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Review

Genetic Components of Self-Incompatibility in Brassica Vegetables

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Abstract: Brassica vegetables are very important to human beings. Self-incompatibility (SI) is a common phenomenon in Brassica. Breeding by SI lines is an important way to utilize heterosis of Brassica vegetables. It is believed that the SI inheritance in Brassica species is controlled by three linkage genes on the S-locus, including SRK (S-locus receptor kinase), SCR (S-locus cystine-rich protein)/SP11 (S-locus protein 11), and SLG (S-locus glycoprotein). SRK is the female determinant and SCR/SP11 is the pollen S gene. The expression of SLG is necessary for SRK, and it enhances the SRK-mediated SI reaction. In addition to these three S-locus genes, some other functional molecules also have significant regulatory effects on SI, such as ARC1 (arm repeat containing 1), MLPK (M-locus protein kinase), Exo70A1 (exocyst compounds), THL1/THL2 (thioredoxin H-like), MOD (aquaporin), SLR (S-locus-related glycoprotein), BPCI (pollen calcium-binding protein I), etc. SI is also associated with the dominant/recessive relationship between S alleles. Here, the genetic elements and molecular mechanisms of SI, mainly in Brassica vegetables, are reviewed.

Keywords: Brassica; self-incompatibility; S gene; S-locus; signal transduction

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1. Introduction

Brassica vegetables, such as cabbage, Chinese cabbage, cauliflower, broccoli, etc., are important foods for people all over the world. Self-incompatibility (SI) is one of the main ways to utilize heterosis of Brassica vegetables. Using SI lines for breeding not only avoids an emasculation operation but also obtains heterosis. SI is widely used in the breeding of Brassica vegetables and has greatly improved the yield and quality of these vegetables. In China, "Jingfeng No. 1" was the first cabbage variety to be developed using SI lines and was developed in 1973. Later, SI lines were used to breed Qingfeng, Muchun, Zhengchun, Wanfeng, 8398, and other varieties. Understanding the molecular mechanisms of the SI recognition reaction in Brassica vegetables is important for utilizing heterosis.

SI refers to a phenomenon that plants have complete flowers and can form normal female and male gametes, but cannot bear seeds through self-pollination or the rate of fruiting is very low. SI is a widespread mechanism that helps prevent detrimental inbreeding and maintains genetic variation within higher plants. According to the morphology of perfect flowers, SI can be classified as heteromorphic SI and homomorphic SI. Homomorphic SI occurs in species with uniform flower morphology and is further divided into gametophytic SI (GSI) and sporophytic SI (SSI) [1]. GSI is controlled by the genotype of the gametophyte and SSI controlled by the genotype of pollen parents. SI in Brassica vegetables belongs to SSI and is sporophytically controlled by the S-locus. Three tightly linked genes are distributed on the S-locus, including SRK (S-locus receptor kinase), SLG (S-locus glycoprotein), and SCR (S-locus cysteine-rich protein)/SP11 (S-locus protein 11). The first two genes are exclusively expressed in the pistil and the last one is expressed in pollen grains [2]. SRK and SCR/SP11 function as receptor–ligand pairs that determine specificity

in the stigma epidermis and pollen [3]. In addition, many other elements also affect SI, such as ARC1 (Aarm repeat containing 1), MLPK (M-locus protein kinase), Exo70A1 (exocyst compounds), THLl/THL2 (thioredoxin H-like), MOD (aquaporin), SLR (S-locus-related glycoprotein), BPCI (pollen calcium-binding protein I), etc.

