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REVIEW ARTICLE

Progress on deciphering the molecular aspects of cell-to-cell communication in *Brassica* self-incompatibility response

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Abstract

The sporophytic system of self-incompatibility is a widespread genetic phenomenon in plant species, promoting out-breeding and maintaining genetic diversity. This phenomenon is of commercial importance in hybrid breeding of *Brassicaceae* crops and is controlled by single *S* locus with multiple *S* haplotypes. The molecular genetic studies of *Brassica* '*S*' locus has revealed the presence of three tightly linked loci viz. S-receptor kinase (*SRK*), S-locus cysteine-rich protein/S-locus protein 11 (*SCR*/*SP11*), and S-locus glycoprotein (*SLG*). On self-pollination, the allele-specific ligand–receptor interaction activates signal transduction in stigma papilla cells and leads to rejection of pollen tube on stigmatic surface. In addition, arm-repeat-containing protein 1 (*ARC1*), M-locus protein kinase (MLPK), kinase-associated protein phosphatase (KAPP), exocyst complex subunit (*Exo70A1*) etc. has been identified in *Brassica* crops and plays a key role in self-incompatibility signaling pathway. Furthermore, the cytoplasmic calcium (Ca^{2+}) influx in papilla cells also mediates self-incompatibility response in *Brassicaceae*, but how this cytoplasmic Ca^{2+} influx triggers signal transduction to inhibit pollen hydration is still obscure. There are many other signaling components which are not well characterized yet. Much progress has been made in elucidating the downstream multiple pathways of *Brassica* self-incompatibility response. Hence, in this review, we have made an effort to describe the recent advances made on understanding the molecular aspects of genetic mechanism of self-incompatibility in *Brassicaceae*.

Keywords Self-incompatibility · *Brassicaceae* · S haplotypes · Molecular mechanism · Ca^{2+} influx

Introduction

The genetic phenomenon of self-incompatibility (SI) has emerged as an indispensable widespread mechanism in the flowering plants causing physiological hindrance to self-pollination, self-fruitfulness, and enforcing out-crossing (Takayama and Isogai 2005). Hence, it allows gene flow through pollen, thus maintaining and enhancing the genetic diversity in plant species. The term self-incompatibility is defined as the inability of plants, producing functional gametes, to set viable seed on self-pollination or when crossed with some of their genetic relatives (Brewbaker 1957). This phenomenon was first described in plants by Koelreuter in 1764 when he observed no seed set after self-pollination in the flowers of purple mullen (*Verbascum phoeniceum*), but produced abundant seeds when crossed with other nearby plants. However, the term 'self-incompatibility' was first coined by Stout (1917) and it is the genetic mechanism used to avoid self-fertilization (Suzuki 2009). Self-incompatibility can be divided into heteromorphic and homomorphic types. In the heteromorphic system, self-incompatibility is associated with differences in floral morphology and pollination is compatible only between the flowers of different morphological types, whereas in the homomorphic type, flowers of the same species have same morphological types (de Nattencourt 2001). The homomorphic system can further be classified into gametophytic self-incompatibility (GSI) and sporophytic self-incompatibility (SSI), based on the genetic control of pollen incompatibility reactions. In the sporophytic system (SSI), the pollen behavior in the SI interaction is determined by the genotype of plant on which it is produced (sporophytic control), whereas in the gametophytic system (GSI), it is determined by the genotype

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of pollen grain or tube itself (Nasrallah 2002; Hiscock and Tabah 2003). The system of self-incompatibility is widely distributed in more than 100 angiosperm families (Igic et al. 2008) and is controlled by multiple alleles, up to 200 SI haplotypes (Lawrence 2000). The gametophytic system is thought to be more common as compared to sporophytic system, but sporophytic type is more characterized, and both systems evidently evolved independently (Kao and McCubbin 1996). Both the genetic systems are controlled by single locus, with multiple S alleles (S haplotypes) (S_1, S_2, S_3, … S_n) in most of the plant species.

Homomorphic sporophytic self-incompatibility system has been reported to be known from ten families of angiosperms (Igic et al. 2008) and has been best characterized and exploited commercially in the family *Brassicaceae*, especially in cole vegetables. Self-incompatibility (SI) system has evolved as models for investigating cell-to-cell signaling, as it involves cell-to-cell interaction between pollen and pistil, and signal transduction in stigma/style (Watanabe et al. 2012). The SSI system has been proved effective for commercial hybrid seed production in *Brassica* crops (Shen et al. 2008). Thus, an understanding of fundamental biology and molecular mechanisms of SSI in *Brassicaceae* vegetables is an important research target. During last two decades, intense molecular studies of self-incompatibility (SI) have focused on identifying and characterizing male and female determinants of SI in different plant families. Currently, both the determinants have been identified in angiosperms along with other players of SI response. Here, in this review, we have discussed the progress made towards characterization and identification of SI-signaling components in *Brassica* crops.

Self-incompatibility system in Brassica vegetables

In all the members of *Brassicaceae* family including cole crops, homomorphic sporophytic self-incompatibility (SSI) system exists, associated with trinucleate pollen and inhibition of self-pollen germination on 'dry' stigmatic surface (Bateman 1952, 1955; Kroh 1964; Ockendon 1972). The SSI system has been confirmed in kale (Thompson 1957), sprouting broccoli (Sampson 1957a), radish (Sampson 1957b), cabbage (Adamson 1965), and in cauliflower (Hoser-Krauze 1979). The exploitation of sporophytic system of self-incompatibility (SSI) for hybrid breeding in *Brassica* was first suggested by Pearson (1932); however, development of hybrids on commercial scale was achieved in Japan in 1950, and where first hybrid in cabbage based on SI system was developed (Odland and Noll 1950). Subsequently, in the United States, commercial hybrid seed production exploiting SSI was achieved in 1954 and in the Europe in 1966 (Haruta 1962; Wallace and Nasrallah 1968; Johnson 1972; Wallace 1979). Thompson and Taylor (1966) reported that ancestral *Brassica oleracea* var. *sylvestris* is highly self-incompatible. On this basis, it is natural that cole vegetables would be self-incompatible. However, there is wide variation present in the level of self-incompatibility (SSI) in *Brassica* vegetables. Among the cole group, Kale (Thompson and Taylor 1965) and round-headed cabbage (Adamson 1965) have high level of self-incompatibility. On the other hand, broccoli has relatively low level of self-incompatibility as compared to kale and cabbage, and very low level of self-incompatibility is present in early summer cauliflower owing to weak S-alleles (Watts 1965). Systematic studies on self-incompatibility in cauliflower (Watts 1963, 1965) suggested that among the European types, the biennial winter- and autumn-type cauliflower has higher level of self-incompatibility, and in European, summer-type cauliflower (snowball, alpha, and erfurt) has low level of SSI (Singh et al. 2013). Investigations on the level of self-incompatibility in Indian cauliflower revealed that varieties/lines under maturity group I have strongest self-incompatibility followed by maturity group II and III and maturity group IV has weak self-incompatibility level (Vidyasagar 1981; Chatterjee and Swarup 1984; Singh et al. 2013).

In the self-incompatible plants of *Brassicaceae* family, following the self-pollination, pollen grains either do not germinate on the stigma or they germinate, but pollen tube fails to penetrate the stigma papillar wall which is having the same S haplotypes as the pollen parent. The self-pollen is recognized during the interaction of pollen grain with stigma papillar cell wall and inhibition occurs at stigmatic surface. The inhibition of pollen tube at stigmatic surface in SSI is due to response of papillae to incompatible pollinations by the deposition of the β-1, 3-glucan callose (Kanno and Hinnata 1969; Roberts et al. 1980, 1983; Sulaman et al. 1997). On the occasions of both germination and penetration of pollen tube, its development halts at the callose barrier synthesized by the cytoplasm of the stigmatic papillar cells (Dickinson and Lewis 1973a; Elleman and Dickinson 1990; Edlund et al. 2004). Thus, failure of pollen grain adhesion, hydration, germination, pollen tube development, and penetration to stigma papillar wall leads to the expression of self-incompatibility (Chapman and Goring 2010; Singh et al. 2013).

