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A REVIEW

Molecular basis of self-incompatibility and its utilization in crop improvement

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Abstract : Self-incompatibility, is a genetically controlled mechanism for rejection of own pollen. It has been a favourite topic for botanists and geneticists since Darwin who first discussed this phenomenon and suggested its central significance during the evolution of flowering plants. Different genetic and mechanistic systems of SI among different plant families suggest either multiple origins of SI or considerable evolutionary diversification. Within the last two decades, molecular and biochemical analyses which have significantly contributed to the elucidation of the complex series of interactions occurring at the pollen-stigma interface. Molecular analyses of self incompatibility systems have focused on identifying and characterizing the pollen and pistil components of the self-incompatible response as well as other proteins and events that lead to pollen rejection. In this review, an attempt has been made to provide a comprehensive insight for the molecular dissection of this important mechanism for its utilization in crop improvement.

Key Words : Self-incompatibility, S-allele, Pollen-stigma interaction, Molecular analysis, Gametophytic, Heteromorphic

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INTRODUCTION

Various bisexual flowering plants evade the harmful effects of inbreeding by employing genetically controlled self-incompatibility (SI) mechanisms to ensure out crossing (Charlesworth and Charlesworth, 1987). SI mechanisms make available the biochemical machinery essential for plants to recognize and discard their own pollen as well as non-self pollen with a genotype amply similar to obtain activation of the SI mechanism. SI plays an important role in determining the spatial and temporal distribution of genetic diversity in plant populations and is thought to influence patterns of lineage diversification in clades within which these mechanisms are utilized (Igic *et al*., 2008). About 96 per cent of flowering plants produce perfect flowers that contain both the male and female reproductive organs in close proximity; accordingly, they would have a strong affinity to self-fertilize if there were no mechanisms to prevent them from doing so. Because inbreeding can result in reduced fitness in the progeny, hermaphroditic plants have adopted a variety of reproductive strategies, including self-incompatibility (SI), by which inbreeding is prevented and outcrosses are promoted (de Nettancourt 2001). SI allows the pistil of a flower to distinguish between genetically related (self) and unrelated (non-self) pollen. This self/non-self recognition results in the inhibition of germination of self-pollen on the stigmatic surface or the inhibition of growth of self-pollen tubes in the style. Self-incompatibility thus, the most sophisticated and widespread in occurrence, has been known in flowering plants for over a century since Darwin's description in 1876 (Darwin, 1876). SI was defined by de Nettancourt (1977) as 'the inability of a fertile hermaphrodite seed plant to produce zygotes after self-pollination'. In other words SI is a prezygotic reproductive barrier by which incompatible pollen/pollen tubes are prevented from delivering the sperm cells to the ovary to affect double fertilization (Sims, 1993; Charlesworth *et al*., 2005). Incompatibility is widespread and present in species of Leguminosae, Solanaceae, Cruciferae, Compositae and

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Gramineae. In cultivated crops, incompatibility is found in red clover, aliska clover, white clover, alfalfa, tall fescue, ryegrass, rye, sugarbeets, sunflower, pearmillet, tobacco, potatotes, bahigrass, bermudagrass, and others (Haring *et al*., 1990; de Nettancourt, 2001; Allen and Hiscock, 2008). Identification of the biochemical components of SI mechanisms has proven to be difficult in most lineages (Clarke and Newbigin, 1993). Within the last two decades, scientists have been able to complement Darwin's genetic observations with molecular and biochemical analyses which have significantly contributed to the explanation of the complex series of interactions occurring at the pollen-stigma interface (Takasaki *et al*., 2000; Silva and Goring, 2001; Franklin-Tong and Franklin, 2003). Molecular analysis of self incompatibility systems have focused on identifying and characterizing the pollen and pistil components of the self-incompatible response as well as other proteins and events that lead to pollen rejection are discussed hereafter.

Incompatibility systems :

Self-incompatibility systems may be classified into two basic types: heteromorphic and homomorphic.

Heteromorphic incompatibility :

In heteromorphic incompatibility, flowers of the same species can have two or three different morphological forms, and successful pollination occurs only between flowers of different morphological forms (Darwin, 1877 and Haldane, 1933). This is caused by differences in the lengths of stamens and style (called heterostyly) and may be of two types: (i) Distylic and (ii) tristylic. In the distylic forms (e.g. *Fagopyrum* sp., *Pulmmaria* sp., *Linus* sp., *Potigorum* sp.) two types of floral structure (short style and high anther, long style and short anther) is found (Fig. 1). In one flower type called the pin, the styles are long while the anthers are short. In the other flower type, thrum, the reverse is true (e.g., in *Primula*). The pin trait is conditioned by the genotype *ss* while thrum is conditioned by the genotype *Ss*. A cross of pin (*ss*) × pin (*ss*) as well as thrum (*Ss*) × thrum (*Ss*) is incompatible. However, pin (*ss*) × thrum (*Ss*) or vice versa, is compatible. In tristylic types (e.g. *Lythrum* sp., *Oxalis* sp.) the population comprise three distinct groups characterized by long, mid and short styled flowers, each flower bearing anther at two different heights which do not correspond to the level of stigma (Li *et al*., 2007).