2. S-Locus Transducers of SSI

2.1. SRK

SRK was the receptor that allowed stigma epidermal cells to discriminate between genetically self- and non-self-pollen in SI responses of Brassicaceae [2]. SRK encoded a trans-membrane protein which contained an S domain, a trans-membrane region, and a cytoplasmic serine/threonine (Ser/Thr) protein kinase domain (Figure 1), and it was proposed to be the female determinant of SI reaction [4]. The SRK gene of *Brassica oleracea* contained seven exons and six introns, and introns varied in size from 76 bp to 896 bp. The derived protein products contained 857 amino acids. Exon 1 encoded the S-region and was composed of 438 amino acids [5]. Its N-terminal contained a signal peptide consisting of 31 hydrophobic amino acids with 67% homologous to SLG. The remaining S-domain contained 12 almost completely conserved cysteine residues and three polytropic regions, with 89% homologous to SLG. The conservative cysteines played an important role in SRK function. The trans-membrane domain was encoded by exon 2 and contained 20 hydrophobic amino acids. The exon 3–7 encoded protein kinase domain, which was the functional site and was used to express and transmit signals from the extracellular domain through phosphorylation of Ser and Thr [5]. The SRK extracellular ligand-binding domain contained several potential N-glycosylation sites. In Arabidopsis, although five of six potential N-glycosylation sites in SRKb were glycosylated in stigma, N-glycosylation was not important for SCRb-dependent activation of SRKb. N-glycosylation functioned primarily to ensure the proper and efficient sub-cellular trafficking of SRK to the plasma membrane [6].

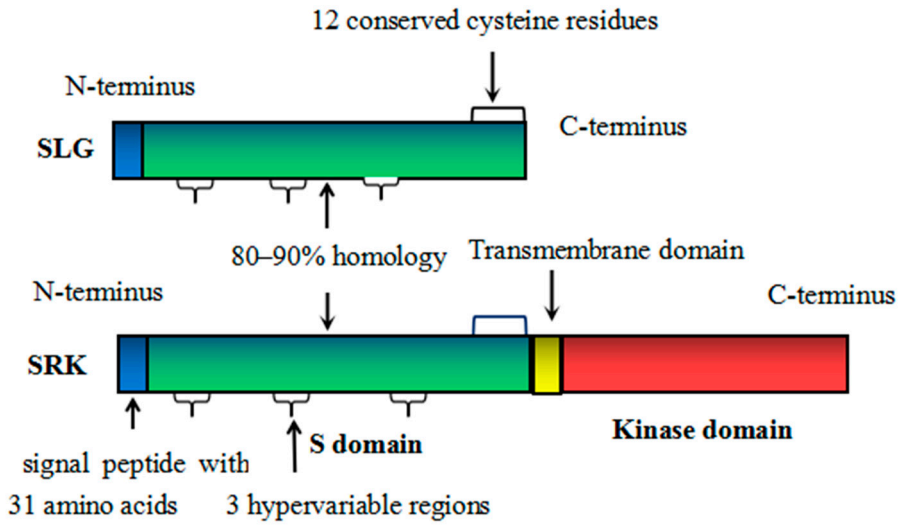

Figure 1. Schematic structure of SLG and SRK of Brassica vegetables. The signal peptide is colored blue. The S domain is colored green. The transmembrane domain is colored yellow. The kinase domain is colored red. SRK consists of an S domain, a transmembrane domain, and a kinase domain. Both SRK and SLG have a signal peptide with 31 amino acids, 3 hypervariable regions and 12 cysteine residues. SRK is similar to SLG in the S domain.

SRK was specifically expressed in the stigma, whose expression level was closely related to SI [7]. Takasaki et al. introduced the SRK^{28} gene of S haplotype (S^{28}) into S^{60} of *Brassica rapa*, and all the 17 transgenic plants obtained were SI. Among them, 3 plants rejected pollen of S^{28} and S^{60}, while the other 14 only rejected S^{60} pollen, which indicated that the stigma SI phenotype of the first 3 plants changed and differed from the parent

S^{60}. Using pollen of S^{24}, S^{43}, S^{45}, S^{52}, and S^{29} for pollination, they obtained a SI phenotype specific to SRK^{28}, with the SRK^{28} transcript being detected in stigma but not in anther [8]. Silva et al. introduced SRK^{910} into SC (self-compatibility) of *Brassica napus* and found that SI occurred [9]. These evidences proved that SRK was not only a determinant of pistil, but also played an important role in pollen recognition. When homologous SCR fell and bound to SRK, its intracellular kinase area activated, Ser and Thr residues phosphorylated, resulting in a cascade reaction that transmitted and amplified signals in mastoid cells and ultimately inhibited pollen germination and pollen tube growth.

