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Self-Incompatibility in Vegetable Crops – A Review

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INTRODUCTION

Self-pollination in hermaphrodite flowers leads to inbreeding depression and decreased genetic variation; therefore plants have evolved several mechanisms to avoid self-pollination. One of the most important mechanisms in many flowering plants is self-incompatibility (SI). Self-incompatibility (SI) is defined as "the inability of a fertile hermaphrodite seed plant to produce zygotes after self-pollination (Nettancourt, 1977). It is the genetic mechanism of avoiding self-fertilization which promotes heterozygosity and prevent inbreeding depression. Self-incompatibility in angiosperms is believed to be a change asset owing to its potency in avoiding inbreeding and promoting out crossing. About 40%-60% of all species of flowering plants are considered to be self-incompatible (Igic *et al.*2008). Self-incompatibility (SI) is one of the critical systems used by many plant species to avoid self-fertilization, developing and sustaining genetic diversity. The SI response is comprised of a self - and non self-recognition process between pollen and pistil that is followed by selective inhibition of the self-pollen (tube) development. In this phenomenon, pollen grains are functional but fail to fertilize egg cell of the same plant. In this case, pollen grains fail to germinate on the stigma of flower that produced them. But if some pollen grains do germinate, they fail to enter the stigma. In some cases, there is the very slow growth of pollen tubes after entering the style that the flower drops before the fertilization occurs. In few cases, fertilization is enforced but the embryos degenerate at an early stage.

Self- incompatibility was first reported by Koelreuter in middle of eighteenth century. It has been extensively studied in different plant families after the first discussion on self-incompatibility by Darwin (1877). Term self-incompatibilty is given by Stout in 1917.

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Bateman reported incompatibility in three brassica plants namely *Iberis amara* L., *Raphanus sativus* L. and *Brassica campestris* L. and now a compelling amount of knowledge is accessible on genes and gene products involved in the expression of SI trait (Dodds *et al* 1997).

SI is classified into two categories viz., heteromorphic system and homomorphic system. In the heteromorphic system, there are morphological differences among the flowers of the same plante.eg., in primula (*Primula* species) and buckwheat (*Fagophyrum* species) there are two types of flowers pin (ss) having long style and short stigma and thrum having a short style and long stigma (Ss). This character is controlled by a single gene S; having two alleles. The alleles for short style are dominant over long style. The only compatible mating is between pin and thrum flowers and progeny have pin and thrum flowers in 1:1 ratio.

Heteromorphic self-Incompatibility

CROSSES		**RESULTS**	
Phenotype	**Genotype**	**Genotype**	**Phenotype**
Pin x Pin	ss x ss	Incompatible mating	
Pin x Thrum	ss x Ss	1 Ss :1 ss	1 Thrum :1 Pin
Thrum x Pin	Ss x ss	1 Ss : 1 ss	1 Thrum :1 Pin
Thrum x Thrum	Ss x Ss	Incompatible mating	

In some plant another situation called tristyly occurs. In this case style are of three length:, style short, stamens medium and long , style medium, stamens short and long, style long, stamens short and medium. Tristylic species are less widely distributed, found in *Lythraceae and Oxalidaceae* family.

In the homomorphic system, there is no association with morphological differences among flowers. The incompatibility reaction may be regulated by the diploid genome pollen producing plant (sporophytic control) or by the haploid genome of the pollen (gametophytic control).

Gametophytic incompatibility was first described by East and Mangelsdorf in 1925 in *Nicotiana sanderae*. In this, the incompatibility reaction of pollen is controlled by its own genotype, not by the genotype of plant on which it is produced. This is because the

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biochemical substances involved in SI reaction are produced after meiosis. This reaction may be controlled by one or two genes. In this, growth of the pollen tube arrests in the style.

Sporophytic SI was first reported by Hughes and Babcock in *Crepisfoetida* and by Grestel in *Parthenium argentatum* in 1950. In this outcome of the interaction between the pollen tube and the style is determined by the genotype of the sporophyte (diploid tissue). In this, growth of the pollen tube arrests at the surface of the stigma. All types of *Brassica oleracea* acquire a sporophytic SI system which diversifies in effectiveness in the different crop types, being strongest in kale and weakest in summer cauliflower (Watts 1963).

