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Transfer of resistance against *Phoma lingam* to *Brassica napus* by asymmetric somatic hybridization combined with toxin selection

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Summary. Irradiated mesophyll protoplasts from nine different accessions of *B. juncea, B. nigra* and *B. carinata,* all resistant to *Phoma lingam,* were used as gene donors in fusion experiments with hypocotyl protoplasts isolated from *B. napus* as the recipient. A toxin, sirodesmin PL, was used to select those fusion products in which the resistant gene(s) was present. In the fusion experiments different gene donors, various irradiation dosages and toxin treatments were combined. Symmetric and asymmetric hybrid plants were obtained from the cell cultures with and without toxin selection. Isozymes were used to verify hybrid characters in the symmetric hybrids, whereas two DNA probes were used to identify donor-DNA in the asymmetric hybrids. Resistance to *P. lingam* was expressed in all symmetric hybrids, and in 19 of 24 toxin-selected asymmetric hybrids, while all the unselected asymmetric hybrids were susceptible.

Key words: *Brassica* – Asymmetric hybrids – In vitro selection – Resistance – *Phoma lingam*

Introduction

Gene exchange between different species is normally restricted by incompatibility barriers. These problems can be overcome by using somatic cells for hybridization or DNA transformation. To transfer single, isolated and cloned genes into plants, methods such as the *Agrobacterium* system (Horsch et al. 1987), direct gene transfer (Potrykus et al. 1987), liposome-meidated transfer (Deshayes et al. 1985) and microinjection (Crossway et al. 1986; Neuhaus et al. 1987) could be used. However, when the goal is to transfer several genes or when the genes of interest are unidentified, asymmetric hybridization mediated by protoplast fusion may be a more efficient technique.

Asymmetric somatic hybrids, carrying the complete genome of the recipient partner plus a few chromosomes or chromosome fragments from a donor partner, can either arise spontaneously from fusions between phylogenetically remote species (Hoffmann and Adachi 1981; Schieder et al. 1985) or be induced. In the latter case, fragmentation of chromosomes in the donor plant material can be obtained after exposure to various physical or chemical mutagens (Blonstein and King 1985).

The most frequently used technique today, for induction of asymmetric somatic hybrids, is the donor-recipient method where the gene donor is irradiated. This method was originally developed for transfer of organelles (Zelcer et al. 1978), but is also used for transfer of nuclear DNA (Dudits et al. 1980). A number of different asymmetric hybrids have been produced by fragmentation of one fusion partner by irradiation (see Hinnisdaels et al. 1988 for a review).

Since the elimination of DNA from the irradiated donor is variable and random, a selection pressure is required to ensure the maintenance of a defined trait in the recipient genome. Asymmetric hybridization in combination with in vitro selection has enabled a transfer of traits such as methotrexate and 5-methyltryptophan resistance from *Daucus carota* to *Nicotiana tabacum* (Dudits et al. 1987), as well as kanamycin resistance and the gene for nopaline synthesis from *N. plumbaginifolia* to wild-type protoplasts of *N. tabacum* (Bates et al. 1987).

The present study describes the transfer of resistance gene(s) against *Phoma lingam* from resistant accessions of *Brassica juncea, B. nigra* and *B. carinata* (Sjödin and Glimelius 1988) into a susceptible cultivar of *B. napus.*

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An in vitro selection was applied on the hybrid cell cultures with the toxin, sirodesmin PL, produced by *P. lingam* (Sjödin et al. 1988). This toxic compound has been shown to express selective properties (Sjödin and Glimelius 1989a) and was used in these experiments as a selective agent.

Materials and methods

Plant material

For the production of asymmetric hybrids, *B. napus* L. ssp. *oleifera* var. *annua* cv Hanna was used as recipient and five accessions of *B. juncea* (L.) Czern. & Coss., one of which was a rapid cycling line, two accessions of *B. nigra* (L.) Koch cv Junius and a rapid cycling line, and two *B. carinata* A. Braun accessions, all resistant to *Phoma lingam* (Sjödin and Glimelius 1988) were used as donor fusion partners. The origins of these have been described earlier by Sjödin and Glimelius (1988).

