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Transformation of *Brassica oleracea* with an *S*-locus gene from *B. campestris* changes the self-incompatibility phenotype

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Summary. An *SLG* gene derived from the *S*-locus and encoding and *S*-locus-specific glycoprotein of *Brassica campestris* L. was introduced via *Agrobacterium*-mediated transformation into *B. oleracea* L. A self-incompatible hybrid and another with partial self-compatibility were used as recipients. The transgenic plants were altered in their pollen-stigma interaction and were fully compatible upon self-pollination. Reciprocal crosses between the transgenic plants and untransformed control plants indicated that the stigma reaction was changed in one recipient strain while the pollen reaction was altered in the other. Due to interspecific incompatibility, we could not demonstrate whether or not the introduced *SLG* gene confers a new allelic specificity in the transgenic plants. Our results show that the introduced *SLG* gene perturbs the self-incompatibility phenotype of stigma and pollen.

Key words: *Brassica campestris* – *B. oleracea* – Self-incompatibility – *SLG* gene – Transgenic plants

Introduction

Many plants possess mechanisms that prevent self-fertilization. One of the barriers to self-pollination, self-incompatibility (SI), is manifested by the inhibition of pollen germination and/or pollen tube growth in the pistil. In self-incompatible strains of the family Brassicaceae, pollen is rejected at the stigma surface. In this family, SI is controlled by alleles at the *S*-locus, and the phenotype of pollen is determined by the diploid genotype of the sporophyte (Bateman 1955). It is observed that an SI response occurs when the same *S*-allele is expressed in pollen and stigma. One hypothesis explains the SI reaction on the basis of the expression of a single *S*-locus gene in anther and stigma (de Nettancourt 1977; Nasrallah and Nasrallah 1989).

DNA sequences that are genetically linked to the *S*-locus have been isolated from *Brassica oleracea* and are designated *SLG* genes (Nasrallah et al. 1985b, 1988). The *SLG* gene was shown to be expressed specifically in the papillar cells of the stigma. The expression of the gene during pollen development is less well characterized and its protein products in anther and/or pollen remain elusive. However, transformation of the related crucifer *Arabidopsis* with a chimeric construct consisting of an *SLG* gene promoter fused to the reporter gene β-glucuronidase (*GUS*) showed the promoter to be active in the tapetal cells of the anther as well as in the stigma (Toriyama et al. 1991). Based on these observations, the *SLG* gene appears to have the requisite expression profile for the determination of the pollen-stigma interaction of incompatibility. It is expected that the transformation of recipient plants with an *SLG* gene will allow the production of *S*-locus specific glycoprotein (SLSG) encoded by the transgene and will affect the SI phenotype of recipient plants.

Preliminary experiments in our laboratory had established that transformation is feasible in a limited number of cultivars of *B. oleracea*. We found that two hybrids, Green Comet and Kairan, were amenable to transformation by agrobacterial infection. However, these commercial hybrids have complex S-genotype constitution and little is known about their endogenous *S*-alleles. Despite these drawbacks, we transformed Green Comet and Kairan with an *SLG* gene isolated from the *B. campestris* S_8 homozygote to test the hypothesis that the transgene could influence the pollen-stigma interaction of incompatibility. In this paper, we report that phenotypic changes in the pollen-stigma response stemming from the

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introduction of the S_8 transgene were evident in the recipient plants, confirming the expectation that the cloned gene plays a role in this cellular response.

