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Distribution of *S*-haplotypes and its relationship with self-incompatibility in *Brassica oleracea*. Part 1. In inbred lines of cauliflower (*B. oleracea* var 'botrytis')

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Abstract Self-incompatibility in the Brassicaceae is controlled by the single multiallelic *S*-locus which contains at least two genes expressed in the stigma, the *SLG* (*S*-locus glycoprotein) and *SRK* (*S*-locus receptor kinase) genes. The presence of two transcriptional units at the *S*-locus led to the use of the term *S*-haplotype to define allelic forms of the *S*-locus. On the basis of sequence data obtained for *SLG* and *SRK* from different *S*-haplotypes, two classes of *S*-haplotypes have been described in *Brassica*. Here, we report the identification of *S*-haplotypes in 126 plants representing 82 open-pollinated cultivars of *B. oleracea* var 'botrytis' (cauliflower) by means of an immunochemical analysis of SLG products. The antibodies used enabled class I and class II *S*-haplotypes to be discriminated. Ten different *S*-haplotypes were identified, eight of class I, only one belonging to class II and another one for which no SLG products were detected by our antibodies. In heterozygous plants containing the class II *S*-haplotype associated with some particular class I *S*-haplotypes, specific modifications of class II SLGs were found. Pollen tube counts and seed set were used and compared to assess the self-incompatibility phenotypes. SC, a class I haplotype, was always found to be associated with a fully self-compatible phenotype. Half of the plants analyzed possessed the class II *S*-haplotype. Plants homozygous for this haplotype showed various levels of self-incompatibility, from highly self-incompatible to fully self-compatible. These results are discussed with specific reference to, F_1 hybrid breeding.

Key words Self-incompatibility · *S*-haplotype · *S*-locus glycoprotein · Immunochemical analysis · *Brassica oleracea* var 'botrytis'

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Introduction

In the Brassicaceae, the sporophytic origin of self-incompatibility, which is controlled by a single genetic locus (the *S*-locus) with a series of alleles, was first demonstrated by Bateman (1954, 1955). Since then, most of the Brassica crops have been investigated for their *S*-allele distribution (Thompson and Taylor 1965; Ockendon 1974, 1980, 1982).

The correlation between stigmatic proteins and specific sporophytic *S*-alleles was first established by Nasrallah and his colleagues using electrophoresis and immunodiffusion (Nasrallah and Wallace 1967; Nasrallah et al. 1972; Nasrallah 1974). All the *S*-allele proteins appeared to be glycoproteins (Nishio et Hinata 1978, 1982) and were designated "*S*-locus glycoproteins" (SLG). The *SLG* gene encoding these glycoproteins has been isolated and sequenced (Nasrallah et al. 1985, 1988). In *Brassica oleracea*, the *SLG* gene has been shown to belong to a multigenic family which consists of approximately 12 members (Nasrallah et al. 1985, 1988). Among them, another gene situated at the *S*-locus, the *S*-locus receptor kinase (*SRK*) gene, encodes a transmembrane protein kinase, that shares sequence homology with *SLG* (Stein et al. 1991; Boyes et al. 1993). Recently, a third *S*-locus-linked gene designated *SLA* (*S*-locus anther), with anther-specific expression, has been described for the *S2* haplotype of *B. oleracea* (Boyes and Nasrallah 1995). Two other genes, the *SLR-1* (*S*-locus related) and the *SLR-2*, have also been isolated in Brassica species, but they segregate independently of the *S*-locus and are therefore not considered to be involved in the self-incompatibility reaction (Boyes et al. 1991; Lalonde et al. 1989). The *SLG* and *SRK* genes are physically linked at the *S*-locus (Boyes and Nasrallah 1993), and data suggest that the self-incompatible phenotype results from the action of both genes (Nasrallah et al. 1994). The "*S*-haplotype" designation is used here instead of the classical *S*-allele designation (Boyes and Nasrallah 1993).

In *Brassica oleracea*, about 60 different *S*-haplotypes have been described (Brace et al. 1994). Nevertheless, little is known about the *S*-alleles found in cauliflower. In this morphotype,

self-incompatibility has been investigated as a hybridization system by several authors (Watts 1963; Nieuwhof 1974; Gray and Taylor 1977) who found a range of incompatibility phenotypes: summer types were mainly self-compatible, whereas autumn and winter types were rather more self-incompatible. In Brassica, *S*-haplotype surveys have classically been carried out by crossing plants against tester lines from a Brassica *S*-haplotype collection (Ockendon 1975). However, cauliflower was not self-incompatible enough to be studied by this method (Ockendon 1980). More recently, genetic analysis of the self-incompatibility system has been achieved via the identification of *S*-gene products by means of an isoelectric focusing (IEF) analysis of stigma extracts (Nishio and Hinata 1977) and, with more accuracy, by identifying SLG glycoproteins using specific anti-SLG antibodies (Kandasamy et al. 1989; Nasrallah 1989; Gaude et al. 1995). Two classes of *S*-haplotypes have been distinguished according to criteria based on polymorphism among SLG sequences and on the dominance or recessive interactions occurring between haplotypes in heterozygous plants (Nasrallah 1989; Nasrallah et al. 1991). Class I haplotypes exhibit a strong incompatibility phenotype and are placed high in the dominance scale of haplotypes, whereas class II haplotypes confer a weaker incompatibility phenotype and show recessive and competitive interactions in the pollen. Among class I haplotypes, the *SLG* genes share over 90% DNA similarity, whereas the *S2* haplotype, which belongs to class II haplotypes, shares less than 70% DNA sequence similarity with class I haplotypes ; 3′ sequences of class I and class II *SLG* genes do not share any homology (Nasrallah et al. 1991).