Isolation and irradiation of protoplasts

Mesophyll protoplasts were isolated from in vitro-grown plants according to Glimelius (1984). The X-rays were generated by a Siemens Stabilipan 200 apparatus. The tube (TR 200f) was operated at 180 kV, 10 mA and the radiation was filtered through 4 mm Al, with a dose rate of 5 Gy/min. Irradiation of the protoplasts was performed directly after enzyme treatment and enrichment of protoplasts, by floating on a CPW 16 solution (Banks and Evans 1976). After irradiation, the protoplasts were further washed with a salt solution W5 (Menczel et al. 1981).

Protoplast fusion, selection and culture of heterokaryons

Mesophyll protoplasts were isolated from in vitro-grown plants of the resistant accessions of *B. nigra, B. juncea* and *B. carinata*. The mesophyll protoplasts were treated with an X-ray dosage of 70, 100, 150, 180 and 200 Gy, as described above, before fusion with hypocotyl protoplasts isolated from *B. napus*. Growth of hypocotyls and isolation of hypocotyl protoplasts was performed according to Sjödin and Glimelius (1989b).

The hypocotyl protoplasts were stained for 20 min by 5(6)-carboxyfluorescein diacetate (Molecular Probes, Inc.) in a concentration of 0.05 m*M* per ml of protoplast suspensions. The stain was dissolved in W5 (Menczel et al. 1981) as a stock solution of 0.22 m*M*.

The protoplast fusions were performed with polyethylene glycol (PEG), both as described by Sundberg and Glimelius (1986), and with the following modification. The protoplasts were mixed to a final concentration of 4.8×10^5/ml of hypocotyl protoplasts and 2.5×10^5/ml of mesophyll protoplasts. The protoplast mixture was heated in a 45 °C water-bath for 5 min before being spaced as droplets in 10-cm petri dishes. The PEG solution and the $CaCl_2$ washing solutions were also heated to 45 °C before addition to the protoplast droplets. A solution with the modified 8 pm culture medium with 0.4 *M* mannitol and W5 (1:1 v/v), containing 4.5 μ*M* 2,4-D, 2.2 μ*M* BAP and 0.5 μ*M* NAA, was finally added to the PEG-treated protoplasts.

Enrichment, selection and culture of heterokaryons

After fusion treatment the cultures were stored in darkness, for at least 4 h, in a refrigerator. Before selection of the heterokaryons with flow sorting (Glimelius et al. 1986), the protoplasts were washed off the petri dishes and centrifuged at 75 g for 5 min. The protoplast pellet was suspended in the 8 pm culture medium to a density of about 75×10^3 protoplasts per ml.

The enriched heterokaryons were cultured after flow sorting in the 8 pm medium. When the cell divisions had started, fresh culture medium without hormones was added. The volumes and the hormone concentrations used were as described above.

When small cell aggregates were obtained (15–20 cells), the cell cultures were embedded according to Sjödin and Glimelius (1989a). After gelling, the beads were placed in 2.0-cm petri dishes with liquid 8 pm medium, containing the same hormones as the beads. Sirodesmin PL was added to the bead cultures to give a final concentration of 3.0 μ*M*.

Differentiation of the small calli was performed according to the methods described by Sjödin and Glimelius (1989b). The hormone concentrations used during these steps were 0.6 μ*M* IAA, 2.2 μ*M* BAP and 2.3 μ*M* zeatin, or 0.6 μ*M* IAA, 4.4 μ*M* BAP and 4.6 μ*M* zeatin. During the whole culture period, 3.0 μ*M* sirodesmin PL was present in the medium. Light and temperature conditions for the cell cultures were as described by Sjödin and Glimelius (1989b). Shoots were transferred to hormone-free medium with salts from the root-inducing medium M4 (Installé et al. 1985), supplemented with vitamins from the K_3 medium, 0.03 *M* sucrose and 0.3% Gellan Gum (Kelco). The shoots were cultured under sterile conditions (Sjödin and Glimelius 1989b) in a temperature of 15 °C. When roots had developed, the established plants were planted in the greenhouse.

