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Transfer of *Brassica tournefortii* (TT) genes to allotetraploid oilseed *Brassica* species (*B. juncea* AABB, *B. napus* AACC, *B. carinata* BBCC): homoeologous pairing is more pronounced in the three-genome hybrids (TACC, TBAA, TCAA, TCBB) as compared to allodiploids (TA, TB, TC)

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Abstract For the transfer of genes from *B. tournefortii* (TT) to the allotetraploid oilseed brassicas, *B. juncea* AABB, *B. carinata* BBCC and *B. napus* AACC, *B. tournefortii* was first crossed with the three basic diploid species, *B. campestris* (AA), *B. nigra* (BB) and *B. oleracea* (CC), to produce the allodiploids TA, TB and TC. These were tetraploidized by colchicine treatment to produce the allotetraploids TTAA, TTBB and TTCC, which were further crossed with *B. juncea* and *B. napus* to produce three-genome hybrids with substitution-type genomic configurations: TACC, TBAA and TCAA. These hybrids along with another hybrid TCBB produced earlier, the three allodiploids, their allotetraploids and the four diploid parent species were studied for their male meiotic behaviour. The diploid parent and the allotetraploids (TTAA, TTBB and TTCC) showed regular meiosis although the pollen viability was generally low in the allotetraploids. In the allodiploids (TA, TB and TC) only some end-to-end associations were observed without any clearly discernible chiasmata or exchange points. Chromosomes involved in end-to-end associations were randomly distributed at the metaphase/anaphase-I stages. In contrast, the three-genome hybrids (TACC, TBAA, TCAA and TCBB) showed normal bivalents whose number exceeded the expected bivalent values. Bivalents arising out of homoeologous pairing were indistinguishable from normal pairs by their disjunction pattern but could be distinguished on the basis of the heteromorphy of the homoeologous chromosomes. The three-genome hybrids could be backcrossed to allotetraploid oilseed brassicas as they had some fertility. In contrast, the allodiploids could neither be selfed nor backcrossed. On the basis of their meiotic stability, in terms of more pronounced homoeologous pairing and fertility for backcrossing, the three-genome configurations provide the best possible situation for the introgression of alien genes from the secondary gene pool to the allotetraploid oilseed crops *B. juncea*, *B. napus* and *B. carinata*.

Key words *Brassica* species · Allodiploids · Three-genome hybrids · Homoeologous pairing · Alien gene introgression

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Introduction

The wild relatives of crop plants often carry many useful agronomic traits for resistance to biotic and abiotic stresses. The utilization of this secondary gene pool is extremely important in crop improvement programmes (Rick et al. 1986; Jena and Khush 1990). Successful gene introgression from wild to cultivated crop species can be brought about by the production of hybrids with genomic configurations that encourage genetic exchange between homoeologous chromosomes by homoeologous pairing.

In general, a substitution-type of genomic configuration would be more conducive for alien gene introgression, as compared to chromosome addition-type configurations, because the absence of homologous chromosomes in the former condition would encourage homoeologous pairing. Based on the genomic relationship of six agronomically important *Brassica* species, *B. campestris* AA (n=10), *B. nigra* BB (n=8), *B. oleracea* CC (n=9), *B. juncea* AABB (n=18), *B. napus* AACC (n=19) and *B. carinata* BBCC (n=17), we earlier proposed a model for the development of substitution-type genomic configurations for the transfer of both nuclear and organellar genes from a wild species to allopolyploid crop brassicas using diploid species as bridging material (Mukhopadhyay et al. 1994). Thus, the transfer of genes from *B. tournefortii* TT to *B. carinata* BBCC, sexual hybrids were produced between

B. tournefortii and *B. oleracea* CC. Protoplasts of TC hybrids were fused with protoplasts of *B. nigra* BB to synthesize TCBB hybrids. In such hybrids one set of chromosomes of the constituent genome of *B. carinata* has been substituted by one set of chromosomes of the alien species, a situation which would be most conducive for an interaction between the homoeologous T and C chromosomes with the other constituent genome providing meiotic stability.