The present investigation was performed with inbred lines of cauliflower belonging to a breeding program in which self-incompatibility is used in F_1 hybrid production. Seeds are harvested on one parent only, which is selected for the strength of its self-incompatibility. Inbred lines can be multiplied by seeds (bud pollination), but more often they are vegetatively propagated, mainly because variations in the level of self-incompatibility have often been observed after selfing. A knowledge of *S*-haplotype variability is important for the breeder, not only in selecting the strongest forms but also in assuring cross-compatibility between lines. This paper reports an *S*-haplotype survey of cauliflower lines that used a combination of an isoelectric focusing (IEF) and immunoblot analysis to identify stigmatic SLGs. At the same time the self-incompatibility phenotypes of plants were investigated using two complementary methods: seed set and pollen tube growth observation. Pollen tube counts constitute a direct and rapid assessment of the strength of self-incompatibility and allow comparison between genotypes. Nevertheless, seed production is the final expression of the character and the more accurate measurement for the breeder.

Materials and methods

Plant material

The *S*-haplotype survey was carried out on 126 plants, representing 82 open-pollinated cultivars of cauliflowers. Distribution of these cultivars between summer, autumn and winter types is given in Table 1. The plants analyzed were chosen in order to give the largest picture of the variability in our breeding material: most of the plants were obtained by selfing (1–14 generations of self-pollination), 4 plants were issued from anther culture and only 5 plants were directly issued from open-pollinated cultivars. The variable level of inbreeding is the consequence of the regular introduction of new genetic material in our breeding program. Selfed progenies were analyzed for plants whose homozygosity or heterozygosity at the *S*-locus needed to be confirmed. Origins of the initial open-pollinated cultivars were: Northern France for summer types, Italy, Australia and the Netherlands for autumn types, Roscoff and Angers for winter types. December and January curd production could be assured by autumn-type varieties as well as winter type. So the designation of the types depends also on the origins and not only on the period of production. The plants were cultivated in insect-proof polythene tunnels as previously described (Ruffio-Châble et al. 1993).

Phenotype determination

Self-incompatible phenotypes were assessed either by counting the number of seeds set following self-pollination or by monitoring the pollen tube growth in pistil tissue through fluorescence miscroscopy after basic anilin blue staining. Seed-set data were obtained from 6 emasculated open flowers which were self-pollinated by brushing dehisced anthers on stigmas. This was repeated three times during the flowering period. For two of the three repetitions, pollen tubes were also counted on 3 extra flowers. The flowers were harvested 24 h after pollination and prepared for microscopic observations according to the method of Martin (1958), which was adapted to our material. Briefly, pistils were fixed in alcohol–acetic acid (3 : 1, v/v) for 24 h and then stored in 70% alcohol. Before observation, the pistils were treated in an aqueous solution of sodium hydroxide (5 *N*) for 12 h, rinsed carefully in water and stained in an 1% solution of water-soluble anilin blue dye in 0.1 *N* K_3PO_4 for 1 h. Pollen tubes were visualized using UV fluorescence microscopy. Each pistil was scored for pollen tube penetration into the upper part of the pistil; 9 scores were defined (Wallace 1979): 0 = 0 tube, 1 = 1–2 tubes, 2 = 3–5 tubes, 3 = 6–9 tubes, 4 = 10–14 tubes, 5 = 15–25 tubes, 6 = 26–50 tubes, 7 = 51–100 tubes, 8 = > 100 tubes. For both techniques, the female fertility was controlled by cross-pollinations and male fertility by test-cross pollinations against male-sterile plants. The *S*-haplotypes of plants used as testers for female and male fertility were also determined. Because a great variability in female fertility was observed, seed-set data were expressed by calculating the self-compatibility index (SCI), which

Table 1 Periods of production and number of cultivars from which the studied plants were issued

	Periods of production											Number of studied plants
	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Total	
Types:												
Summer	1	1									2	3
Autumn			10	8	5	2					25	51
Winter					8	12	14	9	11	1	55	72
Total											82	126

corresponds to the ratio of the number of seeds obtained after self-pollination by the number of seeds set after cross-pollination (Zapata and Arroyo 1978). SCI = 0% for highly self-incompatible plants and SCI = 100% for fully self-compatible plants.