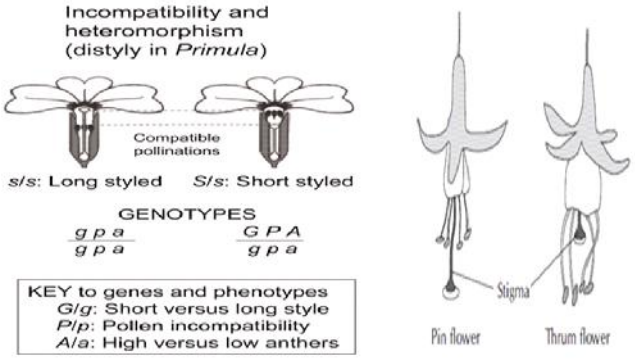

Fig.1 : Heteromorphic incompatibility showing floral modifications in which anthers and pistils are of different lengths in different plants. Long- and short-styled primrose flowers (showing the pollinations that are compatible) from and the three genes hypothesised to control style length, pollen incompatibility type, and anther position. This type of incompatibility is believed to be always of the sporophytictype. Pin and thrum flowers occurs in flowers such as *Primula, Forsythia, Oxalis,* and *Silia*.
Source: Charlesworth *et al*., 2005

Homomorphic incompatibility :

There are two kinds of homomorphic incompatibility – gametophytic and sporophytic (Fig. 2) depending on the genetic control of the pollen behavior during SI interactions. In many cases, SI is controlled by a single multi-allelic locus, the *S* locus, but complementary multiple-locus systems also exist.

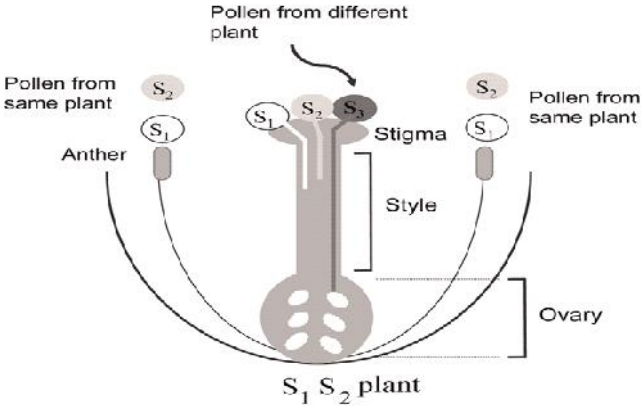

Fig.2: Homomorphic self-incompatibility (SI).Gametophytic control of pollen incompatibility types is shown; the haploid pollen grains express the allele they carry. This system is known in Solanaceae, Papaveraceae, Rosaceae, and Antirrhinum species (in an unrelated angiosperm family, Plantaginaceae). In other families, pollen specificities are controlled by the genotype of the diploid anther tissue (sporophytic system). This is known in Brassicaceae and in Ipomoea, in the family Convolvulaceae. Charlesworth *et al*. (2005).

Gametophytic self- incompatibility :

This type of incompatibility is found in clovers, grasses, sugarbeets, potatotes, and tobacco. In gametophytic incompatibility (originally called the oppositional factor system as the incompatibility allele in the style opposes the penetration of pollen tubes with the same allele), the ability of the pollen to function is determined by its own genotype and not the plant that produces it. Rate of pollen tube growth is controlled by a series of multiple alleles which are designated S_1, S_2, S_3 and so on. The pollen is rejected when the *S* haplotype of the haploid pollen matches either of the two *S* haplotypes

of the diploid pistil (Fig. 3). Unlike sporophytic systems, there is no dominance of S-alleles in style; both operate to oppose the growth of respective pollen tube. The co-dominance in style prevents any self-pollination and leads invariably to heterozygote progeny.

Genetics of gametophytic self- incompatibility :

Gametophytic self- incompatibility is controlled by a single polyallelic S locus. The genetics of incompatibility system based on the one locus system found in tobacco and clovers has been described already above (Fig.3). The number of incompatibility alleles within species may be rather large so that cross-pollinations occur freely. More than 100 S alleles in *Trifolium pretense*, 41 in red clover, and at least 64 alleles in white clover have been estimated in these populations. More complex systems are also known e.g. two polyallelic loci in grasses designated as S and Z (Fig. 4) ; three and four loci each in *Ranunculus ascaris*and *Beta vulgaris* respectively (Lundqvist *et al*., 1973, Igic *et al*., 2003).

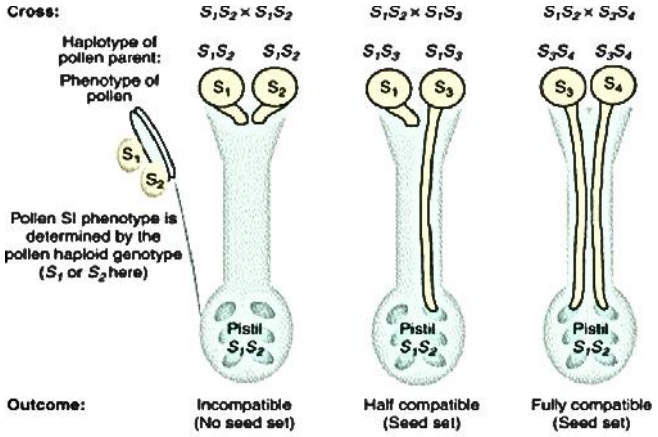

Fig. 3: Gametophytic system of self-incompatibility showing the pollen tube growth, in compatible and incompatible pollinations. In first one, pollen tubes do not grow in styles carrying similar alleles for in compatibility ($S_1S_2xS_1S_2$). In second only pollen grains with different in compatibility alleles from those in the style develop normal pollen tubes ($S_1S_3xS_1S_2$). In Third one all pollen grains carry different incompatibility alleles from those in the styles and develop normal pollen tubes ($S_1S_2xS_3S_4$).
Source: (Franklin-Tong and Franklin, 2003)