Genetics and dominance hierarchy of S haplotypes in *Brassica*

Sporophytic self-incompatibility (SSI) in *Brassicaceae* is governed by the single Mendelian 'S' locus consisting of multiple alleles, referred as S haplotypes (Bateman 1955; Ockendon 1974). So far, more than 100 S-alleles have been reported in *Brassica campestris* (Nou et al. 1991, 1993), 50 in *Brassica oleracea* (Brace et al. 1994; Ockendon 1974,

2000; Nasrallah 1997), and 34 S-alleles in *Raphanus sativus* L. (Sampson 1957b; Karron et al. 1990). The S haplotypes exhibit complex inheritance and show dominant, co-dominant, and recessive allele interactions (Bateman 1955; Brugiere et al. 2000), and thus, four types of S-allele interactions (Types I, II, III, and IV) have been reported in *Brassica* (Mackay 1997; Hoser-Krauze 1979; Wallace 1979). Depending upon the SI phenotype, the *Brassica* S-alleles have been categorized into two classes viz. class-I haplotypes and class-II haplotypes (Nasrallah et al. 1991; Nasrallah and Nasrallah 1993). The high-activity alleles (class I) determining strong self-incompatibility are placed relatively high on the dominance scale, in which on average, development of 0–10 pollen tubes occurs per stigma on self-pollination. On the other hand, the low-activity alleles, exhibiting competitive and recessive interactions in pollen (class II), have weak incompatibility phenotype in which 10–30 pollen tubes develop per self-pollinated stigma (Nasrallah et al. 1991; Sobotka et al. 2000). In case of SSI, all the pollen grains produced on self-incompatible plant exhibit same incompatibility reaction irrespective of S-allele (constitution) assigned to particular pollen grain in the process of male gamete formation. The activity of S-alleles in stigma and pollen of heterozygous plants is based upon the result of complex dominant/recessive allelic interactions (Thompson and Taylor 1966).

The dominance hierarchy of S haplotypes, which is the key feature of SSI in *Brassica* (Bateman 1955; Thompson and Taylor 1966; Hatakeyama et al. 1998b), relies on their relative genetic behavior to other S haplotypes in stigma and pollen of heterozygous plants (Thompson and Taylor 1966; Ockendon 1975). Dominance relationships have been investigated in several species of *Brassicaceae*, like *B. campestris* (Haruta 1962; Hatakeyama et al. 1998b), kale (Thompson and Taylor 1966, 1971), Brussels sprout (Ockendon 1973, 1975), cabbage (Wallace 1979; Negi and Vidyasagar 2008), and *Sinapis arvensis* (Stevens and Kay 1989). The dominance relationships between three S-alleles (S_1, S_2, and S_3) can be linear and non-linear. When $S_1 > S_2$ (S_1 dominant to S_2), $S_2 > S_3$ (S_2 dominant to S_3), and S_1 is also dominant to S_3 ($S_1 > S_3$), there is linear dominance ($S_1 > S_2 > S_3$), in contrast, if S_1 is not dominant to S_3, it is non-linear dominance relationship (Thompson and Taylor 1966). For example, Hatakeyama et al. (1998a, b), Shiba et al. (2002), and Kakizaki et al. (2003) reported linear dominance relationship between four S haplotypes ($S_{44} > S_{60} > S_{40} > S_{29}$) in self-incompatible *Brassica rapa* pollen. Genetic analysis of dominance relationships in *Brassicaceae* unveiled following characteristic features viz. (1) co-dominance is more common than dominance/recessiveness; (2) frequent occurrence of dominance/recessiveness in pollen in contrast to stigma; (3) dominance relationships between pollen and stigma are distinct; and (4) occurrence of non-linear dominance relationships in stigma is more common as compared to pollen (Thompson and Taylor 1966; Ockendon 1975; Hatakeyama et al. 1998b; Shiba et al. 2002). On the basis of these genetic features, the molecular mechanisms of dominance relationships between S haplotypes were revealed.

Genes governing self-incompatibility response in *Brassica*

The cell-to-cell communication of sporophytic self-incompatibility (SSI) system in *Brassicaceae* is governed by single polymorphic 'Mendelian S locus' (Bateman 1955). The molecular genetic analysis revealed that 'S locus' is multigene, multiallelic highly complex locus, which spans many kilobase pairs, and encompasses various physically linked polymorphic genes that co-segregate with SI phenotype (Yu et al. 1996; Boyes et al. 1997; Suzuki et al. 1999; Casselman et al. 2000; Sobotka et al. 2000). Genomic analysis and physical mapping of S locus in *Brassica* have identified three polymorphic physically linked loci (Fig. 1) viz. S-receptor kinase (*SRK*) (Female determinant) (Stein et al. 1991), S-locus cysteine-rich protein/S-locus protein 11 (*SCR*/*SP11*) (Pollen ligand) (Suzuki et al. 1999; Takayama et al. 2000), and S-locus glycoprotein (*SLG*) (Female determinant) (Nasrallah et al. 1985; Suzuki et al. 1995). Besides these polymorphic linked genes, the other candidate genes playing role in downstream SI-signaling pathway in *Brassicaceae* have been identified (Fig. 2). The identification of first S-locus gene in *Brassica oleracea* as S-specific stigmatic proteins, termed S-locus glycoprotein (*SLG*) (Nasrallah and Wallace 1967; Nasrallah et al. 1985; Suzuki et al. 1995) attributed to initiation of molecular investigation on elucidating the mechanism of self-incompatibility in *Brassica*. It is highly expressed specifically in stigmatic papillar cells as revealed by in situ hybridization of *SLG* transcripts (Nasrallah et al. 1988) and GUS (GUS: β-glucuronidase) reporter gene system assay of *SLG* promoter sequence (Sato et al. 1991). In the variant *S*-homozygotes of *Brassica* also S-locus glycoproteins were explored (Nishio and Hinata 1977; Nishio and Kusaba 2000). There is abundance of *SLG* proteins in stigma papilla cells, which is the site of self-incompatibility reaction (Nasrallah et al. 1985, 1987). Identification, cloning, and sequencing of *S*-locus glycoproteins (*SLG*) have been done (Nasrallah et al. 1985, 1987), along with determination of amino-acid sequence of *SLG* by direct sequencing of protein (Takayama et al. 1987). Subsequently, in different *Brassica* crops such as *Brassica oleracea, Brassica rapa*, and *Raphanus sativus*, this sequence information enabled the sequencing of various S haplotypes of *SLG* (Chen and Nasrallah 1990; Kusaba et al. 1997; Sakamoto et al. 1998). For secretion to the outside of cells, *S*-locus glycoproteins (*SLG*) consist of a hydrophobic signal peptide at the N-terminus,

