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Self-Incompatibility: Nature's Breeding Barrier Unveiling the Secrets of Self-Rejection in Plants – A review

Roshin Mariam George^{1*} and M.R. Bindu²

¹Ph.D. Scholar, Department of Genetics and Plant Breeding, College of Agriculture, Vellayani, Thiruvananthapuram (Kerala), India. ²Professor and Head, Department of Genetics and Plant Breeding, Farming Systems Research Station, Sadanandapuram, Kottarakkara, Kollam (Kerala), India.

(Corresponding author: Roshin Mariam George*) (Received: 25 May 2024; Revised: 19 June 2024; Accepted: 19 July 2024; Published: 14 August 2024) (Published by Research Trend)

ABSTRACT: Self-incompatibility (SI) is a sophisticated reproductive strategy employed by numerous flowering plants to prevent self-fertilization and promote outcrossing. This mechanism, crucial for maintaining genetic diversity and adaptability, involves intricate molecular interactions between pollen and pistil components, leading to the rejection of incompatible pollen. This review delves into the evolutionary history, classification, and molecular mechanisms underlying diverse SI systems. We explore the historical journey of understanding SI, from early observations of plant breeding patterns to the current molecular-level insights. We discuss the two primary types of SI: heteromorphic, characterized by distinct floral morphologies, and homomorphic, relying solely on genetic mechanisms. The molecular intricacies of single-locus and two-locus SI systems are examined, highlighting key genes and signaling pathways involved in pollen recognition and rejection. Furthermore, we explore the implications of SI for plant breeding, including its role in hybrid seed production and the development of desirable traits. Understanding and manipulating SI systems hold immense potential for enhancing crop yield, quality, and resilience in the face of changing environmental challenges.

Keywords: Self incompatibility, GSI, SSI, Heterostyly, Molecular basis of SI.

INTRODUCTION

Self-incompatibility (SI) is a widespread mechanism in flowering plants that prevents self-fertilization, thus promoting outcrossing and its associated benefits for genetic diversity and adaptability. While selfpollination might seem advantageous, it often leads to inbreeding depression, reducing the evolutionary fitness of offspring. SI, present in over 6000 plant species, serves as an elegant solution to this problem (Charlesworth, 1985). It ensures that only pollen from genetically distinct individuals is accepted for fertilization, fostering genetic diversity within populations.

SI systems exhibit remarkable diversity, ranging from physical separation of male and female flower parts (Lloyd and Webb, 1992) to sophisticated genetic mechanisms. The two major genetic systems, gametophytic SI (GSI) and sporophytic SI (SSI), differ in how they determine pollen compatibility (de Nettancourt, 2001). GSI relies on the genetic makeup of individual pollen grains, while SSI is determined by the genotype of the entire parent plant.

Despite its importance and widespread occurrence, our understanding of the molecular mechanisms underlying SI remains limited to a handful of model plant families, such as Brassicaceae and Solanaceae (Fujii *et al.*, 2016; Goring *et al.*, 2023). These model systems have illuminated the intricate dance of pollen-stigma interactions that determine compatibility, but much remains to be discovered.

Although significant advancements have been made in comprehending gametophytic and sporophytic selfincompatibility (GSI and SSI) in model plant families, the wide range of SI systems found across angiosperms is still largely unknown (Igic et al., 2008). This highlights a critical research gap: elucidating the molecular basis of SI in other plant families, including those with less characterized systems like those found in the Poaceae family (Wang et al., 2022). Further investigation is also needed to understand the evolutionary dynamics of SI. The frequent gain and loss of SI systems across evolutionary history raises intriguing questions about the selective pressures driving these changes (Allen and Hiscock 2008; Goldberg et al., 2010; Barrett, 2013). A deeper understanding of these dynamics could shed light on SI's contribution to plant diversification. Finally, harnessing the power of SI for crop improvement holds immense potential. Deeper insights into the molecular mechanisms of SI could pave the way for engineering self-incompatible crop varieties, facilitating hybrid breeding programs and ultimately boosting agricultural yields (Zhang et al., 2024).

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A HISTORICAL OVERVIEW OF POLLINATION AND SELF-INCOMPATIBILITY

The human understanding of plant reproduction, particularly the intricacies of pollination and fertilization, has evolved significantly over millennia. Early civilizations recognized the importance of these processes for agricultural productivity. Evidence from ancient Mesopotamia, as reflected in the laws of Hammurabi in 1750 BCE, underscores the appreciation of pollination in agricultural practices (Weiss, 2015). Assyrian reliefs depicting fertilization rituals for date palms further highlight the ancient recognition of plant sexuality (Weiss, 2015).

Despite this early empirical knowledge, a formal understanding of plant reproductive structures remained elusive until the late seventeenth century. Rudolf Jakob Camerarius' groundbreaking work, "De sexu Plantarum epistole" in 1694, provided the first comprehensive description of plant sexual organs, marking a pivotal moment in the history of botany (Abrol, 2012). Building upon this foundation, Joseph Gottlieb Kölreuter made significant contributions to our understanding of plant reproduction in the eighteenth century. His experiments with Verbascum species demonstrated the phenomenon of self-sterility and the potential for creating hybrid offspring through crosspollination (East and Park 1917). Charles Darwin's seminal works in the nineteenth century further advanced our comprehension of plant reproduction. His studies on the effects of self- and cross-fertilization highlighted the importance of outcrossing for plant vigor and adaptation (McClure, 2009). Darwin's work laid the groundwork for subsequent investigations into the mechanisms underlying self-incompatibility (SI), a phenomenon that prevents self-fertilization.

The term "self-incompatibility" was formally coined by A.B. Stout in 1917, marking a significant milestone in SI research. Subsequent studies, particularly those conducted by Bateman on Brassica species, provided valuable insights into the genetic basis of this complex reproductive barrier.

The study of self-incompatibility (SI) has a rich history, evolving from initial observations of plant breeding patterns to the current molecular-level understanding. Early botanical studies in the 18th and 19th centuries laid the groundwork for our comprehension of plant reproduction. The work of Camerarius, Kölreuter, and Darwin established the fundamental concepts of plant sexuality, cross-pollination, and the significance of selfsterility (later termed self-incompatibility) (Abrol, 2012; East and Park 1917; McClure, 2009). The early 20th century witnessed significant advancements in SI research. The discovery of Mendelian inheritance patterns in self-sterility by East and Park (1917) in Reseda and Nicotiana marked a turning point. The subsequent identification of the S-locus and its multiallelic nature in Nicotiana by East and Yarnell (1929) provided crucial insights into the genetic control of SI.

