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MINI REVIEW

Commonalities and differences between *Brassica* and *Arabidopsis* self-incompatibility

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In higher plants, the self-incompatibility mechanism is important for inhibition of self-fertilization and facilitation of out-crossing. In Brassicaceae, the self-incompatibility response is mediated by allele-specific interaction of the stigma-localized *S*-locus receptor kinase (SRK) with the pollen coat-localized ligand (SCR/SP11). All self-incompatible Brassicaceae plants analyzed have been found to have the *SRK* and *SCR/SP11* genes in the *S*-locus region. Although *Arabidopsis thaliana* is self-compatible, transformation with functional *SRK-SCR* genes from self-incompatible *Arabidopsis* species confers the self-incompatibility phenotype to *A. thaliana*. The allele-specific interaction between SRK and SCR activates the downstream signaling cascade of self-incompatibility. Yeast two-hybrid analysis with a kinase domain of SRK as bait and genetic analysis suggested several candidate components of self-incompatibility signaling in *Brassica*. Recently, *A. thaliana* genes orthologous to the identified genes for *Brassica* self-incompatibility signaling were evaluated by using a self-incompatible transgenic *A. thaliana* plant and these orthologous genes were found not to be involved in self-incompatibility signaling in the transgenic *A. thaliana*. In this review, we describe common and different aspects of *S*-locus genomic regions and self-incompatibility signaling between *Brassica* and *Arabidopsis*.

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INTRODUCTION

Higher plants have a self-incompatibility mechanism for preventing self-fertilization and facilitating out-crossing. Self-incompatibility is considered to contribute to the maintenance of genetic diversity and avoidance of inbreeding depression. The Brassicaceae self-incompatibility system is well studied. Recognition specificity of this self-incompatibility system is determined by a diploid genotype of a parent plant. In self-pollination, pollen germination and pollen tube penetration of the cell wall of stigma papillar cells are inhibited.

Self-incompatibility is generally controlled by a single locus, the *S* locus. In Brassicaceae, the *S-locus receptor kinase* (*SRK*) and *S-locus cysteine rich protein/S-locus protein 11* (*SCR/SP11*) genes, which encode highly polymorphic proteins as female and male determinants of recognition specificity, respectively, have been found at the *S* locus.[1–3] Because these two genes are tightly linked with each other and inherited as a single Mendelian locus, a set of alleles of the *S*-locus genes is referred to as *S* haplotype.[4] The *SRK* gene is expressed in the stigma papillar cells and encodes a plasma membrane-localized receptor kinase, which has a highly polymorphic extracellular receptor domain (S domain, hereafter) followed by a transmembrane domain and a serine/threonine kinase domain.[1,5] Some variants of SRK exhibit more than 30% amino-acid sequence divergence in the S domain.[6,7] *SCR/SP11* (*SCR* hereafter) is expressed in anthers and its translational products are secreted to the pollen coat.[8] SCR is a small peptide, ~60 amino acids of mature form, and functions as the ligand for SRK.[2,9,10] SCR is also highly polymorphic and less than 50% amino-acid sequence similarity is shared between *S* haplotypes.[2,7,11–13] Although they have high sequence diversity, all SCR proteins appear to form a typical defensin-like 3D structure consisting of three β-sheets and one α-helix.[14,15] In self-pollination, stigma-localized SRK interacts with SCR of the same *S* haplotype located on the pollen surface and activates a self-incompatibility signaling cascade, resulting in inhibition of self-pollen germination and tube penetration of the stigma papillar cell wall.

Arabidopsis thaliana, which is a model plant belonging to the family Brassicaceae, had not been used for studies of self-incompatibility mechanism because *A. thaliana* is a self-compatible species due to lack of functional *SRK* and/or *SCR*.[16–21] However, transformation with functional *SRK-SCR* genes from self-incompatible *Arabidopsis* and closely related species, such as *Arabidopsis lyrata*, *Arabidopsis halleri* and *Capsella gradiflora*, confers self-incompatibility phenotype to *A. thaliana*,[20,22–26] indicating that *A. thaliana* has the molecular components that are required for self-incompatibility signaling and can be used for studies of the Brassicaceae self-incompatibility mechanism.

