Latent *S* alleles are widespread in cultivated self-compatible *Brassica napus*

U.U. Ekuere, I.A.P. Parkin, C. Bowman, D. Marshall, and D.J. Lydiate

Abstract: The genetic control of self-incompatibility in *Brassica napus* was investigated using crosses between resynthesized lines of *B. napus* and cultivars of oilseed rape. These crosses introduced eight C-genome *S* alleles from *Brassica oleracea* (S_{16}, S_{22}, S_{23}, S_{25}, S_{29}, S_{35}, S_{60}, and S_{63}) and one A-genome *S* allele from *Brassica rapa* (S_{RM29}) into winter oilseed rape. The inheritance of *S* alleles was monitored using genetic markers and *S* phenotypes were determined in the F_1, F_2, first backcross (B_1), and testcross (T_1) generations. Two different F_1 hybrids were used to develop populations of doubled haploid lines that were subjected to genetic mapping and scored for S phenotype. These investigations identified a latent *S* allele in at least two oilseed rape cultivars and indicated that the S phenotype of these latent alleles was masked by a suppressor system common to oilseed rape. These latent *S* alleles may be widespread in oilseed rape varieties and are possibly associated with the highly conserved C-genome *S* locus of these crop types. Segregation for S phenotype in subpopulations uniform for *S* genotype suggests the existence of suppressor loci that influenced the expression of the S phenotype. These suppressor loci were not linked to the *S* loci and possessed suppressing alleles in oilseed rape and non-suppressing alleles in the diploid parents of resynthesized *B. napus* lines.

Key words: self-incompatibility, *B. oleracea*, *B. rapa*, *S* locus, suppression.

Résumé : Le contrôle génétique de l'auto-incompatibilité chez le *Brassica napus* a été examiné en utilisant des croisements entre des lignées nouvellement synthétisées du *B. napus* et des cultivars de colza. Ces croisements ont introduit au sein du colza d'hiver huit allèles *S* du génome C du *Brassica oleracea* (S_{16}, S_{22}, S_{23}, S_{25}, S_{29}, S_{35}, S_{60} et S_{63}) et un allèle *S* du génome A du *Brassica rapa* (S_{RM29}). La transmission des allèles *S* a été suivie à l'aide de marqueurs moléculaires et les phénotypes S ont été déterminés au sein des générations suivantes : F_1, F_2, backcross 1 (B_1) et testcross 1 (T_1). Deux hybrides F_1 ont été employés pour développer des populations de lignées haploïdes doublées, lesquelles ont fait l'objet d'une cartographie génétique et d'un phénotypage pour l'auto-incompatibilité. Ces études ont permis d'identifier un allèle *S* latent chez au moins deux cultivars de colza et elles ont indiqué que le phénotype S de ces allèles latents était masqué par un système de suppression répandu chez le colza. Ces allèles *S* latents pourraient être très communs chez les variétés de colza et sont possiblement associés au locus *S* en provenance du génome C, lequel est hautement conservé chez cette culture. La ségrégation du phénotype S au sein de sous-populations uniformes pour le génotype au locus *S* suggère l'existence de locus suppresseurs qui influencent l'expression du phénotype S. Ces locus suppresseurs n'étaient pas liés aux locus *S* et présentaient des allèles suppresseurs chez le colza et non-suppresseurs chez les parents diploïdes des lignées synthétisées du *B. napus*.

Mots clés : auto-incompatibilité, *B. oleracea*, *B. rapa*, locus *S*, suppression.

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Introduction

Brassica species have a sporophytic self-incompatibility (*S*) system in which the S phenotype of pollen is controlled by the genotype of the pollen parent (de Nettancourt 1977). A single highly polymorphic *S* locus appears to control the specificity of self-incompatibility in diploid *Brassica* species such as *Brassica oleracea* (Ockendon 1974, 1982) and *Brassica rapa* (Takayama et al. 1987; Suzuki et al. 1999). The self-incompatibility phenotype of both the pollen and stigma are influenced by dominant and (or) recessive relationships between *S* alleles of the parent plant (Thompson and Taylor 1966). These dominant and (or) recessive relationships are complex and can be described in terms of codominance and linear and non-linear dominance (Thompson and Taylor 1966; Ockendon 1975; Hatakeyama et al. 2001; Shiba et al. 2002; Kakizaki et al. 2003). The molecular biology of the self-incompatibility systems of *B. oleracea* and *B. rapa* are well characterized, as reviewed by Silva and Goring (2001). In brief, a *Brassica S* locus consists of two tightly linked genes that act together to control the *S*-haplotype specificity of the stigma in self-incompatible interactions; one encodes an *S*-locus glycoprotein (*SLG*) and the other an *S*-locus receptor kinase (*SRK*). *SLG* is secreted on the stigma surface, whereas *SRK* is a membrane-spanning receptor kinase consisting of an extracellular domain, a transmembrane domain, and a serine/threonine cytosolic domain. Although these two genes are associated with self-incompatibility, it has been demonstrated recently that *SRK* can function to control self-incompatibility independent of *SLG* (Takasaki et al. 2000).

A *Brassica S* locus also encodes a pollen ligand called either the *S* locus cysteine-rich protein (*SCR*, Schopfer et al. 1999) or *S* locus protein 11 (*SP11*, Takayama et al. 2000). This gene controls the *S* specificity of pollen in *Brassica* self-incompatibility. The accepted model for self incompatibility is that the *SLG* and *SRK* function together as the receptor for the pollen ligand and that this receptor–ligand interaction sets off a signaling cascade that leads to rejection of pollen (Silva and Goring 2001). *S* haplotypes are classified into classes I and II based on sequence similarity between their SLGs, SRKs (Nasrallah et al. 1991), and, recently, SP11s (Kakizaki et al. 2003). There is about 35% divergence in amino acid sequences between classes and class I *S* haplotypes are dominant over class II *S* haplotypes (Hatakeyama et al. 2001; Nasrallah and Nasrallah 1993; Thompson and Taylor 1966).

