1991

The RSS system of unidirectional cross-incompatibility in maize: control by the interaction of non-allelic male and female genes

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The RSS system of unidirectional cross-incompatibility in maize: Control by the interaction of non-allelic male and female genes

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Iowa State University, 1991
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The RSS system of unidirectional cross-incompatibility in maize: Control by the interaction of non-allelic male and female genes

by

Abdul Rashid

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Agronomy
Major: Plant Breeding and Cytogenetics

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1991
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I. INTRODUCTION

Sexual incompatibility in flowering plants leads to the rejection of viable pollen by a fertile pistil. This rejection may be for its own (self) pollen or for foreign pollen with specified genotypes. The phenomenon of self-incompatibility is common in flowering plants and is known in about half of the total Angiosperm species (Darlington and Mather, 1949; Brewbaker, 1959). Similarly, numerous cases of cross-incompatibility are reported in the literature and most of these involve interspecific or intergeneric crosses (Subramanyam, 1954; Pandey, 1962; Sampson, 1962; Knox et al., 1972; Takahashi, 1973; Heslop-Harrison et al., 1984b; Munoz, 1984; Bob et al., 1986; Sarker et al., 1988; Darnell and Lyrene, 1989). Sometimes, this cross-incompatibility proved to be unilateral in nature (Karpechenko, 1924; Anderson and de Winton, 1931; Pushkarnath, 1953; Lewis and Crowe, 1958; Grun and Aubertin, 1966; Martin, 1967; Townsend, 1971; Pandey, 1981; Le-Guen, 1983; Boyle and Stirmart, 1986). In these cases, the crosses were compatible in one direction but reciprocal crosses were incompatible.

This type of unidirectional cross-incompatibility is not as common in intraspecific crosses and a few cases are reported in the literature (Martin, 1964; Sukhapinda and Peterson, 1983; Keulemans, 1984; Contolini and Hughes, 1989).
In maize, the first such intraspecific unidirectional cross-incompatibility was reported by Demerec (1929). He found that when White Rice popcorn was pollinated by the field corn, there was no seed setting but the reciprocal cross gave normal seed setting. Schwartz (1950) and Nelson (1952) further studied this unilateral cross-incompatibility and found that this condition is controlled by a locus named gal in which gal pollen is unable to fertilize Gal-s pistil. It was also found that most of the field corn contains the gal allele while popcorn contains Gal or Gal-s.

A similar case of unidirectional cross-incompatibility was observed in the maize cytogenetics nursery Ames, Iowa in 1975 (Sukhapinda, 1981). In this case when a specific female (am(pa-pu)/a1 sh2) was crossed by a specific male (a1 et /a1 et), the resultant ears showed reduced seed setting (<25 kernels per ear). The reciprocal cross between these parents resulted in normal seed setting. These and further studies in this cross-incompatibility by Bdliya (1984) revealed that:

1. Two lines in the genetic stock, i.e., am(pa-pu)/a1 Sh2 and a1 sh2/a1 sh2 were similar in behavior as the female component in this incompatible cross.
2. Two other lines, i.e., a1 et/a1 et and A1 et/A1 et were behaving as incompatible males in this incompatible cross.
3. The incompatible condition of the male was recessive to the compatible male condition.
4. The females and males of this incompatible cross were self-compatible.

5. The cross between incompatible males (al et/Al et and A1 et/A1 et) was compatible in both directions.

6. The cross between the incompatible females (am(pa-pu)/a1 sh2 and a1 sh2/a1 sh2) was also compatible in both directions.

7. The factors involved in the incompatibility were independent of the am(pa-pu) allele or the a1 locus.

8. This incompatibility does not involve the gal factor.

9. The incompatibility is prefertilization and the incompatible pollination does not adversely affect the receptivity of the silk.

10. The incompatibility is not complete but few seeds are set in incompatible crosses.

With this background information, the present study was planned to answer the following questions.

1. Is this incompatibility caused by cytoplasmic factors, chromosomal factors, or is it an interaction of cytoplasmic-chromosomal factors?

2. If chromosomal, how many factors are controlling the incompatibility reaction in the female parent of the incompatible cross?

3. How many factors are controlling the incompatibility reaction in the male parent of the incompatible cross?
4. Why is the incompatibility not complete? What causes few seeds to set?

5. What is the time and place of the incompatibility reaction?

6. Are these incompatibility factors prevalent in commercial inbred lines?

7. What are the anatomical and cytological events associated with the incompatibility reaction?
II. LITERATURE REVIEW

A. Genetics of Incompatibility

Sexual incompatibility, in which viable pollen fails to fertilize a viable female gamete is a common occurrence in the plant kingdom (Arasu, 1968). It is a genetic barrier to inbreeding and it also safeguards the genetic integrity of the species by preventing out-crossing, and thus, incompatibility plays an important role in the evolution of the flowering plants (de Nettancourt, 1977). This incompatibility may be for the self pollen or for the foreign pollen. Based on this rejection of self or foreign pollen, the incompatibility can be placed into two categories:

1. **Self-incompatibility**

   Self-incompatibility is the most common form of incompatibility and is known in about half of the Angiosperm families (Darlington and Mather, 1949; Brewbaker, 1959). It has played a major role in the success and evolution of Angiosperms (Clark et al., 1990). According to Whitehouse (1950), the ability of plants to prevent self-fertilization and promote cross-pollination through self-incompatibility mechanisms resulted in a sudden increase of Angiosperms in the cretaceous period. According to Stebbins (1957), only those evolutionary lines which have acquired the mechanisms enforcing sexuality and cross-fertilization have been
Successful over long periods of time in the geological sense.

