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Somatic hybrids between *Brassica oleracea* L. and *Sinapis* alba L. with resistance to *Alternaria brassicae* (Berk.) Sacc.

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Abstract Somatic Hybrids between *Sinapis alba* and rapid-cycling *Brassica oleracea* were generated for transferring of resistance to *Alternaria brassicae* to *B. oleracea*. *A. brassicae* causes the significant disease black spot in cruciferous crops. A total of 27 plants were regenerated from protoplast fusion using 0, 5, 10, 20 and 30 krad γ-irradiation of the resistance donor and iodoacetate treatment of *B. oleracea*. All plants showed intermediate morphology with partially divided leaves and some trichomes on stems and leaves. Flow cytometry and banding patterns of the enzymes leucine amino peptidase (LAP) and phosphoglucose isomerase (PGI) confirmed the hybrid status of the regenerated plants. Some of the plants obtained from cuttings from the somatic hybrids showed a resistance to *A. brassicae* that was similar to that found in *S. alba*. The flowers of the somatic hybrids had reduced anthers with little pollen production.

Key words Black spot · Intergeneric hybridization · protoplast fusion · white mustard

Introduction

Interspecific hybridization within the Brassicaceae family is an attractive strategy for the introduction of desirable characters to the crop species. However, crossing barriers may prevent the generation of hybrids by sexual means. An alternate way of achieving hybridizations among related species is offered by protoplast fusions, which may overcome interspecific and even intergeneric crossing barriers. Resistances to pathogens and pests are among the traits that may be found in related species, when not present in the cultivated ones (Batra et al. 1990; Hagimori et al. 1992; Lelivelt et al. 1993).

The fungal pathogen *Alternaria brassicae* causes blackspot disease in *Brassica oleracea*, which contains many of our most important vegetables, such as cabbage, cauliflower and broccoli. By developing spotting symptoms on any part of the plant, *A. brassicae* is capable of significantly reducing the market value of the product.

Sufficient resistance to *A. brassicae* is not found within *B. oleracea*, nor in species with which it readily crosses. High resistance has been identified in other crucifer species such as *Sinapis alba* (Brun et al. 1988; Zhu and Spanier 1991); *Camelina sativa* (false flax) and *Capsella bursa-pastoris* (shepherd's-purse) (Conn et al. 1988). In addition to resistance to *A. brassicae*, *S. alba* carries resistance to flea beetles (*Phyllotreta cruciferae* and *P. striolata*), a major insect pest of crucifers (Putnam 1977; Lamb 1984; Palaniswamy and Lamb 1992), to the beet cyst nematode (*Heterodera schachtii*), which may utilize *Brassica* crops as hosts for multiplication (Lelivelt and Hoogendoorn 1993), to drought (Primard et al. 1988) and, possibly, to the cabbage maggot (Finch and Ackley 1977). *S. alba* is therefore a very valuable source for introgression of desirable traits into *B. oleracea*.

Hybridizations between *S. alba* and *B. napus* have previously been achieved sexually through the use of embryo rescue techniques (Ripley and Arnison 1990; Chevre et al. 1994; Lelivelt et al. 1993) and by protoplast fusion (Primard et al. 1988; Lelivelt et al. 1993). Sexual hybrids have been produced between *S. alba* and *B. juncea* (Mohapatra and Bajaj 1987; Sharma and

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Singh 1992) and between *S. alba* and *B. campestris* (Jandurova and Dolezel 1995). Harberd and McArthur (1980) obtained sterile, sexual hybrids between *B. oleracea* and *S. alba*. In this paper, we report on the production and analysis of somatic hybrids between *B. oleracea* and *S. alba* with the purpose of transferring resistance to *A. brassicae* into *B. oleracea*.

Materials and methods

Plant material

Thirty-seven lines of *S. alba* obtained from the Israel Gene Bank for Agricultural Crops (Bet Dagan, Israel) were screened for resistance to *A. brassicae*. Four of these genotypes were tested for their performance in protoplast culture. On the basis of good cell division and callus growth, 1 *S. alba* line, Alt 543, was selected and used in protoplast fusions with rapid cycling *B. oleracea* (Crucifer Genetics Cooperative 3-1), for which a good regeneration system is available (Hansen and Earle 1994a). Rapid cycling *B. oleracea*, a white-flowered broccoli selection from cv 'Packman', and a Chinese kale (*B. oleracea* ssp. *alboglabra* NVRS02,006707) with some resistance to *Alternaria* spp. were used in greenhouse pollinations of the somatic hybrids.

