Theor Appl Genet (1994) 89: 19–25

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Somatic hybrids with substitution type genomic configuration TCBB for the transfer of nuclear and organelle genes from *Brassica tournefortii* TT to allotetraploid oilseed crop *B. carinata* BBCC

Received: 22 December 1993 / Accepted: 12 January 1994

Abstract Oilseed crop *Brassica carinata* BBCC is a natural allotetraploid of diploid species *B. nigra* BB and *B. oleracea* CC. To transfer the nuclear and organelle genes in a concerted manner from an alien species, *B. tournefortii* TT, to *B. carinata*, we produced somatic hybrids with genomic configuration TCBB using *B. nigra* and *B. oleracea* stocks that carried selectable marker genes. *B. tournefortii* TT was sexually crossed with hygromycin-resistant *B. oleracea* CC. Protoplasts isolated from shoot cultures of hygromycin-resistant F_1 hybrids of *B. tournefortii*×*B. oleracea* TC were fused with protoplasts of kanamycin-resistant *B. nigra* BB. In two different fusion experiments 80 colonies were obtained through selection on media containing both hygromycin and kanamycin. Of these, 39 colonies regenerated into plants. Analysis of 15 regenerants by random amplified polymorphic DNA (RAPD) markers showed the presence of all three genomes, thereby confirming these to be true hybrids. Restriction fragment length polymorphism (RFLP) analysis of organelle genomes with heterologous chloroplast (cp)and mitochondrial (mt) DNA probes showed that the chloroplast genome was inherited from either of the two parents while mitochondrial genomes predominantly showed novel configurations due to either rearrangements or intergenomic recombinations. We anticipate that the TCBB genomic configuration will provide a more conducive situation for recombination between the T and C genomes during meiosis than the TTCCBB or TCCBB type configurations that are usually produced for alien gene transfer. The agronomic aim of producing TCBB hybrids is to transfer mitochondrial genes conferring cytoplasmic male sterility and nuclear genes for fertility restoration from *B. tournefortii* to *B. carinata*.

Communicated by G. S. Khush

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Key words *Brassica carinata* · *Brassica tournefortii* Cytoplasmic male sterility · Three genome somatic hybrids

Introduction

The three allotetraploid oilseed *Brassica* species namely, *B. juncea* AABB (Indian mustard), *B. napus* AACC (rapeseed) and *B. carinata* BBCC (Ethiopian mustard) are natural allopolyploids of three diploid species namely, *B. campestris* AA, *B. nigra* BB and *B. oleracea* CC. The cytogenetical relationship of these species was first described on the basis of chromosome pairing (Morinaga 1934; U 1935) and was later confirmed by restriction fragment length polymorphism (RFLP) analysis (Song et al. 1988; Hosaka et al. 1990). This unique genomic relationship has been utilised to synthesize allotetraploids from the diploid species to enlarge the gene pool for further breeding (Olsson 1960; Prakash 1973). The close genomic relationship of diploid and allotetraploid species could also be exploited for the transfer of alien genes to allotetraploid Brassicas using diploid species as bridging material.

We propose a model in which diploid species are used to develop a cytogenetic configuration in which one set of chromosomes of one of the constitutive genomes of the allotetraploid species is replaced by one set of chromosomes of the alien species. The generation of such a configuration, i.e. TCBB, is illustrated in Fig. 1: one set of the C genome of *B. carinata* BBCC is replaced by the T genome of alien species *B. tournefortii* TT. Compared to TTBBCC- or TBBCC-type configurations that have been generated in *Brassica* species by sexual (McGrath and Quiros 1990; Struss et al. 1991; Chevre et al. 1991) or somatic cell hybridisation (Sjodin and Glimelius 1989; Primard et al. 1988; Glimelius et al. 1991; Kirti et al. 1992), the TCBB (or TBCC) type configuration would provide a far more conducive situation for interaction between the alien genome (T) and one of the constitutive genomes of *B. carinata* (C or B) when such plants go through the meiotic phase.

