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ORIGINAL PAPER

Genetic analysis of interspecific incompatibility in *Brassica rapa*

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Abstract In interspecific pollination of *Brassica rapa* stigmas with *Brassica oleracea* pollen grains, pollen tubes cannot penetrate stigma tissues. This trait, called interspecific incompatibility, is similar to self-incompatibility in pollen tube behaviors of rejected pollen grains. Since some *B. rapa* lines have no interspecific incompatibility, genetic analysis of interspecific incompatibility was performed using two F_2 populations. Analysis with an F_2 population between an interspecific-incompatible line and a self-compatible cultivar 'Yellow sarson' having non-functional alleles of *S*-locus genes and *MLPK*, the stigmas of which are compatible with *B. oleracea* pollen grains, revealed no involvement of the *S* locus and *MLPK* in the difference of their interspecific incompatibility phenotypes. In QTL analysis of the strength of interspecific incompatibility, three peaks of LOD scores were found, but their LOD scores were as high as the threshold value, and the variance explained by each QTL was small. QTL analysis using another F_2 population derived from selected parents having the highest and lowest levels of interspecific incompatibility revealed five QTLs with high LOD scores, which did not correspond to those found in the former population. The QTL having the highest LOD score was found in linkage group A02. The effect of this QTL on interspecific incompatibility was confirmed by analyzing backcrossed progeny. Based on synteny of this QTL region with *Arabidopsis thaliana* chromosome 5, a possible candidate gene, which might be involved in interspecific incompatibility, is discussed.

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Introduction

"Species" is defined as a population isolated reproductively from other populations, reproductive isolation being a key factor of speciation. As a mechanism of reproductive isolation in plants, abortion of hybrid embryos is commonly observed. Since embryo abortion results in the loss of egg cells, preventing fertilization by pollen of different species by inhibiting pollen tube growth in a pistil is advantageous for plants. Incompatibility between pollen and a pistil of different species is termed interspecific (or interspecies) incompatibility. On the other hand, many plant species have a mechanism to inhibit pollen tube growth on the stigma or in the style to prevent self-fertilization, i.e., self-incompatibility.

Interspecific incompatibility is analogous to self-incompatibility in its biological significance, that is to avoid undesirable fertilization, and pollen tube behaviors are similar between interspecific incompatibility and self-incompatibility. Interestingly, self-incompatible species that reject self-pollen on the stigma surface also inhibit pollen germination or pollen tube growth of different species on the stigma and those rejecting self-pollen in the

style also inhibit growth of pollen tubes of different species in the style (Lewis and Crowe 1958; Hiscock and Dickinson 1993). Genetics of self-incompatibility has been studied using various plant species, and such studies have revealed the participation of the *S* locus having multiple alleles in self-recognition reaction in self-incompatibility (Nettancourt 2001). Molecular genetic studies of the *S* locus have largely contributed to the elucidation of self-recognition mechanisms in various plant species and have revealed that different plant families use different recognition molecules in self-incompatibility. On the other hand, the genetics of interspecific incompatibility has been seldom studied (Bernacchi and Tanksley 1997). Genetic analysis of interspecific incompatibility may help to clarify the molecular mechanism of interspecific incompatibility.

In interspecific pollination between self-incompatible species and self-compatible species, pistils of self-incompatible species generally inhibit growth of pollen tubes of self-compatible species, while fertilization occurs in reciprocal crossings. This one-way interspecifc incompatibility is called unilateral incompatibility (Lewis and Crowe 1958). Unilateral incompatibility is clearly observed in Solanaceae, and participation of a gene of the *S* locus in unilateral incompatibility has been suggested (Bernacchi and Tanksley 1997).

Brassicaceae species have a self-incompatibility system, the molecular mechanism of which has been intensively studied (Kitashiba and Nishio 2009). Rejection of both self-pollen and pollen of different species occurs on stigma surface in Brassicaceae, and both self-incompatibility and interspecific incompatibility can be overcome by bud pollination. The *S* locus of Brassicaceae contains *SCR/SP11*, which determines pollen recognition specificity (Schopfer et al. 1999; Suzuki et al. 1999), and *SRK,* which determines stigma recognition specificity (Stein et al. 1991). As another locus controlling self-incompatibility, the *M* locus has been elucidated by classical genetics (Hinata et al. 1983), and *M*-locus receptor kinase (*MLPK*) has been reported to participate in self-incompatibility (Murase et al. 2004).

