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GAMETOPHYTIC SELF-INCOMPATIBILITY IN ROSACEAE FRUIT TREES

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ABSTRACT

Rosaceae fruit trees are characterized by gametophytic self-incompatibility, with their production typically requiring artificial pollination or pollination tree is required in production. Both of these solutions cause reductions in production efficiency, and self-incompatibility has become a major issue in agricultural biology, and as such, has been extensively studied. In this review, we discuss the relationship between S-RNase content in the style and self-incompatibility, and the role of the SLF gene in stamen-determining factor. Considering mutations in self-compatibility-related genes and self-compatibility in polyploid fruit trees, we discuss the potential mechanisms of self-incompatibility. Based on a preliminary study of the role of pollen tube Ca^{2+} gradients in self-incompatibility in *Pyrus*, we propose a new mechanistic model of self-incompatibility taking into account the effect of Ca^{2+}. We also discuss the potential for hormone regulation to be used to control self-incompatibility in Rosaceae fruit trees.

Key words: Rosaceae fruit tree, self-incompatibility, style, pollen, calcium

In plants, self-incompatibility (SI) refers to a self-incompatibility mechanism acquired over the course of plant evolution, which allows plants to distinguish between self-pollen and non-self-pollen [Silva and Goring 2001]. It involves interactions between pollen and the pistil during pollination and insemination, making it an ideal model system, in which plant cell identification and the temporal and spatial expression of associated genes is studied. As such, SI has become a well-studied topic in plant physiology and molecular biology. Genetically, SI is controlled by multiple alleles in the S-*locus* [Meng et al. 2014b]. Based on morphology and genetics, SI in plants can be classified as either sporophytic self-incompatibility (SSI) or gametophytic self-incompatibility (GSI). In SSI, pollen cannot pass through the papillose cells of the style and stigma and as such it cannot grow towards the seed bud, while in GSI, pollen can pass through these cells but stops growing upon reaching the middle or upper part of the stylus. Phenotypes associated with SSI are determined by the S genes of sporophytes, while phenotypes associated with GSI are determined by the genotype of monoploid pollen.

Many species of Rosaceae fruit trees exhibit gametophytic self-incompatibility, including *Pyrus*, *Malus*, *Prunus avium*, *Prunus armeniaca*, *Prunus salicina*, *Prunus dulcis*, and *Prunus mume* [Sassa et al. 1993, Janssens et al. 1995, Burgos et al. 1997, Sonneveld et al. 2001, Yaegaki et al. 2001, Ortega et al. 2005, Zhang et al. 2015]. As these fruit trees are incapable of self-pollination, artificial pollination or fertilization using pollenizers is required during fruit production to achieve acceptable fruit setting rates. When the weather prevents pollination by pollinating insects, fruit output is reduced. Reports have shown that some fruit trees may not be able to produce self-pollinated seeds *via* traditional self-fertilization methods. For example, in one breed of *Prunus avium*, only two seeds were

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successfully fertilized out of 1429 attempted self-pollinations. Self-compatibility can only be induced once a complete understanding of the mechanisms of SI is reached. In recent 30 years, numerous studies have investigated SI in fruit trees, which have provided theoretical grounds for the development of our understanding of SI in fruit trees, with significant implications for fruit production. This paper provides a summary of current understanding of SI in Rosaceae fruit trees at the molecular and physiological level. Other aspects of SI in this group, including effects of female and male gametophytes on SI, are also discussed. We suggest methods, by which plant growth-regulating substances could be used to overcome the limitations of SI.