2.2. SCR/SP11

SCR/SP11 encoded a small cysteine-rich protein, secreted from the anther tapetum, localized to the pollen surface and functioned as the male determinant of SI reaction [10,11]. In SC mutants of cabbage, SCR will not express, and as a result SI is lost. Analysis showed that SCR and SP11 was the same gene with a single copy, encoding an 8.4–8.6 kD small molecule protein [12]. The protein of SCR was alkaline and hydrophilic, belonged to the pollen coat protein family, and accumulated and was specifically expressed in anthers. The SCR protein contained eight cysteine residues and one glycine residue, which were conserved in most Brassica SCR alleles [13,14]. Analysis of the amino acid sequence of Brassica SCR of S^6, S^8, and S^{13} of *B. oleracea* showed that the similarity was only 30–42%. The high variation indicated that it had specific SI alleles. The pollen of transgenic plants had the S^6 pollen phenotype (constructed expression vectors with the SCR^8 promoter and SCR^6 gene, and transferred into S^2S^2 homozygous of cabbage), which verified that SCR was a pollen S gene [12]. Analysis of SP11 transgenic plants of *B. rape* showed that SI occurred when the pollen of the transgenic plants was pollinated to the stigma of the same haplotype. However, when it was pollinated to different haplotypes, SC happened [14]. Takayama et al. expressed SP11 protein of *Brassica campestris* in vitro, and then treated the same haplotype material stigma mastoid cells with this protein. The results showed that the hydration of pollen was inhibited and incompatibility happened, which also proved that SCR/SP11 was the S gene of pollen [10]. In Brassica, the expression of SP11 can be suppressed by two small RNAs, SP11 methylation inducer (SMI1) and SMI2 [15]. SCR had high homology among some species. Haseyama et al. reported that the SCR of S^{31} in cabbage, S^{69}, and S^{52} in rape were highly homologous to those of S^{22}, S^{25}, and S^{30} in radish with the amino acid identities being 84.6%, 87.7%, and 82.0%, respectively. However, the homology was low between intergeneric pairs [16].

2.3. SLG

The first S-locus product to be identified was SLG, which encoded a secreted glycoprotein and was first isolated from cabbage. In some Brassica genotypes, the highest content of SLG was as much as 5% of the total stigma soluble protein [17]. The accumulation of SLG on the stigma was significantly correlated with SI [18]. The SLG was shown to increase during stigma development and to reach maximal levels simultaneously with the onset of the SI response [19]. The molecular weight of SLG was 57–65 kD, distributed in the cell wall and intercellular region of the stigma papilla cells [20]. The SLG proteins of different genotypes had different molecular weights. In *B. oleracea*, it consisted of 436 amino acids. The functional area comprised 405 amino acid residues. The N-terminal (1–181 amino acids) was hydrophobic and highly conserved (approximately 80%) with a signal peptide of 31 amino acids, which was responsible for guiding SLG into the cellular secretion system. The residues of 182–268 amino acids showed the greatest variation, with only 52% conservative. The C-terminal (269–436 amino acids) contained 12 completely conserved cysteine residues and 12 N-glycosylation sites, with the C-terminal approximately 78% conserved [20] (Figure 1). The Asn and n-GLCNAC on the glycosylation sites of *Brassica oleracea* were connected by N-glucoside bonds [21]. The arrangement of N-glycation sites of different SLG proteins was different. The relationship between the change of this structure and the specific pairing of the S allele product was still unclear [22]. Amino acid sequences

of SLG was highly similar to SRK, with both having 12 conserved cysteine residues and three hypervariable regions [1]. The homology between SLG and SRK was approximately 80–90% in *B. rapa*, *B. oleracea*, and *B. napus* (Figure 1) [4]. It was once thought that SLG was only responsible for pollen adhesion, but now SLG diversity is interpreted as evidence of a role in SI [23]. Furthermore, the degree of S pollen rejection by SRK was correlating with the degree of amino-acid identity in S domain between SRK and SLG; the higher the identity, the stronger the rejection [8].

