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Conservation of *S*-locus for self-incompatibility in *Brassica napus* (L.) and *Brassica oleracea* (L.)

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Abstract Self-incompatibility (SI) in *Brassica* is a sporophytic system, genetically determined by alleles at the *S*-locus, which prevents self-fertilization and encourages outbreeding. This system occurs naturally in diploid *Brassica* species but is introduced into amphidiploid *Brassica* species by interspecific breeding, so that in both cases there is a potential for yield increase due to heterosis and the combination of desirable characteristics from both parental lines. Using a polymerase chain reaction (PCR) based analysis specific for the alleles of the *SLG* (*S*-locus glycoprotein gene) located on the *S*-locus, we genetically mapped the *S*-locus of *B. oleracea* for SI using a F_2 population from a cross between a rapid-cycling *B. oleracea* line (CrGC-85) and a cabbage line (86-16-5). The linkage map contained both RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) markers. Similarly, the *S*-loci were mapped in *B. napus* using two different crosses (91-SN-5263 × 87-DHS-002; 90-DHW-1855-4 × 87-DHS-002) where the common male parent was self-compatible, while the *S*-alleles introgressed in the two different SI female parents had not been characterized. The linkage group with the *S*-locus in *B. oleracea* showed remarkable homology to the corresponding linkage group in *B. napus* except that in the latter there was an additional locus present, which might have been introgressed from *B. rapa*. The *S*-allele in the rapid-cycling *Brassica* was identified as the S_{29} allele, the *S*-allele of the cabbage was the S_5 allele. These same alleles were present in our two *B. napus* SI lines, but there was evidence that it might not be the active or major SI allele that caused self-incompatibility in these two *B. napus* crosses.

Key words Self-incompatibility · *Brassica oleracea* · *Brassica napus* · RFLP · PCR

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Introduction

Self-incompatibility (SI) in flowering plants is one of the mechanisms of sexual reproduction control that prevents inbreeding and promotes outcrossing (de Nettancourt 1977). In the diploid *Brassica* species, it is under the genetic control of a single locus, the *S*-locus, with more than 50 alleles (Ockendon 1974). The SI reaction is manifested during the first steps of pollination by pollen rejection at the stigma surface whenever the pollen and the stigma bear identical *S*-alleles. The SI system in *Brassica*, especially that of *B. oleracea*, has been well-studied (Nasrallah et al. 1985; 1988; Trick and Flavell 1989; Scutt et al. 1990; Boyes et al. 1991; Stein et al. 1991; Scutt and Croy 1992) and has been described as being under sporophytic control, which occurs when the phenotype of the pollen is determined by the diploid genotype of the pollen producer, the sporophyte (Bateman 1955). The molecular basis of SI in *Brassica* was intensely reviewed by Nasrallah et al. (1991) and Trick and Heizmann (1992). The specificity of the *S*-locus is determined by the production of *S*-locus specific glycoprotein (SLSG) through the expression of the *SLG* gene (Nasrallah et al. 1987). *SLG*s from plants with different alleles have been cloned and

sequenced, and extensive sequence divergence has been observed (Nasrallah et al. 1988; Ebert et al. 1989; Yamakawa et al. 1994). *SLG* belongs to a multigene family consisting of approximately 12 members in *B. oleracea* (Nasrallah et al. 1985; 1988; Dwyer et al. 1989), including pseudogenes, the S-receptor kinase gene (*SRK*), which is closely linked to the *S*-locus, and two classes of *S*-locus-related sequences (*SLR*s) unlinked to the *S*-locus. The *SLR*s, unlike *SLG*s, are relatively invariant between the *S*-allele lines (Boyes et al. 1991).

In the amphidiploid *B. napus*, *S*-alleles of *B. rapa* and *B. oleracea* have been introduced into breeding lines (Dwyer et al. 1991; Nasrallah et al. 1991; Toriyama et al. 1991) to prevent self-pollination of this primarily self-pollinating crop and to facilitate the production of F_1 hybrid seeds for higher yields through heterosis (Banks 1988).

Molecular genetic mapping using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers has been carried out on *B. oleracea* and *B. napus* in our laboratory, which has provided an opportunity for comparison of the organization of the genomes of these two species. Recently, a simple method for the identification of *S*-alleles by specific polymerase chain reaction (PCR) based amplification coupled to restriction endonuclease digestion has been devised for *B. oleracea* (Brace et al. 1993). In view of this, we decided to take a closer look at the *S*-locus of *B. oleracea* and of *B. napus* in order to: (1) compare the location of the *S*-locus or loci in *B. napus* with the results from *B. oleracea*; (2) characterize the *S*-alleles introduced into the two different *B. napus* breeding lines and (3) determine the possible origins of the active alleles and the background alleles in the two *B. napus* lines. The comparative mapping employed a F_2 population of *B. oleracea* from the cross of a rapid-cycling *B. oleracea* line (CrGC-85) and a cabbage line (86-16-5), and two *B. napus* F_1-derived doubled haploid (DH) mapping populations which have the same self-compatible male parent (87-DHS-002), crossed to a spring SI breeding line (91-SN-5263) and a winter SI line (90-DHW-1855-4), respectively. The pedigree of 91-SN-5263 suggested that a strong SI allele from *B. rapa* had been deliberately introduced and that possibly an *S*-allele from *B. oleracea* had also been incorporated from the 'Topas' background of 91-SN-5263. Similarly, the winter SI line, 90-DHW-1855-4 might have a *S*-allele from *B. oleracea*, while additional alleles were suspected but remain uncharacterized. We present here a report of the linkage group locations of the *S*-locus in *B. oleracea* and the *S*-loci in *B. napus*; the comparison of these locations in these two *Brassica* species; the origin of at least one *S*-allele of 91-SN-5263 and 90-DHW-1855-4 and preliminary evidence for the characterized *B. oleracea S*-allele not being the active *S*-allele in these two crosses.

