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Molecular Mechanism of The Self-Incompatibility In Flowering Plants

A. Satyaraj Kumar* and K. Geethanjali
Department of Genetics and Plant Breeding and Crop physiology
College of Horticulture, Parvathipuram, Dr YSRHU
Corresponding author: * Teaching Associate, rajkumargpbr08@gmail.com

ABSTRACT
In flowering plants, Self-incompatibility is an important intraspecific reproductive barrier. It is estimated that nearly half of the flowering plants have developed some kind of self-incompatibility. In many species, these mechanisms are controlled by a highly ***polymorphic locus*** *called the* ***S-locus****, which encodes components controlling the pistil and pollen expression of self-incompatibility.In dicots, self-incompatibility maps to a single genetic locus (grasses have 2 unlinked loci!). Molecular dissection of the S-locus in several plant species has shown that the S-locus consists of multiple, tightly-linked genes, encoding male and female compatibility determinants. Divergent mechanisms of self-incompatibility are encoded by the S-loci of different plant species. Molecular studies on several self-incompatible species of Brassicaceae, Solanaceae and Papaveraceae have revealed that the S-locus of these families consists of at least two polymorphic genes, one encoding the male determinant and another female. This multi-gene complex at the S-locus is known to be inherited as one unit and therefore the variants of the gene complex now called 'S-haplotypes'. The molecular interactions between the male and female determinants encoded by the same S-haplophyte are expected to induce the SI response. SI is an important mechanism for preventing inbreeding in plants. It is promising in the creation of new varieties to prevent candidate plants crossing with themselves and thus increasing the chance of creating new desirable outcrosses.There are different mechanisms of SI that have been described at the molecular level in members of different families. Currently, research mainly focus on the finding the biochemical and molecular basis of self-incompatibility.*
Keywords: S-locus, Self-incompatability, Polymorphic locus, Halophytes

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INTRODUCTION

Self Incompatibility enables more efficient breeding techniques to be employed for crop improvement. It was possible to search for the genetic basis of self-incompatibility after the Mendelian laws of heredity had been rediscovered. Carl Correns (1913) performed first studies on this topic. But the breakthrough was achieved by E. M. East and A. J. Mangelsdorf with their studies on *Nicotiana* (1925). Their results show that self-incompatibility is caused by a gene (SI) with numerous alleles: SI_1, SI_2, SI_3, SI_4....SI_n. Incompatibility occurs whenever the two plants to be crossed carry the same alleles. Since pollen is haploid is just one of the self incompatibility alleles expressed at its surface, while the diploid stigma surface bears the products of two alleles. Fertilization occurs only, if the allele product of the pollen and those of the stigma are unlike. The case just mentioned is a gametophytic self-incompatibility (GSI). It is opposed by sporophytic self-incompatibility (SSI) where the components of the pollen exine produced by the tapetal cells are responsible for the stigma's repulsion of the pollen.

The situation of gametophytic self-incompatibility is rarely as simple as in the case of *Nicotiana* (and numerous other *Solanaceous* species). A. Lundquist (1956) and D. L. Hayman (1956) discovered independent of each other a second self-incompatibility locus (Z) in grasses. In such cases is the pollen rejected only, if both plants contain the same alleles in both loci (SI and Z).

All this aggravates a genetic analysis since it is impossible to identify all components of the combination matrix individually. The situation becomes completely confused in case of polyploidy. Probability calculus allows a simple, but far-reaching prediction: the frequency of incompatible combinations decreases rapidly with the increasing number of gene loci. Systems with several genes are therefore weaker self-incompatibility systems than those with just one gene.

Self incompatibility and sterility are the two mechanisms which encourage cross pollination. It was first reported by Koelreuter in the middle of eighteenth century. More than 300 species belonging to 70 families of angiosperms show self incompatibility. Self incompatibility is appeared to be due to biochemical reaction, but precise nature of these reactions is not clearly understood.