Genetic basis of self-incompatibility

The self-incompatibility reaction is controlled by a single locus S having multiple S-alleles. The number of S-alleles in the gametophytic system is reported to be more than the sporophytic system. The alleles show co-dominance in case of gametophytic system and the progeny will be incompatible, half-compatible or fully compatible while in sporophytic system alleles show dominance, co-dominance and sometimes competition and resultant progeny will be compatible or incompatible. The S alleles of brassica have been organized in a dominance series based on their genetic behavior relative to other alleles in heterozygous plants (Thomson and Taylor 1966). According to a classical genetic analysis, brassica S alleles are grouped into two categories which are based on their phenotypic effect on self-incompatibility characteristics. In the first group, the alleles have strong self-incompatibility phenotype with an average of 0 to 10 pollen tubes develop per self-pollinated stigma and are placed relatively high on the dominance scale. The second group's alleles are considered to be recessive and have weak self-incompatibility phenotypic effect with 10 to 30 pollen tubes develop per self-pollinated stigma.

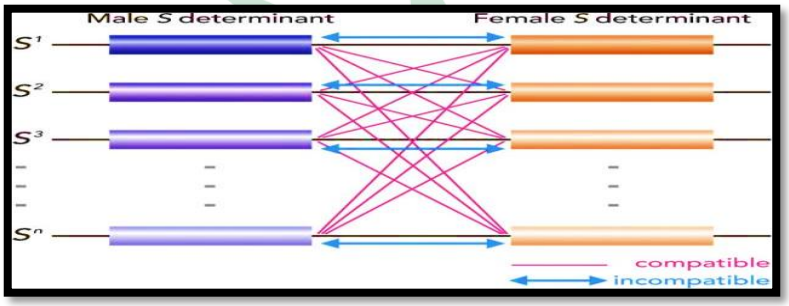

Fig. 1: Schematic model of the S locus

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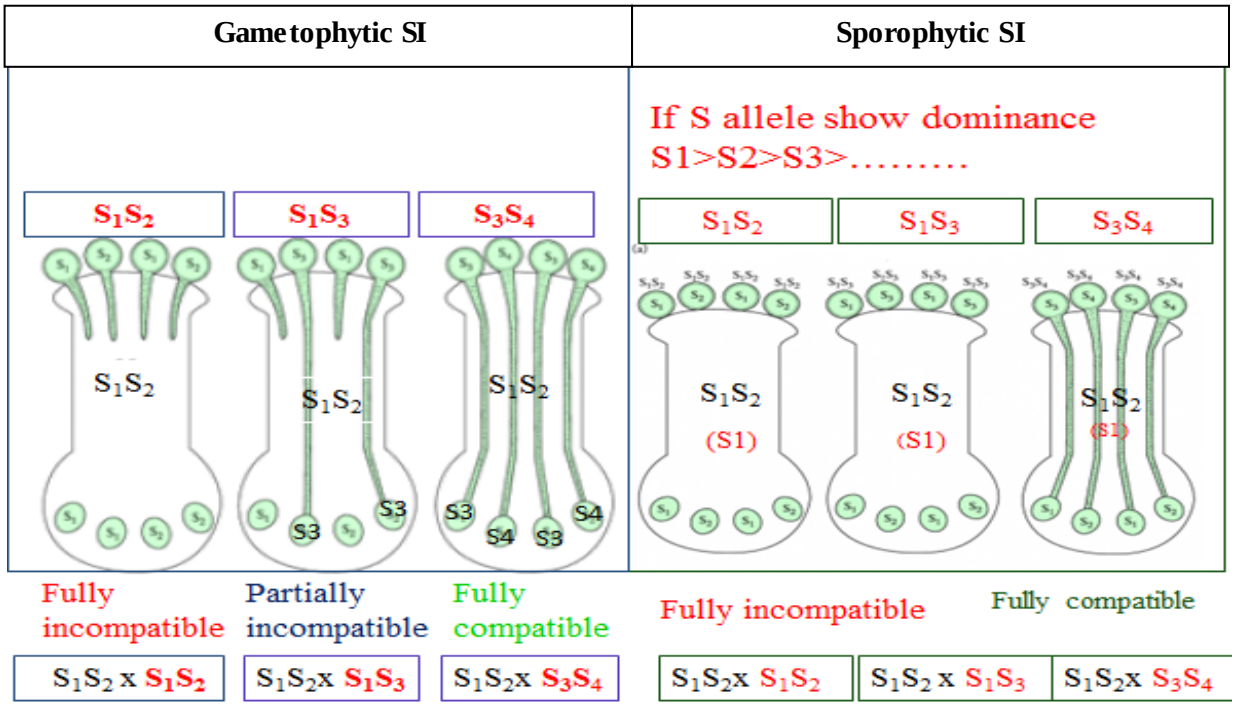