Screening for resistance to *A. brassicae*

The 37 lines of *S. alba* were screened for resistance to *A. brassicae* as described (Hansen et al. 1995). Plants were inoculated when 3–4 weeks old by spraying to run-off with a conidia suspension (5×10^4 spores/ml). They were incubated at 20°C for 5–7 days. Three separate trials were performed, one under mist and two in plastic bags. The disease severity was evaluated on a scale from 1 to 10, where 1 = no disease, 2 = high resistance, 3–4 = moderate resistance, 5–9 = increasing susceptibility and 10 = dead. Analysis of variance was conducted using SAS Statistical Software (SAS Institute, Cary, N.C.).

Protoplast isolation and culture

Culture of plants in vitro and protoplast isolation were carried out as previously described (Hansen and Earle 1994a). For protoplast culture of *S. alba*, the protoplasts were resuspended in liquid medium B (Pelletier et al. 1983) lacking Tween 20 in concentrations of 1×10^5 or 5×10^5 protoplasts per milliliter. The protoplasts were plated on membranes (Millipore, type AA, 0.8 μm) over a feeder cell suspension of *B. napus* on solid medium B lacking Tween 20, according to Walters and Earle (1990). They were cultured on a series of media (B, C, E, F) (Pelletier et al. 1983) as previously described for rapid cycling *B. oleracea* (Hansen and Earle 1994a). For protoplast fusions, the densities of the two fusion partners were adjusted to 2×10^6 protoplasts/ml W5 medium (Menczel et al. 1981).

Protoplast pretreatments and fusion

Prior to fusion, the *B. oleracea* partner was inactivated by treating the protoplast suspension with 3 m*M* iodoacetate for 20 min before processing to prevent the division of unfused protoplasts. This dosage is usually sufficient; however, it failed to eliminate division of *B. oleracea* protoplasts in the fusions with 20- and 30-krad donor pretreatments. Since the *S. alba* fusion partner does not regenerate under the culture conditions used, symmetric hybrids could be produced and selected without any pretreatment of this species. For the generation of asymmetric hybrids, leaf tissue of *S. alba* was exposed to γ-irradiation using a Cesium 137 source (Gamacell 1000, Nordion International, Canada) at rates of 5, 10, 20 and 30 krad prior to initiation of the enzyme treatment. During irradiation, the excised leaves were placed in 3.5 ml 0.5 *M* sorbitol, 10 m*M* $CaCl_2$–$2H_2O$ and 5 m*M* MES, pH 5.8, in 60-mm petri dishes.

Protoplast fusions were performed as previously described (Hansen and Earle 1994b). Following fusion, the concentration was adjusted to 8×10^4 intact protoplasts per milliliter, and approximately 1 ml of protoplast suspension was plated per filter. The protoplasts were cultured as described above.

Plant regeneration

Shoots excised from regenerating calli on medium F were transferred to MS medium containing 3% sucrose and no growth regulators (MS-3,0) (Murashige and Skoog 1962) for rooting. Upon root formation, plantlets were transferred to soil and covered with plastic bags for gradual adaptation to conditions out of culture. When well-established, the plants were moved to the greenhouse, where they were grown to maturity.

Nuclear DNA content

The nuclear DNA content of the fusion products was estimated by flow cytometry. Samples were prepared and analysed with an EPICS Profile Analyzer (Coulter Electronics, Hialeah, Fla.) as described by Arumuganathan and Earle (1991). Chicken red blood cells were used as a standard that was run separately, because the peak of chicken red blood cells coincides with the G0/G1 peak of the somatic hybrids.

Isozyme analysis

Approximately 50 mg young leaf tissue was ground in 200 μl extraction buffer consisting of 0.1 *M* K_2HPO_4, 10% glycerol (v/v), 10% PVP-40 (w/v), 0.1% β-mercaptoethanol (v/v), 0.5% Triton-X 100 (v/v). The samples were run in cellulose acetate gels (76 × 76 mm plates, Titan III, Helena Laboratories, Beaumont, Tex.) and stained according to the instructions of Helena Laboratories with some modifications. The following enzymes were screened: phosphoglucose isomerase (PGI), phosphoglucose mutase, alcohol dehydrogenase, malate dehydrogenase, malic enzyme, 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase, lactate dehydrogenase, fumarate hydratase and leucine amino peptidase (LAP). Electrophoresis was for 20 min at 200 V for all enzymes, except for LAP, which was run for 30 min at 130 V.