MECHANISMS OF SELF INCOMPATIBILITY

2.1.S-RNase degradation model

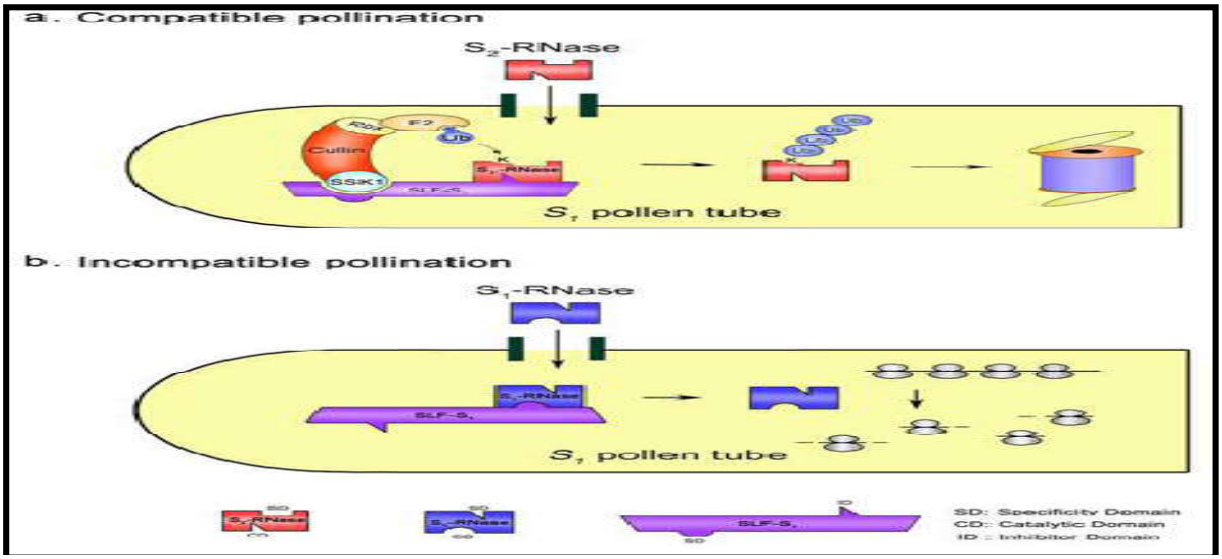
One of the most widely distributed type of self-incompatibility is found in the *Solanaceae, Scrophulariaceae* and *Rosaceae* families.

All S-RNases are proposed to have two domains, a specificity domain (SD) and a catalytic domain (CD). The SD is described to be the recognition portion by the corresponding SLF protein; whereas, the CD is in charge of triggering the next step of the SI response. Similarly, SLF proteins are also supposed to adopt two domains, a specificity domain, used for specific interaction with cognate S-RNase, and an inhibitor domain (ID), for preventing non-self S-RNase catalytic activity.