Fig. 2: Gametophytic and Sporophytic system of self- incompatibility

In gametophytic system, the stigma surface is plumose having elongated receptive cells and is commonly known as wet stigma. Incompatible pollen grains generally germinate on reaching the stigma. The incompatibility reaction occurs at a later stage without the stigma. There are clear cut serological differences among the pollen grains with the different S genotypes.In sporophytic system, pollen grain has an outer lipidic coating superficial layer (CSL), which is thin layer deposited by tapetum on the pollen grain just prior to dehydration. Below the coating superficial layer is the main outer coating of pollen called tryphine, which contain factors that give rise to the maternal control of pollen reaction. The stigma in sporophytic system is papillate and the papillae are covered with a pellicle or sheath. The pellicle is protenaceous in nature; it may be covered with a layer of wax that varies in thickness. When compatible pollen lands on a stigma, pollen CSL fuses with the papillae. The pollen hydrates and germinates on papillae, the pollen release a cutinase, which digest the cuticle and allows the pollen tube to grow inside the cellular pectin layer of papillae.

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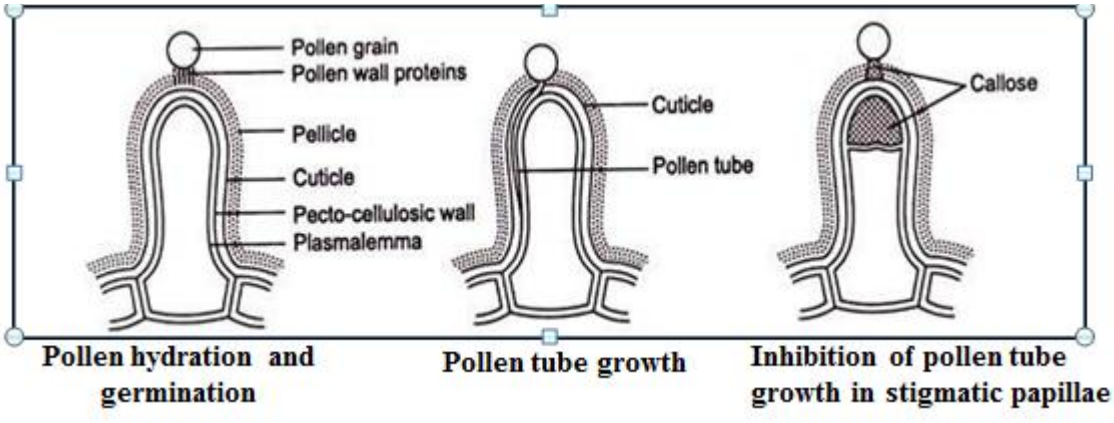

Fig.3: Pollen - Stigma interaction in SSI

Molecular basis of self-incompatibility

Self-incompatibility system has been studied extensively in case of Solanaceae and brassicaceae family of vegetables. It involves two proteins located at S locus. One is male determinant and other is female determinant.

In case of brassica, the self-recognition determinants are SRK (S-locus receptor kinase) present in stigma and SP11/SCR (S-locus cysteine-rich protein) which is present in the pollen. The genes of both the determinants have multiple alleles in a species and closely linked with each other in the S-locus complex. There is higher variability in the structure of S-locus and this high structure polymorphism is studied to be necessary for abolishing the SRK and SP11 recombination, which may result in the breakdown of self-incompatibility. There is another protein located near SRK namely SLG (S-locus glycoprotein; Nasrallah *et al* 1985) which is suggested to arise from duplication of the S domain of SRK. It enhances the SI recognition reaction (Takasaki *et al* 2000) by stabilizing it in transgenic plants. The SP11 protein is expressed in the tapetum cells of anther locules, which accumulates on the pollen surface on the maturation of pollen grains. SP11 molecules enter into the papilla and interact with SRK in a specific manner in case of self-pollination. Then there is phosphorylation of SRK which further attach phosphorus groups to other proteins present in stigma such as MLPK (M-locus protein kinase). After this, there is subsequent signal transduction, which has not yet determined which leads to rejection of self-pollen (Watanable *et al*2012).