Analysis of resistance to *A. brassicae*

Cuttings were taken from the somatic hybrids to produce plants for analysis of resistance to *A. brassicae*. The plants were screened under mist as previously described. Six plants each of rapid cycling *B. oleracea* and *S. alba*, Alt 543, participated in the screen.

Fertility studies

Pollen release was studied in the somatic hybrids every 2–3 weeks for a period of 3 months. As a measure of pollen viability in flowers

from plants releasing pollen, pollen stainability by aceto-carmine (1%) was examined in newly opened flowers. Pollen germination on stigmas of somatic hybrids following pollination with various *B. oleracea* genotypes was studied using aniline blue staining. Pollen was applied to buds as well as open flowers, to styles having their stigmas removed and to stigmas treated with 0.25 *M* NaCl according to Monteiro et al. (1988). Following the NaCl treatment, drying was allowed for approximately 15 min before application of pollen.

The stigmas were fixed in acetic acid and 70% ethanol 1 : 3 for 15 min 5–6 hours after pollination. They were then softened in 1 *N* NaOH overnight, squashed onto a microscope slide in a drop of aniline blue (2 g/l) in 10 m*M* K_3PO_4 and observed under the fluorescence microscope.

Meiotic behaviour was studied in two of the somatic hybrids. Floral buds were fixed in Carnoy's solution (ethanol, chloroform, acetic acid 6 : 3 : 1) for 48 h and stored in 70% ethanol at 4°C. Anthers were squashed in 1% aceto-carmine solution, and pollen mother cells undergoing division were observed in the microscope.

Results

Alternaria resistance screen

The disease severity rating in the 37 *S. alba* lines varied from 2.7 to 4.7 taken as an average over the three independent trials. There was a high plant to plant variability within genotypes, and genotypes also responded differently in the three screens. Therefore, 4 lines were selected at random for the protoplast culture experiment. The average disease severity ratings of these lines are given in Table 1.

Protoplast culture of *S. alba*

Protoplast yield ranged from $1–4 \times 10^7$ protoplasts/g leaf tissue in the 4 *S. alba* lines screened in protoplast culture. Line Alt 543 showed good division and growth, and more than three times as many calli could be isolated of this line as from the second best, line Alt 550 (Table 1). The lower plating density (1×10^5 protoplasts/ml) was better for Alt 543 than the higher (5×10^5 protoplasts/ml), since the latter concentration often resulted in the formation of a mat of microcalli that was very difficult to separate. The calli of Alt 543 grew vigorously on the regeneration medium (medium E) for about 3 weeks, after which the calli turned brown rapidly. No regeneration was seen. On the basis of these observations, line Alt 543 was selected for use as the resistance donor in the protoplast fusions.

Table 1 *S. alba* used in protoplast culture

Genotype	Average disease severity rating	Protoplasts plated[a]	Calli isolated
Alt 543	3.1 ± 0.4[b]	6.5×10^5	447
Alt 550	3.0 ± 0.7	6.5×10^5	136
Alt 565	3.6 ± 0.7	6.5×10^5	28
Alt 568	4.7 ± 1.0	6.5×10^5	124

[a] Data from the two plating concentrations combined
[b] Standard deviation

Protoplast fusions

Fusion products showed good division on medium B in the dark and on medium C after transfer to the light. Individual calli could be moved to regeneration medium (medium E) starting 22 days after protoplast fusion. Beginning 4–5 weeks after fusion, calli with initiated shoots were transferred to medium F for shoot development (Fig. 1). Regeneration results are shown in Table 2. The regeneration frequency was quite low (0.18–0.81%), and highest when no irradiation was applied. In the fusions with 5- and 10-krad irradiation pretreatment, more calli initiated shoots than actually developed full shoots that could be excised (Table 2). In total, 20 shoots from calli originating from symmetric fusions and 7 shoots from calli originating from asymmetric fusions were excised and transferred to MS-3,0 for root formation. The plantlets were cultured on MS-3,0 about 2 weeks before being transferred to soil. In the fusions with irradiation doses of 20 and 30 krad, the iodoacetate treatment failed to eliminate the division of unfused *B. oleracea* protoplasts, and selection of fusion products was based on the morphology of shoots in vitro (Fig. 1). Leaves of somatic hybrid shoots were partially divided and had trichomes like *S. alba*. Shoots of rapid cycling *B. oleracea* have undivided leaves lacking trichomes. Leaves from an adult hybrid