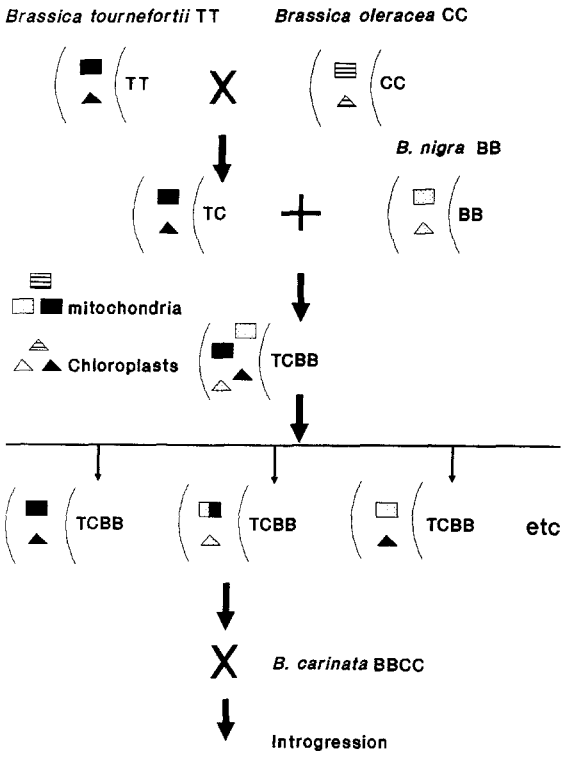

Fig. 1 A model for the transfer of nuclear and organelle genes from an alien species to allotetraploid *Brassica* species through sexual and somatic cell hybridization. The model is illustrated here using *B. tournefortii* TT as the alien parent and *B. carinata* BBCC as the recipient species. *B. tournefortii* TT is sexually crossed with *B. oleracea* CC to produce TC hybrids. Protoplasts from TC plants are then fused with protoplasts of *B. nigra* BB to produce TCBB hybrids

Further, in the TCBB hybrids generated by somatic cell hybridisation, additional variability would be generated through mitochondrial recombination and organelle substitution (Fig. 1). A generalised scheme for the *in vitro* selection of such three-genome hybrids has been developed by the introduction of unique selectable marker genes into the three diploid species: *hpt* (encoding hygromycin phosphotransferase for resistance to hygromycin), and *bar* (encoding phosphinotricin acetyl transferase conferring resistance to herbicide phosphinotricin) in *B. oleracea* (Mukhopadhyay et al. 1991), *npt* (encoding neomycin phosphotransferase conferring resistance to kanamycin) and *hpt* in *B. campestris* (Mukhopadhyay et al. 1992; unpublished); *npt* in *B. nigra* (Gupta et al. 1993).

In this paper we report the production of TCBB hybrids through the use of diploid stocks carrying selectable marker genes, *B. oleracea* with the *hpt* gene and *B. nigra* with the *npt* gene. TCBB hybrids have been analysed for their nuclear and organelle genome composition by random amplified polymorphic DNA (RAPD) and RFLP analysis. Alloplasmic lines of *B. juncea* and *B. napus* with *B. tournefortii* cytoplasm are male sterile (Pradhan et al. 1991). The agronomic aim in producing TCBB hybrids is to transfer mitochondrial genes conferring the trait of cytoplasmic male sterility and nuclear genes involved with fertility restoration from *B. tournefortii* TT to *B. carinata* BBCC, the latter a drought-tolerant crop that holds considerable promise for increasing oilseed production in rainfed agriculture areas of the Indian subcontinent and parts of Africa.