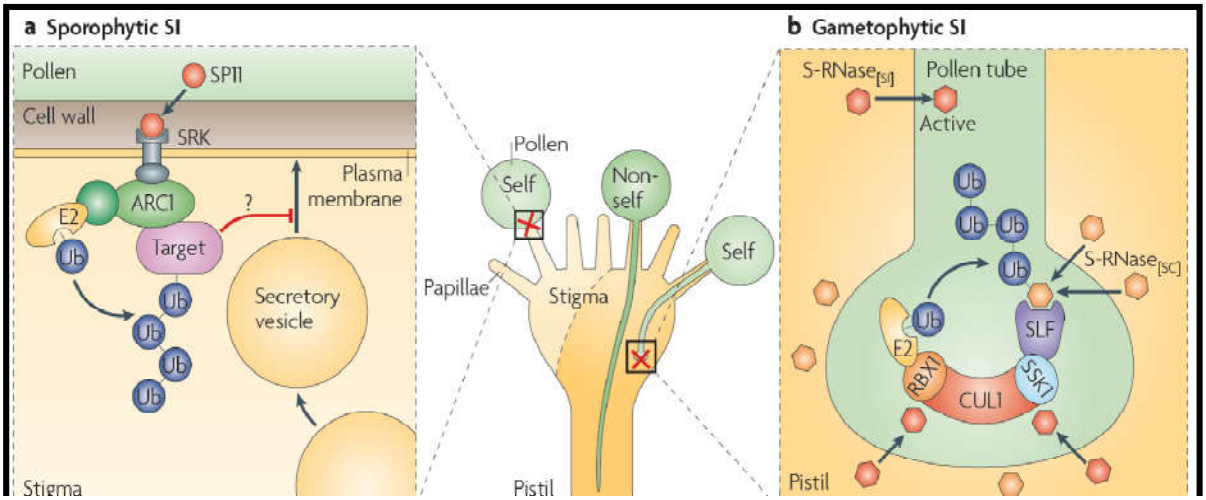
Although previous studies have proved that **S-RNase** is the ***female determinant*** for the pistil component of self-incompatibility of flowering plants, the male determinant, which determines the pollen component of self-incompatibility, remained elusive for a long time. It is generally accepted that **S-RNase *acts to degrade RNA of self-pollen tubes***, therefore stopping the process of self-pollination. Earlier research suggested that the pollen-*S* component encodes an S-RNase inhibitor in non-self pollen tubes to block S-RNase from functioning, hence promoting the non-self pollination process. For the first time ever, the team succeeded in cloning a *pollen-S* gene, the ***SLF*** **(S-Locus F-box)-*S_2*** from *Antirrhinum*, and advanced to demonstrate its direct interactions with the S-RNase as well as SSK-1 and Cullin-1like proteins of the SCF (Skp1/Cullin or CDC53/F-box) complex. Their follow-up studies revealed that the S-RNase could only be ***ubiquitinated and degraded in a compatible pollination***. Integrating the above discoveries, the team proposed an S-RNase degradation model of self-incompatible pollination.

This discovery proves to have solved an important problem in botany, marking an extraordinary breakthrough in the field of plant sexual reproduction. This achievement also has a strong potential in applied research for breeding of hybrids and generating compatible varieties.

Figure 1: The S-RNase degradation model. In a compatible pollination process (a), an SLF protein forms an active SCF complex, which unibiquitinates and degrades S-RNase.



While in an incompatible pollination (b), an **SLF *protein fails to form a functional SCF complex to inactivate* S-RNase,** resulting in its degrading RNA and inhibiting the growth of pollen tubes.



a. Control of sporophytic self-incompatibility (SI) by the ARMADILLO REPEAT-CONTAINING 1 (ARC1) U-box E3. Recognition of the S-LOCUS PROTEIN 11 (SP11) from pollen by its cognate S-LOCUS RECEPTOR KINASE (SRK) on the papillae surface of the stigma triggers the phosphorylation and presumably the activation of ARC1. Ubiquitylation by ARC1 might block the fusion near self pollen of secretory vesicles needed to provide water and nutrients that are essential for pollen germination, thereby preventing self-fertilization.

b. Control of gametophytic SI by an S phase kinase-associated protein 1 (SKP1)-cullin 1 (CUL1)-F-box (SCF) E3 assembled with polymorphic S-locus F-box proteins (SLFs). As pollen tubes grow down the pistil toward the ovary, the growth of self pollen is halted by the import of a cognate S-RNase expressed in the transmitting tract. However, for non-self pollen, the imported *S*-RNase is degraded following ubiquitylation by the SCF^{SLF} E3 assembled in the pollen tube along with a unique SI-SPECIFIC SKP1 PROTEIN 1 (SSK1). It has been proposed that the SCF^{SLF} complex only recognizes and removes S-RNases from non-self pistils but not from self pistils, thus allowing self S-RNases to specifically inhibit self-pollen tube growth. The central diagram locates where on the stigma or pistil sporophytic and gametophytic SI occurs. RBX1, RING BOX 1; SC, self compatible.