Our preliminary investigation showed that stigmas of a self-compatible cultivar, 'Yellow sarson', in *Brassica rapa* are compatible with *Brassica oleracea* pollen, while those of self-incompatible cultivars are generally incompatible with *Brassica oleracea* pollen, suggesting possible participation of the *S* or *M* locus in interspecific incompatibility. Therefore, in the present study, we investigated interspecific incompatibility of an F_2 population between 'Yellow sarson' and a self-incompatible cultivar and found no effect of *S* or *M* genotypes on interspecific incompatibility in the stigma. Interspecific incompatibility was found to be a quantitative trait. Selecting and using a line having strong interspecific incompatibility and a line completely compatible with *Brassica oleracea* pollen as parental lines, we analyzed QTLs for the strength of interspecific incompatibility.

Materials and methods

Plant materials

A doubled haploid line 'P11' of a Komatsuna cultivar 'Osome', a Japanese leafy vegetable of *rapifera* group in *Brassica rapa*, whose stigmas are incompatible with pollen of *Brassica oleracea*, and an inbred line 'C634' of an Indian oilseed cultivar 'Yellow sarson', whose stigmas are compatible with pollen of *B. oleracea*, and their F_2 populations were used for linkage analysis of a gene or genes responsible for interspecific incompatibility with the *S* and *M* loci and for QTL analysis. An *S* tester inbred line of *S-32* haplotype named 'STS32', which is compatible with *B. oleracea* pollen; a doubled haploid line 'P04' of a Chinese cabbage cultivar 'Harusakari', which is incompatible with *B. oleracea* pollen; and 94 plants of their F_2 progeny were also used for QTL analysis. Pollen donors in *B. oleracea* were a Chinese kale line 'B479' and '*B. oleracea S-32*' in the linkage analysis with *S* and *M* and in QTL analysis, respectively. The self-recognition specificity of *S-32* in *B. oleracea* is different from that of *S-32* in *B. rapa* (Sato et al. 2003). These plants were cultivated using 24 cm pots in an unheated greenhouse.

Observation of interspecific incompatibility

Pollen-tube behavior after interspecific pollination was observed under a fluorescent microscope. Flowers were emasculated just after anthesis and placed on an agar plate. Pollen grains of 'B479' and '*B. oleracea S-32*' were pollinated, and pollen tubes were observed 6 h after pollination. Stigmas were hydrolyzed in 1 N NaOH at 55°C for 1 h and pollen tubes were stained in aniline blue solution (0.1% aniline blue in 2% K_3PO_4). The number of pollen tubes in a stigma was rated using the following indices: 1 (completely incompatible), no pollen tube in a stigma; 2 (strongly incompatible), 1–2 pollen tubes entering a stigma; 3 (weakly incompatible), 3–9 pollen tubes per stigma; 4 (partially compatible), 10–29 pollen tubes per stigma; 5 (compatible), 30–ca. 100 pollen tubes per stigma; 6 (fully compatible), more than ca. 100 pollen tubes per stigma. Three flowers were used for each test, and the test was repeated three times for evaluating the strength of interspecific incompatibility of each plant.

DNA preparation

Plant genomic DNA was isolated by a modified CTAB method (Doyle and Doyle 1990). A 0.1 g piece of leaf was pulverized in liquid nitrogen and suspended in 2× CTAB solution (2% cetyltrimethyl ammonium bromide, 100 mM Tris–HCl buffer pH 8.0, 1.4 M NaCl, 20 mM EDTA). After chloroform/isoamylalcohol (24:1) extraction, DNA was precipitated by the addition of isopropanol. DNA was dissolved in 1× TE buffer and treated with RNase.