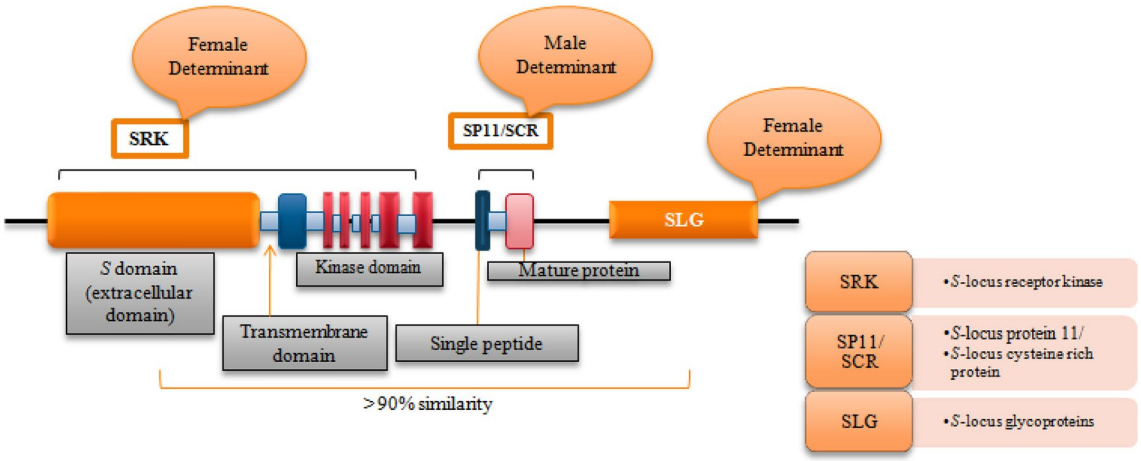

Fig. 1 Structure of *S* locus comprising three tightly linked polymorphic loci: *SRK, SCR/SP11*, and *SLG*. *SRK* and *SLG* share high sequence identity and act as female determinants of Brassica SI response. On interaction with pollen ligand (*SCR/SP11*), the kinase domain of *SRK* gets activated and signal is transduced to stigma papilla cell and causes self-pollen rejection (Nasrallah 1997; Watanabe et al. 2012; Kitashiba and Nishio 2009)

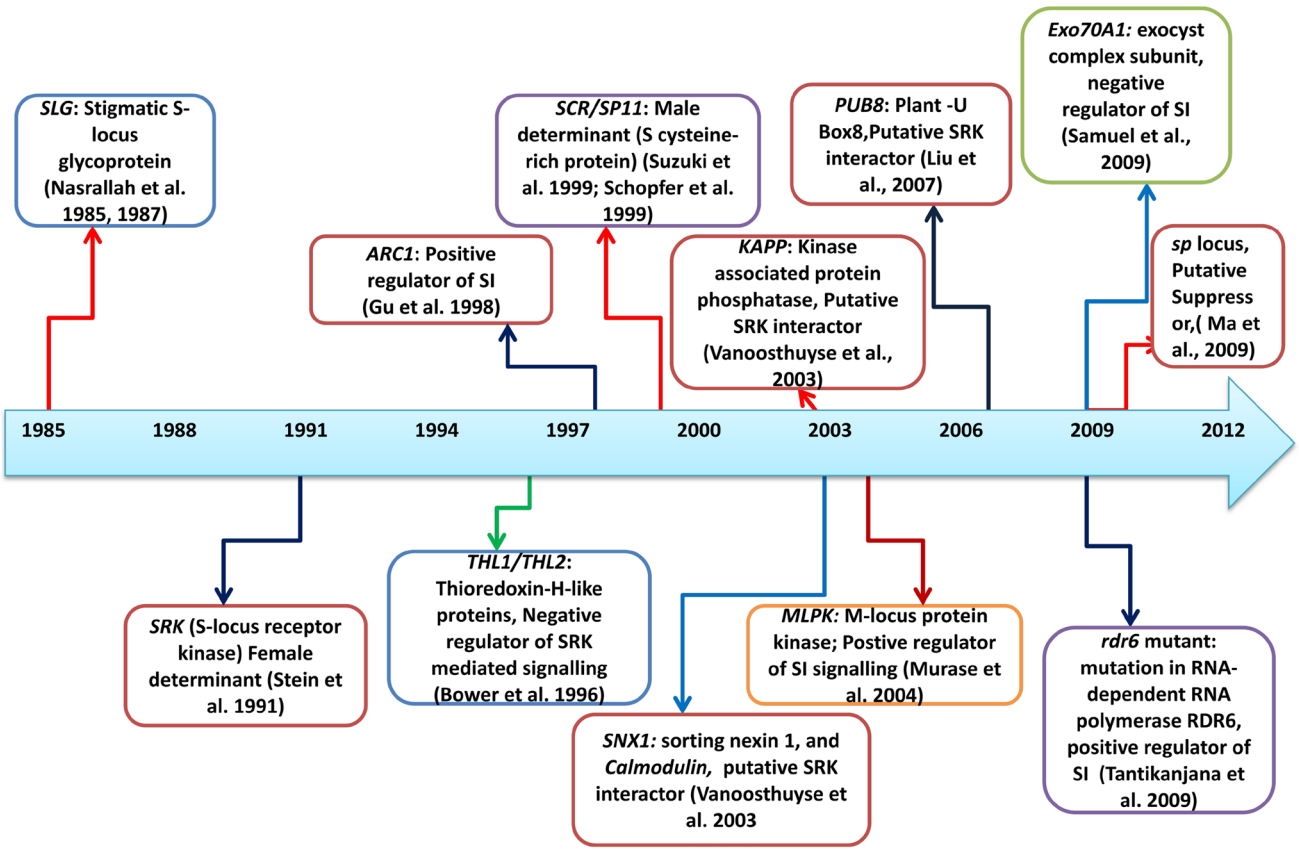

Fig. 2 Timeline of identification of SI-signaling components in Brassica

various *N*-glycosylation sites and 12 conserved cysteine residues (Kitashiba and Nasrallah 2014).

The identification of *SLG* led to the isolation of another S-locus gene, S-locus receptor kinase (*SRK*) having extracellular transmembrane *S* domain, depicting serine/threonine kinase activity (Stein et al. 1991). Both genes, *SLG* and *SRK*, known as female determinants of SI response in *Brassica*, exhibit high polymorphism (Stein et al. 1991;

Kusaba et al. 1997) and are expressed particularly at the mature stigmatic surface (Sobotka et al. 2000). However, some transcripts of *SLG* and *SRK* have been diagnosed in anther tissue, but detection of both proteins could be done only in stigmatic surface (Stein et al. 1996; Delorme et al. 1995). The *SRK* gene encompasses three domains: an extracellular *S* domain, which is the center for recognition of pollen ligand gene and shares high degree of sequence similarity to *SLG* gene; the other domain which passes through the plasma membrane is a transmembrane domain (encoding exon 2) and an intracellular kinase domain (exons 4–7), which plays role in signal transduction in stigma cells (Takayama and Isogai 2005; Edh et al. 2009). The female determinant, *SRK* gene consists of 12 conserved cysteine residues in the *Brassicaceae* (Jung et al. 2013) and its coding region spans a length of 2.6 kb which is divided by six introns (Sato et al. 2002).