Materials and methods

Isolation of the SLG gene

A *B. campestris* L. S_8 homozygote (Takayama et al. 1987) was used as a gene-donor plant. The *SLG* gene was identified by hybridization to a gene-specific probe derived from the untranslated 3′ end of the SLSG-encoding cDNA from the *B. olereacea* S_{13} homozygote (Nasrallah et al. 1988). A 20-kb EcoRI restriction fragment was identified on genomic DNA blots as containing the *SLG* gene. A subgenomic library enriched in this fragment was constructed in the bacteriophage vector lamda GEM11. Positive clones were identified by hybridization to the gene-specific probe. The region containing the SLSG-encoding sequence was determined by probing with S_{13} cDNA. A 6.8-kb EcoRI-KpnI fragment containing the S_8 SLSG-encoding region, 1.6 kb of 5′ upstream flanking sequence, and 3.6 kb of 3′ downstream sequence was inserted into the Ti binary vector pBIN19 (Bevan 1984). A selectable marker consisting of the Cauliflower Mosaic Virus (CaMV) 35S promoter, the hygromycin phosphotransferase coding region, and the CaMV 35S terminator was isolated from pCIB709 (Rothstein et al. 1987) as a 2.2-kb HindIII-KpnI fragment and also inserted into the Ti vector. The resulting vector, pKTS8 (Fig. 1), was mobilized into *Agrobacterium tumefaciens* strain pCIB542 (derived from helper plasmid pEHA101; Hood et al. 1986) and MP90 (Koncz and Schell 1986) by triparental mating (Rogers et al. 1988).

Plant transformation

Two *B. oleracea* commercial F_1 hybrids were used as gene-recipient plants: "Kairan" [Chinese Kale, *B. oleracea* L. var. *alboglabra* (L. H. Bailey) Musil cv Hakushin; Sakata Seed, Japan] and broccoli (*B. oleracea* L. var. *botrytis* L. cv Green Comet; Takii Seed, Japan). Flower stem disks were transformed as described by Fry et al. (1987) with the following modifications. Hygromycin (20 mg/l) was used for selecting transformants instead of kanamycin. Hygromycin was added to the medium for 3 weeks starting from a week after coculture. Hygromycin resistance was confirmed by placing leaf segments of regenerated shoots on an expression assay medium (Fry et al. 1987) containing 50 mg/l hygromycin. Untransformed leaf segments were bleached in a week, while transformed leaves remained green and produced callus. The transgenic plants thus identified were grown in a greenhouse with supplemented illumination (day: 14 h, 21 °C; night: 10 h, 18 °C).

DNA gel blot analysis

DNA was isolated from leaves by the cetyltrimethylammonium bromide (CTAB) method (Rogers and Bendich 1988) and digested with EcoRI. Following electrophoresis on a 0.7% (w/v) agarose gel, the DNA was transferred to a Gene-Screen Plus (New England Nuclear) membrane. The probe was a ^{32}P-labeled 1.6-kb ClaI-BamHI fragment of pKTS8, which contained the SLSG-encoding sequences (Fig. 1). In the remainder of the paper, this probe will be designated as the "S_8 probe." Hybridization conditions were as described previously (Nasrallah et al. 1988). The membrane was washed in 0.1 × SSC, 0.1% SDS at 60 °C.

Chromosome counting

Somatic chromosomes from root tips were prepared by an enzyme-maceration and air-dry method, and stained with Giemsa as described in Toriyama and Hinata (1988).

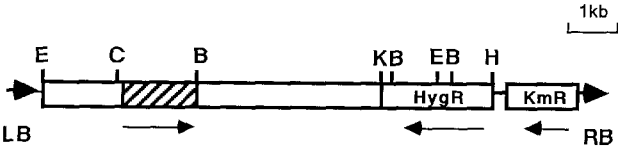

Fig. 1. A partial restriction map of the transformation vector pKTS8. The coding region of the S_8 *SLG* gene is indicated by the hatched area. KmR: the Kanamycin-resistance gene of pBIN19; HygR: the Hygromycin-resistance gene from pCIB709; LB: left border; RB: right border; B: BamHI; C: ClaI; E: EcoRI; H: HindIII; K: KpnI. *Arrows* indicate the 5′ to 3′ orientation of the genes

Protein immunoblot analysis

Protein was extracted from stigmas and other plant tissues in 10 m*M* TRIS-HCl buffer, pH 7.2. Protein concentration was determined using a Coomassie protein assay reagent (Pierce). The extract was subjected to SDS polyacrylamide gel electrophoresis on a 10% resolving gel or to thin-layer polyacrylamide gel isoelectric focusing (IEF; pl 3.5–9.5), as previously described (Nasrallah et al. 1985a). After blotting onto nitrocellulose membrane, the *S*-locus specific glycoprotein (SLSG) was detected by the anti-SLSG monoclonal antibody, MAb/H8, as described in Kandasamy et al. (1989).