Sporophytic self- incompatibility

This system is found in sunflower, cabbage, broccoli, cacao, buckwheat, and other species. In sporophytic incompatibility, the incompatibility characteristics of the pollen are determined by the plant (sporophyte) that produces it and thus dominance interactions between *S* alleles are possible. When both pollen and stigma *S* alleles are codominant, the pollen is recognized as self and rejected if either of the two *S* haplotypes

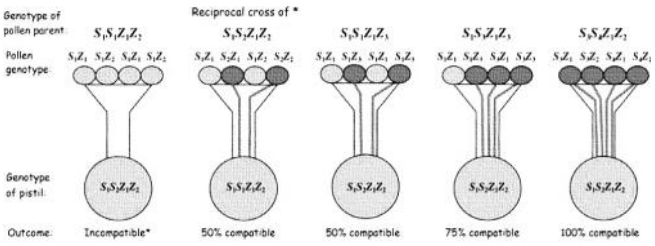

Fig. 4: Genetic control of gametophytic self-incompatibility (GSI) by two multiple-allelic loci *S* and *Z*. When both pollen *S* and *Z* alleles are matched in the pistil, in compatibility occurs, and pollen growth is inhibited. Otherwise pollen is compatible. The degree of compatibility can be 0, 50, 75 and 100% compatible, depending on the genotypes of pollen and stigma. Reciprocal crosses (marked with '*') between plants of the genotypes $S_1S_2Z_1Z_2$and $S_1S_2Z_1Z_2$ produce pollen of different proportions of compatible pollen. If a $S_1S_1Z_1Z_2$ genotype is the pollen donor and crossed with $S_1S_1Z_1Z_2$ genotype pistils, the pollen is incompatible, while, if a $S_1S_2Z_1Z_2$ genotype is the pollen donor and pollinated on $S_1S_1Z_1Z_2$ pistils, 50% of the pollen is compatible.
Source: (Franklin-Tong and Franklin, 2003).

of the pistil (Li *et al*., 2007and 2011). Incompatible pollen may be inhibited on the stigma surface. For example, a plantwith genotype S_1S_2 where S_1 is dominant to S_2, will produce pollen that will function like S_1. Furthermore, S_1 pollen will be rejected by an S_1 style but received by an S_2 style. Hence, homozygotesof *S* alleles are possible. However, interactions of *S* alleles can occur independently for pollen and stigma, leading to complicated compatibility/incompatibility patterns and differences in reciprocal compatibility.

Sporophytic system of incompatibility differs from gametophytic system in that S alleles exhibit dominance, the dominance being determined by plant producing the pollen, hinderance to pollen germination or pollen tube growth is localized in the surface of the stigma in contrast to the gametophytic system in which hindrance to pollen tube growth is in the style. Furthermore, in sporophytic system plants may be produced that are homozygous for an *S* allele either by by passing the self-incompatibility barrier or through pseudo-self- incompatibility. This feature has been utilized in the production of hybrids in self-incompatible species.

Incompatibility is expressed in one of three generalways, depending on the species. The germination of thepollen may be decreased (e.g., in broccoli). Sometimes, removing the stigma allows normal pollen germination. In the second way, pollen germination is normal, but pollen tube growth is inhibited in the style (e.g., tobacco). In the third scenario, the incompatibility reaction occurs after fertilization (e.g., in *Gesteria*). This third mechanism is rare.

Self incompatibility pathways :

It is expected that the amino acid stretches between the

cysteine residues, varying in length and composition between SCR alleles to form loops at the surface of folded protein. By imparting extensive structural diversity on the small SCR polypeptide molecules, such loops could mediate specificity in the SI recognition reaction.

Having established that the SCR gene determines pollen SI specificity, it is suggested that the SCR gene product represents the pollen-borne ligand pastulated to activate the stigmatic SRK recepter. The small hydrophilic polypeptide predicted by the SCR sequence is expected to localiza to the pollen coat after its secretion from developing microspores (similar to the secretion of other gametophytically expressed components of the pollen coat (Doughty *et al*., 1998) and also possibly from cells of tapetum. In either case, SCR molecules would mix readily within the anther locule and consequently, the pollen coat of all pollen grains in an S-locus heterozygote would incoporate SCR protein encoded by each of the two parental S-haplotypes, as predicted by sporophytic control of SI in *Brassica* (SI in sporophic system, the pollen phenotype in S locus heterozygotes is determined by the two S alleles carried by the diploid parent plant and not by the single S allele carried by the haploid pollen grain). SCR would translocate into the cell walls of the stigma epidermal cell through the pollen coat-stigma contact zone.