Materials and methods

Plant materials

Seeds of the three *Brassica napus* parental lines (87-DHS-002, 91-SN-5263 and 90-DHW-1855-4) and the mapping population of 89 F_1-derived DH from Cross 1 (91-SN-5263 × 87-DHS-002) and that of 95 F_1-derived DH from Cross 2 (90-DHW-1855-4 × 87-DHS-002) were obtained from Pioneer Hi-Bred Production Ltd. Georgetown Research Station, Canada. The source of *B. oleracea* parental lines (CrGC-85 and 86-16-5) and the F_2 mapping population used is as described previously (Landry et al. 1992). All plants were grown in the greenhouse at 18–24°C with a photoperiod of 14–18 h. For each parental or DH line, tissues from four plants were pooled for DNA extraction.

Isolation of genomic DNA

Plant genomic DNA used for RFLP analyses was isolated as described previously by Landry et al. (1991) for all the *B. oleracea* samples, and for the *B. napus* samples with one modification: purification by CsCl gradient was replaced with two successive chloroform/isoamylalcohol (24 : 1, v/v) extractions. The genomic DNA samples used for RAPD and specific PCR analyses were isolated from single leaf discs taken at the two- to four-leaf stage using the DNA micro-extraction method as described previously by Cheung et al. (1993).

Source of probes for RFLP mapping

The RFLP markers mapped in Fig. 2 were obtained with a subset of polymorphic probes which had been tested on the parental lines of each cross. These probes were selected from 341 *B. napus* cDNA clones from either Harada et al.(1988) or from C. Quiros (University of California, Davis, USA.). Markers detected by the former probes were numbered according to the codes of the probe, and those detected by the latter probes were labelled with the prefix "pX" (Fig. 2). The marker loci DP319 and DP447 (Fig. 2) were derived from *B. oleracea* genomic RFLP probes generated by representational difference analysis (RDA) (Hubert et al. unpublished). The cDNA inserts were isolated from *Pst*I digests of the clones by agarose gel electrophoresis, and were further purified using the Sephaglas™ BandPrep Kit (Pharmacia). The "DP" probes were isolated from *Eco*RI digests of the clones and further purified similarly as for the cDNA inserts.

Southern blot hybridizations

DNA samples of the parental lines and the segregation populations were digested with *Bam*HI, *Eco*RI, *Eco*RV and *Hin*dIII in separate reactions (5 U/ μg DNA). Digested DNA samples (3 μg/lane for *B. napus*; and 5 μg/lane for *B. oleracea*) were electrophoresed in 0.8% agarose gels and then transferred onto Hybond N^+ membranes (Amersham) by the alkaline transfer method recommended by the manufacturer. Probes were radioactively labelled with [^{32}P] using the T7 Quick Prime™ labelling kit (Pharmacia). Conditions for hybridizations and autoradiography were as described by Landry et al. (1991).

RAPD and sequence-tagged site (STS) analyses

The RAPD markers labelled with the prefix "OP"in Fig. 2 were obtained from screening 140 RAPD primers (Operon 10-mers, sets

A–G) against the parental lines. Primers which were found to yield polymorphic amplified DNA fragments in the parental lines were subsequently used for DNA amplification of the segregating populations.

For each RAPD reaction, 25 ng of DNA (or 1/50 of the DNA sample from the micro-extraction of a single 5 mm leaf disc) was used in 25 ul of a reaction mixture containing 10 m*M* Tris-HCl (pH 8.2), 50 m*M* KCl, 2.5 m*M* $MgCl_2$, 200 u*M* of each dNTP (Pharmacia) 0.2 u*M* of primer and 0.5 U of AmpliTaq™ DNA polymerase (Perkin-Elmer Cetus). Amplification was carried out in a Hybaid thermal reactor programmed as follows: 30 s at 94°C and 1 s at 42°C for 1 cycle; 1 s at 50°C, 45 s at 72°C, 5 s at 94°C, 30 s at 42°C for 45 cycles, followed by 7 min at 72°C. The amplified products were analysed in a 1.4% agarose gel as described previously (Cheung et al. 1993).

The STSs for SI (labelled SIEF in Fig. 2) were obtained using a set of two specific primers [CAGCATCTACTCGAGATTGAC and AAA(A/C/G)CCATCTCCACTGCAGCT; primers E and F, Brace et al. 1993] for *SLG* of *B. oleracea*. The reaction was carried out with 100 ng of DNA in 25 ul of the same reaction mixture described above for RAPD reactions, except with 1 U of AmpliTaq™ DNA polymerase. Amplification was carried out with a 'hot start' programme as follows: 30 s at 94°C and 10 s at 58°C for 1 cycle, with the temperature being held at 58°C for the addition of AmpliTaq™ DNA polymerase; 1.5 min at 72°C, 20 s at 94°C, 2 min at 58°C for 30 cycles, followed by 5 min at 72°C. The amplified products were digested with 1 U of the restriction endonuclease *Dde*I for 2 h, and then analysed in a 3% Nusieve 3:1 agarose gel.