SLG gene encodes a 55 kDa glycoprotein secreted into the stigmatic papillar cell wall, is about 1.3 kb in length, and contains no introns (Sobotka et al. 2000; Jung et al. 2013). *SLG* and extracellular domain (*S* domain) of *SRK* derived from the same S-allele shares about 90% nucleotide sequence homology and in some cases exceeds 98% (Watanabe et al. 1994; Hatakeyama et al. 1998c). The consistent genetic experiment indicates the essentiality of both stigmatic proteins for the operation of SI response (Toriyama et al. 1991; Nasrallah et al. 1992; Takasaki et al. 1999) and suggesting that *SLG* work as the co-receptor of male determinant and important for *SRK* stabilization (Dixit et al. 2000; Takasaki et al. 2000; Hiscock and Allen 2008; Watanabe et al. 2003). Although the essentiality of *SLG* for the activation of SI response is more equivocal and has been questioned (Cabrillac et al. 1999; Nishio and Kusaba 2000; Silva et al. 2001), in particular, after the report of high level of class-II type *SLG* proteins expression in naturally self-compatible line of *B. oleracea* (Gaude et al. 1995). In an experiment, Suzuki et al. (2000) reported the occurrence of frame-shift mutation and non-sense mutation in *Brassica oleracea SLG* haplotypes, *S-18* and *S-60* respectively. Sato et al. (2002), observed lack of *SLG* protein in *S-24* haplotype of *Brassica oleracea* and *S-32, S-33*, and *S-36* haplotypes in *Brassica rapa*. The absence of *SLG* gene also recorded in another self-incompatible species of *Brassicaceae, Arabidopsis lyrata* (Kusaba et al. 2001; Goubet et al. 2012). These results indicate the dispensability of *SLG* female determinant in SI reaction and essentiality of *SRK* proteins rather than SLG in playing a key role in the SI reaction of *Brassica*. The evolution of *SLG* stigmatic protein has been suggested to be related to *SRK* gene duplication (Tantikanjana et al. 1993). The other *S*-locus glycoprotein (*SLG*)-related genes (*SLR1, SLR2*, and *SLR3*) have been identified, which shows no genetic linkage to *S* locus, but exhibit sequence similarity with *S*-locus glycoprotein (Scutt et al. 1990; Boyes et al. 1991; Watanabe et al. 1992; Cock et al. 1995; Tantikanjana et al. 1996; Hatekeyama et al. 1998c). Of these, *SLR2* has been shown to exhibit extensive sequence similar to *SLGs* detected from pollen-recessive haplotypes (Tantikanjana et al. 1996). Depending upon the degree of sequence similarity among *SLGs* and dominance relationships among their respective *S* haplotypes, *SLGs* were grouped into two classes (Nasrallah and Nasrallah 1993). The class-I *SLG* haplotypes show dominance in pollen, whereas *SLG* haplotypes in class II show recessiveness.

The *S*-receptor kinase (*SRK*) is essential for SI response as loss of the function experiments of *SRK* were reported to result in breakdown of self-incompatibility (Goring et al. 1993; Nasrallah et al. 1994; Sato et al. 2002), and furthermore, the importance of *SRK* in SI response was also supported by the genetic analysis of self-compatible variants of cabbage (*Brassica oleracea* var. *capitata* L.) and oilseed rape (Nasrallah et al. 1994; Goring et al. 1993). The expression level of *SLG* gene in self-compatible variants of both the species was found to be normal, in both the species mutations led to the lack of *SRK* transcripts or to the generation of truncated transcripts; thus, no functional *SRK* can occur in both the variants. These findings suggest that *SRK* gene alone plays role in SI reaction. On the other hand, the role of *SLG* in stimulating SI response might be restricted to some *S* haplotypes exhibiting extensive homology between *SLG* and *SRK*, for example, *S-29* haplotype in *Brassica rapa*.

After the identification of female determinant, *SRK*, it took a long gap of about 8 years for the detection of male determinant (*SCR*/*SP11*) of SI reaction, when two different group of researchers by employing the cloning and sequencing of *Brassica S*-locus region, reported the isolation of another polymorphic gene expressed in anther tapetum (Suzuki et al. 1999; Schopfer et al. 1999). The male determinant was first identified in *Brassica rapa* as S_9-haplotype-specific gene expressed in anther tapetum in an *SLG*/*SRK* flanking region of S_9-haplotype (Suzuki et al. 1999) and named *S*-locus protein 11 (*SP11*). Concurrently, in *Brassica rapa* itself, Schopfer et al. (1999), independently identified the different allele of the same gene in the corresponding region of S_8-haplotype as potential pollen ligand of *SRK*, and named it as *SCR* (*S*-locus cysteine-rich protein). *SCR* is basic cysteine-rich protein and is predicted to have N-terminal signal peptide (Schopfer et al. 1999). The *SCR*/*SP11* locus is adjacent to locus of female determinants (*SLG*/*SRK*) and sequence comparison of mature paternal *SCR*/*SP11* protein demonstrated that *SCR*/*SP11* exhibit extensive allelic diversity within species (with less than 50% amino-acid similarity shared by variants) (Sato et al. 2002; Okamoto et al. 2004; Kitashiba and Nasrallah 2014). The *SCR*/*SP11* variants have C1–C8 conserved cysteine residues, a glycine residue between C1 and C2 cysteines, and an aromatic amino-acid residue between C3 and C4 cysteines

(Schopfer et al. 1999; Takayama et al. 2000; Watanabe et al. 2000; Kitashiba and Nasrallah 2014). All eight cysteines play role in intramolecular disulphide bonds (Takayama et al. 2001). In addition, the gain-of-function experiments, for example, GUS reporter analysis of promoter region of *SCR*/*SP11* (Schopfer and Nasrallah 2000), RNA gel blot and in situ hybridization of transcripts of *SCR*/*SP11* protein (Takayama et al. 2000; Kusaba et al. 2002), also revealed that *SCR*/*SP11* gene is specifically expressed in anther tapetal cells at early developmental stages and in the microspores later (Takayama et al. 2000; Shiba et al. 2001). Hence, *SCR*/*SP11* act as direct pollen ligand for *SRK* specifically (Watanabe et al. 2003; Shimosato et al. 2007). Jung et al. (2012) also proved the essentiality of *SP11*/*SCR* gene as potential ligand for self-incompatibility response in *Brassica*. They obtained the self-compatible *Brassica rapa* line with the RNAi-mediated gene silencing of S_{60}-haplotype of *SCR*/*SP11* of cv. 'Osome'. Male determinant (*SCR*/*SP11*) has been proved to exhibit high allelic diversity (19.5–94% amino-acid identity) (Watanabe et al. 2003; Hiscock and Mclnnis 2003), and so far, 22 alleles, encoding small basic cysteine-rich proteins, of *SCR*/*SP11* have been reported in *Brassica* species like, *B. rapa, B. oleracea* var. *botrytis* and oilseed rape (Bi et al. 2000; Watanabe et al. 2000; Shiba et al. 2001; Hiscock 2002). Despite having high sequence variability, all *SCR* male determinants form a typical defensin-like 3D structure comprising three β-sheets and one α-helix (Mishima et al. 2003; Chookajorn et al. 2004; Yamamoto and Nishio 2014). Most of the sequence diversity in male determinant *SRK* exists within its receptor domain having hyper-variability regions for *S* specificity (Hiscock and Tabah 2003; Nasrallah 2002; Watanabe et al. 2003; Hiscock and Mclnnis 2003). The DNA sequence is now available in the NCBI database for the 20, 23, 2, and 10 *SCR* variants of *Brassica oleracea, Brassica rapa, Brassica napus*, and *Raphanus sativus*, respectively (http://www.ncbi.nlm.nih.gov/unigene) (Naithani et al. 2013).

Other players of SI response in *Brassica*

In addition to male and female determinants of SI reaction in *Brassica*, some other downstream signaling factors acting as positive or negative regulator of self-incompatibility in *Brassicaceae* have been identified (Fig. 2). Although, the exact signaling pathway of some molecules is yet obscure. Below, we have provided a brief account of other genes regulating SI response in *Brassica*.

THL1 and *THL2*

The yeast-two-hybrid assay in the *SRK* kinase domain has been instrumental in the identification of various *SRK* interacting proteins. Employing this approach, Bower et al. (1996) reported the identification of two thioredoxin-h-like proteins *THL1* and *THL2*, as putative *SRK* interactor using SRK_{910} protein kinase domain of *B. napus* as bait. When the *SRK* activating pollen coat protein consisting self-*SCR* is absent, the *THL1* protein keeps *SRK* receptor in inhibited state and autophosphorylation of receptor is impeded (Cabrillac et al. 2001). Thus, *THL1* and *THL2* are potential negative regulator of *Brassica* SI response. Furthermore, the antisense suppression of *THL1*/*THL2* mRNA in the stigmas of *Brassica napus* cv. Westar, resulted in the low level of constitutive pollen inhibition response typical to *Brassica* SI rejection response (Haffani et al. 2004).