A stigma cell is capable of discriminating between two pollen grains, one self and one cross, placed touching each other on its surface. The cross grain will germinate and penetrate the sytle tissues within 40 minutes, whereas the self pollen barely begins to germinate (Dickinson, 1995). Self pollen rejection requires protein synthesis in the stigma (Sarker *et al.*, 1988), and can be overcome by creation of high humidity, which stimulates rapid pollen germination. Analysis of self-incompatible mutants suggests that the self pollen rejection mechanism may involve the participation of a specific aquaporin (water transport channel) (Ikeda *et al.*, 1997).

In their study by Stone *et al.* (1999) investigated the stigma protein that interact with kinase domain of SRK of three candidate protein one, ARCI (a protein that binds to the SRK kinase domain) became phosphorylated on the binding SRK (Gu *et al*., 1998). Encouragingly ARCI is expressed in the stigma, but until now evidence for its involvement in SI has been circumstantial. These investigators report the use of antisense oligonurcleotides to block expression of ARCI in *Braassica*. Strikingly, both pollination and seed set studies showed that the SI system broke down in plants that did not express ARCI, confirming ARCI as a key component of the self-pollen rejection response. Of coure how the activation of ARCI is linked to the interruption of stigma's water supply to the pollen grains remains to be determined and SI mechanisms occurs in some of species with dry stigma also.

Molecular basis of self-incompatibility :

Since the beginning of the 1980s, the rapid expansion of new techniques in molecular biology and protein chemistry, and their use to study SI systems, has allowed significant advances in our knowledge of which molecules are involved in male–female recognition in flowering plants. Four of the families that display gametophytic self incompatibility (GSI), Solanaceae, Rosaceae, Scrophulariaceae and Papaveraceae and one of the families that exhibits soporophytic self incompatibility (SSI), Brassicaceae, have been extensively studied at the molecular level. These families often contain plant species of substantial interest for horticulture or agriculture, and thus, have been the object of intense classical genetic work in the past. For all these five families, SI is controlled by a single polymorphic S-locus. During the past two decades, much progress has been made in identifying and characterizing the S-locus genes that control the specificity of the SI interaction in the five families mentioned above. Comparisons of the S-locus genes expressed in the pistil among the different families have revealed three biochemically distinct mechanisms. The Solanaceae, Rosaceae and Scrophulariaceae use the same mechanism, the Papaveraceae uses another, and the Brassicaceae uses a third. For the Solanaceae and Papaveraceae mechanisms, the gene that controls female specificity has been identified; these genes were named the S-RNase gene and the S-gene, respectively. The Solanaceae mechanism involves S-RNase–mediated degradation of RNA in self-pollen tubes. The Papaveraceae mechanism is mediated by a signal transduction cascade in pollen that involves a number of known components of signal transduction (e.g., Ca^{2+}, phosphoinositides, protein kinases, and phosphatases). For the SSI mechanism found in the Brassicaceae, both the gene that controls male specificity, S-locus cysteine-rich protein (SCR)/S-locus protein-11 (SP11), and the gene that controls female specificity, S-locus receptor kinase (SRK), have been identified. The SI response is mediated via a signal transduction cascade in the stigmatic papilla, which is elicited by the interaction of a pollen-borne ligand, SCR/SP11, and SRK, a receptor kinase in the stigmatic papilla (Table 1). Gametophytic self-incompatibility (GSI) is employed by a number of families, however, only a few have been studied at molecular level. The most extensively studied families are the Solanaceae, Rosaceae, Scrophulariaceae and Papaveraceae. In most families, GSI is controlled by a single locus (S-locus, termed after the word "sterility") with multiple alleles. However, there are more complex systems involving several gene loci, for example, some grass species have two loci (Lundqvist, 1956) or *Beta vulgaris* has four loci (Larsen, 1977). Sporophytic self-incompatibility (SSI) is not as widespread as GSI and it is largely studied in the Brassicaceae and Asteraceae. Various mechanisms providing an insight to molecular basis of self-incompatibility are briefly described below.

Table 1: Scheme of the *S*-locus and a list of the identified female and male determinant genes

Plant family	Type of SI	Genetic locus	Female determinant	Male determinant	Mechanism	References
Solanaceae, Rosaceae, Scrophulariaceae	GSI	S-locus	RNase	SLF/SFB?	RNase–mediated degradation of pollen tube RNA	Vieira *et al*. (2007)
Papaveraceae	GSI	S-locus	S-gene	Unknown S-	Protein–mediated signaling cascade in pollen	Franklin-Tong (2007)
Brassicaceae	SSI	S-locus	SRK	SCR/SP11	Receptor-kinase–mediated signaling in stigma	Sherman-Broyles and Nasrallah (2008)

Table 1: A scheme of the *S*-locus and a list of the identified female and male determinant genes. The *S*-locus contains at least two genes, one encoding the male determinant that is carried by the pollen grain, and the other encoding the female determinant that is expressed in the pistil. Both the male and female determinants are polymorphic and inherited as one segregating unit. The variants of this gene complex are called *S*-haplotypes. The recognition of self/non self operates at the level of the protein-protein interactions between the two determinants and an incompatible response occurs when both determinants are issued from the same *S*-haplotype. Thus far, both determinants have been identified in the Brassicaceae and Solanaceae.