Brassica napus is grown as a major oilseed crop species in Canada (canola), Europe (oilseed rape), China, and Australia. It is an amphidiploid species that arose from interspecies hybridization between *B. oleracea* (the *Brassica* C genome) and *B. rapa* (the *Brassica* A genome) (U 1935; Parkin et al. 1995). Both A- and C-genome *S* haplotypes (Kott 1995; Thompson et al. 1978) can function to determine self-incompatibility in *B. napus*. However, the vast majority of winter oilseed rape and canola varieties are self-compatible (SC) despite the fact that resynthesized lines of *B. napus* (new chromosome-doubled, interspecies hybrids between *B. oleracea* and *B. rapa*) are typically self-incompatible (SI) (Beschorner et al. 1995).

Self-incompatibility is an important characteristic in *B. oleracea* and *B. rapa* vegetables and genetic markers have been developed that allow marker-assisted selection for specific *S* haplotypes in crop breeding (Brace et al. 1993, 1994; Nishio et al. 1996). In *B. napus*, the application of self-incompatibility and its suppression has been proposed as a mechanism for producing hybrid varieties (Thompson 1978; Werner et al. 1995). Genetic analysis has mapped the *S* locus of *B. oleracea* (Bohuon 1995; Ramsay et al. 1996; Camargo et al. 1997) and physical maps containing the *B. rapa* and *B. napus S* loci have been described (Conner et al. 1998; Ciu et al. 1999).

It has been suggested that a 1-bp deletion in the gene encoding SRK at least contributes to making *B. napus* self compatible (Goring et al. 1993) and a transgenic mechanism for engineering self compatibility in *B. napus* has been devised (Stahl et al. 1998). However, the genetic and (or) molecular basis for self compatibility in cultivated *B. napus* has received relatively little attention and the genetic analysis of crosses between SI-resynthesized *B. napus* and SC-domesticated *B. napus* described here has provided insights into the genetic control of self incompatibility and self compatibility in *B. napus*.

Materials and methods

S-allele nomenclature

The *S*-allele nomenclature used to describe and discuss the *S* alleles of *B. napus*, *B. oleracea*, and *B. rapa* was as follows: the letter in superscript before an *S* allele was used to denote its genomic origin (^{O}S, C genome; ^{R}S, A genome) and the combinations of letters and (or) numbers in subscript after an *S* allele were used to distinguish specific alleles. The established *B. oleracea S* alleles were identified using the previously assigned numbers (Ockendon 1974, 1982), whereas combinations of letters specific for the genotype of origin were used to identify previously uncharacterized *S* alleles. For example, the A-genome *S* allele from *B. napus* 'Tapidor' had the following nomenclature: $^{R}S_{Tap}$.