Protein extraction and immunochemical analysis

Proteins were extracted, separated on isoelectric focusing (IEF) and electrotransferred onto nitrocellulose membranes as previously described (Gaude et al. 1993). *S*-locus gene products were detected on protein blots by using two types of antibodies : a polyclonal serum raised in rabbit and specific for SLGs of class I (anti-class I SLG antibody) and a monoclonal mouse (Mab 157-35-50) antibody specific for class II SLGs (anti-class II SLG antibody). These antibodies were raised against synthetic peptides corresponding to the N-termini of SLGs of class I and II, respectively. Details on how these antibodies were obtained and on their reactivities towards SLGs have been described elsewhere (Gaude et al. 1993, 1995). A third antibody was used to detect SLR1 proteins and was obtained by immunizing a rabbit with the peptide TNTLSPNEALTISSY cross-linked to ovalbumin as a carrier protein by bisdiazobenzidine. This sequence corresponds to the N-terminus of SLR1 proteins (Gaude et al. 1991). The immunodetection procedure was performed as in Gaude et al. (1993). Either nitroblue tetrazolium and 5-bromo-4-chloro-indolyl phosphate (Gaude et al. 1993) or ECL detection kit reagents (Amersham, Les Ulis, France) were used as color development substrates.

Results

S-haplotype survey

S-haplotype designation was based on the identification of bands detected by anti-SLG antibodies (Fig. 1). Conversely to what was observed in *Brassica oleracea* var 'acephala', anti-class I antibody revealed a few bands which were common to all stigma extracts and that could not be associated with the specific expression of *S*-gene products. Separate from this background, among the 126 plants analyzed by the immunostaining of protein blots, ten different antigenic patterns were detected. According to the *S*-haplotype specificity of the antibodies (Gaude et al. 1995), only one *S*-haplotype belonged to class II (haplotype sx), eight others were of class I (haplotypes SA, SC, SD, SE, SF, SG, SH, SJ) and one extract (SB) presented no specific compounds detected by antibodies. Whatever the haplotype class, multiple bands were revealed by antibodies and probably reflected the presence of glycoforms of *SLG* gene products, as has already been reported (Isogai et al. 1987; Kandasamy et al. 1989). The use of the anti-SLR1 antibody allowed the characterization of two antigenic patterns, one consisting of a major band located at pI = 7.50 and the other one of a major band of pI = 7.35. Analysis of an F_2 population of 34 plants segregating for two SLG-haplotypes (SG and sx) indicated that these proteins segregated independently of the SLG products (Fig. 2) and, consequently, were not products of genes linked to the *S*-locus. The two distinct anti-SLR1 antigenic patterns