Materials and methods

Plant material

Brassica tournefortii accession BEC187 TT (2n=20) plants were sexually crossed to hygromycin-resistant (Hm^+) *B. oleracea* cv 'Early Kunwari' CC (2n=18) (Mukhopadhyay et al. 1991). The developing siliqua were collected 10 days after pollination, surface sterilized in 0.2% w/v mercuric chloride and cultured on ER medium [MS (Murashige and Skoog 1962) with 1.0 mg/l NAA (α-naphthalene acetic acid), 1.0 mg/l Kn (kinetin), 1.0 mg/l GA_3 (gibberellic acid)]. Seeds were dissected from the siliqua after 3–4 weeks of growth *in vitro* and germinated on MS medium. Seedlings were screened for resistance to the antibiotic by re-rooting on RI medium [MS with 0.1 mg/l IBA (indole-3-butyric acid)] containing 30 mg/l hygromycin]. Shoots of TC plants that rooted on 30 mg/l hygromycin were also tested for resistance to the antibiotic by culturing the leaf discs on CI medium [MS with 1.0 mg/ l 2, 4-D (2,4-dichlorophenoxyacetic acid), 1.0 mg/l Kn] with 30 mg/l hygromycin. The TC Hm^+ plants were micropropagated through nodal cuttings on SM medium (MS with 0.005 mg/l NAA, 0.05 mg/l Kn, 50 mg/l casein hydrolysate). *B. nigra* cv 'IC257' BB (2n=16) plants resistant to kanamycin (Km^+) (Gupta et al. 1993) were maintained by backcrossing. The seeds were surface sterilized, germinated *in vitro*, tested for resistance to 50 mg/l kanamycin on RI and CI media and subsequently multiplied on SM medium. Sensitivity assays of TC Hm^+ and BB Km^+ shoots on RI and CI media showed these to be susceptible to 30 mg/l kanamycin and 10 mg/l hygromycin, respectively.

Isolation, fusion and culture of protoplasts

Shoots of TC Hm^+ and BB Km^+ plants were maintained on SM medium and transferred to RI medium 15 days prior to the isolation of protoplasts. Protoplasts were isolated from leaf petioles and soft internodes as described earlier (Mukhopadhyay et al. 1991) except that the concentrations of cellulase R-10 and Macerozyme R-10 (Yakult, Honsha Co, Japan) were reduced from 1.0% to 0.5% w/v.

Protoplast fusions were carried out by a high pH/Ca^{++} treatment of protoplasts following the method of Keller and Melchers (1973) with some modifications. A mixture of TC Hm^+ and BB Km^+ protoplasts was suspended in 0.5 ml CPW medium (Frearson et al. 1973) with 9% mannitol (CPW9M), 3% sucrose, 1.0 mg/l Kn and 1.0 mg/l 2,4-D. Four milliliters of fusogen (0.05 M glycine-NaOH buffer, 1.1% $CaCl_2.6H_2O$ and 9% mannitol, pH 10.4) was gently added to the protoplast mixture, which was subsequently incubated at 45 °C for 10 min. Following incubation, the protoplasts were pelleted by centrifugation at 500 *g* for 5 min and resuspended and washed in CPW9 M with 0.74% $CaCl_2.6H_2O$. This post-incubation centrifugation step was found to be essential for the recovery of hybrid colonies. Both fusogen-treated and untreated protoplasts were plated [3.5 cm Nunc petri-dishes (Nunclon, Roskilde, Denmark), 5 ml medium in each dish] at a density of $4–5\times10^4$/ml in PC1 medium [-Kao's basal medium (Glimelius 1984) with 0.5 M glucose, 1.0 mg/l 2,4-D, 1.0 mg/l Kn]. After 10 days of culture the medium was diluted with PC2 medium (PC1 medium modified by replacing 0.5 M glucose with 0.1 M sucrose).

Selection of hybrid colonies, regeneration and establishment of plants

A two-step selection procedure was followed. In the first step, microcolonies grown on PC2 medium for 3 weeks were plated on SL1 medium [K_3 medium (Nagy and Maliga 1976) with 1.0 mg/l 2,4-D, 1.0 mg/l Kn], supplemented with 15 mg/l hygromycin, 30 mg/l ka-

namycin and solidified with 0.25% low melting agarose (Seakem, Rockland, USA). In the second step, the colonies growing on this medium for 3–4 weeks were picked individually and transferred to SL2 medium (MS with 1.0 mg/l 2,4-D and 1.0 mg/l Kn) supplemented with 30 mg/l hygromycin and 50 mg/l kanamycin.

For each fusion experiment the following treatments were applied as controls: (1) TC Hm^+ on hygromycin, (2) BB Km^+ on kanamycin, (3) TC Hm^+ on kanamycin, (4) BB Km^+ on hygromycin and (5) a physical mixture of TC Hm^+ and BB Km^+ protoplasts on medium containing both antibiotics. The selected colonies were grown on SL2 medium (four subcultures, each with a duration of 15–20 days) before transfer to the SR medium (MS with 2.5 mg/l BAP, 0.5 mg/l NAA, 70 μm silver nitrate) for shoot regeneration.