Model for the S-RNase-dependent mechanism of gametophytic self-incompatibility (GSI) :

In gametophytic SI, the phenotype of the pollen is determined by its own haploid genotype. In the Rosaceae family, GSI is controlled by the single, polymorphic S-locus. Fertilization is prevented when the S-allele expressed by the haploid pollen grain matches one of the S-alleles expressed in the pistil. Pollen grains from the S_1S_2 anther are incompatible with the S_1S_2 pistil. If two different cultivars have identical S-genotypes, it presents an incompatible combination in each direction (Kozma *et al.*, 2003; Nyéki and Szabó, 1995). These cultivars are mutually self-incompatible, in other terms cross- or inter-incompatible. First report about RNases in plant style described that their activity varied greatly among species (Schrauwen and Linskens, 1972). A discovery, which gave further background for the hypothesis that RNases may have more diverse functions in plants was made in 1989 by Clarke's and team at the University of Melbourne (McClure *et al*., 1989). They were the first who clarified that the basic S-glycoproteins associated with gametophytic self incompatibility (GSI) in *Nicotiana alata* possessed inherent ribonuclease activity. The stylar S-glycoproteins can penetrate the pollen tube and degrade RNA in the cytoplasm. This would interfere with protein synthesis; however, no *in vivo* evidence had been known to support this hypothesis. Half a year later, the same laboratory was able to present the adequate proof (McClure *et al*., 1990): it was revealed by the use of 32P isotope that ribosomal RNAs from styles of compatible crosses are intact, but degraded in incompatible crosses. The Solanaceae, Rosaceae, and Scrophulariaceae families all share a female *S*-determinant, an S-RNase and an F-box protein, suggesting the involvement of RNA and protein degradation in the system (Kao and Tsukamoto, 2004; Franklin -Tong and Franklin, 2003). The S-RNase was first identified in the Solanaceae and thus, referred as S-RNase-mediated type of SI as Solanaceae type SI. The Solanaceae-type SI is under gametophytic control (GSI) and the rejection ofself-pollen occurs during pollen tube growth in the style. S-RNase is the sole female factor determining the *S*-haplotype specificity ofthe pistil. The conclusive evidence that SLF/SFB (*S*-haplotype-specific F-box) that encodes the pollen *S*-determinant was finally obtained from transformation experiments in *Petunia inflata* (Sijacic *et al*., 2004). One plausible model that represents the mechanism of this type is the "inhibitor model," in which the pollen *S*-determinant was postulated to be an inhibitor that could inhibit all S-RNases with the exception of the cognate S-RNase (Kao and McCubbin, 1996). Thus, once in the pollen tube cytosol, S-RNases sharing no S-allele specificity with the pollen S-locus F-box (SLF) protein will interact with a general pollen RNase inhibitor that inactivates the S-RNases. By contrast, if the S-RNase and SLF share the same S-allele specificity (here S_1), the general pollen RNase inhibitor will not be able to inactivate the self S_1-RNase.The Mechanisms of *S*-haplotype-Specific Pollen Inhibition is presented diagrammatically (Fig. 5). Several cDNA cloning, sequencing or genomic PCR based experiments were carried out in case of several S-alleles from more and more rosaceous species, including Japanese pear (Sassa and Hirano, 1997), apple (Kitahara and Matsumoto, 2002), almond (López *et al*., 2004), sweet cherry (Sonneveld *et al*., 2003), sour cherry (Hauck *et al*., 2002) and apricot (Halász *et al*., 2005). The increasing abundance of data confirmed the rosaceous S-RNase gene structure being completely identical (Kubo *et al*., 2010).

However, this has also several unclear details and further biochemical investigations are required to shed light on the mechanism of S-RNase inactivation and the precise role of SFB in protecting self S-RNases from degradation.

Model for the S-glycoprotein-dependent mechanism of gametophytic self-incompatibility (GSI) system in *Papaver rhoeas* :

SI in the field poppy, *Papaver rhoeas*, is also under gametophytic control (GSI) in that the *S* phenotype of pollen

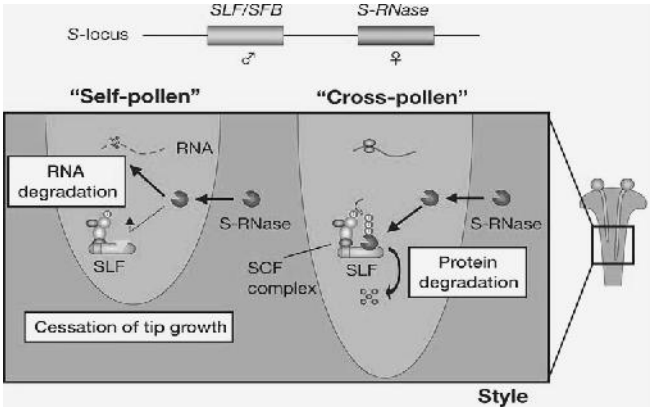

Fig.5: Molecular model of the self-incompatibility response in the Solanaceae, Rosaceae, and Scrophulariaceae.The *S*-locus consists of two genes, *S-RNase*and *SLF/SFB*. S-RNase is the female determinant and is secreted in large amounts into the extracellular matrix of the style. In a pollinated style, S-RNase is incorporated into the pollen tubes and functions as a cytotoxin that degrades pollen RNA. Although the S-RNase enters the pollen tubes regardless of their *S*-haplotypes, RNA degradation occurs only in self-pollen tubes. SLF/SFB is the male determinant and is a member of the F-box family of proteins, which generally function as a component of an E3-ubiquitin ligase complex. Thus, SLF/SFB is expected to be involved in ubiquitin-mediated protein degradation of nonself-S-RNases. (Source: Kubo *et al.*, 2010)