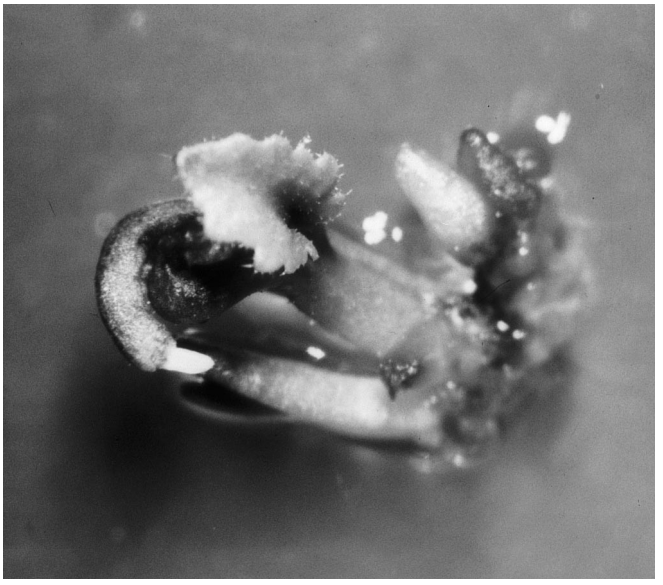

Fig. 1 Shoot regeneration of somatic hybrid on medium F. Leaf has hybrid morphology with trichomes (4 ×).

Table 2 Callus isolation and plant regeneration from protoplast fusions between RC[a] *B. oleracea* and resistance donor *S. alba*

Recipient	Donor	Donor irradiation (krad)	Calli isolated	Shoot-forming calli	Regeneration frequency[b] (%)	Plants
RC[a] *B. oleracea*	*S. alba*	0	743	6	0.81	20
RC[a] *B. oleracea*	*S. alba*	5	225	1 (5)[c]	0.44	2
RC[a] *B. oleracea*	*S. alba*	10	559	1 (3)[c]	0.18	3
RC[a] *B. oleracea*	*S. alba*	20	352	0	0	0
RC[a] *B. oleracea*	*S. alba*	30	454	1[d]	0.22	2

[a] Rapid cycling
[b] Based on number of calli yielding shoots large enough to be excised
[c] Figure in parentheses is number of calli with shoot initiation. Not all of these formed shoots that could be excised
[d] Selection based on hybrid morphology

Fig. 2 Leaves of (from *left* to *right*) rapid cycling *B. oleracea*, somatic hybrid and *S. alba*

plant compared to leaves from the parental genotypes are shown in Fig. 2.

Analysis of somatic hybrids

All 27 plants regenerated from fusion between *S. alba* and rapid cycling *B. oleracea* showed morphology intermediate to that of the two parents, with partially divided leaves (Fig. 2) and intermediate amounts of trichomes on the stems and leaves. *S. alba* has divided leaves, and the entire plant is densely covered with trichomes. Rapid cycling *B. oleracea* has oval waxy leaves and no trichomes. The height of the somatic hybrids (Fig. 3b) varied between 40 and 125 cm. In comparison, rapid cycling *B. oleracea* (Fig. 3a) grew to 30–40 cm, and *S. alba* (Fig. 3c) grown in pots under similar conditions as the somatic hybrids grew no taller than 50 cm. Pods were larger than in either of the parents with little hairiness and long beaks. Apart from plant height, there was little morphological difference between the somatic hybrids.

Nuclear DNA content (2C) of rapid cycling *B. oleracea* was estimated by flow cytometry to be 1.29 ± 0.07 pg and that of *S. alba* to be 1.07 ± 0.08 pg. The 2C nuclear DNA content of the somatic hybrids is given in Table 3. For plants from the 0-krad fusions, the average DNA content was 2.51 ± 0.10 pg. The average DNA contents in plants from fusions with irradiation pretreatments of 5, 10 and 30 krad were 2.32 ± 0.19 pg, 2.46 ± 0.22 pg and 2.39 ± 02 pg, respectively. Thus, there was no significant reduction in the DNA content after irradiation. However, somatic hybrids 18-4-2 (5 krad) and 19-4-1 (10 krad) apparently had a slightly reduced DNA content compared to the sum of the two diploid parental species. Figure 4 shows the flow cytometric profile of somatic hybrid 18-1-3 mixed with nuclei of the two parents.

