Theor. Appl. Genet. 59, 49-52 (1981)

Chromosome Pairing in Haploids of *Brassica campestris*

K.C. Armstrong and W.A. Keller
Ottawa Research Station, Research Branch, Agriculture Canada, Ottawa, Ontario (Canada)

Summary. The maximum chromosome pairing observed in haploids of *Brassica campestris* was two bivalents plus one trivalent but differences were observed in the chromosome pairing frequencies of the four haploids studied. This pairing supports the theorem that the species is hexasomic for one chromosome, tetrasomic for two and disomic for three others but it is emphasized that some of the observed pairing might be explained by a phenomenon other than homology.

Key words: *Brassica* – Haploids – Meiosis

Introduction

The *Brassica* spp., *B. nigra* Koch (2n = 16), *B. oleracea* L. (2n = 18) and *B. campestris* L. (2n = 20) are believed to be secondary polyploids which have evolved from a common genome with X = 6. The mitotic (Richharia 1937) and pachytene karyotypes (Röbbelen 1960; Venkateswarlu and Kamala 1971) suggested that *B. nigra* was tetrasomic for two and *B. oleracea* tetrasomic for three linkage groups, while *B. campestris* was postulated to be tetrasomic for two linkage groups and hexasomic for one. The possible mechanisms involved include autopolyploidy followed by subsequent loss of selected chromosomes and differentiation of the remaining ones, or chromosome loss from an alloploid (Sikka 1940; Venkateswarlu and Kamala 1971).

Secondary pairing at metaphase I and II of meiosis tends to support the secondary polyploid theorem (Catcheside 1934, 1937). Chromosome pairing in haploids might also reflect this evolutionary derivation. In *B. nigra* (2n = 16, genomes *BB*) the theoretical maximum of two bivalents was found (Prakash 1973); in *B. oleracea* var. 'acephala' D.C. (2n = 18, genomes *CC*) a maximum of two bivalents occurred where three could have been expected (Thompson 1956); in *B. tournefortii* Gouan (2n = 20, genomes *DD*) the theoretical maximum of one trivalent plus two bivalents was observed (Prakash 1974) but in *B. campestris* (2n = 20, genomes *AA*) a maximum of only one bivalent (Ramanujam 1941) was detected. Anther culture of *B. campestris* has been successful in producing a high frequency of haploids (Keller and Armstrong 1979). Chromosome pairing has been studied in several of these to determine if the theoretical maximum pairing of two bivalents plus one trivalent could be found.

Materials and Methods

Haploids of *B. campestris* were produced from anther culture of Torch × R500 F_1 plants by the techniques previously outlined (Keller and Armstrong 1979). Flower buds were fixed in 6:3:1, containing ferric chloride as a mordant, and anthers were squashed in 1% aceto-orcein. In general, only chromosomal configurations which became oriented on a metaphase plate were scored. Therefore the majority of cells scored were in late metaphase to early anaphase (meta-anaphase). End-to-end or side-to-side associations were not scored although the distinction between these and true chiasmate associations was difficult in some cases because of the small chromosome size.