The plant family Brassicaceae contains 338 genera and 3709 species, 308 of the 338 genera being assigned to 44 tribes.[27,28] These tribes are grouped into three major linages.[29–32] *Arabidopsis* and *Brassica* belong to lineage I and II, respectively,[33] and these two genera were separated approximately 15 million years ago. Whole genome duplication or triplication has occurred only in the *Brassica* lineage but not in *Arabidopsis* since their separation. These observations suggest that *Brassica* and *Arabidopsis* would have different genetic backgrounds, although both self-incompatible plants of these two genera possess the *SRK* and *SCR* genes for recognition specificity of self-incompatibility. In this mini-review, we describe the molecular components functioning in SRK-mediated self-incompatibility signaling in *Brassica* and *in planta* evaluation results of these identified molecular components by using self-incompatible transgenic *A. thaliana*. We also discuss common and different aspects of self-incompatibility between *Brassica* and *Arabidopsis*.

THE *S*-LOCUS IN *BRASSICA* AND *ARABIDOPSIS*

Although introduction of *Arabidopsis SRK-SCR* genes confers the self-incompatibility response to *A. thaliana*,[20,22–26] construction of

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self-incompatible transgenic *A. thaliana* plants by introduction of the *Brassica SRK-SCR* gene pair has not succeeded.[34] One possible explanation for this failure is that *Brassica* SRK and/or SCR are too greatly differentiated to function in *Arabidopsis*.

Molecular genetic studies have elucidated an interesting difference of the *S*-locus regions between *Brassica* and *Arabidopsis*. In *Brassica*, three genes are generally found in the *S* locus. In addition to the *SRK* and *SCR* genes, the *S-locus glycoprotein* (*SLG*) gene is located at the *S* locus. The *SLG* gene encodes a stigma soluble glycoprotein showing high similarity to the S-domain of SRK. Like SRK, SLG is a highly polymorphic protein between *S* haplotypes. The role of SLG in self-incompatibility remains unclear. Because some *S* haplotypes lack the functional *SLG* gene at the *S* locus,[35] the *SLG* gene is not considered to be an essential component in the self-incompatibility in *Brassica*. The S domain of *SRK* of a self-compatible *Brassica rapa S-54* mutant has been found to be 100% identical to the *S-54 SLG* gene,[36] suggesting that gene conversion between *SRK* S domain and *SLG* occurred, although this gene conversion caused the loss of the SRK function. This observation indicates one possible role of *SLG* in self-incompatibility, namely that the *SLG* gene contributes to production of a new *SRK* allele by gene conversion.

The *SLG* gene has not been found at the *S* locus of any *A. lyrata S* haplotypes. Instead of the *SLG* gene, the *ARK3* gene, which is closely related to the *SRK* gene and contains the S domain, transmembrane domain and kinase domain, is located at the *A. lyrata S* locus. The *ARK3* gene, as well as *SRK* and *SCR*, has been affected by positive selection.[37] In addition, gene conversion between *SRK* and *ARK3* was detected as observed in the *Brassica S* locus. This gene conversion occurred in the region of the kinase domain,[37] and possibly functioned to promote *SRK* evolution to produce new substrate specificity.

POSITIVE REGULATORS IN THE SELF-INCOMPATIBILITY SIGNALING PATHWAY IN *BRASSICA*

M-locus protein kinase (*MLPK*) has been identified in self-incompatible *B. rapa* plants as a positive regulator of *Brassica* self-incompatibility.[38] MLPK is a protein kinase and a self-compatible *mlpk* mutant of *B. rapa* has a G194R mutation in its kinase domain.[38] *In vitro* kinase assay has shown that MLPK has autophosphorylation activity, whereas MLPK protein of the self-compatible *mlpk* mutant has no activity.[38] The *MLPK* gene produces two alternative transcripts (*MLPKf1* and *MLPKf2*) from distinct transcription initiation sites.[39] *MLPKf1* and *MLPKf2* produce proteins of 404 and 410 amino acids, respectively, and only their N-terminus sequences are different between them.[39] *MLPKf2* transcripts are more abundant than *MLPKf1* transcripts in the stigma.[39] Both MLPK forms have an N-myristoylation motif, which functions as a plasma membrane targeting motif.[38,39] Bimolecular fluorescence complementation assay has indicated that MLPK interacts with SRK at the plasma membrane and that this interaction is independent of the SCR peptide, suggesting that MLPK might form hetero-oligomers with SRK on the plasma membrane.[39] In addition, *in vitro* kinase assay has revealed that MLPK is phosphorylated by SRK, suggesting that MLPK could be a substrate of SRK.[40] However, a complementation experiment with the *MLPK* gene to demonstrate the function of MLPK has not been performed. Further analysis is required to confirm that MLPK functions in *Brassica* self-incompatibility signaling.