Recognizing the potential of SI as a breeding tool, researchers and breeders began exploring its applications in various crop species. The John Innes Horticultural Institution, for instance, initiated studies on SI in fruit trees, laying the foundation for breeding programs aimed at developing self-compatible cultivars (Crane and Lewis, 1942; Crane and Brown, 1937; Lewis and Crowe, 1954). Similar efforts were undertaken in other crop species, including cabbage, potato, sunflower, rye, cocoa, and pummelo. Despite these early successes, the underlying molecular mechanisms of SI remained elusive until the mid-20th century. It wasn't until the 1980s that significant breakthroughs in molecular biology began to unravel the complexities of this reproductive barrier.

The enduring questions surrounding selfincompatibility (SI) include how it is maintained and how the transition from one SI type to another occurs (Barrett, 2013). Recent advancements in molecular biology, particularly phylogenetics and genome-wide analysis of S-determinants, have significantly improved our comprehension of these issues, especially concerning the evolution of two specific SI systems: S-RNase-based gametophytic SI (GSI) and Brassicaceae sporophytic SI (SSI) (Zhang *et al.*, 2024).

SI AS A REPRODUCTIVE BARRIER

Self-incompatibility functions as a critical reproductive barrier, ensuring outcrossing and promoting genetic diversity within plant populations. By preventing selffertilization, SI safeguards against inbreeding depression, a decline in fitness due to the accumulation of deleterious recessive alleles (Mudhalvan *et al.*, 2024). This mechanism is particularly important in plant species with hermaphroditic flowers, where selfpollination is a constant threat.

The incompatibility reaction is a dynamic process involving complex molecular interactions between pollen and pistil components. When compatible pollen grains land on the receptive stigma, a series of signaling events is initiated, culminating in pollen tube growth and fertilization. In contrast, incompatible pollen is recognized and rejected, preventing fertilization. This pollen rejection can occur at different stages, including pollen hydration, pollen tube growth, and pollen tube penetration of the ovules.

MODELS OF SELF-INCOMPATIBILITY

Two primary models have been proposed to explain the phenomenon of self-incompatibility in angiosperms: the oppositional and complementary models.

Oppositional models suggest that SI is a result of an antagonistic interaction between pollen and pistil components. This interaction leads to the inhibition of pollen tube growth. Constituents of the male gametophyte and pistil interact or combine to produce a substance that adversely affects the development of the male gametophyte. The majority of self-incompatibility cases align with this model.

Complementary models propose that successful fertilization requires specific complementary factors present in both pollen and pistil. The absence of these factors in self-pollination leads to incompatibility. This model is less common and has been observed in specific plant groups, such as Dendrobium orchids,

where incompatibility reactions can progress to flower abscission.

While significant progress has been made in understanding the molecular mechanisms of SI, many aspects remain to be elucidated. Future research will focus on identifying novel SI genes, deciphering the complex signaling pathways involved, and exploring the evolutionary implications of SI diversity.

CLASSIFICATION OF SELF-INCOMPATIBILITY SYSTEMS

The concept of self-incompatibility (SI) was further refined by H. Lewis in 1949, who categorized SI systems into distinct groups based on their genetic and physiological characteristics. This classification laid the foundation for subsequent research into the molecular mechanisms underlying SI.

Criteria	Types	
Flower Morphology	Heteromorphic Self	Distyly
	incompatibility	Tristyly
		Sporophytic
		Gametophytic
Loci involved	Monofactorial (Single locus - S)	
	Bifactorial (Two locus – S, Z)	
Pollen Cytology	Binucleate (pollens with two nuclei)	
	Trinucleate (pollens with three nuclei)	
Expression site	Ovarian (expression site is ovary)	
	Stylar (expression site is style)	
	Stigmatic (expression site is stigma)	

Table 1: Classification of Self incompatibility.

CYTOLOGICAL LOCALIZATION OF SELF-INCOMPATIBILITY RESPONSES

The site of pollen tube inhibition within the pistil provides valuable insights into the nature of self-incompatibility (SI) systems. Tonosaki *et al.* (2016) classified SI systems based on the location of pollen tube arrest:

Stigmatic Inhibition: The most common type, stigmatic inhibition occurs in plants with trinucleate pollen and is associated with sporophytic SI. It involves the prevention of pollen germination or early pollen tube growth on the stigma surface. Examples include cabbage, radish, sunflower, and Limonium.

Stylar Inhibition: Predominant in plants with binucleate pollen, stylar inhibition occurs when pollen tubes grow through the stigma but are arrested within the style. This is often associated with gametophytic SI and is observed in species like Bougainvillea and Fagopyrum.

Ovarian Inhibition: A less common type, ovarian inhibition occurs when pollen tubes reach the ovary but are unable to fertilize ovules. This is exemplified by cocoa and plants with hollow styles, such as Narcissus and Lilium (Arasu, 1968).

Understanding the cytological basis of SI is essential for elucidating the molecular mechanisms underlying this complex reproductive barrier.

HETEROMORPHIC SELF-INCOMPATIBILITY: A MORPHOLOGICAL BARRIER TO SELFING

Homomorphic SI is characterized by indistinguishable floral structures between individuals within a species. Determining incompatibility types in homomorphic systems necessitates laborious cross-pollination experiments. In contrast, **heteromorphic SI** involves distinct floral morphs with specific arrangements of reproductive organs. These morphological differences, often termed heterostyly, serve as visual indicators of incompatibility types (Ernst, 1955).

Heterostyly, a prime example of heteromorphic SI, is found in species with two (distvlv) or three (tristvlv) floral morphs. These morphs differ in the relative positions of styles and stamens, ensuring that pollen from one morph can only fertilize ovules of another. This morphological differentiation, coupled with genetic incompatibility systems, reinforces outcrossing. Distyly: A Morphological and Genetic System for Promoting Outcrossing. Distyly, a form of heteromorphic self-incompatibility (HSI), represents a striking example of evolutionary adaptation to promote outcrossing. In distylous species, individual plants exhibit one of two floral morphs: long-styled (pin) or short-styled (thrum). This morphological differentiation, coupled with genetic incompatibility systems, ensures that pollen from one morph can only fertilize ovules of the other, preventing self-fertilization (Shivanna et al., 1981).