In the present study *B. tournefortii* TT was crossed with two other diploid species, *B. campestris* AA and *B. nigra* BB, to synthesize TA, TB hybrids. These were then used to produce hybrids with three-genome configurations, i.e. TBAA, TCAA and TACC, by sexual crosses (see Table 1). We have compared the meiotic stability and the extent of homoeologous pairing in the two-genome allodiploids (TA, TB and TC), the allotetraloids (TTAA, TTBB and TTCC) and the three-genome hybrids (TACC, TBAA, TCAA and TCBB).

Table 1 The genomic configurations produced and studied for meiotic stability and the extent of pairing. The different species used in the crosses are – *B. tournefortii* TT (2n=20), *B. campestris* AA (2n=20), *B. nigra* BB (2n=16), *B. oleracea* CC (2n=18), *B. juncea* AABB (2n=36) and *B. napus* AACC (2n=38)

Cross	Genome	2n	References
TT × AA	TA	20	Present study
TT × BB	TB	18	Present study
TT × CC	TC	19	Mukhopadhyay et al. (1994)
Doubling of TA	TTAA	40	Present study
Doubling of TB	TTBB	36	Present study
Doubling of TC	TTCC	38	Present study
TTAA × AABB	TBAA	38	Present study
TTAA × AACC	TCAA	39	Present study
TTCC × AACC	TACC	38	Present study
TC + BB	TCBB	35	Mukhopadhyay et al. (1994)

Materials and methods

Plant material

A list of the parental species and hybrids is given in Table 1. All the hybrids with the exception of TCBB were produced by sexual crosses. TCBB hybrids were produced by somatic cell hybridization (Mukhopadhyay et al. 1994).

Sequential ovary culture for the production of hybrids

B. tournefortii BEC-187 was crossed with *B. campestris* cv Pusa Kalyani, *B. nigra* IC-257 and *B. oleracea* cv Early kunwari, respectively. Ovaries were harvested 6, 10 and 16 days after pollination, sterilized in 0.02% mercuric chloride for 15 min followed by three washes in sterile distilled water, and then cultured on MS medium (Murashige and Skoog 1962) containing various combinations of α-napthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin (Kn), 6-benzylaminopurine (BAP), gibberellic acid (GA_3) and casein hydrolysate (CH). The best regime to recover hybrid embryos was when ovaries were cultured 10 days after pollination on MS medium with NAA (0.1 mg/l) and GA_3 (1.0 mg/l). Ovaries were dissected 15–18 days after culture and the developing ovules were inoculated on MS medium containing NAA (0.1 mg/l), Kn (1.0 mg/l) and GA_3 (1.0 mg/l). Embryos dissected from the ovules and transferred to basal MS medium after 10–14 days in culture developed shoots at very high frequencies. TA, TB and TC shoots were multiplied and maintained on MS medium containing Kn (0.05 mg/l), NAA (0.05 mg/l) and CH (50 mg/l) and subsequently rooted on MS with indole-butyric acid (IBA) (1.0 mg/l). In vitro tetraploidization on TA, TB and TC shoots was done according to Nanda Kumar et al. (1988).

Molecular confirmation of the interspecific hybrids

DNA was isolated from the leaves of field-grown TTAA, TTBB and TTCC plants following Dellaporta et al. (1984), and purified in a cesium chloride density gradient. For RFLP analysis the purified DNA samples were restricted with *Eco*RI and hybridized to a 18*S* rDNA sequence cloned from the wheat nuclear genome (Gerlach and Bedbrook 1979) following the method described by Pradhan et al. (1992). RAPD analysis of total DNA was done with four different oligonucleotide primers, OPD8, OPD10, OPD3 and OPD13 (Operon Technologies), following Quiros et al. (1991). For checking the nature of the organellar genomes in the hybrids, *Eco*RI-digested total DNA was probed with two chloroplast-specific probes, rbcL (Zurawski et al. 1981) and psbD (Alt et al. 1984).

