 I.

Table 1. *Showing meiotic behaviour, seeds set and types of single plant progenies in the hybrids between Brassica campestris sub.sp. oleifera var. toria × Brassica nigra and B. c. sub.sp. japonica × B. nigra*

Hybrids	Meiotic behaviour at M I	Hybrid no.	No. of seeds set	No. of normal amphidiploids	No. of homozygous homoeologous recombinants with their characteristics	
<i>B.c. sub.sp. oleifera var. toria × B. nigra</i>	18 I-8 II 2 I	TN 1	37	29	0	—
		TN 2	9	9	0	—
		TN 3	22	19	0	—
		TN 4	18	8	1	Radical leaves obovate, almost wingless with curled margins, thick and crisp (Fig. 1)
		TN 5	31	27	2	(a) Plant very tall with a large number of primary and secondary branches and very high seed yielder. (b) Radical leaves lyrate pinnatisect divided into a number of smaller lobes. Upper lobes strongly curled (Fig. 2)
		TN 6	29	14	0	—
		TN 7	4	4	0	—
		TN 8	7	6	1	Radical leaves lyrate pinnatisect with strongly curled margins (Fig. 3)
		TN 9	25	11	0	—
		TN 10	34	27	1	Plant bushy with radical leaves very large and very high yielding.
<i>B.c. sub.sp. japonica × B. nigra</i>	18 I-6 II 6 I	JN 1	13	9	0	—
		JN 2	74	13	0	—
		JN 3	34	31	0	—
		JN 4	19	6	1	Plant bushy with profuse vegetative growth
		JN 5	27	24	0	—
		JN 6	69	63	1	Radical leaves large, obovate with a white flattened midrib and short stout and strongly grooved petiole (Fig. 4)
		JN 7	3	0	0	—
		JN 8	53	51	0	—
		JN 9	17	16	0	—
		JN 10	61	61	0	—

gives no indication whether the structurally heterozygous chromosomes belong to the same or different genomes. The absence of any quadrivalents in the triploid hybrid demonstrated that translocation could not have originated by autosyndetic recombination. On the other hand the presence of trivalents in these

Table 2. *Frequency of different configurations at first metaphase in the 36-chromosome hybrid between non-homologous homozygous recombinant and normal homozygous amphidiploid of Brassica juncea*

Configurations	Number of cells
2 IV + 14 II	32
1 IV + 16 II	45
18 II	23
Total	100

Table 3. *Frequency of different configurations at first metaphase in the 28-chromosome hybrid between 36-chromosome non-homologous homozygous recombinant of Brassica juncea and 20-chromosome B. campestris*

Configurations	Number of cells
2 III + 8 II + 6 I	37
1 III + 9 II + 7 I	41
10 II + 8 I	22
Total	100

hybrids shows that the translocation might have derived by homoeologous recombination between the chromosomes of the two genomes.

The mechanism in the production of 36-chromosome plants from 18-chromosome hybrids involves the formation of unreduced gametes through the process of meiotic restitution following failure of the first division and their subsequent doubling. During metaphase I in some of the cells of 18-chromosome hybrids, univalents move away from the polar regions and accumulate at the equatorial plate. It has been observed that in the cells undergoing restitution, metaphase I is generally prolonged. At anaphase I, the bivalents separate and move towards opposite poles while univalents remain at the plate instead of dividing in a mitotic fashion. At this point, there is a deviation in the behaviour of chromosomes, from the one normally occurring in hybrids with meiotic irregularities and the restitution sets in. Chromosomes become diffuse and a new nuclear membrane forms. Second division occurs very regularly resulting in the formation of unreduced egg cells which on doubling give rise to $2n$ eggs and ultimately develop into $2n$ sporophytes. Such a mechanism of chromosome doubling has been termed Pseudo-diploid parthenogenesis by Nei (1963) and has been quoted by him as occurring in *Brassica*. It has also been observed that high bivalent frequency is not conducive to restitution because of the greater dispersion of chromosomes away from the plate which leads to non-inclusion in the restitution nuclei. It appears that cells with only one or two bivalent, undergo restitution which can be confirmed from the actual recovery of a maximum of two quadrivalents and two trivalents in the homozygous allosyndetic recombinant \times homozygous amphidiploid and homozygous allosyndetic recombinant \times *B. campestris* hybrids respectively.

As has earlier been pointed out, pairing and crossing over between non-homologously paired *campestris* and *nigra* chromosomes, would constitute a reciprocal

translocation. The formation of a single restitution nucleus at the end of first division in the 18-chromosome F_1 hybrids will have two translocated and 16 normal chromosomes. The occurrence of regular second division will give, due to random segregation of four chromatids of the bivalents, one-fourth of the gametes which would have two interchanged chromosomes, another one-fourth would be normal and the rest or one-half of the total eggs would contain one normal and one interchanged chromosome. The egg cells of the last group will give homozygous duplication-deficiency types on chromosome doubling, and may be the source of morphological and physiological variability in the present investigation put under class 2 and listed in Table 1. When one of these morphological deviants was crossed with *B. juncea* and *B. campestris* having standard karyotypes, two chains of four chromosomes were recovered in the first hybrid and two trivalents in the second hybrid. This is consistent with the presence of duplication-deficiency interchange types. The egg cells of the first type will give amphidiploids homozygous for a segmental exchange between *campestris* and *nigra* chromosomes and were not distinguishable from normal plants.

The non-homologous recombination between the *campestris* and *nigra* chromosomes, besides releasing morphological variability, also yielded some very high yielding physiological variants (Table 1). Consequently the phenomenon may have value in the genetical improvement of *B. juncea*.

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