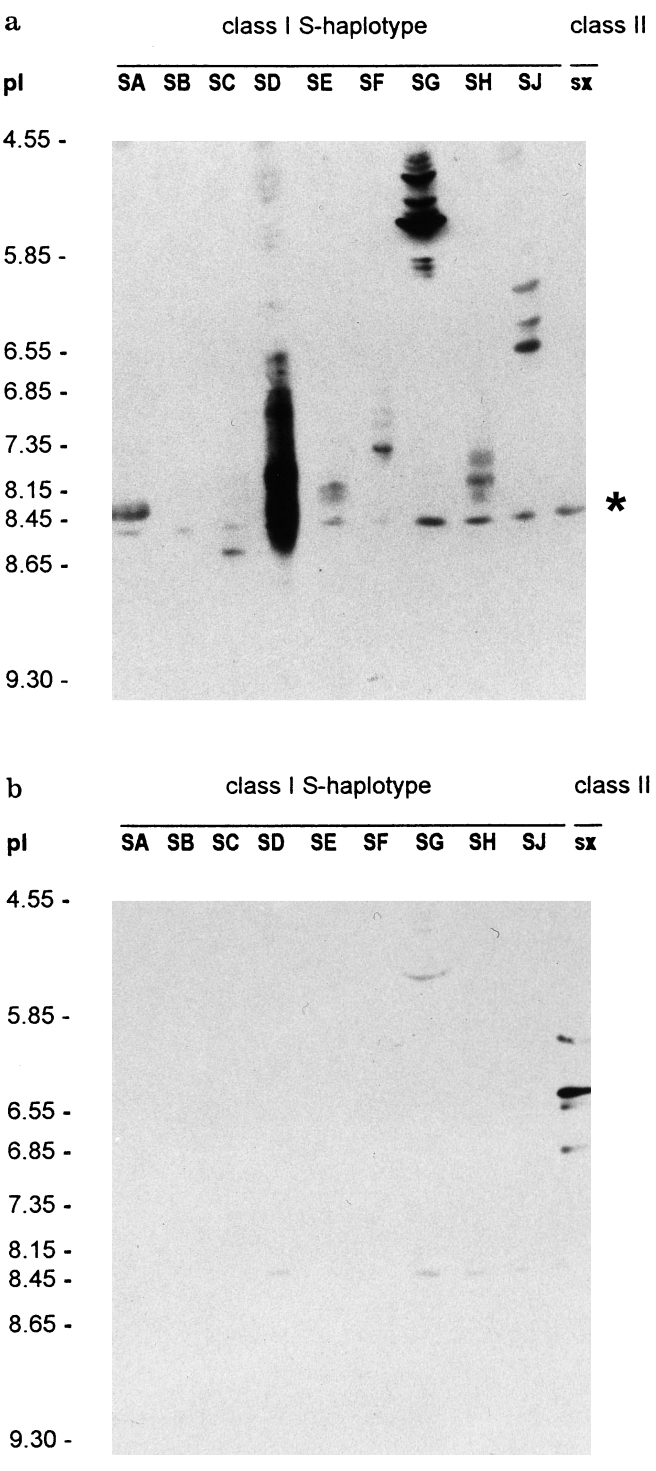

Fig. 1a, b Identification of the ten *S*-haplotypes found in cauliflower based on SLG glycoprotein immunodetection. Stigma proteins were separated by IEF and then electrotransferred onto a nitrocellulose membrane. The membrane was immunostained first with anti-SLG class I (**a**) and secondly with anti-SLG class I (**b**) antibodies using the ECL detection kit reagents. The pI values are indicated on the *left*. The asterisk indicates the non-specific band. **a** The anti-class I antibody revealed the SLG bands of the SA, SC, SD, SE, SF, SG, SH and SJ haplotypes. **b** The anti-class II antibody revealed only one class II *S*-haplotype, sx

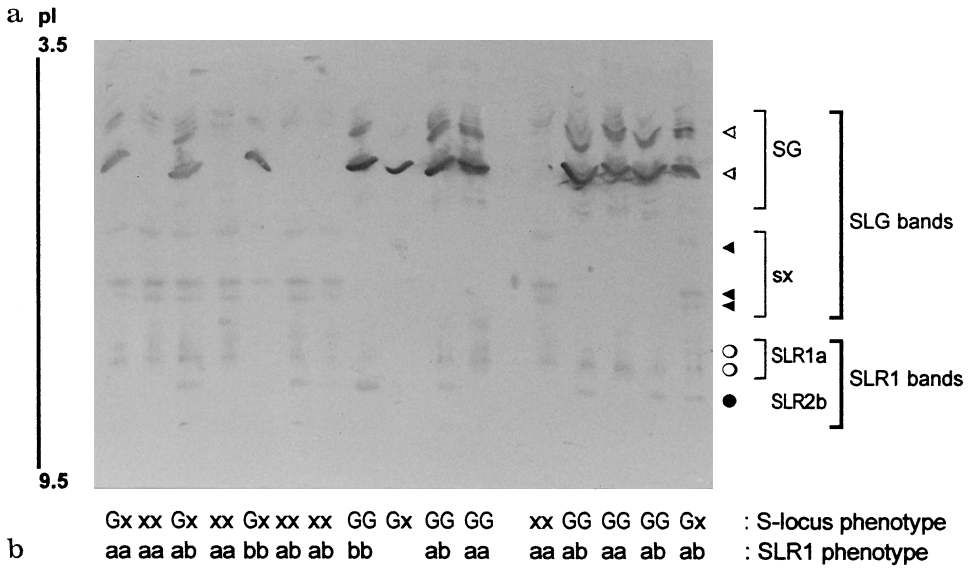

Fig. 2a, b Segregation of SLG and SLR1 proteins in an F_2 progeny issued from the self-pollination of a plant heterozygous both at the *S*-locus (*SGsx*) and for the *SLR1* gene (*SLR1a* and *SLR1b* alleles). **a** IEF immunodetection patterns of 15 F_2 plants (first 15 lanes) and the parental F_1 heterozygous plant (*right lane*) after staining the blot with the anti-class I (*white arrowheads*), anti-class II (*dark arrowheads*) and anti-SLR1 (*circles*) antibodies. *Open* and *dark circles* correspond to *SLR1a* and *SLR1b* allelic bands, respectively. The color reaction used to detect antigen-antibody complexes was based on NBT/BCIP reagents. The pI values are indicated on the *left*. **b** *S*-haplotypes and *SLR1* genotypes of the plants analyzed in **a** as deduced from the immunostaining patterns. The presence of the SG and/or sx haplotype is indicated by *G* and *x*, respectively. The presence of the *SLR1a* and/or *SLR1b* allele is indicated by *a* and *b*, respectively. SLG and SLR1 segregated independently (contengency test: $\chi^2 = 3.5$, $df = 4$, $P = 0.48$) in the F_2 progeny of 34 plants. The distribution of the three *SLG* genotypes (SGSG, SGsx, sxsx) fitted the ratio 1:2:1 ($\chi^2 = 3.19$, $df = 2$, $P = 0.20$), and the distribution of the 3 *SLR1* genotypes (SLR1aSLR1a, SLR1aSLR1b, SLR1bSLR1b) fitted the ratio 1:2:1 ($\chi^2 = 1.29$, $df = 2$, $P = 0.52$)

were designated SLR1a and SLR1b, respectively. For some extracts, anti-class I antibody faintly revealed SLR1 bands. This cross-reactivity may be assigned to the high level of sequence homology observed between *SLR1* and some *SLG* sequences.