is determined by its haploid *S*-genotype. However, the identified S-protein (female determinant) and the mechanisms involved in pollen inhibition differ dramaticallyfrom those in the Solanaceae. In the Papaveraceae, the only identified female determinant induces a Ca^{2+}-dependent signaling network that ultimately results in the death of incompatible pollen (Lane and Lawrence, 1993). Self–incompatibility (SI) involves the recognition and rejection of self– or incompatible pollen by the pistil. In *Papaver rhoeas*, SI uses a Ca^{2+}–based signalling cascade triggered by the *S*–protein, which is encoded by the stigmatic component of the *S*–locus. This results in the rapid inhibition of incompatible pollen tube growth (Franklin- Tong *et al*., 2002). The possible mechanism is represented diagrammatically below (Fig. 6). The Papaveraceae self-incompatibility system also involves signaling pathways which are, however, quite different from the *Brassica* system. A small ligand-like S protein has been found to be secreted by stigmatic cells at the top of the pistil. The stigmatic S protein is thought to bind to an unidentified pollen S receptor to initiate signalling inside the pollen. Several signalling events have been observed including a rapid increase in Ca^{2+} levels, protein phosphorylation, and depolymerization of the actin cytoskeleton resulting in growth arrest of the self-pollen (Franklin-Tong and Franklin, 2003). Recently, programmed cell death has also been identified as the definitive contributor in this rejection response. Key features of programmed cell death including nuclear DNA fragmentation, leakage of cytochrome c from the mitochondria, and cleavage of poly (ADP-ribose) polymerase were all observed during self-pollination in *Papaver* (Bosch and Franklin-Tong 2008). The result of programmed cell death is an irreversible rejection of the self-incompatible pollen (Thomas and Franklin-Tong, 2004).

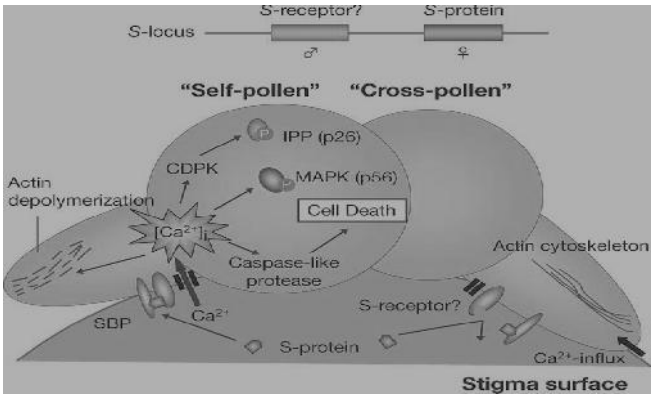

Fig. 6: Molecular model of the self-incompatibility response in the *Papaveraceae*. Only the female determinant gene has been identified, which encodes a secreted stigma protein named S-protein. S-protein interacts with the assumed *S*-haplotype-specific pollen receptor (the putative male determinant) and induces Ca^{2+} influx in the shank of the pollen tube. SBP is an integral proteoglycan of the pollen plasma membranes and is expected to function as an accessory receptor. Ca^{2+}-influx stimulates increases in $[Ca^{2+}]_i$, with some contribution from the intracellular stores as well as from extracellular sources. These increases in $[Ca^{2+}]_i$ trigger the downstream signaling cascades that result in rapid growth inhibition and ultimately the death of incompatible pollen tubes.
(Source : Paape *et al.*, 2011)

The recent identification of the pollen S protein as the S locus F-box (SLF) protein fits nicely with this model (Sijacic *et al*., 2004, Bosch and Franklin-Tong, 2008). F boxes are members of the larger protein complexes, the SCF complexes, which are also involved in targeting proteins for degradation by the proteasome. One can speculate that SLF may fit into the inhibitor model by mediating the degradation of all non-self S RNases, and therefore allow the continued growth of compatible pollen tubes. Following a self-incompatible pollination, an allelic match between SLF and S RNase would somehow prevent the degradation of S RNase (Kubo *et al*., 2010), and pollen tube growth would be arrested by the degradation of the pollen RNA. *Papaver rhoeas* possesses a gametophytic self-incompatibility (SI) system not homologous to any other SI mechanism characterized at the molecular level. Four previously published full length stigmatic *S*-alleles from the genus *Papaver* exhibited remarkable sequence divergence, but these studies failed to amplify additional *S*-alleles despite crossing evidence for more than 60 *S*-alleles in *Papaver rhoeas* alone (Paape *et al*., 2011).