The regenerated shoots were numbered to denote the petri-dish, the colony and the shoot number in a sequential manner, e.g. in the shoot designated as 17.6.1, 17 – denotes the plate number, 6 – denotes the colony number and 1 – denotes the first shoot regenerated from this colony. Regenerated shoots were multiplied and maintained on SM medium, rooted on RI medium with 30 mg/l hygromycin and 50 mg/l kanamycin and subsequently transferred to the field.

Characterization of somatic hybrids

Total DNA was isolated from the parents and 15 independently regenerated hybrids following the protocol of Dellaporta et al. (1983) and subsequently purified on CsCl gradients. To determine the polymorphism among parental types, DNAs isolated from *B. oleracea*, *B. nigra*, *B. tournefortii* and the *B. tournefortii*×*B. oleracea* hybrid were tested by RAPD analysis using 60 10-mer primers (kit B to E, Operon Technologies, Alameda, Calif., USA). DNA amplification was performed using Taq DNA polymerase (Perkin-Elmer/Cetus) in a thermal cycler for 45 cycles following conditions reported earlier (Quiros et al. 1991) except that in the first cycle denaturation was for 2 min at 92 °C. Amplified products were electrophoresed at 3V/cm on a 1.8% agarose gel and detected and analysed by staining with ethidium bromide. Amplification with each primer was repeated at least twice to ascertain the reproducibility of the profiles.

Total DNA isolated from 10 out of the 15 somatic hybrids was used for analysing chloroplast and mitochondrial genomes. For the chloroplast genome, total DNAs were digested with *Eco*RI, electrophoresed and transferred to nylon membranes (Hybond-N, Amersham, UK) and hybridised with two heterologous probes of chloroplast origin, namely *rbc* L (Zurawski et al. 1981) and *psb* D (Alt et al. 1984) following the method of Pradhan et al. (1992). Chloroplast (cp) DNAs were also isolated from the parents and 4 selected hybrids, 19.13.36, 17.19.1, 17.6.1 and 23.1.1 following Kemble (1987), digested with *Bam*HI and *Hin*dIII and electrophoresed on 0.8% agarose gels.

For mitochondrial genome analysis, total DNAs digested with *Eco*RI and *Hin*dIII were electrophoresed on 0.8% agarose gel, transferred to nylon membranes, and hybridised with 7 overlapping cosmid clones covering about 162 kb of the mitochondrial (mt) DNA of *B. oxyrrhina* (a detailed map of these clones will be published elsewhere). These clones hereafter are referred to as pCos 13, 17, 24, 61, 88, 131 and 175 and have mtDNA inserts of 33.9, 41.8, 36.6, 32.7, 32.4, 34.8 and 31.7 kb, respectively.

Results

Protoplast fusion, recovery of hybrid colonies and plant regeneration

Protoplasts of the two parents, i.e. TC Hm^+ and BB Km^+, showed colony growth only on the permissive media. In cultures with a physical mixture of the parental protoplasts, a few escapes were observed on SL1 medium supplemented with 15 mg/l hygromycin and 30 mg/l kanamycin, but all such colonies died upon transfer to SL2 medium containing 30 mg/l hygromycin and 50 mg/l kanamycin. Hence, the two step selection eliminated any colony growth in cultures with a mixed population of protoplasts of TC Hm^+ and BB Km^+ parents. From two different fusion experiments, 80 colonies were obtained, and these were carried through to plant regeneration stage (Table 1).

Table 1 Fusion of TC Hm^+ and BB Km^+ protoplasts and recovery of hybrids

Exp. no.	Number of protoplasts used for fusion		Number of hybrid colonies	Number of colonies regenerating shoots
	TC Hm^+	BB Km^+		
1	0.6×10^6	1.2×10^6	52	28
2	1.0×10^6	1.5×10^6	28	11

In preliminary experiments it was observed that no shoot morphogenesis occurred on SR medium without silver nitrate. In the presence of silver nitrate the regeneration frequency increased from 0% to 46%. Several shoots were obtained from each colony. Regenerated shoots, in general, were green and showed vigorous growth *in vitro* and in soil. However, 3 colonies produced only purple shoots, and 2 differentiated both green and purple shoots. All of the shoots were maintained on SM medium and rooted on RI medium containing the antibiotics. A total of 44 green plants, obtained from 5 colonies of independent origin, were transferred to soil during the growing season of 1992–1993. Of these, hybrids 19.13.18, 19.13.19, and 19.13.24 were successfully backcrossed to *B. carinata* accession HC-25. Viable seeds obtained through ovary culture and embryo rescue on ER medium were germinated to produce the BC_1 progeny. Due to the late transplantation of the hybrids and consequent non-availability of the pollinator parent, the other hybrids could not be backcrossed.