2.4. Genomic Organization of S-Locus Genes

The genomic organization and transcription direction of three S-locus genes were different among species. For example, in *B. campestris* S^8, S^9, and S^{12}, SCR/SP11 gene was located between SLG and SRK [10]. In radish WK10039, the SRK gene was located between SCR/SP11 and SLG. However, in Aokubi, the SCR/SP11 gene was located between SLG and SRK [24]. The direction of transcription of these three S-locus genes were divergent as shown in Figure 2. The length of the S-locus in Brassica was usually 200–400 kb [10,12]. However, the physical distance between SLG and SRK varied greatly, from as little as a few kb in *Brassica campestris* (syn *B. rapa*) to as much as several hundred kb in *B. oleracea*. In cabbage, it was more than 200 kb in S^2 and S^6, less than 50 kb in S^{13}, and about 13 kb in rape S^8 [25]. It was believed that SRK and SLG may co-evolve through gene conversion. The co-evolution of SLG and SRK suggested that SLG was related to S haplotypes [26]. In the case of *Brassica napus*, the S-locus region was found to originate from the introgressed *Brassica rapa* genome and not from *Brassica oleracea* [4].

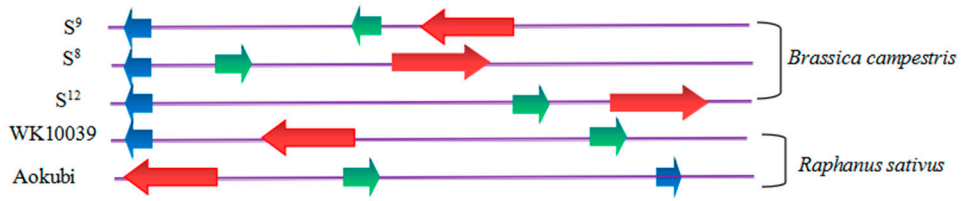

Figure 2. Schematic organization of S-locus genes. The arrow shaped boxes indicate S genes and the direction of transcription. The SLG is colored blue. The SCR/SP11 is colored green. The SRK is colored red. SCR/SP11 gene is located between SLG and SRK in *Brassica campestris* and *Raphanus sativus* Akokubi, while SRK gene is located between SLG and SCR/SP11 in *Raphanus sativus* WK10039. The direction of transcription of these genes were divergent in *B. campestris* and *Raphanus sativus*.

2.5. The Relationship of S-Locus Genes in SI Reaction

During self-pollination, pistil factor SRK localized on the plasma membrane of the papilla cell, specifically recognized its cognate pollen factor SCR/SP11 which was released from the pollen surface, triggered self-phosphorylation of the kinase domain and transduction the SI signal to intracellular downstream effector. SLG can form a complex with SCR. Then, the signal peptide guided the SCR-SLG complex to pass through the intercellular space and reach the cell wall of the stigma epidermal cell. Here, the signal peptide dissociated, the complex conformation changed, and the SCR ligand bound specifically to the extracellular domain of the homologous SRK in the stigma and activated intracellular kinase. The SRK conformation was affected by SLG which functioned in the stabilization and proper processing of SRK [1]. The expression of SLG was necessary for the physiological activity of SRK. SLG and SRK must be expressed together, otherwise SRK forms a polymer with high molecular weight. SRK and SLG may interact through the same receptor or ligand [27,28]. How did SRK discriminate self or non-self SCR/SP11? Murase et al. established a model of self/non-self-discrimination in Brassica. They thought that the binding free energies were most stable for cognate SRK-SP11 combinations, and the modes of SRK–SP11 interactions differed between intra- and inter-subgroup combinations [15].

3. Other Regulating Transducers of SSI

In addition to the three closely linked encoding genes on the S-locus, some other genetic regulators also play important roles in SI, such as ARC1, MLPK, Exo70A1, THL1/THL2, MOD, SLR, BPCI, etc.

3.1. ARC1

ARC1 was an important protein factor downstream of the SSI signal transduction pathway. Although it was not a protein encoded by a S-locus gene, it can be bound to the SRK kinase domain in a phosphorylated manner. ARC1 was known to be a positive regulator of the SI system because the suppression of ARC1 transcripts in the pistils of *Brassica napus* resulted in a partial breakdown of SI [29].

ARC1 contained an open reading frame (ORF) with no introns. The ARC1cDNA homology of cabbage between Arabidopsis and rape was 29% and 94%, respectively. The number of amino acids that encoded ARC1 of cabbage, rape, and Arabidopsis was also different, with 663, 661, and 729, respectively [30]. The ARC1 protein of Brassica was composed of three typical domains: the leucine zipper and coiled-coil domain at the N-terminus, the middle U-box, and ARM (the arm repeat domain) at the C-terminus. In cabbage, one NLS (nuclear localization signal) and two NES (nuclear export signals) were also involved (Figure 3) [31,32].