Self-incompatibility systems are usually classified as heteromorphic and homomorphic. In the heteromorphic system, self-incompatible species produce morphologically distinct types of flowers while homomorphic species produce flowers morphologically identical to each other. The distinct features of flowers in a heteromorphic system are their relative style length and anther level. These features present a mechanical barrier to the self-pollination. This type of self-incompatibility is known in 24 families and 164 genera (Ganders, 1979).

Darwin (1880) was first to establish the relationship between heteromorphic flowers and incompatibility in genera such as *Fagopyrum*, *Pulmonaria*, *Linum*, *Hottonia* and *Polygonum*. He reported that flowers of one form were fully fertile only when pollinated with pollen from flowers of the other form. The flowers showed reduced fertility when pollinated with their own pollen or pollen from other flowers of the same form.

Lewis (1979) described in detail many examples of the heteromorphic system. The most quoted example is the common European primrose, *Primula vulgaris*. In this species about half of the plants have long-styled flowers called pin flowers which have a round stigma at the mouth of the corolla tube and the anthers are attached to the tube at about the mid level. The other half of the plants in this species have flowers with
short styles called thrum flowers in which the anthers are at
the mouth of the corolla tube but the stigma is at the mid
point. These two types of flowers also differ in some other
characteristics, e.g., pollen grain size, pollen surface
sculpturing and stigma surface. These morphological
differences in two types of flowers help in preventing self-
pollination and favor cross-fertilization. In some cases, the
flowers are trimorphic with three different levels of style
and anthers.

In the homomorphic system of self-incompatibility, there
is no difference in flower morphology in a species and most of
the time, the flowers are hermaphrodite. The mechanism to
prevent self-fertilization is physiological rather than
morphological.

The first attempt to explain the genetic basis of self-
incompatibility was the work of Prell, 1921 cited in Arasu
(1968) who proposed the hypothesis of "oppositional factors"
but unfortunately his paper was not available or ignored by
most of the workers of that time. East and Mangelsdorf (1925)
reported their findings on Nicotiana and proposed the S symbol
for sterility alleles. They did not mention Prell's paper but
gave a similar interpretation of self-sterility on the basis
of "oppositional hypothesis". According to their
interpretation, self-sterility is governed by an allelomorphic
series of alleles (S1 - Sn) and pollen tubes having the same
allele as the style grow slowly (or are inhibited) but grow
rapidly when growing in styles with different S-alleles. They also assumed that the incompatibility reaction of the pollen grain is determined by the S-allele carried by the pollen (gametophyte) itself and not by the S-allele make-up of the pollen parent.

Following the publication of the East and Mangelsdorf paper, attempts were made by other workers to see if that hypothesis explains the data for all self-incompatibility (SI) cases. It explained most of the cases but workers like Kakizaki (1930) and Beatus, 1934 cited in Arasu (1968) were not able to explain the data for their experiments on Brassica oleracea and Cardamine pratensis respectively on the basis of oppositional hypothesis.

Gerstel (1950) and Hughes and Babcock (1950) working with Parthenium argentatum and Crepis foetida respectively, gave a modified explanation of SI called the sporophytic system. They suggested that though the SI is controlled by a series of alleles at a single locus, the incompatibility reaction of the pollen is determined by the S genotype of the diploid pollen parent (sporophyte) and not by the pollen genotype itself. So all the pollen grains produced by a plant have a similar incompatibility reaction. The S-alleles may also show dominant or co-dominant expression for incompatibility effects.

From the previous discussion, it is clear that the mechanism of SI is not uniform in all the plant species. In
some cases, the incompatibility reaction of the pollen depends on the allele it carries while in others it depends on the $S$-genotype of the pollen mother plant. The former system is called the gametophytic system while the later is known as the sporophytic system.

a. **Gametophytic system** This is the most common and wide-spread system of self-incompatibility and is recorded in over 60 families of Angiosperms (Crowe, 1964). It occurs in families such as Solanaceae, Liliaceae, Leguminoseae, Poaceae, Commelinaceae, Onagraceae, Papaveraceae, Rosaceae, Rubiaceae and Gramineae. In most cases studied, gametophytic self-incompatibility is controlled by a single $S$-gene with multiple alleles. In these cases, self-pollen or pollen bearing an $S$-allele identical to either of the $S$-alleles of the diploid pistil tissue, is unable to affect fertilization.