Segregation and linkage analyses

Polymorphic cDNA probes were identified by differences in the banding patterns between the parental lines on autoradiographs. Segregation analysis was done by hybridizing each of the selected probes to DNA samples from individual DH lines of the mapping populations digested with the selected restriction enzyme that displayed the maximum degree of polymorphisms. Similarly, RAPD primers and the SI-specific primers E and F that revealed polymorphic amplification profiles between the SI and self-compatible lines were used for amplification involving the whole segregation population.

Multi-point linkage analysis of the marker loci was performed with the computer program MAPMAKER Macintosh V2.0 (Dupont). RFLP, RAPD and the SI-specific STS were assigned to linkage groups. In *B. napus*, the linkage criteria used were a LOD score of 7.0 and a maximum recombination fraction (RF) threshold of 0.3 for Cross 1; and a LOD score of 5.0 and a maximum RF threshold of 0.4 for Cross 2. With *B. oleracea*, the linkage criteria was a LOD score of 4.5 and a maximum RF of 0.3. All map distances were expressed in Kosambi centiMorgans.

Evaluation of self-incompatibility (SI) on the parents and the segregating progeny

Parents and the progeny of the two *B. napus* crosses were grown in the greenhouse and bagged at flowering time. The setting of selfed seeds was scored as self-compatible (SC) in phenotype.

