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ORIGINAL PAPER

Distribution of *S* haplotypes and its relationship with restorer–maintainers of self-incompatibility in cultivated *Brassica napus*

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Abstract *Brassica napus* (AACC, $2n = 38$) is a self-compatible amphidiploid plant that arose from the interspecies hybridization of two self-incompatible species, *B. rapa* (AA, $2n = 20$) and *B. oleracea* (CC, $2n = 18$). Self-incompatibility (*S*) haplotypes in one self-incompatible line and 124 cultivated *B. napus* lines were detected using *S*-locus-specific primers, and their relationships with restorer–maintainers were investigated. Two class I (*S*-$I_{SLG}a$ and *S*-$I_{SLG}b$) and four class II (*S*-$II_{SLG}a$, *S*-$II_{SLG}b$, *S*-$II_{SP11}a$ and *S*-$II_{SP11}b$) *S* haplotypes were observed, of which *S*-$II_{SP11}b$ was newly identified. The nucleotide sequence of *SP11* showed little similarity to the reported *SP11* alleles. The lines were found to express a total of eleven *S* genotypes. The self-incompatible line had a specific genotype consisting of *S*-$II_{SP11}a$, similar to *B. rapa S-60*, and *S*-$II_{SLG}a$, similar to *B. oleracea S-15*. Restorers expressed six genotypes: the most common genotype contained *S*-$I_{SLG}a$, similar to *B. rapa S-47*, and *S*-$II_{SLG}b$, similar to *B. oleracea S-15*. Maintainers expressed nine genotypes: the predominant genotype was homozygous for two *S* haplotypes, *S*-$II_{SLG}a$ and *S*-$II_{SP11}b$. One genotype was specific to restorers and four genotypes were specific to maintainers, whereas five genotypes were expressed in both restorers and maintainers. This suggests that there is no definitive correlation between the distribution of *S* genotypes and restorer–maintainers of self-incompatibility. The finding that restorers and maintainers express unique genotypes, and share some common genotypes, would be valuable for detecting the interaction of *S* haplotypes in inter- or intra-genomes as well as for developing markers-assisted selection in self-incompatibility hybrid breeding.

Key words *Brassica napus* · Self-incompatibility · *S* haplotype · Restorer · Maintainer

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Introduction

In *Brassica*, self-incompatibility is controlled sporophytically by the multiallelic *S*-locus (Bateman 1955), which consists of three highly polymorphic genes: the *S*-locus receptor kinase (*SRK*) gene (Stein et al. 1991), the *S*-locus protein 11 (*SP11*) gene (Schopfer et al. 1999), and the *S*-locus glycoprotein (*SLG*) gene (Nasrallah et al. 1985). Recognition specificity is determined by SRK and SP11 in the stigma (Takasaki et al. 2000; Silva et al. 2001) and pollen (Shiba et al. 2001), respectively. The function of SLG is still controversial (Takasaki et al. 2000; Silva et al. 2001).

S-locus genes are transmitted to the progeny as one segregation unit; hence the classical '*S*-allele' is termed the '*S* haplotype' (Nasrallah and Nasrallah 1993). On the basis of dominant/recessive interaction and the nucleotide sequences of the *SLG* and *SRK* alleles, *S* haplotypes can be divided into two classes: class I and class II. Class I *S* haplotypes are generally dominant over class II *S* haplotypes in the pollen and exhibit a strong self-incompatibility phenotype (Nasrallah and Nasrallah 1993). Many *SRK*, *SP11*, and *SLG* alleles have been characterized (Robert et al. 1994;

Suzuki et al. 1999; Sato et al. 2002; Fukai et al. 2003; Fujimoto et al. 2006; Okamoto et al. 2007). Their sequences are useful for identifying *S* haplotypes (Nishio et al. 1996, 1997; Möhring et al. 2005). The distribution of *S* haplotypes in most *Brassica* vegetables has been investigated (Ockendon 1980; Ruffio-Châble et al. 1997; Sakamoto and Nishio 2001; Wang et al. 2007), but little is known in *Brassica napus*.

Self-incompatibility is one of the most important pollination systems for hybrid seed production in *B. napus*. When compared to cytoplasmic male sterility (CMS), self-incompatibility hybrids have higher F_1 seed production, no obvious negative cytoplasm effect, and an abundance of restorers (Fu et al. 1995). However, *B. napus* (AACC, $2n = 38$) is normally self-compatible, unlike the diploid species *B. oleracea* (CC, $2n = 18$), *B. rapa* (AA, $2n = 20$), and artificially synthesized amphidiploids, which are typically self-incompatible (Gowers 1989). Naturally self-incompatible *B. napus* lines occur rarely, but self-incompatibility can be introduced into the species by introgressing (MacKay 1977; Goring et al. 1992) or resynthesis from *B. oleracea* and *B. rapa* (Gowers 1989; Rahman 2005). Self-incompatible line '271' was produced by introgressing an *S* haplotype of *B. rapa*, called Xishuibai, into a *B. napus* line through interspecific hybridization (Fu and Liu 1975). A double-low (low erucic acid, low glucosinolates) self-incompatible *B. napus* line called S-1300 was derived from crossing '271' with a double-low self-compatible *B. napus* line (Ma et al. 1998). Most cultivated *B. napus* lines are capable of restoring self-incompatibility, but some lines are capable of maintaining the self-incompatibility of '271' and S-1300. Lines with these characteristics were therefore referred to as restorers and maintainers, respectively (Liu et al. 1981; Ma et al. 2003). The distribution of *S* haplotypes in restorers and maintainers or whether the *S* haplotype is related to the restoration or maintenance of self-incompatibility has not yet been reported.