2.2.Mechanisms of Self-Incompatibility by protein – protein interaction

The best studied mechanisms of self incompatibility act by inhibiting the germination of pollen on stigmas, or the elongation of the pollen tube in the styles. These mechanisms are based on ***protein-protein interactions***, each mechanism being controlled by a single locus termed **S**, which has many different alleles in the species population. Despite their similar morphological and genetic manifestations, these mechanisms have evolved independently, and are based on different cellular components; therefore, each mechanism has its own, unique S-genes.

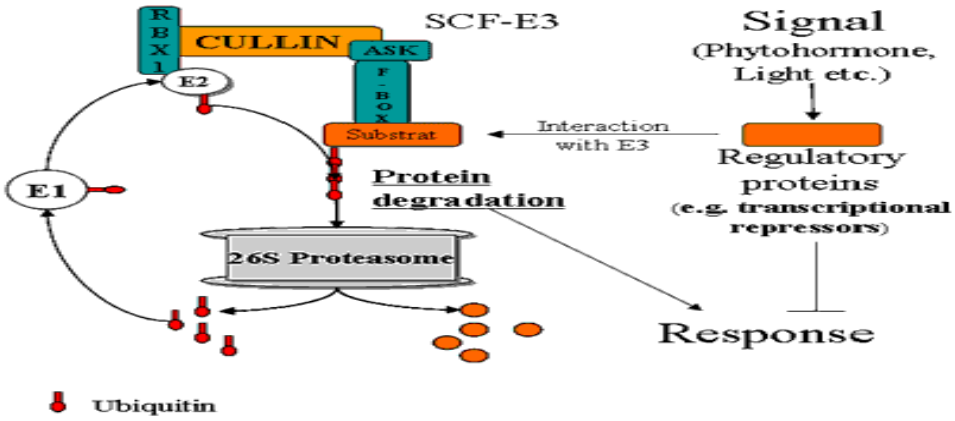
The S-locus contains two basic protein coding regions - one expressed in the pistil, and the other in the anther and/or pollen (referred to as the ***female*** and ***male determinants***, respectively). Because of their physical proximity, these are genetically linked, and are inherited as a unit. The units are called ***S-haplotypes. The translation products of the two regions of the S-locus are two proteins which, by interacting with one another, lead to the arrest of pollen germination and/or pollen tube elongation, and there by generate self incompatibility response, preventing fertilization.*** However, when a female determinant interacts with a male determinant of a different haplotype, no self incompatibility is created, and fertilization ensues. This is a simplistic description of the general mechanism of self incompatibility, which is more complicated, and in some species the S-haplotype contains more than two protein coding regions.

2.3. F-box proteins:

The female component of genotypic self incompatibility in the *Solanaceae* was found in 1989. Proteins in the same family were subsequently discovered in the *Rosaceae* and *Plantaginaceae*. Despite some early doubts about the common ancestry of genotypic self incompatibility in these distantly related families, phylogenetic studies and the finding of shared male determinants **(F-box proteins)** clearly established homology. Consequently, this mechanism arose approximately 90 million years ago, and is the inferred ancestral state for approximately 50 % of all plants. In this mechanism, ***pollen tube elongation is halted when it has preceded approximately one third of the way through the style.*** The female component ribonuclease, termed **S-RNase** probably causes degradation of the ribosomal RNA (rRNA) inside the

pollen tube, in the case of identical male and female S alleles, and consequently pollen tube elongation is arrested, and the pollen grain dies.