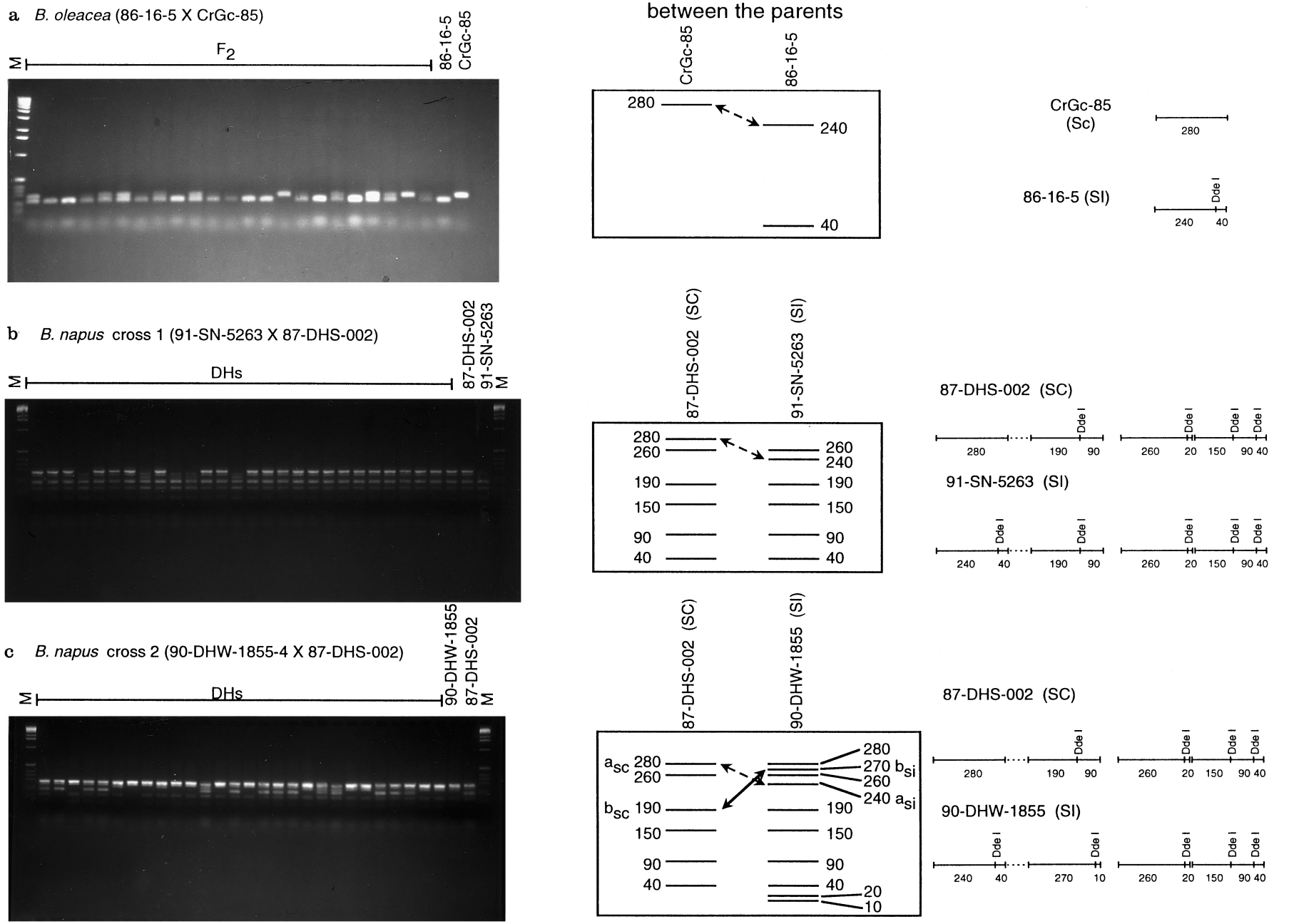
Results and discussion

Specific PCR amplification of *SLG* in *B. oleracea* and *B. napus*

Among all the PCR primers specific for *SLG* (Brace et al. 1993), only the pair of primers E and F revealed polymorphisms between the parental lines of both *B. oleracea* and *B. napus* after the amplified products were digested with the restriction endonuclease *Dde*I. In *B. oleracea*, a 280-bp fragment was amplified from the *SLG* using primers E + F, as expected from the sequence data. There was no polymorphism observed between the rapid-cycling *Brassica* line CrGC-85 and the cabbage line 86-16-5. Similarly, with *B. napus*, a 280 bp band was observed when the amplified products from the parental lines were analysed by agarose gel electrophoresis, and no polymorphism was observed. However, this 280-bp amplified product was found to be heterogeneous, and in fact contained four amplified fragments of the same size which became distinguishable after digestion with *Dde*I. The fact that the amplified product was similar in size to the product seen in *B. oleracea* and that it was observed with both the SC and the SI parents indicated that the allele(s) revealed in *B. napus* might not be the active or major allele for SI in these two crosses.

In *B. oleracea*, after the amplified products of the two parents were digested with *Dde*I, a polymorphism was revealed between the parents, CrGC-85 and 86-16-5. The 280-bp-long fragment remained with CrGC-85, while the digest from 86-16-5 showed a 240-bp and a much smaller 40-bp fragment in place of the 280-bp amplified fragment (Fig. 1a). These results indicated the presence of a *Dde*I site in the amplified *SLG* fragment from 86-16-5 which was absent in CrGC-85. From the sizes of the fragments in the digests and the location of the *Dde*I site in the *SLG*-specific amplified fragments using primers E + F, the allele present in 86-16-5 could be the S_5 allele of *B. oleracea* (Scutt and Croy 1992), and the allele in CrGC-85 could be the S_{29} allele (Trick and Flavell 1989).

In *B. napus*, after the digestion with *Dde*I, it was deduced from the restriction products that there were originally four different amplified fragments present in each of the SI parents (91-SN-5263 and 90-DHW-1855-4) and the SC parent (87-DHS-002). Moreover, polymorphisms between the parental lines were observed among the digests. In the case of Cross 1, there were six monomorphic *Dde*I fragments and only one set of polymorphic fragments (a 280-bp fragment in 87-DHS-002 and a 240-bp fragment in 91-SN-5263) among the SI and SC parents (Fig. 1b). The organization of the restriction fragments in the two parents is summarized diagramatically in Fig. 1b. With Cross 2, when the restriction products of the parents (90-DHW-1855-4 and 87-DHS-002) were compared, two sets of polymorphic fragments (a 280-bp fragment in 87-DHS-002 and a 240-bp fragment in 90-DHW-1855-4; a 190-bp fragment in 87-DHS-002 and a 270-bp fragment in 90-DHW-1855-4) were found among four monomorphic fragments (Fig. 1c). The former polymorphism corresponded to the polymorphism observed both in *B. oleracea* and in Cross 1 of *B. napus*. The latter polymorphism was unique to Cross 2, since the corresponding


a B. oleacea (86-16-5 X CrGc-85)
M
F2
86-16-5
CrGc-85
b B. napus cross 1 (91-SN-5263 X 87-DHS-002)
M
DHs
87-DHS-002
91-SN-5263
M
c B. napus cross 2 (90-DHW-1855-4 X 87-DHS-002)
M
DHs
90-DHW-1855
87-DHS-002
M
Polymorphism(s) between the parents
Amplified products
CrGc-85
86-16-5
280
240
40
CrGc-85 (Sc)
280
86-16-5 (SI)
Dde I
240 40
87-DHS-002 (SC)
91-SN-5263 (SI)
280
260
190
150
90
40
87-DHS-002 (SC)
Dde I
280 190 90
260 20 150 90 40
91-SN-5263 (SI)
240 40 190 90
260 20 150 90 40
87-DHS-002 (SC)
90-DHW-1855 (SI)
asc 280
260
bsc 190
150
90
40
280
270 bsi
260
240 asi
190
150
90
40
20
10
87-DHS-002 (SC)
280 190 90
260 20 150 90 40
90-DHW-1855 (SI)
240 40 270 10
260 20 150 90 40

fragments in Cross 1 were monomorphic. The organization of the restriction fragments in the parents of Cross 2 is summarized in Fig. 1c. The diagrams in Fig. 1 also illustrate clearly the relationships between different polymorphic fragments observed in the *B. oleracea* and *B. napus* parental lines.

The four monomorphic *Dde*I fragments of sizes 260 bp, 150 bp, 90 bp and 40 bp that originated from two 280-bp *SLG*-specific amplified fragments were observed in all three *B. napus* parents irrespective of whether the line was SI or SC. Obviously, these invariant fragments were not linked to the active *S*-allele, but they could be possible candidates for the SLRs (*S*-locus related sequences), which belong to the same multigene family as *SLG* owing to their shared homology but are unlinked to the *S*-locus (Lalonde et al. 1989; Boyes et al. 1991).

Segregation analysis of *SLG*-specific amplified fragments in *B. oleracea* and *B. napus*

The polymorphisms identified between the parents of the *B. oleracea* cross and the parents of the two *B. napus* crosses were followed in the segregating populations of *B. oleracea* and *B. napus*, respectively, in order to map the *S*-loci on the corresponding linkage maps.