In other cases, the gametophytic system of SI is more complex and is controlled by more than one locus. One such system was explained by Lundqvist (1954, 1956, 1961, 1962, 1965, 1968) in grasses. In this system, self-incompatibility is controlled by two loci named $S$ and $Z$ segregating independently of each other with multiple alleles at each locus. Identity of alleles between pollen and pistil at either of the two loci does not cause incompatibility but the alleles at both of these loci must match between pollen and pistil to condition incompatibility. Lundqvist also suggested that the $Z$ locus originated by the duplication of the $S$-locus
and the complementary interaction between these two loci was
developed during the course of evolution. Similarly, self-
incompatibility (SI) in *Briza media* (Murray, 1974) and in
*Lolium multiflorum* Lam. (Fearon et al., 1983) is also
controlled by two loci.

In other cases such as *Borago officinalis* (Crowe, 1971)
and *Beta vulgaris* (Larsen, 1977a), the SI is controlled by
more than two loci. Larsen found 4 loci, with a complementary
interaction, controlling the SI in *Beta vulgaris*. Both of
these authors (Crowe, 1971 and Larsen, 1977b) reported that
the strength of incompatibility in these species decreased
with the decrease in frequency of homozygosity at these loci;
the more the number of the S-loci homozygous, the stronger
will be the self-incompatibility reaction.

Mulcahy and Mulcahy (1983) proposed another explanation
of self-incompatibility. They argued that the oppositional
hypothesis proposed by Prell, 1921 cited in Arasu (1968) and
East and Manglesdorf (1925) did not satisfactorily explain
several cases of SI. They cited the cases studied by
Lundqvist (1958), Crowe (1971) and Larsen (1977b) in which the
authors noted the weakening of the incompatibility reaction
with an increase in heterozygosity at the S-loci. To explain
this, they suggested a new model called the heterosis model of
self-incompatibility. This model was based on the assumption
that if pollen and style carry dissimilar alleles, there will
be heterotic interaction between them and pollen tube growth
rate will be increased. If the style is homozygous for a deleterious recessive allele and the pollen carries the same allele, pollen tube growth rate will be reduced. The actual growth rate of the pollen tube will be the sum of all pollen style interactions. Their model suggests that the $S$-gene is a supergene composed of a group of linked loci. Each supergene contains one dominant locus and the rest are deleterious recessive, such as $S_1$ is $Abcd_-$, $S_2$ is $aBcd_-$, $S_3$ is $abCd_-$, $S_4$ is $abcD_-$ and so on and the genotype of the $S_1S_2$ and $S_1S_3$ plants in reality is $ABcd_-$ and $AbCd_-$ respectively.

From the above discussion, it is evident that gametophytic self-incompatibility is a complex system and there is no single model which explains all the cases. Only a very few systems have been studied in any detail and according to Bernatzky et al. (1988), generalization from a narrow data base should be viewed cautiously.

The best studied examples in the gametophytic system of incompatibility are in the members of the Solanaceae family, e.g., *Lycopersicon peruvianum* and *Nicotiana alata* (Bernatzky et al., 1988). In these species, the SI phenomenon is developmentally regulated and the $S$-gene expression is only in the mature flowers and not in immature flowers. Thus, self-pollination of immature flowers is used to produce plants homozygous for particular $S$-alleles (Gradziel and Robinson, 1989). The inhibition of incompatible pollen tube is normally in the style. The incompatibility phenotype of the pollen is
determined by the genotype of the pollen and the crosses in which the pollen carries an allele identical to any of the alleles present in the diploid pistil are incompatible. The other feature which is general in the gametophytic system is that the pollen grains are binucleate whereas in the sporophytic system, these are generally trinucleate.

b. **Sporophytic system** The sporophytic system of self-incompatibility, first studied in *Parthenium argentatum* and *Crepis foetida* and explained by Gerstel (1950) and Hughes and Babcock (1950) is not as widely distributed in the plant kingdom as the gametophytic system of incompatibility. It has never been found in monocots (Larsen, 1977a), and in dicots, it is reported in *Cruciferae, Compositae, Convolvulaceae, Betulaceae, Caryophylaceae* and *Sterculiaceae* families (Charlesworth, 1988 and references therein). The genetic control is mostly through a single $S$-locus with multiple alleles (Nasrallah and Nasrallah, 1989). The incompatibility response is determined by the alleles carried by the pollen producing plant rather than the $S$-allele of the individual pollen grains. This means that all the pollen grains produced by a plant will have the same incompatibility phenotype irrespective of the $S$-allele they carry.

The alleles in the sporophytic system show a complex relationship of dominance or codominance in their expression. The same allele may show different dominance or codominance relationships in the style and pollen (Bateman, 1954; Eenink,
1981; Nasrallah and Nasrallah, 1986). Richards and Thurling (1973) observed that the dominance relationship was more frequent in the pollen while in the stigma, codominance was predominant in Brassica campestris.

Lewis (1979) proposed a system to indicate whether a particular allele is dominant or recessive in the pollen mother cell or style or in both tissues. He illustrated this system with an example of a genotype S1S2, as if the S1 allele is dominant over the S2 in the pollen mother cell, it will be indicated by placing a dot over the name of that allele as S1 S2 and if the S1 is dominant over the S2 in the style, then a line underneath will indicate the genotype as S1 S2. If the same S1 allele is dominant over the S2 in both pollen mother cell as well as in the style, then its genotype will be written as S1 S2. But if the S1 is dominant over the S2 in the pollen and the S2 is dominant over the S1 in the style, then the genotype will be indicated as S1S2.