Pollination tests

Stigmas were pollinated and incubated overnight. Following fixation in ethanol/acetic acid (3:1), the stigmas were softened in 1 *M* NaOH, stained with decolorized aniline blue, and squashed (Kho and Baer 1968). Pollen tube growth was directly monitored by UV fluorescence microscopy, and the number of pollen tubes penetrating the papillar cells was counted and recorded on a per stigma basis. Pollination tests were repeated on several flowers over a period of at least 2 days.

Results

Transgenic Kairan

Three independent hygromycin-resistant (HygR) plants were obtained following transformation of the self-incompatible Kairan F_1 hybrid with pKTS8. These plants were analyzed for the integration and expression of the transgene. In these experiments, the original plants from which our tissue explants were derived served as controls. Each untransformed control plant was given a designation consisting of the letter "N" (for negative control) followed by the number of the transgenic plant derived from that plant. The results of DNA blot analysis of the HygR and control plants are shown in Fig. 2a. The untransformed control, no. N1, exhibited a 7.8-kb and a 7.0-kb restriction fragment that hybridized with the S_8 probe. The HygR plant no. 1 showed a strong hybridization signal at 7.8 kb that corresponded to the introduced gene (EcoRI fragment of pKTS8; Fig. 1). The hybridization patterns of HygR plants no. 2 and no. 3 were not different from the untransformed control. These plants apparently did not contain the coding region of the S_8 transgene although they were hygromycin-resistant.

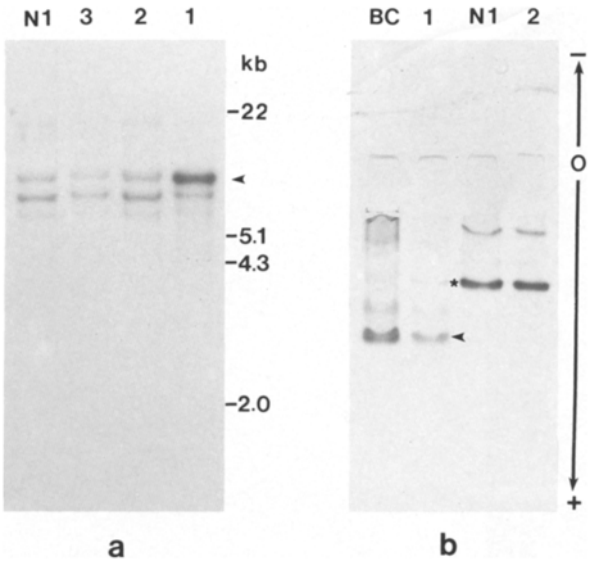

Fig. 2 a, b. DNA-gel blot (**a**) and protein IEF-gel blot (**b**) of transgenic Kairan (1, 2, and 3), untransformed control (N1) and *B. campestris* homozygous for the S_8 allele (BC). **a** Two micrograms of DNA was loaded in each lane. Molecular weight markers in kilobases (kb) are indicated to the *right*. The *arrowhead* points to the restriction fragment containing the introduced *SLG* gene. **b** The amount of protein loaded was 30 μg for the BC lane and 40 μg for the other lanes. o, −, and + indicate the origin, the cathode, and anode, respectively. The * marks the reduced endogenous SLSG and the *arrowhead* points to the SLSG encoded by the S_8 *SLG* gene in transgenic plant no. 1

Chromosome counts on the root tips of the transgenic plants showed 18 chromosomes, confirming that they were diploids.

The analysis of the expression of the S_8 transgene was complicated by the observation that untransformed control Kairan plants synthesized an endogenous stigma protein that reacted with MAb/H8 and migrated on SDS gels with nearly the same mobility as the SLSG of the *B. campestris* S_8 donor. However, the SLSG of untransformed Kairan consisted of two bands, while the SLSG of the transgenic plant exhibited an additional higher molecular weight form as shown in Fig. 5. This pattern is typical of the SLSG encoded by the *B. campestris* S_8 allele. The transgene-encoded SLSG and the endogenous SLSG could be unequivocally distinguished from one another following IEF-gel electrophoresis, since they exhibited distinct pI points of 8.4 and 6.3, respectively. As shown in Fig. 2 b, transgenic plant no. 1 showed a strongly immunoreactive band at pI 8.4 and a faint band at pI 6.3. This result indicates that the S_8 SLG transgene was expressed, but that the expression of the endogenous *SLG* gene was reduced. Plants no. 2 and no. 3 exhibited the same protein profile as their respective control plants (no. N2 and no. N3) and control plant no. N1, consistent with the results of our DNA blot analysis.