Confirmation of hybrid character

Isozyme analysis

Isozyme analyses were performed according to Sundberg and Glimelius (1986), using the buffer G and N according to Shields et al. (1983). The enzymes examined were phosphoglucomutase (PGM), leucine aminopeptidase (LAP), phosphoglucose isomerase (PGI), aspartate aminotransferase (AAT), 6-phosphogluconate dehydrogenase (6-PGDH), glucose-6-phosphate dehydrogenase (G-6-PDH), shikimate dehydrogenase (SHDH), malate dehydrogenase (MDH), and triosephosphate isomerase (TPI), which gave distinct isozyme patterns between *B. napus* and the other fusion parents used.

RFLP analysis

DNA isolation from plants. Total DNA was isolated according to a modified method of Dellaporta et al. (1983). One gram of tissue from each of the young greenhouse-grown plants of *B. napus* cv Hanna, the 9 donor accessons and 23 putative hybrid plants was homogenized in liquid nitrogen. The homogenate was suspended in a buffer (15% sucrose, 50 m*M* TRIS-HCl pH 8, 50 m*M* Na_2EDTA), and 200 μl of a 10% SDS solution was added to the suspension. The sample was incubated at +70 °C for 20 min before addition of 1 ml 5 *M* KAc. After incubation on ice for 60 min, the sample was centrifuged at 1,000 × g for 10 min, and the supernatant was collected and filtered through one layer of Miracloth. DNA was ethanol-precipitated, dried and dissolved in 900 μl TE-buffer (1 m*M* NA_2EDTA, 10 m*M* TRIS-HCl, pH 7.5) and 100 μl 3 *M* NaCl. The sample was then treated with RNAse, precipitated again and the final pellet was dissolved in 100–200 μl TE-buffer.

Southern blot and hybridization. About 10 μg DNA of each sample was digested with *Bam*HI, *Eco*RV and *Hae*III (Pharmacia), and fragments were separated by electrophoresis in 0.5% agarose gel (40 V, 40 mA, 20 h).

The Southern blot analysis was a modified method of Maniatis et al. (1982). The DNA fragments were transferred

to Pall Biodyne membranes by capillary transfer. A 570-bp *Hind*III- *Xho*I fragment from a napin gene, prepared according to Ericsson (1988), and a 6.7-kb *Eco*RI rDNA fragment from *Vicia faba* (Yakura et al. 1984) were used as probes. The probes were radiolabeled with 50 μCi (α-^{32}P)dCTP by random priming, using an oligolabeling kit (Pharmacia). After inhibition of the reaction, the sample was extracted with 100 μl phenol and centrifuged for 5 min. The membranes were prehybridized for 1 h in 20 ml of a solution containing 40% formamide, 5 × SSC, 50 m*M* Na_2PO_4 pH 6.5, 5 × denhardts, 0.1% SDS, 10% dextran sulphate, and 0.25% milk powder at 42 °C.

Hybridization was performed overnight at 42 °C and then the filter was washed twice with 2 × SSC + 0.2% SDS for 5 min at room temperature and twice with 0.7 × SSC + 0.5% SDS at 58 °C. The filters were exposed to Kodak XAR-5 films, using intensifying screens at −70 °C, for 5–116 h.

Analysis of chromosome number, ploidy level, seed set, morphology and resistance to Phoma lingam

The chromosome number in cells from root tips was determined by the Feulgen method according to Sundberg et al. (1987). For each hybrid plant, about 50 flowers were self-pollinated and about 50 flowers were pollinated with pollen from *B. napus* cv Hanna. The number of seeds obtained per pollination procedure was determined.