Smith et al. (1983) investigated the effect of dominant or recessive S-alleles on the strength of incompatibility reaction in Brassica oleracea var. Gemmifera. They constituted the inbred lines homozygous for dominant or recessive S-alleles and tested their self-incompatibility strength. They reported that there was no difference in the strength of self-incompatibility between the lines with dominant alleles and the lines with recessive S-alleles.

Sarker et al. (1988) studied the pollen hydration and
pollen tube growth in *Brassica*. They reported that in contrast to the gametophytic system of SI, in which the pollen tube growth is arrested usually in the style, pollen tube arrest in the sporophytic system characteristically occurs at an early stage of germination, usually on the stigma surface. Initially this growth inhibition is reversible, in that pollen grains that will not germinate or even hydrate on incompatible stigma, will begin to germinate after a short lag period when transferred to the compatible stigma. They also reported that the inhibition of protein synthesis by Cycloheximide, inhibited the pollen hydration. The inhibition of glycosylation of proteins by the application of Tunicamycine to the stigma, suppressed the self-incompatibility reaction of the stigma and the pollen tube growth was normal.

This system is most extensively studied in *Brassica* species (Nasrallah and Nasrallah, 1989) and it represents the general aspects of the sporophytic system which are given as:

i. The incompatible phenotype of the pollen is determined by the genotype of the pollen producing plant (sporophyte), therefore all the pollen grains produced by a plant will exhibit the same incompatibility reaction.

ii. The alleles determining the incompatibility reaction show dominance or codominance and this interaction may be different in the pollen than in the pistil.

iii. In general, the inhibition of the incompatible pollen tube is at the stigma surface.
iv. The pollen grains are generally trinucleate compared with binucleate pollen grains in the gametophytic system.

2. **Cross-incompatibility**

In cross-incompatibility, the foreign pollen is unable to affect fertilization of a viable female gamete. This foreign pollen could be from a different variety within a species, or it could be from a different species within the same genus, or it may be from a different genus in the same family. The most common among these is the interspecific or intergeneric cross-incompatibility (Anderson and de Winton, 1931; Subramanyam, 1954; Pandey, 1962; Sampson, 1962; Knox et al., 1972; Takahashi, 1973; Heslop-Harrison et al., 1984b; Munoz, 1984; Bob et al., 1986; Sarker et al., 1988; Laurie and Bennett, 1989).

Sometimes this cross-incompatibility could be unilateral in nature. It is a unique situation in which the cross is compatible in one direction but the reciprocal cross is incompatible (Harrison and Darby, 1955). This unilateral cross-incompatibility could be interspecific or intraspecific.

a. **Unilateral interspecific cross-incompatibility**

The first case of unilateral interspecific cross-incompatibility was reported by Anderson and de Winton (1931), though they did not use the term unilateral incompatibility (UI). They found a biotype of *Nicotiana alata* which rejected the pollen of *Nicotiana longsdorfii* but the reciprocal cross was compatible.
Similarly, Pushkarnath (1953) studied the cross-incompatibility in potato and reported that the species *Solanum aracc-papa* never set berries when crossed with other species as a female parent but when used as a male, it set berries containing viable seeds. This species itself was SI. The F1s' of the compatible crosses were also self-incompatible. From the data of the backcrosses, they concluded that this unilateral incompatibility of the *S. aracc-papa* was conditioned with a modifying factor named *R*. When this is present in the style either in homozygous or heterozygous condition, it prevents all pollen tubes carrying *S*-alleles from affecting fertilization.

The term unilateral hybridization was first used by Harrison and Darby (1955) while reporting the results of interspecific incompatibility in *Antirrhinum*. They noticed that some crosses were compatible in one direction but reciprocal crosses were incompatible. They observed that in general, the crosses were compatible only if the self-compatible (SC) species of the pair was used as a female parent and self-incompatible (SI) species as a male parent. They also reported that the *Antirrhinum* species differing in chromosome number were always cross-incompatible in both directions. They made a special mention about the species *A. majus* which has been selected for self-fertility during intensive cultivation. They mentioned that though this species has been bred for self-fertility, its intrinsic self-
incompatibility was apparent from interspecific crosses in which it behaved like SI species and the crosses were compatible only when this species was used as a male parent and not when used as a female parent. Similarly, Lewis and Crowe (1958) reported the unilateral incompatibility in SI and SC species of Crucifereae, Onagraceae and Solanaceae. However, they found several exceptional SC species which behaved like SI species when crossed to other SC species. They explained these unexpected results by assuming that SC species had evolved from SI species. The unusual self-compatible species represent an intermediate stage in the evolution of SC species. They suggested a sequence of events for such evolution as SI --> Sc --> Sc' --> SC. The first step in this series of events is the mutation which changes the SI species into Sc species. This new Sc species retains its cross-incompatibility reaction both in the style and the pollen when crossed on or with a normal SC species. During the 2nd step, this Sc species loses its stylar incompatibility reaction but its pollen still behaves like SI pollen, so this 2nd step Sc' species accepts the pollen from the normal SC species but its pollen is also accepted by the SI styles. During the 3rd step, the Sc' species loses its pollen incompatibility reaction also and now this species becomes a normal SC species. This SC species can not pollinate the SI species and its style accepts pollen with any S genotype. However, they did not find a representative of the Sc'
species.