Of the ten enzymes screened, only PGI and LAP showed polymorphism between *B. oleracea* and *S. alba*. Hybrid identity was confirmed in all transferred plants from the 0-, 5- and 10-krad fusions by analysis of these two enzymes (Table 3). The plants obtained from callus 1 and 2 from fusion 18 (no irradiation pretreatment) expressed only the PGI enzyme from the *S. alba* parent; however, strong bands of LAP were present from both parents. All other tested regenerants showed the presence of isozymes from both parental species as well as a heterodimer in the case of PGI (Table 3, Fig. 5).

The reaction of 25 somatic hybrids and 6 plants each of the two parental genotypes to inoculation with *A. brassicae* is shown in Fig. 6. Some plants showed high resistance (rating of 2 or 3), similar to the *S. alba* parent. Others had intermediate resistance, and 2 were as susceptible as the *B. oleracea* parent (rating of 7 or 8). There was no apparent difference in the response of plants from fusions with different irradiation doses (Table 3). Average disease severity ratings were as follows: somatic hybrids: 3.8 ± 1.5, rapid cycling *B. oleracea*: 7.5 ± 0.4, *S. alba*: 2.2 ± 0.6.

All somatic hybrids showed very little male fertility. The anthers were reduced, and most did not release any pollen. Fourteen of the plants at times released a small amount of pollen from some flowers (Table 3). These plants were all from fusions without irradiation.

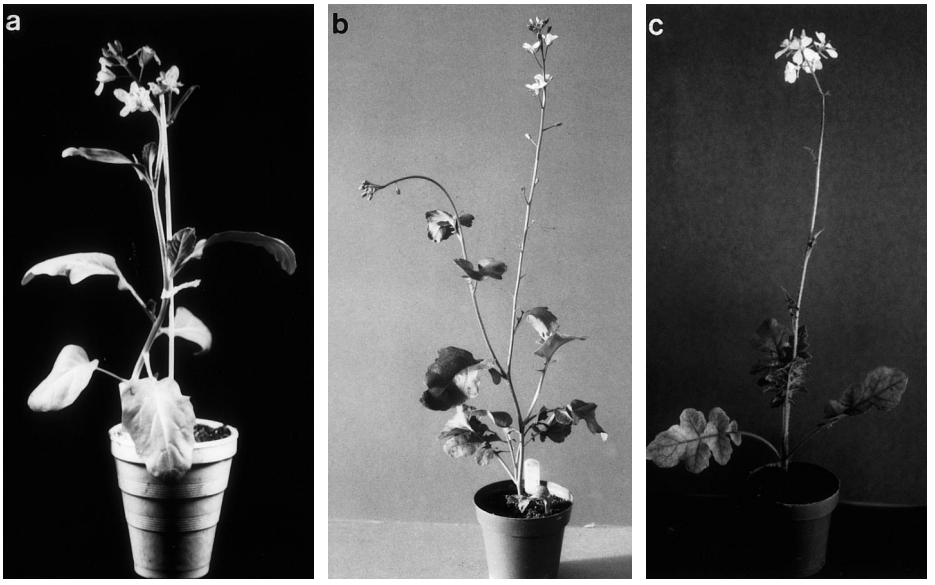

Fig. 3 **a** Rapid cycling *B. oleracea*; plant height is 30 cm. **b** Somatic hybrid plant; plant height is 50 cm. **c** *S. alba*, Alt 543; plant height is 40 cm

Table 3 Analysis of regenerants from protoplast fusions between RC[a] *B. oleracea* and *S. alba*