Genotyping of *S* haplotype and *MLPK*

S genotypes of plants were identified using PCR–RFLP analysis of *SLG* alleles (Nishio et al. 1996) and dot-blot analysis of *SP11* alleles (Takuno et al. 2010). An SNP in *MLPK* was analyzed by dot-blot-SNP analysis according to Shirasawa et al. (2006) to identify *MLPK* genotypes. Genomic DNA of *MLPK* was amplified using a primer pair (TTCATTTTATCTGGTAACTCGC and GTTCTGT GATCATGTCAATGAG) and probed with a wild-type sequence (GTGCAAAAgGTCTAGCT) or a sequence of 'Yellow sarson' (GTGCAAAAcGTCTAGCT).

DNA marker production

SCAR, CAPS, PCR-RF-SSCP (Inoue and Nishio 2004), and dot-blot-SNP markers showing polymorphism between 'Harusakari P04' and 'STS32', i.e., the parental lines of F_2 plants used for QTL analysis, were newly developed using EST sequences available at NCBI (http://www.ncbi.nlm.nih.gov/) and DDBJ (http://www.ddbj.nig.ac.jp/). Among previously reported SSR (simple sequence repeat) markers for *B. rapa* and *B. oleracea* (Suwabe et al. 2006, Tamura et al. 2005; Iniguez-Luy et al. 2008), primer pairs of 143 markers were examined for selection of DNA markers. Dot-blot-SNP markers, 114 markers, developed for *B. rapa* (Li et al. 2009) were also used. To relate the linkage group of our DNA markers to the standard reference map of *B. rapa* (Kim et al. 2006) and to further increase the number of DNA markers, CAPS and dot-blot-SNP markers were developed using BAC sequences published by the Multinational Brassica Genome Project (http://www.brassica.info/).

Construction of a linkage map and QTL analysis

Linkage analysis and map construction were performed using Antmap version 1.2 (Iwata and Ninomiya 2006). Linkage groups were identified in the threshold range of 0.3, and the Kosambi mapping function was used to convert recombination frequencies into map distances (cM). QTL analysis was performed using composite interval-mapping (CIM) analysis with Windows QTL Cartographer v2.5. A permutation test was applied to each data set (1,000 repetitions) to determine the LOD thresholds ($P = 0.05$).

Results

Effects of *S* and *MLPK* genotypes on interspecific incompatibility

Pollen tubes of *B. oleracea* 'B479' did not enter stigma papilla cells of 'Osome P11', whose incompatibility index was 2 (Fig. 1a), while 'Yellow sarson C634' showed full compatibility with index 6 (Fig. 1b). Stigmas of 'Osome P11' 2 days before anthesis were compatible with *B. oleracea* 'B479' pollen (Fig. 1c).

'Yellow sarson' has a non-functional *S* haplotype, *S-f2* (Fujimoto et al. 2006), and therefore is self-compatible. 'Osome P11' having *S-60* haplotype is self-incompatible. Two F_2 populations derived from a cross between 'Yellow sarson C634' and 'Osome P11' grown under different culture conditions and at different seasons were used for investigating the effect of the *S* locus on interspecific incompatibility. There was no significant difference of interspecific incompatibility levels between *S-f2* homozygotes and *S-60* homozygotes by the *T* test nor was any significant difference found between different genotypes of *MLPK* (Table 1).

The strength of interspecific incompatibility of F_2 plants was distributed continuously (Fig. 2), suggesting that interspecific incompatibility is a quantitative trait. Therefore, QTL analysis was conducted using 52 F_2 plants and their genotyping data of 241 DNA markers obtained in our previous study (Li et al. 2009). The *S* locus and the *M* locus have been previously mapped in linkage groups A07 and A03, respectively (Li et al. 2009). The *S* locus region showed no peak of LOD score. The region having the *M* locus showed a minor LOD score peak, being ca. 0.5; it was much lower than the threshold value of 2.5. Three QTLs were detected in linkage groups A04, A06, and A09, and the additive effect of the QTL in A09 was negative and those in A06 and A04 were positive, indicating that the allele of 'Osome P11' in A09 strengthens interspecific incompatibility and that 'Osome P11' alleles in A06 and A04 weaken interspecific incompatibility. However, their LOD scores were not high, scarcely exceeding the threshold value, and the variance explained by each QTL was small. For further study of QTL analysis of interspecific incompatibility, we used another F_2 population.