Plant height of the hybrids was measured during seed set and the plant morphology was determined on actively growing plants. The DNA-content of the hybrid plant cells was determined as the relative fluorescence normalized to *B. campestris* (Fahleson et al. 1988) using flow cytometry. Resistance to *P. lingam* was tested by inoculation of adult leaves and stems with pycnospores from the pathogen, according to Sjödin and Glimelius (1989b).

Results

Protoplast fusion and culture of heterokaryons

Based on the dose-response effects on protoplast cultures, doses between 70 and 200 Gy were chosen for treatment of the donors in the fusion experiments. The cell aggregate formation of the protoplast cultures was reduced to 93%, 96% and 99% by an exposure of 70 Gy, 100 Gy and 200 Gy, respectively. No significant differences in sensitivity to the irradiation were found between the *Brassica* accessions of different ploidy level.

In fusion experiments where the radiation dosage was higher than 150 Gy, only a few cell divisions occurred in some hybrid combinations, but no cell aggregates were formed. When 150 Gy was used, more cell divisions were found, but calli were only obtained at very low frequencies, less than 0.001%. Thus, the irradiation dose was further reduced to 100 and 70 Gy.

By performing the PEG-induced fusions at 45 °C, a higher fusion frequency, determined as heterokaryons in percentages of viable protoplasts (18%–31%), was obtained than when performed at room temperature (11%–15%). However, the protoplasts isolated from *B. nigra* were found to be heat-sensitive, thus the heat treatment was excluded from fusions involving *B. nigra*.

Between 3×10^4 and 15×10^4 fusion products of each fusion combination were selected by flow sorting. The enriched cultures contained 79%–93% hybrids when calculated 1 day after sorting.

Recovery of selected calli

Fusion experiments. When the toxin was applied to the bead cultures, 0.4%–6% of the small cell aggregates survived the treatment. Out of these, 55%–77% continued to grow. The calli that survived the toxin treatment stayed much greener on the regeneration medium, in contrast to the unselected hybrid cultures, among which browning rapidly started. Shoots, in frequencies between 1% and 11%, emerged from the calli, but only about 50% of the shoots developed into plants (Table 1, Fig. 1).

Shoots and plants obtained in the different fusion experiments are presented in Table 1, and each group is designed with a capital letter, which will be referred to in the following text.

Control cultures. No toxin-resistant calli were recovered from regular protoplast cultures of *B. napus,* whereas an average of 86% of the donor cell aggregates survived the toxin treatment. From the irradiated donor cultures not exposed to the toxin, 0%–3% calli developed. However, when the irradiated donor cultures were treated with toxin, no calli developed. In cultures of a mixed but unfused population of rapeseed and irradiated donor protoplasts, without toxin, few delayed cell divisions occurred and a browning of the cultures started almost immediately. The irradiated protoplasts exerted a negative influence on the viability of the rapeseed protoplasts, which resulted in a low frequency of callus formation (12%). No calli developed from the toxin-selected mixed cultures.

Confirmation of hybrid character

Isozyme analysis. When the enzymes PGI, 6-PGDH, PGM, MDH and TPI were analysed in the symmetric hybrids between *B. napus* and *B. juncea,* hybrid characters were found in 23 plants, whereas two plants showed hybrid patterns only for PGI and PGM and one plant had hybrid bands only for PGI.

Forty-two out of 45 putative asymmetric hybrid plants analysed did not show any hybrid character for the 9 enzymes used. Only *B. napus* bands were found. Even though hybrid bands were detected in five young shoots, the analyses showed that these had disappeared in the fully developed plants.