Table 1. Pollination tests among transgenic (no. 1, no. 2, and no. 3) and control plants (no. N1, no. N2, and no. N3). The number of pollen tubes per stigma is shown as a mean ± SD. The number of stigmas observed is shown in parenthesis. BC: *B. campestris* homozygous for the S_8 allele

	Pollen						
	N1	N2	N3	2	3	1	BC
Stigma							
N1	3±3 (20)					4±6 (25)	2±5 (12)
N2		1±1 (5)		1±3 (5)			
N3			2±3 (9)				
2		4±2 (7)		7±6 (5)			3±4 (12)
3					2±2 (10)		
1	210±92 (34)					203±80 (42)	2±3 (11)

Phenotypic analysis was carried out by observing pollen tube growth on stigmas following pollination and incubation for 6 h or overnight. The results of these pollination tests are presented in Table 1 and are illustrated by the photographs of pollen tubes shown in Fig. 3. Self-pollination of untransformed Kairan resulted in the growth of fewer than ten pollen tubes in the stigma, confirming that this cultivar was self-incompatible (Table 1, Fig. 3). In contrast, self-pollination of transgenic plant no. 1 resulted in the growth of 203 ± 80 (mean ± SD) pollen tubes, and this plant was therefore self-compatible. In addition, the stigmas of this transgenic plant were compatible with the pollen of the untransformed control no. N1 plant since an average of 210 ± 92 was produced. The pollen phenotype of transgenic plant no. 1 was unchanged, however, and its pollen was incompatible with the stigma of the untransformed control no. N1 plant, with an average of only 4 ± 6 pollen tubes produced. Thus, the plant became self-compatible due to a change in the reaction of the stigma but not in that of pollen. Pollination tests revealed that HygR plants no. 2 and no. 3, which did not possess S_8 antigen, behaved identically to their respective untransformed controls no. N2 and no. N3 and were cross-incompatible. It is concluded that only the plant with an integrated functional S_8 *SLG* gene was modified in its stigma phenotype. The contribution of the *SLG* transgene to allelic specificity could not be tested due to interspecific incompatibility between *B. campestris* and *B. oleracea*. The stigmas of Kairan con-