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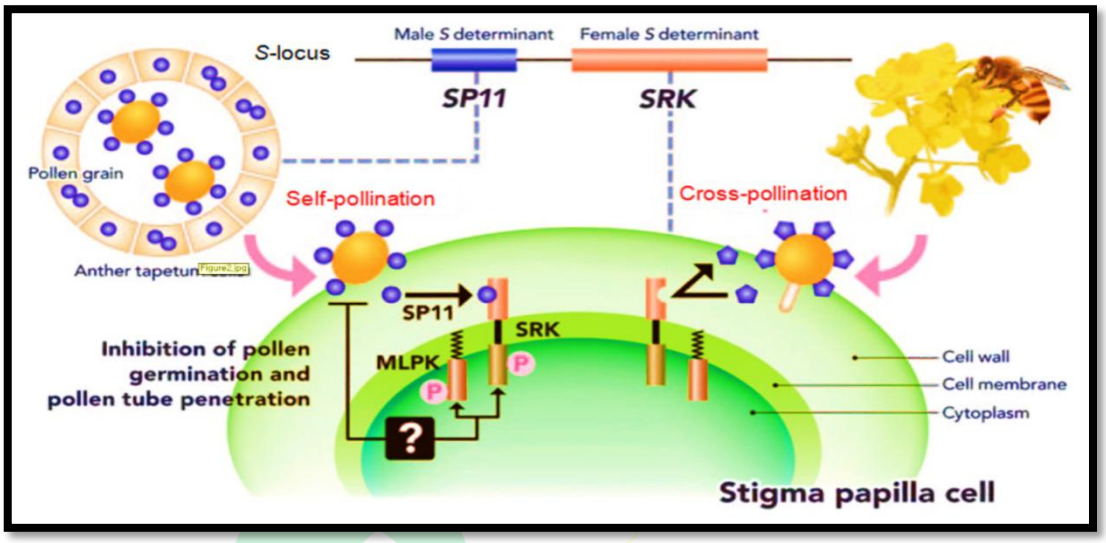

Fig.4: Mechanism of Sporophytic SI in Brassicaceae

Molecular mechanisms of dominance relationships at the pollen side

Dominance relationships of SI are regulated by transcription of SP-11. In Sprorophytic heterozygous plant,SP-11 derived from dominant allele is normally expressed whereas expression of recessive SP-11 is suppressed and not detected on RNA gel blot analysis. It was also demonstrated that small RNA produced from dominant allele could activate methylation of recessive allele and repress SP-11 at transcriptional level.

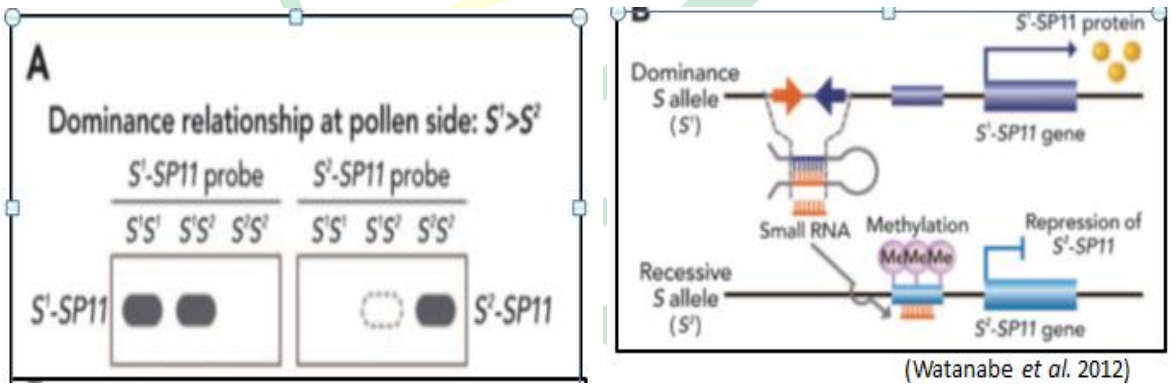

Fig.5: dominance relationships at the pollen side in SSI

In case of Solanaceae, there is S-RNase-Based SI compatibility system. In this the incompatible pollen grains germinates on the stigma surface and grows through the

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transmitting tract of style and this growth is arrested when it has occupied the one-third of the way through the style. There is SFB (S-locus F Box protein) present in the pollen tube which is said to the male determinant. When pollen tube grows through the style, the S-RNase present in the stigma enters into the pollen tube and then degrades its rRNA arresting pollen tube growth. But when there is compatible pollen grain the S-RNase binds with the pollen SFB on which ubiquitin is attached and form SFB complex. The ubiquitin destroys the damaged protein present in living tissues and helps in synthesizing new proteins (Takyama and Isogai, 2005).