MLPK, ARC1, and *Exo70A1*

A recessive *mod* mutation of the modifier (*m*) gene, which leads to elimination of *Brassica* self-incompatibility response, facilitated to the discovery of cytoplasmic protein kinase, *M*-locus protein kinase (*MLPK*) encoded by the *mod* locus (Murase et al. 2004). In case of *Brassica rapa*, the two distinct transcript isoforms of *MLPK* (*MLPKf1* and *MLPKf2*) are generated through alternate transcription initiation sites (Murase et al. 2004; Kakita et al. 2007a). The positional cloning of *M* locus from a self-compatible line 'Yellow Sarson' of *B. rapa* revealed that *MLPK* is having serine/threonine protein kinase activity, and membrane localization of *MLPK* by different mechanisms restores the self-pollen rejecting ability of *mm* papilla cells, confirming MLPK as essential positive regulator of *Brassica* self-incompatibility (Murase et al. 2004; Kakita et al. 2007a). The transcript *MLPKf1* localizes to papillae cell membrane via N-terminal myristoylation motif, while localization of other transcript *MLPKf2* depends upon its N-terminal hydrophobic region (Kakita et al. 2007a). Recently, the genome-wide survey and synteny analysis revealed the identification of new *MLPKf1* homologous gene *MLPKn1* in *Brassica oleracea* and both shares as high as 84.3% sequence identity (Gao et al. 2016). Both, *B. oleracea* and *B. rapa* genomes, contain three *MLPK* homologous genes, *BoMLPKf1/2, BoMLPKn1, Bol008343n* and *BrMLPKf1/2, BraMLPKn1, Bra040929*, respectively (Gao et al. 2016).

Arm-repeat-containing protein 1 (*ARC1*) was identified as another positive regulator of SI response in *Brassica* by employing yeast-2-hybrid approach with the *SRK* kinase domain as bait (Gu et al. 1998). *ARC1* is a stigma-specific plant U-Box protein having E3 ubiquitin ligase activity and is phosphorylated by *SRK–MLPK* complex in vitro (Gu et al. 1998; Stone et al. 2003; Samuel et al. 2008; Yee and Goring 2009). Thus, *ARC1* shows high specificity to *SRK* and interacts with female determinant in phosphorylation-dependent manner (Stone et al. 1999). The role of *ARC1* in SI response has been proved by different studies, suggesting *ARC1* as

potential player of self-incompatibility reaction. Stone et al. (1999) reported partial breakdown of SI response in transgenic *Brassica* with the antisense *ARC1* and this breakdown was attributed to incomplete silencing or the activity of another component of SI reaction (Stone et al. 1999). Recently, *ARC1* was also discovered in the self-incompatible species *Arabidopsis lyrata*, playing role in self-pollen rejection (Indriolo et al. 2012). Furthermore, the strong self-incompatible phenotypes were obtained in self-compatible *A. thaliana*, with the introgression of *ARC1* of *B. napus* or *A. lyrata* with SCR_b–SRK_b into the *Col-0* or *Sha* ecotypes of *A. thaliana* (Indriolo et al. 2014). Ubiquitination has been proved to control many processes in plant system including inhibition of self-pollen in self-incompatibility response of *Brassica* (Indriolo and Goring 2014). A new compatibility factor, termed negative regulator of SI response in *Brassica, 'Exo70A1'*, was identified in *Brassica* and *Arabidopsis* by using yeast-2-hybrid analysis with *ARC1* as bait (Samuel et al. 2009). *Exo70A1* has been suggested to be the substrate for *ARC1*'s ubiquitination mediated degradation pathway degrading the proteins of compatible reaction in the SI response (Samuel et al. 2009). The antisense knockdown of *Exo70A1* gene using RNA interference (RNAi) technique in the stigma of self-compatible *B. napus* cultivar, 'Westar' resulted reduction in pollen grains count on the stigmatic surface after pollination (Samuel et al. 2009). Furthermore, the control of *Exo70A1* by *SLR1* promoter (Franklin and Centre 1996) resulted in partial breakdown of self-incompatibility in the SI line of *B. napus*, 'W1' (Samuel et al. 2009). The *Exo70A1* has been suggested to play role in pollen grains hydration, germination, and then pollen tube penetration (Samuel et al. 2009). Eventually, it was suggested that *Exo70A1* protein acts at the intersection of two cellular pathways, where it is essential in the stigma for the acceptance of compatible pollen in both *Arabidopsis* and *Brassica* and is negative regulator of self-incompatibility in *Brassica*.

JDP1

By employing yeast-2-hybrid approach against cDNA library from stigma of ornamental kale (*Brassica oleracea* var. *acephala*), another protein was identified recently by Lan et al. (2015), which is J domain protein 1 (*JDP1*) that interacts with *ARC1*. *JDP1*, a member of heat shock protein 40 (*Hsp40*) family, having 344 amino acids is a 38.4-kDa protein. The N-terminus of *JDP1* ($JDP_{1–68}$) and C-terminus of *JDP1* ($JDP1_{69–344}$) contains a J and X domain, respectively (Lan et al. 2015). The C-terminus of *JDP1* is sufficient for interaction with *ARC1* and Tyr^8 in the *JDP1* N-terminal region is the specific site for *JDP1* and *ARC1* interaction. However, the exact role of *JDP1–ARC1* complex in regulating the SI response in *Brassica* is yet to be elucidated.

KAPP, Snx1, and *Calmodulin*

Besides above-stated positive and negative regulators of *Brassica* SI response, *KAPP* (kinase-associated protein phosphatase), sorting-nexin1 (*Snx1*), and calmodulin have been reported to be other putative interactors of *SRK* (Braun et al. 1997; Vanoosthuyse et al. 2003). *KAPP* is a membrane-anchored type 2C protein phosphatase, which by its kinase interaction domain interacts with phosphorylated receptor-like kinase (RLK) of plants (Braun et al. 1997; Shah et al. 2002; Vanoosthuyse et al. 2003; Manabe et al. 2008). Furthermore, *KAPP* is suggested to inactivate the functioning of *RLK* by dephosphorylation process (Tichtinsky et al. 2003), and thus, different genetic studies speculate *KAPP* to be a negative regulator of *RLK* pathways (Williams et al. 1997; Stone et al. 1998; Vanoosthuyse et al. 2003; Manabe et al. 2008). Eventually, it was suggested that *Brassica* female determinant *SRK* interact with *Brassica* homolog of *KAPP* and this interaction leads to dephosphorylation of *SRK*, indicating downregulation of *SRK* proteins by *KAPP* (Vanoosthuyse et al. 2003). Thus, *SRK* acts as a substrate for phosphatase activity of *KAPP*. *Brassica* homolog *KAPP* protein extracted from *Brassica* stigma cDNA library shares 80.1% amino-acid identity with Arabidopsis *KAPP* (Vanoosthuyse et al. 2003). The other two proteins, *Snx1* and calmodulin, also interact with *SRK* and these have been reported to downregulate *RLK* in animals. The interaction of calmodulin and kinase domain of *SRK* was confirmed by calmodulin-Sepharose affinity binding approach (Vanoosthuyse et al. 2003). It was reported that calmodulin is not phosphorylated in this calmodulin–*SRK* interaction and has no direct effect on *SRK* kinase activity, implicating that calmodulin does not act as a substrate for *SRK* kinase activity. Furthermore, the *Brassica* sorting nexin homolog (*BoSNX1*) was isolated as SRK_{29} kinase domain interactor via yeast-2-hybrid screening (Vanoosthuyse et al. 2003). The genome-wide sequence analysis showed great similarity of *Brassica SNX1* with *Arabidopsis* and *Human SNX1* protein. The *BoSNX1* was suggested to interact with *kinase* domain of *SRK* indicating that *SNX1* regulate many plant receptor kinase (*PRK*) signaling pathways.

rdr6 mutation enhancing SI

Using transgenic self-incompatible *A. thaliana* model and mutants screening, a recessive mutation was identified in the RNA-dependent RNA polymerase *rdr6* producing trans-acting short interfering RNA (ta-siRNA), which results in exsertion of stigma and enhancing SI response, without simultaneous increase in level of *SRK* transcripts. This stigma exsertion in the *rdr6* mutant background is attributed to pistil elongation due to S-locus receptor kinase (SRK) catalytic activity (Tantikanjana et al. 2009). Thus, trans-acting short interfering

RNA pathways regulate the potential positive regulators of SI response and pistil development depicting dual role of *SRK*, in SI response and pistil development owing to coordinate evolution of these mechanisms.