Analysis of the different immunostaining patterns showed that among the 126 plants analyzed, 101 were homozygous and 25 heterozygous at the *S*-locus. Among the heterozygous plants, only 3 were heterozygous for two class I haplotypes whereas 22 were heterozygous for one class I and one class II haplotype. As has already been shown, the plants heterozygous at the *S*-locus presented immunostaining patterns generally corresponding to the sum of the immunostaining patterns of the homozygous *S*-haplotype (e.g. SCsx, SDsx SEsx, SGsx). However, Fig. 3 shows, in some particular heterozygous combinations, new specific bands (e.g. SBsx, SFsx, SHsx) or a slight modification of the pI value of one of the SLG associated bands (e.g. SJsx). Thus, two types of heterozygotes could be distinguished according to the presence or absence of these modifications. Modified or new bands were specific for given couples of *S*-haplotypes. In our material, these

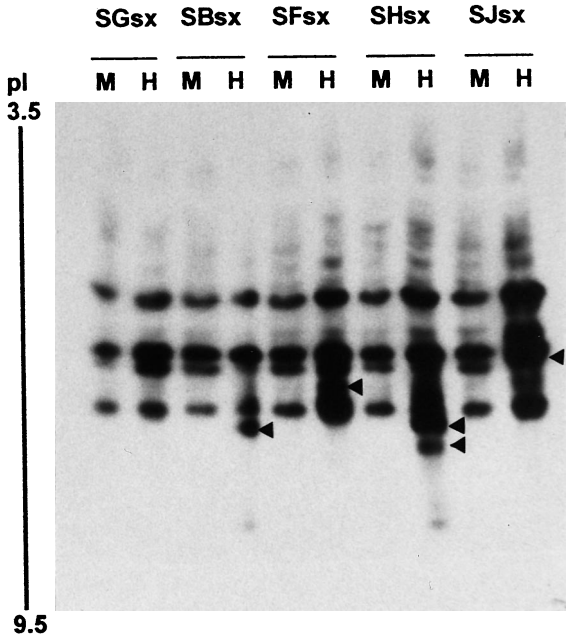

Fig. 3 IEF immunoblot patterns of plants heterozygous for one class I (SB, SF, SH or SJ) haplotype and the class II sx haplotype and showing new or modified bands in the heterozygous situation. *S*-haplotypes of the heterozygous plants analyzed are indicated at the *top* of the blot. Immunostaining patterns obtained by mixing stigma extracts (*M*) from the two parental homozygous plants are compared with those of extracts from the corresponding heterozygous (*H*) plants. The same amount of total protein was loaded per deposit. The nitrocellulose membrane was immunostained with the anti-class II antibody and ECL detection kit. The pI values are indicated on the *left*. *Dark arrowheads* indicate the new specific or modified band in the heterozygous pattern

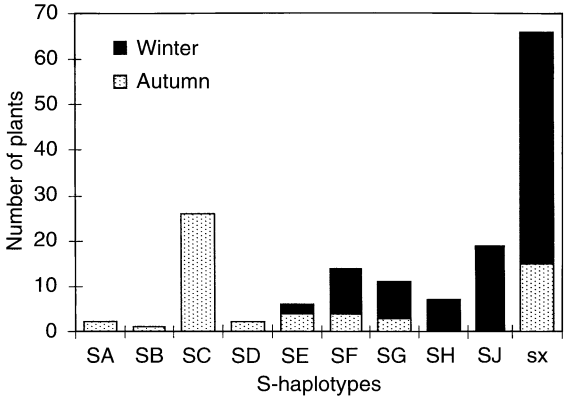

Fig. 4 Distribution of *S*-haplotypes in autumn and winter types of cauliflower. SA to SJ are class I haplotypes, sx is the class II haplotype. SA, SB, SC and SD were only found in autumn cauliflower, whereas SH and SJ only in winter cauliflower

modifications were only observed in heterozygotes containing the sx haplotype and were revealed only by the anti-class II antibody. Interestingly, evidence of the existence of the SB haplotype, which does not present any specific SLG immunodetected band, was demonstrated by the presence of a new band in the antigenic pattern of SBsx heterozygous plants. In fact, an immunochemical analysis of the F_2 progeny derived from self-pollination of the SBsx heterozygote led to the identification of three haplotypes: SBSB, SBsx and sxsx (data not shown).

Distribution of *S*-haplotypes among the various cultivars studied is illustrated in Fig. 4. Some *S*-haplotypes were specifically found in autumn (SA, SB, SC, SD) or winter (SH, SJ) types, while others were found in both types (SE, SF, SG and sx). Class II sx haplotype was the most common and was found in half of the original varieties (43/82). With respect to class I haplotypes, SC was found in the 3 summer cauliflower plants analysed, was more frequently encountered among the autumn cauliflowers than in the other types (24 out of 54) and was absent in the winter type. SJ was the most common for winter cauliflowers (19 out of 72) and SH was found only in late winter types (i.e. March and April).