Primula vulgaris has become a classic model system for studying distyly. Pin and thrum morphs differ not only in style and stamen length but also in pollen size, exine ornamentation, and stigmatic papillae structure. Genetic studies have shown that distyly in Primula is controlled by a single locus with two alleles (S and s). where the Ss genotype produces thrum flowers and the ss genotype produces pin flowers (Ernst, 1955). The discovery of self-compatible long-styled variants led to the development of the supergene model, positing that multiple tightly linked genes control the suite of traits associated with distyly (Lewis and Jones 1992). Subsequent research refined this model, suggesting the involvement of three linked loci (G, P, and A) governing gynoecium characteristics, pollen size, and anther height, respectively (Charlesworth and Charlesworth 1979).

Recent genomic studies have provided new insights into the genetic basis of distyly. In Primula vulgaris, the S-locus is absent in pin plants and hemizygous in thrum plants, highlighting the importance of gene dosage in determining floral morphology (Cocker *et al.*, 2018). Similarly, studies in Linum tenue have revealed the hemizygous nature of the S-locus in thrum plants, emphasizing the conserved genetic architecture of distyly (Gutiérrez-Valencia *et al.*, 2022). The hemizygous nature of the S-locus in distylous species has significant implications for the evolution and maintenance of this system. It suggests that the loss of an S-allele could lead to the breakdown of distyly and the emergence of self-compatible homostyle variants.

In short-styled Primula flowers, the female selfincompatibility (SI) type is determined by the expression of a specific gene, CYP734A50, which inhibits cell elongation in the style. This inhibition is caused by the inactivation of brassinosteroids (BRs)

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(Huu et al., 2022). The GLOBOSA2 (GLO2) gene may influence the position and height of the anther (Huu et al., 2020), but the gene responsible for male incompatibility types remains unknown. A recent study revealed that silencing the CYP734A50 gene in Smorph plants altered both style length and female compatibility, leading to homostyled flowers (Huu et al., 2022). The study also found that BRs play a crucial role in determining female SI, affecting both pollen germination and pistil tube (PT) growth. S- and Lmorph pollen responded differently to BR treatment. This system of female SI and style-length determination by a BR-inactivating enzyme has also been observed in Turnera. The BR-inactivating activity of BAHD, an Slocus gene, controls style length and female mating specificity (Shore et al., 2019; Matzke et al., 2020). BRs mediate these traits by differentially regulating gene expression in the style (Matzke et al., 2021).

A recent study by Gutiérrez-Valencia et al. (2024) has shed light on the evolutionary transition from distyly to homostyly in the genus Linum. Unlike the distylous Linum tenue, the homostylous Linum trigynum lacks the typical morphological and genetic distinctions associated with distyly. The research suggests that the shift towards homostyly in L. trigynum is not primarily due to the loss of function of genes within the S-locus, as previously hypothesized. Instead, it is likely caused by alterations in gene expression, particularly that of the S-linked gene LtWDR-44. These changes in gene expression have disrupted the complex genetic network underlying distyly, leading to the development of a selfcompatible, homostylous floral morphology. The transition to self-fertilization in L. trigynum has resulted in reduced genetic diversity compared to its distylous counterpart, L. tenue. This study underscores the importance of studying the genetic and molecular mechanisms underlying floral development and selfincompatibility to fully understand the evolutionary pathways leading to different reproductive strategies in flowering plants.

Understanding the genetic and molecular basis of distyly is crucial for comprehending the evolutionary forces that shape plant reproductive systems. By unraveling the complexities of this fascinating phenomenon, researchers can gain insights into the mechanisms underlying plant speciation and adaptation. Tristyly: A Complex System for Promoting Outcrossing. Tristyly represents a more intricate form of heteromorphic self-incompatibility (HSI) compared to distyly. This system involves three distinct floral morphs: long-styled (L), mid-styled (M), and shortstyled (S). Each morph exhibits specific arrangements of styles and stamens designed to prevent selffertilization and promote outcrossing. For instance, in the short-styled morph, the two sets of stamens are positioned at the mid and long levels, effectively preventing self-pollination (Franklin-Tong, 2008).

The genetic control of tristyly is complex and involves multiple loci. Two primary loci, S and M, have been identified, each with two alleles. The combination of these alleles determines the floral morph. The S locus is epistatic to the M locus, meaning that the presence of the S allele masks the effect of the M allele, resulting in the short-styled morph (*Ssmm* or *SsMm*). The midstyled morph is characterized by the presence of the dominant M allele (*ssMm*), while the long-styled morph is homozygous recessive for both loci (*ssmm*).

The concept of a supergene, a cluster of tightly linked genes controlling a complex phenotype, has been proposed to explain the inheritance of tristyly (Lewis and Jones 1992). However, direct genetic evidence for supergenes in tristylous systems is limited. While the supergene model provides a framework for understanding the coordinated inheritance of traits associated with tristyly, it is likely that a more complex genetic architecture underlies this phenomenon. Recent studies have highlighted the role of regulatory genes in modulating the expression of floral traits within tristylous species. These genes may influence pollen size, incompatibility responses, and stamen position, contributing to the overall complexity of tristyly (Weller *et al.*, 2015).

A study on Oxalis psoraleoides has uncovered deviations from the typical tristylic pattern, revealing an atypical form of heterostyly. The population exhibits variations in the proportions of long-, mid-, and shortstyled morphs, with the mid-styled morph being particularly prevalent. Furthermore, while the species exhibits self-incompatibility, there is evidence of fruit formation from both legitimate and illegitimate crosses. This suggests a mixed mating system, with both selfand cross-pollination occurring within the population. Bees and lepidopterans are primarily responsible for pollen transfer, contributing to the observed deviations from the typical tristylous pattern. These findings highlight the complex interplay between genetic factors, floral morphology, and pollinator behavior in shaping the reproductive system of Oxalis psoraleoides. The atypical heterostyly observed in this species challenges the traditional understanding of tristyly and underscores the need for further research to elucidate the evolutionary forces driving these deviations (Lima et al., 2024).

Tristyly represents a fascinating example of evolutionary adaptation, where multiple genetic and morphological factors interact to promote outcrossing and maintain genetic diversity. Understanding the genetic basis of tristyly is essential for comprehending the evolutionary forces shaping plant reproductive systems.