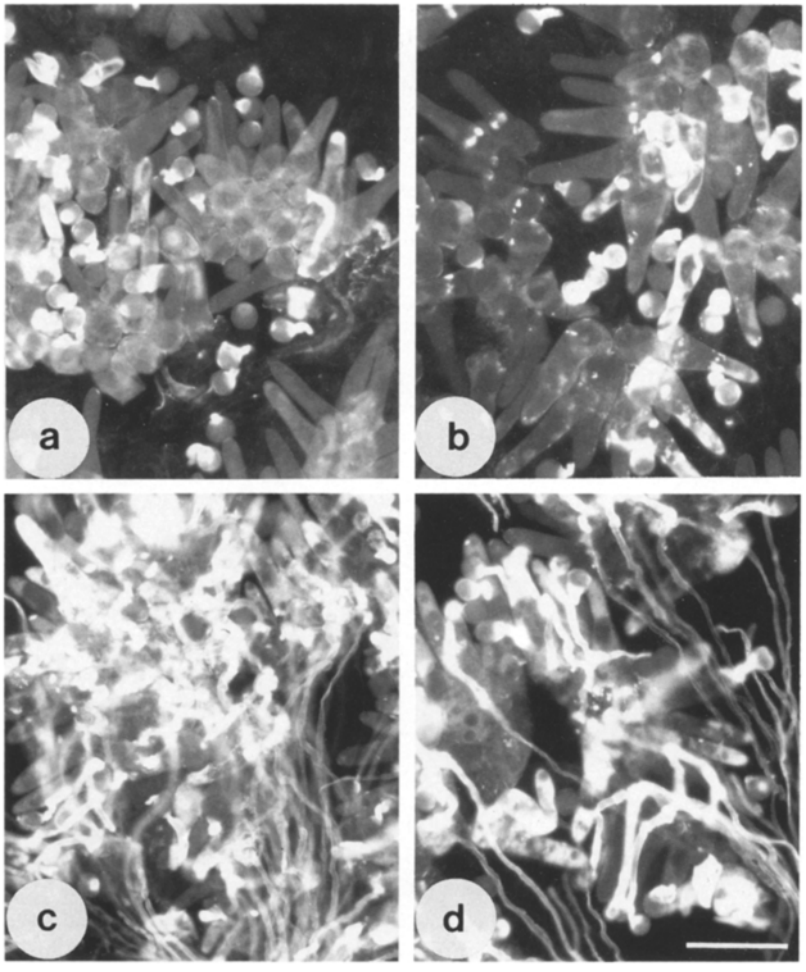

Fig. 3a–d. Pollen tube growth in diallel crosses between transgenic Kairan and a control untransformed plant. The photographs were taken under UV fluorescence microscopy. **a** Self-pollination of the control plant. **b** Cross-pollination of a control stigma with transgenic pollen. **c** Cross-pollination of a tansgenic stigma with control pollen. **d** Self-pollination of the transgenic plant. Note the incompatible reaction in **a** and **b** and the compatible reaction in **c** and **d**. *Bar* = 100 μm

trol no. N1 were found to reject (be incompatible with) *B. campestris* S_8 pollen which germinated but failed to invade the papillar cells. Transgenic plant no. 1 showed the same reaction, indicating that the transgenic stigma retained the potential to reject pollen tube penetration into papillar cells.

Transgenic broccoli

A commercial F_1 hybrid of broccoli "Green Comet" was also used as recipient. Although an F_1 hybrid is expected to contain a single genotype, we noticed that our lot of seed was heterogeneous and produced two types of plants differing in S-genotype, suggesting that more than two alleles (parental genotypes) were used in the breeding of Green Comet. This conclusion was based on immunoblot analysis of stigma extracts with MAb/H8. In previous work (Kandasamy et al. 1989), we had reported that this monoclonal antibody reacted with the SLSG of a group of *S*-alleles, which we designated CRM^+ (cross-reacting material positive), but failed to react with the SLSG of another group of *S*-alleles, namely, pollen recessive alleles, which we designated CRM^- (cross-reacting material negative). Among the F_1 hybrid population, we found plants that reacted with MAb/H8 and plants that did not, indicating that CRM^+ and CRM^- alleles were represented.

CRM^+ and CRM^- plants were transformed with pKTS8, and a number of HygR regenerants were obtained. Chromosome counts on root-tip cells showed that some regenerated plants were tetraploid. Since tetraploidly often results in pollen abortion, only the diploid regenerants were characterized further. Seven independent diploid HygR plants were obtained from the CRM^- recipients, while no diploid plants were obtained from the CRM^+ recipients. As negative controls, and since the recipient plants were not saved, we used hygromycin-sensitive "soma sib" plants regenerated from the same stem disks that produced the HygR plants. The hygromycin-sensitive soma sibs were designated by the letter "N" followed by the number of the HygR transformant originating from the same disk.

Out of seven HygR plants, four contained the S_8 *SLG* transgene as demonstrated by DNA gel blot analysis. In

Table 2. Pollination tests among transgenic Green Comet (no. 26, no. 27, no. 29, no. 24, no. 37, no. 30, and no. 32) and control plants (nos. N24 and N37). The number of pollen tubes per stigma is shown as a mean ± SD. The number of stigmas observed is shown in parenthesis. S2; *B. oleracea* homozygous for the S_2 allele, BC: *B. campestris* homozygous for the S_8 allele