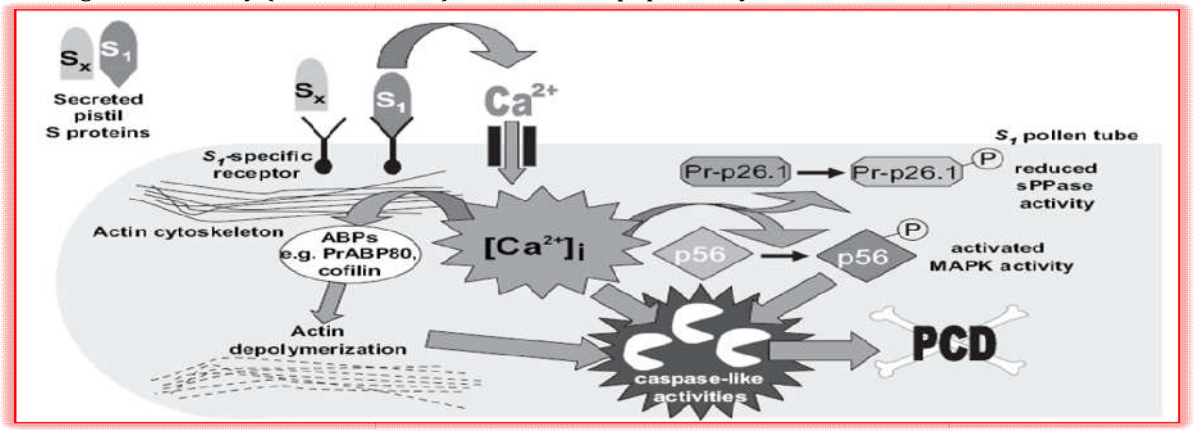
The ***male component*** was only recently putatively identified as a member of the **"F-box" protein family**. The members of this group typically function as ***ubiquitin ligases,*** and they may function by recognizing the matching S-RNase molecules and sending them to proteosomal degradation. Despite some fairly convincing evidence that it may be the male component, several features also make it an unlikely candidate.



Characterization of SCF complexes (structure, localization, dynamics).Identification of interacting F-box-proteins (approx. 700 in *Arabidopsis thaliana.*Identification of substrate proteins and target genes, whose stability and transcription is directly or indirectly influenced by SCF-complexes

2.4.The S-glycoprotein mechanism

The following mechanism was described in detail in *Papaver rhoeas*. In this mechanism, pollen growth is inhibited within minutes of its placement on the stigma. The female determinant is a small, ***extra cellular molecule,*** expressed in the stigma. The identity of the male determinant remains elusive, but it is probably some cell membrane receptor. The interaction between male and female determinants transmits a cellular signal into the pollen tube, resulting in ***strong influx of calcium cations***; this interferes with the intracellular concentration gradient of calcium ions which exists inside the pollen tube, essential for its elongation. ***The influx of calcium ions arrests tube elongation within 1-2 minutes***. At this stage, pollen inhibition is still reversible, and elongation can be resumed by applying certain manipulations, resulting in ovule fertilization. Subsequently, the cytosolic protein **p 26**, a pyrophosphatase, is inhibited by phosphorylation, possibly resulting in arrest of synthesis of molecular building blocks, required for tube elongation. There is depolymerization and reorganization of actin filaments, within the pollen cytoskeleton. Within 10 minutes from the placement on the stigma, the pollen is committed to a process which ends in its death. At 3 to 4 hours past pollination, fragmentation of pollen DNA begins, and finally (at 10-14 hours), the cell dies apoptotically.



A proposed model for the self-incompatibility mechanism in Papaver rhoeas.

Incompatible pollen undergoes an S haplotype-specific interaction. Secreted stigmatic S-proteins interact with the pollen S receptor. An haplotype-specific interaction such as binding S_1 protein to S_1 pollen results in triggering an intracellular Ca^{2+} signalling cascade(s), involving large-scale Ca^{2+} influx and increases in