HOMOMORPHIC SELF-INCOMPATIBILITY: GENETIC CONTROL OF POLLEN REJECTION

Homomorphic self-incompatibility (HSI) operates within plants possessing indistinguishable floral structures, relying solely on genetic mechanisms to prevent self-fertilization. Unlike heteromorphic systems, HSI plants do not exhibit morphological differences to facilitate outcrossing. The genetic control of HSI can manifest in two primary forms: gametophytic and sporophytic. In gametophytic selfincompatibility (GSI), the pollen's ability to fertilize an ovule is determined by its own genotype (East and Mangelsdorf 1925). In contrast, sporophytic selfincompatibility (SSI) is influenced by the genotype of

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the pollen parent plant. A cornerstone of HSI is the Slocus, a genetic region containing multiple alleles that govern self-recognition. In GSI, the S-locus typically comprises a pair of linked genes encoding a female determinant and a male determinant (Muthuselvi and Praneetha 2019). Self-incompatibility arises when the S-alleles of the pollen and pistil are identical, leading to pollen rejection. The molecular basis of S-locus genes varies across plant species, reflecting the diversity of SI systems. While the S-locus has been extensively studied in several plant families, the complete understanding of its structure and function remains a complex challenge. Self-incompatibility (SI) systems can be categorized into two primary types based on the genetic basis of pollen rejection: gametophytic self-incompatibility (GSI) and sporophytic self-incompatibility (SSI).

Gametophytic Self-Incompatibility (GSI). In GSI, the ability of a pollen grain to fertilize an ovule is determined by its own haploid genotype, rather than the genotype of the pollen parent plant. The S-locus, a complex genetic region, plays a pivotal role in GSI. Alleles at this locus encode proteins involved in pollenpistil recognition and rejection. The pollen tube growth is arrested within the style when the S-allele of the pollen matches an S-allele in the pistil (Franklin-Tong, 2008). This pollen-pistil interaction is characterized by a co-dominant relationship between the S-alleles in the pistil. For instance, a pollen grain carrying the S1 allele will be rejected by a pistil with the S₁S₂ genotype, but will be compatible with a pistil carrying the S3S4 genotype.

GSI was first described by East and Mangelsdorf in 1925 in *Nicotiana sanderae* and is prevalent in families such as Solanaceae, Scrophulariaceae, Poaceae, Fabaceae, Campanulaceae, Onagraceae, Papaveraceae, and Rosaceae (Muthuselvi and Praneetha 2019).

Sporophytic Self-Incompatibility (SSI). In contrast to GSI, SSI is determined by the genotype of the pollen parent rather than the pollen itself. The S-locus in SSI also plays a crucial role, but the incompatibility reaction is governed by the products of both S-alleles expressed in the pollen grain. This leads to a dominant interaction, where the pollen phenotype is determined by the most dominant S-allele.For example, a pollen grain from a plant with the S_1S_2 genotype will behave as if it carries only the S_1 allele if S_1 is dominant over S2. Consequently, this pollen grain will be rejected by a pistil carrying the S1 allele (Takayama and Isogai 2005). Hughes and Babcock first described sporophytic self-incompatibility (SSI) in Crepis foetida, while Gerstel independently documented the phenomenon in Parthenium argentatum in 1950. It is commonly found in families such as Brassicaceae, Polemoniaceae, Carvophyllaceae, Betulaceae, Asteraceae, Sterculiaceae, and Convolvulaceae (Muthuselvi and Praneetha 2019).

Understanding the distinctions between GSI and SSI is crucial for comprehending the diversity of selfincompatibility mechanisms across plant species. These systems have evolved as effective barriers to selffertilization, promoting outcrossing and genetic diversity.

MOLECULAR MECHANISMS OF SINGLE LOCUS SELF-INCOMPATIBILITY

Scientists have uncovered the molecular details of three mechanisms that prevent self-fertilization in plants, each controlled by a single gene. Two of these, found in families like nightshades (Solanaceae) and roses (Rosaceae), operate through a system called gametophytic self-incompatibility (GSI). These two GSI systems differ: one uses S-RNase proteins, while the other, found in poppies (Papaver), triggers programmed cell death. The third mechanism, sporophytic self-incompatibility (SSI), is well-characterized in the mustard family (Brassicaceae).

MOLECULAR MECHANISMS OF GSI

The Solanaceae GSI System: A Molecular Perspective. The mystery of how certain plants recognize and reject their own pollen, a process called self-incompatibility, has intrigued scientists for years. Gametophytic self-incompatibility (GSI) systems utilizing female-produced S-RNase toxins are found in diverse plant families, including nightshades (Solanaceae), roses (Rosaceae), and figworts (Scrophulariaceae). In this system, ribonucleases play a crucial role in recognizing and rejecting incompatible pollen, preventing self-fertilization. Beyond these wellknown examples, S-RNase-based GSI has been identified in a broader range of plant families, such as Plantaginaceae, Rubiaceae, Cactaceae, and Rutaceae (Sijacic et al., 2004; Asquini et al., 2011; Li et al., 2019; Liang et al., 2020; Ramanauskas and Igic 2021; Vieira et al., 2021). This system, known as S-RNasemediated gametophytic self-incompatibility (GSI), blocks self-fertilization during pollen tube growth within the flower's style.

Early research identified S-RNases, enzymes that break down RNA, as the key players in this process (Takayama and Isogai, 2005). These diverse proteins, unique to each plant, act as lethal gatekeepers, allowing only compatible pollen to fertilize the egg. However, the identity and role of the male counterpart in this interaction remained elusive for a long time (Franklin-Tong, 2008).

Scientists initially hypothesized that the male determinant acted as an inhibitor, selectively blocking the action of all but its own S-RNase. However, studies revealed that all S-RNases could enter the pollen tube, regardless of compatibility. The breakthrough came from analyzing the S-locus, the specific region on the plant's genome responsible for self-incompatibility. Research in species like petunias (Petunia) and tobacco (Nicotiana) pointed to a new candidate: a gene encoding an F-box protein, named AhSLF-S2, found in the figwort species Antirrhinum hispanicum (Wang et al., 2003; Lai et al., 2002). This protein, specifically produced in the anther and pollen, offered the first concrete lead in identifying the elusive male determinant, although its exact role in the S-RNasemediated rejection process is still being investigated.