Cytology and DNA estimation

Meiotic preparations were made from the anthers fixed in alcohol-chloroform-acetic acid (6:3:1) for at least 24 h and stained in 1% acetocarmine. Pollen viability was tested using fluorescein diacetate (Heslop-Harrison et al. 1984).

DNA measurements in the 2C nuclei were carried out as described previously (Raina and Rees 1983). About 35 2C nuclei were measured in three replicates in every species. In each case, root tips of *B. campestris*, 2C=1.64 pg (Verma and Rees 1974), processed along with the samples were used as a control to correct experimental errors as well as to convert extinction values in arbitrary units to absolute amounts (pg). Measurements were also made for ten 4C nuclei (nuclei entering, or at, early prophase) in each replicate to verify the estimated 2C DNA amounts.

Results

Fertility and seed set in the hybrids

All the three allodiploids (TA, TB and TC, Table 1) were completely male sterile. Although, occasional pod formation occurred on open pollination, no seed set was observed. These plants also did not set seed after and pollination with their respective crop parents. Therefore, these allodiploids could be maintained only by *in vitro* multiplication.

Hybrids TTAA, TTBB and TTCC (Table 1) produced functional pollen, but showed a variable number of anthers (3–6 per flower). However, the percentage of viable pollen varied from plant to plant within each type of hybrid. Pollen viability in TTAA and TTBB hybrids was as high as 76% and 93%, respectively. However, in TTCC hybrids the viability was low, around 10%. TTCC hybrids had mostly sterile flowers. Pod formation occurred in all the three allotetraploids both on selfing and cross pollination. However, seed set was generally low.

TTAA, TTBB and TTCC plants were characterised for their hybrid nature by RAPD analysis (Fig. 1). All the hybrids carried the specific bands characteristic of their respective parents. RFLP analysis with chloroplast DNA probes showed that all the hybrids had the organellar genome of the female parent *B. tournefortii*.

The three-genome hybrids (TACC, TBAA, TCAA and TCBB, Table 1) showed normal floral morphology but extremely poor pollen fertility. However, some seed set could be obtained by back crossing either by hand pollination or on open pollination. No seed set was obtained by selfing.

Determination of genome size

The 2C DNA content of the three diploid cultivated species ranged narrowly between 1.64 (*B. campestris*) and 1.76 pg (*B. oleracea*). Clear morphological and cytogenetical differences have been reported between *B. tournefortii* (2n=20) and *B. campestris* (2n=20) (Olsson 1954; Prakash and Narain 1971). *B. tournefortii* (1.12 pg) has about 47% less DNA than *B. campestris*.

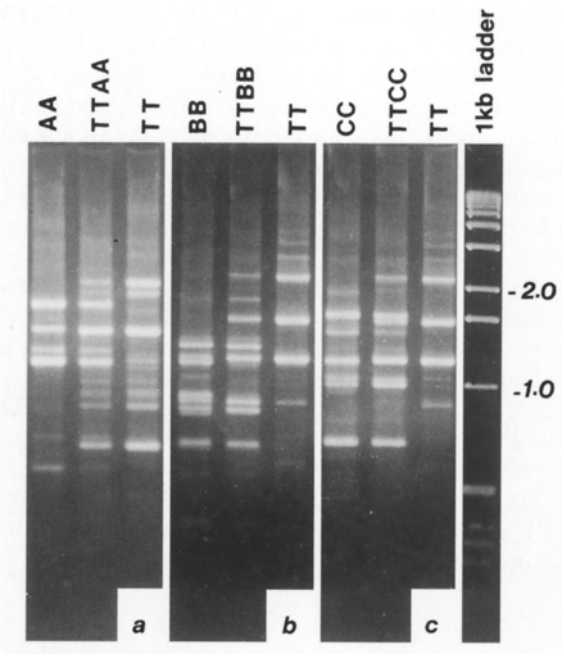

Fig. 1a–c RAPD analysis of three synthetic allotetraploids TTAA, TTBB and TTCC using different primers. **a** OPD8; **b, c** OPD13

Meiosis

In the diploid parents

All the PMCs analysed at metaphase-I in *B. nigra*, *B. oleracea*, *B. campestris* and *B. tournefortii* had a normal number of 8, 9, 10 and 10 bivalents (II), respectively. The distribution of chromosomes at anaphase-I was also normal resulting in high pollen fertility (90–98%).