By using yeast two-hybrid approach with the SRK kinase domain as bait, Arm repeat-containing protein (ARC1) has been identified as a positive regulator of the SRK-mediated signaling cascade.[41] ARC1 is a plant U-Box E3 ubiquitin ligase, which functions to attach ubiquitin to target proteins. *ARC1* is predominantly expressed in the stigma, and ARC1 is phosphorylated by SRK and MLPK *in vitro*.[41,42] ARC1 has been observed in both cytosol and nuclei when expressed in tobacco BY-2 cells, and it was relocated to the ER-localized proteasomes when ARC1 and SRK_{910} were coexpressed.[42,43] The knockdown of the *ARC1* gene in a self-incompatible *B. napus* 'W1' line has been found to result in a partial breakdown of self-incompatibility phenotype,[44] suggesting that the *ARC1* gene is required for the *Brassica* self-incompatibility.

Further analysis by using yeast two-hybrid analysis with ARC1 as bait has identified Exo70A1 to be an interactor with ARC1.[45] Exo70A1 was ubiquitinated by ARC1 *in vitro*.[45] Exo70A1 is a subunit of the exocyst complex, and mutation of the *A. thaliana* orthologous gene affected fertility.[45,46] Knockdown of *EXO70A1* by RNAi in the stigma of self-compatible *B. napus* 'Westar' showed a reduced number of pollen grains on the stigma surface after pollination,[45] and, in contrast, expression of *Exo70A1* by SLR1 promoter, which is a stigma specific promoter,[47] in the self-incompatible *B. napus* 'W1' line partially overcame self-incompatibility.[45] In addition, co-expression of the *SRK* and *ARC1* genes caused redistribution of Exo70A1 from cytosol to ER-associated proteasomes in tobacco BY-2 cells.[45] In a current model, activated SRK (and MLPK) phosphorylates ARC1, and then the phosphorylated ARC1 ubiquitinates Exo70A1 for proteasome-mediated degradation, resulting in inhibition of pollen germination in self-pollinated stigmas of self-incompatible *Brassica* plants (Figure 1).

THE OTHER SRK INTERACTORS IN *BRASSICA*

By using yeast two-hybrid screening with the SRK kinase domain as bait, Thioredoxin H-Like proteins (THL1 and THL2) have been identified as interactors of SRK.[48] THL1/2-SRK interaction was mediated by a cysteine residue at a transmembrane domain of SRK protein.[49] *In vitro* analysis suggested that the addition of recombinant THL1/2 proteins inhibited autophosphorylation activity of SRK, and that this inhibition was suppressed by a pollen coat fraction of the same *S* haplotype.[50] Suppression of *THL1/2* gene expression in self-compatible *B. napus* 'Westar', which has functional *SRK* that is identical to *B. oleracea* SRK_{15},[51] showed spontaneous inhibition of pollen germination and pollen tube elongation.[51] These results suggest that THL1/2 proteins function as inhibitors of SRK-mediated signaling in *Brassica* plants.

In addition to the ARC1 and THL1/2 proteins, kinase-associated protein phosphatase (KAPP), sorting nexin 1 and calmodulin have been identified as SRK interactors.[52] Yeast two-hybrid experiments have shown that KAPP interacted with the SRK kinase domain.[52] *In vitro* experiments have revealed that SRK phosphorylated KAPP and KAPP dephosphorylated SRK,[52] suggesting that KAPP might function in attenuation of SRK signaling. Calmodulin has been identified by yeast two-hybrid analysis with the kinase domain of a kinase-dead mutant of SRK as the bait, and interacted with SRK in a Ca^{2+}-dependent manner.[52] Sorting nexin 1 has also been also found to interact with the kinase-dead mutant.[52] However, the necessity and role of these proteins in self-incompatibility remain unclear.

SELF-INCOMPATIBILITY SIGNALING PATHWAY IN *ARABIDOPSIS*

Although *A. thaliana* is a self-compatible plant, self-incompatible transgenic *A. thaliana* plants have been successfully constructed by introducing the *SRK-SCR* genes of closely related species, such as *A. lyrata*, *A. halleri* and *C. gradiflora*, into *A. thaliana*.[20,22–26] These results indicate that *A. thaliana* has all the molecular components required for self-incompatibility signaling other than SRK and/or SCR. Because of a highly efficient and easy transformation protocol of *A. thaliana* and many genetic resources, the transgenic *A. thaliana* plants enable *in planta* evaluation of the molecular components in the self-incompatibility mechanism that were identified in *Brassica* plants.