Selection of parents and DNA marker production

For QTL analysis of the strength of interspecific incompatibility, we selected *B. rapa* lines having the highest and

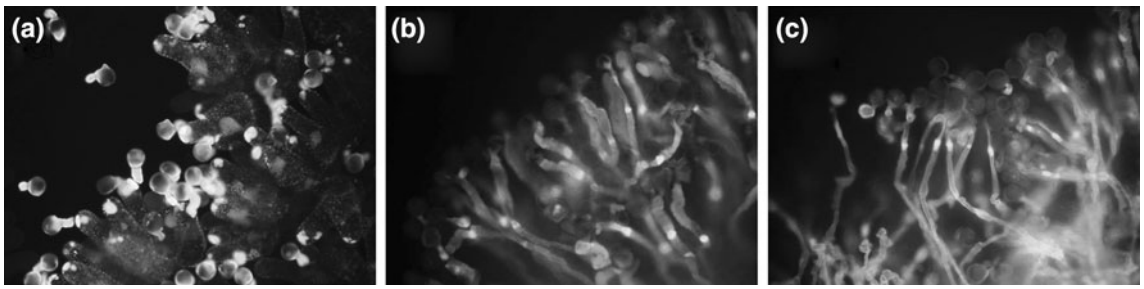

Fig. 1 Pollen tube behavior in the stigmas after interspecific pollination between *B. rapa* × *B. oleracea*. *B. oleracea* pollen tubes in a stigma of an open flower of 'Osome P11' (**a**), in a stigma of an open flower of 'Yellow sarson' (**b**), in a stigma of a bud 2 days before anthesis of 'Osome P11' (**c**)

Table 1 Strength of interspecific incompatibility of *B. rapa* plants with *S* and *M* genotypes

Genotypes		No. of plants	Index ± SE[a]
2007 autumn ($n = 25$)			
	S-60/S-60	4	4.0 ± 0.4
	S-60/S-f2	16	3.9 ± 0.3
	S-f2/S-f2	5	4.0 ± 0.3
2008 spring ($n = 52$)			
	S-60/S-60	8	4.9 ± 0.1
	S-60/S-f2	29	4.8 ± 0.1
	S-f2/S-f2	15	4.7 ± 0.2
2007 autumn ($n = 25$)			
	M/M	7	3.4 ± 0.5
	M/m	11	4.3 ± 0.3
	m/m	7	3.9 ± 0.3
2008 spring ($n = 52$)			
	M/M	17	4.6 ± 0.2
	M/m	23	4.8 ± 0.1
	m/m	12	5.0 ± 0.1

[a] Strength of interspecific incompatibility is shown by indices from 1 (completely incompatible) to 6 (fully compatible)

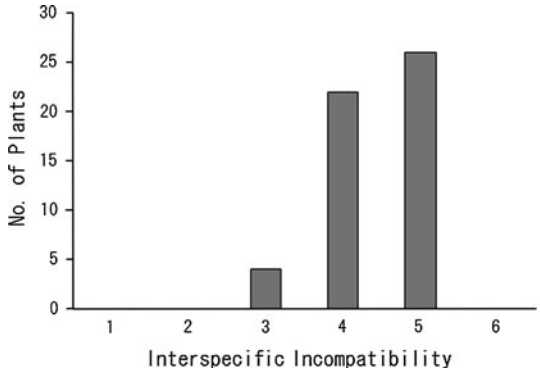

Fig. 2 Frequency distribution of the strength of interspecific incompatibility in 52 F_2 plants derived from 'OsomeP11' × 'Yellow sarson'

lowest levels of interspecific incompatibility from six lines (Supplementary Table S1). The selected line having the highest level of incompatibility was a doubled haploid line 'P04' of a Chinese cabbage cultivar 'Harusakari', showing an incompatibility index of 1, and the line having the lowest level of incompatibility was a homozygous line of *S-32* haplotype, 'STS32', showing an incompatibility index of 6. F_1 hybrids between them showed strong interspecific incompatibility, the index being 2. The levels of interspecific incompatibility of these lines were constant in pollination tests using pollen grains of eight different lines of *B. oleracea* (Supplementary Table S2), suggesting no variation in interspecific incompatibility on the male side in the *B. oleracea* lines used in this study.