In the allodiploids (TA, TB and TC)

The meiotic configurations of the allodiploid plants TA, TB and TC showed the presence of 20, 18 and 19 univalents (I), respectively at metaphase-I. No bivalents were observed at the prometaphase/metaphase-I stage. Chromosomes were not aligned at the equatorial plate, but were randomly distributed. Most of the univalents from *B. tournefortii* could be easily recognized by their considerably smaller size (Figs. 2a, b). Occasional secondary associations were observed. These secondary associations between the T-genome and the A/B/C-genome chromosomes could be identified due to heteromorphy of the T and A/B/C chromosomes. As the smaller univalents were more or less round, classification of associations into end-to-end, end-to-side and side-to-side was not possible for all the chromosomes. The average frequency per cell of these pairs of associations was estimated to be 1.9, 2.6 and 1.4 in TA, TB and TC hybrids, respectively. Secondary associations between more than two chromosomes (3–7) were also observed occasionally in the three allodiploids. These associations were always terminal and even at the diplotene/diakinesis stage no clearly observable exchange points were evident.

In the allotetraploids (TTAA, TTBB and TTCC)

The five plants analysed within each combination did not show much variation in their meiotic details. Most (91.1–99.8%) of the chromosomes in the three allotetraploids were paired as bivalents (Figs. 2e, f). Occasional univalents were present in a few cells.

In the three-genome hybrids (TACC, TBAA, TCAA and TCBB)

The trigenomic tetraploid hybrids TACC (TTCC×AACC), TBAA (TTAA×AABB) and TCAA (TTAA×AACC) showed the expected chromosome numbers of 38 (T10+A10+CC18), 38 (T10+B8+AA20) and 39 (T10+C9+AA20), respectively. In addition, all the six TCBB hybrids produced by a combination of sexual and somatic hybridization methods also showed a stable chromosome number of 35 (T10+C9+BB16) (see Tables 1, 2). In TACC, TBAA, TCAA and TCBB combinations, the average number of chiasmate bivalents per cell exceeded the expected values (9, 10, 10 and 8 II, respectively) by 2.00, 2.71, 1.95 and 1.44, respectively (Table 2). The appearance of some near-terminal associations suggested that the chiasmata (or chiasma) was in the process of terminalization, which is evidence that these were true bivalents and not quasibivalents. Chromosome heteromorphy in some of the bivalents also indicated pairing of T chromosomes with the chromosomes of the other haploid set present in the hybrids. All the bivalents were properly arranged at the equatorial plate and the disjunction pattern of the homoeolo-

Table 2 Mean number of associations per cell at metaphase-I in trigenomic tetraploid hybrids

Hybrid combination	Genomic constitution	2n	No. of cells analysed	Chromosome associations											
				IV		III		II				I			
								Ring		Rod		Total			
				Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
TTCC × *B. napus*	TACC	38	50			0.15 ± 0.36	0–1	6.55 ± 2.03	3–11	4.30 ± 2.12	1–8	11.0 ± 1.04	9–13	15.55 ± 2.59	12–18
TTAA × *B. juncea*	TBAA	38	50			0.24 ± 0.42	0–1	7.09 ± 2.30	3–11	5.48 ± 2.17	1–9	12.7 ± 2.05	10–15	11.85 ± 3.35	8–16
TTAA × *B. napus*	TCAA	39	50			0.15 ± 0.36	0–1	6.05 ± 2.29	1–11	5.90 ± 2.46	2–10	11.95 ± 1.43	9–14	14.65 ± 2.41	11–19
TC[a] + *B. nigra*	TCBB	35	50	0.01 ± 0.11	0–1	0.41 ± 0.47	0–2	5.59 ± 1.73	2–11	3.85 ± 1.56	0–8	9.44 ± 1.39	7–13	14.82 ± 2.51	9–21

