

# Recent Transposition of an LTR-Retrotransposon in the Gene Coding for S Receptor Kinase is Responsible for a Novel Self-Compatible Phenotype of Radish (*Raphanus Sativus L.*)

So-Hyeon Bong

Chonnam National University

Ganghee Cho

Chonnam National University

Dong-Seon Kim

FarmHannong Co Ltd

Sunggil Kim (✉ [dronion@jnu.ac.kr](mailto:dronion@jnu.ac.kr))

Chonnam National University <https://orcid.org/0000-0001-8555-2995>

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## Research Article

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## Abstract

Self-incompatibility (SI) responses of radish (*Raphanus sativus* L.) are determined by two tightly linked genes encoding an *S*receptor kinase (SRK) and an *S*locus cysteine-rich protein/*S*locus protein 11 (*SCR/SP11*), respectively. A radish showing an almost self-compatible (SC) phenotype was identified in this study. Inheritance patterns showed that this SC phenotype was dominant over an SI phenotype. In addition, this SC phenotype co-segregated with an *Shaplotype* in an *F*<sub>2</sub> population. This SC radish contained an *RsS-26* haplotype in which duplicate *SRK*-like genes were previously identified. Full-length sequences of two *SRK*-like genes of 18,133-bp and 6,200-bp in length were obtained from radish with the *RsS-26* haplotype (designated as *RsSRK-26-1* and *RsSRK-26-2*, respectively). Duplicate *SCR/SP11*-like genes were also identified in the radish with the *RsS-26* haplotype. Phylogenetic analyses indicated that both duplicate *SRK*-like and *SCR/SP11*-like genes were closely related to other known *SRK* and *SCR/SP11* genes, respectively. No critical mutation was found in the coding region of *SRK*-like or *SCR/SP11*-like gene. However, a 4,146-bp intact LTR-retrotransposon was identified in the third intron of *RsSRK-26-1* of the SC radish. Interestingly, this LTR-retrotransposon was not detected in three other breeding lines containing the same *RsS-26* haplotype. Except for this LTR-retrotransposon, only two single nucleotide polymorphisms (SNPs) were identified in intronic regions between normal and mutant *RsSRK-26-1* alleles. While normal transcription was observed for radish showing *RsSRK-26-1* and SI phenotypes in these three breeding lines, no transcript of *RsSRK-26-1* was detected in the SC radish, suggesting that recent transposition of an LTR-retrotransposon in the *RsSRK-26-1* gene might be responsible for the SC phenotype of radish.

## Introduction

Radish (*Raphanus sativus* L.) is one of the most important root vegetables in East Asian countries such as China, Japan, and Korea. As a cross-pollinated crop, radish shows hybrid vigor. Its *F*<sub>1</sub> hybrid varieties that harness such hybrid vigor have been developed (Curtis 2011). Self-incompatibility (SI) is defined as inhibition of pollen tube growth in pistils during self-pollination. It has been commercially used for the production of *F*<sub>1</sub> hybrid seeds in radish (Singh et al. 2001). SI systems are widespread in plants. They can promote outcrossing and increase genetic diversity. More than 100 families and almost 40% of angiosperms have adopted SI systems (Igic et al. 2008).

Generally, a single locus initially named as *S* (Sterility) is involved in SI responses. Two tightly linked genes consisting of male and female determinants of SI are positioned in the *S*locus. Nucleotide sequences of these two SI determinants are highly polymorphic and multi-allelic. Since at least two genes are present in the *S*locus, combinations of these genes can lead to *Shaplotypes* (Stone and Goring 2001; Watanabe et al. 2012). Types of SI systems are largely categorized into heteromorphic and homomorphic SI. In the case of heteromorphic SI, cross-pollination is possible between different floral morphs. Candidate genes responsible for distyly have been reported in *Primula vulgaris* (Li et al. 2016) and *Fagopyrum esculentum* (Yasui et al. 2012).

Homomorphic SI systems are generally classified into gametophytic SI (GSI) and sporophytic SI (SSI) depending on inheritance patterns of SI phenotypes. Such inheritance patterns are determined by genotypes of haploid pollen in GSI and diploid pollen parents in SSI (Silva and Goring 2001; Muñoz-Sanz et al. 2020). GSI systems based on RNase and S-locus F-box proteins have been extensively studied in Solanaceae, Plantaginaceae, and Rosaceae families (Franceschi et al. 2012). The *Papaver* system based on programmed cell death has been mainly studied in poppy (Wheeler et al. 2009). Meanwhile, SSI has been extensively studied in Brassicaceae including radish (Sobotka et al. 2000; Takayama and Isogai 2003; Tantikanjana et al. 2010; Watanabe et al. 2012).

Two tightly linked genes encoding *S* receptor kinase (SRK) and *S* locus cysteine-rich protein (SCR)/*S* locus protein 11(SP11) or SP11/SCR have been revealed as female and male determinants of SSI, respectively (Schopfer et al. 1999; Takasaki et al. 2000; Takayama et al. 2000). Another gene encoding *S* locus glycoprotein (SLG) has been identified in the *S* locus, although its precise role remains uncertain (Nasrallah et al. 1985; Watanabe et al. 2012). Sequences of *SLG* and *S* domain of *SRK* genes are highly homologous within the same *S*haplotypes (Sato et al. 2002; Lim et al. 2002; Okamoto et al. 2004). Based on sequence diversity of *SLG* and *SRK* genes, *S*haplotypes are classified into class I and class II (Nasrallah et al. 1991; Sato et al. 2002). Generally, *S*haplotypes of class I are dominant over those of class II and small noncoding RNAs and DNA methylation are involved in such dominance relationships (Tarutani et al. 2010; Yasuda et al. 2016).