In this study, the distribution of *S* haplotypes in cultivated *B. napus* lines and their relationship with restorer–maintainers were investigated using *S*-locus-specific primers. The results will be helpful in developing markers-assisted selection for breeding self-incompatibility hybrids and for defining the genetic mechanism responsible for self-incompatibility/self-compatibility in cultivated *B. napus*.

Materials and methods

Plant material

The self-incompatible *B. napus* line, S-1300, and 124 cultivated self-compatible *B. napus* lines conserved in Huazhong Agricultural University, Wuhan, China, were used in this study (Supplementary Table 1). The S-1300 line was crossed as the female parent with the self-compatible lines to produce 124 F_1 generation hybrids.

Calculation of SCI and determination of the *S* phenotype

Fifteen F_1 plants from each cross were analyzed for the determination of their *S* phenotype. When three to five flowers were present on the major inflorescence of each plant, the top buds were cutoff to inhibit indefinite flowering. The major inflorescence and two or three branches were then bagged. The bags were tapped gently every two days to ensure sufficient self-pollination. The bags were removed approximately 2 weeks later in order to allow the seeds to develop in a more natural environment. After the seedpods matured, the seeds and flowers produced from each bag were counted, and the self-compatibility index (SCI) was calculated as the number of seeds per number of flowers (Yang et al. 2001). Approximately 100–150 flowers from each F_1 plant were investigated. Previous reports (Li et al. 2007; Zhang et al. 2008) grouped the SCI of *B. napus* into two distinct classes, SCI ≥ 2 and SCI < 2. Plants with a SCI ≥ 2 were referred to as self-compatible and plants with a SCI < 2 were referred to as self-incompatible. The same grouping was used in this study.

PCR primers

DNA fragments corresponding to the region of the genome containing the *SLG* alleles were amplified with a class I *SLG*-specific primer pair, PS5 and PS15, resulting in a 1,300 bp fragment, or a class II *SLG*-specific primer pair, PS3 and PS21, resulting in a 1,000 bp fragment (Nishio et al. 1996). Two primer pairs, SP11a-L with SP11a-R (SP11a-L: 5′-CATAAGTCATGAGATATGCTAC-3′; SP11a-R: 5′-CCGTCGTATATTGCATAGAGTA-3′) and SRKa-L with SRKa-R (SRKa-L: 5′-CTGAGGAATAA-TAGGAGATACG-3′; SRKa-R: 5′-CGTATCTCCTATT ATTCCTCAG-3′) were designed to specifically amplify 420–440 bp of *SP11* and 1,100 bp of *SRK*, respectively (Zhang et al. 2008).

DNA isolation and PCR

Genomic DNA was isolated from young leaves according to the CTAB method (Doyle and Doyle 1990), and DNA from three individuals in each line was mixed for PCR analysis.

PCR was performed with 100 ng of genomic DNA as the template in a total reaction volume of 25 µl, including 0.2 mM dNTP mix (Sangon, China), 0.5 µM of each primer, 0.025 U *Pyrobest* DNA polymerase (TaKaRa),

and 2.5 µl 10× *Pyrobest* Buffer II. The mixture was covered with 20 µl mineral oil and PCR was performed using an MJ research thermocycler model PTC-225 (MJ Research). The samples underwent 30 cycles of 94°C for 30 s, 55 or 59°C for 30 s, 72°C for 1 min and one cycle of 72°C for 5 min. PCR products were subjected to 6% polyacrylamide gel electrophoresis (PAGE) and visualized with silver staining system (Promega, Madison, WI, USA).

CAPS analysis

CAPS analysis was performed at 37 or 65°C for 2 h, using six restriction enzymes (*Hpa*II, *Mbo*I, *Hae*III, *Rsa*I, *Alu*I, *Mse*I; MBI Fermentas, Lithuania) in a digestion mix containing 5.0 µl PCR product, 1.0 µl of 10× buffer and 0.2 U restriction enzyme in a final volume of 10 µl. The products were subjected to 6% PAGE.