$[Ca^{2+}]_i$. A series of events then occur in the incompatible pollen. Within 1 min there is a dissipation of the tip-focused calcium gradient that is required for continued pollen growth and the activation of calcium-dependent protein kinase (CDPK). The CDPK phosphorylates Pr-p26.1, a soluble inorganic pyrophosphatase (sPPase). Both calcium and phosphorylation inhibit sPPase activity, resulting in a reduction in the biosynthetic capability of the pollen, thereby inhibiting growth. Dramatic changes to pollen cytoskeleton organization are apparent within 1 min, with extensive depolymerization of the F-actin accompanying this, also predicted to cause rapid arrest of tip growth. p56-MAPK is activated and may signal to PCD. PCD is triggered, involving key features of PCD including caspase-like activity, cytochrome c leakage and DNA fragmentation. This ensures that incompatible pollen does not start to grow again. Adapted from McClure and Franklin-Tong (2006). With kind permission of Springer Science and Business Media

2.5.Other mechanisms of self-incompatibility

2.5.1.Cryptic Self Incompatibility (CSI)

Cryptic self-incompatibility (CSI) exists in a limited number of taxa (example: Silene vulgaris, Caryophyllaceae). In this mechanism, the simultaneous presence of cross and self pollen on the same stigma, results in higher seed set from cross pollen relative to self pollen. However, as opposed to 'complete' or 'absolute' self-incompatibility, in cryptic self-incompatibility, self-pollination without the presence of competing cross pollen, results in successive fertilization and seed set; in this way, reproduction is assured, even in the absence of cross-pollination. Cryptic self-incompatibility acts, at least in some species, at the stage of pollen tube elongation and leads to faster elongation of cross pollen tubes relative to self pollen tubes. The cellular and molecular mechanisms of Cryptic self-incompatibility have not been described.

2.5.2.Late-acting self-incompatibility (LSI)

Late-acting self-incompatibility (LSI) is also termed ovarian self-incompatibility (OSI). In this mechanism, self pollen germinates and reaches the ovules, but no fruit is set. Late-acting self-incompatibility can be pre-zygotic (e.g. deterioration of the embryo sac prior to pollen tube entry, as in Narcissus triandrus) or post-zygotic (malformation of the zygote or embryo, as in certain species of Asclepias and in Spathodea campanulata).

The existence of the Late-acting self-incompatibility mechanism among different taxa and in general is subject for scientific debate. Criticizers claim, that absence of fruit set is due to genetic defects (homozygosity for lethal recessive alleles), which are the direct result of self-fertilization (inbreeding depression).

DISCUSSION

Ed Newbigin *et al.* (1993) studied the molecular genetics of gametophytic self- incompatibility and opened up the observation that the SI phenotype of pollen is determined by its own haploid genotype and the sequence of the allelic series of S-RNases expressed in female tissue, which degrades the RNA of growing pollen tube in incompatible pollination.

Allen *et al*. (2011) studied the Pollen–pistil interactions and self-incompatibility in the Asteraceae and concluded that the stigma structure and the molecular mechanism of SSI in the Asteraceae and Brassicaceae are different. The Senecio transcriptome analysis showed that its pistil contains many novel pistil-specific genes, but also pistil-specific genes previously shown to play a role in pollen–pistil interactions in other species.

Seiji Takayama and Akira Isogal (2003) studied the molecular mechanism of self-recognition in Brassica and revealed the presence of three highly polymorphic genes SRK, SLG and SP11, and their complex interaction and receptor activation occur in a S- halophyte specific manner as the basis of self-incompatibility.

Teh-hui Kao and Tatsuya Tsukamoto (2004) revealed the molecular and genetic basis of S-RNase-based self- incompatibility by studying RNase activity of S-RNases that plays a major role in degradation of RNA inside the self-pollen tubes results in growth inhibition.

Maurice Bosch and Vernonica E. Franklin-Tong (2008) focused on self-incompatibility in Papaver and revealed that SI specific events triggers Ca^{+2} dependent signaling cascade leads to programmed cell death in self-incompatible pollen.

CONCLUSION

SI is an important mechanism for preventing inbreeding in plants. It is promising in the creation of new varieties to prevent candidate plants crossing with themselves and thus increasing the chance of creating new desirable outcrosses.There are different mechanisms of SI that have been described at the molecular

level in members of different families. Currently, research mainly focus on the finding the biochemical and molecular basis of self-incompatibility.

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