SI was first used in production of  $F_1$  hybrid seeds of cabbage in 1940s by Japanese seed companies. Since then, SI has been exploited for economical production of  $F_1$  hybrid seeds of radish and *Brassica* crops (Muñoz-Sanz et al. 2020). To implement SI systems in radish  $F_1$  hybrid breeding programs, specific *S*haplotypes of parental lines should be identified to avoid cross-incompatibility between parental lines. At least 35 *S*haplotypes have been identified in radish (Haseyama et al. 2018). More than 50 and 100 *S*haplotypes have been identified in Chinese cabbage (Nou et al. 1993) and cabbage (Ockendon 2000), respectively.

For efficient identification of *S*haplotypes, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods have been developed for radish and *Brassica* crops (Brace et al. 1993, 1994; Nishio et al. 1994, 1996, 1997; Sakamoto et al. 2000; Lim et al. 2002). Although PCR-RFLP is more efficient than laborious pollination tests, it has pitfalls such as complicated band patterns of digested PCR products and difficulty in specific PCR amplification of *SLG* or *SRK* genes due to the presence of multiple homologous *SRK*-like genes (Boyes et al. 1991; Kim and Kim 2018). To overcome these problems, a new *S*hapotyping system has been developed based on *SLL2* and *SP6* genes positioned at borders of *S*core regions and 31 *S*haplotypes have been identified from diverse breeding lines in our previous studies (Kim et al. 2016; Kim and Kim 2019).

Self-compatible (SC) mutants have been found naturally or induced by irradiation in crops normally showing SI phenotypes (Muñoz-Sanz et al. 2020). In some fruit tree species, such SC phenotypes are sometimes advantageous for better fruit set and yield (Claessen et al. 2019). A radish breeding line

showing a SC phenotype was identified and the critical mutation responsible for the SC phenotype was revealed in this study. In addition, efficient application of this SC phenotype in radish breeding programs is discussed.

## Materials And Methods

### Plant materials

A radish breeding line (JNUR1537) showing an SC phenotype was introduced from a seed company (Farm Hannong Co., Ltd., Republic of Korea). Detail pedigree of this breeding line is unknown. JNUR1537 was crossed with JNUR1123 to produce F<sub>1</sub> hybrids by hand-pollination. An F<sub>2</sub> population was produced by self-pollination of F<sub>1</sub> hybrids. Three breeding lines (DBRL294-2, DBR2085 and DBR2086) containing the *RsS3* haplotype were used to compare SI phenotypes with JNUR1537. Shaplotypes of these three breeding lines have been identified in a previous study (Kim and Kim 2019). Evaluation of SI phenotypes were performed in greenhouses. Single plants were covered with mesh cages to prevent cross-pollination. No insect pollinator was introduced to mesh cages.

### PCR amplification, genome walking, and sequencing of PCR products

Total genomic DNAs were extracted from leaf tissues using a cetyl trimethylammonium bromide (CTAB) method (Doyle and Doyle 1987). PCR amplification and sequencing of PCR products of *SLL2*, *SP6*, and *SRK* genes of parental lines of the F<sub>2</sub> population were carried out following methods described in a previous study (Kim and Kim 2019). The detail protocol for high-resolution melting (HRM) analysis has been described in a previous study (Seo et al. 2020). SYTO®9 green fluorescent nucleic acid stain (Thermo Fisher Scientific, Waltham, MA, USA) was used as a dye. HRM peaks were obtained using a LightCycler® 96 system (Roche Molecular Systems, Pleasanton, CA, USA). Primer sequences of HRM markers are shown in Supplementary Table 1.

To obtain full-length genomic DNA sequences of duplicated *SRK* homologs, long PCR and genome walking were performed. Long PCR reactions were carried out in 50 µL reaction mixtures containing 0.25 µg DNA template, 5 µL 10× PCR buffer, 1.0 µL forward primer (10 µM), 1.0 µL reverse primer (10 µM), 8.0 µL dNTPs (10 mM each), and 0.5 µL Taq polymerase (TaKaRa LA PCR™ Kit Ver. 2.1; Takara Bio, Shiga, Japan). Long PCR amplification was performed with 40 cycles of 98°C for 10 s and 68°C for 15 min. Genome walking was performed using a Universal GenomeWalker kit (Takara Bio) according to the manufacturer's instructions. Total genomic DNAs used to construct genome walking libraries were isolated from young seedlings using a DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA). All PCR products were visualized on 1.5% agarose gels after ethidium bromide staining. Subsequently, PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN). Sequencing was performed by a specialized company (Macrogen, Seoul, Republic of Korea).

# **RNA extraction and reverse transcription polymerase chain reaction (RT-PCR)**

Total RNAs were extracted from floral buds using a RNeasy Plant Mini Kit (QIAGEN). cDNAs were synthesized using a cDNA synthesis kit (SuperScript™ III first-strand synthesis system for RT-PCR, Invitrogen, Carlsbad, CA, USA). RT-PCR amplification was performed with an initial denaturation step at 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 68°C for 30 s, and 72°C for 2 min. It was finished with a final 10-min extension step at 72°C. Primer sequences used in RT-PCR are listed in Supplementary Table 1. Radish tubulin sequence (Rs395780) identified from a draft genome sequence (Jeong et al. 2016) was used as a control.