	Pollen										
	N24	N37	26	27	29	24	37	30	32	S2	BC
Stigma											
N24	14±18 (18)	29±14 (8)				212±105 (15)	284±94 (8)				0±0 (5)
N37	12±10 (19)	24±22 (33)		29±28 (24)	8±12 (14)	227±80 (13)	205±76 (12)	244±52 (26)	240±110 (23)	0±2 (18)	0±0 (4)
26			5±10 (12)								0±0 (5)
27		21±28 (20)		18±24 (23)				182±73 (11)	101±89 (11)	0±0 (5)	
29					3±4 (17)	158±116 (7)				0±0 (5)	
24	16±13 (16)	40±22 (11)		24±24 (4)	16±12 (5)	230±112 (16)					0±0 (4)
37	43±24 (9)	34±27 (15)					247±100 (16)				
30		35±30 (32)		22±27 (19)				329±86 (8)		1±2 (16)	0±0 (5)
32		43±35 (28)		26±19 (23)					330±88 (6)		
S_2	33±46 (15)	64±47 (19)	43±37 (6)	99±91 (6)		207±149 (16)	194±134 (13)	268±144 (6)		0±0 (4)	
BC	2±3 (14)	1±1 (18)				1±2 (20)	2±3 (10)	3±4 (5)		7±7 (13)	1±2 (30)
128	284±110 (12)	393±121 (11)	311±104 (6)	223±82 (13)	269±134 (8)					161±91 (12)	

transgenic plants no. 24, no. 37, no. 30, and no. 32 the expected 7.8-kb EcoRI restriction fragment hybridized with the S_8 probe (Fig. 4). Plants no. 26, no. 27, no. 29, and a negative soma sib (no. N37) contained no bands that hybridized with the S_8 probe. Immunoblot analysis of stigma proteins demonstrated that three plants carrying the S_8 transgene produced SLSG with the apparent MW and pI expected for the S_8 encoded molecules (Fig. 5). Plant no. 37 produced the highest level of SLSG, which amounted to approximately 50% of the level observed in the *B. campestris* S_8 donor. A lower level of SLSG was found in plants no. 30 and no. 32, and no SLSG could be detected in plant no. 24, even though 40 μg of protein was loaded in one test. Similarly, plants no. 26, no. 27, and no. 29, and two negative sibs (no. N24 for no. 24, and no. N37 for no. 37) contained no immunoreactive proteins. MAb/H8 did not detect any antigens in anther extracts when 40 μg of protein was loaded per lane.

Phenotypic analysis of the transgenic plants and their negative controls was carried out following self-pollination and in reciprocal cross-pollinations. The results of this analysis are presented in Table 2. We will describe the phenotype of the negative control plants first. Following self-pollination of the negative soma sibs, a few dozen pollen tubes, usually less than 50, were observed per stigma. This number is greater than expected for a fully self-incompatible strain, but significantly lower than expected for a self-compatible strain. This phenotype cannot be ascribed to the general inability of pollen from these plants to efficiently germinate and invade stigmas, since this pollen was shown to be fully functional in

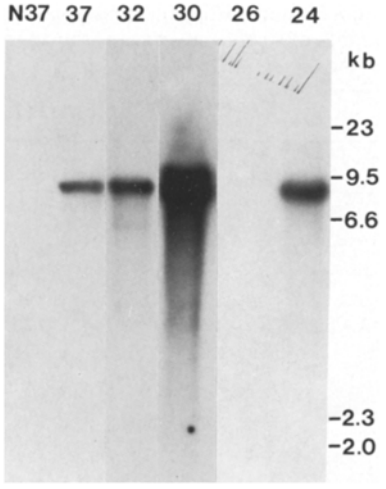

Fig. 4. DNA-gel blot analysis of HygR Green Comet plants (24, 26, 30, 32, 37) and untransformed control (N37). Two micrograms of DNA was loaded in each lane. Molecular weight markers in kilobases (kb) are shown to the *right*