[a] Somatic hybrids

Fig. 2a–f Metaphase-I stage of male meiosis in different hybrids. **a, b** Allodiploids (TA) showing 20 I; note secondary associations. **c, d** Trigenomic tetraploid (TCBB) showing 1 III+10 II+12 I (**c**) and 2 III+9 II+11 I (**d**). **e** Allotetraploid (TTAA) showing 20 II. **f** Allotetraploid (TTBB) showing 17 II+2 I. TT (*B. tournefortii*), AA (*B. campestris*), BB (*B. nigra*), CC (*B. oleracea*). Bar: 10 μm

gous pairs was indistinguishable from that of bivalents produced by the pairing of homologous chromosomes. Associations of more than two chromosomes were also observed in the three-genome hybrids leading to the formation of trivalents and quadrivalents in some of the PMCs (Figs. 2c, d).

Discussion

We had earlier proposed that for the transfer of genes from wild species to oilseed allotetraploid *Brassica* species, namely *B. juncea* AABB, a substitution-type genomic configuration, i.e. XABB or XBAA, would be the best possible situation for homoeologous pairing between the alien genome and one of the constituent genomes of the allotetraploid. All four different three-genome hybrids (TACC, TBAA, TCAA and TCBB), whether produced by sexual hybridization or by somatic cell fusion, had the anticipated

chromosome numbers. Numerous other studies on the chromosome numbers of somatic hybrids in the family Brassicaceae have reported instability in the chromosome numbers of the hybrids (Schenck and Röbbelen 1982; Terada et al. 1987; Chatterji et al. 1988; Jourdan et al. 1989; Fahleson et al. 1994). If the culture periods are kept short and efficient regeneration protocols are used, as was done in the case of the TCBB hybrids, it may be possible to avoid drift in the chromosome number of somatic hybrids.

There are two major prerequisites for the transfer of alien genes to a crop plant. The hybrids should have some fertility, so that they could be either selfed or backcrossed, and the genomic configuration should promote homoeologous pairing. The more pronounced these two factors are, the higher would be the chances of alien gene transfer. The allodiploid hybrids TA, TB and TC could not be crossed with either diploid or allotetraploid species. By contrast, the three-genome hybrids could be crossed with allotetraploids, and progeny plants could be recovered by embryo rescue. Meiotic studies of the allodiploids (TA, TB and TC) showed only some end-to-end associations amongst a few chromosomes. Such associations need not necessarily be due to homology (Orellana 1985). Our observations suggest that homoeologous exchange between the T and A/B/C genomes in TA, TB and TC hybrids was non-existent. No chiasmata could be observed in such end-to-end associations. The associations were always terminal and even at the diplotene/diakinesis stage no clearly observable exchange points were evident. The end-to-end associations could not be explained as products of early terminalization as the chromosomes in many cases were seen associated even at anaphase-I. Another notable feature of these associations was their total lack of congression at the equatorial region at metaphase-I. In many other systems end-to-end associations have also been found to be non-chiasmatic (Östergren and Vigfusson 1953; Slizynski 1964; Southern 1967; Sadasivaiah and Kasha 1971; Manga and Pantulu 1971; Sadasivaiah 1974; Ashley 1979; Driscoll et al. 1979; Ashley and Moses 1980; Orellana 1985). In some of these studies where specialised staining techniques were used to reveal the telomeric C bands, it was shown that most of the end-to-end associations between wheat and rye chromosomes were non-chiasmatic (Orellana 1985).