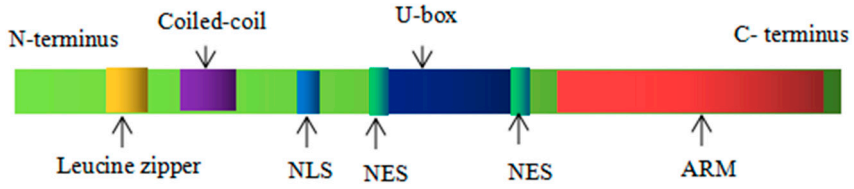

Figure 3. Schematic structure of ARC1 protein in cabbage. Leucine zipper is colored yellow. The coiled-coil is colored purple. The NLS is colored blue. The NES is colored dark green. The U-box is colored dark blue. The ARM is colored red. ARC1 protein in cabbage contains these domains: leucine zipper, coiled-coil, U-box, ARM, NLS, and NES.

ARM played important roles in SI response and signal transduction, whose amino acid was homologous to that of the Armadillo protein family [33,34]. The R1, R2, and R5 of ARC1 was homologous to R12, R2, and R10 of Armadillo protein by 29%, 19%, and 24%, respectively, while the homology of R3, R4 of ARC1, and R6 of Armadillo was 33% and 29% [33]. ARM can bind to the SRK kinase domain in a phosphorylated manner, initiating signal transmission without interacting with the kinase domains of Arabidopsis receptors RLK4 (receptor-like kinase) and RLK5 [33,34], which indicated that ARM determined the specificity of binding to the SRK kinase domain.

The U-box domain was the second-most important function domain of ARC1, as it endowed ARC1 E3 ubiquitin ligase activity and enabled ARC1 to regulate ubiquitination and protein degradation of unknown SI transduction elements in stigma mastoid cells, and the action must be completed on 26 S proteasome/CSN (COP9 signalosome) [35]. In transgenic tobacco cells, which was transformed with myc-ARC1 (the myc epitope tag was fused to the N terminus of ARC1) and GST-SRK (glutathione S-transferase-SRK), ARC1 can shuttle between the nucleus, cytoplasm, stroma, and 26 S proteasome/CSN. However, when all or part of the U-box structure was removed or the conservative Pro residues were replaced with Ala residues, ARC1 cannot be positioned on 26 S proteasome/CSN. This indicated that the U-box was necessary for ARC1 localization to 26 S proteasome/CSN [32,35]. ARC1 localization to 26 S proteasome/CSN provided a guarantee for ubiquitinated protein degradation and indicated that a ubiquitinated proteasome pathway was involved in SI reaction. A novel plant U-box protein (BoPUB3) was identified from the stigma of cabbage. BoPUB3 was highly expressed in stigma, and its expression increased with the stigma development and reached the highest level in the mature-stage stigma. BoPUB3, a 76.8 kDa protein with 697 amino acids, was a member of the PUB-ARM family. BoPUB3 contained three

characteristic domains of BoARC1, including leucine zipper and coiled-coil, U-box, and ARM [36]. Leucine zipper and coiled-coil motifs of ARC1 were required for the interaction with Exo70A1. The leucine zipper and coiled-coil motifs of ARC1, hypervariable region, and SUMO modification motifs of Exo70A1 were the core interaction domains between ARC1 and Exo70A1 [37].

3.2. MLPK

MLPK encoded a membrane-anchored cytoplasmic protein kinase and composed of 404 amino acids with a typical structure of N-myristoylation. The amino acid sequence was Met-Gly-XXX-Ser/Thr (Arg) (x was an arbitrary amino acid residue) and consisted of a region with 30 amino acids enriched in Ser (33%) and 11 subdomains of protein kinase. MLPK had eight exons and seven introns. In Arabidopsis, MLPK was most similar to *APK1b* (76% amino acid identity) [38]. The MLPK homology was high among species; for example, it was up to 98% between cabbage and rape [39]. In the SI signal transduction pathway of Brassica, MLPK was highly expressed in the part of plasma membrane differentiated from the pistil cells. The plasma membrane localization of MLPK suggested that it may function in the vicinity of SRK, and an in vitro phosphorylation assay indicated that the kinase domain of SRK can efficiently phosphorylate MLPK [40]. Murase et al. confirmed that the MLPK mutant of Brassica showed SC, while the mutants restored SI when the MLPK gene was introduced [38]. MLPK can be phosphorylated by SRK and form an allogenic complex, which indicated that MLPK was one of the downstream signal elements of SRK [40,41]. Since 24% of the 610 kinase receptors found in Arabidopsis were RLCK proteins (receptor-like cytoplasmic kinase), MLPK most likely belonged to the RLCK protein family [38].

