Current Agriculture Trends: e-Newsletter

A Review on Self Incompatibility and Genetic & Molecular Mechanisms Involved.

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INTRODUCTION

What is self-incompatibility?

Genetic mechanisms that prevent self-fertilization are widespread throughout the flowering plants. In many plant families, male and female parts are presented simultaneously, and a genetic self-incompatibility (SI) mechanism ensures that pollen landing on a stigma of the plant that produced it will not be able to sire a seed (Stone 2002). SI mechanisms prevent self-fertilization in flowering plants based on specific discrimination between self- and non-self pollen. Based on flower morphology Lewis (1954) has classify SI into or hetero-morphic. In heteromorphic system, flowers of different incompatibility groups are different in morphology. For example, phenomenon of distyly in primula shows two types of flowers, pin (long styles and short stamens) and thrum (short styles and long stamens). A condition tristyly is known in some plant species, e.g. Lythrum; where, the style of a flower may be either short, long or of medium length. In distyly, the only compatible mating is between pin and thrum flowers. This characteristic is governed by a single gene s; Ss produces thrum, while ss produces pin flowers. The incompatibility reaction of pollen is determined by the genotype of the plant producing them. Allele S is dominant over s. The incompatibility system, therefore, is heteromorphic sporophytic. The pollen grains produced by pin flowers; would all be s in genotype as well as incompatibility reaction. The pollen produced in thrum flowers would be of two types genotypically, S and s, but all of them would be S phenotypically. The mating between pin and thrum plants would produce Ss and ss progeny in equal frequencies.

SI is caused primarily by a reaction between haploid pollen grains or pollen tubes and diploid stigmas or styles, and can be classified as either gametophytic where SI reaction is controlled by the genotype of pollen grain or sporophytic in which SI reaction is determined by the genotype of its diploid parent.

East and Mangelsdorf (1925) first described gametophytic incompatibility in *Nicotiana sanderae.* Generally, incompatibility reaction is determined by a single gene having multiple alleles, e.g., Trifolium, Nicotiana, Lycopersicon, Solanum, etc. Sporophytic system was first reported by Hughes and Babcock in 1950 in *Crepis foetida*, and by Gerstel in *Parthenium argentatum* (1950). In this system, incompatibility reaction of pollen is governed by the genotype of the plant on which the pollen is produced, is governed by a single gene, S, with multiple alleles (e.g.; > 30 alleles in *Brassica oleracea*). Lewis has summarized the following characteristics of this system. 1. There are frequent reciprocal differences. 2. Incompatibility can occur with the female parent 3. A family can consist of three incompatibility groups. 4. Homozygotes are a normal part of the system.

Relevance in Plant Breeding:

SI is important for maintaining genetic diversity, but it is often an obstacle to establishing new pure lines (Matsui and Yasui 2020). It promotes outbreeding and reduces the likelihood of adverse effects caused by homozygosity of recessive alleles at multiple loci (Goldberg et al. 2010). Genetic and molecular basis of SI provides new opportunities to exploit this trait for crop improvement by breeding and biotechnology-based approaches. A better understanding of the genetic control of S loci offers new insight into SI systems, their evolutionary origins and their reproductive significance (Cropano et al. 2021). Lines developed from SI are suitable for hybrid breeding of cauliflower and appears to be more effective than CMS in seed production (Kucera, 2006). In recent years, highly diversified group such as brassica are being grown as F_1 hybrids varieties. However, the progress is slow owing to instability and complex inheritance of the SI. Self-incompatibility has a profound effect on breeding approaches and objectives. In self-incompatible fruit trees, it is necessary to plant two cross-compatible varieties to ensure fruitfulness. Self-incompatibility may be used in hybrid seed production. For this purpose, (1) two self-incompatible, but cross-compatible, lines are interplanted; seed obtained from both the lines would be hybrid seed. (2) Alternatively, a self-incompatible line may be interplanted with a self-compatible line. From this scheme, seed from only the self-incompatible line would be hybrid. (3) Schemes for the production of double cross and triple cross hybrids have also been proposed and their feasibility has been demonstrated in the case of brassicas. Therefore, keeping in view the importance of self incompatibility in plant breeding it is necessary to reveal its mechanism at genetic, biochemical and molecular levels for better understanding.