In comparison to the allodiploids, the three-genome hybrids showed a limited number of normal bivalents between homoeologous chromosomes. Such pairs were indistinguishable in their configurations from normal homologous pairs and showed proper orientation at the equatorial plate leading to their normal disjunction. It can be concluded that the three-genome configurations provide better scope for introgression of alien genes into one of the constituent genomes of allotetraploid oilseed *Brassica* species due to better fertility and more pronounced homoeologous pairing in comparison to the situation in allodiploids. Clearly, the presence of a complete diploid genome in addition to the presence of two divergent haploid genomes enhances homoeologous pairing between the chromosomes of the two haploid genomes.

In our earlier work (Pradhan et al. 1991) we had shown that alloplasmic lines of *B. napus* and *B. juncea* with *B. tournefortii* cytoplasm are male sterile. An important use of the synthesized three-genome hybrids will be to introgress the restorer gene(s) for "tour" CMS into *B. juncea* and other allotetraploid oilseed *Brassica* species. While success has been achieved in locating restorer functions amongst some *B. napus* lines for "tour" cytoplasm (Sodhi et al. 1994), no restorer genes have been identified in *B. juncea* despite extensive germ plasm screening. The transfer of the restorer gene(s) from *B. tournefortii* to *B. juncea*, therefore, assumes significance for developing a hybrid seed production capability in *B. juncea*. The three-genome hybrid TBAA reported in this study and a newly synthesized TABB hybrid (unpublished) are now being backcrossed to *B. juncea* for the transfer of the restorer gene(s) from *B. tournefortii* to *B. juncea*.

In general, due to their more pronounced fertility and chromosome pairing, the substitution-type three-genome configurations should provide ideal genomic configurations for alien gene transfer to allopolyploid oilseed *Brassica* species.

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References

Alt J, Morris J, Westhoff P, Hermann RG (1984) Nucleotide sequence of the clustered genes for the 44-Kda chlorophyll a apoprotein and the "32Kda"-like protein of the photosystem II reaction center in the spinach plastid chromosomes. Curr Genet 8:597–606

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

Ashley T, Moses MJ (1980) End associations and segregation of the achiasmatic X and Y chromosomes of the Sand Rat *Psammomys obesus*. Chromosoma 78:203–210

Chatterji G, Sikdar SR, Das S, Sen SK (1988) Intergeneric somatic hybrid production through protoplast fusion between *Brassica juncea* and *Diplotaxis muralis*. Theor Appl Genet 76:915–922

Dellaporta SL, Wood J, Hicks JB (1984) Maize DNA miniprep. In: Molecular biology of plants: a laboratory course manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 36–37

Driscoll DJ, Palmer CG, Melman A (1979) Non-homologous associations of C-heterochromatin at human male meiotic prophase. Cytogenet Cell Genet 23:23–32

Fahleson J, Eriksson I, Landgren M, Stymne S, Glimelius K (1994) Intertribal somatic hybrids between *Brassica napus* and *Thlaspi perfoliatum* with a high content of the *T. perfoliatum*-specific nervonic acid. Theor Appl Genet 87:795–804

Gerlach WL, Bedbrook JR (1979) Cloning anad characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res 7:1869–1885

Heslop-Harrison J, Heslop-Harrison Y, Shivanna KR (1984) The evaluation of pollen quality, and a further appraisal of the fluorochromatic (FCR) test procedure. Theor Appl Genet 67:367–375

Jena KK, Khush GS (1990) Introgression of genes from *Oryza officinalis* Well ex Watt to cultivated rice, *O. sativa* L. Theor Appl Genet 80:737–745

Jourdan PS, Earle ED, Mutschler MA (1989) Atrazine-resistant cauliflower obtained by somatic hybridization between *Brassica oleracea* and ATR-*B. napus*. Theor Appl Genet 78:271–279

Manga V, Pantulu JV (1971) The meiotic behaviour of a haploid pearl millet. Genetica 42:319–328