While scientists have successfully pinpointed the key molecules responsible for self-recognition in certain plants – the female S-RNase and the male SLF/SFB –

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the precise mechanisms governing their interaction and the subsequent blocking of self-pollen remain a puzzle.

We know that S-RNases, with their RNA-degrading activity, act as toxins within pollen tubes, selectively targeting and destroying the RNA of self-pollen. This suggests a model where S-RNases are taken up by all pollen tubes, but only exert their toxic effect in selfpollen. The male determinant, SLF/SFB, possesses an intriguing feature: an F-box motif. This motif is known to interact with other proteins, forming a complex called the E3 ubiquitin ligase. This complex plays a crucial role in tagging proteins for destruction by the cell's waste disposal system, the 26S proteasome. This suggests a possible scenario where the SLF/SFB, in compatible pollen, might neutralize the S-RNase, perhaps by targeting it for degradation, thus allowing the pollen tube to grow normally. However, the exact details of this interaction and how self-pollen is specifically targeted remain to be fully elucidated (Franklin-Tong, 2008).

A research Zhao et al. (2022) offers significant insights into the evolution and function of SI systems in angiosperms. A study by Claessen et al. (2019) delved into the intricacies of self-incompatibility in European pear (Pyrus communis), aiming to unravel its genetic basis, molecular mechanisms, and effects on fertilization and fruit production. Their findings confirmed that, like in some other plant families, a single location on the genome, the S-locus, governs self-incompatibility in pears. This locus houses two tightly linked genes with multiple forms (polymorphic): the S-RNase gene, active in the pistil (female part), and a cluster of SFB (S-locus F-Box) genes, expressed in the pollen (male part). These genes act as the determinants of self-recognition, ensuring that only pollen with a different S-haplotype can successfully fertilize the flower. Interestingly, the researchers discovered that self-compatibility in certain pear cultivars stemmed from mutations in either the S-RNase or SFB genes. This highlights the importance of both components for a functional self-incompatibility system. Furthermore, the study emphasized the need for suppressed recombination (gene shuffling) within the Slocus. This ensures that the pistil S-gene and its corresponding pollen S-gene are inherited as a single unit, maintaining the integrity of the self-recognition mechanism.

A research Zhao et al. (2022) offers significant insights into the evolution and function of SI systems in angiosperms. The study underscores the prevalence of type-1 SI, characterized by multiple S-locus F-box genes (SLFs) interacting with S-RNases. This system effectively promotes outcrossing by allowing for the detoxification of non-self S-RNases while maintaining self-incompatibility. The evolutionary trajectory of this system is proposed to involve the expansion of SLFs from an ancestral state with a single SLF. This adaptation likely enhanced the ability of plants to recognize and reject a wider range of incompatible pollen. The study also highlights the impact of wholegenome duplications (WGDs) on the maintenance or loss of SI systems. The retention or loss of SI following WGD appears to be influenced by complex interplay of George & Bindu

selective pressures. By integrating phylogenetic analyses with functional studies, researchers provide a comprehensive framework for understanding the evolution and diversification of SI systems in angiosperms.

The Papaveraceae GSI System: A Molecular Perspective. Unlike the extensively studied Solanaceae and Rosaceae GSI systems, the Papaveraceae family exhibits a distinct mechanism of self-incompatibility (SI). While also classified as gametophytic selfincompatibility (GSI), as the pollen's ability to fertilize is determined by its own genotype, the molecular interactions involved differ significantly.

In Papaver rhoeas, the S-locus comprises two tightly linked genes: PrsS encoding the female determinant, a secreted stigma protein, and PrpS or SBP, a putative pollen receptor. The S-protein, a small, highly polymorphic molecule, is secreted by stigmatic papillae and interacts with its cognate pollen receptor (Wheeler *et al.*, 2003). This interaction triggers a signaling cascade, leading to increased cytosolic calcium, reactive oxygen species (ROS) production, and nitric oxide generation (Narayanapur *et al.*, 2018).

A key player in this process is the soluble organic pyrophosphatase p26, whose inactivation is linked to pollen tube growth inhibition. The role of the mitogenactivated protein kinase (MAPK) p56 in the early stages of SI is less clear, but it is implicated in later stages, potentially activating programmed cell death (PCD) (Wang *et al.*, 2019). The involvement of caspases, key executioners of apoptosis, supports the hypothesis that PCD is a critical component of the SI response in Papaver rhoeas (Riedl and Shi 2004).

A proteomic study conducted by Haque et al. (2020) delved into the molecular consequences of selfincompatibility (SI)-induced pollen rejection. The researchers found that incompatible pollen undergoes significant oxidative stress, resulting in irreversible modifications to numerous proteins. Crucially, proteins involved in essential cellular functions, such as cytoskeletal organization and energy metabolism, were identified as primary targets of reactive oxygen species (ROS). These oxidative modifications are believed to compromise protein function and contribute to the overall decline in pollen tube viability. The study provides compelling evidence that the oxidative burst triggered during SI is not merely a byproduct of the incompatibility reaction, but rather a key mechanism contributing to pollen tube abortion. The irreversible damage inflicted upon critical proteins ultimately leads to a reduction in cellular activity and, consequently, programmed cell death (PCD), culminating in the failure of the pollen tube to reach the ovule. These findings underscore the complex interplay between signaling pathways, oxidative stress, and protein modification in the context of SI. By elucidating the molecular consequences of incompatible pollen-pistil interactions, this research offers valuable insights into the mechanisms underlying SI rejection.

highlights the impact of whole-WGDs) on the maintenance or retention or loss of SI following Iuenced by complex interplay of *Biological Forum – An International Journal* 16(8): 262-274(2024) 267 death (PCD) in incompatible pollen tubes (Wilkins et al., 2015). Research indicates that this rapid acidification is likely linked to vacuolar disruption. Pollen tube vacuoles undergo a dramatic transformation following SI induction. Initially, a rapid reorganization of the vacuolar structure occurs, followed by extensive permeabilization and eventual breakdown of the tonoplast (vacuolar membrane) (Wang et al., 2019). The release of vacuolar contents, including hydrolytic enzymes, into the cytosol contributes to the acidic environment that promotes PCD. This temporal sequence of events suggests a causal relationship between vacuolar disruption and cytosolic acidification in the SI response. The acidic environment, in turn, activates downstream processes, such as caspase activation and DNA fragmentation, leading to pollen tube degeneration. Understanding the mechanisms underlying cytosolic acidification and vacuolar dynamics is crucial for elucidating the complex signaling pathways involved in SI.