Cloning and sequence analysis

After silver staining, the PCR products were excised from the dried polyacrylamide gel, dissolved in 40 µl of deionized water, boiled for 15 min, and centrifuged at 10,000 rpm for 1 min (Cho et al. 1996). The supernatant was used for PCR amplification using the same conditions as previously described. The bands corresponding to the amplified material were excised from 1.5% agarose gels and the DNA fragment was purified using the UNIQ-10 column Gel Recovery Kit (Sangon). Each fragment was then ligated into the pGEMT-Easy vector (Promega) and the transformed clones were screened by PCR analysis with commercially available M13 primer (Sangon). Three positive clones were sequenced with an ABI 3730 automatic sequencer (Sangon) and DNA sequence analysis was performed using the BLAST search tool (NCBI) and CLUSTAL X program (Thompson et al. 1997).

Results

The *S* phenotype of F_1 plant

The F_1 generation of *B. napus* plants had two obviously different *S* phenotypes (Fig. 1, Supplementary Table 1). The offspring of crosses between S-1300 and 83 of the self-compatible lines were completely self-compatible with an average SCI of more than 10. These lines were therefore considered restorers for S-1300. The F_1 plants of the remaining 41 crosses were completely self-incompatible, setting very few seeds and having an average SCI of less than 1. These male parents were therefore referred to as maintainers for S-1300.

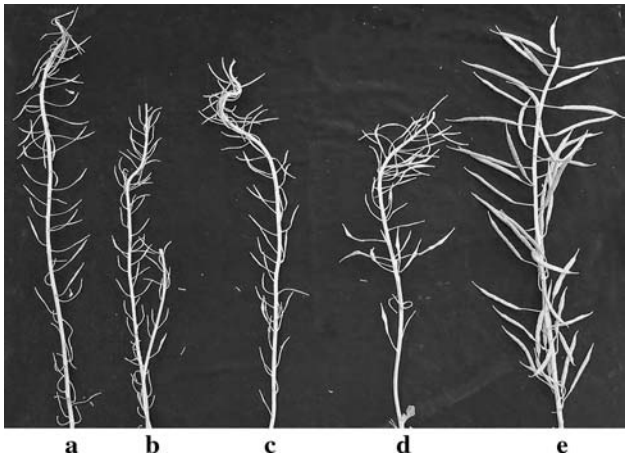

Fig. 1 A visualization of the *S* phenotype in F_1 plants. **a–d** Self-incompatible F_1 plants from crossing S-1300 with a maintainer. The siliques were short and devoid of seeds. **e** A self-compatible F_1 plant from crossing S-1300 with a restorer. The siliques were long and full of seeds

PCR amplification of DNA fragments

The results from PCR amplification of genomic DNA obtained from the *B. napus* parental lines with four primer pairs corresponding to *SLG*, *SRK*, and *SP11* are shown in Supplementary Table 1.

A DNA fragment of approximately 1,300 bp corresponding to the class I *SLG* alleles was present in 89 lines (81/83 restorers and 8/41 maintainers), but no amplification was seen with primers PS5 and PS15 in the other 36 lines, including S-1300. The use of class II *SLG*-specific primers PS3 and PS21 resulted in a fragment of approximately 1,000 bp, with DNA from S-1300 as well as all 124 male lines.

The primers SRKa-L and SRKa-R amplified an approximately 1,100 bp fragment in only two lines, S-1300 and 06-9-4114-1 (#81). The corresponding *S* haplotype was named as $S\text{-}II_{SRK}a$. A fragment between 430 and 440 bp was amplified from S-1300 and 06-9-4114-1 (#81) DNA with the primers SP11a-L and SP11a-R. This *S* haplotype was named $S\text{-}II_{SP11}a$. Thirty-four lines (6 out of 83 restorers and 28 out of 41 maintainers) had an amplification of a slightly smaller fragment, approximately 420–430 bp. This *S* haplotype was named $S\text{-}II_{SP11}b$. There was no amplification in the other 89 lines. None of the 124 cultivated self-compatible *B. napus* lines exhibited the same amplification pattern as the self-incompatible S-1300 line.

CAPS analysis

The class I *SLG* fragments present in 89 lines (81 restorers and 8 maintainers) were subject to CAPS. The lines were grouped into two types on the basis of their restriction

analysis profiles obtained by gel electrophoresis after digestion with each enzyme; the classification was consistent across the six enzymes. Eighty-six lines (78/81 restorers and 8/8 maintainers) had the same profile and the *S* haplotype was named *S-I*$_{SLG}$*a*. Three restorers, designated 'Profit' (#105), 'Maskot' (#108) and 'Puma' (#114), exhibited another profile, which was named *S-I*$_{SLG}$*b*. Figure 2 shows the restriction pattern of enzyme *Hpa*II. *SLG-I*$_{SLG}$*a* was digested into four bands (560, 390, 220, and 160 bp), whereas *SLG-I*$_{SLG}$*b* showed an obviously different restriction profile. The sum of the sizes of the digested DNA fragments, calculated from the electrophoretic mobility of the fragments for each of the six enzymes, did not exceed the size of the fragment amplified with PS5 and PS15 (1,300 bp), indicating that a single fragment was amplified.