## **Construction of phylogenetic trees**

Genomic DNA, cDNA, or deduced amino acid sequences of *SLL2*, *SP6*, *SRK*, and *SCR/SP11* genes were aligned using a BioEdit software (Hall 1999). Large gaps in alignments were removed using Gblocks program (Castresana 2000). Phylogenetic trees were produced using MEGA version X (Kumar et al. 2018) with a neighbor-joining method. Node support of the phylogenetic tree was assessed using 1,000 bootstrap replicates.

## **Results**

### **Discovery of an SC radish breeding line and inheritance patterns of the SC phenotype**

Radishes derived from JNUR1537 showed an almost SC phenotype. Compared with an SI radish accession (Fig. 1A), a large number of pods were formed in the SC radish without any insect pollinators (Fig. 1B). This SC plant was cross-pollinated with a SI breeding line (JNUR1123). F<sub>1</sub> hybrids showed the SC phenotype, indicating that the SC phenotype was dominant over the SI phenotype. Subsequently, an F<sub>2</sub> population was produced from self-pollination of F<sub>1</sub> hybrids. Ten individuals of the F<sub>2</sub> population were grown in a greenhouse. Each individual plant was separately covered with mesh cages. SI phenotypes clearly segregated among F<sub>2</sub> plants (Table 1).

Table 1  
Number of seeds produced from 10 randomly selected F<sub>2</sub>  
individuals grown in isolated greenhouses

Plant code	Number of seeds	Genotype of Shaplotypes
F2-1	540	<i>RsS3 / RsS3</i>
F2-2	693	<i>RsS3 / RsS32</i>
F2-3	74	<i>RsS32 / RsS32</i>
F2-4	2,330	<i>RsS3 / RsS32</i>
F2-5	1,483	<i>RsS3 / RsS32</i>
F2-6	174	<i>RsS3 / RsS3</i>
F2-7	1,012	<i>RsS3 / RsS32</i>
F2-8	576	<i>RsS3 / RsS3</i>
F2-9	86	<i>RsS32 / RsS32</i>
F2-10	1,086	<i>RsS3 / RsS32</i>

To test whether the SC phenotype was related to Shaplotypes, specific Shaplotypes of both SC and SI parental lines were identified using an Shaplotyping system developed in a previous study (Kim and Kim 2019). From the SI parent, novel sequences of *SLL2*, *SP6*, and *SRK* genes closely related to *RsSLL2-25*, *RsSP6-8*, and *RsSRK9*, respectively (Supplementary Figs. 1, 2, 3), were isolated. These novel allele sequences were designated as *RsSLL2-31*, *RsSP6-26*, and *RsSRK21*, respectively. Their nucleotide sequences were deposited into GenBank under accession numbers of MZ383797, MZ383798, and MZ383799, respectively. Since *RsSRK21* was clustered with class II *SRK* genes (Supplementary Fig. 3), *RsSK21* was considered as a member of class II *SRK* genes. The new class II Shaplotype consisting of three novel sequences was designated as *RsS32*. Nucleotide sequences of both *SLL2* and *SP6* genes in the SC parent were identical to those of the class II *RsS3* haplotype. As expected, a mixture of duplicate *SRK*-like genes was amplified. In a previous study (Kim and Kim 2019), closely related duplicate *SRK*-like genes have also been detected in the *RsS3* haplotype.

For efficient genotyping of Shaplotypes of F<sub>2</sub> individuals, two HRM markers were developed based on polymorphic sequences of *SLL2* and *SP6* alleles of parental lines, respectively (Supplementary Fig. 4). After genotyping F<sub>2</sub> seedlings, five individuals of each genotype were separately grown in cages. While SC phenotypes were observed in both homozygous *RsS3* and heterozygous individuals, few pods and seeds were formed in homozygous *RsS32* individuals (Table 2). In addition, SI phenotypes of 10 F<sub>2</sub> individuals previously grown in isolated greenhouse were matched with genotypes of Shaplotypes (Table

1). These results implied that the SC phenotype was probably related to *S* core genes such as *SRK* and *SCR/SP11*.

Table 2  
Numbers of pods and seeds produced from  $F_2$  individuals representing three genotypes of *S* haplotypes

<b>Genotype</b>	<b>Plant code</b>	<b>Number of pods</b>	<b>Number of seeds</b>
<i>RsS3 / RsS3</i>	A1	328	844
	A2	115	136
	A3	232	257
	A4	396	761
	A5	124	245
	A6	76	136
<i>RsS3 / RsS32</i>	H1	280	705
	H2	36	67
	H3	24	19
	H4	192	610
	H5	59	161
	H6	48	122
<i>RsS32 / RsS32</i>	B1	9	10
	B2	12	7
	B3	16	13
	B4	0	0
	B5	0	0
	B6	8	7

## Assembly of full-length genomic DNA sequences of *S* core genes in the SC radish

To identify any defects in *SRK* and *SCR/SP11* genes in the SC radish, full-length sequences of *SRK* and *SCR/SP11* genes were analyzed. To identify the authentic *SRK* among duplicate *SRK*-like genes, full-

length genomic DNA sequences of closely related *SRK*-like genes were obtained in this study. Using primers designed based on conserved regions among radish and three *Brassica* class II *SRK* genes (Supplementary Fig. 5), 5' *S* domain sequences of *SRK* were isolated. After obtaining sequences of the last exon7 using genome walking, a full-length *SRK* sequence was assembled by connecting 5' *S* and 3' kinase domains through long PCR amplifications (Fig. 2). In addition, approximately 2-kb 5' sequences containing a putative promoter was obtained by genome walking, resulting in a 18,133-bp full-length sequence. Meanwhile, a 6,200-bp full-length sequence of another putative *SRK* gene was obtained using genome walking PCRs with primers designed based on dissimilar sequences between two putative *SRK* genes (Fig. 2).