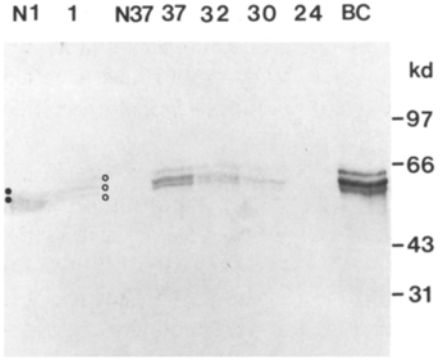

Fig. 5. Protein SDS-immunoblot analysis of stigma proteins from transgenic and untransformed plants. Shown are the patterns obtained for *B. campestris* homozygous for the S_8 allele (BC), the transgenic Green Comet plants (24, 30, 32, 37), the untransformed control (N37), transgenic Kairan (1), and the untransformed control Kairan (N1). Twenty micrograms of total protein was loaded per lane. The *open circles* represent the three moleular weight forms typical of the S_8 allele, and the *closed circles* represent the two molecular weight forms endogenous to Kairan. Molecular weight markers in kilodaltons (kd) are shown to the *right*

crosses to a broccoli plant belonging to the CRM^+ group (plant no. 128 in Table 2). We designate this phenotype as partially self-compatible according to Hodgkin's nomenclature (1978).

The analysis of the transgenic plants demonstrated that they were altered in their pollination phenotype. The self-pollinated stigmas of transgenic plants no. 24, no. 37, no. 30, and no. 32, all of which carried the S_8 transgene, supported the growth of more than 200 pollen tubes per stigma. These plants were therefore fully self-compatible. In contrast, plants no. 26, no. 27, and no. 29, which did not contain the S_8 transgene, remained partially self-compatible. The integration of the S_8 transgene was also associated with altered phenotype in cross-pollinations to the negative soma sibs. In Table 2, we present the data from reciprocal cross-pollinations between transgenic plants no. 24 and no. 37 and their respective negative soma sibs no. N24 and no. N37, but similar results were also obtained with transgenic plants no. 30 and no. 32. Transgenic stigmas supported the growth of less than 50 pollen tubes when pollinated with pollen from the negative sibs, and their phenotype was therefore unchanged. In contrast, the reciprocal pollination showed that the pollen of transgenic plants was fully compatible with the stigmas of negative soma sibs, since more than 200 pollen tubes were observed per stigma. Plants no. 26, no. 27, and no. 29, which did not contain the S_8 transgene, behaved as the negative sibs.

Additional pollinations were carried out to further define the genetic constitution of the recipient plants and the phenotype of the transgenic plants. As described earlier, the fact that the host plants that gave rise to our transgenic plants were CRM^- suggested that these plants carried one or more pollen-recessive *S*-allele. One such allele that is commonly found in commercial cultivars is the S_2 allele. We therefore performed testcrosses with a *B. oleracea* S_2 homozygote (Thompson and Taylor 1966). We found that the stigmas of the control soma sib plant (no. N37) were cross-incompatible with pollen of the S_2 homozygote (Table 2), indicating that this plant carried an S_2 allele. The stigmas of transgenic plants no. 29 and no. 30 were likewise incompatible with S_2 pollen. However, when the pollen of the transgenic plants (no. 24, no. 30, and no. 37) was used to pollinate S_2 stigmas, a larger number of pollen tubes were observed than in the corresponding control pollination (Table 2). This result further supports the conclusion that the pollen phenotype was modified in these transgenic plants.

Discussion

We used an *SLG* gene isolated from a self-incompatible *B. campestris* S_8 homozygote to transform plants of the related species *B. oleracea*. The cloned gene was identified as an *SLG* gene based on its hybridization to a gene-specific probe derived from the 3′ untranslated region of SLG-13 (Nasrallah et al. 1988) and on nucleotide sequence data (Dwyer et al. 1991). Evidence that the *SLG* gene carried on the pKTS8 vector contained all of the regulatory sequences required for its correct expression was determined in transgenic tobacco plants (Umbach et al. 1990). *Nicotiana tabacum* plants harboring the S_8 gene synthesized immunoreactive SLSG in cells of the stigma and style, in a manner similar to that reported for

tobacco plants transformed with other *SLG* genes (Moore and Nasrallah 1990; Kandasamy et al. 1990).