Molecular characterization of the nuclear genome

In RAPD analysis, polymorphism amongst the parental lines was detected with all of the 60 primers that were tested. Twelve primers that showed distinct polymorphism among the parents, OPB8, OPB10, OPB11, OPB12, OPD1, OPD2, OPD3, OPD13, OPE1, OPE5, OPE9 and OPE20, were selected to confirm the genetic contribution of the three genomes to the somatic hybrids. Most of the hybrids showed bands characteristic of all three parental genomes (Fig. 2a) confirming the hybrid nature of the analysed plants. However, some of the major amplified products were found to be missing in a few hybrids (Fig. 2b).

In order to determine the stability of TCBB hybrids, a total of 111 polymorphic bands were scored among the three parents from the amplified products using the 12 primers mentioned above. Out of these, 36 belonged to the *B. oleracea* parent 32 to *B. tournefortii* and 43 to *B. nigra*.

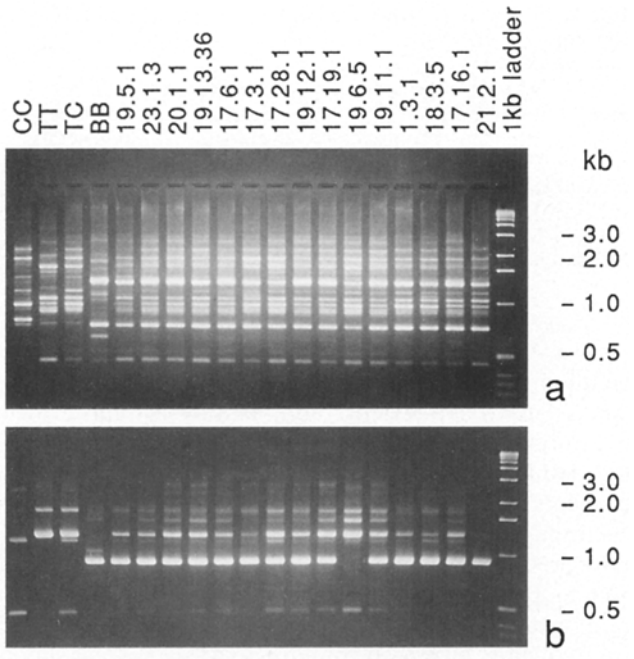

Fig. 2a, b RAPD analysis of parents (CC *B. oleracea*, TT *B. tournefortii*, TC F_1 of *B. tournefortii*×*B. oleracea*, BB *B. nigra*) and 15 TCBB somatic hybrids using 10-mer oligonucleotides. **a** With primer OPD3 all of the hybrids show all of the bands that are specific to the three genomes; **b** with primer OPB8, hybrid 19.6.5 shows the absence of a 0.9-kb band that is specific to *B. nigra*