Since partial *S* domain sequences of the larger putative *SRK* gene containing a large-sized 11,865-bp intron 3 were identical to those of the *SRK*-26 deposited in the GenBank (LC341218), the *S*haplotype of the SC radish might be identical to the *RsS*-26 haplotype designated by Haseyama et al. (2018). Indeed, nucleotide sequences of *SLG* of the SC radish were identical to those of *SLG*-26 (LC341241) of the *RsS*-26 haplotype. In addition, partial sequences identical to those of *RsSCR*-26 (LC325812) were amplified in the SC radish. However, additional 552-bp full-length *SCR/SP11* gene sharing 91.0% sequence identities with *RsSCR*-26 was obtained by genome walking in the SC radish, implying that both *SRK* and *SCR/SP11* genes might be duplicated in the *RsS*-26 haplotype. The novel *SCR/SP11* gene was closely related to other known *SCR/SP11* genes (Supplementary Fig. 6). This novel gene was designated as *RsSCR*-26-2. Its sequence was deposited into GenBank under the accession number of MZ383800. The full-length *RsSCR*-26-2 contained intact exons. Following the unified nomenclature suggested by Haseyama et al. (2018), the *RsS*3 haplotype was renamed as *RsS*-26 to avoid confusion.

Coding sequences of two putative *SRK* genes were also intact without any premature stop codons. They shared 91.7% nucleotide sequence identities with each other. Since *S*core gene sequences were identical to those of the *RsS*-26 haplotype, the large and small putative *SRK* genes were designated as *RsSRK*-26-1 and *RsSRK*-26-2, respectively. Full-length sequences of both *SRKs* were deposited into GenBank under accession numbers of MZ383801 and MT241389, respectively. The phylogenetic tree of radish *SRK* genes showed that *RsSRK*-26-1 and *RsSRK*-26-2 were closely related to each other (Fig. 3). Similar to other class II *SRK* genes, the *RsSRK*-26-1 gene contained a large-sized intron 3. However, a relatively small intron 3 was identified in the *RsSRK*-26-2 gene (Supplementary Fig. 7), suggesting that the *RsSRK*-26-1 gene might be the genuine *SRK* in the *RsS*-26 haplotype.

## **Identification of a critical mutation responsible for the radish SC phenotype**

Interestingly, a transposable element-like sequence was found in the large-sized intron 3 of *RsSRK*-26-1 (Fig. 2). A 4,146-bp intact open reading frame (ORF) was identified in the transposable element-like sequence. Five typical domains of long terminal repeat (LTR)-retrotransposon were identified in the polyprotein region (Fig. 2). Since the integrase (INT) domain was positioned upstream of the reverse transcriptase (RT) domain, this LTR-retrotransposon belonged to *Copia* superfamily (Wicker et al. 2007).

Target site duplication (TSD) of 4-bp ('GGAC') was found at flanking regions of this element. LTR sequences of 284-bp in length positioned at both ends were perfectly identical to each other. This novel LTR-retrotransposon was designated as *RsCopia1*.

Since the ORF encoding a polyprotein was intact and both LTR sequences were identical to each other, *RsCopia1* was assumed to be recently transposed into the *RsSRK-26-1* gene. To investigate whether all *RsS-26* haplotypes contained *RsCopia1* in the *SRK* gene, three breeding lines (DBRL294-2, DBR2085 and DBR2086) found to contain *RsS-26* haplotypes in the previous study (Kim and Kim 2019) were analyzed. Results of PCR amplification and sequencing showed no *RsCopia1* insertion in any of these three breeding lines (Fig. 4A). Except for the *RsCopia1* insertion, only two single nucleotide polymorphisms (SNPs) were identified in the intron 3 between normal and mutant *RsSRK-26-1* alleles. Nucleotide sequence of the normal allele was deposited into GenBank with accession number of MT241388.

Unlike SC JNUR1537 containing the mutant *SRK* allele, three breeding lines harboring normal *SRK* alleles showed SI phenotypes (Fig. 4C). Significantly reduced numbers of pods and seeds were produced in these three SI breeding lines (Fig. 4C, Supplementary Fig. 8). Transcripts of the mutant *RsSKR-26-1* were not detected in the SC radish compared with the normal *RsSRK-26-1* in three SI breeding lines (Fig. 4B). There was only a single SNP in putative promoter regions between normal and mutant *RsSRK-26-1* alleles. These results suggest that transposition of *RsCopia1* in the *RsSRK-26-1* gene might be responsible for the inactivation of *SRK* and the resulting SC phenotype.

## Discussion

### Identification of duplicate *SRK*-like genes in the radish *RsS-26* haplotype

Full-length genomic DNA sequences of duplicated putative *SRK* and *SCR/SP11* genes were obtained from the radish *RsS-26* haplotype in this study (Fig. 2). Duplication of *S*core genes including *SCR/SP11* and *SRK* and its effects on SI responses have been reported in *Brassica rapa* (Takada et al. 2005, 2017) and *Leavenworthia alabamica* (Chantha et al. 2013), a member of Brassicaceae family. Takada et al. (2017) have demonstrated that duplicated *SUI1* and *PUI1* genes corresponding to *SRK* and *SCR/SP11*, respectively, control intraspecific unilateral incompatibility in *B. rapa*. In the case of *Leavenworthia*, a novel SI system might have evolved from paralogs (*LaLal2* and *LaSCL*) of *SRK* and *SCR/SP11* genes after loss of the original *S*locus, which is common in *Arabidopsis*, *Brassica*, and *Leavenworthia* (Chantha et al. 2013). The phylogenetic tree indicated that duplication of *SRK* homologs in the radish *RsS-26* haplotype occurred more recently than that in *SUI1* and *LaLal2* genes (Fig. 3).