RFLP analysis. Total-DNA from 23 putative asymmetric hybrids was hybridized with two DNA probes. In nine of ten hybrids from group H, two bands in the hybrids (4.5 kb and 4.8 kb) co-migrated with bands found in *B.*

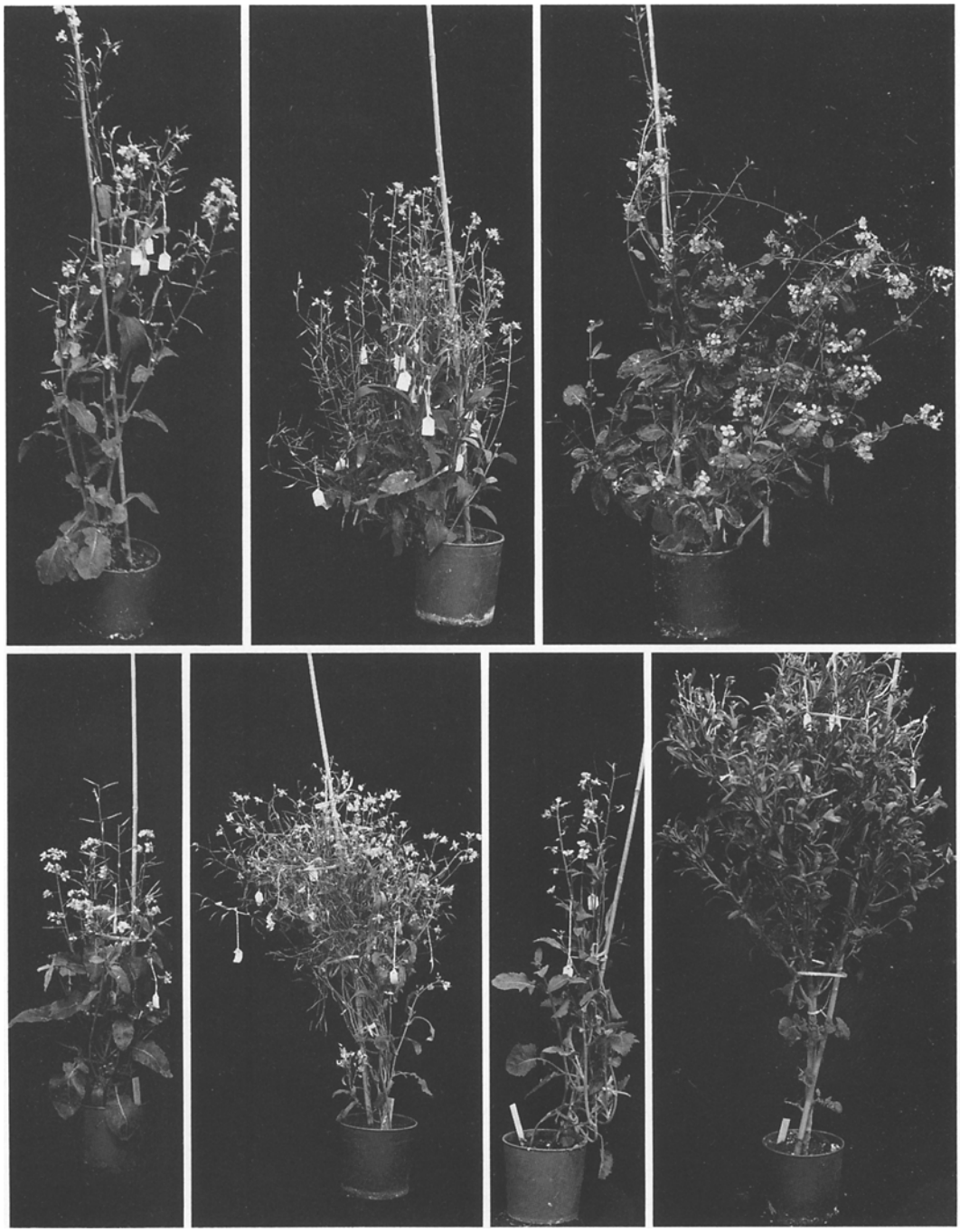

Fig. 1. Representatives of different hybrid combinations. First row (left to right): *B. napus* (+) *B. nigra* (group B), *B. napus* (+) *B. juncea* (groups D and E). Second row (left to right): *B. napus* (+) *B. juncea* (two representatives of group F with different donor accessions, group H), *B. napus* (+) *B. carinata* (group L)

juncea when hybridized to the rDNA probe. This fragments were not present in *B. napus*. In Fig. 2, five of these hybrids are shown. In addition, the 10-kb band seen in *B. napus* is absent in some of the hybrids.