Significantly, the introduction of this gene resulted in the perturbation of the self-incompatibility phenotype. In contrast to the Kairan and Green Comet untransformed plants which were self-incompatible and partial-compatible, respectively, transgenic plants carrying the S_8 transgene were fully self-compatible. Self-compatibility was associated with a change in the stigma phenotype in the case of the Kairan recipient, and with a change in the pollen phenotype in the case of the Green Comet recipient.

One explanation for the compatibility of the transgenic plants is suggested by the observation that the expression of the S_8 *SLG* gene was associated with a drastic reduction in the level of the endogenous SLSG in transgenic Kairan. At the present time, the molecular basis of this reduction is unknown. Reductions in endogenous gene expression are a well-documented effect of introduced antisense genes (van der Krol et al. 1988; Smith et al. 1988). However, we have found no evidence for gene rearrangements in transgenic DNA, and the introduced S_8 *SLG* gene was functional and produced SLSG with the expected electrophoretic properties. The effect we describe here appears to be similar to recently reported examples of sense inhibition of gene expression in transgenic plants, such as the cosuppression of introduced and endogenous flavonoid genes in *Petunia* (Napoli et al. 1990; van der Krol et al. 1990), and the suppression of one copy of T-DNA by the introduction of another copy (Matzke et al. 1989). In any event, concomitant reductions in stigma SLSG and loss of self-incompatibility phenotype have been reported in the analysis of self-compatible mutant strains of *Brassica* (Nasrallah 1974, 1989). It is interesting that in these mutants and in the transgenic Kairan described here, only stigma phenotype, and not pollen phenotype, was affected. Because interspecific incompatibility precluded the analysis of allelic specificity, however, we were unable to determine if the self-compatible phenotype of transgenic Kairan was a direct effect of endogenous *SLG* gene suppression or if it was caused by the acquisition of S_8 specificity.

The phenotype of the transgenic Green Comet plants in which pollen phenotype but not stigma phenotype was modified has not been previously described in *Brassica*. It is unlikely that this phenotype was caused by somaclonal variation, since it was perfectly correlated with the integration of the S_8 *SLG* gene. Rather, we believe this phenotype to be due to the expression of the transgene during pollen development. Recently, plant transformation experiments in our laboratory have demonstrated that *SLG* genes contain regulatory elements that allow their expression in the anthers of transgenic crucifers and solanaceous plants (Toriyama et al. 1991; Thorsness et al. 1991). In particular, a chimeric gene consisting of an *SLG* gene promoter fused to the reporter gene β-glucuronidase was expressed in the tapetal cells of the anther in transgenic *Arabidopsis* (Toriyama et al. 1991). Thus, the phenotypic modification of pollen in transgenic Green Comet may be ascribed to the expression of the transgene in the anther. The elucidation of the molecular basis of this modification has to await the identification of *SLG* gene products in anther tissue. A testable hypothesis is that the endogenous anther products are reduced due to the suppression of the endogenous *SLG* genes or to the dominant action of the S_8 transgene over the pollen-recessive allele(s) of the CRM^- Green Comet recipient plants. As stated above, and due to interspecific incompatibility, it was not possible to determine whether S_8 specificity was acquired by transgenic pollen. It should be noted, however, that the transgenic Green Comet plants were cross-compatible as pollen parents with the transgenic Kairan plant, indicating that S_8 specificity was not imparted simultaneously on the pollen of the former and the stigma of the latter. Transformation experiments in which recipient plants of defined S-genotype and the *SLG* gene-donor plant belong to the same species will be required to test whether or not the *SLG* gene is a determinant of *S*-allelic specificity.

The observation that the phenotypic effects of the introduced S_8 *SLG* gene differed in Kairan and Green Comet indicates that the genetic background and/or the S-genotype of the recipient plant can influence the expression of the transgene. In addition, the fact that the stigma and pollen phenotypes were modified independently of one another, together with the apparent uncoupling of *SLG* gene expression in stigma and anther in transgenic Green Comet plants, suggests the existence of distinct controls on *SLG* gene expression in the two tissues. In any event, the mutant phenotypes generated by the introduction of the S_8 transgene provide direct evidence that the pollen-stigma interaction of incompatibility is influenced by the expression of the *SLG* gene.

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