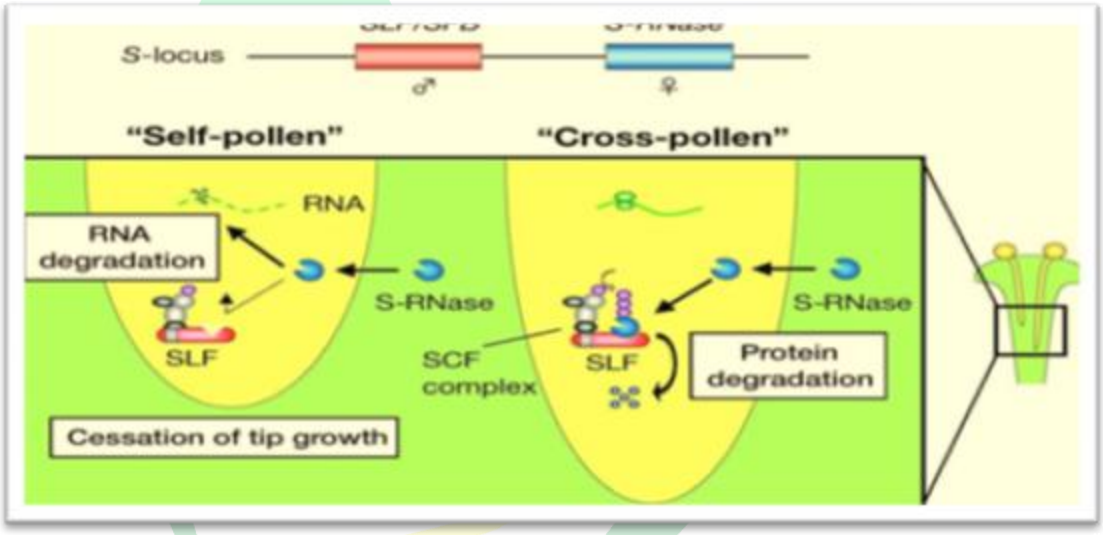

Fig. 6 : Model for Molecular Mechanism of SI in Solanaceae

Need to use of self-incompatibility in the hybrid breeding of vegetable crops

Hybrids are of great economic importance in various cross-pollinated crops but due to higher seed cost of seed production, it is less used in self-pollinated crops.Self-incompatibility in the hybrid breeding is used to increase the productivity, to reduce the cost of hybrid seed, to develop hybrids easily and to reduce the duration of hybrid seed production. A number of reasons witness the evolution of self-fertilized species from cross-fertilized and rarely, if ever, the reverse. Hence, it is a good possibility to find self-incompatibility among the relatives of self-fertilized crops e.g., tomatoes, lettuce, beans etc. It is present in some wild species of tomato and its genetics has been extensively studied by McGuire and Rick (1954) and Martin (1968). Martin (1968) reported the incorporation of

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self-incompatibility in Tiny Tim cultivar of *Lycopersicon esculentum* which was originally a self-compatible. However, in this line, there were several crossing barriers which do not allow its commercial potential. It was reported by Whitaker and Jagger (1939) the species perennis of Lactuca genera probably contain self-incompatible individuals. The chromosome number (n=9) of *L. perennis* and commercial lettuce *L. sativus*, is same but when these were crossed, the seed produced was not viable. The possible existence of unilateral incompatibility, it appears advisable to transfer it from one species to another. But the presence of modifiers genes can affect the expression of the major incompatibility alleles (Martin 1968; Thompson and Taylor 1966). Also, the dominance the relationship between alleles of the sporophytic system creates complications (Sampson 1957; Thompson and Taylor 1966). So, there should be breeding work with a broad genetic base and rather than backcross method, pedigree selection method should be used.

In the cole crops like cabbage, cauliflower, broccoli etc., sporophytic self-incompatibility mechanism is has been used for hybrid seed production at several places including India (Singh 2000) and it was first observed by Stout(1920). In 1921, first hybrid of chinese cabbage was produced named Nagaoka Kohai I Go by a Japanese company and later in 1961 a radish F_1 hybrid variety 'Harumaki Minowase' was produced. The self-incompatibility system has been confirmed in various cole crops like in kale (Thompson 1957), broccoli (Sampson 1957 and Odland 1962), cabbage (Adamson 1965) and cauliflower (Hoser-Krauze 1979).