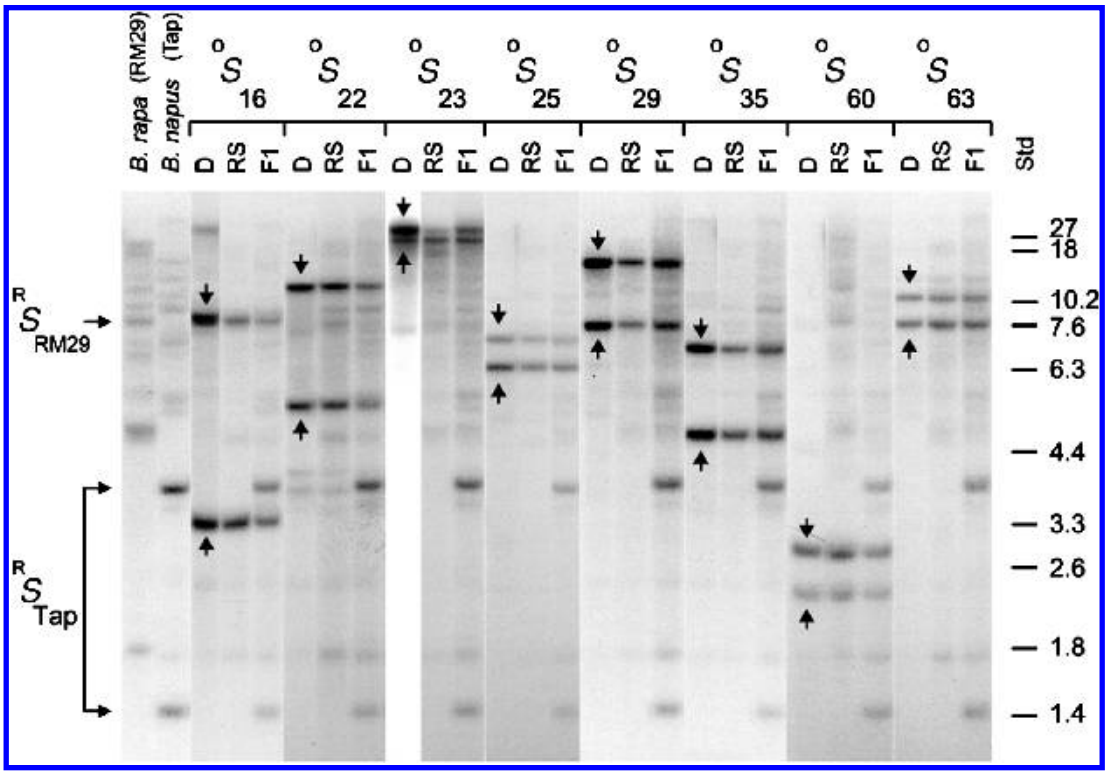
Plant material

Eight inbred *B. oleracea* lines, each homozygous for a previously defined *S* allele, were obtained from D. Ockendon (Horticultural Research International, Wellsbourne, U.K.). These lines were as follows: 0-38, $^{O}S_{16}$; 0-39, $^{O}S_{22}$; 0-40, $^{O}S_{23}$; 0-47, $^{O}S_{25}$; DJ4148, $^{O}S_{29}$; 0-43, $^{O}S_{35}$; 0-46, $^{O}S_{60}$; and DJ4103, $^{O}S_{63}$). RM29.2 was a self-incompatible inbred line of *B. rapa* provided by R. Mithen (John Innes Centre, Norwich, U.K.). The *B. oleracea* lines were each used to pollinate RM29.2 and the resulting hybrid embryos were rescued in tissue culture, propagated, and colchicine doubled as described by Crouch et al. (1994). This produced resynthesized *B. napus* plants to act as donors of specific C-genome *S* alleles in future *B. napus* crosses.

The *B. napus* winter oilseed rape cultivars 'Apache', 'Apex', 'Bristol', 'Express', 'Mandarin', and 'Navajo' were obtained from commercial sources. TapidorDH1 was a microspore-derived, doubled-haploid line developed from 'Tapidor' (Howell et al. 1996).

The *B. napus* mapping populations, N-fo-61-9 (Parkin et al. 1995) and N-fo-61-13 (Parkin 1995), were populations of microspore-derived, doubled-haploid lines. Each population was developed from a single F_1 plant produced from a cross

Fig. 1. Monitoring *S* alleles using Southern hybridization of *Eco*RI-digested plant DNA probed with pSLG29. Tracks 1 and 2 represent the common *Brassica rapa* (RM29.2) and *Brassica napus* ('TapidorDH1') parents, respectively. Tracks 3–26 represent the *Brassica oleracea S*-allele donor (D), the resynthesized *Brassica napus* line (RS), and the F_1 from the cross with 'TapidorDH1' (F_1) for each of the eight introduced C-genome *S* alleles in turn. The RFLPs representing each of the eight *Brassica oleracea S* alleles are identified with pairs of arrows. Std, size standard with values in kilobases.



between a resynthesized *B. napus* line (SYN1) and a doubled-haploid line of winter oilseed rape (N-o-9) (Parkin et al. 1995).

DNA extraction and Southern hybridization technology

DNA extraction, restriction enzyme digestion, Southern blotting, and Southern hybridization were all performed as described by Sharpe et al. (1995). A *Brassica* cDNA clone, SLG29 (Trick and Flavell 1989), and a genomic clone, pW150 (Sharpe et al. 1995), were used as probes. SLG29 was a clone of the gene encoding the *B. oleracea* S_{29} glycoprotein (Trick and Flavell 1989). This gene was known to be intimately associated with the *S* locus (Trick and Flavell 1989). pW150 identified *Brassica* RFLP loci closely linked to the *S* loci (Parkin 1995). The *pW150a* locus of *B. napus* was 1.5 cM below the C-genome *S* locus on linkage group N16 (Parkin 1995), whereas the pW150b locus was 3 cM below the A-genome *S* locus of *B. napus* on linkage group N7 (Parkin 1995).

Pollen-tube fluorescence microscopy

The *S* phenotypes of plants were determined using pollen tube fluorescence microscopy as described by Kho and Baer (1968). To determine the S phenotype of a given plant, four newly opened flowers were self-pollinated and labeled during the morning of the first day and subsequently harvested for pollen tube staining the following day. Pollen tube counts of over 30 pollen tubes/style were taken to indicate compatible pollen–stigma interactions, whereas counts of fewer than 5 pollen tubes/style indicated incompatible interactions and counts of 5–30 pollen tubes/style indicated intermediate interactions.

Results

Self-incompatibility in resynthesized *B. napus*

RFLP analysis was used to confirm the *S* genotype of the resynthesized *B. napus* plants developed as donors of specific C-genome *S* alleles. DNA was extracted from the eight *B. oleracea* parents of the resynthesized lines (each carrying one of the following C-genome *S* alleles: 16, 22, 23, 25, 29, 35, 60, or 63) and the DNA was digested with *Eco*RI and subjected to Southern hybridization analysis using SLG29 as a probe. Each of the eight lines exhibited a distinct restriction fragment length polymorphism (RFLP) pattern, suggesting that the eight ^{O}S alleles could each be uniquely identified using this single molecular assay (Fig. 1). The ^{O}S allele composition of each of the eight resynthesized *B. napus* lines was then tested using Southern hybridization analysis of *Eco*RI-digested DNA probed with SLG29 and all eight lines exhibited the alleles expected of the specific hybrid combinations; that is, $^{R}S_{RM29.2}$ with each of the eight ^{O}S alleles (Fig. 1). The S phenotype of each of the resynthesized *B. napus* lines was tested using pollen-tube fluorescence microscopy and all eight lines were clearly self-incompatible.

F_1 hybrids formed by pollinating a doubled-haploid line of domesticated *B. napus* (TapidorDH1) with each of the eight resynthesized *B. napus* lines were also assayed for *S*

Table 1. The *S* genotypes and S phenotypes of T_1 and B_1 plants derived from crosses between eight F_1 *S*-allele donor plants and three *Brassica napus* winter oilseed rape cultivars ('Apache', 'Apex' and 'TapidorDH1').