With *B. oleracea*, DNA samples from 90 individuals in the segregating F_2 population were amplified with the *SLG*-specific primers E +F followed by *Dde*I digestion. The results of the segregation analysis are summarized in Table 1. An example of the segregation of the polymorphic alleles in the mapping population is shown in Fig. 1a. Similar analyses were carried out in *B. napus* with 89 individuals in the DH population of Cross 1 and with 95 individuals in the DH population of Cross 2. These results are also summarized in Table 1. Examples of segregation analyses of 28 individuals of the DH population of Cross 1 and 28 individuals of Cross 2 are shown in Fig. 1b and c, respectively. The segregation of the *S*-alleles revealed by the *SLG*-specific amplification-*Dde*I digestion in *B. oleracea* showed unbiased Mendelian segregation. However, the same sets of alleles were found to have a significant segregation distortion ($\chi^2 = 23.01$, $P < 0.005$) in Cross 1 with a bias towards alleles from the SC parent, while they segregated normally in Cross 2 (Table 1).

Segregation of the SI/SC phenotypes in the two *B. napus* DH populations

The phenotype of each individual in the segregating DH populations of both Cross 1 and Cross 2 was assayed as to whether or not the plants produced selfed seeds. The plants that produced selfed seeds were considered to be SC in phenotype, while the plants lacking selfed seeds were classified as SI. The results are shown in Table 2. The segregation of the SI/SC phenotypes in Cross 1 was significantly biased towards the SC phenotype ($\chi^2 = 28.89$, $B < 0.005$). Very few SI phenotypes were recorded in this case. A large number of the plants in this population showed an intermediate or partial SC phenotype in which there was either a significantly reduced number of seeds or some branches of the plant showed the SC phenotype and other branches of the same plant showed the SI phenotype; and these plants were not included for linkage analysis. Segregation for the same phenotypes (SC : SI) in Cross 2 followed the 1 : 1 Mendelian ratio expected for DH populations.

Linkage mapping of *S*-loci in *B. oleracea* and in *B. napus*

Using the segregation data of the *S*-alleles revealed by the *SLG*-specific amplification-*Dde*I digestion, and based on the frequency of recombination of this *SLG*-specific STS marker with the RFLP and RAPD loci already mapped in the *B. oleracea* F_2 population of the cross 86-16-5 × CrGC-85, we mapped the *S*-locus in *B. oleracea* on linkage group (LG) 8 of the published *B. oleracea* map (Landry et al. 1992). The map of this LG covered 79.5 cM and included 14 marker loci in addition to the *S*-locus located by the *SLG*-specific STS marker and labelled as STS-EF. (Fig. 2). Four more RFLP (606, 658, DP319 and DP447b) and 2 more RAPD (OP113, OP511a) loci have been mapped on this LG since the last published map (Landry et al. 1992). All markers on this LG except 357 ($\chi^2 = 6.933$, $P < 0.05$) showed normal segregation ratios.

Fig. 1a–c Segregation analyses and diagramatic representation of the *SLG*-specific STS in *B. oleracea* and *B. napus*. **a** An example of 23 individuals of the *B. oleracea* F_2 segregating for the *SLG*-specific STS, STS-EF, and the polymorphism detected between the two *B. oleracea* parents, CrGC-85 and 86-16-5 (*left*). The *Dde*I restriction patterns of the *SLG*-specific amplified fragment from CrGC-85 and 86-16-5 are represented diagramatically (*centre*). The origin of the polymorphism between these two parents are illustrated (*right*). **b** An example of 28 individuals of the *B. napus* DH population from Cross 1 segregating for the *SLG*-specific STS, SIEF, and the polymorphism observed between the two *B. napus* parents of Cross 1, 91-SN-5263 and 87-DHS-002 (*left*). The *Dde*I restriction patterns of the *SLG*-specific amplified fragments from 87-DHS-002 and 91-SN-5263 are represented diagramatically (*centre*). The origin of the polymorphism between the *B. napus* parents is illustrated (*right*). **c** An example of 28 individuals of the *B. napus* DH population from Cross 2 segregating for the *SLG*-specific STSs, SIEFa and SIEFb, and the polymorphisms observed between the two *B. napus* parents of cross 2, 90-DHW-1855-4 and 87-DHS-002 (*left*). The *Dde*I restriction patterns of the *SLG*-specific amplified fragments from 87-DHS-002 and 90-DHW-1855-4 are represented diagramatically (*centre*). The origin of the polymorphism between the *B. napus* parents is illustrated (*right*). The sizes of the fragments in base-pairs are estimated with reference to the 1 kb DNA ladder (BRL)

Table 1 Segregation of *SLG*-specific amplified allele in *B. oleracea* and *B. napus*

Population	Marker	Number of individuals with alleles			Unknown	Total	χ^2
		AA	AB	BB			
B. oleracea F_2	STS-EF	21	35	18	16	90	0.459
B. napus DH (Cross 1)	SIEF	67	–	21	1	89	23.01**
B. napus DH (Cross 2)	SIEFa	55	–	39	1	95	2.39
	SIEFb	51	–	43	1	95	0.52

** Significant at $P < 0.01$

Table 2 Segregation of SI/SC phenotypes in two *B. napus* crosses

Population	No. of individuals		Unknown	Total	χ^2
	SC	SI			
B. napus DH (Cross 1)	54	10	25	89	28.89**
B. napus DH (Cross 2)	57	37	1	95	3.84

** Significant at $P \leqslant 0.01$

A similar approach was employed to map the *S*-loci revealed by the *SLG*-specific STS onto the linkage maps of *B. napus* based on the Cross 1 and Cross 2 populations, respectively. With each of these two populations, a linkage map based on both RFLP and RAPD markers had already been constructed (Cheung et al. unpublished results). With Cross 1, the *SLG*-specific STS, labelled as SIEF, was mapped on LG1 of the *B. napus* map cosegregating with two other RFLP markers (727 and 381) and a RAPD marker (OP110a). As expected from the distorted segregation ratio of SIEF with an excess of SC alleles, SIEF was mapped onto a region with markers also showing significant bias towards the alleles of the SC parent.