Pandey (1962) studied the genetic basis of interspecific incompatibility in a diallel cross involving a number of SI and SC species of tuberous Solanum. He found that it is not a general rule that SI X SC species cross will always be incompatible in Solanum. He reported that the pollen from a Mexican SC species (*S. verrucosum*) was able to fertilize a South American SI species (*S. vernei*). On the other hand, SC species *S. poladenium* was cross-incompatible with all other species in both directions. He proposed that interspecific incompatibility may not be controlled only by the *S*-locus. The alleles at another locus may be interfering with the *S*-locus and thus result in the failure of unilateral incompatibility principle in Solanaceae. Martin (1961a&b, 1963, 1967, 1968) made intensive studies into the SI and UI relationships in Lycopersicon species and reported that two major genes were controlling the SI and UI in progenies of the cross between *L. esculentum* (SC) and *L. hirsutum* (SI).

Through a series of backcrosses, Martin (1968) transferred SI alleles from *L. peruvianum* var. dentatum (SI) to *L. esculentum* (SC) and confirmed his earlier findings that two major genes from the SI parent were controlling unilateral incompatibility. Similar results were also observed by Grun and Aubertin (1966) when they studied the genetics of unilateral incompatibility in Solanum species. They reported that no cytoplasmic factors were involved in the control of
the unilateral incompatibility but 2-4 dominant nuclear genes were responsible for this unilateral incompatibility. They also reported that these genes are different from the SI genes.

Pandey (1968) studied the role of the S-gene complex in the incompatibility relationships in 17 species of Nicotiana, 9 of them from South America and 8 of Australian origin. He found that pollen and style compatibilities in these species were inversely related; the species with more pollen compatibilities have less stylar compatibility, and vice versa. Thus, the pollen of SI species N. alata is able to grow into all styles, whereas its style rejects pollen of all other species except of its own species. On the other hand, SC Australian species show opposite behavior, pollen of these species is rejected by all other species, while their styles accepted pollen from all other species. He suggested that different patterns of interspecific pollen-style compatibility relationships are highly specific and are mainly controlled by different forms of S-alleles which have arisen from mutations of various genetic elements of the primitive S-gene complex. Unilateral incompatibility owes its origin primarily to the mutational independence of the pollen and style controlling elements of S-gene complex. He argued that the supremacy of the S-gene in the determination of compatibility behavior is dependent upon the maintenance of the native poly-genic background in which it normally acts. A hybrid or disturbed
genetic background may lead to the loss of this supremacy; as a result, major genes or polygenic combinations, which could modify the compatibility behavior of the species but which are normally incapable of expression may now be able to express themselves.

b. **Unilateral intraspecific cross-incompatibility**

A few reports of this type of unilateral incompatibility are available in the literature in which different lines within a species show unusual incompatibility relationships, i.e., the crosses are compatible in one direction but reciprocals are incompatible. Martin (1961b, 1964) reported one such case in *Lycopersicon hirsutum*. He found that this species has all types of lines showing self-compatibility, self-incompatibility and unilateral incompatibility. He analyzed the backcross and F2 populations of the crosses between these lines and suggested that both SI and UI were controlled by two dominant genes present in the SI line Cajamarca and polygenic modifiers were responsible for some pseudocompatibility. He also reported that the environment especially temperature, caused variation in pollen-style interaction leading to pseudocompatibility.

Another case of unidirectional cross-incompatibility within a species was reported by Keulemans (1984). He presented the results of pollination experiments in sweet cherries and showed that some varieties show unidirectional incompatibility in their crosses. The cross between the
varieties Early River and Buttners was compatible when the variety Buttners was used as female but was incompatible when used as male. He explained these results on the basis of the bipartite nature of $S$-gene. He said that the mutation in the stylar component of the $S$-gene in variety Buttners was responsible for this unidirectional cross-incompatibility because its style lost the ability to reject the SC pollen.

c. Unilateral cross-incompatibility in maize Self-incompatibility has never been reported in maize (Heslop-Harrison and Heslop-Harrison, 1985) but some cases of cross-incompatibility have been reported in the literature. The first such case was reported by Demerec (1929) when he found that White Rice popcorn was not producing any seed when crossed with field corn pollen while the reciprocal cross was showing normal seed setting. He analyzed the F2 populations of the crosses between White Rice popcorn and various sweet corn varieties and noted a deficiency of the sugary kernels (12% instead of expected 25%), therefore, he suggested that there may be a relationship between this cross-sterility of White Rice popcorn and the gametophytic factor ($gal$) which had earlier been reported by Emerson (1925) and Mangelsdorf and Jones (1926). A similar case of unidirectional cross-incompatibility between two popcorn varieties was reported by Burnson (1937) after he found that the variety South American did not set any seed when pollinated by the variety Supergold but the cross was compatible when South American was used as a
male parent in the cross.