DNA markers showing polymorphism between 'Harusakari P04' and 'STS32' were produced. Primer pairs were designed from 251 EST sequences of *B. rapa* and *B. napus* and used for genomic DNA amplification by PCR. Single DNA fragments were amplified from both 'Harusakari P04' and 'STS32' by 143 primer pairs, and four showed polymorphism by agarose gel electrophoresis, yielding SCAR markers. By polyacrylamide gel electrophoresis after digestion with *Mbo*I or *Msp*I, 49 amplified DNA fragments showed polymorphism, yielding CAPS markers (Supplementary Table S3). Forty-one DNA fragments showed polymorphism by PCR-RF-SSCP analysis. Among them, 26 PCR-RF-SSCP markers were sequenced, and SNPs of 12 markers were identified. Based on these SNPs, 11 dot-blot-SNP markers were produced (Supplementary Table S3). Reported SSR markers were applied to these parental lines, and 8, 5, and 2 SSR markers among 38 (21.0%), 9 (55.9%), and 96 (2.1%) markers reported by Suwabe et al. (2006); Tamura et al. (2005), and Iniguez-Luy et al. (2008), respectively, showed polymorphism between them. SCAR, CAPS, and dot-blot-SNP markers having polymorphism between 'Yellow sarson' and 'Osome P11' (Li et al. 2009) were also tested, and 2, 26, and 17 markers in SCAR, CAPS, and dot-blot-SNP markers, respectively, showed polymorphism between 'Harusakari P04' and 'STS32'.