Restriction analysis was performed on the PCR products obtained with primers PS3 and PS21 for all 124 self-compatible lines and S-1300. The same restriction profiles were obtained from digestion with one of four enzymes: *Hpa*II, *Hae*III, *Rsa*I, and *Alu*I. Digestion with *Mbo*I or *Mse*I resulted in two different profiles for the 125 lines, and classification was uniform. Thirty-eight lines including S-1300, 34 maintainers, and three restorers with the same pattern had an *S* haplotype which was named *S-II*$_{SLG}$*a*. The remaining 87 lines (80 restorers and 7 maintainers) had an *S* haplotype that was named *S-II*$_{SLG}$*b*. When compared with *S-II*$_{SLG}$*b*, *S-II*$_{SLG}$*a* had additional fragments of 690 bp (*Mse*I) and 400 bp (*Mbo*I, Fig. 3). The sum of the DNA fragment sizes after digestion was larger than the size of the PCR product, suggesting that more than one fragment was amplified.

Distribution of *S* haplotypes in *B. napus*

On the basis of PCR and CAPS analysis, the *S* haplotypes and genotypes of 125 *B. napus* lines are presented in Table 1. Eighty-six lines (86/125) had the *S-I*$_{SLG}$a haplotype and three lines (3/125) had the *S-I*$_{SLG}$*b* haplotype. Approximately 70% of the lines (87/125) had the *S-II*$_{SLG}$*b* haplotype, and 30% lines (38/125) had the *S-II*$_{SLG}$*a* haplotype. Thirty-four lines (34/125) exhibited the *S-II*$_{SP11}$*b* haplotype, but both the *S-II*$_{SRK}$*a* and *S-II*$_{SP11}$*a* haplotypes were found in only two lines, S-1300 and 06-9-4114-1 (#81).

The *B. napus* lines were found to express 11 different genotypes. Six genotypes (B, C, E, F, J, and K) were class I/II homozygous, meaning that they were positive for amplification with both class I and class II *SLG*-specific primer pairs. Genotype A was class II/II homozygous and was only found in the S-1300 line. The other four genotypes (D, G, H, and I) exhibited no amplification with the class I *SLG*-specific primers, but contained at least one class II *S* haplotype.

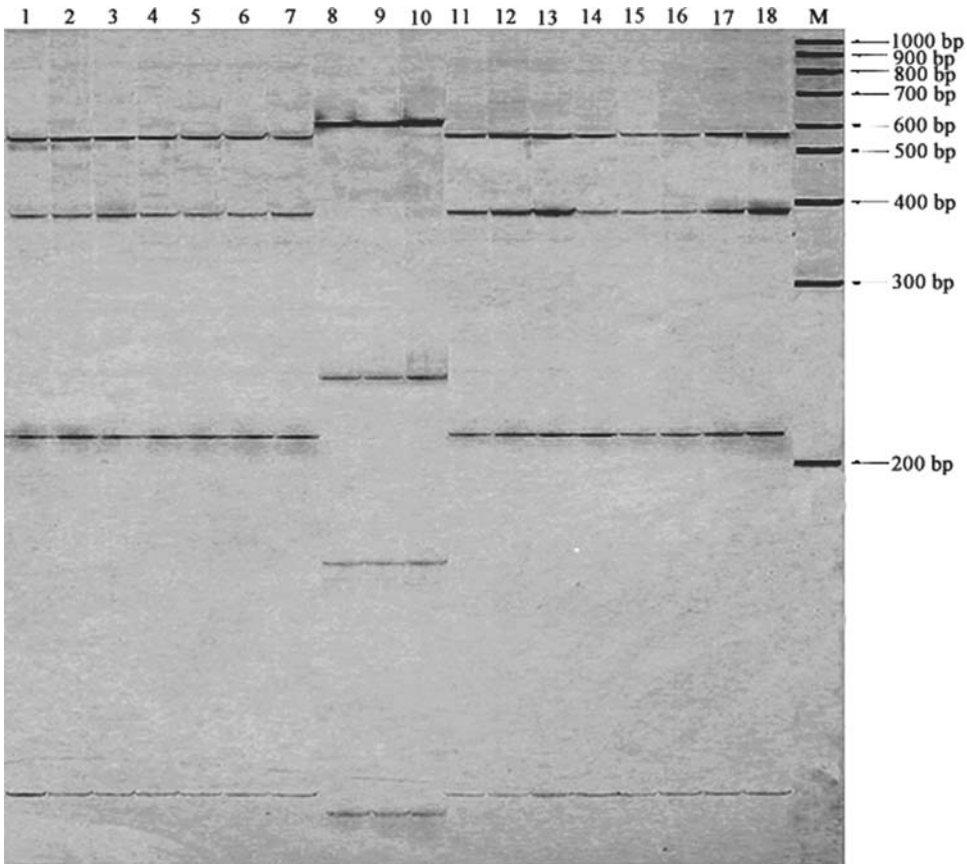

Fig. 2 The restriction patterns of class I *SLG* alleles by enzyme *Hpa*II. *Lanes 1–7*, Restorers with *SLG-I*$_{SLG}$*a*; *Lanes 8–10*, Restorers with *SLG-I*$_{SLG}$*b*; *Lanes 11–18*, Maintainers with *SLG-I*$_{SLG}$*a*; *M*, DNA ladder