A southern blot of asymmetric hybrids from groups E, F and L hybridized with the napin probe is shown in Fig. 3. Fragments from the different donor fusion partners could also be found in some of the hybrid plants with this DNA probe. These bands had sizes of 7, 12, 14 and 18 kb. Moreover, two of the hybrids showed an additional band (7 kb) not detected in their parental accessions. The number of plants containing DNA from the donor accessions determined by RFLP analysis with the two DNA probes is presented in Table 1.

Table 1. A summary of different fusion combinations, with the dose of irradiation prior to fusion, toxin selection, number of shoots and plants obtained. The chromosome range, resistance to *P. lingam* and RFLP were analysed in the developed plants

Fusion combinations		X-ray (Gy)	Toxin (3 μ*M*)	No. of shoots	No. of plants	Chromosome range	No. of plants resistant (R) and susceptible (S) to *P. lingam*		No. of plants with DNA from the donor genome, according to RFLP analysis
							R	S	
Group									
A	*B. napus* (+) *B. nigra*	70	–	7	1	50		1	0 (1)[a]
B	*B. napus* (+) *B. nigra*	70	+	10	1	48	1		1 (1)
C	*B. napus* (+) *B. juncea*	–	–	9	0				
D	*B. napus* (+) *B. juncea*	–	+	38	31	40–74	28		
E	*B. napus* (+) *B. juncea*	70	–	19	11	38–66		10	1 (1)
F	*B. napus* (+) *B. juncea*	70	+	24	12	38–42	9	2	5 (7)
G	*B. napus* (+) *B. juncea*	100	+	3	2	nd	nd	nd	
H	*B. napus* (+) *B. juncea*	150	–	28	10	54–56		10	9 (10)
I	*B. napus* (+) *B. carinata*	–	–	4	0				
J	*B. napus* (+) *B. carinata*	–	+	2					
K	*B. napus* (+) *B. carinata*	100	–	3	1	44		1	
L	*B. napus* (+) *B. carinata*	100	+	20	12	38–66	9	3	1 (3)
M	*B. napus* (+) *B. carinata*	150	–	3	1	40		1	
Parental material									
B. napus						38		S	
B. nigra						16	R		
B. juncea						36	R		
B. carinata						34	R		

[a] The total number of plants investigated is given in the brackets
nd – Not determined

Chromosome number and DNA content

The chromosome numbers (Table 1) and the DNA content varied widely between the hybrids obtained. Twenty-three out of 26 analysed plants obtained from fusions without irradiation between *B. napus* and *B. juncea* (group D) had the expected chromosome number of 74, whereas the asymmetric hybrids had between 38 and 66 chromosomes. The asymmetric hybrids between *B. napus* and *B. nigra* and between *B. napus* and *B. carinata* also had a high variation of chromosomes, 48–50 and 38–66, respectively. No divergent values of the DNA content, compared to the chromosome numbers, were found among the different hybrids, except in group D, where two chimaeric plants were revealed by the flow cytometric determination of the nuclear DNA content.

Hybrid fertility and morphology

The fertilities of the hybrid plants were calculated as the seed set of selfed flowers and expressed in percentage of seed set of *B. napus*. According to this determination, the fertility was found to be rather high in groups D (18%), E (9%), F (11%) and L (8%), but low in groups H (0.8%) and K (0.5%). Backcrosses with pollen from *B. napus* cv Hanna have, however, increased the seed set in some plants. The fertility among the other hybrids has not yet been determined.