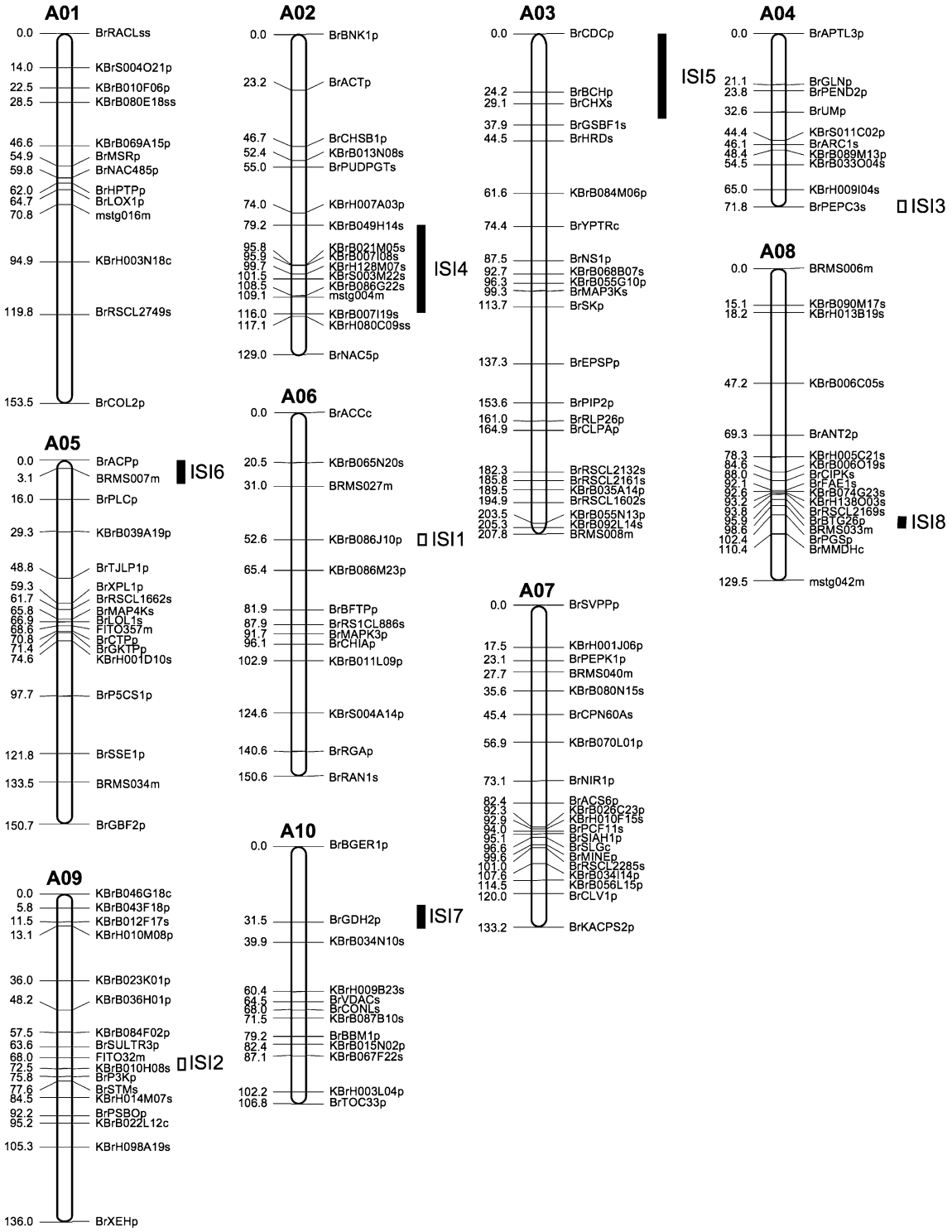

Fig. 3 A linkage map of DNA markers and detected QTLs for the strength of interspecific incompatibility. Map distances are shown to the *left* of *vertical lines* of linkage group in cM, and marker names are shown on the *right*. *White bars* indicate QTLs detected in F_2 between 'OsomeP11' × 'Yellow sarson', and *black bars* indicate QTLs detected in F_2 between 'STS32' × 'Harusakari P04'

QTL analysis

Using 94 F_2 plants derived from a cross between 'Harusakari P04' and 'STS32', linkage analysis of the 125 DNA markers, i.e., 6 SCAR, 75 CAPS, 15 SSR, 28 dot-blot-SNP, and 1 PCR-RF-SSCP markers, was carried out. In total, 101 DNA markers were mapped on the 11 linkage groups of *B. rapa*, but 24 DNA markers were not linked to

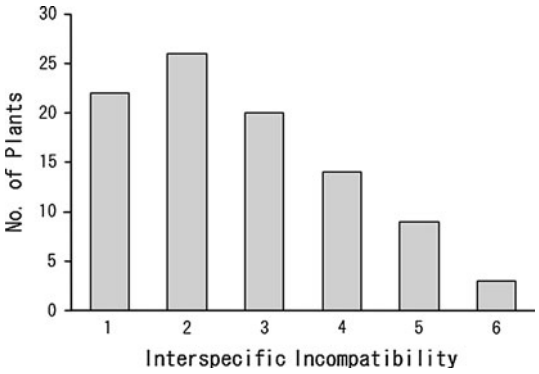

Fig. 4 Frequency distribution of the strength of interspecific incompatibility in 94 F_2 plants derived from 'STS32' × 'Harusakari P04'

the linkage groups. To fill in the gap of the map, DNA markers were further produced using the BAC clone sequences on the linkage map (http://www.brassica.info/). Fifty-four DNA markers, i.e., 3 SCAR, 21 CAPS, 28 dot-blot-SNP, and 2 PCR-RF-SSCP markers, were newly developed. The linkage map, including the newly developed BAC-sequence-based markers, consisted of ten linkage groups with 158 markers and had 1374.6 cM in total, the average distance between DNA markers being 8.7 cM (Fig. 3). The longest distance between DNA markers was 31.5 cM.