The SI/SC phenotypic marker, labelled as SELFIN, was also mapped based on the segregation of the phenotype to LG1 at a distance of 17.5 cM from SIEF (Fig. 2). These results showed that another allele, probably originating from *B. rapa*, as suggested by the pedigree, was the active *S*-allele in this cross. This further confirmed our earlier speculations of SIEF being a defunct *S*-locus originating from *B. oleracea*. The closest markers bordering the active SELFIN locus were two RAPD markers, OP118c and OP104. There were altogether 28 RFLP and 11 RAPD loci located on this LG spanning 141.9 cM (Fig. 2). Out of these 39 markers located on this linkage group, 31 of them showed significant segregation distortion in favour of the alleles from the SC parent. It is not unusual to find clusters of loci with biased segregation in linkage maps generated using DH populations, and they are probably a result of factors operating in gametic selection during in vitro androgenesis or subsequent plant regeneration (see Orton and Browers 1985; Guiderdoni et al. 1991; Thompson et al. 1991; Foisset et al. 1993; Cloutier et al. 1995).

With Cross 2, the two *SLG*-specific STSs (SIEFa and SIEFb) were mapped on the same linkage group, LG1 of the *B. napus* map (Cheung et al. 1997) based on the DH population from Cross 2 (Fig. 2). This LG consisted of 37 other RFLP loci and nine RAPD loci covering a genetic distance of 319.8 cM. In contrast to the map of LG1 based on Cross 1, only five marker loci showed significantly biased segregation. SIEFa corresponded to the STS-EF mapped in *B. oleracea*, and SIEFb was located close to the terminal part of LG1 of the Cross 2 map on an intra-chromosomal duplicated segment consisting of three other intra-linkage group duplicated markers (Fig. 2, 3). The SI/SC phenotypic marker failed to map to any of the 19 LGs at LOD 5.0 and a maximum RF of 0.4, the criteria used to construct the linkage map of *B. napus* based on Cross 2 data. This suggested again that the active allele did not originate from *B. oleracea* and that the *SLG*-specific STS mapped on LG1 (SIEFa and SIEFb) were not the major active alleles. Since the mapping of the SI/SC phenotypic marker had assumed a single active locus, the failure to map the phenotypic marker on the existing linkage map might also suggest that there might be more than one active *S*-allele operating in this cross. More quantitative data may be necessary to resolve this question.

Comparison of LG1 of the Cross 1 map with its counterpart in the Cross 2 map clearly showed that there was significant homology between the two maps. Fourteen of the marker loci spanning the whole LG1 of Cross 1 were also found in the LG1 of Cross 2. The two maps can be aligned with two inversions. In addition, there was an intra-chromosomal inverted duplication of the segment marked by the loci 381a-SIEFb-479b, which is found at the distal end of LG1 of Cross 2 (Fig. 3). The genetic distances between the common markers were diminished in the LG1 map of Cross 1 compared to that of Cross 2, and this was probably due to the effects of segregation distortions of the marker loci in the former case.