	S genotype of F_1 gametes[b]		'Apache'			'Apex'			'TapidorDH1'		
Introduced *Brassica oleracea S* alleles[a]	^{R}S	^{O}S	Si[c]	INT[c]	Sc[c]	SI	INT	SC	SI	INT	SC
$^{O}S_{16}$	RM29	16	NT	NT	NT	3	1	—	2	—	—
	Tap	16	NT	NT	NT	—	1	—	—	2	2
	RM29	Tap	NT	NT	NT	—	1	3	—	—	2
	Tap	Tap	NT	NT	NT	1	—	—	—	—	1
$^{O}S_{22}$	RM29	22	7	1	—	1	—	—	3	—	—
	Tap	22	—	—	—	3	—	—	4	—	—
	RM29	Tap	—	—	—	2	—	1	—	—	2
	Tap	Tap	—	1	1	1	—	3	1	—	—
$^{O}S_{23}$	RM29	23	3	—	—	2	—	—	2	—	—
	Tap	23	1	—	—	2	—	—	2	—	—
	RM29	Tap	—	1	—	—	—	1	2	—	—
	Tap	Tap	1	—	2	—	—	3	1	2	1
$^{O}S_{25}$	RM29	25	1	—	—	—	—	—	3	—	—
	Tap	25	—	—	—	—	2	1	1	3	6
	RM29	Tap	—	—	—	—	1	4	—	—	4
	Tap	Tap	—	1	4	—	—	3	—	—	7
$^{O}S_{29}$	RM29	29	—	1	—	6	—	—	7	—	—
	Tap	29	9	—	—	—	—	—	5	—	—
	RM29	Tap	—	1	4	2	—	1	—	—	9
	Tap	Tap	—	—	3	—	1	1	—	—	5
$^{O}S_{35}$	RM29	35	—	2	—	1	—	1	1	1	2
	Tap	35	—	3	—	2	—	—	1	—	1
	RM29	Tap	—	2	2	—	1	2	1	1	1
	Tap	Tap	—	—	2	—	—	3	—	—	1
$^{O}S_{60}$	RM29	60	NT	NT	NT	4	—	—	1	—	—
	Tap	60	NT	NT	NT	2	1	—	—	2	—
	RM29	Tap	NT	NT	NT	2	—	1	2	1	2
	Tap	Tap	NT	NT	NT	—	—	1	—	1	2
$^{O}S_{63}$	RM29	63	2	1	—	1	—	—	7	—	—
	Tap	63	2	1	—	1	—	—	1	—	—
	RM29	Tap	—	1	1	1	—	1	1	1	—
	Tap	Tap	—	2	2	2	—	—	—	1	—
	Total		26	18	21	39	9	30	48	15	48

[a]*Brassica oleracea S* alleles introduced into the different F_1 plants from their respective resynthesized *Brassica napus* parents.

[b]^{R}S, the A-genome *S* locus; ^{O}S, the C-genome *S* locus.

[c]The number of plants exhibiting different S phenotypes. SI, self incompatible (≤5 pollen tubes/style); INT, intermediate self-incompatibility (6–20 pollen tubes/style); SC, self compatible (≥30 pollen tubes/style); NT, not tested.

genotype, based on Southern hybridization, using SLG29 (Fig. 1) and pW150 as probes, and S phenotype, using pollen-tube fluorescence microscopy. All eight F_1 genotypes inherited the A- and C-genome *S* loci from both TapidorDH1 and the corresponding resynthesized *B. napus* parent. Six of the eight F_1 genotypes (those carrying $^{O}S_{16}$, $^{O}S_{22}$, $^{O}S_{23}$, $^{O}S_{29}$, $^{O}S_{60}$, and $^{O}S_{63}$) were clearly self-incompatible, whereas the remaining two (those carrying the $^{O}S_{25}$ and $^{O}S_{35}$ alleles) exhibited an intermediate S phenotype (6–20 pollen tubes/style). This intermediate phenotype had not been observed in any of the parental material, which was either SC (>30 pollen tubes, as in the case of TapidorDH1) or SI (<5 pollen tubes, as in the case of the eight resynthesized *B. napus* lines).

Latent self-incompatibility in domesticated *B. napus*

Eight F_1 plants, one representing each of the eight TapidorDH1 × resynthesized *B. napus* combinations described above, were each bud pollinated to produce F_2 progeny. The same eight F_1 plants were also used to pollinate the *B. napus* winter oilseed rape varieties 'Apache' and 'Apex' to produce

Table 2. Summary of the frequency with which specific *S* genotypes exhibited particular S phenotypes.

S genotype of F_1 gametes[a]		S phenotype of T_1 and B_1 plants[b]			
R*S*	^{O}S	SI	INT	SC	Total
RM29	X[c]	57	7	3	67
Tap	X[c]	36	15	10	61
RM29	Tap	13	11	41	65
Tap	Tap	7	9	45	61

[a]^{R}S, the A-genome *S* locus; ^{O}S, the C-genome *S* locus.
[b]Number of plants exhibiting each S phenotype. SI, self-incompatible; INT, intermediate self-incompatibility; SC, self-compatible.
[c]X, the eight introduced *Brassica oleracea*, C-genome *S* alleles ($^{O}S_{16}$, $^{O}S_{22}$, $^{O}S_{23}$, $^{O}S_{29}$, $^{O}S_{60}$, and $^{O}S_{63}$).

segregating testcross (T_1) populations and to pollinate TapidorDH1 to produce segregating first backcross (B_1) populations.