Model for the SRK-dependent mechanism of sporophytic self-incompatibility (SSI) in Brassicaceae :

The various naturally occurring, classically defined S-alleles that have been described in Brassica have been arranged in a dominance series based on their genetic behaviour relative to other alleles in heterozygous plants (Thomson and Taylor, 1966). A classical genetic analysis has grouped the *Brassica* S alleles into two categories based on their phenotypic effect on self-incompatibility characteristics. The first group of alleles (high-activity) is placed relatively high on the dominance scale and exhibit a strong self-incompatible phenotype in which an average of 0 to 10 pollen tubes develop per self-pollinated stigma. The second group of alleles (low-activity) demonstrates a weak or leaky self-incompatible phenotypic effect in which 10 to 30 pollen tubes develop per self-pollinated stigma and they are considered to be recessive (Nasrallah *et al*., 1991). Molecular analysis of the S-locus region shows that this locus is a complex locus spanning many kilobases and containing several physically linked transcriptional units that co-segregate perfectly with SI phenotype (Boyes *et al*., 1997, Casselman *et al*., 2000; Hiscock and McInnis, 2003). A subset of genes within the S-locus complex ("S haplotype") is highly polymorphic as expected for genes involved in recognition, and specific combinations of allelic forms of each of these genes are thought to define different SI specificities. Thus, the S-locus may be viewed as a master recognition locus that encodes the function(s) required for the stigma to distinguish self related from self-unrelated pollen.

The SI in the Brassicaceae belongs to SSI and, so far, is the only SSI system in which the mechanism has been characterizedat the molecular level (Kachroo *et al*., 2002; Hiscock and McInnis, 2003). *Arabidopsis halleri* and *Alternaria lyrata* are two Brassicaceae species with functional sporophytic SI (Schierup *et al*., 2001; Llaurens *et al*., 2008) that diverged approximately 2 Ma (Koch and Matschinger, 2007; Castric and Vekemans, 2007). Two genes have been identified as essential for determination of incompatibility mating types in the Brassicaceae: S-receptor kinase SRK (Stein *et al*., 1991) and S locus protein 11/S locus cystein rich protein (SP11/SCR, hereafter referred to as SCR (Schopfer *et al*., 1999; Suzuki *et al*., 1999). The female determinant, SRK, is expressed in the stigma as a trans membrane receptor kinase, which recognizes the gene product of SCR (the male determinant) located on the pollen surface. Recognition of self-SCR by SRK receptors leads to haplotype-specific rejection of self-pollen (Kachroo *et al*., 2001, Sherman-Broyles and Nasrallah 2008). The SRK and SCR genes are typically inherited as a single unit, as recombination between the two determinant genes would disrupt the SI response (Uyenoyama and Newbigin, 2000). The possible mechanism is represented diagrammatically (Fig. 7). Despite recent progress in our understanding of the molecular basis of flower development and plant SI systems, the molecular mechanisms underlying heteromorphic SI remain unresolved. By examining differentially expressed genes from the styles of the two floral morphs, Yasui *et al.* (2012) recently identified a gene that is expressed only in short-styled plants. The novel gene identified was completely linked to the *S*-locus in a linkage analysis of 1,373 plants and had homology to *EARLY FLOWERING 3*. They named this gene as *S-LOCUS EARLY FLOWERING 3* (*S-ELF3*).

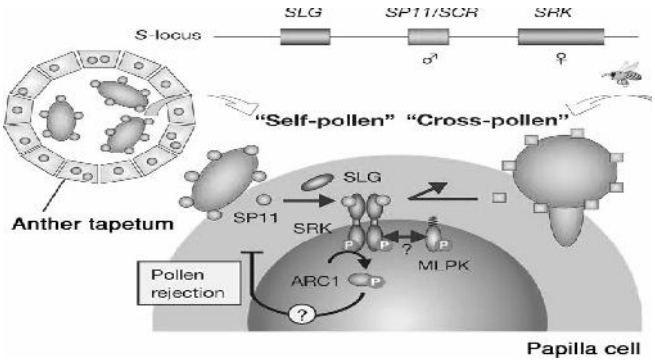

Fig.7: Molecular model of the self-incompatibility (SI) response in the *Brassicaceae*, The *S*-locus consists of three genes, *SRK*, *SP11*, and *SLG*. The SRK receptor kinase is the female determinant and spans the plasma membrane of the stigma papilla cell. SP11 is the male determinant and is predominantly expressed in the anther tapetum and accumulates in the pollen coat during pollen maturation. Upon pollination, SP11 penetrates the papilla cell wall and binds SRK in an *S*-haplotype-specific manner. This binding induces the autophosphorylation of SRK, triggering a signaling cascade that results in the rejection of self-pollen. SLG is not essential for the self-/nonself-recognition but localizes in the papilla cell wall and enhances the SI reaction in some *S*-haplotypes. The signaling cascade downstream of SRK has not yet been characterized, but the essential positive effectors include MLPK and ARC1. MLPK localizes papilla cell membrane and may form a signaling complex with SRK. ARC1, an E3 ubiquitin ligase, binds to the kinase domain of SRK in a phosphorylation-dependent manner and may target unknown substrates for ubiquitination. The proteasomal degradation of these substrates could result in pollen rejection.
Source: Castric and Vekemans, 2007)

Plant breeding implications of self-incompatibility :

Infertility of any kind hinders plant breeding. However, this handicap may be used as a tool to facilitate breeding by certain methods. Self-incompatibility may be temporarily overcome by techniques or strategies such as the removal of the stigma surface (or application ofelectric shock), early pollination (before inhibitory proteins form), or lowering the temperature (to slow down the development of the inhibitory substance). Self-incompatibility promotes heterozygosity. Consequently, selfing self-incompatible plants can create

significant variability from which a breeder can select superior recombinants. Self-incompatibility may be used in plant breeding (for F_1 hybrids, synthetics, triploids), but first homozygous lines must be developed. Self-incompatibility systems for hybrid seed production have been established for certain crops (e.g., cabbage, kale) that exhibit sporophytic incompatibility (Fig. 8). Inbred lines (compatible inbreds) are used as parents. These systems are generally used to manage pollinations for commercial production of hybrid seed. Gametophytic incompatibility occurs in vegetatively propagated species. The clones to be hybridized are planted in adjacent rows.