Genetic mechanism involved in self incompatibility

In **homomorphic self incompatibility** a single locus S having multiple S alleles controls the incompatibility reaction. Generally, the number of S-alleles in the gametophytic system is more than the sporophytic system (Thakur and Dhiman 2021). In gametophytic system S alleles show co-dominance inheritance and results in incompatible, half-compatible or fully compatible reactions. If same allele as that of pollen is present in the stylar tissues, it opposes the growth of pollen tube in the style, due to which it is termed as 'oppositional factor system'. In a single gene system, there are three types of mating; **1**). Fully incompatible, e.g., S1S2 × S1S2) **2**). Fully compatible, e.g., S1S2 × S3S4 3). Partially (i.e., 50% of the pollen) compatible, e.g., S1S2 × S2S3 (fig 1)
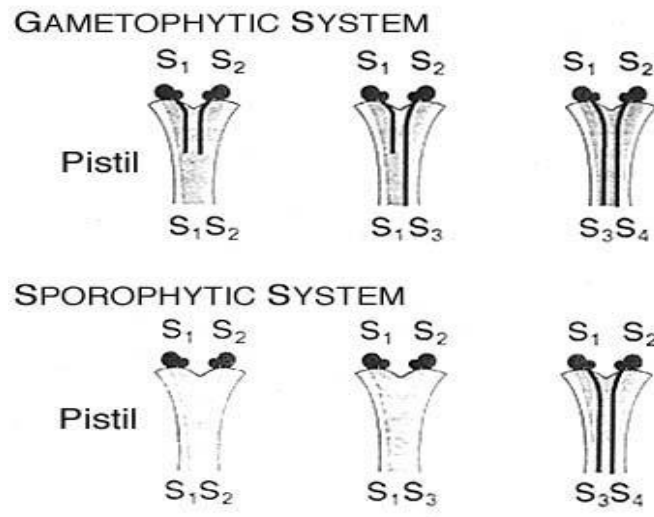
Figure1: Homomorphic system of self incompatibility

In gametophytic system, the wet stigma surface is plumose having elongated receptive cells. Incompatible pollen grains generally germinate on reaching the stigma. The incompatibility reaction occurs at a later stage (pollen-stylar region) without the stigma. In the sporophytic system, the S alleles may exhibit dominance, individual action (codominance) or competition. Consequently, there may be many complex incompatibility relationships. In this system, pollen grain has an outer lipidic coating superficial layer (CSL), which is thin layer deposited by tapetum on the pollen grain just prior to dehydration. Below the coating superficial layer is the main outer coating of pollen called tryphine, which contain factors that give rise to the maternal control of pollen reaction. The stigma in sporophytic system is papillate covered with a pellicle or sheath which is proteinaceous in nature. When compatible pollen lands on a stigma, pollen CSL fuses with the papillae. The pollen hydrates and germinates on papillae, the pollen release a cutinase, which digest the cuticle and allows the pollen tube to grow inside the cellular pectin layer of papillae.

Heteromorphic self incompatibility: Buckwheat is a distylous having long-styled flowers with short stamens (pin flowers) and short-styled flowers with long stamens (thrum flowers). Pollen grains of thrum are larger than those of pin plants (Matsui and Yasui 2020). Both SI and flower morphology in buckwheat are controlled by a single locus (S locus); thrum plants are heterozygous (Ss), whereas pin plants are homozygous (ss), and the SS genotype does not exist (Garber and Quisenberry 1927, Lewis and Jones 1992). In thrum × thrum plants, thrum pollen tube growth is inhibited in the upper part of the style with hyper-trophy at the tips of pollen tubes, whereas in pin × pin plants, pin pollen tube growth is inhibited in the middle part of the style with no hypertrophy (Hirose et al. 1995). Similar studies has been conducted in primula and observed that the S locus in Primula contains five genes (S supergene complex locus) for the different traits viz., the style length gene (G for short style, g for long style), style incompatibility gene (I S for style incompatibility of short style, i s for style incompatibility of long style), pollen incompatibility gene (I P for pollen incompatibility of short anther, i p for pollen incompatibility of long anther), pollen size gene (P for large pollen grain, p for small pollen grain) and anther height gene (A for long anther, and a for short anther). The S supergene complex locus consists of GIS I

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ISSN (E): 2583 – 1933

PPA gene cluster (haplotype) which would be inherited without recombination in most cases. Sharma and Boyes (1961) postulated that heteromorphic SI in common buckwheat is also controlled by the S super- gene. However, unlike in Primula, recombinations within the supergene complex have not been observed in buckwheat so far.