The *S* genotype and S phenotype of 6–18 T_1 progeny from each testcross involving 'Apache' or 'Apex' and of 9–26 B_1 progeny from each backcross involving TapidorDH1 were determined and the results of these analyses are presented in Table 1 and summarized in Table 2. RFLP analysis using pW150 and SLG29 as probes unambiguously identified the *S* genotype of each of the 254 T_1 and B_1 individuals and suggested that none of the individuals assayed exhibited recombinant genotypes resulting from crossovers between either the pW150a locus and the linked ^{O}S locus on *B. napus* linkage group N16 or the pW150b locus and the linked ^{R}S locus on N7. Of the 254 T_1 and B_1 plants tested, 113 were clearly self-incompatible, 99 were clearly self-compatible, and the remaining 42 plants exhibited an intermediate S phenotype (Table 2).

The C-genome *S* allele inherited from the F_1 parent played a prominent role in determining the S phenotype of the 254 T_1 and B_1 plants tested (Table 2). Of the 113 self-incompatible plants, 93 had inherited one of the C-genome *S* alleles introduced from *B. oleracea*, whereas only 20 had inherited the TapidorDH1 C-genome *S* allele from the F_1 parent. In contrast, of the 99 self-compatible plants, only 13 had inherited an *S* allele introduced from *B. oleracea*, whereas 86 had inherited the TapidorDH1 C-genome *S* allele from the F_1 parent. The association between the C-genome *S* allele from the F_1 parent and the S phenotype of a plant was therefore highly significant ($\chi^2 = 101.1$; $p \leq 0.0001$). However, the *S* genotype did not allow the accurate prediction of S phenotype in all plants (Tables 1 and 2), suggesting that loci other than the *S* loci also played a role in determining the S phenotype.

Contrary to the general trend, 3 of the 67 plants that inherited the *B. oleracea* C-genome *S* allele and the $^{R}S_{RM29}$ allele from the F_1 parent exhibited a self-compatible phenotype (Table 2). Each of these self-compatible plants carried the $^{O}S_{35}$ allele (Table 1), suggesting that it was less effective at conferring self-incompatibility on *B. napus* than the other seven *S* alleles introduced from *B. oleracea*. Interestingly, there were also seven plants that exhibited a self-incompatible phenotype, but carried only native winter oilseed rape *S* alleles (Tables 1 and 2). Self-incompatible T_1 individuals carrying only winter oilseed rape *S* alleles were also recovered in each case where the F_1 donors of $^{O}S_{22}$, $^{O}S_{23}$, and $^{O}S_{29}$ were crossed with four additional winter oilseed rape varieties ('Bristol', 'Express', 'Mandarin', and 'Navajo'). One explanation for this finding is that TapidorDH1 and possibly the majority of oilseed rape varieties carry a latent, functional *S* allele. If this is the case, the function of this *S* allele must be suppressed in normal oilseed rape varieties.

S allele interactions and the suppression of self incompatibility

Thirty-three F_2 individuals derived from the F_1 donor of the $^{O}S_{25}$ allele were probed with SLG29 and pW150 to determine their *S* genotypes and tested for self-incompatibility. The results of these analyses are summarized in Fig. 2. Figure 2 also includes data for 24 B_1 individuals derived from the same F_1 and described previously in Table 1.

The data summarized in Fig. 2 can be explained by a model that evokes a gene at the A-genome *S* locus of TapidorDH1 that can interfere with the action of functional *S* alleles and the existence of suppressor loci unlinked to the *S* loci. Suppressing alleles at these suppressor loci (presumably common in oilseed rape) would act in concert with the interfering ^{R}S gene to reduce self-incompatibility and non-suppressing alleles (presumably common in *B. oleracea* and *B. rapa*) would act as antagonists to the interfering ^{R}S gene.

The uniform self-incompatibility of the plants homozygous for the RM29 *S* allele ($^{R}S_{RM29/RM29}$; Fig. 2, top row) can then be explained by the absence of the interfering gene and the action of the $^{R}S_{RM29}$ allele. When the A-genome *S* locus is heterozygous ($^{R}S_{RM29/Tap}$: middle row Fig. 2) or homozygous for the TapidorDH1 *S* allele ($^{R}S_{Tap/Tap}$; Fig. 2, bottom row) the situation is more complex. The interfering gene appears to completely disrupt the function of the $^{R}S_{RM29}$ and $^{O}S_{Tap}$ alleles causing uniform self-compatibility (Figs. 2*f* and 2*i*). In contrast, the interaction of the interfering gene with the $^{O}S_{25}$ allele can result in the full range of S phenotypes (SI, INT, or SC: Figs. 2*e* and 2*h*) with the actual phenotype of a given plant controlled by the genotype at hypothetical suppressor loci. The relative abundance of SC plants in Fig. 2 cell h (genotype $^{R}S_{Tap/Tap}$, $^{O}S_{25/Tap}$) compared with the relative abundance of SI plants in Fig. 2*e* (genotype $^{R}S_{RM29/Tap}$, $^{O}S_{25/Tap}$) can be explained by the suppression of self incompatibility requiring the action of suppressor alleles at more loci when the interfering gene is heterozygous ($^{R}S_{RM29/Tap}$) compared with when the interfering gene is homozygous ($^{R}S_{Tap/Tap}$), either because of the dosage effect of the interfering allele or because of synergy between the $^{O}S_{25}$ and $^{R}S_{RM29}$ alleles. The number of plants homozygous for the C-genome S_{25} allele (Figs. 2*a*, 2*d*, and 2*g*) were too few to distinguish their phenotypes from the phenotypes of the corresponding genotypes heterozygous at the C-genome *S* locus (Figs. 2*b*, 2*e*, and 2*h*, respectively).

Latent self incompatibility and the suppression of self incompatibility in the N-fo-61-9 and N-fo-61-13 populations of *B. napus*

The model developed to explain the variation in S phenotypes observed in the F_2 and B_1 populations represented in

Fig. 2. The distribution of S phenotypes associated with nine *S* genotypes in a combined F_2 and B_1 population of *Brassica napus*. *x* axis, genotype at the C-genome *S* locus; *y* axis, genotype at the A-genome *S* locus. (*a–i*) Cells for each of the nine classes of genotype. Numbers on the left of each histogram represent the number of individuals with a particular phenotype. SI, self incompatible; INT, intermediate; SC, self compatible.