The first step in the production hybrid seed using self-incompatibility is identification of SI plants from diverse population or genotypes. It is done by making different types of pollination i.e., self-pollination in freshly opened flowers (SP), pollination with unrelated S-allele pollen (CP), and bud pollination to assure whether the plant is male or female fertile and also to self-seed to get its progeny. The plants are grouped as self-incompatible/compatible based on SP in relation to CP (Watts 1963). Fertility index (FI) values can be calculated for assessment of self-incompatibility. But this method has some disadvantages because one has to wait for sixty days till the maturity and also the set can be reduced due to environmental factors.

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$$\text{Fertility index (FI)} = \frac{\text{Average number of seeds per siliqua from CP}}{\text{Average number of seeds per siliqua from OP}}$$

If value >2 = SI, <1= self-compatible, 1-2= pseudo-compatibility

Pollen tube growth method is also used in which fluorescent microscopy is used which provides results within 24-48 hours of pollination (Vidyasagar and Chatterjee 1984). The penetration of 6-9 pollen tubes through style is considered as an incompatible reaction. A more advanced and quick method to know the presence of self-incompatibility genes is the use of molecular markers. It provides results at a very early stage of plant growth.

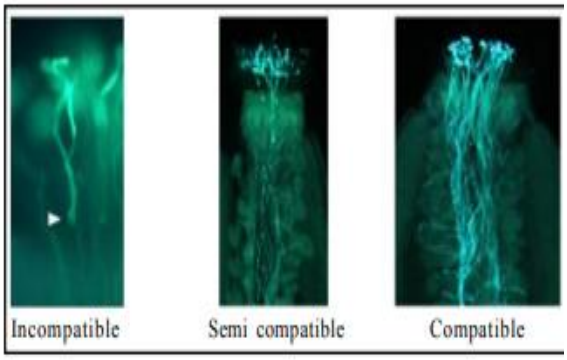

Fig.7 : Assessment of self-incompatibility in cabbage by cytological method

PCR-RFLP has been utilized in radish for the identification of S-alleles by Niikura and Matsuura (1998). In this study, a single DNA fragment of about 1.16kb was amplified which was expected from the original sequence of *B.oleracea*.

After identification of self-incompatible plants, the next step is the development of homozygous self-incompatible plants by using at least seven plants from the progeny of self-incompatible plants and making intra progeny crossings in freshly opened flowers in full diallel (Mackey, 1977). The intra crossings are done to know the heterozygous nature of the self-incompatible plants. Based on this, plants are categorized into compatible and incompatible. Then we get one homozygous and one heterozygous or both homozygous and one heterozygous phenotypic groups. From the homozygous group, plants are selected and homozygous lines are produced for next 2-3 generations.

Next, after producing homozygous self-incompatible lines the S-alleles are identified in the lines. It is done to know the level of dominance and S-allele interactions. The presence

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of highly dominant alleles leads to the very low production of sibs and selves in hybrid seed. To know the S-allele interactions seed set method, as well as fluorescent microscopy, are used for the pollinations carried out reciprocally between a heterozygote and its two corresponding homozygotes. It is reported that there are four types of S-allelic interactions viz., type I (same S-allele dominant over others in both pollen and stigma), type II (one S-allele dominant over in pollen but co-dominant in stigma), type III (ones-allele dominant over other in stigma but co-dominant in pollen) and type IV (both S-alleles co-dominant in pollen as well as stigma).

The inter-allelic relationships among S-alleles are established and best-combining lines are identified. For having heterotic hybrids, it is necessary to identify best specific combining S-allele lines. It can be evaluated from SCA studies and per se performance. Hybrid seed can also be produced by combining self-incompatible line with the self-compatible line but the seed produced is very less as it collected from only self-incompatible plants.

The next step is the maintenance of parental hybrid lines which is a very costly affair. Methods followed on large scale are manual self-pollination at bud stage, treatment of carbon dioxide at 3-5% conc. for 8-24 hours at 100% relative humidity in air-tight growth chambers and tissue culture in which meristemis used as an explant. The sprays of sodium chloride at 3-5% are also reported to be effective for temporary suppression of self-incompatibility (Kucera 1990, Yang *etal* 1995, Kucera *et al*2006).