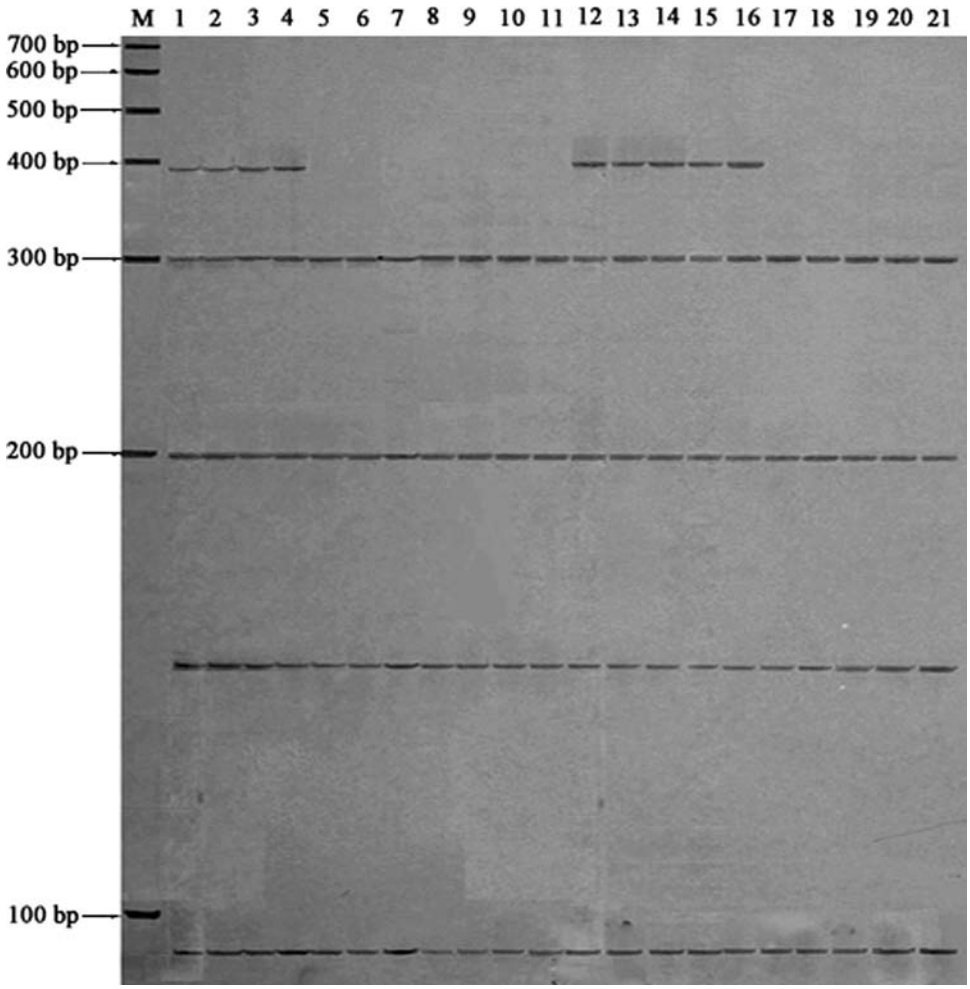

Fig. 3 The restriction profiles of class II *SLG* alleles by enzyme *Mbo*I. *Lane 1*, S-1300 with *SLG-II*$_{SLG}$*a*; *Lanes 2–4*, Restorers with *SLG-II*$_{SLG}$*a*; *Lanes 5–11*, Restorers with *SLG-II*$_{SLG}$*b*; *Lanes 12–16*, Maintainers with *SLG-II*$_{SLG}$*a*; *Lanes 17–21*, Maintainers with *SLG-II*$_{SLG}$*b*; *M*, DNA ladder