However, it was unlikely that duplication was very recent since significant sequence and length polymorphisms existed between duplicated *SRKs* (Fig. 2). Since duplicate *SCR/SP11* genes were also identified, the entire *S*core region might be duplicated in the *RsS-26* haplotype. Alternatively, two separate *S*core regions might have been merged by homologous recombination-mediated translocation. Further

studies are needed to elucidate the exact duplication event and effects of *S*core region duplication. Isolation of full-length *S*core regions of the *RsS-26* haplotype might provide a clue to resolve these issues.

As shown in *B. rapa* (Takada et al. 2005, 2017) and *Leavenworthia* (Chantha et al. 2013), SI responses in the Brassicaceae family are complex processes. However, they are flexible enough to adopt duplicate paralogous *SRK* and *SCR/SP11* pairs and restore SI systems after losing the original *S*locus under sufficient selection pressure. Multigene family of *SRK* homologs in Brassicaceae (Cock et al. 1995; Suzuki et al. 1997; Pastuglia et al. 1997; Kai et al. 2001) represents a potential source of such plastic evolution of SI systems. In radish, a total of 61 *SRK* homologs have been identified from two draft genome sequences (Kim and Kim 2018). Further studies such as functional characterization of duplicate *SRK* and *SCR/SP11* genes and analysis of their effect on the strength of SI responses are needed to determine implications of *S*core region duplication in the evolution of SI systems in Brassicaceae family. The radish *RsS-26* haplotype harboring duplicate *S*core genes represent a valuable material for such studies in the future.

## Identification of a radish breeding line showing a SC phenotype and its application in radish breeding

A radish showing a SC phenotype was identified in this study. To the best of our knowledge, this is the first study to report an SC radish. Among *Brassica* species, several SC mutants have been previously reported. In *Brassica rapa*, SC mutants have been identified from two cultivars, Yellow Sarson (Fujimoto et al. 2006a) and Dahuangyoucui (Zhang et al. 2013), containing similar mutant Shaplotypes. Another SC phenotype induced by gene conversion from *SLG* to *SRK* has been reported (Fujimoto et al. 2006b). In addition, an SC *B. rapa* has been artificially developed by silencing of *SCR/SP11* using RNAi (Jung et al. 2012). In the case of *B. oleracea*, deletion of exon1 and 2 of *SRK* is responsible for an SC phenotype (Nasrallah et al. 1994). Recently, eight quantitative trait loci (QTLs) controlling a SC phenotype have been identified in an inbred line of *B. oleracea* (Xiao et al. 2019).

Duplicate *SRK*-like genes were identified from the SC radish in this study. Some evidences indicated that the large-sized *RsSRK-26-1* might be a genuine *SRK*. First of all, transcription of *RsSRK-26-1* was inactivated in the SC radish in contrast to three other SI breeding lines containing normal *RsSRK-26-1*. These results showed a direct relationship between SI phenotypes and *RsSRK-26-1*. In addition, the *RsSRK-26-1* gene contained a large-sized intron 3 as shown in other *Brassica* class II *SRK* genes (Supplementary Fig. 7). Further functional studies are needed to clarify exact roles of both duplicate *SRK* genes. Since the *RsSRK-26-2* gene contained intact exons and its transcripts were more abundant than those of *RsSRK-26-1* (Fig. 4B), further functional characterization of *RsSRK-26-2* might be an intriguing topic.

An intact LTR-retrotransposon was identified in the large-sized intron 3 of *RsSRK-26-1* of the SC radish in this study. Since this element was transposed into an intronic region, this insertion might not have any effect on transcription of *SRK*. Transcripts of *RsSRK-26-1* were not detected in the mutant allele. Because there was only one single SNP in approximately 2.0 kb putative promoter regions between mutant and normal *RsSRK-26-1* alleles, insertion of *RsCopia1* might be responsible for blockage of transcription. DNA methylation of promoter regions of *RsSRK-26-1* is assumed to be induced by transposition of *RsCopia1*, although further functional analyses are required. DNA methylation is known to be involved in silencing of transposable elements in plants (Bartels et al. 2018). Similarly, transcripts of the *SRK* gene are not detected in SC Yellow Sarson probably due to insertion of an LTR-retrotransposon in the intron 1, although there is no critical mutation in their promoter regions (Fujimoto et al. 2006a). In another case, reduced expression of the *FLC* gene is caused by insertion of an LTR-retrotransposon in the first intron (Michaels et al. 2003).

The SC phenotype observed in this study was apparent compared with phenotypes of SI breeding lines and  $F_2$  individuals. The SC phenotype was detected in  $F_1$  and heterozygous  $F_2$  individuals, suggesting that SC was dominant over SI in this population. This result indicates that the class II *RsS-26* haplotype is dominant over a novel class II *RsS32* haplotype. Despite a conspicuous SC phenotype, there were significant variations of pod and seed numbers produced by SC plants in this study. In addition to effects of minor modifying genes, environmental effects might play a significant role in the expression of SC phenotypes. High temperature is known to cause breakdown of SI phenotypes in Brassicaceae (Yamamoto et al. 2019). However, variations observed in this study might be largely derived from growth conditions where single plants were covered with mesh cages in the greenhouse. Large variations might result from such inferior growth conditions. When SC plants were grown in open field conditions, seed settings were significantly improved (Supplementary Fig. 9).