Relationship between *S*-haplotype and self-incompatibility phenotype

Plants homozygous for the sx haplotype (class II) showed a large degree of variation in their pollen tube growth counts throughout the range of highly self-incompatible plants to self-compatible plants (Table 2). They were equally distributed between the different levels of self-incompatibility. Except for SC homozygous plants, which were always self-compatible, plants homozygous for class I haplotypes were self-incompatible, although they presented various levels of self-incompatibility, varying from highly to partially self-incompatible. Plants heterozygous at the *S*-locus

Table 2 *S*-haplotype distribution and pollen-tube mean score range for 128 plants of cauliflower

Haplotype	Locus state	Total	Pollen tube mean score range				Not observed
			0.0 Highly self-incompatible	0.1–1.0 Self-incompatible	1.1–4.0 Partial self-incompatible	4.1–8.0 Self-compatible	
SA	Homozygous	2	2	–	–	–	–
SB	Homozygous	1	–	1	–	–	–
SC	Homozygous	23	–	–	–	22	1
	Heterozygous	3	–	–	1	2	–
SD	Homozygous	2	–	–	1	–	1
SE	Homozygous	4	3	1	–	–	–
	Heterozygous	2	–	2	–	–	–
SF	Homozygous	13	6	6	1	–	–
	Heterozygous	2	–	–	2	–	–
SG	Homozygous	5	–	3	2	–	–
	Heterozygous	6	1	2	2	1	–
SH	Homozygous	1	–	1	–	–	–
	Heterozygous	6	1	4	1	–	–
SJ	Homozygous	9	7	1	–	–	1
	Heterozygous	10	3	4	1	1	1
sx	Homozygous	40	10	11	10	8	1
	Heterozygous	22	3	7	7	4	1
Total	Homozygous	101	29	24	14	30	4
Total	Heterozygous	25	3	10	7	4	1
Total summer + autumn		54	10	12	4	25	3
Total winter		72	22	22	17	9	2
Total		126	32	34	21	34	5

were mainly self-compatible or partially self-incompatible. Four heterozygous plants were totally self-compatible. Thus, the self-compatible phenotype was found in three kinds of situations: in all SC homozygous plants, in some sx homozygotes and in some heterozygotes. Self-compatible phenotypes were frequently observed in summer and autumn types (54% of plants analyzed) but far less frequently encountered in winter types (12% of plants analyzed).

Comparison of seed set and pollen tube score

To estimate the level of self-incompatibility by seed set independently of fertility problems, we calculated the SCI index, which corresponds to the ratio between seeds obtained after selfing and seeds obtained after cross-pollination. The maximum SCI value accepted by a breeder is 5%; above this value self-incompatibility is not strong enough for hybrid seed production. For class I *S*-haplotypes, Table 3a represents SCI values from plants which were defined as highly self-incompatible when pollen tubes were counted and Table 3b represents SCI values from plants considered to be self-incompatible (with pollen tube mean scores ranging from 0.1 to 2.0). In the first case, most of the plants (10/12) would fulfill the breeder's requirement; in the second case, only 2 of 10 were self-incompatible enough for seed production. For the sx class II haplotype, SCI varied from 4% to 46% even with plants in which no pollen tube was ever observed 24 h after pollination. When the average pollen mean score is at the most 1.0, only an average of 1 pollen tube is detected at the most in the upper part of the style 24 h after pollination in each flower. From that point of view, self-incompatibility remains strong at this time, compared to cross-pollination for which more than 100 pollen tubes could be counted only 5 h after pollination. For some of these plants, we regularly followed pollen tube growth in the pistil, from 24 h to 7 days after pollination (data not shown, Ruffio-Châble 1994). We observed that the number of pollen tubes counted increased with time after pollination and the age of the flower. Thus, a plant determined initially, to be self-incompatible plant on the basis of pollen tube counting 24 h after pollination appeared to be self-compatible on the basis of seed set assessment (SCI value of 71%).

With respect to plants homozygous for SC, their phenotype was defined as fully self-compatible whatever the method used (number of seeds/pollinated flower or pollen tube counts), and no statistical differences were noted between the two types of pollination (self-or cross pollination).

Discussion

S-haplotype identification

The immunostaining method we used in this study to determine *S*-haplotypes in cauliflower, which was based on the identification of stigmatic SLG glycoproteins by specific anti-SLG antibodies, allowed the identification of ten *S*-haplotypes among the 128 plants tested. Since 28% of the plants we analyzed were self-compatible (pollen tube mean score > 4.0), it is clear that the method previously used to identify *S*-haplotypes and based on observation of the self-incompatibility reaction (pollen tube arrest on the stigma surface) with tester plants was not adapted to cauliflower (Ockendon

Table 3 SCI[a] index (mean, minimum and maximum) calculated with plants homozygous for class I *S*-haplotypes, for two phenotypes determined by pollen tube counts 24 h after pollination: for plants were highly self-incompatibles (**a**) and for plants were self-incompatibles (**b**)

a:	SCI of plants a highly self-incompatible phenotype when measured by pollen tube counts: mean score = 0.0		
Haplotype	SCI (%)		
	Mean	Min	Max
SA	0	0	0
	0	0	0
SF[b]	0	0	0
	0	0	0
	2	2	2
	3	0	7
	5	0	7
	5	3	6
SG[b]	9	4	14
SJ[b]	0	0	0
	2	0	6
	7	6	7

b:	SCI of plants a self-incompatible phenotype when measured by pollen tube counts: mean score range = 0.1–1.0		
Haplotype	SCI (%)		
	Mean	Min	Max
SB	15	4	25
SE	0	0	0
SF	5	0	11
	6	3	15
	24	3	29
	53	43	63
SG	2	0	10
	39	35	46
	74	32	89
SH	57	19	90