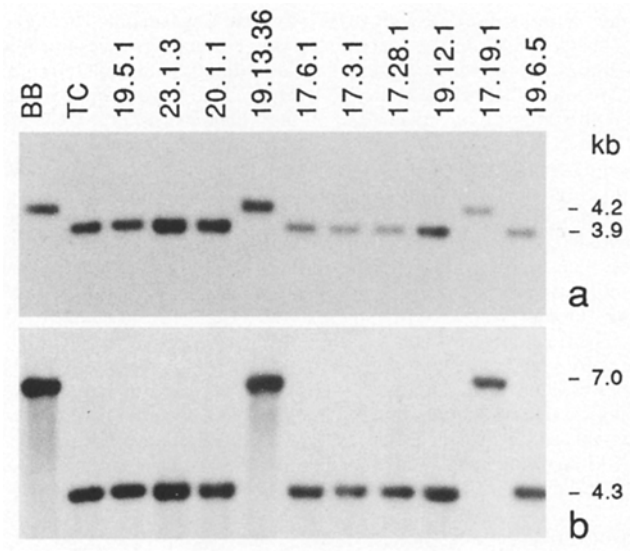

Fig. 3 Southern hybridization of *Eco*RI digested total DNAs of the two parents (BB *B. nigra*, TC F_1 of *B. tournefortii*×*B. oleracea*) and 10 TCBB somatic hybrids to radioactively labelled cpDNA probes *rbc* L **a** and *psb* D **b**. Hybrids 19.13.36 and 17.19.1 show 4.2-kb and 7.0-kb *B. nigra* BB-specific bands, whereas all the other hybrids have 3.9-kb and 4.3-kb TC-specific bands (TC plants have organelle genomes of *B. tournefortii)* when hybridized to *rbc* L and *psb* D probes, respectively

Table 2 Genomic constitution of 15 TCBB somatic hybrids based on RAPD analysis with 12 different primers

	Polymorphic bands			
	C	T	B	Total
Parents				
B. oleracea	36	–	–	
B. nigra	–	–	43	111
B. tournefortii	–	32	–	
Hybrids				
19.5.1	36	32	43	111
23.1.3	34	32	43	109
20.1.1	35	32	43	110
19.13.36	34	32	43	109
17.6.1	35	32	43	110
17.3.1	36	32	43	111
17.28.1	36	32	43	111
19.12.1	33	32	43	108
17.19.1	36	32	43	111
19.6.5	36	32	37	105
19.11.1	36	32	43	111
1.3.1	36	32	43	111
18.3.5	36	32	43	111
17.16.1	36	32	43	111
21.2.1	36	32	43	111

Out of the 15 hybrids 9 had all 111 parental polymorphic bands (Table 2). Of the remaining 6 hybrids, 5 had lost a few *B. oleracea*-specific bands and 1 had lost *B. nigra* specific bands; none of the hybrids showed any loss of *B. tournefortii* specific bands (Table 2).

Table 3 Organelle composition of 10 TCBB somatic hybrids (T *B. tournefortii* type; B *B. nigra* type, B^R rearranged mitochondrial genome with predominance of *B. nigra*-type pattern; T^R rearranged mitochondrial genome with predominance of *B. tournefortii*-type pattern)

Hybrids	Organelle genome	
	Chloroplast	Mitochondria
19.5.1	T	T^R
23.1.3	T	B^R
20.1.1	T	T^R
19.13.36	B	T^R
17.6.1	T	T^R
17.3.1	T	T^R
17.28.1	T	T
19.12.1	T	T
17.19.1	B	B^R
19.6.5	T	B^R

Analysis of organelle genomes

RFLP analysis of total DNAs with heterologous cpDNA probes showed that 2 of the hybrids, 19.13.36 and 17.19.1, had the *B. nigra* type chloroplast, while the others had the *B. tournefortii* type chloroplast (Fig. 3, Table 3). This was further confirmed by analysing the profiles of cpDNA of 4 selected hybrids, 19.13.36, 17.19.1, 17.6.1, 23.1.1, digested with *Bam*HI and *Hin*dIII. The restriction profiles of

19.13.36 and 17.19.1 were exactly similar to that of *B. nigra* and the profiles of 17.6.1 and 23.1.1 matched that of *B. tournefortii*.

RFLP analysis of total DNAs with 7 cosmid clones containing mtDNA of *B. oxyrrhina* showed that only 2 of the 10 analysed hybrids, namely 17.28.1, and 19.12.1 had the strictly *B. tournefortii* type mitochondrial genome inherited from the TC parent (Fig. 4, Table 3). The remaining 8 hybrids had novel mitochondrial genomes that showed the presence of some bands characteristic of both the parents and/or novel bands (Fig. 4, Table 3). None of the hybrids had the strictly *B. nigra*-type mitochondrial genome.

Discussion

The importance of an allotriploid (sesquidiploid) genomic configuration for the transfer of genes from alien species to crop species has been highlighted by O'Mara (1940) and Rick et al. (1986). This type of genomic configuration can be utilised for generating alien addition lines by repeated backcrossing to the crop species. Gene introgression may occur in alien addition lines either by translocations or by pairing of the homoeologous chromosomes (Sears 1956; Rick et al. 1988). The production of alien substitution lines could increase the probability of genetic exchange between the homoeologous chromosomes. However, alien substitution lines have not been reported in basic diploid (2n=2x) and tetraploid (2n=4x) species (Khush 1973), including *Brassica* species.