The SC radish identified in this study would be a valuable material for radish  $F_1$  hybrid breeding. Due to unstable SI phenotypes of some inbred lines, inadvertent self-pollination of maternal lines of  $F_1$  hybrids frequently can result in a low genetic purity of  $F_1$  cultivars. For this reason, male-sterility has replaced SI systems as a more stable genetic emasculation tool. Although male-sterility is used for  $F_1$  hybrid breeding, propagation of inbred parental lines should be performed by self-pollination of parental lines using high concentrations of CO<sub>2</sub>. However, if the SC phenotype is introgressed to parental lines, such expensive treatment with CO<sub>2</sub> might be unnecessary. Regarding fixed varieties, the SC phenotype might greatly improve seed yields. Taken together, the SC radish identified in this study will become an important material for radish breeding programs.

## Declarations

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## Authors' contribution

So-Hyeon Bong and Ganghee Cho performed experiments and drafted the manuscript. Dong-Seon Kim performed phenotypic analyses. Sunggil Kim organized and coordinated this research project and edited the final manuscript.

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## Ethics approval

All experiments were performed in compliance with current laws of the Republic of Korea.

## Consent for publication

Not applicable.

## Competing interests

The authors declare no competing interest.

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## Figures

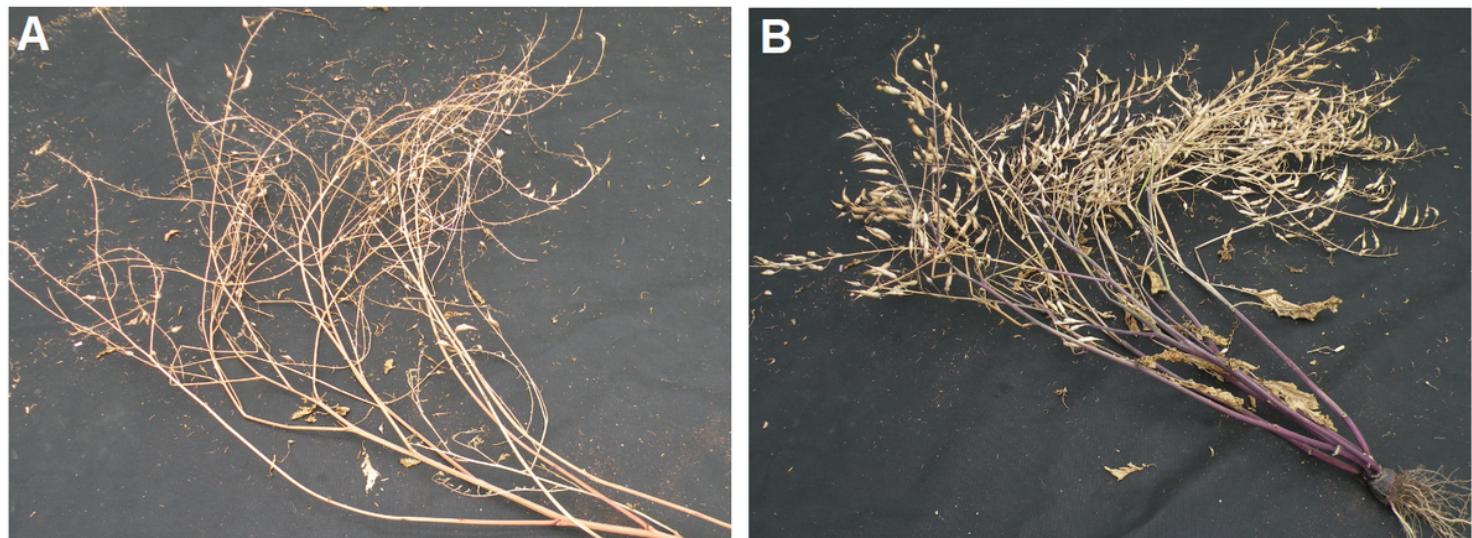
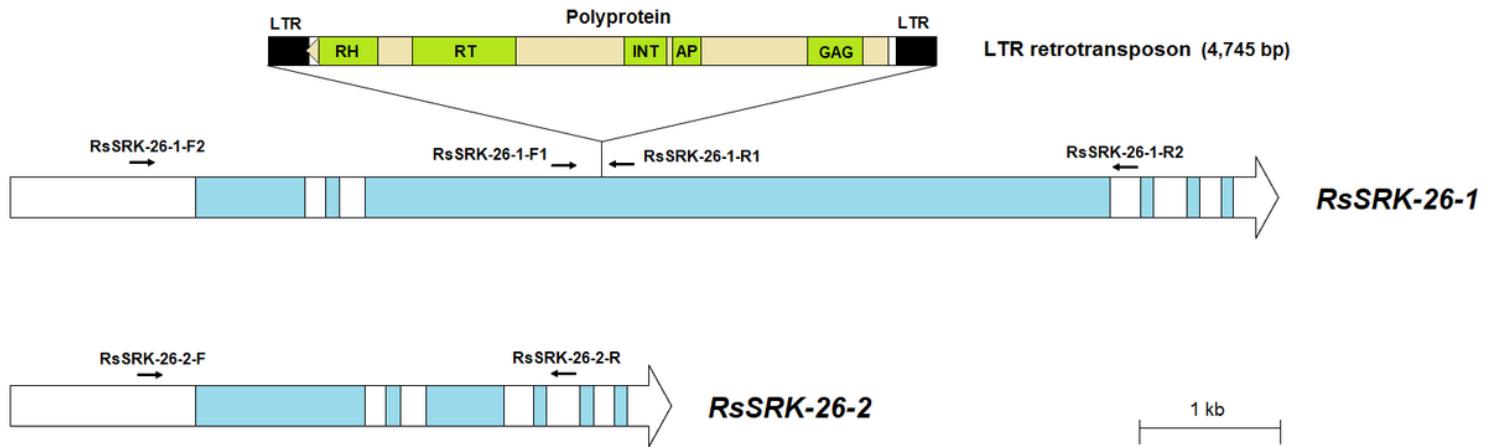