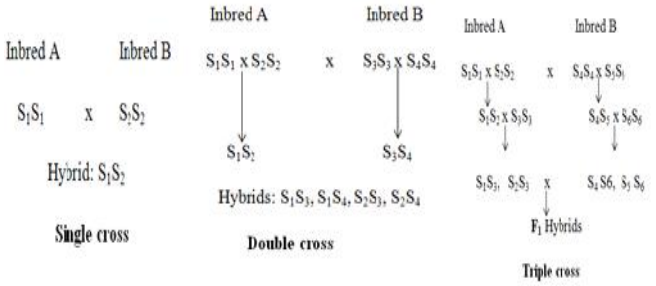

Fig. 8 : Application of self-incompatibility in practical plant breeding. Sporophytic incompatibility is widely used in breeding of cabbage and other Brassica species. The single-cross hybrids are more uniform and easier to produce. The top cross is commonly used. A single self incompatible parent is used as female, and is open pollinated by a desirable cultivar as the pollen (source. Castric and Vekemans, 2007)

Breakdown of self incompatibility

There are a number of factors and environmental circumstances which in most species can prevent incompatibility reaction to occur or enable the incompatible pollen tube, to escape the pistil barrier and accomplish self-pollination. Such factors are of importance for breeder engaged in the production of hybrid based on self-incompatibility. The effects these factors can induce may perhaps correspond to: (i) inhibition of S-gene action, (ii) inactivation of S-gene products and (iii) transmission of growth stimulus enabling the incompatible pollen tube to effect fertilization before floral abscission. Although a number of factors/environmental conditions result in the breakdown of SI (de Nettancourt, 1977). These may be bud pollination, high or low temperature, use of stored pollen, end of season effects, high relative humidity, irradiation of styles, hormone treatment of pollen or pistils, increased CO_2 content, application of NaCl, double pollination, use of pollen mixtures, treatment of stigma with ether soluble pollen coat material, very early and late flowers pollination, mechanical methods such as steel brush or cotton pad rub stigma and then pollination, electrically aided and thermally aided pollination, clipping of the style and then pollination fertilization *in vitro*. The effectiveness of these methods depend on the nature of S alleles, genetic background, the age and vigour of plant and flower, type of crop and the incompatibility system. Mutagens (agents of mutation) such as X-rays, radioactivesources such as P_{32} and certain chemicals have been used to make a self-infertile genotype self-fertile. Such a change is easier to achieve in gametophytic systems than sporophytic systems. Furthermore, the effect of incompatibility alleles in gametophytic incompatibility is not so great as to prohibit self –fertilization entirely; for most species an occasional seed may set from pollen carrying the same allele that is present in the stylar tissue. This condition is referred to as 'pseudo-self-compatibility'. In addition, 'self-fertility alleles (*Sf*)' may be present, which render the alleles for incompatibility ineffective. The *Sf* allele is a part of the S allele series and may arise by mutation from an S allele. Sometimes, incompatible diploid species become self-compatible with induction of polyploidy, yet some polyploidy species, like white clover, possess alleles for self-incompatibility.

Conclusion and future outlook :

Despite a number of setbacks and encounters with unexpected complexities a picture is starting to emerge of how rejection of self-pollen is mediated at the molecular level, at least on the female side. However, several questions remain to be answered, the most important being the nature of the male component of the SI response. On pollen- stigma interactions, several groups described their ongoing efforts to identify the male component using approaches ranging from differential screening, to mutagenic approaches and chromosome walking at the S locus, to biochemical approaches and bioassays. One important aspect of the SI response, about which very little is known, is the mechanism of self-pollen rejection following recognition by the stigma. Some recent advances in this area have been described above. Many other questions will become easier to address as we learn more about how SI works at the molecular level. An obvious example is the molecular basis of dominance between S haplotypes. The evidence that SRK molecules are able to associate with each other in an oligomeric complex suggests a possible mechanism for dominance in stigmas. Current research aimed at understanding the molecular mechanism of SI builds on genetic and physiological studies that have been carried out over the past few decades. Present review provided an exciting forum to discuss the recent developments in this area. Further analysis of the recognition reaction of Sl will be conducted through a combination of a broad spectrum of approaches including genetics, genomics, molecular biology, biochemistry, and biophysics. Though a large amount of information is now known about each of the different self-incompatibility systems, many pieces of the puzzles are still missing. All three known molecular mechanisms which plants have adopted to prevent inbreeding differ greatly with the only commonality between the S RNase and *Brassica* SRK systems being the employment of

ubiquitin-mediated protein degradation. Recent findings have furthered our understanding of these systems, but it will be exciting to follow how these stories continue to unfold, and to see what new systems will be uncovered as other self-incompatible plant families are studied.

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