[a] SCI, Self-compatibilty index = number of seeds after self-pollination/number of seeds after cross-pollination
[b] Some plants did not figure in this table because of their low fertility

1974, 1975, 1980, 1982; Stevens and Kay 1989). A similar immunochemical approach was performed by Nou et al. (1991) to study *S* haplotype distribution in *Brassica campestris*. They compared their immunostaining method with three other approaches (staining of glycoproteins with Concanavalin A-peroxidase, staining of proteins with Coomassie Brilliant Blue or with silver nitrate) to determine the *S*-genotypes and concluded that the immunochemical method was the most sensitive. Nevertheless, they also observed that for a few plants no stainable band was detected by antibodies. This absence of SLG detection might be due to a very low amount of SLGs, one that is below the threshold of sensitivity of the immunostaining technique. This could be the case for the SF haplotype (class I), where immunostained material was only weakly revealed. Gaude *et al.* (1995) have shown that the S2 haplotype (class II), which reacted very faintly to anti-SLG antibody, produced a very low amount of SLG glycoproteins in the stigma; this was associated with a low level of expression of *SLG* transcripts. Similarly, the absence of detection of SLG in stigmas of the SB haplotype may be due to a very weak expression of the SLG-SB gene. However, we cannot rule out the possibility that the SLG-SB glycoprotein may be sufficiently different from the others at its N-terminus to be not recognized by the antibodies used. In this latter case, this may indicate the existence of more than only two classes of *S*-haplotype in *Brassica*.

As was previously observed in *Brassica campestris* (Nou et al. 1991), we showed in cauliflower that heterozygous plants may present SLG-specific bands not detected in each of the two homozygous parent patterns. The significance of this at the phenotype level remains to be studied in heterozygous plants. It would be particularly interesting to determine whether the presence of modified or new bands in heterozygous plants may reflect an *S*-haplotype interaction (dominance, mutual weakening . . .). Interestingly, these bands were only detected by the anti-class II antibody, which suggests that they result from modifications in the expression of class II *S*-haplotype genes.

Comparison with other *Brassica oleracea* *S*-haplotypes surveys

In spite of the great number of cultivars (82) represented in our cauliflower plants, the total number of *S*-haplotypes (ten) we found is lower than the one reported in Brussels sprouts (19 among 488 plants studied representing 16 cultivars) and Cape broccoli (20 among 182 plants studied representing 7 cultivars). In kale and cabbage, the number of *S*-haplotypes is even higher (more than 30). Ockendon (1982) suggested that the variability observed in cabbage and in kale at the morphological level, which is greater than that observed in Brussels sprouts, Cape broccoli and cauliflower, may also be found at the *S*-locus. In our experiment, the most common *S*-haplotype found was sx, defined as a class II haplotype according to its immunochemical reactivity. Interestingly, the immunostaining pattern obtained for sx stigma extracts was similar to that reported for the *S15* class II haplotype in kale (Gaude et al. 1995). Thus, sx in cauliflower may correspond to the *S15* haplotype. In *Brassica* crops, the most common *S*-haplotypes found in natural populations are usually the pollen-recessive ones (Thompson and Taylor 1966; Ockendon 1974, 1980, 1982). Our investigation confirms this observation for cauliflower, although only one recessive *S*-haplotype (sx) was detected in our analysis among those commonly described (S2, S5, S15, S45 . . .).

Self-compatibility in cauliflower

We found self-compatible phenotypes in homozygous plants exhibiting either the sx (class II) or SC (class I) haplotype. In plants expressing the sx haplotype, the self-incompatibility character was variable according to the genetic background, ranging from self-incompatibility to self-compatibility, whereas all plants carrying the SC haplotype were strictly self-compatible. The presence of self-compatible plants among the homozygous class II haplotype has already been described in kale (Thompson and Taylor 1971), Brussels sprouts and cabbage (Hodgkin 1980), where a wide range of levels of self-incompatibility has also been described. Recently, a self-compatible line homozygous for a class II *S*-haplotype (Sc), has been described in kale (Gaude et al. 1995). These plants showed a normal level of expression of their SLG gene and, from analysis of an F_2 progeny, only homozygous Sc plants exhibited a self-compatibility phenotype. This data suggested that the self-compatibility character was associated with the expression of a gene (or genes) other than *SLG* and was present at the *S*-locus or genetically very close to it. Here, among the numerous sx plants we analyzed, we always detected SLG-sx proteins, whether the plants were self-incompatible or not. Thus, the self-compatible phenotype associated with sx homozygous plants did not appear to rely on a reduction in the SLG glycoprotein level. Moreover, only some of the sx homozygous plants presented a self-compatible phenotype, and this suggests that genetic control of this character was not dependent on the expression of *S*-locus genes. Rather, we may suppose the involvement of "modifier" genes unlinked to the *S*-locus, as already reported in kale by Thompson and Taylor (1971). The molecular action of these genes is poorly understood except for a self-compatible variant *B. oleracea* strain in which mutations in these 'modifier' genes led to a reduction in SLG in the stigma (Nasrallah 1974). Such reduced levels of stigma SLG associated with the loss of the incompatibility response has also recently been described in *B. campestris* (Nasrallah et al. 1992). In this latter example, a single recessive mutation occurring at a locus unlinked to the *S*-locus, designated *SCF1*, is