The hybrid plants in group D had an intermediate plant height and leaf morphology compared to their different parents, whereas a larger variation was found among the asymmetric hybrids. In comparison with the parental accessions, they were all shorter, except for three plants in group L, and in group H all plants had thicker and waxier leaves. In general, the majority of the asymmetric hybrids had a leaf morphology different to that of the parental species and to the corresponding symmetric hybrid. Five of the asymmetric plants had a morphology similar to *B. napus*. Occasionally, flowers with short stamens or clustered flowers appeared (mainly in groups H and L), but in other respects the flowers had a morphology typical for the *B. napus* type. In all asymmetric hybrid groups, some plants developed thick pods after pollination, and several aborted embryos were found when the pods were harvested.

Resistance to P. lingam

Cuttings were made from each hybrid plant, and after about 6 weeks these were analysed for disease resistance.

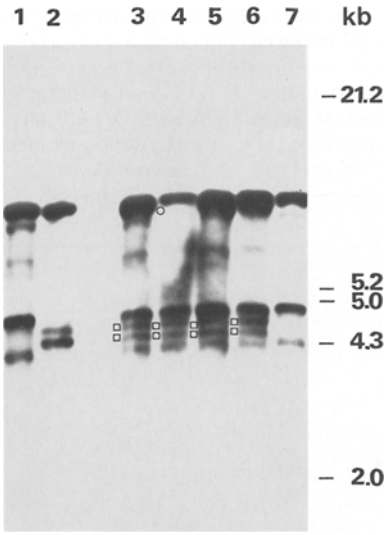

Fig. 2. Total DNA from five putative asymmetric hybrids from group H and the fusion parentals were isolated, digested with BamHI and hybridized to a rDNA probe. The different lanes represent the following: *1* – *B. napus* cv Hanna (the recipient); *2* – *B. juncea* (the donor); *3–7* – five putative asymmetric hybrids. The donor fragments in the asymmetric hybrids are indicated by (□), and (○) indicates absence of *B. napus* fragments. Lambda-DNA digested with EcoRI/HindIII was used as the size marker

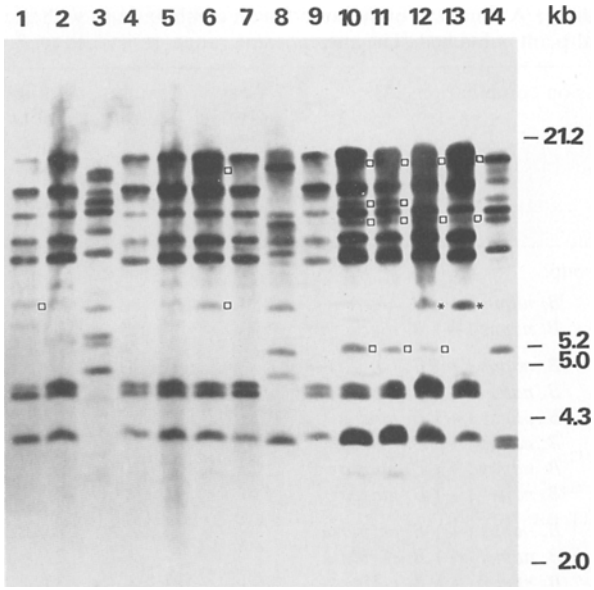

Fig. 3. Total DNA from nine putative asymmetric hybrids and their parental accessions was isolated, digested with EcoRV and hybridized to a DNA probe coding for a part of the storage protein napin. The different lanes represent the following: *Lanes 1 and 2* – hybrids from group L; *lane 3* – *B. carinata*, donor to group L; *lanes 4 and 9* – *B. napus* cv Hanna, recipient; *lanes 5–7, 10–13* – hybrids from group F; *lane 8* – one accession of *B. juncea*, donor to hybrids in *lanes 5–7; lane 11* – hybrid from group E; *lane 14* – one accession of *B. juncea*, donor to hybrids in *lanes 10–13*. The donor fragments in the asymmetric hybrids are indicated by (□), and (*) indicates a "new" fragment. Lambda-DNA digested with EcoRI/HindIII was used as the size marker

All of the *B. napus* (+) *B. juncea* hybrids from group D were resistant, as were 19 out of the 24 asymmetric and toxin-selected hybrids. All unselected asymmetric hybrids were susceptible (Table 1, Fig. 4).