Proteomics in understanding of SI

There are a few other different stigmatic proteins and have also been identified in *Brassicaceae* with respect to SI response. Proteomics approaches have been widely used in unravelling the SI response in *Brassica* and have extended our understanding of this enigma to great extent. The proteome research in SI has progressed from the use of isoelectric focusing in last three decades to the current third generation technique of comparative isobaric tag for relative and absolute quantification (iTRAQ) (Sankaranarayanan et al. 2013a, b). The role of multiple modifier genes in weakening the self-incompatibility or causing pseudoincompatibility has been reported by genetic studies of transgenic self-incompatible *A. thaliana* (Nasrallah et al. 2004; Boggs et al. 2009b). Samuel et al. (2011), utilized three-dimensional difference gel electrophoresis technique (2-D DIGE) and compared the stigmatic proteins of self-incompatible *Brassica napus* at 0 and 60 min after self-pollination and observed that tubulin and microtubule network were linked to SI reaction. They further observed the breakdown of microtubule network (MT) in the compatible pollinations, which was suggested to be mediated by exocyst component, *Exo70A1*, leading to successful fertilization and seed set. Recently, Wang and associates (2014) identified 25 protein spots with distinct activity in self-incompatible and compatible lines of non-heading Chinese cabbage by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF/TOF MS) and peptide mass fingerprinting (PMF), out of which 22 protein spots were confidently established. Then, by employing quantitative RT-PCR technique, mRNA levels of the corresponding genes were identified and suggested that UDP–sugar pyrophosphorylase could have role in sucrose degradation to affect pollen germination and growth. Furthermore, they also suggested the role of glutathione S transferases in pollen grains' maturation, and affecting the pollen fertility and concurrently, the senescence-associated cysteine protease could be related to self-pollen recognition of non-heading Chinese cabbage (Wang et al. 2014). Thus, there are several lines of evidence which support the role of multiple signaling pathways in *Brassica* SI response (Tantikanjana et al. 2010).

Molecular mechanism and interaction of Brassica SI-signaling components

A schematic model of interaction of *Brassica* SI-signaling components is presented in Fig. 3. When there is self-pollination, the pollen ligand (*SCR*/*SP11*) and receptor (*SRK*) interaction from the same *S* haplotype induces autophosphorylation of *S*-receptor kinase in an *S*-haplotype-specific manner (Takayama et al. 2001; Kachroo et al. 2001, 2002; Watanabe et al. 2012). The autophosphorylated *SRK* elicit a signal transduction cascade in stigma papilla cells that results in the pollen rejection and inhibition of pollen tube penetration into the epidermal cell wall of stigma and induces SI response (Kao and McCubbin 2000; Ivanov and Gaude 2009; Watanabe et al. 2012; Nasrallah and Nasrallah 2014; Yamamoto and Nishio 2014). In contrast, when there is cross pollination, the pollen ligand (*SCR*) can neither bind nor activate *SRK* kinase domain and no pollen rejection signaling cascade is triggered, hence leads to development and penetration of pollen tube into the stigma papillae. It is the extracellular *S*-domain region of *SRK* which comprises hypervariable subdomains for haplotype-specific pollen ligand (*SCR*/*SP11*) binding (Kemp and Doughty 2007; Shimosato et al. 2007; Boggs et al. 2009a; Jung et al. 2013). Despite the identification of both ligand and receptor, the downstream mechanism behind the *SRK* signal transduction was not understood. To elucidate the *SRK*-signaling cascade, various *SRK* interactors were identified and characterized (Gu et al. 1998; Stone et al. 1999, 2003; Cabrillac et al. 2001; Murase et al. 2004; Kakita et al. 2007a, b; Leducq et al. 2014). Two thioredoxin-h-like proteins, *THL1* and *THL2* (Bower et al. 1996; Cabrillac et al. 2001), are putative interactor of *SRK* which maintain *SRK* in an inactive state in unpollinated stigmas (Cabrillac et al. 2001; Haffani et al. 2004; Jung et al. 2013). Furthermore, *MLPK* (*M*-locus protein kinase) was identified as another positive interactor of *SRK* (Murase et al. 2004). The kinase domain of female determinant, *SRK*, interacts with *MLPK* and forms *SRK–MLPK* complex. This *SRK–MLPK* complex induces signal transduction to stigma papilla cell and causes self-pollen rejection (Gao et al. 2016). Thus, *MLPK* act as positive regulator of SI response. Another positive regulator of SI response is *ARC1* (Gu et al. 1998; Stone et al. 2003; Samuel et al. 2008), which shows high specificity to *SRK*. When *ARC1* interact with *SRK*, the *SRK–MLPK* complex induces phosphorylation of *ARC1* via kinase domain of *SRK*. This phosphorylated *ARC1* interact with *Exo70A1*, which is putative component of exocyst complex (Samuel et al. 2009). This phosphorylated *ARC1* degrade the *Exo70A1* and inhibit exocytosis. Thus, it is speculated that these phosphorylated pathways prevent or degrade compatibility