The *A. thaliana APK1b* gene (*At2g28930*) shows the highest similarity to the *B. rapa MLPK* gene. Like the *MLPK* gene, *APK1b* produced two transcripts from two distinct initiation sites.[39,53] In addition, the *B. rapa* chromosomal region containing *MLPK* shows the highest synteny with an *A. thaliana* chromosomal region con-

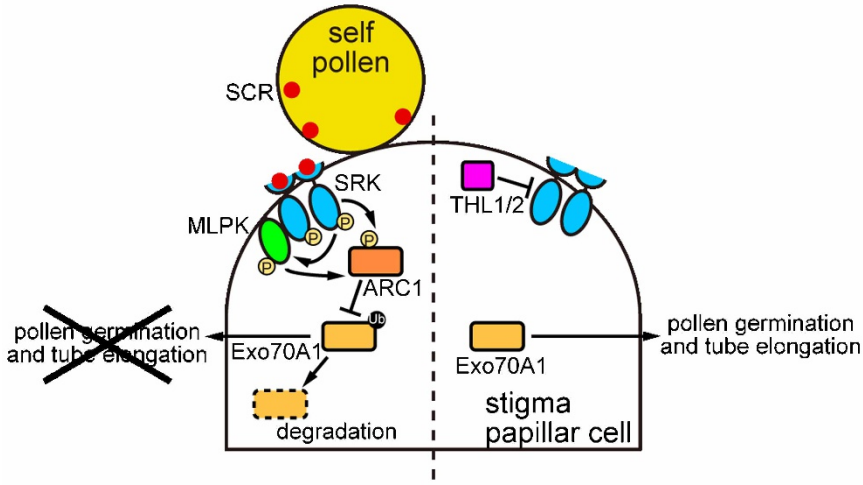

Figure 1. A current model of the self-incompatibility signaling cascade in *Brassica*.

taining *APK1b*.[53] The transgenic *SRKb-SCRb A. thaliana* carrying the T-DNA insertion mutation in *APK1b* (SALK_055314), which is a null mutation,[39,54] showed a self-incompatibility response toward self pollen, indicating that the *apk1b* mutation did not affect the self-incompatibility response in transgenic *SRKb-SCRb A. thaliana* plants.[53] Recently, it has been reported that an *apk1b* mutation in *A. thaliana* affected stomatal conductance.[55] These reports suggest that APK1b does not function in the self-incompatibility signaling, but functions in another signaling cascade.

The *B. rapa* genome contains three putative orthologous genes of *A. thaliana APK1b*, i.e., *MLPK* (*Bra000478*), *Bra035659* and *Bra040929*, because of an extra genome triplication in *Brassica* species. Although the *B. rapa* genomic region containing the *MLPK* gene shows the highest similarity to that containing *APK1b*, synteny analysis using the *A. thaliana* genomic region containing *APK1b* as a query revealed that the *Brassica* genomic region containing *Bra035659* has the highest synteny among the three genomic regions of *Brassica*, indicating that *Bra035659* is the orthologous gene of *APK1b* and *A. thaliana* does not contain an orthologous gene of *Brassica MLPK*. These observations may suggest that, if MLPK is required for the self-incompatibility signaling in *Brassica* plants, the *MLPK* gene would have appeared as a positive regulator of self-incompatibility signaling after species differentiation between *Brassica* and *Arabidopsis*.

A survey of the *A. thaliana* genome revealed that *A. thaliana* does not have an orthologous gene of the *Brassica ARC1* gene.[53,56] In contrast to *A. thaliana*, *A. lyrata*, which is a self-incompatible *Arabidopsis* species, has an ortholog of *ARC1*.[56] Knockdown of the *ARC1* gene in the *A. lyrata* stigmas has been reported to cause partial breakdown of the self-incompatibility response.[56] In addition, the *A. lyrata ARC1* and *B. rapa ARC1* genes have been found to confer strong self-incompatibility phenotype to *SRKb-SCRb* transgenic *A. thaliana* Col-0,[57] which shows a transient self-incompatibility phenotype, suggesting that ARC1 plays an important role in *Arabidopsis* self-incompatibility signaling. However, *SRKb-SCRb* transgenic *A. thaliana* C24 plants show a strong self-incompatibility response, although the *ARC1* gene is not found in the *A. thaliana* C24 genome nor in the *A. thaliana* Col-0 genome.[56]