Figure 1

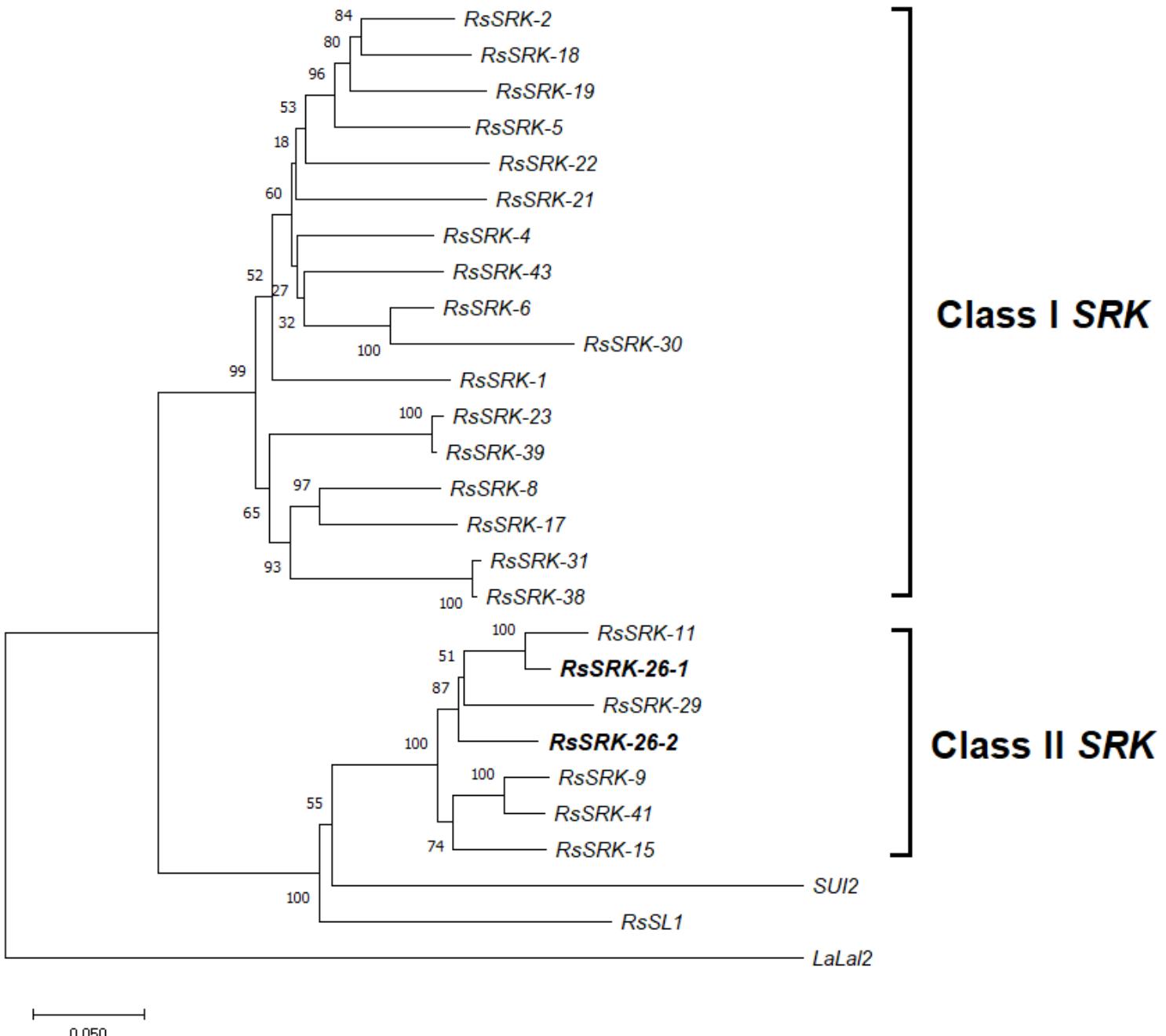
Phenotypes of self-incompatible and self-compatible radish breeding lines at the stage of seed harvest.