Pistil-specific determinative factors

The role and structural features of pistil-specific determinants, including S-RNase, have been discussed more frequently in recent years [Franklin-Tong and Franklin 2003, Kao and Tsukamoto 2004, Wu et al. 2013b], and as such are not discussed here. Cultivars often differ in their s-RNase content according to measurements of the volume of S-glycoprotein per microgram of soluble protein. Zhang et al. [2002] classified the degree of SI in *Pyrus* into three groups based on this measure: strong (≥1.3 ng), medium (0.7–0.9 ng), and weak (≤0.7 ng) [Zhang et al. 2002]. Total S-glycoprotein volume within the style is significantly and positively correlated with the degree of self-incompatibility ($r = 0.906$, $n = 20$). Suassuna et al. [2003] reported that S-RNase content in the styles of bud-stage *Pyrus* was low, such that seeds can be formed during fructification following self-pollination. In *Solanum chacoense*, a S-RNase expression threshold has been reported. S-RNase accumulation differs among styles. Within the styles, self-compatibility is typically characterized by the accumulation of less than 68 ng of S-RNase, with self-incompatibility being characterized by the accumulation of more than 160 ng of S-RNase [Qin et al. 2006]. If S-RNase accumulation in the matrix outside of the style transfer tissue cells is lower than a certain threshold, then the transfer of RNA of the same genotype present in the pollen tube is prevented. This process supports the growth of the pollen tube into an embryo, thus completing fertilization. It has been suggested that the volume of S-RNase affects the SI. However, some reports also show that, although S-protein concentrations and S-protein quantities differ among different breeds, these differences have no effect on the growth of self-incompatible pollen tubes. S_4-protein content in 'Nijisseiki' (*Pyrus pyrifolia*) and 'Kikusui' is greater than that in 'Yakumo,' with 'Nijisseiki' and 'Kikusui' exhibiting medium SI and 'Yakumo' exhibiting strong SI [Zhang and Hiratsuka 1999]. Qin et al. [2006] observed that, upon averaging S-RNase content values from a single *Solanum chacoense* style, the levels of the S11- and S12-RNases differed by up to 10-fold, even within a single genotype. Additionally, S12-RNase levels can differ by over 3-fold among genotypes. Surprisingly, S12-RNase abundances in different styles from the same plant can differ by over 20-fold. Thus, S-RNase is responsible for more than just the degradation of RNA during the SI response. Further study is required to accurately determine the role of S-RNase in the SI response.

Determinative factors in controlling the pollen S unit specificity

Studies by classic geneticists show that the self-incompatibility of a plant is controlled by multiple alleles at the S-*locus*. There are at least two types of genes that participate in the specific identification of genes, which facilitates the self-incompatibility. One is a S-glycoprotein specifically expressed in the pistil transfer tissue, while the other is a pollen S-specific determinative factor linked with the S-*locus*. Concerning pollen S genes, in recent years, breakthroughs have been made concerning the gametophytic self-incompatibility of certain breeds in the Scrophulariaceae, Solanaceae, and Rosaceae families. The results of these studies culminated in the identification of a likely candidate gene, SFB/SLF (haplotype-specific F-box gene) [Kirch et al. 1989]. F-box gene expression products controlling pollen self-incompatibility have not been isolated or identified at present. Ushijima et al. [2004] screened a SFB (S-haplotype-specific F-box) that exhibited high sequence polymorphism in its F-box genes, and a SLF (S-*locus* F-box) exhibiting low sequence polymorphism in F-box gene ORFs (Open Reading Frames) in almond, and also a SLFL (S-*locus* F-box-like gene), which was highly expressed in multiple tissues. Sonneveld et al. [2001] induced self-compatible responses in 2 pollen grains using X rays and found that pollen SFB genetic sequences in

2 types of pollen were abnormal and dysfunctional. They also demonstrated that SFB/SLF is the best candidate factor for pollen S-determinative factors.

Self-compatibility mechanisms in Rosaceae fruit trees

During self-pollination, pistil and pollen S genes induce SI as part of their normal function, with SI occurring less often when their function is disrupted.