Schwartz (1950) found another allele of the gal factor. The new allele designated Gal-s (super gametophyte factor) was different from the Gal allele in the sense that gal pollen completely fails to function on the homozygous Gal-s style even in the absence of competing pollen. He also reported that the gal pollen yields partial seed setting on a heterozygous Gal-s style. Less seed is set when the female parent is heterozygous as Gal-s/Gal than when it is Gal-s/gal. In the case of the Gal style, if it is pollinated by a mixture of Gal and gal pollen, the gal pollen fails to compete against the Gal pollen and all the seed set are from the Gal pollen. The gal/gal style does not inhibit the pollen tube of any genotype. He suggested that the cross-sterility cases reported by Demerec (1929) and Burnson (1937) may be due to the action of Gal-s allele.

Nelson (1952) investigated the inheritance of this cross-sterility in popcorn. He used different popcorn and field corn inbred lines including Schwartz's Gal-s line in his experiments. He classified the cross as incompatible if the seed setting was less than 10% of the normal. He analyzed the backcross and F2 data and found that cross-sterility was caused by Gal-s allele which is wide-spread in the commercial popcorn lines of USA and South America. The cross-neutral allele, Gal, was found only in White Rice popcorn and all the field corn lines showed the constitution at this allele as
gal/gal. He also found that cross-sterility may be relative rather than absolute because he observed some differences in degree of sterility during different years of his study. His finding of the cross-neutral allele Gal in White Rice popcorn disagree with the Demerec’s (1929) finding that White Rice popcorn was cross-incompatible with field corn when used as female. Nelson discussed this point and said that Demerec’s photographs of White Rice popcorn show that in fact the variety was not White Rice but was a White Pearl type which is quite distinct from White Rice type.

Ashman (1975) while investigating the gal locus observed an unusual behavior of this locus. He crossed two gal/gal lines (Sg1533 and Hy) as female parents with pollen from a Gal-s/Gal-s line SA24. The crosses were compatible as expected but when he backcrossed these two F1s’ \{gal(Sg1533)/Gal-s and gal(Hy)/Gal-s\} with gal/gal parents, he noted a clear cut difference in the strength of cross-incompatibility. The F1 hybrid Sg1533 X SA24 gave normal seed setting in the backcross while the other hybrid (Hy X SA24) gave partial sterility. The partial sterility of the F1 was expected but not the normal seed setting. To further investigate this difference, he produced the F2 of these two hybrids and noted that the F2 population from the cross of Sg1533 and SA24, which showed unusual normal seed setting in a backcross, contained the gal/gal genotype among 50% of the plants. This was not expected at all because according to
Nelson (1952), gal pollen can not compete with Gal-s pollen in the mixture. The other surprise was that no plant with a genotype homozygous for the Gal-s allele was found though 25% plants with this genotype were expected. Ashman explained this situation by assuming that the gal/gal line Sgl533 contained a dominant modifier which suppresses the action of the Gal-s, and this is why the F1 between the line Sgl533 and SA24 did not show partial sterility in a backcross. The reason for finding 25% plants with the gal/gal genotype was also that the same modifier suppressed the competitive advantage of the Gal-s allele over the gal. He also suggested that the cross neutral allele Gal may in fact be Gal-s plus a modifier.

Sukhapinda (1981) and Sukhapinda and Peterson (1983) studied another case of unidirectional cross-incompatibility in maize. They reported that when the derivatives of the line am(pa-pu) containing the transposable element En were crossed by a specific male parent al et/ al et, ears with reduced seed setting (RSS) were produced. In the reciprocal cross with the same genetic stocks used as a male parent, the seed setting was normal (NSS). The RSS effect was heritable and does not involve En or the etched (et) allele. They also observed that the incompatibility effect was recessive to the compatibility effect. They tested the possibility of Gal-s allele involvement and reported that the factor(s) causing this cross-incompatibility were independent of the gal locus. They
hypothesized that the genetic control of this cross incompatibility reaction is influenced by a cytoplasmic-chromosomal factors interaction in the female and chromosomal factor in the male. They also reported that the site of the incompatibility reaction is not the silk surface because the pollen did germinate and the pollen tube penetrated the silk body.

Bdliya (1984) further studied this case of cross-incompatibility and found that it was under the control of a gametophytic system. He also reported that the incompatible pollination does not affect fertilization nor does it alter the receptivity of the silk for further pollination. He also noted that afternoon pollination gave more ears with reduced seed setting compared with morning pollination.