Fusion	Callus	Regenerant	Donor treatment (krad)	DNA content (pg)	Isozyme pattern		Disease severity rating	Pollen release[b] (+/−)
					PGI	LAP		
18	1	1-1	0	2.55	S.a.[d]	B.o.[e] + S.a.	5	+
		1-2	0	2.49	S.a.	B.o. + S.a.	5	+
		1-3	0	2.41	S.a.	B.o. + S.a.	4	+
		1-4	0	2.47	S.a.	B.o. + S.a.	4	+
		1-5	0	2.48	S.a.	B.o. + S.a.	3	+
		1-6	0	NT[c]	NT	NT	NT	NT
		1-7	0	NT	NT	NT	NT	NT
	2	2-1	0	2.46	S.a.	B.o. + S.a.	4	+
		2-2	0	2.36	S.a.	B.o. + S.a.	3	+
	3	3-1	0	2.52	B.o. + S.a. + heterodimer	B.o. + S.a.	3	+
		3-2	0	2.55	B.o. + S.a. + heterodimer	B.o. + S.a.	2	+
	4	4-1	5	2.45	B.o. + S.a. + heterodimer	B.o. + S.a.	3	−
		4-2	5	2.18	B.o. + S.a. + heterodimer	B.o. + S.a.	5	−
19	1	1-1	0	2.48	B.o. + S.a. + heterodimer	B.o. + S.a.	3	−
		1-2	0	2.50	B.o. + S.a. + heterodimer	B.o. + S.a.	4	+
		1-3	0	2.44	B.o. + S.a. + heterodimer	B.o. + S.a.	4	+
		1-4	0	2.56	B.o. + S.a. + heterodimer	B.o. + S.a.	7	−
	2	2-1	0	2.57	B.o. + S.a. + heterodimer	B.o. + S.a.	3	−
	3	3-1	0	2.78	B.o. + S.a. + heterodimer	B.o. + S.a.	8	+
		3-2	0	2.55	B.o. + S.a. + heterodimer	B.o. + S.a.	3	−
		3-3	0	2.42	B.o. + S.a. + heterodimer	B.o. + S.a.	2	+
		3-4	0	2.62	B.o. + S.a. + heterodimer	B.o. + S.a.	3	+
	4	4-1	10	2.24	B.o. + S.a. + heterodimer	B.o. + S.a.	2	−
		4-2	10	2.47	B.o. + S.a. + heterodimer	B.o. + S.a.	2	−
		4-3	10	2.67	B.o. + S.a. + heterodimer	B.o. + S.a.	4	−
20	1	1-1	30	2.40	NT	NT	4	−
		1-2	30	2.37	NT	NT	4	−

[a] Rapid cycling
[b] +, Occasional low-quantity release; −, no release
[c] Not tested. Plants did not survive transfer to soil
[d] *S. alba* banding pattern
[e] *B. oleracea* banding pattern

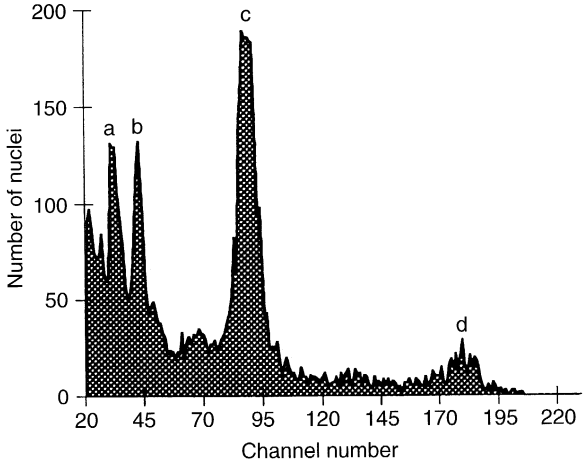

Fig. 4 Flow cytometric estimation of nuclear DNA content in one of the symmetric somatic hybrids (18-1-3) and its two diploid parents. Peaks are: **a** *S. alba*, 2C = 1.07 pg; **b** rapid cycling *B. oleracea*, 2C = 1.29 pg; **c** somatic hybrid, 2C = 2.41 pg; **d** somatic hybrid, G2

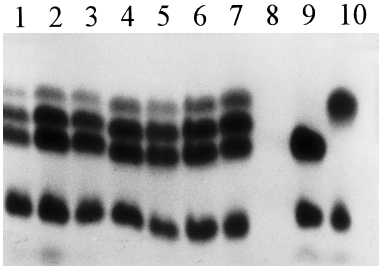

Fig. 5 Isozyme analysis of 4 symmetric and 3 asymmetric somatic hybrids. The enzyme is phosphoglucose isomerase. *Lanes 1–3*. Somatic hybrids from the 10-krad fusion, *lanes 4–7* symmetric somatic hybrids, *lane 8* empty, *lane 9 S. alba*, Alt 543, *lane 10* rapid cycling *B. oleracea*