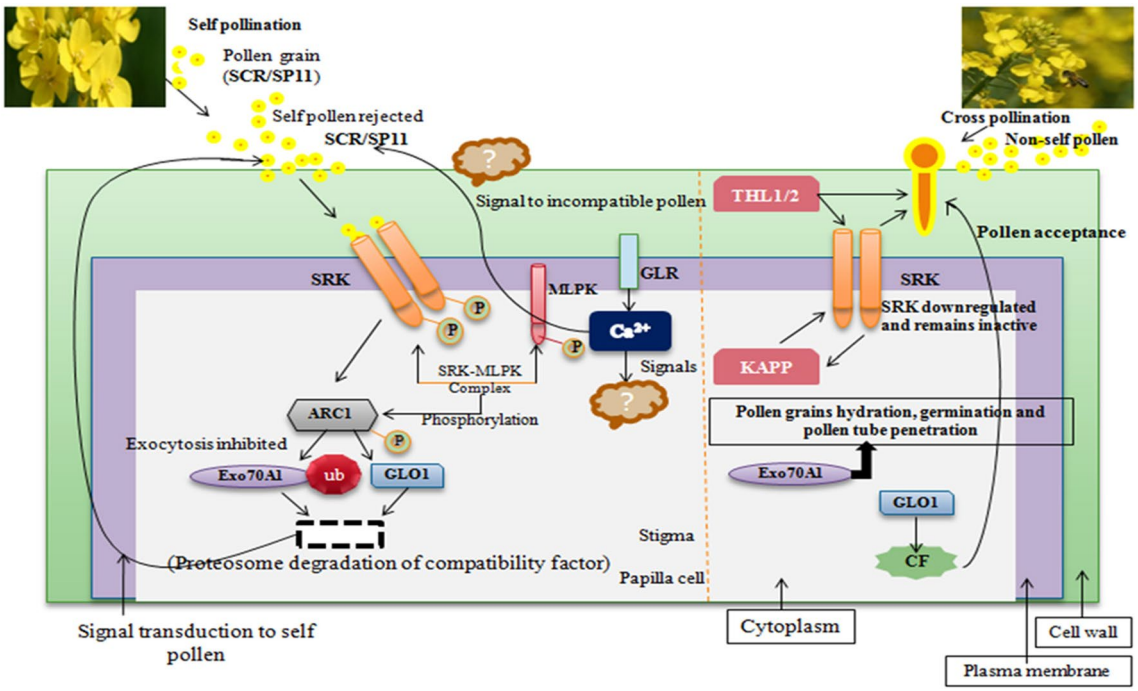

Fig. 3 Proposed schematic model of interaction of SI-signaling components in *Brassicaceae*. *SCR/SP11* S-locus cysteine-rich protein/S-locus protein 11 (Suzuki et al. 1999; Takayama et al. 2000); *SRK* S-receptor kinase (Stein et al. 1991); *SLG* S-locus glycoprotein (Nasrallah et al. 1985; Suzuki et al. 1995); *THL1/THL2* thioredoxin-h-like proteins (Cabrillac et al. 2001); *ARC1* armadillo-repeat containing protein 1 (Gu et al. 1998); *MLPK M*-locus protein kinase (Murase et al. 2004); *Exo70A1* Exocyst subunit exo70 family protein A1 (Samuel et al. 2009); *KAPP* Kinase-associated protein phosphatase (Braun et al. 1997; Vanoosthuyse et al. 2003); *GLO1* Glyoxylase 1 (Sankaranarayanan et al. 2015); *GLR* glutamate-like receptor (Iwano et al. 2015); *CF* compatibility factors for compatible pollination (Sankaranarayanan et al. 2015)

factors secretion to stigma and result in inhibition of pollen tube penetration (Ivanov et al. 2010; Lan et al. 2015). Another protein, a kinase-associated protein phosphatase (*KAPP*), also interacts with phosphorylated *SRK* kinase domain (Braun et al. 1997) and leads to dephosphorylation of *SRK*, thus downregulating the *SRK*. Hence, *KAPP* act as a negative player of self-incompatibility response. Sankaranarayanan et al. (2015) reported 'glyoxalase 1 (*GLO1*)', as another compatibility factor which is downregulated by *ARC1*-mediated reaction in SI-signaling pathway. *GLO1* is a compatible protein in stigma required for pollination to occur and its suppression leads to reduction in compatibility, and thus leads to SI response. On the other hand, the over expression of *GLO1* induces partial breakdown of self-incompatibility, as reported in self-incompatible stigma of *B. napus* (Sankaranarayanan et al. 2015). Recently, the role of cytoplasmic Ca^{2+} was reported in self-incompatibility reaction in *Brassicaceae* (Iwano et al. 2015; Franklin-Tong 2015). When there is self-pollination, incompatible pollen lands on stigma and the ligand–receptor interaction lead to increase in cytoplasmic Ca^{2+} in stigma papilla cells. Therefore, stigma papilla cells use this cytoplasmic calcium influx to give signal to incompatible pollen to inhibit hydration, germination and pollen tube growth on stigmatic surface. However, it is still obscure that how this cytoplasmic Ca^{2+} influx in stigma papilla cells triggers signal transduction to incompatible pollen which results in self-pollen rejection and ultimately leads to self-incompatibility response.

Molecular genetics of dominance relationships between S haplotypes

In the stigma of self-incompatible *Brassica*, the dominance relationships between S haplotypes are determined by stigmatic female determinant, S-receptor kinase (*SRK*) by post-transcriptional regulation (Hatakeyama et al. 2001). Regarding dominance relationships on pollen side, the pollen ligand S-locus protein 11 (*SP11*)/S-locus cysteine-rich protein (*SCR*) from pollen-recessive S haplotypes have not been observed yet. Although, it has been suggested that a set of *SLG* and *SRK* Class-II S haplotypes is present in pollen-recessive S haplotypes, while a set of class-I S haplotypes is present in pollen-dominant S haplotypes (Nasrallah and Nasrallah 1993; Shiba et al. 2002). The class-II SLGs pollen-recessive S haplotypes have been found in *Brassica rapa* (S_{29}, S_{40}, S_{44}, S_{60}) (Hatakeyama et al. 1998c; Takasaki

et al. 2000) and *Brassica oleracea* (S_2, S_5, S_{15}) (Chen and Nasrallah 1990; Scutt and Croy 1992; Cabrillac et al. 1999). Shiba et al. (2002) and Kakizaki et al. (2003) suggested that dominance/recessive relationships of S haplotypes in *Brassica* pollen are regulated by transcription of pollen ligand *SP11*/*SCR*. They observed significant reduction in the expression (mRNAs) of recessive pollen ligand *SP11* in the anther tapetum cells of *S*-heterozygous plants, while dominant *SP11* predominantly expressed. Furthermore, Kakizaki et al. (2003) depicted the role of epigenetic regulation, as the dominance/recessiveness of S_{44}, $S_{40,}$ and S_{60} S haplotypes could be altered. Furthermore, the role of DNA methylation, which is instrumental in modifying eukaryotic genomes and altering gene expression, was confirmed in governing dominance relationship between S-alleles by Shiba et al. (2006). They reported *de novo* DNA methylation at the promoter region of recessive allele-specific pollen ligand *SP11* in anther tapetum before initiation of its transcription. Thus, suggested the role of DNA methylation in silencing of recessive allele-specific gene *SP11*, hence controlling dominance relationship between S haplotypes in *Brassica*. This DNA methylation of promoter regions of recessive pollen ligand *SP11* is activated by 24-nucleotide trans-acting small non-coding RNA (sRNA) produced by dominant allele and causes mono-allelic gene silencing (Tarutani et al. 2010; Durand et al. 2014). Recently, Yasuda et al. (2016) put forward a 'polymorphic dominance modifiers' model as the mechanism responsible for dominance hierarchy of S haplotypes produced by receiving polymorphic sRNA (small RNA) and their targets. They proved that single polymorphic sRNA, '*Smi2*' (*SP11* methylation inducer 2), and its polymorphic targets govern the linear dominance relationship of class-II S haplotypes ($S_{44} > S_{60} > S_{40} > S_{29}$) in *Brassica rapa*. Hence, as the genetic elements, *SP11* methylation inducer (*Smi*) and *SP11* methylation inducer 2 (*Smi2*) correspond to 'dominance modifiers' in *Brassica rapa* and control the dominance hierarchy of S haplotypes (Tarutani et al. 2010; Yasuda et al. 2016).

Molecular markers and S-haplotype identification

The genetic mechanism of SSI has been commercially utilized for hybrid seed production in *Brassicaceae*. Thus, the discrimination of S-alleles is useful not only for the study of self-incompatibility, but also for the commercial hybrid seed production. Traditionally, S haplotypes have been identified by controlled pollination with S-allele tester lines, followed by fluorescence microscopic observation of pollen tube growth through stigma or by seed set data. The technique of counting seed set data is labor and time consuming, though the fluorescent microscopy is relatively fast, but it is essential to have the plants in flowering stage. In addition, the ideal plant selection having recessive traits, such as SI, is often difficult and thus a potential barrier in accelerating plant breeding. In this regard, the use of molecular marker technology for the S-haplotype identification is advantageous that it could be done on young plants and no need to have the S-allele plants in flowering phase (Brace et al. 1994). Hence, the development and identification of molecular markers linked with self-incompatible genes are of utmost value for geneticist and plant breeders to discriminate between class-I and class-II S haplotypes (Havlickova et al. 2014). Different kinds of molecular markers have been reported for the identification of S haplotypes in *Brassica* species by different groups of researchers (Table 1), although all the S haplotypes have not been identified yet.