responsible for the self-compatible phenotype by down-regulating the RNA levels of the *SLG*, *SLR1* and *SLR2* genes but not the *SRK* gene.

The class I SC haplotype may be considered to be a self-fertility haplotype as no differences were observed between self- and cross-pollination data, whatever the method of measurement used to assess the self-incompatibility response. Consequently, the expression of self-compatibility in homozygous SC plants seems to be directly dependent on the SC locus. Since SLG products are detected and are abundant in the stigma extract, it is likely that self-compatibility is associated with mutations occurring at the level of the *SRK* gene or of another *S*-locus-located gene, like the *SLA* gene. Indeed, mutations of the *SRK* gene, which result in either a non functional protein kinase or in the absence of *SRK* expression, have been associated with a self-compatibility phenotype in *B. napus* (Goring et al. 1993) and *B. oleracea* (Nasrallah et al. 1994). Moreover, the presence of a large insertion in the *SLA* sequence of a self-compatible *B. napus* strain, leading to a nonfunctional *SLA* gene, supports the idea that a functional *SLA* gene may be required for the operation of self-incompatibility. However, so far, the *SLA* gene has been identified only in the *S2* haplotype, and it remains to be demonstrated that an *SLA* homolog is also present in the class I *SC* haplotype.

Self-incompatibility and creation of F_1 hybrid varieties in cauliflower

Genetic variability for the level of self-incompatibility appeared to differ according to the method of measurement. When plants homozygous for class I *S*-haplotypes, were examined, pollen tube countings showed a narrower variability than seed set data. Thus, plants were determined to be self-compatible on the basis of seed set results, whereas the same plants were only partially self-incompatible based on pollen tube counting. When the SCI index (calculated with seed set data) is used to measure self-incompatibility, we assess two different components: the strength of the interaction and its duration. Pollen tube counts allow the evaluation of the strength of the self-incompatibility response 24 h after pollination, whereas the presence of seeds accounts for the competition of the duration of the *S*-genes activity and the longevity of the ovule and pollen functions. Thus, many genes and regulation systems could be involved in the final expression of self-incompatibility; consequently, the same *S*-haplotype may not have the same expression according to the genetic background (Nasrallah and Wallace 1968). Pollen tube counting is necessary to analyze incompatibility genetics, but the breeder needs seed set data for a more precise measurement of the degree of self-incompatibility conferred by a given *S*-haplotype.

Our experiments have shown that it is difficult to associate the identity of one *S*-haplotype to one level of self-incompatibility reaction. Plants homozygous for the sx haplotype could show all level of self-incompatibility, and most of the plants with the class I haplotype varied from the highly self-incompatible to the partially self-incompatible phenotypes. Nevertheless, SA and SJ haplotypes could be more frequently associated with high self-incompatibility reactions than the others. Once more we verified that a high level of self-incompatibility in cauliflower is relatively more frequent among plants homozygous for class I haplotypes than among the class II haplotype; nevertheless, very few plants presented a level of self-incompatibility that was sufficiently strong enough to be used for hybrid F_1 seed production. Thus, for a breeder, the probability of finding lines possessing both a good combining ability for agronomical characters and a high level of self-incompatibility is low. Consequently, for more than 10 years, male sterility has been used in numerous breeding programs (Ruffio-Châble et al. 1993).

The detection of S products by immunostaining allowed the identification of *S*-haplotypes of plants which may be selected to produce parents for F_1 hybrid production because of their cross-compatibility (differing in their *S*-haplotype). The problem is more striking for winter cauliflowers in which only two *S*-haplotypes (SJ and sx) have been found in 80% of the tested plants. As sx confers a variable level of self-incompatibility, the presence of this haplotype may allow some possibilities of crossing two lines with the same haplotype. For summer and autumn cauliflowers, the high frequency of the SC haplotype (in 45% of plants) makes hybrid combinations easier.

In conclusion, *S*-haplotype detection remains interesting for the breeder. It enables the isolation of plants with SA or SJ haplotypes amongst which the probability of finding female lines is the greatest. *S*-haplotype variability can also be controlled in order to maintain a good level of cross-compatibility between lines. Our experiment has also confirmed the genetic complexity of self-incompatibility. If the SLG products detected by the antibodies are correlated to the recognition system between pollen and stigma, the duration of the rejection reaction on the stigma depends on the genetic background, the influence of which is different according to class I or class II haplotypes.

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