Discussion

Resistance to *Phoma lingam* was obtained in most of the toxin-selected asymmetric plants produced via the donor-recipient method, whereas all unselected asymmetric hybrids were sensitive. It is, thus, obvious that the selection performed in vitro was of fundamental importance to ensure that the desired gene(s) was retained in the asymmetric hybrid.

The use of the toxin, sirodesmin PL, produced by *P. lingam* made it possible to successfully obtain disease-resistant asymmetric hybrid plants. The selective capacity of the toxin was most clearly demonstrated when the putative asymmetric hybrids with and without toxin selection were compared. All plants arising from cultures without the toxin selection were susceptible to *P. lingam*, whereas the majority of plants emerging from calli cultured under a selection pressure of the toxin were resistant to *P. lingam*. However, 5 of 24 toxin-selected asymmetric plants were susceptible to the pathogen. These susceptible plants might have been derived from calli resistant to the toxin during the cell culture phase, when

Fig. 4. *P. lingam* inoculated leaves (left to right): a resistant symmetric hybrid between *B. napus* and *B. juncea*; a susceptible asymmetric hybrid between *B. napus* and *B. juncea*, not selected with the toxin; a resistant asymmetric hybrid between *B. napus* and *B. nigra*, selected with the toxin

cultured in presence of the toxin. Since the resistance test was performed on cuttings of the original hybrid plant, an elimination of donor DNA might have occurred during the time elapse between the in vitro culture of the calli and shoots in presence of the toxin, and the period with-

out a selection, before the diseases screening event. That an elimination of donor DNA can actually take place during development of the plants was revealed by the isozyme analysis. In five asymmetric hybrids where hybrid bands were present in the young shoots, all were lost in the developed plants.

A majority of the putative asymmetric hybrids contained more nuclear DNA than *B. napus,* but less than the sum of the two fusion partners according to the flow cytometric measurements. To determine whether extra DNA from the donor genomes was present in the hybrids, isozymes and DNA probes were used as markers. The isozyme analysis was shown to be a fast and easy way to verify hybrid characters when the non-irradiated hybrid plants were analysed. However, when analysing the asymmetric hybrids, the isozymes, with few exceptions, did not show presence of the donor DNA. The use of DNA probes, on the contrary, revealed specific RFLP patterns of the different *Brassica* species, and DNA from the donor partners present in the asymmetric hybrids could clearly be visualized.

One of the DNA probes consists of a part of a rDNA coding sequence from *Vicia faba* and represents a non-translated part of the genome. The other probe codes for the storage protein napin, and is encoded by a multigene family comprising a minimum of 16 genes (Scofield and Crouch 1987). This may explain why the DNA probes could identify donor-DNA in the asymmetric hybrids not detected with the isozymes used, although the precise distribution in the *Brassica* genome of the two DNA probes and the isozymes is unknown. However, not all putative asymmetric hybrids could be verified using only two DNA probes. More markers are needed to prove whether donor DNA is present in all asymmetric hybrids or not.

"New" DNA fragments were found in the hybrid plants as well as loss of *B. napus* bands, indicating that rearrangements of the DNA might have occurred. In spite of the fact that variation of the RFLP pattern has been found within the rapeseed cultivar Hanna, the 10-kb fragment was always present in all individual plants investigated (data not shown). Thus, the loss of this fragment in some of the asymmetric hybrids most probably reflects rearrangement events. Regarding the "new" bands in the hybrids, it cannot be excluded that these may be due to variation in the RFLP pattern within the different donor accessions used. The variation among these has not yet been investigated.

In conclusion, by using the donor-recipient method, the agronomically important trait, resistance to *P. lingam,* was transferred from resistant *Brassica* accessions to a susceptible cultivar of *B. napus*. This was only the case when the toxin, sirodesmin PL, was used as a selective agent on cell aggregates and calli of the different fusion products.

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