Mutation of pistil S genes. Mutations associated with SI are common in Rosaceae fruit trees, resulting in the dysfunction of S-RNase. The pistil S-RNase of mutated breeds is unable to identify pollen of the same genotype, such that a self-compatible reaction between the pistil and the pollen will occur. Most self-compatible fruit trees are S gene mutants. S-RNase-deficient plants are found in breeds of sweet cherry, pear, and almond. The Japanese pear (Osa-Nijisseiki) genotype $S_2S_4^{SM}$ is self-compatible pear variety and is a mutant of the Japanese pear (Nijisseiki) genotype S_2S_4. In the L2 layer of the mutated pear, a 236 kb segment, which usually includes S_4-RNase genes, is missing, resulting in the loss of S-RNase's identification ability and the induction of the self-compatible response [Okada et al. 2008]. The self-fertilization seed set propagation coefficient of the Japanese pear (Osa-Nijisseiki) is 98%. The self-fertilization seed set propagation coefficient of the 'Yanzhuang' pear, which expresses self-compatibility and is a bud variant of the 'Yali' pear (*Pyrus bretschneideri* Rehd.), is 56.68% and the average seed quantity of fruits is 8.64. Pollination experiments have shown that, when the maternal plant is a 'Yanzhuang' pear and the paternal plant a 'Yali' pear, the pollination seed set propagation coefficient can reach 39.3%, and the average seed quantity of fruits is 3.36. Offspring of this cross also express self-compatibility. In the reverse cross, the seed set propagation coefficient is 2%, with offspring expressing self-incompatibility. It has been proven that self-compatibility in the 'Yanzhuang' pear may be caused by mutation and the low expression of S-RNase in the style [Li et al. 2008]. Self-compatibility mutation in the 'Katy' apricot is also caused by the reduced activity or non-expression of S genes in the style [Zhang et al. 2001]. Qi et al. [2011] found, upon identification of S genotypes normally expressed in 'Zaoguan' pears in descendants of 'Zaoguan' pears, that S_4-RNase is expressed normally in the style but not in the pistil. This suggests that the absence of S_{34}-RNase in the style prevents the pistil from identifying the S_{34} unit of its own pollen tube, allowing it exhibit self-compatibility.

Mutation of pollen S genes. The self-incompatibility of plants is controlled by multiple S-*locus* alleles. In other words, mutation of the pollen F-box genes that control self-incompatibility also interrupt associated self-incompatibility responses. Among the 3 self-compatible breeds of *Prunus persica*, in one of the breeds, the self-incompatibility response is caused by the mutation of style S genes, while in the other two breeds it is caused by the mutation of pollen S genes [Tao et al. 2007]. Yang et al. [2015] studied a self-compatible cherry breed (*Cerasus pseudocerasus*) and a self-incompatible cherry breed ('Rainier') and found that the 'Lapins' cherry exhibited normal pollen fertility, while RT-PCR and sequencing showed that both 'Rainier' and 'Lapins' SFB4 can be transduced normally. Sequencing showed that the pollen SFB4′ of 'Lapins' is missing 4 basic groups at the 911 bp position, which makes C-end translation stop ahead of time. Prokaryotic expression of SFB4 and SFB4′ proteins showed that SFB4 is slight greater than SFB4′. This shows that the self-incompatibility of 'Lapins' might be due to the missing amino acids at the SFB4′C end, which perhaps inhibits its ability to identify S-RNase. The Japanese apricot (*Prunus mume* Sieb. et Zucc.) is a fruit crop that exhibits GSI. Some self-compatible varieties have evolved during the course of the species' evolution. Wang et al. [2012] compared the typically self-incompatible Japanese apricot breed 'Nanko' and the self-compatible breed 'Koshinoume'. They also studied the self-compatibility of two other breeds, 'Sichuan Baimei' and 'Changnong 17', both of which are native to China, and their results suggested that there was an insertion mutation in the pollen SFB genes of both breeds, which possibly resulted in their acquisition of self-compatibility. 'Jin Zhui' is a spontaneous self-compatible mutant of 'Yali' (*Pyrus bretschneideri* Rehd. $S_{21}S_{34}$) that displays typical S-RNase-based GSI. The pollen-part mutation (PPM) of 'Jin Zhui' might be due to a natural mutation in the pollen-S gene (S34 haplotype). However, the molecular mechanisms behind these phenotypic changes are still unclear. Five SLF (S-*locus* F-box) genes in 'Yali' have been identified, while no nucleotide differences

were found in the SLF genes of 'Jin Zhui' [Wu et al. 2013a]. Therefore, it is needed to further investigate the pollen S gene in these breeds.