Table: Heteromorphic sporophytic system of incompatibility (Singh 2015)

Mating		Progeny	
Phenotype	Genotype	Genotype	Phenotype
Pin x Pin	ss x ss	Incompatible mating	
Pin x Thrum	ss x Ss	1 Ss : 1 ss	1 Thrum : 1 Pin
Thrum x Pin	Ss x ss	1 Ss : 1 ss	1 Thrum : 1 Pin
Thrum x Thrum	Ss x Ss	Incompatible mating	

Molecular mechanism involved in self incompatibility

S locus controlling SI reaction encodes two proteins male determinant and female determinant which interact in S-haplotype specific manner. The two genes involved in interaction are very tightly linked so that they are inherited as one segregating unit. These two have coevolved to give rise to large number of S allele. E.g. 30 in *Brassica campestris*, 50 in *Brassica oleracea* etc. (Singh 2015). The interaction between male and female determinant initiates various activities which hinder the growth of pollen grains, arrest the development, causes degradation of essential proteins, affect metabolic pathways, failure of pollen hydration, activation of signalling pathways, phosphorylation of cascades etc. Three homomorphic SI systems have been extensively studied at the molecular level (Iwano and Takayama 2012): (1) gametophytic SI based on the SLF (SFB)/S locus RNase system in Solanaceae, Rosaceae and Plantaginaceae, (2) gametophytic SI based on the PrpS/PrsS system in Papaveraceae, and (3) sporophytic SI based on the SP11 (SCR)/SRK system in Brassicaceae. However, considerable progress has been made to understand molecular basis of heteromorphic SI in heterostylous plant species including common buckwheat (*Fagopyrum esculentum*) (Takeshima et al. 2019).

1. Gametophytic SI in Solanaceae:


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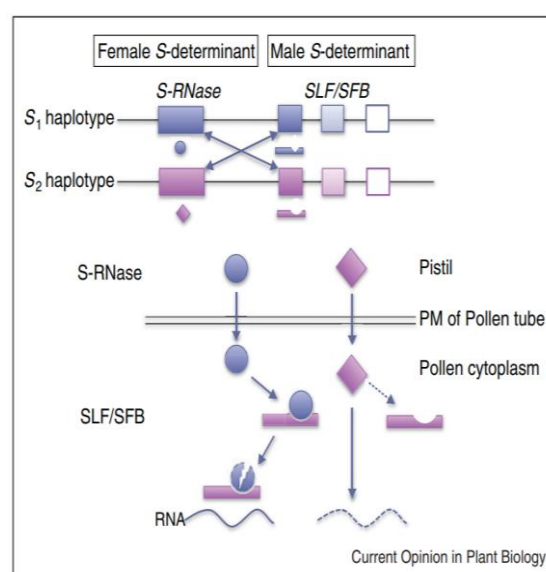
This is most wide spread and common form of SI mediated by S-RNase (S locus RNase) found in families Solanaceae, Rosaceae and Plantaginaceae etc. In this system S-RNase is female determinant and expresses exclusively in pistil and localized in extracellular matrix of upper region of style (Iwano and Takayama 2012). S-RNase are basically glycoproteins of approx. 30 KDa which has highly specific cytotoxic activity inhibiting growth of incompatible pollen tubes. S-RNase enter both compatible and incompatible pollen tubes but degrade only RNA of incompatible pollen tubes. However, the exact mechanism is still unknown. The male determinant here encodes for **SLF (S-locus F-box protein) or SFB (S-haplotype-specific F box protein)**. The proteins

functions as ubiquitin ligase which contributes in self incompatible response by proteasomal mediated degradation.

2. Gametophytic SI in Papaveraceae:

In this mechanism female determinant encode small extracellular molecule S-protein (15 KDa) which expressed in stigma. It appears that amino acid residues Asp-77, Asp-78 and Asp-79 od S proteins are critical for SI response. Male determinant is a cell membrane receptor most likely PrpS1 encoded by gene tightly to the S gene. However, a receptor called SBP (S-protein binding protein) located in pollen plasma membrane and binds S-protein in non -S-haplotype specific manner. The S-haplotype specific interaction between stigmatic S protein and its cognate receptor leads to a rapid increase in Ca^{2+} dependent signalling cascade in associated with rapid depolymerisation of microtubules and actin cytoskeleton, and phosphorylation of the inorganic pyrophosphatase PRp26.1. these events lead to inhibition of pollen tube growth and ultimately death of incompatible pollen by a process of programmed cell death.

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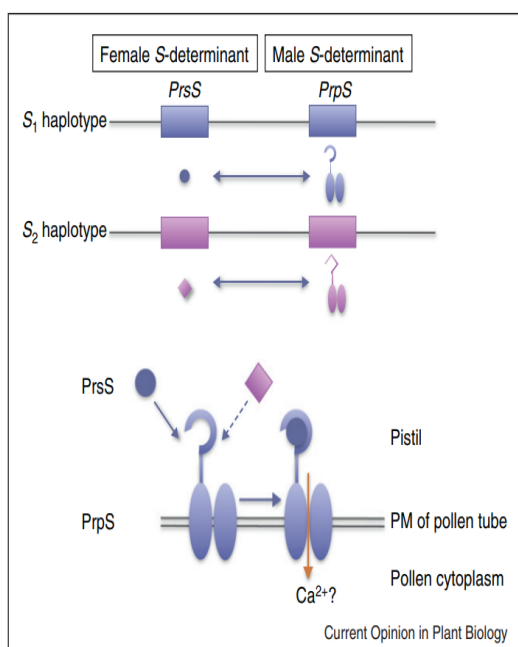
3. Sporophytic SI in Brassicaceae:

Brassicaceae is only sporophytic system of self incompatibility successfully explained at molecular level which indicates probably there

are other different mechanisms operating in different species showing sporophytic system of self incompatibility. In Cruciferae/ Brassicaceae, **SRK (S locus receptor kinase)** functions a female determinant controlling the incompatibility reaction of stigma/ style. This gene product predominantly expressed itself as **transmembrane receptor** in plasma membrane of stigma papilla cells just prior to flower opening when stigma acquire their SI reaction (Iwano and Takayama 2012). The male determinant is **SCR (S locus Cysteine Rich protein) or SP11 (S locus protein 11)** a small protein of size less than 10 KDa, predominantly expressed in anther tapetum and get accumulates in pollen coat during pollen maturation. When a pollen lands on stigma the protein SP11 present in coat crosses papilla cell walls and binds to female gene product SRK and results in autophosphorylation of the system and initiating signalling cascade. SRK phosphorylates the kinase MLPK, which positively regulate SI signalling. The protein ARC1 is phosphorylated and functions a E3 ligase which degrade Exo70A, a potential pollen compatibility factor. All these events prevent pollen hydration and pollen germination/ pollen growth at sigma surfaces thus ultimately causing self incompatibility.

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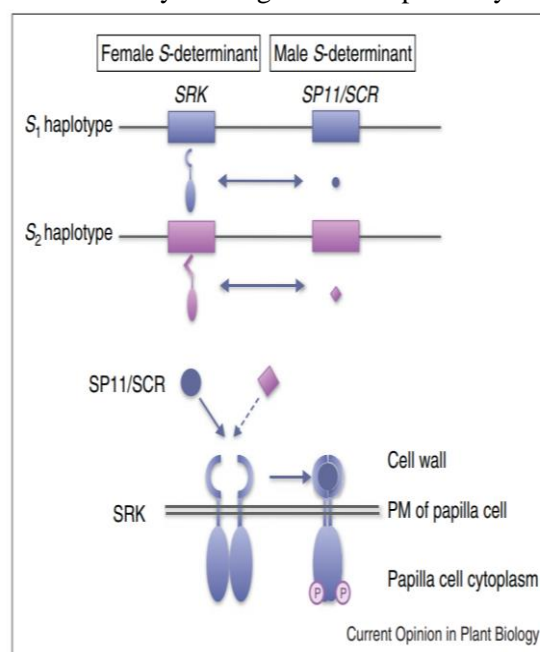
4. Heteromorphic SI in Buckwheat:

Factors related to homomorphic SI systems in Brassicaceae, Solanaceae and Papaveraceae have been identified by searching for specific proteins in different *S* haplotypes, and the finding of proteins segregating with S haplotypes has led to the isolation of S genes (Takayama and Isogai 2005). In distylous plants including buckwheat, the dominance of the S allele over the s allele suggests that a specific gene is expressed only in the flowers of thrum plants therefore, Yasui et al. (2012) isolated mRNAs separately from pistils of thrum as well as from pin plants, and examined high-throughput sequencing of expressed genes. The results indicated four specific transcripts viz., SSG1, SSG2, SSG3 and SSG4 were detected in the style of thrum plants. Simultaneously they found complete genetic linkage between the S locus and SSG2 or SSG3. However, SSG3 was found to be a homolog of Arabidopsis EARLY FLOWERING 3 (ELF3) and was termed as S-LOCUS EARLY FLOWERING 3 (S-ELF3). The gene ELF3 is a component of core circadian clock which forms complex with ELF4 and LUX ARRHYTHMO (LUX). This complex binds to the promoters of circadian clock genes viz., PHYTOCHROME-INTERACTING FACTOR 4 (PIF4), PIF5 and PSEUDO-RESPONSE REGULATOR 9 and regulates their expression in a circadian manner (Herrero et al. 2012).

CONCLUSION

Plant reproduction is essential for crop breeding & production and self-incompatibility systems are target traits for modulating reproductive behaviour as it avoids self-fertilization and promotes outcrossing. Recent advances in understanding mechanism of SI have already allowed intra- and interspecific barriers to be overcome and facilitated development of cultivars, hybrids, Inbred lines and populations etc. ensuring maintenance of crop diversity. For better understanding of SI, emerging powerful tools such as 'omics approaches', CRISPR technology, genome editing tools can probably be helpful in dissecting SI mechanisms. Moreover, future SI studies will further assist breeders addressing crop production challenges by identification and characterization of the S-alleles in the germplasm and improvements in plant genetic resources utilization.

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