Table 1 The distribution of *S* haplotypes and genotypes in *B. napus*

Genotype[a]	Class I *SLG*	Class II *SLG*	Class II *SRK*	Class II *SP11*	Number of lines				*S* haplotype in genome
					S-1300	Restorer	Maintainer	Total	
A (II/II)	–	*II*$_{SLG}$*a*	*II*$_{SRK}$*a*	*II*$_{SP11}$*a*	1	0	0	1	$A^{II}A^{II}C^{II}C^{II}$
B (I/II)	*I*$_{SLG}$*a*	*II*$_{SLG}$*b*	–	–	0	74	1	75	$A^{I}A^{I}C^{II}C^{II}$
C (I/II)	*I*$_{SLG}$*b*	*II*$_{SLG}$*b*	–	–	0	3	0	3	$A^{I}A^{I}C^{II}C^{II}$
D (?/II)	–	*II*$_{SLG}$*b*	–	*II*$_{SP11}$*b*	0	1	1	2	$A^{II}A^{II}C^{II}C^{II}$
E (I/II)	*I*$_{SLG}$*a*	*II*$_{SLG}$*a*	–	*II*$_{SP11}$*b*	0	2	3	5	$A^{I}A^{I}C^{II}C^{II}$
F (I/II)	*I*$_{SLG}$*a*	*II*$_{SLG}$*b*	–	*II*$_{SP11}$*b*	0	2	1	3	$A^{I}A^{I}C^{II}C^{II}$
G (?/II)	–	*II*$_{SLG}$*a*	–	*II*$_{SP11}$*b*	0	1	23	24	$A^{II}A^{II}C^{II}C^{II}$
H (?/II)	–	*II*$_{SLG}$*a*	–	–	0	0	6	6	$A^{?}A^{?}C^{II}C^{II}$
I (?/II)	–	*II*$_{SLG}$*b*	–	–	0	0	3	3	$A^{?}A^{?}C^{II}C^{II}$
J (I/II)	*I*$_{SLG}$*a*	*II*$_{SLG}$*a*	–	–	0	0	2	2	$A^{I}A^{I}C^{II}C^{II}$
K (I/II)	*I*$_{SLG}$*a*	*II*$_{SLG}$*b*	*II*$_{SRK}$*a*	*II*$_{SP11}$*a*	0	0	1	1	$A^{I}A^{I}C^{II}C^{II}$
Total	89	125	2	36	1	83	41	125	

[a] Designated genotype with deduced class of *S* haplotype: I, Class I *S* haplotype; II, Class II *S* haplotype; ?, Unidentified class of *S* haplotype

– indicates that there has been no amplification

Six genotypes (B, C, D, E, F, and G) were identified in the restorers and 89% lines (74/83) expressed the most common restorer genotype, B. Nine genotypes (B, D, E, F, G, H, I, J, and K) were identified in the maintainers. Approximately 56% of the maintainers (23/41) expressed the most common maintainer genotype, G, and 15% of the

lines (6/41) expressed genotype H. Genotype C was specific to restorers, and four genotypes (H, I, J, and K) were specific to maintainers.

Nucleotide sequence analysis

Select lines expressing different genotypes were chosen for nucleotide sequence analysis. In this part of the study, line 05-9-1124-3 (#3) with genotype B, for example, was termed #3B. *S*-locus genes such as *SRK* of the *B. rapa S-60* haplotype was denoted similarly to *BrSRK60*, whereas *Bn* and *Bo* refer to *B. napus* and *B. oleracea*, respectively.

Eleven lines expressing five genotypes (B, E, F, J, and K) with the class I *S-I*$_{SLG}$*a* haplotype had *SLG-I*$_{SLG}$*a* fragments amplified for sequencing, including five restorers (#3B, #6B, #23B, #35E, and #52F) and six maintainers (#67E, #68B, #70J, #81K, #87E, and #89E). The length of the *SLG-I*$_{SLG}$*a* fragment was 1,336 bp and the nucleotide sequences differed by a single base pair. The sequences were 99 and 97% similar to that of *BnSLG-A10* (Robert et al. 1994) and *BrSLG47* (Sato et al. 2002), respectively. Genotype C, the only one expressing the class I *S-I*$_{SLG}$*b* haplotype, was sequenced from three restorers (#105C, #108C, and #114C) for the *SLG-I*$_{SLG}$*b* fragment, which was 1,345 bp. When compared with the 1,336 bp *SLG-I*$_{SLG}$*a* fragment, *SLG-I*$_{SLG}$*b* has one 6 bp and two 3 bp insertions and a deletion of 3 bp (Fig. 4a). The nucleotide sequences of the three *SLG-I*$_{SLG}$*b* fragments were 99% similar, and between 24 and 1,318 bp was 99% similar to nucleotides 1–1,295 bp of *BnSLGBn-2* (Okamoto et al. 2007) and *BrSLG21* (Kusaba et al. 1997).

Amplification with the class II *SLG*-specific primers PS3 and PS21 resulted previously in more than one fragment. Therefore, only the *SLG* allele of the *S-II*$_{SLG}$a haplotype from S-1300 (#1A) and the *S-II*$_{SLG}$b haplotype from 'Defender' (#113B) were sequenced. For the three S-1300 clones, the length of the resulting fragment was 1,025 bp, and the sequence similarity between them was 99%. Two of the fragments exhibited 99 and 96% similarity to *BoSRK15* (Cabrillac et al. 1999) and *BrSRK60* (Fukai et al. 2003), respectively. The remaining fragment exhibited 97% similarity to both *BrSLG60* (Fukai et al. 2003) and *BoSLG15B* (Cabrillac et al. 1999). Of the three fragments sequenced from 'Defender' (#113B), two were 1,025 bp with 99% nucleotide sequence similarity and were found to be 99% similar to *BoSLG15B*. The other fragment was 1,024 bp with the deletion of 1 bp compared to the other two fragments, and exhibited 95% similarity to *BoSLG15B*.