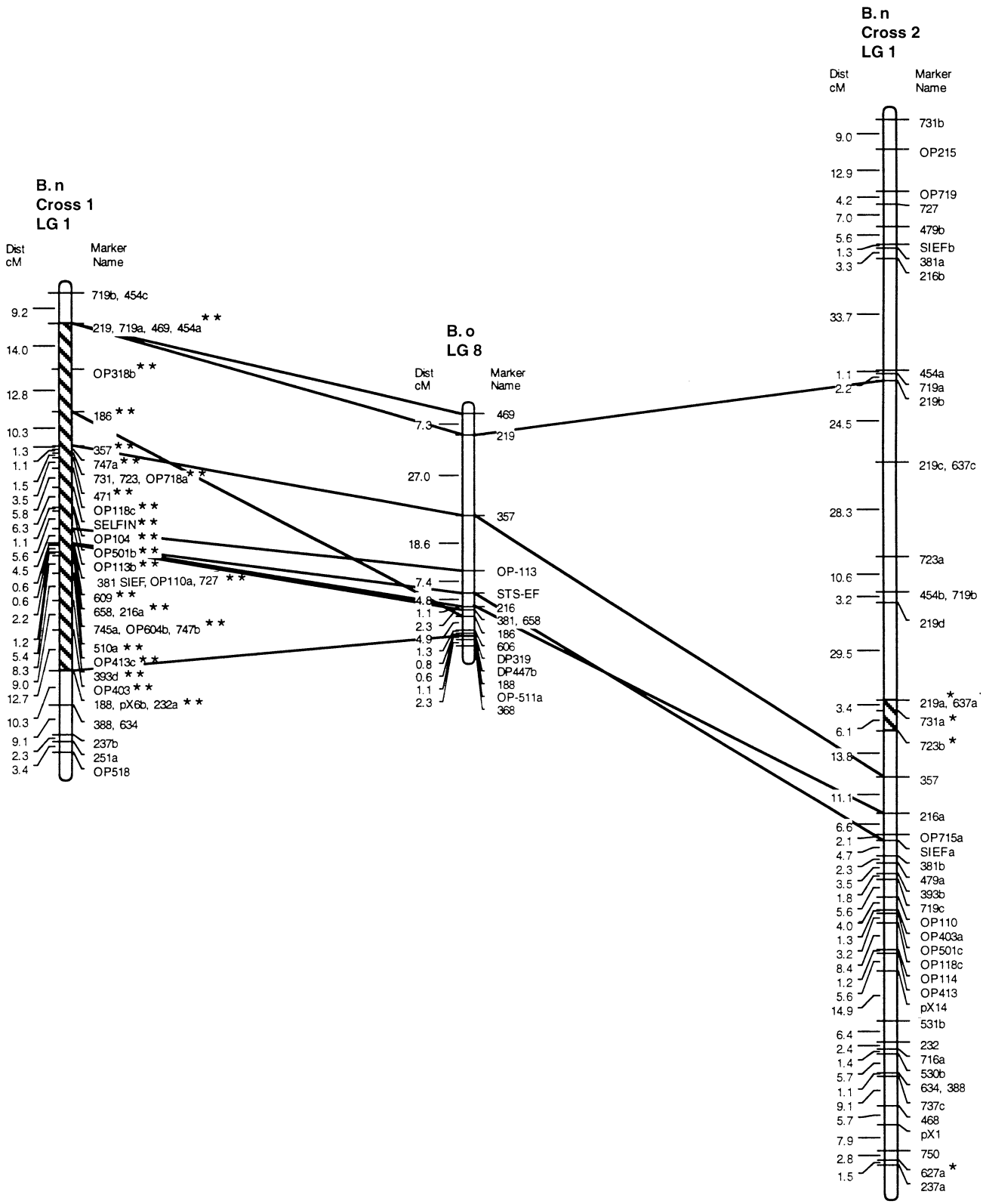


Fig. 2 Conservation between *B. oleracea* and *B. napus* of the linkage groups containing the *B. oleracea S*-locus. Linkage maps of LG1 of *B. napus* based on Cross 1 (*left*) and Cross 2 (*right*) DH populations, and that of LG 8 of *B. oleracea* (*centre*). Duplicated loci detected by the same probe or primer are labelled with a different *lowercase suffix* following the code of the probe or the Operon primer. STS-EF is the *S*-locus detected by the *SLG*-specific STS in *B. oleracea*. Its counterparts in *B. napus* are labelled SIEF on the Cross 1 linkage map and as SIEFa on the Cross 2 linkage map. The duplicated *S*-locus on the Cross 2 map is named SIEFb. SELFIN is the active *S*-locus detected by segregation of the SC/SI phenotypes. Common markers between the different linkage maps are linked by *solid lines*. Marker loci showing segregation distortions are indicated with * for $P < 0.05$, and ** for $P < 0.01$. Regions of segregation distortion towards the SC 87-DHS-002 alleles are indicated by ▨.