Kermicle and Allen (1990) reported another system of cross-incompatibility between dent corn and some accessions of teosinte ssp. Mexicana (Central Plateau 48703 and Chalco). They observed that the dent corn pollen was not able to affect fertilization of teosinte accessions but the reciprocal cross was compatible. They transferred the teosinte incompatibility trait (TIC) to dent corn inbred line W22 (gal/gal) by backcrossing using teosinte as the female parent. The dent corn was used as a recurrent male parent with selection for the teosinte incompatibility trait. Their results showed that the Chalco-derived strains behaved similarly to the cross-incompatible Gal-s/Gal-s popcorn. Pollen from the other
accession (Central Plateau) derived stock fertilized the Gal-s/Gal-s silk but the reciprocal cross as well as the pollination with gal/gal failed to set seed. Hybrids of this central plateau derived stock with dent corn (gal/gal) also do not set seed when pollinated by gal or Gal-s pollen. They named this incompatibility factor of Central Plateau accession as TIC-CP. The lines with TIC-CP were compatible with all other lines as male but were incompatible as female.

B. Cytological and Morphological Features of Incompatibility.

Scott (1865) appears to be the first who observed the pollen tube behavior in incompatible pollinations. He reported that on self-pollination of Oncidium, the pollen grains germinated and pollen tubes penetrated the stigma but did not fertilize the ovule.

Sears (1937) presented a detailed account of incompatible pollinations and grouped plants into following three classes based on the site of pollen tube inhibition in the pistil.

i. Plants in which pollen tube is inhibited before penetration in the stigma (Brassica, Raphanus sativus, Secale cereale).

ii. Plants in which the incompatibility reaction occurs while pollen tube is growing in the style (Petunia violacea, Nicotiana sanderae).

iii. Plants in which the incompatible pollen tube is stopped when it reaches the ovule (Gasteria
The third class is rare in the plant kingdom and few species show this type of incompatibility reaction. Brewbaker (1957, 1959) pointed out that most species in which incompatible pollen tubes reach down to the ovary have hollow styles and suggested that intimate contact between pollen tubes and stylar tissue is necessary for the inhibition to occur.

In most of the species, the incompatible pollen tube does not penetrate the stigma or if it penetrates, its growth is slowed down in the style and it never reaches the ovule. The former situation (inhibition at the stigma) is common in the sporophytic system while the later (inhibition in the style) is a general feature of the gametophytic system of incompatibility, though some exceptions to both of these cases are reported in the literature. Hayman (1956) reported that in some crosses of grass species, e.g., *Gamdinia fragilis* L. X *Dactylis glomerata*, the inhibition of the pollen tube is at or near the stigma surface though the incompatibility system in these species is gametophytic.

Rosen and Gawlik (1966) studied the pollen tube growth both *in vitro* and *in vivo* and reported that the tip of a Lily pollen tube looks much different when growing in the compatible pistil from the tip of the tube growing *in vitro*. The tube in the compatible pistil shows a series of deep, irregular embayments (invaginations) at its tip and in the
subjacent cytoplasm, large, irregular bodies occur whose contents resemble that of the embayments. This appearance is suggestive of material moving into the tube from outside. The pollen tubes grown in vitro are characterized by a compartmented cap over their growing tips. They suggested that in vitro growth appears to be autotrophic, with new growth at the tip derived from stored material which is transformed and transferred to the wall via the vesicles. In the compatible pistil, it appears that the tube is taking up material from the stylar canal and is growing largely by a heterotrophic mode. They also reported that the pollen tube growth in vitro and in the incompatible pistils ceases when stored reserves are exhausted but in the compatible pistil it switches its growth from an autotrophic to a heterotrophic mode and it is manifested through the switching of the pollen tube tip from compartmented cap to embayments. Similarly, Kroh (1967) also noted in Petunia that pollen tubes growing in the compatible pistil have much more elaborate and irregular lateral walls than tubes growing in vitro and she suggested that this may facilitate the uptake of material from the pistil. Mulcahy and Mulcahy (1982) suggested that the pollen tube growth in Petunia is completed in two phases. The first phase is autotrophic and lasts about 7 hours from pollination. The second phase is heterotrophic and is concomitant with the appearance of callose plugs in the pollen tube and lasts from 7 to 27 hours after pollination.
Heslop-Harrison et al. (1984b) studied the pollen-stigma interaction after pollinating maize silk with maize, sorghum and millet pollen. They reported that in the stigma axis, maize pollen tubes reached the transmitting tract by step wise progression through the intercellular spaces of the cortex. Sorghum tubes were frequently disorientated in the stigma axis failing to locate the transmitting tract; yet they were able to grow to a considerable length. Millet pollen tubes entered the maize stigma with greater difficulty and in the axis, tend to grow slowly through the cortical tissue without seeking the transmitting tract.

Clarke et al. (1985) reported that in Solanaceae, the incompatible pollen tube growth was arrested in the upper 1/3 of the style. The tips of the arrested pollen tubes were swollen and sometimes bursted. Similarly, Gaude and Dumas (1987) reported that the incompatibility reaction was associated with abnormalities in wall formation, taking the form of distorted or retarded growth, thickening of the cell wall and sometimes with branching of the tubes. The rejection of incompatible pollen grains or pollen tubes was also characterized by unusual callose synthesis response. In the sporophytic system, this response was observed in both stigma and pollen but in the gametophytic system, the response was seen only in the pollen and pollen tubes.
C. Molecular Biology of Incompatibility.