3.3. MOD

The SI reaction occurred on the surface of the dry stigma, so water was necessary for pollen germination. Water channels were essential for SI and also a part of the SRK signaling pathway. Pollen grains must absorb water and perhaps other substances from the receptive stigmatic surface. Therefore, water transfer from stigmatic papilla cells to pollen grains may be a key point of pollen–stigma interaction [42]. Since the amino acid sequence of MOD protein was very similar to the MIP (major intrinsic protein) whose main function was to transport water molecules, it was speculated that MOD may regulate SI reaction by regulating water transportation between pollen and stigma [43]. MOD had six trans-membranes to facilitate the transport of water and other small molecules across membranes [43]. Two tandem repeats of Asn-Pro-Ala (NPA) and three Ser residues exited in plant MOD. NPA was a characteristic sequence of the MIP family, and Ser was the potential phosphorylation sites and may be the target protein of the SRK-mediated signaling pathway [43]. In self-pollination, phosphorylated SRK activated MOD protein through a series of signal transduction pathways and stimulated water molecules to enter stigma cells and stay away from pollen. In mod mutants of Brassica, SI was shown to be associated with the absence of transcripts encoded by an MOD-related gene, and the recessive mutation mod eliminated SI in the stigma [42].

3.4. THLl/THL2

THLl/THL2 belonged to the H family of thioredoxin, which was widely involved in physiological processes such as enzyme activity regulation and signal transduction [44]. In Brassica, the thioredoxin H proteins, THL1 and THL2, were previously found to be potential inhibitors of SRK in SI response. THL1/THL2 was required for full pollen acceptance in *B. napus cv*. Westar, and SRK may be the target of regulation by THL1/THL2 [45]. THLl/THL2 interacted in a reversible manner with SRK in stigmas and prevented spontaneous activation of the SI signaling pathway. In this model, SRK thioredoxin inhibition was released by PCPs (pollen coat protein) after self-pollination. In the absence of this regulation, the SI system was constitutively active, blocking both self-pollination and cross-pollination, and thus preventing seed set [46].

3.5. Exo70A1

Exo70A1 was an essential sub-unit of the exocyst complex and involved in vesicle transport and secretion, tube development, cell plate formation, seed mucilage formation, and stigma–pollen interaction [47]. In Brassica, the adhesion and hydration of self-pollen increased with the overexpression of the Exo70A1 protein on the stigma, and the chance of pollen tubes passing through stigma also increased and resulted in the reduction of SI [48]. These results suggested that Exo70A1 acted as a negative mediator of SI signaling. Exo70A1 of *Brassica oleracea* was divided into four subdomains, including leucine zipper (Leu128–Leu149), hypervariable region (Ser172-Leu197), SUMO modification motif (Glu260-Ile275), and pfamExo70 domain (His271-Phe627) [32]. Unlike in Brassica, Exo70A1 did not participate in SI reaction in Arabidopsis [49].

3.6. Other Signal Transducers

SLR, a secretory glycoprotein, was abundant in stigmata mastoid cells. When the SLR content in stigma decreased, pollen adhesion decreased greatly in the transgenic plants with the antisense SLR gene of *Brassica napus*. The amino acid sequence of SLR was very similar to that of SLG with the same conservatism, so it was speculated that SLR participated in the SI process [23,50]. GATA transcription factor BnA5.ZML1 was found as a stigma compatibility factor in *Brassica napus*. BnA5.ZML1 suppressed SI response and was induced by compatible pollination. The loss-of-function mutant bna5.zml1 displayed reduced SC. In contrast, overexpression of BnA5.ZML1 in SC led to a partial breakdown of SI, which suggested that BnA5.ZML1 was a stigmatic compatibility factor. Furthermore, the expression levels of SRK and ARC1 were up-regulated in bna5.zml1 mutants and down-regulated in BnA5.ZML1 overexpression lines. SRK affected the cellular localization of BnA5.ZML1 through direct protein–protein interaction [51].