Hybrid seed is then produced by way of the single cross, double cross or triple cross method. In the single cross, two self-incompatible but cross-compatible lines are planted in an alternate row in an isolated plot. Seed harvested from both the lines is hybrid seed. It gives maximum degree of heterosis and produces uniform plants. In the double cross, two single crosses are used. Triple cross was recommended by Thompson (1964) in kale which can be produced only with sporophytic SI. In the three-way cross, one single cross and a self-incompatible line are planted in alternate rows. In this six inbred lines having proper self-incompatibility alleles are used. Top cross is also being used for hybrid seed production in the USA. In this, self-incompatible line and a pollen pollinated cultivar as pollen parent are planted in 2-3:1 and seed obtained from self-incompatible lines is hybrid seed.

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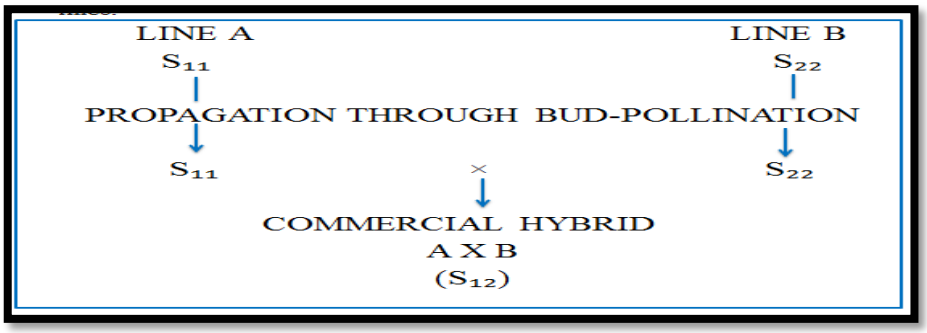
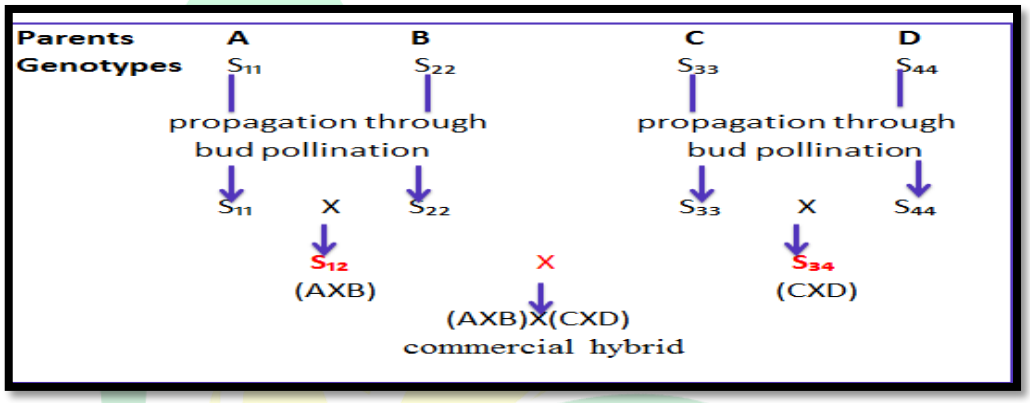
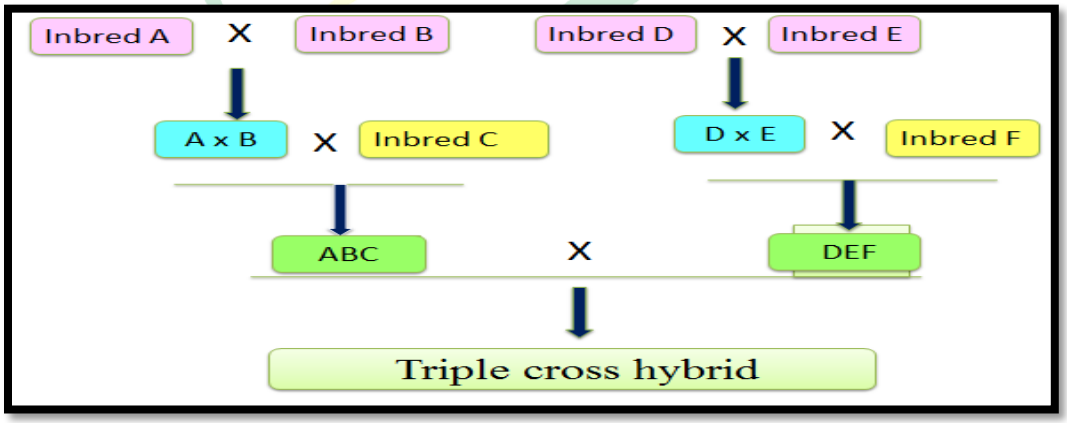