Recent research highlights the crucial role of glycosylphosphatidylinositol-anchored proteins (GPI-APs) in the self-pollen rejection process (Lin et al., 2022; Goring et al., 2023). Specifically, the remodeling of GPI-APs and their subsequent cleavage by the GPIinositol deacylase enzyme, HLD1/AtPGAP1, appear essential for this process. This finding suggests that GPI-APs might function as coreceptors, modulating the interaction between the PrpS and PrsS proteins involved in self-incompatibility recognition. This distinct GSI mechanism in Papaveraceae highlights the diversity of evolutionary strategies employed by plants to prevent self-fertilization. While sharing some common features with other GSI systems, such as the role of S-locus-encoded proteins, the Papaveraceae model offers a unique perspective on the molecular basis of pollen-pistil interactions.

Molecular Model of Sporophytic Self-Incompatibility in Brassicaceae. The Brassicaceae family has served as a model system for understanding the molecular basis of sporophytic self-incompatibility (SSI). Early genetic studies by Bateman in the mid-20th century laid the foundation for our current understanding of this complex trait.

Bateman proposed a single-locus model for SSI, suggesting that a multi-allelic locus governs the incompatibility reaction in Brassicaceae species such as *Iberis, Capsella, Brassica*, and *Raphanus* (Watanabe *et al.*, 1994). Unlike gametophytic self-incompatibility (GSI), where the pollen's own genotype determines its fate, SSI is controlled by the genotype of the pollen parent plant.

The S-locus in Brassicaceae contains multiple alleles, each encoding a unique specificity. When pollen from a plant carrying a specific S-allele lands on a stigma with the same S-allele, an incompatibility reaction is triggered, preventing pollen tube growth. In contrast, pollen from a plant with a different S-allele can successfully germinate and penetrate the stigma, leading to fertilization. This sporophytic control of pollen behavior, as opposed to the gametophytic control seen in GSI, has been a cornerstone in the study of selfincompatibility mechanisms. The Brassicaceae model has provided invaluable insights into the genetic and molecular basis of SSI, paving the way for further research in this field (Watanabe *et al.*, 1994).

Early genetic studies established the S-locus as the central determinant of SI specificity, with multiple alleles controlling the incompatibility reaction (Watanabe *et al.*, 1994). Subsequent research has identified key components of the SSI signaling pathway. The S-locus receptor kinase (SRK), a transmembrane protein located on the stigma papillae, serves as the female determinant of specificity. The male determinant, S-locus cysteine-rich protein (SCR) or S-locus protein 11 (SP11), is a pollen-coat-associated ligand that interacts with SRK (Stone *et al.*, 2003).

Upon self-incompatible pollen-stigma interaction, the binding of SCR/SP11 to SRK triggers a signaling cascade. This involves the activation of the kinase domain of SRK, leading to the phosphorylation of downstream signaling components, including the Armadillo repeat-containing protein 1 (ARC1) and the M-locus protein kinase (MLPK). ARC1, an E3 ubiquitin ligase, is believed to be a key player in the SI response, promoting the ubiquitination and degradation of proteins essential for pollen hydration and germination (Nasrallah, 2019).

Recent investigations in Brassica rapa have revealed an additional signaling pathway, independent of the known MLPK and ARC1 pathways, that contributes to the self-incompatibility (SI) response. This pathway, mediated by the receptor-like kinase FERONIA (FER), triggers a rapid increase in reactive oxygen species (ROS) production via the FER-Rac/Rop-Rboh module (Zhang et al., 2021; Huang et al., 2023). This ROS burst effectively leads to self-pollen rejection. Furthermore, suppressing ROS production, either through scavenger molecules or by inhibiting the FER signaling pathway, can disrupt SI, highlighting the critical role of ROS in this process (Zhang et al., 2021). However, how the FER-ROS pathway is activated downstream of the SRK receptor and its potential interplay with the MLPK and ARC1 pathways in degrading compatibility factors remains unclear. Further research focusing on these interactions will be crucial to unravel the complex intracellular events driving pollen rejection in Brassica SI.

TWO-LOCUS GAMETOPHYTIC SELF-INCOMPATIBILITY (GSI) IN POACEAE

Unlike the single-locus GSI systems found in Solanaceae and Papaveraceae, the grass family Poaceae exhibits a more complex two-locus GSI system. Pioneering studies by Lundqvist (1954) in *Secale cereale* and Hayman (1956) in *Phalaris coerulescens* revealed the involvement of two unlinked, multi-allelic loci, designated S and Z, in controlling selfincompatibility (Baumann *et al.*, 2000). This two-locus system necessitates the presence of matching S and Z alleles in both pollen and pistil for an incompatible reaction to occur. Consequently, reciprocal crosses between plants can yield varying degrees of compatibility, ranging from full compatibility to

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complete incompatibility, depending on the specific allele combinations (Leach, 1988).

Heslop-Harrison (1982) proposed a model where pistilspecific factors, potentially glycoproteins, interact with carbohydrate moieties on the pollen tube wall to trigger incompatibility. This interaction is hypothesized to disrupt pollen tube growth, leading to its arrest.

A research by Chen *et al.* (2019) has unveiled a more complex picture of the GSI signaling pathway in grasses. A comprehensive analysis of gene expression revealed the involvement of numerous genes associated with calcium signaling, protein phosphorylation, reactive oxygen species (ROS), nitric oxide (NO), cytoskeleton dynamics, and programmed cell death (PCD). These findings suggest a complex interplay of molecular events leading to pollen tube rejection in incompatible pollinations.

A key characteristic of the grass self-incompatibility system is its reliance on precise genotype matching between pollen and pistil. Specifically, selfincompatibility is activated only when both the S and Z alleles of the pollen grain perfectly match those present in the pistil (Rohner *et al.*, 2023). While the exact downstream mechanisms of pollen rejection are still being elucidated, they appear to involve complex interactions of calcium signaling, protein phosphorylation, and proteolysis pathways (Yang *et al.*, 2008; Klaas *et al.*, 2011; Chen *et al.*, 2019).