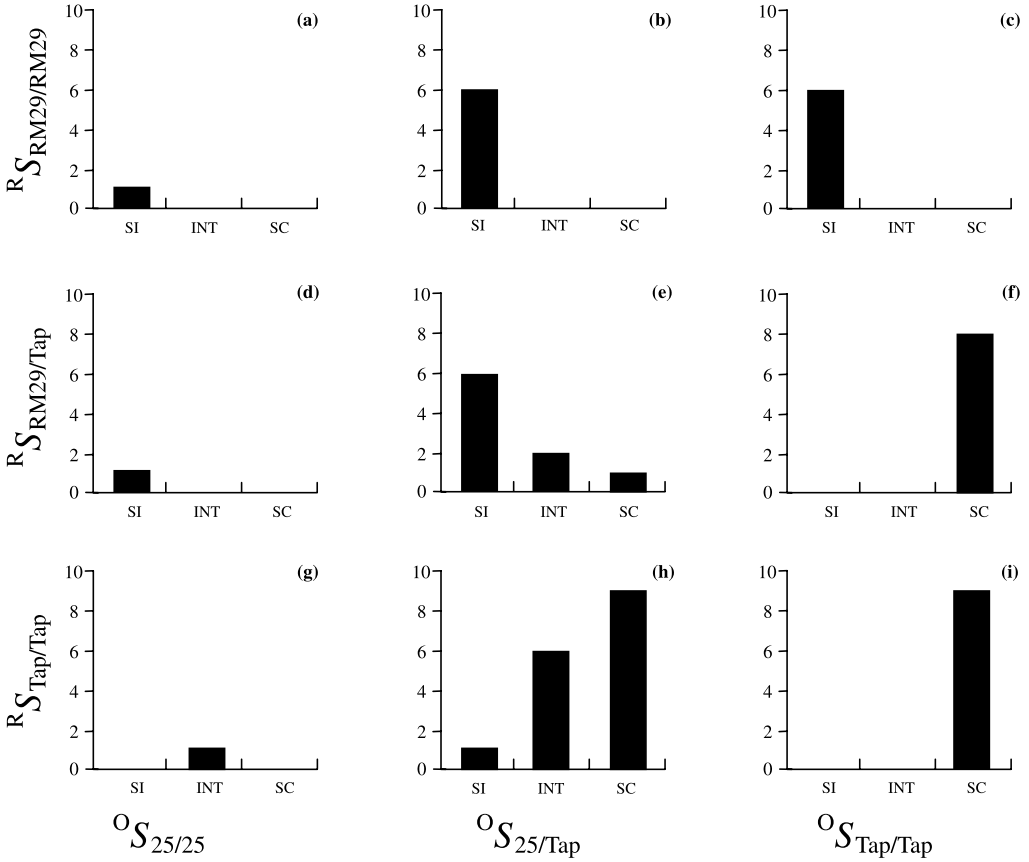


Fig. 2 needed testing in other populations of *B. napus* that segregated for S phenotypes.

The N-fo-61-13 and N-fo-61-9 *B. napus* mapping populations of doubled haploid lines, which have been described previously (Parkin 1995; Parkin et al. 1995), both segregated for S phenotype (Parkin 1995). The relationship between *S* genotype and S phenotype in the N-fo-61-13 population is summarized in Table 3. Three of the four possible genotypes ($^{R}S_{SYN1}$, $^{O}S_{SYN1}$; $^{R}S_{N\text{-}o\text{-}9}$, $^{O}S_{SYN1}$; and $^{R}S_{SYN1}$, $^{O}S_{N\text{-}o\text{-}9}$) only produced lines that were SI (although, in the case of the $^{R}S_{SYN1}$, $^{O}S_{SYN1}$ and $^{R}S_{N\text{-}o\text{-}9}$, $^{O}S_{SYN1}$ genotypes, the sample sizes were too small to be confident that lines with other phenotypes were not possible). In contrast, the $^{R}S_{N\text{-}o\text{-}9}$, $^{O}S_{N\text{-}o\text{-}9}$ genotype produced lines with any one of the three S phenotypes (SI, INT, or SC; Table 3). This finding again suggested the existence of latent *S* alleles in winter oilseed rape cultivars and added extra evidence for the existence of suppressor loci that interact with *S* alleles to control the S phenotype.

Genetic analysis of self-incompatibility using the N-fo-61-9 population was more complex because of cytological abnormalities resulting from the fact that the F_1 parent of the population contained normal copies of N7 and N16 from its oilseed rape parent, but carried two copies of N7 and no copies of N16 from its resynthesized *B. napus* parent (Parkin et al. 1995). N7 and N16 are the chromosomes that carry the A- and C-genome *S* loci, respectively (Parkin 1995). However, the N-fo-61 9 population provided evidence for a gene at or very close to the A-genome *S* locus of oilseed rape (in this case, N-o-9) that interfered with the action of functional *S* alleles. The N-fo-61-9 population contained a subset of lines with one of two related genotypes, $^{R}S_{SYN1}$, $^{R}S_{SYN1}$, $^{O}S_{N\text{-}o\text{-}9}$ and $^{R}S_{SYN1}$, $^{R}S_{N\text{-}o\text{-}9}$, $^{O}S_{N\text{-}o\text{-}9}$ (Table 3), that were both homo-

Table 3. The *S* genotypes and S phenotypes of lines in the N-o-61–9 and N-o-61–13 mapping populations of *Brassica napus*.