Self-compatibility caused by the "competitive effect". The polyploidy of species and the repetition of pollen S genes inhibits the GSI mechanisms of self-incompatible species due to the "competitive effect" mode. Pollen grains containing two different S alleles are compatible with their own S-RNase. Such phenomena have been thoroughly studied in plants within the Solanaceae and Scrophulariaceae. Two pollen grains possessing the same pistil S genes (S_1+S_1/S_2+S_2) are incompatible with their own pistil (S_1S_2). Only two pollen grains with different pistil S genes (S_1+S_2) are compatible with their own pistils (S_1S_2). Qi et al. [2011] studied the self-incompatible diploid 'Kuerlexiangli' (*Pyrus sinkiangensis*)' and the self-compatible tetraplont variant 'Sha01,' and found that $S_{22}S_{28}$ pollen is compatible with its own pistil in the allodiploid 'Sha01.' This proves that there is also a "competitive effect" which can lead to compatibility in Rosaceae plants. The self-compatibility of tetraplont individuals in 'Nanjing Chuisi' cherry is also caused by this "competitive effect" [Huang et al. 2008].

The role of calcium signal transduction in self-incompatibility

Calcium ions are the second most important signaling molecules in cells. The growth of the pollen tube is limited by the polar distribution of calcium ions within the cell. In growing pollen tubes, Ca^{2+} concentration gradient is often created, reduced from the tip to the base [Qu et al. 2016b]. This Ca^{2+} concentration gradient is beneficial in the directional control of Golgi vesicle secretion, operation, and integration, and facilitates the constant creation of new cell wall and plasma membrane and maintains the normal growth of the pollen tube. In the self-incompatible response of *Papaver rhoeas*, the Ca^{2+} concentration gradient in the incompatible pollen tube is broken down rapidly, such that the pollen tube stops growing [Franklin-Tong et al. 1997]. In *Pyrus*, after the pollen tube is treated with its own S-RNase, the Ca^{2+} concentration gradient also dissipates. However, when the pollen tube is treated with exogenous S-RNase, the Ca^{2+} concentration within the pollen tube persists at a normal level. This shows that Ca^{2+} participates in the self-incompatibility response in *Pyrus*. In the self-incompatibility response of Papaveraceae and Rosaceae plants, the Ca^{2+} concentration gradient present in the pollen tube dissipates. However, in these plants, the Ca^{2+} concentration gradient dissipates completely. In the pollen tube of plants in the Papaveraceae family, the dissipation of the concentration gradient was induced by self-S-glycoprotein, which promotes extracellular Ca^{2+} influx and thus interrupts calcium gradients within the pollen tube. The calcium concentration gradient in the pollen tube of Rosaceae plants was interrupted by the self-S-RNase inhibition of pollen tube tip Ca^{2+} channel activity, thus inhibiting extracellular calcium influx. In addition, we found that Ca^{2+} concentrations in the pollen tube of *Pyrus* are associated with pollen tube RNA concentrations. Reducing the intracellular Ca^{2+} concentrations will thus reduce associated RNA concentrations, and vice versa [Qu et al. 2016a].