Comparative genomic analyses suggest that this duallocus system originated from a duplication event, with the S and Z loci sharing similar gene composition, structure, and synteny across the Poaceae family (Rohner et al., 2023). At the S-locus, two DUF247 family genes (SDUF247-I and SDUF247-II) act as the male determinants, while a small extracellular peptide encoded by the HPS10 gene functions as the female determinant (Manzanares et al., 2016; Lian et al., 2021; Wang et al., 2022). Similarly, the Z-locus also utilizes two DUF247 family genes (ZDUF247-I and ZDUF247-II) as male determinants, but the female determinant (sZ gene) remains unidentified. Although two glycerol kinase-like genes (LpGK1 and LpGK2) have been proposed as candidates for sZ, further validation is needed (Zhang et al., 2024).

Notably, the specific linkage between HPS10 and DUF247 genes appears exclusive to the Poaceae, suggesting a distinct evolutionary origin for this S-Z GSI system (Wang *et al.*, 2022). Further research, aided by advancements in gene editing and the increasing availability of high-quality grass genome assemblies, will be crucial to fully decipher the intricacies of the S-Z GSI system in grasses and other lesser-studied plant families.

TEMPORARY SUPPRESSION OF SELF-INCOMPATIBILITY

To overcome the barriers imposed by selfincompatibility (SI) and facilitate self-pollination, particularly for the development of inbred lines, various techniques have been employed. These methods aim to temporarily suppress the SI response, allowing for selffertilization.

Techniques for Overcoming SI:

• **Bud pollination:** Pollinating immature flower buds with mature pollen can sometimes bypass the SI mechanism.

• Mixed pollination: Introducing a large amount of pollen can overwhelm the SI response, increasing the chances of successful self-pollination.

• **Delayed pollination:** Pollinating flowers at the end of their receptive period may coincide with a decline in SI effectiveness.

• **Irradiation:** In some species, such as Solanaceae, exposing flowers to irradiation (e.g., X-rays or gamma rays) can temporarily suppress SI.

• **Surgical techniques:** Removing the stigma or style can bypass the SI response, allowing for direct pollen application to the ovules.

• **High temperature treatments:** Elevated temperatures have been shown to temporarily inactivate the SI response in some species, including Trifolium and Lycopersicon.

• Increased CO_2 concentration: In certain cases, elevated CO_2 levels can weaken the SI response, promoting self-pollination.

While these methods can be effective in overcoming SI, they often result in reduced seed set or progeny vigor. Additionally, the temporary nature of these techniques requires careful timing and application. It's important to note that the success of these methods varies depending on the plant species, the specific SI system involved, and environmental conditions (Muthuselviand Praneetha 2019). By understanding the mechanisms underlying SI and employing appropriate techniques, researchers and breeders can effectively overcome this reproductive barrier to achieve desired breeding goals.

Physiological Basis for Overcoming Self-Incompatibility (SI). While the techniques for temporarily suppressing SI have been widely employed, the underlying physiological mechanisms are not fully understood. However, some insights can be inferred from our current knowledge of SI.

Impact on SI Proteins and Signaling:

• **Irradiation:** It's possible that irradiation induces damage to SI proteins or interferes with the signaling pathways involved in the incompatibility response.

• **High temperature:** Elevated temperatures might alter the conformation or activity of SI proteins, affecting their ability to function effectively.

• **Increased CO₂:** While the exact mechanism is unclear, increased CO_2 levels could potentially influence hormone levels or metabolic processes, indirectly affecting the SI response.

Alteration of Stigma and Pollen Physiology:

• **Bud pollination and delayed pollination:** These techniques may influence the development and physiology of the stigma and pollen, affecting their compatibility.

• **Mixed pollination:** A high pollen load might saturate the stigma, reducing the effectiveness of the SI response.

Overcoming Pollen Tube Inhibition:

• **Surgical techniques:** By bypassing the stigma and style, these methods directly challenge the SI mechanism at the site of pollen tube growth inhibition. It's essential to note that the physiological basis for these techniques is often complex and multifaceted. Multiple factors, including changes in gene expression, protein activity, and cellular processes, likely contribute to the temporary suppression of SI. Further research is needed to elucidate the precise mechanisms underlying these techniques and to develop more targeted approaches for overcoming SI (Ahmad *et al.*, 2022).

INSTANCES WHERE SI IS PREFERRED FOR PLANT BREEDING

Self-incompatibility (SI) plays a pivotal role in plant breeding by preventing self-pollination and promoting outcrossing. This reproductive barrier has significant implications for hybrid seed production, among other breeding objectives.

• Hybrid Seed Production: The exploitation of SI is fundamental to efficient hybrid seed production. By utilizing self-incompatible lines as parental materials, the laborious process of emasculation can be eliminated. Furthermore, the ability to conduct both forward and reverse crosses between self-incompatible lines facilitates the development of complex hybrid combinations, such as double and three-way crosses (Narayanapur *et al.*, 2018). The utilization of SI in hybrid seed production offers several advantages, including increased efficiency, reduced labor costs, and the potential for higher hybrid vigor.

The phenomenon of hybrid vigor, also known as heterosis, occurs when two parents with distinct genetic backgrounds are crossed, resulting in offspring with improved traits such as increased yield and enhanced resistance to environmental stresses. This phenomenon is widespread and has led to the development of hybrid cultivars in many crop species, including maize, sorghum, tomato, and pepper (Kempe and Gils 2011). However, producing hybrid seed requires a controlled pollination system to prevent self-fertilization in selfcompatible (SC) crops. Various methods are used to control pollination, including mechanical emasculation, chemical gametocide agents, and male sterility (Kempe and Gils 2011). Self-incompatibility (SI) is sometimes preferred over male sterility in crops with insectmediated pollination, as bees tend to avoid male-sterile. Nevertheless, SI can also have drawbacks, such as the production of SI F₁ hybrids, which can hinder seed or fruit production in certain crops. As a result, breeding programs often aim to develop SI female lines that produce SC F₁ hybrids (Kaothien-Nakayama et al., 2010).

• **Clonal Gardens:** The establishment of clonal gardens can be facilitated by utilizing self-incompatible superior clones. The resulting hybrid progeny, while lacking precise parental information, offer opportunities for selecting desirable traits. Examples include the production of hybrid cocoa (Junaedi *et al.*, 2017).

• Seedless Fruit Production: In crops like pineapple, the combination of parthenocarpy (seedlessness) and self-incompatibility ensures the production of highquality, consumer-preferred fruits. The absence of selfcompatibility prevents the development of undesirable seeds, enhancing fruit quality.