A substitution-type genomic configuration was used by Paulman and Robbelen (1988) to transfer fertility restorer gene(s) from a *Raphanus sativus* $R_{Rf}R_{Rf}$ line to *B. napus* by sexual crosses. *R. sativus* $R_{Rf}R_{Rf}$ was crossed with *B. napus* AACC, and the resulting $R_{Rf}AC$ plants were then treated with colchicine to produce allohexaploid $R_{Rf}R_{Rf}AACC$ plants that were subsequently crossed with *B. campestris* AA to produce $R_{Rf}CAA$ plants. From such material, *B. napus* lines with the introgression of Rf genes from *R. sativus* were recovered. In such a strategy, the F_1s of alien and crop parents have first to be diploidized with colchicine and then to be crossed with a diploid species. In another study, in which somatic cell hybridisation was applied, the deficiencies of '*Ogura*' *B. napus* lines, i.e. poor nectary formation and chlorosis due to an alien chloroplast genome, were rectified by generating cybrids with recombinant mitochondrial genomes and by substituting the '*Ogura*' chloroplast genome with that of *B. napus* (Pelletier et al. 1983).

By using the fusion strategy outlined in Fig. 1, it should be possible to transfer both the nuclear and organelle genes in a concerted manner.

A cytoplasmic male sterility system in *B. juncea*, earlier reported to be spontaneous in origin, has been found to be alloplasmic in nature with *B. tournefortii* as the most likely donor of the cytoplasm (Pradhan et al. 1991) by RFLP analysis of both cpDNA and mtDNA. No restorer lines have been identified for this cytoplasm amongst *B.*