Levels of interspecific incompatibility with *B. oleracea* pollen in the 94 F_2 plants were also continuously distributed (Fig. 4). QTL analysis was performed using the data on incompatibility levels of the 94 F_2 plants and the 158 mapped DNA markers. Five QTLs having LOD scores exceeding the threshold value, 2.5, were detected in A02, A03, A05, A08, and A10 (Fig. 3). The QTL having the highest LOD score, i.e., 8.9, was found between KBrS003M22s and KBrB086G22s in A02 (Table 2). The additive effect of the QTL was −1.0, and the explained variance was 32.3%. The genotype of 'Harusakari P04' of this QTL strengthened interspecific incompatibility. The total of the explained phenotypic variances of the five QTLs exceeded 75%.

Interspecific incompatibility in progeny

The effect of the QTL of A02 on the strength of interspecific incompatibility was investigated using 55 BC_1F_2 plants, a parent (BC_1F_1) of which was obtained by backcrossing an F_1 plant with 'STS32'. Genotypes of this QTL were inferred from genotypes of the two closely linked DNA markers, i.e., KBrS003M22s and KBrB086G22s. Excluding ten recombinants between KBrS003M22s and KBrB086G22s, 11 homozygotes of 'Harusakari P04' alleles, 11 homozygotes of 'STS32' alleles, and 23 heterozygotes were obtained. Indices of interspecific incompatibility of 'Harusakari P04'-allele homozygotes and 'STS32'-allele homozygotes were 3.4 and 4.4, respectively. The *T* test indicated a significant difference ($P < 0.05$) in the strength of interspecific incompatibility between groups of these two homozygotes. The index of interspecific incompatibility of heterozygotes was 3.7.

Discussion

Pollen tube behavior after interspecific pollination of *B. rapa* x *B. oleracea* was quite similar to that after self-pollination in these species. Observing pollen tube behavior after interspecific pollination within Brassicaceae and treatment for overcoming self-incompatibility, Hiscock and Dickinson (1993) have suggested involvement of the *S* locus in unilateral incompatibility, which is one type of interspecific incompatibility. In Solanaceae, linkage of a QTL for interspecific incompatibility with the *S* locus has been revealed (Bernacchi and Tanksley 1997), and

Table 2 QTLs for the strength of interspecific incompatibility

Population	QTL (linkage group)	LOD	Additive effect	Phenotypic variance explained (%)	Markers
F_2 of 'Osome P11' × 'Yellow Sarson C634'					
	ISI1 (A06)	2.8	0.34	14.2	KBrB086J10s-BrRS2CL2148 s
	ISI2 (A09)	2.7	−0.25	12.8	BrRS2CL2795s-BrCRY1s
	ISI3 (A04)	2.6	0.23	12.2	BrPEPC3s-BrCPNs
F_2 of 'STS32' × 'Harusakari P04'					
	ISI4 (A02)	8.9	−1.00	32.3	KBrS003M22s-KBrB086G22s
	ISI5 (A03)	3.8	−0.17	17.0	BrCDCp-BrBCHp
	ISI6 (A05)	3.5	−0.65	11.4	BrACPp-BRMS007m
	ISI7 (A10)	2.6	−0.20	8.0	BrBGER1p-BrGDH2p
	ISI8 (A08)	2.5	−0.39	7.4	BrBTG26p-BRMS033m

participation of *S* RNase, which is the pistil recognition molecule encoded by the *S* locus, in interspecific incompatibility has been reported (Murfett et al. 1996). Although the stigmas of a self-compatible cultivar 'Yellow sarson' in *B. rapa,* which has a nonfunctional *SRK* allele due to retrotransposon insertion (Fujimoto et al. 2006), were compatible with *B. oleracea* pollen, the present study revealed that this interspecific compatibility locus is not linked to the *S* locus, indicating no participation of *SRK* in interspecific incompatibility. Since SRK is the receptor molecule for a pollen ligand encoded by the *S* locus, i.e., SCR/SP11, it is reasonable that *SRK* is not involved in interspecific incompatibility.