According to the results of existing research, we believe that the self-compatibility mechanism, in which Ca^{2+} participates is as follows: pollen and stigma recognize each other following pollination; if the pollen is not self-pollen, the activity of the Ca^{2+} channels at the apical pollen tube is not affected, and a normal Ca^{2+} concentration gradient is maintained. Vesicles move to the tip of the pollen tube and fuse with the plasma membrane to create more plasma membrane. Cell wall precursors are released, leading to pollen tube elongation, surrounded by vesicles containing S-RNase that is excreted extracellularly (Fig. 1A). However, if the stigma receives self-pollen, self-S-RNase will reduce the activity of Ca^{2+} channels at the tip of the pollen tube to inhibit extracellular Ca^{2+} influx. The Ca^{2+} concentration gradient is broken, and as such, growth of the pollen tube is slowed. Vesicles within the pollen tube are ruptured last, and S-RNase degrades RNA as a toxic cellular substance, which leads to the complete ceasing of pollen tube growth (Fig. 1B). Relevant studies also show that Ca^{2+} within self-incompatible pollen tubes also mediate other downstream processes, which induces programmed cell death in the pollen tube tip (PCD) [Franklin-Tong and Franklin 2003, Wang et al. 2010].

Problems and future considerations

Although style S genes have been identified [Liu et al. 2014], the S genes of pollen remain controversial.

Studies show that SLF genes determine the specificity of male self-incompatibility. However, speculative GSI models based on SLF are often paradoxical. For example, in the Solanaceae, polyploidy directly causes a transition from SI to self-compatibility due to the compatibility of heteroallelic pollen. In *Prunus*, polyploidy does not directly result in the breakdown of SI. Although it has been proven that SLFS forms SCF complexes such as E3 ubiquitin ligase, which interact with S-RNase and cause the degradation of non-self-S-RNase; S-RNase is known to still enter the pollen tube *via* the style. Thus, determining the exact concentrations of S-RNase required to induce a response is difficult. In *in vitro* experiments, differences between the amount of S-RNase within the pollen tube after treatment with self-S-RNase and non-self-S-RNase are not detected when using the immune colloidal gold technique [Qu et al. 2016a]. A large amount of S-RNase can be found in self-compatible and self-incompatible potato pollen tubes using immune-histo-