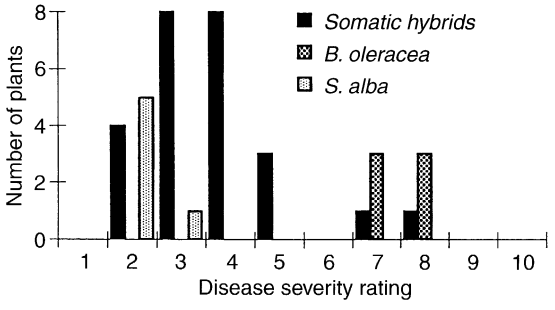

Fig. 6 Reaction to inoculation with *A. brassicae* of 25 somatic hybrids and 6 plants each of rapid cycling *B. oleracea* and *S. alba* tested together with the somatic hybrids

The pollen only showed 1–5% stainability with aceto-carmine. Cytogenetic studies of meiosis in pollen mother cells from 2 of the somatic hybrids (18-1-3 and 18-3-2) revealed that bivalent formation was the most common; however, univalents were also observed

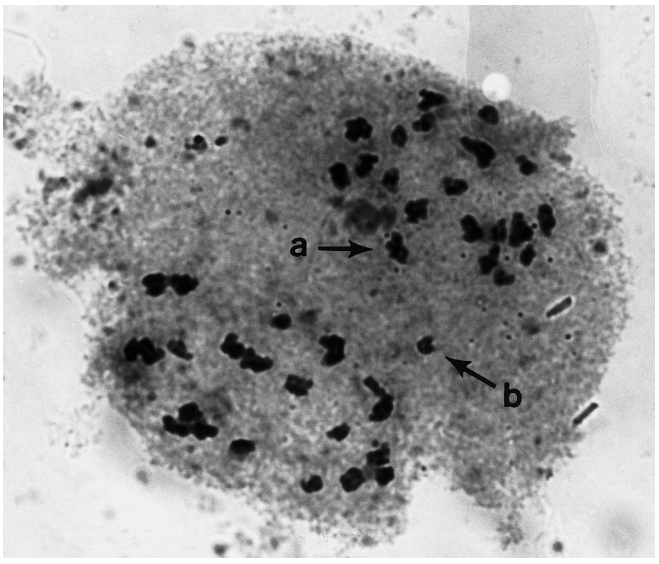

Fig. 7 Meiosis in a pollen mother cell of a somatic hybrid (1000 ×). *Arrows* are: *a* bivalent, *b* univalent

occasionally (Fig. 7). The number of chromosomes observed support the measured DNA content being close to the sum of *B. oleracea* (n = 9) and *S. alba* (n = 12). As expected from the low pollen viability, self pollinations did not yield any seeds. Crosses to the somatic hybrids using three different *B. oleracea* genotypes as pollinators were largely unsuccessful. No pollen germination was observed on untreated stigmas of somatic hybrids, nor on styles with removed stigmas. In one cross using the white-flowered broccoli line as the pollinator after treating the stigma of a bud with 0.25 *M* NaCl, approximately 25% of the pollen grains germinated, although with curled, stunted tubes.

Discussion

Protoplast fusions between rapid cycling *B. oleracea* and *S. alba* yielded some somatic hybrids with high resistance to *A. brassicae*. Regeneration frequencies of 0.18–0.81% were obtained using the regeneration system previously described for rapid cycling *B. oleracea* (Hansen and Earle 1994a). One reason for the relatively low values is that many of the calli were unfused *S. alba*, which were non-regenerable. In addition, irradiation may have reduced regeneration, since the highest regeneration frequency was seen when no irradiation was applied. As is characteristic for the rapid cycling genotype, several shoots could be excised from each regenerating callus, making the generation of plants very efficient. In comparison, Primard et al. (1988) regenerated hybrids from approximately 0.1% of the isolated calli in fusions between *B. napus* and *S. alba*

without the use of irradiation. Toriyama et al. (1987) fused a different *Sinapis* species, *S. turgida*, with *B. oleracea*. Although only 6 calli were isolated, all regenerated shoots. However, no whole plants were obtained.

There was no apparent reduction in the DNA content of the somatic hybrids generated from fusions involving irradiation of the resistance donor. The plant material was irradiated as whole leaves prior to enzyme treatment. If repair mechanisms are functional during the overnight enzyme treatment and not in the isolated protoplasts, the irradiation treatment could be less efficient when applied to the intact leaves. However, Hall et al. (1992) found no difference in the amount of DNA damage in gamma-irradiated sugarbeet (*Beta vulgaris* L.) suspension culture protoplasts treated before or after enzyme digestion.