The similarity of pollen tube behavior of self-pollen and that of pollen of different species may suggest that the same rejection mechanism functions after recognition to inhibit pollen tube growth between self-incompatibility and interspecific incompatibility. Although the *S* locus has been intensively studied, there are few reports on the genes participating in the downstream pathway after self-recognition reaction in self-incompatibility. A point mutation in an *MLPK* allele of 'Yellow sarson' resulting in a single amino acid substitution from G to R has been reported to be the cause of the loss of function of the *M* locus (Murase et al. 2004). In the present study, the strength of interspecific incompatibility of F_2 plants between 'Yellow sarson' and 'Osome P11' did not depend on the genotypes of the *M* locus, and a significant QTL was not detected at the *M* locus. Thus, it can be concluded that the *M* locus is not involved in interspecific incompatibility, either.

Another gene participating in self-pollen rejection in self-incompatibility may be involved in interspecific incompatibility. ARC1 (Stone et al. 1999) has been reported to be involved in the pathway for self-rejection downstream of self-pollen recognition (Gu et al. 1998). A gene encoding ARC1 was mapped on linkage group A04, and a small peak of LOD score, which was not significant, was detected near the *ARC1* locus in QTL analysis using the two populations. This result might suggest involvement of *ARC1* in interspecific incompatibility. However, parental lines used for QTL analysis were not self-compatible lines caused by the loss of *ARC1* gene function. There were 15 SNPs in *ARC1* between 'Harusakari P04' and STS32, including seven non-synonymous substitutions. To study involvement of *ARC1*, analysis using an *ARC1* mutant is required. Although other genes participating in the downstream signaling pathway for self-pollen rejection have not been reported in *Brassica*, such genes may also function in interspecific incompatibility.

Different QTLs not shared by different incompatible parents used in this study were detected between different F_2 populations, indicating that there are several genes contributing to the strength of interspecific incompatibility. Since the LOD scores of all the QTLs detected in F_2 between 'Yellow sarson C634' and 'Osome P11' were as high as the threshold value, we analyzed another F_2 population and found a major QTL, which explains 32.3% phenotypic variance, in linkage group A02. Backcross F_2 plants having 'Harusakari P04' alleles of DNA markers in this QTL region showed stronger interspecific incompatibility than those having 'STS32' alleles, confirming the effect of this QTL on interspecific incompatibility.

Synteny analysis between *B. rapa* and *Arabidopsis thaliana* revealed that the QTL region in linkage group A02 has synteny with chromosome 5 of *A. thaliana*. The syntenic region of *A. thaliana* contains seven genes specifically expressed in the stigma (Tung et al. 2005). Among them, there is a gene encoding a putative receptor-like protein kinase, At5g59700. Although the function of At5g59700 in *A. thaliana* has not been elucidated, its structure is similar to that of SRK participating in *Brassica* self-incompatibility. However, the receptor-like protein kinase (RLK) is one of the largest gene families with more than 600 members with diverse function, such as control of plant growth and development, disease resistance, stress response, and so on (Shiu and Bleecker 2003). The gene most similar to At5g59700 is *HERCULES1*, which is involved in brassinosteroid-mediated cell elongation (Guo et al. 2009). Another gene similar to At5g59700 is *FERONIA,* which participates in male–female interaction for fertilization. Involvement of *FERONIA* in the failure of interspecific fertilization between *A. thaliana* and *Arabidopsis lyrata* or *Cardamine flexuosa* has been suggested (Escobar-Restrepo et al. 2007). A *FERONIA*-like gene specifically expressed in the stigma might function in acceptance or rejection of pollen tubes on the stigma surface, but further study is required.

For identification of the gene responsible for interspecific incompatibility in the QTL region, it would be necessary to narrow down the QTL region by developing a near-isogenic line having only the QTL region of the dominant parent, 'Harusakari P04', in the genetic background of the recessive parent, 'STS32', and recombinants within the QTL region. We are backcrossing 'STS32' to BC_1F_1 to develop a near-isogenic line, and producing SNP markers using the sequences of the BAC clones in the QTL region.

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