As the second messenger in plant cells, Ca^{2+} played an important role in pollen germination and pollen tube elongation which was correlated with SI [52]. Self-pollination specifically induced an increase in cytoplasmic Ca^{2+} concentration in papilla cells, and the Ca^{2+} influx was mediated by GLR (glutamate receptor-like channel) [53]. Ca^{2+} concentration was also regulated by BPCI, which contained two EF-hand domains that may be associated with calcium binding. In *Brassica napus*, residues of Asp, Gly, and Glu at sites 10, 15, and 21 of BPCI were highly conserved [54]. These conservatively suggested that BPCI played an important role in the mutual recognition of pollen and stigma. In addition to these genetic factors, other external factors such as climate, hormones and some chemicals can also affect SI signaling.

4. Molecular Mechanism of SI in Brassica Vegetables

It was widely believed that SI signals transmitted along the pathway of SRK-ARC1-Exo70A1 in Brassica. When self-pollen fell on the stigma, it was recognized by the stigmatic papilla, and the SP11/SCR binding triggered homodimerization of the extracellular domain of SRK (eSRK), leading to the formation of a heterotetrameric complex (2:2 eSRK: SP11/SCR) [55]. The kinase domain of SRK interacted with ARM of ARC1, resulting in phosphorylation of ARC1 [33]. Subsequently, the phosphorylated ARC1 bound to Exo70A1 and led to a series of cascade reactions. Leucine zipper and coiled-coil motifs of ARC1 mediated the interaction with the N-terminal domain of Exo70A1. The interaction strength between SRK and ARC1 was less than that of ARC1 and Exo70A1. It was speculated that the ARC1-Exo70A1 signaling pathway may only be a branch of the SRK signaling pathway, and there were other signal elements and branches involved in the downstream signaling of Brassica plants [56,57]. MLPK was also involved in the SI reaction, because MLPK mutation or deletion resulted in the loss of SI, and MLPK directly acted with SRK to transmit SI signals on the cell membrane [38,40]. MLPK was also phosphorylated by SRK. Both MLPK and SRK can phosphorylate ARC1, but the phosphorylation effect of MLPK was stronger than that of SRK [40,41]. It was not clear whether ARC1 was phosphorylated by both SRK and MLPK, or by phosphorylated MLPK which was first phosphorylated by

SRK. In SRK-mediated SI, activity of SRK can be inhibited by THL and activated by pollen coat proteins [46]. A molecular process of SI reaction in Brassica was shown in Figure 4, according to Zhu et al., with some modifications [58].