A. thaliana has an orthologous gene (*At5g03540*) of *Brassica EXO70A1* encoding Exo70A1,[53] which has been identified as a putative substrate of ARC1 in *Brassica*.[45] Unlike self-incompatible *B. napus* 'W1' plants, overexpression of *EXO70A1* in *SRKb-SCRb* transgenic *A. thaliana* was not found to affect the self-incompatibility response.[53] This result suggests that Exo70A1 has no effect on self incompatibility in *A. thaliana*. In contrast to the result of Samuel *et al.*,[45] Li *et al.*[46] have recently reported that *A. thaliana exo70a1* mutant did not show defects in pollen germination and pollen tube elongation, when hand-pollinated, supporting the conclusion of Kitashiba *et al.*[53] However, to answer the discrepancy between the result of Samuel *et al.*[45] and Li *et al.*,[46] Safavian *et al.* have reported that the pollination defect in the *A. thaliana exo70a1* mutant was observed at 40% humidity, but the defect was rescued at 80% humidity, suggesting that the pollination defect of the *A. thaliana exo70a1* mutant was humidity-dependent.[58] To conclude whether Exo70A1 functions in self-incompatibility, further studies are required on degradation of EXO70A1 in self-pollinated stigmas of self-incompatible plants and effect of high humidity, such as 80% humidity, on *Brassica* self-incompatibility response.

THL1/2 were identified as negative regulators of SRK-mediated self-incompatibility signaling.[50,51] The effect of THL proteins on self-incompatibility was examined by using *A. thaliana* T-DNA insertion mutants of the *AtTRX3* and *AtTRX4* gene, which are orthologous genes of *Brassica THL1* and *THL2*, respectively.[59] Unlike the *THL1/2* genes, the *attrx3*, *attrx4* and *attrx3attrx4* mutations did not affect the *A. thaliana* self-incompatibility response.[59] The effect of thioredoxin H–SRK interaction on self-incompatibility was also examined by using a transgenic *A. thaliana* plant expressing an SRKb (C463W) mutant gene, which has a mutation at the Cys463 residue that corresponds to the Cys residue required for the thioredoxin H–SRK interaction.[49] The phenotype of the transgenic *A. thaliana* plants expressing the *SRKb* (*C463W*) mutant gene was indistinguishable from that of *SRKb* transgenic *A. thaliana* plants under both self- and cross-pollination, indicating that the replacement of the Cys residue does not affect the self-incompatibility response in transgenic *A. thaliana*.[59] In addition, the fact that the Cys residue is not conserved among some SRK haplotypes of not only *Arabidopsis* but also *Brassica* plants, such as *A. lyrata* SRKa and SRK_3, *A. halleri* SRK_3 and *B. oleracea* SRK_{68}.[59]

PERSPECTIVES

After identification of the *SRK* gene in *Brassica*, subsequent research has presented several candidates as possible components of self-incompatibility signaling in *Brassica*. *In vitro* experimental results have provided an interesting model of a signaling cascade in self-incompatibility in *Brassica* (Figure 1). However, because of the difficulty of transformation and gene targeting disruption in self-incompatible *Brassica* plants, the constructed model for self-incompatibility has not been confirmed by *in planta* experiments using gene-knockout self-incompatible mutant plants.

Because the gene targeting deletion method of non-model organisms including *Brassica* has not been developed, it has been difficult to examine the necessity and roles of the identified candidates in self-incompatibility mechanism in *Brassica*. However, recently, new genome editing methods, such as TALEN and CRISPR/Cas,[60] have been developed. The development of these methods should enable us to construct null mutants of non-model self-incompatible plants to examine the effects of the candidate genes on self-incompatibility signaling. In turn, molecular components that function in *Arabidopsis* self-incompatibility signaling have not been identified. Therefore, identification of the molecular components in *Arabidopsis* self-incompatibility is also required. In conclusion, *in planta* evaluation of candidate genes in the *Brassica* self-incompatibility signaling and the identification of molecular components of the *Arabidopsis* self-incompatibility signaling contribute to the knowledge of not only the molecular mechanism of self-incompatibility, but also evolutionary aspects of the self-incompatibility mechanism in Brassicaceae.

COMPETING INTERESTS

The authors declare no conflict of interest.

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