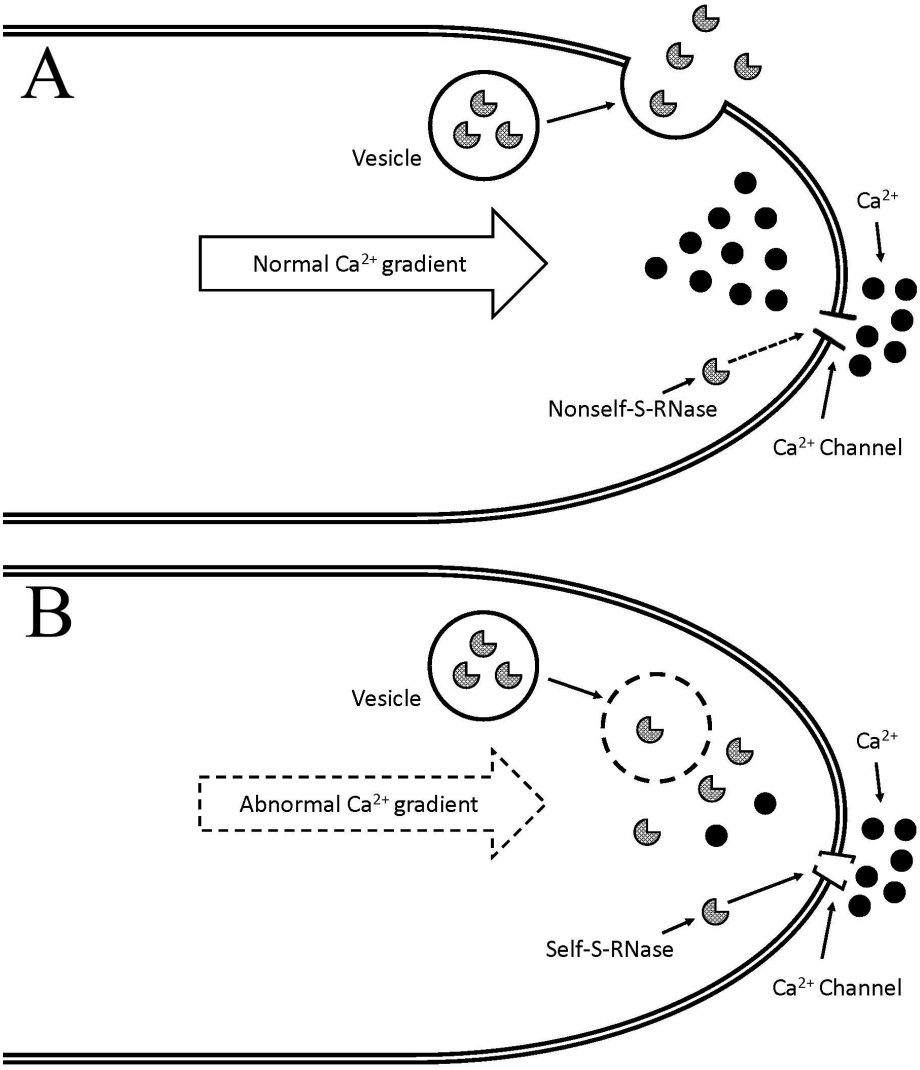

Fig. 1. Model for the inhibition of pollen-tube growth by self-S-RNase through Ca^{2+} transduction [Qu et al. 2016]. (A) Compatibility. (B) Incompatibility

chemical study [Luu et al. 2000]. MdABCF is localized on the pollen tube membrane and interacts with S-RNase. MdABCF provides assistance in delivering self or non-self S-RNase to the pollen tube [Meng et al. 2014a]. Moreover, the degradation of S-RNase cannot be detected after S-RNase is marked with ^{3}H in the pollen tube of *Nicotiana alata* (*in vitro* growth) [Gray et al. 1991]. Therefore, McClure [2006] has proposed that S-RNase compartmentalization allows pollen with compatible vesicles to surround S-RNase and reduce its toxic effect. When an incompatible pollination occurs, the vesicles rupture, and S-RNase is released into the cytoplasm, acting as a cytotoxin that inhibits the growth of the pollen tube. Growth of an incompatible pollen tube is inhibited completely 6 days after pollination in tobacco and 80% of pollen tubes exhibited abnormal actin structure, despite the fact that all vesicle membranes had remained intact [Roldán et al. 2012]. Therefore, these two studies' results cannot suitably explain self-incompatibility at present. Sundberg and Stergaard [2009] have proposed that three obvious classifications of auxin concentration gradient: high concentration of auxin/low concentration of mitogen on the top, low concentration of auxin/low concentration of mitogen in the middle and low concentration of auxin/high concentration of mitogen at the bottom. Many researchers have successfully used plant hormones to overcome the GSI response, and in lily plants the external application of auxin can cause self-fruiting [Xue et al. 2008]. Low concentration of NAA, IAA, 6-BA, and 2,4-D can promote pollen germination and pollen tube growth in peach, apple, and pear, but only when high concentrations are used [Xue et al. 2008, Yang et al. 2010]. It has been reported that the application of plant growth regulator can promote pollen germination during the flowering period, increase the growth speed of the pollen tube in the style, and improve fruit setting rate. Based on our preliminary experimental results, we can confirm that exogenous auxin application can promote the growth of pollen tubes in the style following self-fertilization (in press). However, how plant regulators regulate the transport of S-RNase and influence Ca^{2+} concentrations in the pollen tube remains unknown, and thus to be able to properly control SI in fruit trees, further work is required.

If it becomes possible to control SI in cultivated plant species, plant production will be made much more efficient, solving many issues afflicting farmers, and may even allow the breeding of new varieties.

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