SINGLE CROSS HYBRID SEED PRODUCTION



DOUBLE CROSS HYBRID SEED PRODUCTION



TRIPLE CROSS HYBRID SEED PRODUCTION

Hybrid seed production by use of self-incompatibility faces several problems such as pseudo-fertility, depression of S-alleles by continuous inbreeding, the effect of environmental

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factors like high temperature and high humidity on the level of self-incompatibility and a higher proportion of sibs in due to improper synchronization in flowering. This can be managed by using S-allele lines stable under diverse environmental conditions, by vegetative propagation and using parental lines having synchrony in flowering.

Level of SI in *Brassicaoleracea* L.

Crop	**Level of SI**
Kale and round headed cabbage	High
Broccoli	High to moderate
Autumn and winter cauliflower	Moderate
White sprouting broccoli and brussels-sprout	Moderate to low
Green kohl rabi and purple sprouting broccoli	Low
Early summer cauliflower	Very low

Characteristics of superior SI lines

It should have stable self- incompatibility. It should be easy to develop and maintain. It should have desirable combining ability. There should be high seed set on self- pollination at bud stage. It should have uniform economic characters.

Kucera *et al.*(2006) used SI line Montano (MT) x SP Fortuna (FT13). Reproduction of SI lines was done by spraying 3% NaCl solution in evening and using bumblebees as pollinators.F_1 hybrid of MT x FT 13 were characterized by good uniformity, high curd quality, good curd covering by inner leaves and satisfactory disease resistance.

Park *et al.*(2007) worked on development of uniform F_1 hybrid varieties of Korean Radish using self- incompatibility in double crossing. In this, 45 commercial radish varieties were identified and classified by PCR-RFLP in order to select parental material possessing different S haplotypes. Two set of parents were selected from summer (population 1) and autumn varieties (population 2). Hundreds of inbreds developed from two parental sets after one parental cross and five generation of inbred.In each generation PCR based selection performed to select SI heterozygotes.These SI heterozygotes were selected to develop cross compatible near isogenic lines (CCNILs) .Double cross using similar inbred how low uniformity, therefore they used CCNILs to produce increase and uniform seed. NILs derived

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SI plants produced more seeds (4.88) per flower pollination as compared to inbred line (1.5) of radish. Parental seed for double cross can be produced with bee pollination using CCNILs derived from an inbred line possessing different S haplotypes.

S allele lines of cabbage I-4-6 and II-12-4-7 were tested for strength of self-incompatibility. The seeds from these two lines obtained as a result of NaCl solution sprays at 3% & 5 % followed by manual self -pollination in freshly opened flowers. The seeds obtained were sown to assess the strength of SI. The plants were enclosed with nylon net to prevent outcrossing. The OP (selfing in open flowers) and BP (Bud pollination) was carried out. All the plants set seeds in BP treatment confirming the viability of male and female gametes of test plants. However, no seed-set was observed in OP treatments in both S allele lines. They showed that common salt (NaCl) solution sprays resulted temporary breakdown of self-incompatibility, which will have implications in the maintenance of S-allele lines, which are to be used for hybrid seed production (Singh and VidyaSagar,2015).

Singh *et al.* (2016) studied self-incompatibility and its stability in cauliflower under sub temperate condition of western Himalayas. The aim of study was to exploit hybrid vigor the identification of SI lines plants and their progeny is of prime importance.

Conclusion

Self-incompatibility is a system to avoid self-fertilization and thereby promoting outcrossing and it is present in many flowering plants species. Over the years, in vegetable crops, considerable research work has done for the underlying mechanism of gametophytic self-incompatibility in Solanaceae and sporophytic self-incompatibility in case of brassicas. A good combination of molecular and genetic studies has led to identification and characterization of genes involved in this response. Furthermore, the components of signaling cascade of both the families need to be investigated carefully to completely understand the self-incompatibility response. Also, there is a great need of sexually maintaining and increasing highly self-incompatible lines. Bud pollination is most practically used for overcoming self-incompatibility but it works for some crops but not for others. So, hopefully, current research in the physiology of SI may lead to a simple chemical treatment for this purpose. In vegetable crops, for commercial hybrid development a number of methods and mechanisms have not been exploited yet and among that SI is of prime importance. There is

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need to identify and characterize precisely the S-alleles in the germplasm and utilize the strong alleles to develop stable self-incompatible parents.

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