The somatic hybrids showed a variety of responses to inoculation with *A. brassicae*, but most had good to moderate resistance. In contrast, none of the somatic hybrids between *B. napus* and *S. alba* (Primard et al. 1988) had resistance to *A. brassicae* as high as in *S. alba*. A factor that may have contributed to the variable reactions of our somatic hybrids is that the plants tested were grown from cuttings, which gave them variable growth habits and leaf ages. Another possibility is that the various somatic hybrids developed from fusions involving protoplasts from different *S. alba* plants with various levels of resistance, since protoplasts were isolated from more than 1 plant in each fusion. This, however, does not explain the different reactions seen in somatic hybrids coming from the same callus. It is possible that variations in organellar composition might have influenced the expression of resistance.

The male fertility of the somatic hybrids was very low. Only a few plants shed a limited amount of pollen at certain times, which in part may have been determined by environmental factors. One reason for low fertility may be alloplasmic male sterility caused by incompatibility between the nuclear and the cytoplasmic genomes. Male sterility or low fertility is often seen in interspecific somatic hybridizations in the Brassicaceae (e.g., Chiang and Crete 1987; Kirti et al. 1992; Lelivelt and Krens 1992). Lelivelt et al. (1993) found low pollen production in somatic hybrids between *B. napus* and *S. alba*, and observed disturbed meiosis. In contrast, Primard et al. (1988) obtained male-fertile somatic hybrids between *B. napus* and *S. alba* and were able to backcross the hybrids to *B. napus*. Sexual hybrids between these two species were best obtained using *S. alba* as the female parent (Ripley and Arnison 1990; Chevre et al. 1994; Lelivelt et al. 1993). Both sterile (Ripley and Arnison 1990) and fertile (Chevre et al. 1994; Lelivelt et al. 1993) F_1 plants were generated. However, pollen fertility was improved significantly by chromosome doubling (Chevre et al. 1994). The somatic hybrids produced in this study have two full sets of chromosomes, and chromosome doubling therefore should not help fertility. The sexual hybrids between *B. oleracea* and *S. alba* (Harberd and MacArthur 1980) were sterile.

Although *S. alba* and *B. oleracea* belong to the same tribe (*Brassiceae*), using RAPD markers Demeke et al. (1992) found that *S. alba* is not closely related to the *Brassica* species. Its closest relative in the *Brassica* triangle (U 1935) is *B. juncea*, which is related to *S. alba* through *Raphanus sativus* (Demeke et al. 1992). This may explain the feasibility of sexual crosses between *B. juncea* and *S. alba* even without the use of embryo rescue techniques (Bijral et al. 1991) as well as the difficulties of hybridizing *B. oleracea* and *S. alba*.

In addition to protoplast fusions between rapid cycling *B. oleracea* and *S. alba*, intertribal protoplast fusions were carried out between rapid cycling *B. oleracea* and *Camelina sativa*, which is highly resistant to *A. brassicae*. *C. sativa* belongs to the tribe *Sisymbrieae*. Plants have successfully been obtained in intertribal somatic hybridizations between *B. napus* and *Arabidopsis thaliana*, which also belongs to the *Sisymbrieae* (Gleba and Hoffmann 1980; Bauer-Weston et al. 1993; Forsberg et al. 1994), between *Thlaspi perfoliatum* and *B. napus* (Fahleson et al. 1994b), between *Barbarea vulgaris* and *B. napus* (Fahleson et al. 1994a), and between *C. sativa* and *B. carinata* (Narasimhulu et al. 1994). In the latter two cases the plants did not survive transfer out of culture. In our fusions between *C. sativa* and *B. oleracea*, 1 shoot was initiated, but no plants were recovered. It is possible that further work and adjustments could yield somatic hybrids between the two species. It was essential to use very young plants of *C. sativa* for protoplast isolation (2–3 weeks old), since the plants could not be maintained in vitro, either on MS-0,3 or White's medium (Singh and Krikorian 1981).

This work demonstrates the generation of intergeneric somatic hybrids between *B. oleracea* and *S. alba* expressing resistance to *A. brassicae*. Further attempts to recover progeny from the resistant somatic hybrids are in progress.

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