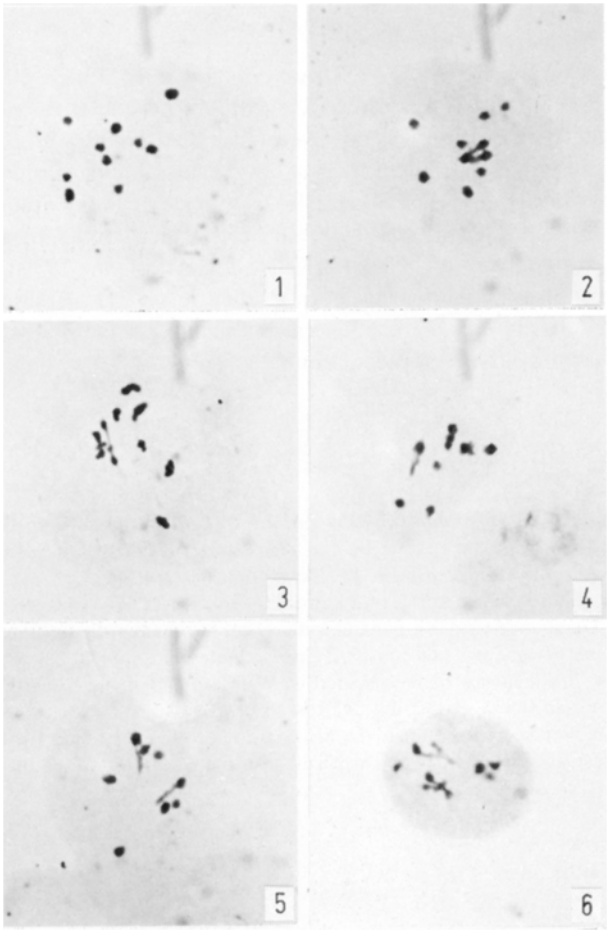
Results

Chromosome pairing was studied in four haploids derived from Torch × R500 F_1 plants. The total number of PMCs studied varied from 211 to 482 per plant (Table 1). Trivalents and bivalents were found in all four haploids (Figs. 2-5), and no configurations other than univalents, bivalents and trivalents occurred. The maximum amount of pairing observed in an individual cell was one trivalent plus two bivalents but this was found only in haploid 914-9A-26. This haploid had a higher frequency of chromosome pairing than the other three haploids which was illustrated by the percentage of cells containing the vari-

0040-5752/81/0059/0049/$ 1.00

Table 1. Number and percentage of PMCs with various pairing configurations in four haploids (x = 10) of *Brassica campestris*

Configuration	*B. campestris* haploid			
	900-23	900-14	825-5	914-9A-26
10 I	171(81.04)	238(72.12)	321(66.60)	218(54.22)
8 I + 1 II	34(16.11)	70(21.21)	127(26.35)	134(33.33)
6 I + 2 II	3(1.42)	14(4.24)	27(4.24)	27(6.78)
4 I + 3 II	0(0.00)	0(0.00)	1(0.21)	5(1.24)
7 I + 1 III	3(1.42)	7(2.12)	4(0.83)	13(3.23)
5 I + 1 II + 1 III	0(0.00)	1(0.30)	2(0.41)	3(0.75)
3 I + 2 II + 1 III	0(0.00)	0(0.00)	0(0.00)	2(0.50)
Total PMCs	211	330	482	402



Figs. 1-6. Meta-anaphase in haploids of *B. campestris*. 1 10I; 2 7I + 1 III; 3 6I + 2 II; 4 4I + 3 II; 5 5I + 1 II + 1 III; 6 4I + 2 III

ous associations (Table 1) and the mean frequency of various configurations per cell (Table 2). Similarly, only two plants contained cells with three bivalents and these were also the two plants with the highest frequency of pairing.

No attempt was made to determine if the difference in chromosome pairing among the haploids was a result of genetic variation. This would not be surprising since the maternal parent (Torch) used in producing the F_1 hybrids (Torch × R500) is a self-incompatible cultivar and the four haploids were not from the same donor plant. Since anther culture allows the production of large numbers of haploids from *B. campestris* it would be possible to study this in more detail.

Discussion

The pairing frequency found in *B. campestris* supports the theorem that *B. campestris* is hexasomic for one chromosome and tetrasomic for two others (Catcheside 1934, 1937; Richharia 1937; Röbbelen 1960; Venkateswarlu and Kamala 1971). More precisely, it would suggest that there are three homoeologues of one chromosome, two homoeologues of two, and one homoeologue of each of the remaining three. The homoeology of the triplicated and duplicated chromosomes is then expressed as a low degree of pairing in the haploids. It might be suggested that the bivalent and trivalents resulted from interchanges arising on the anther culture or regeneration medium. However, in haploid 900-14, 49 diploid sporocytes were observed as well as the 330 haploid sporocytes. Chromosome pairing in these was strictly as bivalent which argues against interchanges of substantial size. However, is it possible that other haploids with a higher frequency of