In certain crops, such as citrus (e.g., orange, mandarin, lemon), seedless fruits are highly desirable, making SI a preferred condition. Many citrus cultivars exhibit parthenocarpy, resulting in normal, but seedless, fruits without fertilization. However, when these seedless cultivars are grown near cross-compatible cultivars, undesirable seeded fruits may still be formed (Vardi et al., 2008). Therefore, breeding for SI in citrus is considered a target trait to reduce seed number, especially when combined with parthenocarpy. Studies have demonstrated the role of gametophytic selfincompatibility (GSI) in seedlessness in several citrus cultivars. For instance, a mutant mandarin (Citrus reticulata Blanco) cv. 'Wuzishatangju' exhibited seedlessness due to GSI (Ye et al., 2009). Similar findings have been reported in other citrus cultivars, including mandarin cv. 'Afourer' (Gambetta et al., 2013), lemon (Citrus limon [L.] Burm. F.) cultivars 'Xiangshui' (Zhang et al., 2012) and 'Kagzi kalan' (Kakade et al., 2017). Research has identified styleexpressed S-like-RNases and SKP1-like genes as potential key players in the SI response of the mandarin cv. 'Wuzishatangju' (Miao et al., 2011; Li et al., 2015). However, further investigation is necessary to fully understand the Citrus SI system before engineering SI for seedlessness can become a practical reality.

SI AND EVOLUTION

Despite independent origins in various lineages, selfincompatibility (SI) loss is common (Barrett, 2013). Once lost, SI cannot be regained, as seen in families with different SI forms (Igic et al., 2008; Ferrer and Good 2012). S-RNase-based GSI has been lost frequently, with over 60 instances reported in Solanaceae alone (Igic et al., 2008). Brassicaceae genera also have numerous secondarily evolved selfcompatible (SC) species. Understanding the causes and consequences of SI to SC transitions is essential for comprehending this significant mating-system shift in plants. While the mechanisms behind SI to SC transitions may differ across taxa, several common pathways can be identified through comparative and functional studies. One pathway involves accumulated mutations in both male and female determinants. Lossof-function mutations and reduced variation at the Slocus can lead to SC, especially when SC offers advantages in mating success, transmission, and reproductive assurance (Shimizu and Tsuchimatsu 2015).

The breakdown of self-incompatibility (SI) systems, leading to self-compatibility (SC), often results from mutations in either male or female SI determinants. In S-RNase based GSI, studies on Solanaceae and Citrus have linked the transition to SC to deletions or dysfunction of S-RNase genes, often followed by mutations in pollen S-factors (Broz *et al.*, 2017, 2021; Markova *et al.*, 2017; Liang *et al.*, 2020). Similarly, in Brassicaceae, the predominantly selfing *Arabidopsis thaliana* arose from SI ancestors through independent

loss-of-function mutations in both SRK and SCR genes (Tsuchimatsu *et al.*, 2017). These transitions highlight the evolutionary plasticity of SI and the potential for its manipulation in crop improvement strategies.

FUTURE SCOPE

Deciphering the complex molecular mechanisms governing self-incompatibility (SI) in plants has unlocked exciting possibilities for targeted crop improvement and breeding strategies. However, to fully harness the potential of SI for agricultural advancement, a deeper dive into its intricacies is crucial.

While our current understanding of SI has facilitated progress in crop production, delving deeper into the molecular architecture of known SI systems can unveil novel gene targets for engineering self-compatibility, as exemplified by the successful manipulation of the Glyoxylase I gene in canola. Moreover, exploring the uncharted territories of uncharacterized SI systems, particularly in crops like tea, cocoa, and olive, holds immense promise for practical applications. This includes leveraging S-genotyping for precise pollenizer selection, developing self-compatible cultivars for ease of breeding, and facilitating hybrid breeding programs for enhanced crop performance.

A key focus for future research should be on the strategic transfer of SI-related genes across plant species to harness hybrid vigor, or heterosis, through the production of hybrid seeds. This endeavor necessitates the meticulous identification and characterization of S-alleles within diverse germplasm collections, with a particular emphasis on identifying strong alleles that can be utilized to develop stable selfincompatible parental lines for hybrid breeding programs. The implications of SI research extend across a wide array of crops. For instance, in naturally selfcompatible cereal crops like wheat, efforts are underway to introduce SI from related species to enable the development of hybrid varieties. In contrast, for vegetable crops like tomatoes, the focus lies in overcoming interspecific reproductive barriers imposed by SI, thereby facilitating the transfer of desirable traits from wild relatives into cultivated varieties. Similarly, investigations in self-incompatible Brassica vegetables aim to identify genes that can be manipulated to induce self-compatibility, simplifying breeding processes. The diverse range of SI systems and their potential applications highlight the need for continued research in this field to unlock the full potential of SI for maximizing agricultural productivity and ensuring global food security (Muñoz Sanz et al., 2020).

CONCLUSIONS

This comprehensive review explored the multifaceted phenomenon of self-incompatibility (SI) in flowering plants, tracing its historical understanding from early botanical observations to the latest molecular insights. While the concept of SI emerged from recognizing patterns of self-sterility in crops, it has evolved into a rich field encompassing genetics, molecular biology, and evolutionary ecology. The review highlighted the diversity of SI systems, ranging from the morphologically distinct heteromorphic systems like distyly and tristyly to the more cryptic homomorphic systems governed by complex genetic interactions at the S-locus. Although model systems in families like Solanaceae, Brassicaceae, and Papaveraceae have illuminated key molecular mechanisms of SI, vast gaps remain in our understanding of the diverse array of SI strategies employed across the plant kingdom.

This incomplete knowledge represents a critical barrier to fully harnessing the power of SI for crop improvement. Future research should prioritize elucidating the molecular basis of SI in understudied plant families, particularly those with agricultural importance like Poaceae. Unraveling the evolutionary dynamics of SI, including the selective pressures driving its gain and loss, is crucial for understanding its broader role in plant diversification.

Finally, the potential applications of SI in crop improvement are vast. By leveraging advancements in gene editing and genomic resources, we can engineer self-incompatible lines, facilitate hybrid breeding programs, and even manipulate SI to control seed production in fruits. A deeper understanding of SI will be paramount for maximizing agricultural productivity and ensuring global food security in the face of a changing climate.

Conflict of Interest. None.

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