Mukhopadhyay A, Arumugam N, Pradhan AK, Murthy HN, Yadav BS, Sodhi YS, Pental D (1994) Somatic hybrids with a substitution-type genomic configuration TCBB for the transfer of nuclear and organelle genes from *Brassica tournefortii* TT to allotetraploid oilseed crop *Brassica carinata* BBCC. Theor Appl Genet 89:19–25

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473–497

Nanda Kumar PBA, Shivanna KR, Prakash S (1988) Wide hybridization in *Brassica*: crossability barriers and studies on the F_1 hybrid and synthetic amphidiploid of *B. fruticulosa*×*B. campestris*. Sex Plant Reprod 1:234–239

Olsson G (1954) Crosses within the campestris group of the genus *Brassica*. Hereditas 40:398–418

Orellana J (1985) Most of the homoeologous pairing at metaphase-I in wheat-rye hybrids is not chiasmatic. Genetics 111:917–931

Östergren G, Vigfusson E (1953) On position correlations of univalents and quasi-bivalents formed by sticky univalents. Hereditas 39:33–50

Pradhan AK, Mukhopadhyay A, Pental D (1991) Identification of the putative cytoplasmic donor of a CMS system in *Brassica juncea*. Plant Breed 106:204–208

Pradhan AK, Prakash S, Mukhopadhyay A, Pental D (1992) Phylogeny of *Brassica* and allied genera based on variation in chloroplast and mitochondrial DNA patterns; molecular and taxonomic classifications are incongruous. Theor Appl Genet 85:331–340

Prakash S, Narain A (1971) Genomic status of *Brassica tournefortii* Gouan. Theor Appl Genet 41:203–205

Quiros CF, Hu J, This P, Chevre AM, Delseny M (1991) Development and chromosomal localization of genome-specific markers by the polymerase chain reaction in *Brassica*. Theor Appl Genet 82:627–632

Raina SN, Rees H (1983) DNA variation between and within chromosome complements of *Vicia* species. Heredity 51:335–346

Rick CM, DeVerna JW, Chetelat RT, Stevens MA (1986) Meiosis in sesquidiploid hybrids of *Lycopersicum esculentum* and *Solanum lycopersicoides*. Proc Natl Acad Sci USA 83:3580–3583

Sadasivaiah RS (1974) Haploids in genetic and cytogenetical research In: Kasha KJ (ed) Haploids in higher plants: advances and potential. University Guelph Press, Guelph, pp 355–386

Sadasivaiah RS, Kasha KJ (1971) Meiosis in haploid barley – an interpretation of non-homologous chromosome associations. Chromosoma 35:247–263

Schenk HR, Röbbelen G (1982) Somatic hybrids by fusion of protoplasts from *Brassica oleracea* and *B. campestris*. Z Pflanzenzucht 89:278–288

Slizynski BM (1964) Chiasmata in spermatocytes of *Drosophila melanogaster*. Genet Res 5:80–84

Sodhi YS, Pradhan AK, Verma JK, Arumugam N, Mukhopadhyay A, Pental D (1994) Identification and inheritance of fertility restorer genes for 'tour' CMS in rapeseed (*Brassica napus* L.). Plant Breed 112:223–227

Southern DI (1967) Pseudomultiple formation as a consequence of prolonged non-homologous chromosome association in *Metrioptera brachyptera*. Chromosoma 21:272–284

Terada R, Yamashita Y, Nishibayashi S, Shimamato K (1987) Somatic hybrids between *Brassica oleracea* and *B. campestris*: selection by the use of iodoacetamide inactivation and regeneration ability. Theor Appl Genet 73:379–384

Verma SC, Rees H (1974) Nuclear DNA and evolution of allotetraploid Brassicae. Heredity 33:61–68

Zurawski G, Perrot B, Bottomley W, Whitfeld PR (1981) The structure of the gene for the large subunit of ribulose-1,5-biphosphate carboxylase from spinach chloroplast DNA. Nucleic Acids Res 9:3251–3270