Population[a]	*S* genotype[b]	SI[c]	INT	SC
13	$^{R}S_{SYN1}$, $^{O}S_{SYN1}$	3	—	—
13	$^{R}S_{N\text{-}o\text{-}9}$, $^{O}S_{SYN1}$	3	—	—
13	$^{R}S_{SYN1}$, $^{O}S_{N\text{-}o\text{-}9}$	18	—	—
13	$^{R}S_{N\text{-}o\text{-}9}$, $^{O}S_{N\text{-}o\text{-}9}$	5	3	9
9	$^{R}S_{SYN1}$, $^{R}S_{SYN1}$, $^{O}S_{N\text{-}o\text{-}9}$[d]	5	—	—
9	$^{R}S_{SYN1}$, $^{R}S_{N\text{-}o\text{-}9}$, $^{O}S_{N\text{-}o\text{-}9}$[d]	6	6	5

[a]13, the N-o-61-13 population; 9, the N-o-61-9 population.
[b]Every locus is homozygous in the doubled haploid lines of the N-o-61-9 and N-o-61-13 populations; $^{R}S_{N\text{-}o\text{-}9}$, the A-genome *S* allele from line N-o-9; $^{R}S_{SYN1}$, the A-genome *S* allele from line SYN1: $^{O}S_{N\text{-}o\text{-}9}$, the C-genome *S* allele from line N-o-9; $^{O}S_{SYN1}$, the C-genome *S* allele from line SYN1.
[c]The number of doubled haploid lines exhibiting a specific S phenotype. SI, self-incompatible; INT, intermediate self-incompatibility; SC, self-compatible.
[d]These lines were tetrasomic for N7, the *Brassica napus* chromosome carrying the A-genome *S* locus (Parkin 1995; Parkin et al. 1995).

zygous for $^{O}S_{N\text{-}o\text{-}9}$ at the C-genome *S* locus and that both contained four copies of the A-genome *S* locus because they are tetrasomic for N7. However, whereas the first genotype had four copies of the $^{R}S_{SYN1}$ allele and produced only lines that were SI (analogous to the $^{R}S_{SYN1}$, $^{O}S_{N\text{-}o\text{-}9}$ genotype in the N-fo-61-13 population), the second genotype had two copies of the $^{R}S_{SYN1}$ allele and two copies of the $^{R}S_{N\text{-}o\text{-}9}$ allele and produced lines exhibiting each of the three *S* phenotypes (Table 3).

Discussion

Eight defined C-genome *S* alleles were introduced into *B. napus* using a combination of resynthesis (chromosome doubled *B. rapa* × *B. oleracea* interspecies hybrids) and conventional breeding. The inheritance of each newly introduced ^{O}S allele was monitored using RFLP analysis with SLG29 as a probe. SLG29 exhibited a high degree of polymorphism in *B. oleracea* that distinguished a large number of *S* alleles (Fig. 1; Ekuere 1997). RFLP analysis using SLG29 also monitored the oilseed rape ^{O}S genotype but, in contrast to the diploid C genome, SLG29 revealed a strikingly monomorphic RFLP phenotype at the ^{O}S locus in winter oilseed rape and spring canola, suggesting selection for a particular ^{O}S genotype in these crops (Ekuere 1997; D.J. Lydiate, unpublished).

The eight resynthesized *B. napus* lines carrying the *B. rapa* A-genome *S* allele, $^{R}S_{RM29}$, and one of the eight introduced *B. oleracea* C-genome *S* alleles, $^{O}S_{16}$, $^{O}S_{22}$, $^{O}S_{23}$, $^{O}S_{25}$, $^{O}S_{29}$, $^{O}S_{35}$, $^{O}S_{60}$, or $^{O}S_{63}$, were all self incompatible. Self-incompatible plants were also recovered after crossing the resynthesized plants with a winter oilseed rape cultivar and either backcrossing or testcrossing with another oilseed rape cultivar. However, the effects of particular introduced *S* alleles differed from those of other introduced *S*-alleles in the B_1 and T_1 generations. In every case where a B_1 or T_1 plant inherited $^{O}S_{22}$, $^{O}S_{23}$, $^{O}S_{29}$, $^{O}S_{60}$, or $^{O}S_{63}$, the phenotype was most often SI, occasionally INT, but never SC (Table 1). In contrast, only approximately one third of B_1 or T_1 individuals with $^{O}S_{16}$, $^{O}S_{25}$, $^{O}S_{35}$, and $^{R}S_{RM29}$ were SI, whereas approximately one third of such plants were SC (Table 1). These observations suggested that $^{O}S_{22}$, $^{O}S_{23}$, $^{O}S_{29}$, $^{O}S_{60}$, or $^{O}S_{63}$ were functioning in a way similar to class I dominant *S* alleles in the diploid species (Thompson and Taylor 1966; Nasrallah and Nasrallah 1993; Shiba et al. 2002), whereas $^{O}S_{16}$, $^{O}S_{25}$, $^{O}S_{35}$, and $^{R}S_{RM29}$ were functioning like class II recessive *S* alleles (Thompson and Taylor 1966; Nasrallah and Nasrallah 1993; Kakizaki et al. 2003). The variation in the S phenotypes produced by $^{O}S_{16}$, $^{O}S_{25}$, $^{O}S_{35}$, and $^{R}S_{RM29}$ also suggested that these *S* alleles were sensitive to suppression by genetic factors present in oilseed rape genotypes of *B. napus*.