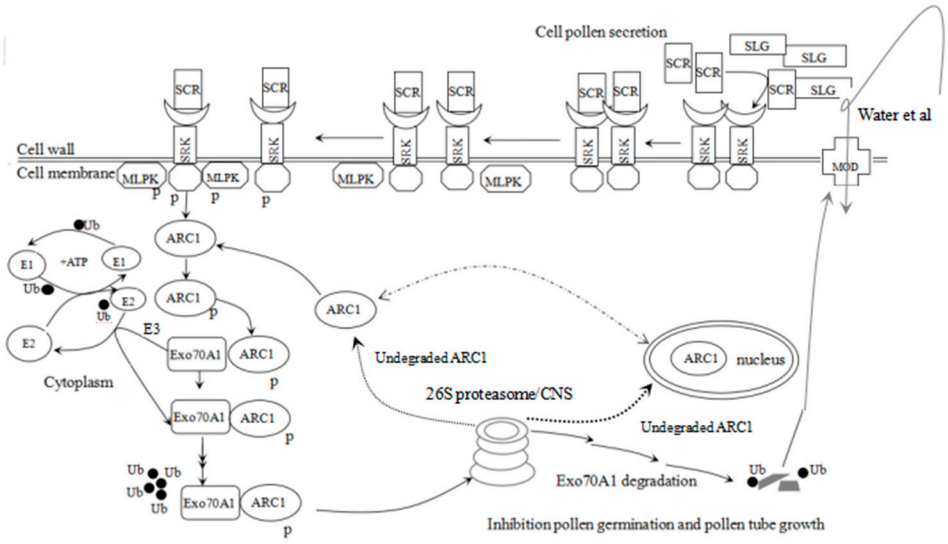

Figure 4. Molecular process of SI signaling pathway in Brassica vegetables. In the absence of SCR, SRK binds to the same SRK or THL and forms an SRK-SRK or SRK-THL complex. When self-pollen falls, SCR is released from the pollen coat, activates SRK, SRK-SRK and SRK-THL depolymerize. SRK is released and bound to SCR to form a heterotetrameric complex (2:2 eSRK: SCR) with the help of SLG. SRK is phosphorylated (p), then MLPK is also phosphorylated. These two phosphorylation processes may happen at the same time. ARC1 shuttles from nucleus to cytoplasm, the phosphorylated molecules both interact with ARM of ARC1, ARC1 is phosphorylated and activated. ARC1 N-terminal attaches to Exo70A1 by means of Ub-activation enzymes (E1) and Ub ligase (E2, E3) to form an ARC1-Exo70A1 complex. Then the complex moves to 26 S proteasome/CNS where it will be degraded. When Exo70A1 is degraded, channels of MOD open. Water and other materials enter into stigma cells and stay away from pollen. Thus, pollen hydration is prevented, the germination and tube growth are inhibited, and SI occurs. Those undegraded molecules may shuttle back into nucleus or be reused again.

5. Dominant/Recessive Relationship between S Alleles

S alleles were divided into two groups, class I and class II, based on the sequence similarity between allelic S-locus genes. In the case of SLG and SRK, allele diversity ranged from a few percent to approximately 20% within a group. In contrast, between groups, the diversity was more than 30%. The diversity of SP11/SCR was higher than that of SLG and SRK [4]. The essence of SI reaction was the mutual recognition and selection between two S haplotypes of pollen-producing cells and two S haplotypes of stigmata mastoid cells. There was a dominant/recessive relationship between the two S haplotypes of stamens. In the stigma of *Brassica campestris*, codominance occurred frequently with dominance or recessiveness seeming to appear according to the combination of S alleles [59]. In pollen, codominance was less frequent and seemed to be a certain hierarchy of the dominance relationship. The S-haplotype in class I was dominant, but it was recessive in class II. Both class I and class II had a set of their own SLG and SRK genes. SLG and SRK gene sequences were similar in either class I or class II, respectively. However, the similarity was low between class I and class II [59].

In pollen of *B. rapa* and *B. oleracea*, the dominant/recessive relationship was regulated at the transcriptional level. The SP11 of class II was mainly expressed in homozygous anther tapetum and greatly inhibited in heterozygous of class I and class II, which indicated that the transcription level of SP11 in pollen determined the dominant/recessive relationship [14]. The dominant relationship between SRK^{28} transgenic and endogenous

S haplotypes was the same as that between S^{28} and endogenous S haplotypes. In $S^{43}S^{43}$ homozygotes which carried SRK^{28} transgenic gene, the phenotype of S^{43} was concealed by SRK^{28} in the stigma, but the transcription level of SRK^{28} was much lower than that of SRK^{43}. These results showed that the dominance relationship between S haplotypes in the stigma was determined by SRK, but not by virtue of its relative expression level [60].

6. Prospects in the Future

The progress of SI breeding depends on the study of the molecular mechanism of SI. Although remarkable progress has achieved in the molecular regulation of Brassica SI, there are still many unanswered questions. For example, how does the synergistic change between pollen specificity and stigma specificity occur? How is the SI signal captured and passed down from molecule to molecule? Moreover, there may be many unknown components involved in SI of Brassica species. Therefore, it is necessary to explore novel signaling transducers, study the protein structures, elaborate the interactions among proteins to reveal the essence of the physiological reaction between stigma and pollen. Now, new research methods have been applied to study SI of Brassica, for example, CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein), and remarkable progress has been achieved [61,62]. It is believed that, with the development of molecular biology, plant genomics, and proteomics, these problems will be solved gradually, and the molecular mechanism of SI in Brassica vegetables will also be clarified in the future.

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