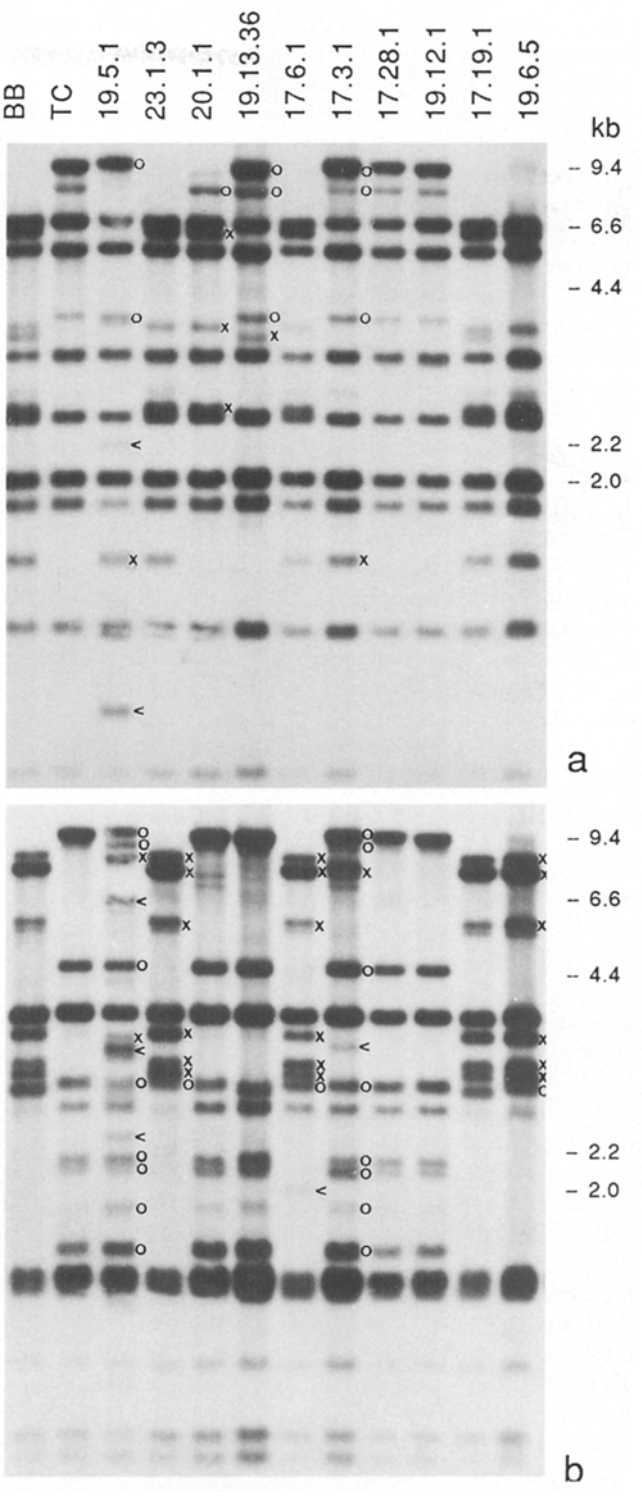

Fig. 4 Southern hybridization of *Hind*III digested total DNAs of parents (BB *B. nigra*, TC F_1 of *B. tournefortii* × *B. oleracea*) and 10 TCBB somatic hybrids to radioactively labelled mtDNA probes, pCos175 **a** and pCos88 **b**. Hybrids having both *B. nigra*-(*x*), *B. tournefortii*-(*o*) specific and novel (<) bands are indicated in the figure

juncea cultivars. *B. juncea* lines with *B. tournefortii* cytoplasm suffer from floral abnormalities and show a high suscpetibility to *Albugo candida* infection (unpublished observations). No report exists on the influence of *B. tournefortii* cytoplasm on *B. carinata.*

The TCBB hybrids, besides providing a conducive genomic configuration for interaction between the T and C nuclear genome for the transfer of restorer genes during the generative phase, also provide a whole gamut of variability for the organelle genomes that is usually observed in somatic hybrids (Kumar and Cocking 1987). RAPD analysis showed that the TCBB somatic hybrids have a stable nuclear genomic constitution. Preliminary cytological analysis of 6 somatic hybrids showed that all of them have 35 chromosomes (T10+C9+BB16). The limited amount of backcrossing to *B. carinata* that was possible during the 1992-1993 growing season indicates that TCBB hybrids can be backcrossed to *B. carinata*, thereby providing an opportunity to develop *B. carinata* lines with variant mitochondrial genomes and the *B. nigra* chloroplast genome. With respect to the transfer of nuclear genes for fertility restoration, stable hybrids with the *B. nigra* chloroplast genome and *B. tournefortii* mitochondrial genome could be used for backcrossing with *B. carinata.* Preliminary analysis of meiosis in the three TCBB hybrids has shown the presence of 12–13 bivalents at the metaphase I stage, indicating some pairing between T and C genomes.

The selectable marker genes used in this study enabled a very efficient selection of hybrid colonies and plants. As marker stocks are available for all three diploid species, *B. campestris* (*hpt* and *npt*), *B. nigra* (*npt*) and *B. oleracea* (*bar* and *hpt*), any combination of diploids could be used for hybridisations. While the A and C genomes are closely related, the B genome is disparate from the other two and belongs to *Sinapis* lineage (Song et al. 1990; Pradhan et al. 1992). Depending upon the genetic relatedness of wild species with the constitutive genomes of allotetraploid species, the initial sexual crossing of the wild species could be done with the most related diploid parent.

The cultivated allotetraploid oilseed *Brassica* species suffer from a number of fungal diseases and abiotic stresses like drought and high temperature stress prevalent in rainfed agriculture areas. Many of the wild species carry resistance to these biotic and abiotic stresses. Using the substitution type of genomic configuration outlined in this paper, useful nuclear and organelle genes could be transferred from alien species to the three allotetraploid oilseed crops *B. napus* (rapeseed), *B. juncea* (Indian mustard) and *B. carinata* (Ethiopian mustard).

Acknowledgements The first three authors have contributed equally to this paper. The authors wish to thank Ms. R. Nagpal and Dr. S. N. Raina for chromosome studies, Prof. R. G. Herrmann and Dr. A. Tyagi for the chloroplast DNA probes, Mr. J. Verma for technical assistance and Ms. S. Gupta for secretarial help. Dr. H. N. Murthy was supported by an Associateship from the Department of Biotechnology, Government of India. This work was supported by grants from the Department of Science and Technology, Government of India (Grant no. SP/SO/D-79/87) and Commission of European Communities [Grant no. EC11-0193-IND(BA)]

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