When the *B. oleracea S* alleles $^{O}S_{16}$ or $^{O}S_{25}$ were present in combination with $^{R}S_{RM29}$, the S phenotype was always SI or INT, suggesting an additive interaction between the introduced *S* alleles at the A- and C-genome loci. This observation was contrary to previous reports of intergenomic *S*-allelic interactions that resulted in mutual weakening of the S phenotype (Beschorner et al. 1995; Kott 1995). This might indicate that the intergenomic interactions between dominant *S* alleles in *B. napus* tend to be antagonistic, whereas intergenomic interactions between recessive *S* alleles tend to be synergistic.

Two independent experiments both produced SI plants that carried only oilseed rape *S* alleles at both the A- and C-genome *S* loci. Of the 61 B_1 or T_1 plants identified in Tables 1 and 2 as inheriting the $^{R}S_{Tap}$ and $^{O}S_{Tap}$ alleles from their F_1 parent, 7 were clearly SI. Similarly, of the 17 doubled haploid lines of the N-fo-61-13 population that were fixed for winter oilseed rape alleles at the A- and C-genome *S* loci ($^{R}S_{N\text{-}0\text{-}9}$, $^{O}S_{N\text{-}0\text{-}9}$), 5 consistently exhibited an SI phenotype (Table 3). These observations suggest the existence of a latent oilseed rape *S* allele present in at least two unrelated oilseed rape cultivars and possibly ubiquitous in oilseed rape varieties. Furthermore, the activity of the proposed latent *S* allele must be suppressed in the normal genetic background of oilseed rape.

Three of the experiments described above gave rise to subpopulations of plants or subpopulations of doubled haploid lines that possessed uniform *S* genotypes, but which segregated for S phenotype. The first examples were the B_1 and T_1 plants described in Table 1 that were heterozygous for $^{O}S_{16}$, $^{O}S_{35}$, or $^{R}S_{RM29}$ at one *S* locus and homozygous for oilseed rape *S* alleles at the other *S* locus. The second example is represented in Figs. 2*e* and 2*h*, where subpopulations uniform for the *S* genotypes $^{R}S_{RM29/Tap}$, $^{O}S_{25/Tap}$ and $^{R}S_{Tap/Tap}$, $^{O}S_{25/Tap}$, respectively, clearly segregate for all three S phenotypes. The third example is represented in the fourth and sixth rows of Table 3, where subpopulations of doubled-haploid lines uniform for the *S* genotypes $^{R}S_{N\text{-}o\text{-}9}$, $^{O}S_{N\text{-}o\text{-}9}$ and $^{R}S_{SYN1}$, $^{R}S_{N\text{-}o\text{-}9}$, $^{O}S_{N\text{-}o\text{-}9}$, respectively, again clearly segregate for all three S phenotypes. In every case, the observed segregation for S phenotype can most easily be explained by invoking the action of suppressing oilseed rape alleles at suppressor loci that act to disrupt the ability of recessive *S* alleles such as $^{O}S_{16}$, $^{O}S_{25}$, $^{O}S_{35}$, $^{R}S_{RM29}$, and presumably $^{O}S_{N\text{-}o\text{-}9}$ to confer an SI phenotype on *B. napus*. If these suppressors exist, it follows that at least some of the suppressor loci are unlinked or only weakly linked to the *S* loci and that the diploid parents of the resynthesized *B. napus S* allele donors carry nonsuppressing alleles at the suppressor loci and that these alleles are segregating in the populations segregating for S phenotype.

There is circumstantial evidence for a gene that is either or close to the A-genome *S* locus, which interacts with the hypothesized suppressors and is essential for the disruption of self incompatibility. This gene would have alleles in normal oilseed rape that interfere with self incompatibility and noninterfering alleles in *B. rapa* lines that normally express self-incompatibility. The strongest evidence for the existence of this gene are the contrasting phenotypes of the $^{R}S_{SYN1}$, $^{R}S_{SYN1}$, $^{O}S_{N\text{-}o\text{-}9}$ and $^{R}S_{SYN1}$, $^{R}S_{N\text{-}o\text{-}9}$, $^{O}S_{N\text{-}o\text{-}9}$ genotypes of doubled-haploid lines of the N-fo-61-9 population (Table 3). The experiments described above indicate one other interesting property of the hypothesized *S*-suppression system; that is, that the system is sensitive to the dominance of the *S* alleles with which it interacts and appears incapable of suppressing the activity of strong *S* alleles including $^{O}S_{22}$, $^{O}S_{23}$, $^{O}S_{29}$, $^{O}S_{60}$ or $^{O}S_{63}$.

Self incompatibility in *B. napus* is of interest as a mechanism for producing hybrid canola seed (Kott 1995; Werner et al. 1995). Breeding systems that employ self incompatibility

as a mechanism for hybrid production and subsequently suppress self incompatibility to produce SC F_1 hybrids have also been proposed (Thompson 1978; Werner et al. 1995). The research described above will assist both of these strategies. Oilseed rape germplasm carrying new *S* alleles ($^{O}S_{16}$, $^{O}S_{22}$, $^{O}S_{23}$, $^{O}S_{25}$, $^{O}S_{29}$, $^{O}S_{35}$, $^{O}S_{60}$, $^{O}S_{63}$, $^{O}S_{SYN1}$, $^{R}S_{RM29}$, and $^{R}S_{SYN1}$) and informative genetic markers (SLG29 and pW150) will all contribute to the manipulation and control of self incompatibility in winter oilseed rape and canola. However, perhaps the most important contribution is the development of a genetic model to explain the suppression of self-incompatibility in *B. napus*. Further research will test the proposed model and map the suppressor loci if they exist. It will then be possible to develop genetic markers to allow the selection of defined genotypes at the suppressor loci as well as the *S*-loci, making possible the precise manipulation of self-incompatibility in oilseed rape and (or) canola breeding programs.

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