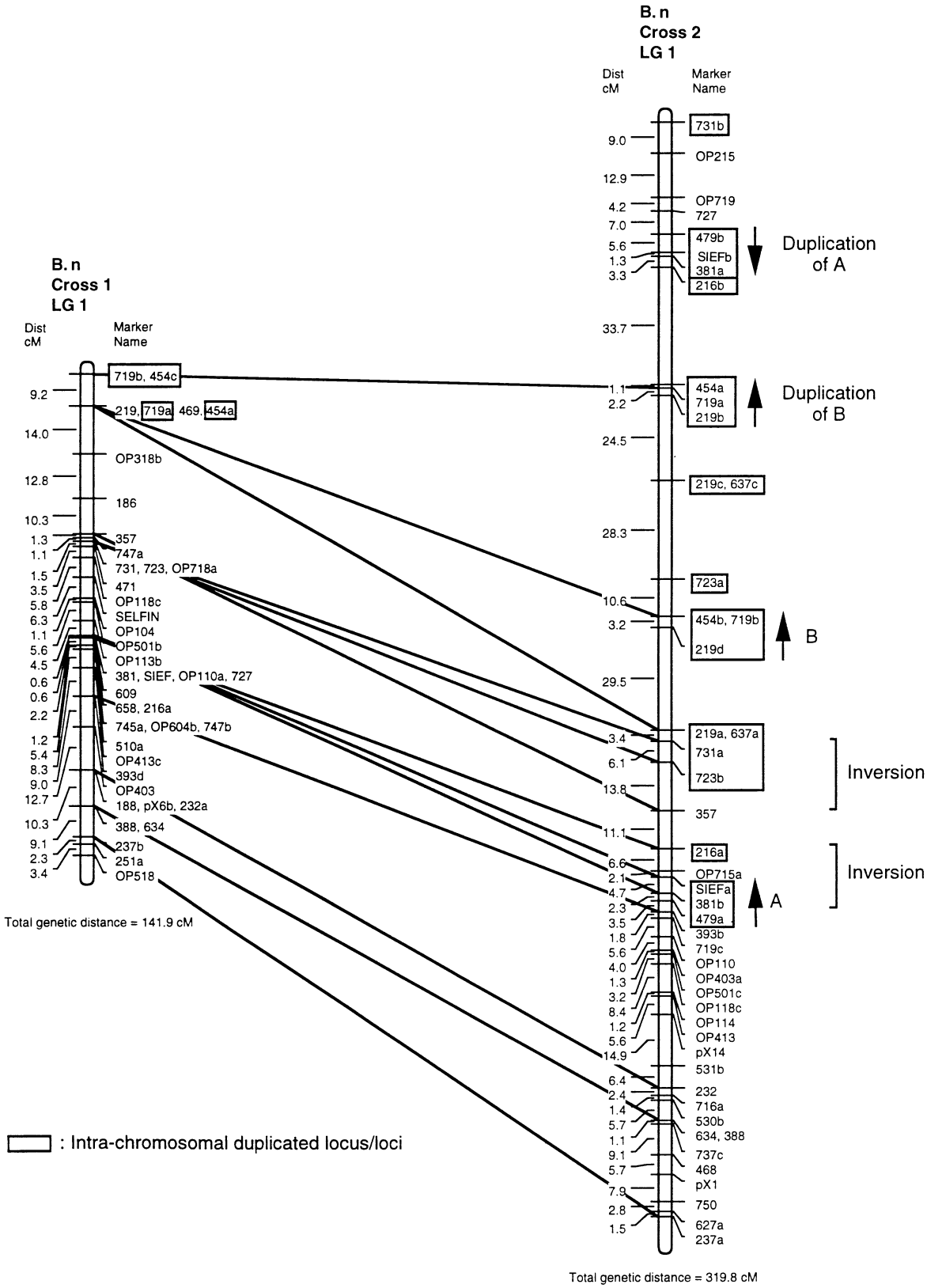


Fig. 3 Comparison of the linkage maps of LG1 of *B. napus* from two different crosses showing the homology between the two maps and the additional intra-chromosomal duplications in the Cross 2 map. Intra-chromosomal duplicated markers are indicated by a *box* around the name(s) of the loci. The common markers between the two maps are linked by *lines*. The two intra-chromosomal duplication segments on the Cross 2 map are marked *A* and *B* with *arrows* showing whether they are direct (*B*) or inverted (*A*) duplications

A Comparison of the LG1 maps from the two *B. napus* crosses with LG8 of *B. oleracea* revealed substantial homology shared by these two linkage groups. This was particularly apparent with 10 of the 15 marker loci of *B. oleracea* LG8 being also found in the *B. napus* LG1 map based on Cross 1. The two maps could be aligned with one translocation (Fig. 2). Four common markers were found between the LG 1 of the Cross 2 map and LG8 of the *B. oleracea* map even though the former shares extensive homology with the entire length of LG1 of the Cross 1 map (Fig. 3). This decrease in common loci could be explained by the fact that some of the markers mapped on the *B. oleracea* map were not polymorphic between the parents used in Cross 2. The extensive conservation of this LG, and with it the *S*-locus from *B. oleracea* in *B. napus*, suggested that LG1 of *B. napus* originated from the C genome of *B. oleracea*. Whether this extent of homology also applied to the other eight linkage groups of *B. oleracea* will only emerge upon comparison of the maps of these two species. Comparison of the *B. napus* genome with those of *B. rapa* and *B. oleracea* has previously been performed by Lydiate et al. (1993), and showed that seven out of nine of the *B. oleracea* LGs and eight out of ten *B. rapa* LGs were conserved in *B. napus*. However, the *B. napus* map used for the comparison was based on a DH population from a cross of a synthetic *B. napus* with winter oilseed rape. The evidence for LG conservation was less apparent when the data from a winter oilseed rape × spring oilseed rape cross were used (Lydiate et al. 1993). Our comparison here and further comparison of the *B. napus* and *B. oleracea* are based on a winter oilseed rape × spring oilseed rape cross and a spring oilseed rape × spring oilseed rape cross, which will help to shed further light on the stability of diploid genomes in the amphidiploid *B. napus*, and will provide a more general view on the conservation of the *B. oleracea* genome in *B. napus*.

Conclusion

In this study, the use of the *SLG*-specific STS marker (STS-EF) to map the *S*-locus in *B. oleracea* and the identification of alleles in the two SI parents have demonstrated the efficiency of this PCR-based marker. This same marker (STS-EF) can also be used to detect the same *S*-locus originating from *B. oleracea* in *B. napus*. However, the specificity of the marker for *SLG* seemed to diminish in *B. napus*, where it also detected other invariant amplified fragments unlinked to the *S*-locus in both the SI and SC parents. Therefore, special attention must be paid to the interpretation of the results obtained when transferring such markers across species.

The comparative study of SI in two different *B. napus* crosses has indicated that the active *S*-alleles are different in the two crosses, and are different from the *S*-alleles originating from *B. oleracea*. In at least one of the crosses (Cross 1:91-SN-5263 × 87-DHS-002) the active allele, probably originating from *B. rapa*, was mapped at close proximity to the *S*-locus from *B. oleracea* on the same linkage group (LG1). Two bordering RAPD markers or their derived SCAR (sequence characterized amplified region) markers identified for the *S*-allele of this cross can now be used to follow this allele in other *B. napus* crosses.

Substantial homology between the two *B. napus* maps and the *B. oleracea* map for the linkage group with the *S*-locus from *B. oleracea* has demonstrated extensive conservation of this *Brassica oleracea* linkage group in the *B. napus*.

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