A. A self-incompatible breeding line, JNUR1123, B. A self-compatible breeding line, JNUR1537.



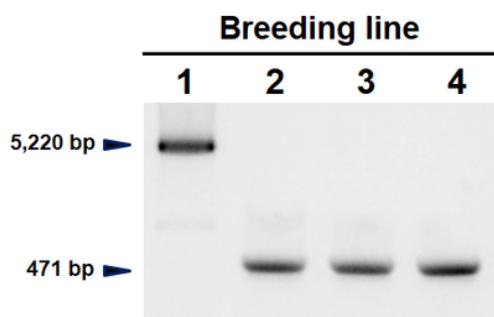
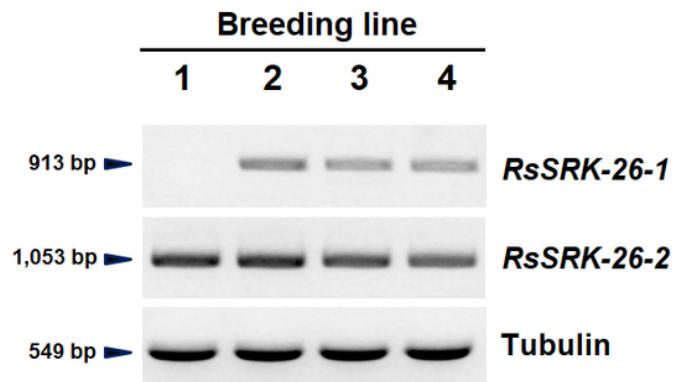
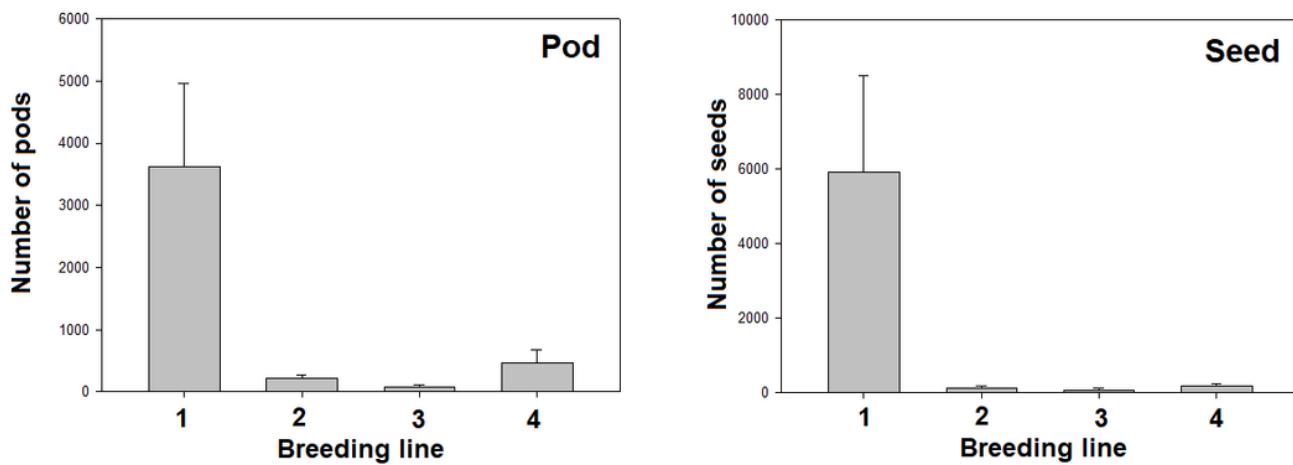
**Figure 2**

Genomic DNA structures of duplicated putative SRK genes in the radish Rss3 (RsS-26) haplotype. Empty and blue-colored boxes indicate exons and introns, respectively. Arrow-shaped boxes indicate 5'-to-3' direction. A rectangular box above the *RsSRK-26-1* indicates a transposed LTR-retrotransposon. Arrow-shaped box in the rectangular box indicates an ORF encoding a polyprotein and the 5'-to-3' direction. LTR: long terminal repeat, RH: RNase H, RT: Reverse transcriptase, INT: Integrase, AP: Aspartic proteinase, GAG: Capsid protein. Horizontal arrows indicate positions of primer binding sites.



**Figure 3**

Phylogenetic relationship of S domain sequences of radish SRK genes. The tree was constructed with nucleotide sequences. Duplicate putative SRK genes positioned in the *RsS3* (*RsS-26*) haplotype are shown in boldface. The *RsSL1* is a radish SRK-homologous gene, which is most closely related to SRK genes (Kim and Kim 2018). *SUI1* (GenBank accession: LC088714) and *LaLal2* (GenBank accession: KC981242) are duplicated SRK genes identified from *Brassica rapa* (Takada et al. 2005, 2017) and *Leavenworthia alabamica* (Chantha et al. 2013), respectively.

**A****B****C****Figure 4**

Comparison of breeding lines containing mutant and normal *RsSRK-26-1* alleles. A. PCR products of *RsSRK-26-1* amplified using a primer pair flanking the LTR-retrotransposon. B. RT-PCR products of *RsSRK-26-1* and *RsSRK-26-2* amplified from cDNAs of floral tissues. C. Comparison of pod and seed numbers produced from four breeding lines. Averages of four plants are shown in graphs. 1: JNUR1537, 2: DBRL294-2, 3: DBR2085, 4: DBR2086.

## Supplementary Files

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- [SupplementaryFig.2.SP6tree.tif](#)
- [SupplementaryFig.3.SRKtree.tif](#)
- [SupplementaryFig.4.HRMmarkers.tif](#)

- SupplementaryFig.5.SRKalignment.tif
- SupplementaryFig.6.SCRtree.tif
- SupplementaryFig.7.FulllengthSRKs.tif
- SupplementaryFig.8.SIandSCphenotypes.tif
- SupplementaryFig.9.SCradishinthefield.tif
- SupplementaryTable1.Primersequences.xlsx