Table 2. Mean frequency of various chromosome configurations at metaphase I in four haploids of *Brassica campestris*

Haploid	II	III	No. of cells
900-23	0.19	0.01	211
900-14	0.31	0.02	330
825-5	0.36	0.01	482
914-9A-26	0.52	0.04	402

pairing could be found and that they would contain a frequency of cells with chromosome pairing not conforming to the hypothesis (e.g. four bivalents or two trivalents)? In one haploid not studied in detail a cell which could be interpreted as containing two trivalents was found (Fig. 6).

Chromosome pairing is observed in presumed monoploids of many species (Sadasivaiah 1974) but its interpretation is controversial. Rieger (1957) suggested that all chromosomes have a tendency to pair and will pair with a non-homologous chromosome in the absence of a homologue or homoeologue. This possibility could be considered in monoploids of *Hordeum vulgare* where pachytene cells have been found with complete pairing (Sadasivaiah and Kasha 1971; Sadasivaiah 1974). In monoploids where this does not occur, genetic factors may limit or completely prevent pairing. It has also been suggested that recombination can occur between non-homologous chromosomes. Shaw and Wilkinson (1978) found a high frequency of bivalents of non-homologous chromosomes of the same genome in inter-racial hybrids of *Caledia captiva*. Since it would have been necessary to propose a very high frequency of duplicated segments or repeated sequences widely scattered over the genome to explain the frequency of pairing observed on the basis of homologies, they preferred to conclude that an unknown mechanism which normally prevents pairing and recombination between non-homologues had broken down in the hybrids.

The production of some bivalent or trivalent-like structures might occur without chromosome pairing and recombination if the proposed molecular model of terminal DNA synthesis is valid (Dancis and Holmquist 1977; Holmquist and Dancis 1979). The model proposed is that all telomeres are similar, they recombine before replication, and subsequently separate. A failure of the mechanism which cuts the telomere fusions after replication would result in the production of chromosomes fused end to end. Fusions would not have to be between homologues although the process might not be random if, as has been proposed, the chromosomes are organized in the nucleus in a particular spatial arrangement (Ashley 1979). In haploids which may be genetically unbalanced the mechanism may function irregularly producing true dicentrics and tricentrics, etc. which might behave like bivalents or trivalents at metaphase of meiosis. White (1961) has suggested that telomere base-sequence homology could cause occasional end-to-end pairing associations of meiotic chromosomes.

In a species such as *Brassica campestris* with very small chromosomes it would be difficult to identify specific chromosomes at meiotic metphase. However, for the meiotic pairing data to be considered valid it should be shown that the observed meiotic pairing is specific (non-random) and that it involves the chromosomes proposed to be homologues (homoeologues) by Röbbelen (1960) and Venkatswarlu and Kamala (1971) on the basis of pachytene morphology. Similarly, it should be shown that the same chromosomes are involved in secondary association. The meiotic univalents can roughly be divided into three size classes; three larger univalents, two intermediate size univalents and five small univalents (Fig. 1) although this is not possible in all cells. From the pairing observed in some cells it would appear that trivalent formation occurs among the three larger chromosomes (Fig. 2) and possibly a bivalent occurs between the two intermediate size chromosomes and two of the small chromosomes (Fig. 3). However, this may not always be the case since the bivalents and trivalents often appear to be assymetrical. The pachytene chromosomes have been described and defined as *AA B C DD E FFF* (Röbbelen 1960; Venkateswarlu and Kamala 1971). It is worth noting that the longest pachytene chromosome is *B*. The length ascribed to the others varies between the two authors but generally the *E* and one *D* chromosome are the next longest. The *F* chromosomes are intermediate to the shortest in length. While pachytene and somatic lengths may not be correlated one hundred percent, there may be a general correspondence which would on final analysis be determined in part by the amount of eu- and heterochromatin in the chromosome (Ramanna and Prakken 1967). The observation on size of meiotic chromosomes involved in the trivalent suggest that the *F* pachytene chromosomes are not involved in the trivalent formation.