The *S-II*$_{SRK}$*a* and *S-II*$_{SP11}$*a* haplotypes expressed by genotypes A and K were separately sequenced from S-1300 (#1A) and 06-9-4114-1 (#81 K). The 1,058 bp fragments from *SRK* were identical and were found to be 99% similar to *BrSRK60*. The 435 bp *SP11* fragments were also identical to each other and were 100 and 98% identical to *BrSP11-60* (Fukai et al. 2003) and *BoSP11-15* (Fujimoto et al. 2006), respectively. As the *SRK* and *SP11* sequences from 06-9-4114-1 (#81 K) and S-1300 (#1A) were identical for the *S-II*$_{SRK}$*a* and *S-II*$_{SP11}$*a* haplotypes, they were referred to as the same *S* haplotype, *S-II*$_{SP11}$*a*. Fragments of the *S-II*$_{SP11}$*b* haplotype, expressed by four genotypes (D, E, F, and G), were amplified from seven lines (#31G, #35E, #41D, #52F, #67E, #77G, and #87E) for sequencing. The fragments were 424 bp and, compared with *SP11-II*$_{SP11}$*a*, had two deletions of 2 and 9 bp (Fig. 4b). The nucleotide sequences were identical except for one or two base pair differences and exhibited the highest similarity (90%) to *BoSP11-2* (Shiba et al. 2002) and *BoSP11-5* (Shiba et al. 2002). The low similarity to reported *SP11* alleles suggested that *S-II*$_{SP11}$*b* was a new class II *S* haplotype.

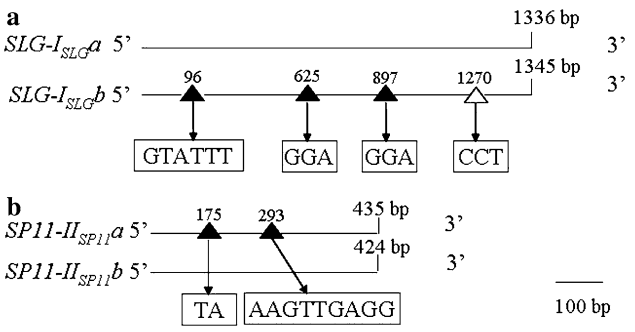

Fig. 4 A comparison of the nucleotide sequences of *SLG-I*$_{SLG}$*a* and *SLG-I*$_{SLG}$*b* (**a**), and *SP11-II*$_{SP11}$*a* and *SP11-II*$_{SP11}$*b* (**b**). The *black* and *white triangles with numbers* indicate the positions of insertions and a deletion, respectively. The nucleotide sequence of the particular insertion or deletion is indicated by an *arrow and box*

Discussion

Determination of *S* haplotypes

CAPS analysis of amplified *SLG* fragments has been widely used to identify *S* haplotypes (Nishio et al. 1996; Sakamoto and Nishio 2001) and detect the F_1 seed purity (Sakamoto et al. 2000). In this study, CAPS analysis found that a single fragment was isolated with the class I *SLG*-specific primers, PS5 and PS15, from 89 lines. The reasons for no amplification in some lines can be attributed to three aspects: there exists no class I *S* haplotype in the line; there exists a class I *S* haplotype, but it lacks *SLG* (Sato et al. 2002); or there is sequence diversity at the primer annealing site. CAPS analysis also found that more than one fragment was amplified from all *B. napus* lines with the class I *SLG*-specific primers PS3 and PS21. The high sequence similarity between *SLG* and the extracellular domain of *SRK* may have resulted in non-specific amplification. Cabrillac et al. (1999) previously

reported that two *SLG* alleles, *SLGA* and *SLGB*, are located at the *S*-locus *BoS-15*. Two other *SLG* alleles in *B. oleracea* cannot be distinguished from each other by CAPS because of their high sequence similarity (Kusaba and Nishio 1999). Moreover, some *S* haplotypes share the same self-recognition specificity, but the CAPS profiles of their *SLG* alleles are different and show only 92.5% nucleotide identity (Kusaba et al. 2000). All these facts suggest that *SLG* alone is not ideal for identifying *S* haplotypes.

The *SRK* and *SP11* gene products are determinants for self-recognition in *Brassica* (Takasaki et al. 2000; Shiba et al. 2001). Analysis of the alleles may be more advantageous for the identification of *S* haplotypes than using *SLG*. Both SRKa-L with SRKa-R and SP11a-L with SP11a-R primer pairs were designed based on sequence of *SRK* and *SP11* at the class II locus *B. rapa S-60*, and were likely to specifically amplify *SRK* and *SP11* in the S-1300 line (Zhang et al. 2008). In this study, we also successfully identified some *S* haplotypes that could not be differentiated by *SLG*-specific primers, such as genotypes B and F, or genotypes A, G, and H. Therefore, combining the analyses of three *S*-locus genes will increase the possibility of the successful identification of *S* haplotypes in *Brassica*.