Maintenance of S-allele lines

The genetic mechanism of sporophytic self-incompatibility has been proved effective for commercial hybrid seed production in *Brassica* crops across the world (Wang et al. 2007; Koprna et al. 2005; Hamid et al. 2010; Kucera et al. 2006). For the continuous development of F_1 hybrids using SI phenomenon, the maintenance of parental S-allele lines is one of the basic requirements (Singh and Vidyasagar 2012). The maintenance of SI lines is a costly affair. In the course of time, the number of techniques has been identified and utilized commercially to overcome the SI reaction for the successful maintenance of parental S-allele lines. The method being followed usually for the large-scale seed production is manual sib mating in bud stage or bud pollination (Cabin et al. 1996; Hamid et al. 2010). However, bud pollination is time consuming, tedious, and labor intensive method. The other techniques being adopted are exposing the plants to high concentration of CO_2 (3–5%) and relative humidity (100%) in air-tight growth chamber for a period of 8–24 h (Palloix et al. 1985; Nikura and Matsuura 2000; Kwun et al. 2004), chemical treatments such as plant lectin (Sharma et al. 1984), phytohormones (Matsubara 1985), KOH (Tatebe 1968), and common salt (NaCl) solution sprays (Wang et al. 2012; Singh and Vidyasagar 2012). The other alternative methods being suggested are electric aided pollination (Roggen et al. 1972), thermally aided pollination (Roggen and Van Dijk 1976), steel-brush pollination (Roggen and Van Dijk 1972), pollen coat extracts (Roggen 1975), high-temperature treatments (Gonai and Hinata 1971), double pollination, use of mixed pollen, and delayed pollination (Kakizaki 1930). The efficiency of these methods depends upon the level of self-incompatibility, activity of S-alleles, genetic background, flower age, type of plant, species, and incompatibility system. All these treatments lead to temporary breakdown of self-incompatibility in *Brassica*.

Table 1 Molecular markers for S-haplotype identification in *Brassica* species

Brassica crop	S haplotype Types/SI Gene	Type of marker	Population used	References
Brassica oleracea L.	*SLG, SRK and SLR*	PCR-RFLP	*B. oleracea* homozygous S-allele lines	Brace et al. (1994)
Chinese cabbage	Two Class-I *SRK* haplotypes (P1, P2) and Two Class-II *SRK* haplotypes (P3, P4)	PCR-RFLP	24 DH lines	Tingting et al. (2013)
Broccoli, cabbage	Class-II and Class-I *SLG* alleles	PCR-RFLP	31 F_1 hybrid cultivars of broccoli and cabbage	Sakamoto et al. (2000)
Radish	Class-I and Class-II *SLG*/*SRK* haplotypes	PCR-RFLP	24 homozygous breeding lines belong to 10 S haplotypes	Lim et al. (2002)
Chinese cabbage, *B. rapa* ssp. *chinensis* var. *purpurea*, cauliflower, cabbage, mustard	Class-I and Class-II *SLG* Class-I and Class-II *SRK*	PCR-RFLP	72 genotypes of 5 Brassica vegetables	Wang et al. (2007)
B. rapa	Class-I and Class-II *SLG* haplotypes	PCR-RFLP	F_1 and open pollinated cultivars of *B. rapa*	Sakamoto and Nishio (2001)
Broccoli	Class-I and Class II *SRK* Class-I and Class-II *SCR*	PCR based gene specific SRK and SCR/SP11 primers	18 DH lines	Yu et al. (2014)
Cabbage, broccoli	Class-I and Class-II *SRK*	PCR-CAPS marker	Inbred lines of cabbage and broccoli	Park et al. (2002)
Brassica napus	*SLG, SLR*	PCR-CAPS	F_2 population and *B. napus* SI lines	Mohring et al. (2005)
Brassica napus	Class-I and Class-II *SRK, SLG, SP11*	CAPS, SCAR	F_2 population of SI and self-compatible lines	Zhang et al. (2008)

The in vitro tissue culture technique can accelerate the F_1 hybrid breeding programme in *B. oleracea* vegetables by employing this technique in maintenance of parental inbred lines of F_1 hybrids. Different researchers have reported the use of in vitro tissue culture techniques for the maintenance and multiplication of SI lines in *Brassica* (Sanghera et al. 2012; Bhatia et al. 2013).

Recently, Lao et al. (2014) carried out the physiological and genetic analysis of CO_2-induced breakdown of SI in *Brassica rapa*. With the use of X-ray microanalysis, they suggested that self-incompatibility breakdown in S-allele line was accompanied by significant accumulation of calcium (Ca) at the pollen–stigma interface. The genetic analyses using F_1 and F_2 progeny of a cross between CO_2-sensitive × CO_2-insensitive line suggested that CO_2 sensitivity is a semi-dominant and quantitative trait, which is under the control of more than one gene. Furthermore, two major loci, *BrSIO1* and *BrSIO2*, were identified using QTL analyses, which act additively in overcoming SI response during CO_2 treatment (Lao et al. 2014).

Recently, Tantikanjana and Nasrallah (2015) suggested a new alternative technique, ligand-mediated cis-inhibition of receptor signaling for the control of SI mechanism in *Brassica*. The use of cis-*SCR* transgenes, or *SRK* off switches, is suggested to have practical implication for both maintenance of parental S-allele lines and commercial hybrid seed production. The cis-inhibition of *SRK* is mediated by allele-specific ligand–receptor interaction (Tantikanjana and Nasrallah 2015). The *SRK* receptor is entrapped in the endoplasmic reticulum by the stigma-expressed *SCR* and thus inhibits the exact targeting of *SRK* to the plasma membrane, where receptor–ligand interaction takes place.

Conclusion

As outlined in this review, the *Brassica* SI response is controlled by multiple signaling pathways and is a complex phenomenon. The mechanism of SSI is most interesting phenomenon in the angiosperms which promote out-breeding in plants. The molecular genetics and physiology of SI pathway in *Brassicaceae* have been extensively studied. As we presented, much progress has been made towards the understanding of downstream complex SI-signaling pathways. However, still, there are many aspects which have not been elucidated yet and current research is going on side by side to understand the downstream SI-signaling events occurring in stigmatic papillae following the pollination. The use of proteomic approach has also been reported to better understand the mechanism of SI response in *Brassica*

vegetables and potential accumulating pistil proteins have been identified especially in Chinese cabbage. Recently, the role of glutamate receptor-like channel (*GLR*) gene mutants in mediating enhancement of cytoplasmic Ca^{2+} influx in stigma papilla cells was reported, which transmit signal to incompatible pollen and causes self-pollen rejection. However, this signaling pathway via cytoplasmic Ca^{2+} to incompatible pollen is yet to be elucidated. Then, further elucidation of interaction of *GLR* and Ca^{2+} with other *Brassica* SI-signaling components will help in understanding other molecular pathways involved in *Brassica* self-incompatibility. Furthermore, the other genomics and next generation sequencing (NGS) approaches can be extended to different *Brassicaceae* crops for the identification of candidate proteins functioning in the SI-signaling pathways. In addition, the downstream molecular events involved in overcoming or temporary breakdown of SI response in *Brassica* with various techniques are still not elucidated. With the progress in use of advanced biotechnological, physiological, and molecular approaches, it would be possible to elucidate the multiple complex mechanisms involved in SI response and it will further enhance our understanding of multiple *Brassica* SI-signaling pathways to new heights in near future. In addition, it will act as a catalyst to boost the F_1 hybrid seed production of *Brassicaceae* crops.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest with respect to this paper.

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