Other approaches to study the evolutionary relationship of chromosomes should be taken. It would be of considerable value if it could be shown for example that some enzyme systems were under triplicate or duplicate gene control as has been found in allopolyloids like wheat (Hart 1979).

Literature

Ashley, T. (1979): Specific end-to-end attachment of chromosomes in *Ornithogalum virens*. J. Cell Sci. 38, 357-367

Catcheside, D.G. (1934): The chromosomal relationships in the swede and turnip groups of *Brassica*. Ann. Bot. 48, 601-633

Catcheside, D.G. (1937): Secondary pairing in *Brassica oleracea*. Cytologia, Fujii Jubilee pp. 366-378

Dancis, B.M.; Holmquist, G.P. (1977): Fusion model of telomere replication and its implications for chromosomal rearrangements. Chromosomes Today 6, 95-104

Hart, G.E. (1979): Evidence for a triplicate set of glucosephosphate isomerase structural genes in hexaploid wheat. Biochem. Genet. 17, 585-598

Holmquist, G.P.; Dancis, B. (1979): Telomere replication, kinetochore organizers, and satellite DNA evolution. Proc. Natl. Acad. Sci. (Wash.) 76, 4566-4570

Keller, W.A.; Armstrong, K.C. (1979): Stimulation of embryogenesis and haploid production in *Brassica campestris* anther cultures by elevated temperature treatments. Theor. Appl. Genet. 55, 65-67

Prakash, S. (1973): Haploidy in *Brassica nigra* Koch. Euphytica 22, 613-614

Prakash, S. (1974): Haploid meiosis and origin of *Brassica tournefortii* Gouan. Euphytica 23, 591-595

Ramanna, M.S.; Prakken, R. (1967): Structure of and homology between pachytene and somatic metaphase chromosomes of the tomato. Genetica 38, 115-133

Ramanujam, S. (1941): A haploid plant in Toria (*Brassica campestris* L.). Proc. Indian Acad. Sci. Sect. B. 14, 25-34

Rieger, R. (1957): Inhomologenpaarung und meioseablauf bei haploiden formen von *Antirrhinum majus* L. Chromosoma 9, 1-38

Richharia, R.H. (1937): Cytological investigation of *Raphanus sativus*, *Brassica oleracea* and their F_1 and F_2 hybrids. J. Genet. 34, 19-44

Röbbelen, G. (1960): Beiträge zur Analyse des *Brassica* – Genoms. Chromosoma 11, 205-228

Sadasivaiah, R.S. (1974): Haploids in genetic and cytological research. In: Haploids in Higher Plants – Advances and Potential. (ed.: Kasha, K.J.) pp. 355-386. Guelph, Ontario: Univ. Guelph Press

Sadasivaiah, R.S.; Kasha, K.J. (1971): Meiosis in haploid barley – An interpretation of non-homologous chromosome associations. Chromosoma 35, 247-263

Shaw, D.D.; Wilkinson, P. (1978): 'Homologies' between non-homologous chromosomes in the grasshopper *Caledia captiva*. Chromosoma 68, 241-259

Sikka, S.M. (1940): Cytogenetics of *Brassica* hybrids and species. J. Genet. 40, 441-509

Thompson, K.F. (1956): Production of haploid plants of marrow-stem kale. Nature 178, 748

Venkateswarlu, J.; Kamala, T. (1971): Pachytene chromosome complements and genome analysis in *Brassica*. J. Indian Bot. Soc. 50A, 442-449

White, M.J.D. (1961): The role of chromosomal translocations in urodele evolution and speciation in the light of work on grasshoppers. Am. Nat. 95, 315-321

Received September 4, 1980
Communicated by P. Maliga and G. Wenzel

Dr. K.C. Armstrong
Dr. W.A. Keller
Ottawa Research Station
Research Branch, Agriculture Canada
Ottawa, Ontario (Canada) K1A OC6