Distribution of *S* haplotypes in *B. napus*

Two class I (*S-I*$_{SLG}$*a* and *S-I*$_{SLG}$*b*) and four class II (*S-II*$_{SLG}$*a*, *S-II*$_{SLG}$*b*, *S-II*$_{SP11}$*a*, and *S-II*$_{SP11}$*b*) *S* haplotypes were identified in *B. napus*. The nucleotide sequences of *SLG* of *S-I*$_{SLG}$*a* and *S-I*$_{SLG}$*b* exhibited high similarity to that of *B. rapa S-47* and *B. rapa S-21*, respectively. This suggested that both the class I *S* haplotypes may be derived from the A genome. The sequence of *SLG* of both *S-II*$_{SLG}$*a* and *S-II*$_{SLG}$*b* was more similar to the sequence of *B. oleracea S-15* than to that of *B. rapa S-60*, suggesting that both of the haplotypes may be derived from the C genome. The *SRK* and *SP11* sequences of *S-II*$_{SP11}$*a* exhibited 99% similarity to *B. rapa S-60*. The *SP11* sequence of the *S-II*$_{SP11}$*b* haplotype was found to have only 90% homology to *SP11* alleles of the class II *S* haplotypes identified so far (Sato et al. 2006), and was therefore deduced to be a new class II *S* haplotype, which would be a valuable resource for studying self-incompatibility in *Brassica*.

Eleven *S*-locus genotypes were identified in this study and were found to all possess a class II *S* haplotype similar to *B. oleracea S-15*. *B. napus* was inferred to be homozygous at two *S*-loci, one *S* haplotype is derived from *B. rapa* and the other from *B. oleracea*. This suggested that all materials in this study possess the C^{II} genome. The S-1300 line expressed genotype A and was $A^{II}A^{II}C^{II}C^{II}$ homozygous. The *S* haplotype in the A genome determined the self-recognition specificity (Zhang et al. 2008). Six genotypes (B, C, E, F, J, and K) were deduced to be $A^{I}A^{I}C^{II}C^{II}$ homozygous. The most common genotype, B, as well as other four genotypes (E, F, J, and K) contained a class I *S* haplotype similar to *B. rapa S-47*, consistent with what was reported by Okamoto et al. (2007). Genotype C had a class I *S* haplotype similar to *B. rapa S-21*. Two genotypes, D and G, were referred to be $A^{II}A^{II}C^{II}C^{II}$ homozygous and contained the new class II *S* haplotype, *S-II*$_{SP11}$*b*. The remaining two genotypes, H and I, were deduced to be $A^{?}A^{?}C^{II}C^{II}$, meaning that the *S* haplotype in the A genome was unidentified in this study.

Relationship between the distribution of *S* haplotypes and restoration–maintenance capabilities

Six genotypes were expressed by restorers, most (81/83, genotype B, C, E, and F, Table 1) being class I/II homozygous ($A^{I}A^{I}C^{II}C^{II}$). In the class I/II heterozygote of *Brassica*, gene expression of class II *SP11* is suppressed by the non-functional class I *SP11* (Fujimoto et al. 2006; Okamoto et al. 2007). In this manner, F_1 plants ($A^{I}A^{II}C^{II}C^{II}$) from the crossing of self-incompatible line S-1300 ($A^{II}A^{II}C^{II}C^{II}$) and a restorer ($A^{I}A^{I}C^{II}C^{II}$) were self-compatible, most likely due to the A^{I} from restorers suppressing the A^{II} from S-1300. This is supported by reports that *S* phenotypes in F_2 and BC_1 populations (backcrossed with S-1300) generated from crossing S-1300 with two restorers segregated 3 (SC):1 (SI) and 1 (SC):1 (SI) (Zhang et al. 2008), respectively.

Nine genotypes were identified in maintainers, most (24/41, genotype D and G, Table 1) being $A^{II}A^{II}C^{II}C^{II}$ homozygous, similar to S-1300. F_1 plants resulting from crossing S-1300 ($A^{II}A^{II}C^{II}C^{II}$) with a maintainer ($A^{II}A^{II}C^{II}C^{II}$) had the genotype $A^{II}A^{II}C^{II}C^{II}$ (*S* haplotypes in the A genome were heterozygous, containing each of the parental lines) and were self-incompatible. The class II *S* haplotype similar to *B. oleracea S-15* in the C genome is functional (Okamoto et al. 2007), but why the maintainer itself is self-compatible whereas its offspring from crossing with the self-incompatible line displays self-incompatibility is unanswered. The suppressor locus (*sp*) supposedly suppresses the *S*-locus (Yang et al. 2001) and co-suppression of the *sp* loci is proposed as an explanation of the suppression interaction (data not shown). Molecular evidence supporting the proposal is not yet available.

Some genotypes were found to be specific for restorers or maintainers, though others were expressed in both groups. This suggests there is no definitive correlation between *S* genotypes and restoration–maintenance capabilities for self-incompatibility. This is the first report on the relationship between the distribution of *S* haplotypes and restorer–maintainers in *B. napus*. This relationship would be valuable in detecting the interaction of *S* haplotypes in inter- or intra- genomes, especially the dominant/recessive interaction of different class *S* haplotypes.

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