Attempts to understand the molecular basis of incompatibility started with the antigen-antibody concept borrowed from the animal biologists. Lewis (1952) injected the pollen extract into the rabbit and the resultant antiserum was tested by precipitin rings against pollen proteins. Specific precipitin reactions occurred only when the pollen protein carried the same allele as the pollen injected into the rabbit. He used the four incompatibility S-allele of *Oenothera* and found complete identity in the genetic tests and antisera tests. He also reported that the stylar incompatibility substance was preformed and was not the result of an antigenic stimulus from the pollen tube. Later, Nasrallah and Wallace (1967) identified the S-allele specific antigens in the stigma extract proteins in *Brassica oleracea*. They also reported that genotype specific antigens of the stigma were not detected in the pollen or in other tissues of the same plant.

With the development of electrophoretic techniques such as SDS-PAGE and isoelectric focussing, Nasrallah et al. (1970) detected the proteins corresponding to particular S-alleles in stylar extracts of *Brassica oleracea* var. Capitata. Later, Hinata and Nishio (1978) showed that the protein corresponding to a particular S-allele of *Brassica campestris* segregates with that allele, indicating that the protein was either the product of the S-gene or of a gene closely linked to it.
Roberts et al. (1979) detected a particular glycoprotein by isoelectric focusing, the appearance of which coincided precisely with the development of the self-incompatibility response in the maturing stigma in *Brassica oleracea*.

Mau et al. (1982) isolated and characterized the components of *Prunus avium* L. (cv. Lambert S3S4) stylar extract and reported that the major components were; a glycoprotein with MW 90Kd, a sticky Uronic-acid containing component and an Arabinogalactain. Among the minor components, one was an antigen (MW 37-39Kd) named Antigen S associated with the self-incompatible genotype and the other component named Antigen P (MW 32Kd) was present in all the *Prunus* species. Williams et al. (1982) tested these major and minor components of *Prunus avium* L. stylar extracts for their effect on the *in vitro* growth of pollen tubes. They found that antigen S was a potent inhibitor of *in vitro* pollen tube growth causing a 65% reduction in the pollen tube length at a concentration of 20 µg/ml. None of the other stylar components was effective inhibitor of pollen tube growth.

Sharma and Shivanna (1982, 1983) incorporated the pistil extract in the pollen germination medium and observed that it selectively inhibits the incompatible pollen but not the compatible pollen of *Petunia hybrida*. They further noted that this inhibition of incompatible pollen (self) *in vitro* can be effectively blocked either by the incorporation of lectin in the germination medium or by treating pollen grains with
sugars before culture. They suggested that these results strongly indicate the involvement of lectin-like components of pollen and specific sugar moiety of the pistil in self-incompatibility recognition. Sharma et al. (1985) tested this hypothesis in vivo and reported that treating the stigma with a lectin (Con A/PHA) before pollination was effective in overcoming SI in *Petunia hybrida*, a gametophytic self-incompatible species, and in *Eruca sativa*, a sporophytic self-incompatible species. They also reported that treatment of pollen with glucose/N-acetyl-D-galactosamine (tested only with *Petunia*) was also effective in overcoming self-incompatibility. Studies of Sharma and Shivanna (1986) showed similar results in *N. alata* also and they concluded their studies with the hypothesis that self-incompatibility recognition is established as a result of complementation between lectin-like components of pollen and specific sugar moieties presumably of glycoproteins in the pistil.

With the availability of recombinant DNA and peptide sequencing technology, the efforts were made to clone and sequence the S-gene and its products. Major work for gametophytic self-incompatibility system in *Nicotiana alata* was pursued at the University of Melbourne in Australia and for the sporophytic system of self-incompatibility in *Brassica oleracea* and *B. campestris* at Cornell University in the United States, and at Tokohu and Sendai University in Japan. The first report of isolating a cDNA clone encoding part of an
S-locus specific glycoprotein (SLSG) was from Nasrallah et al. (1985), working with Brassica oleracea. This clone was obtained by differential screening of a cDNA library prepared from poly(A+) RNA from mature stigma, using cDNA probe synthesized from mature stigma mRNA and whole seedling mRNA. This clone proved to be part of the S6 allele. Subsequently, two more cDNA clones corresponding to S13 and S14 alleles of Brassica oleracea were sequenced (Nasrallah et al., 1987). They compared the amino acid sequence of S6, S13 and S14 alleles and found that the sequences were highly conserved, but there were regions that were more variable than the others. The cysteine residues were clustered at the carboxy-terminal region and might be functionally significant.

In the gametophytic system of incompatibility, Anderson et al. (1986) were the first to isolate and sequence a cDNA clone. First they isolated a glycoprotein from the style of N. alata and showed that this protein (MW 32Kd) was cosegregating with S2 allele. They also showed that the appearance of this glycoprotein in the style extract was similar to the self-incompatibility behavior of the style, i.e., it was not present in immature flower style but present in mature style. They also isolated the cDNA clone by differential screening of cDNA library prepared from mRNA from mature style of N. alata genotype S2S3. The clones which hybridized to the $^{32}$P labeled cDNA of mature style but not to the ovary or green bud style cDNA were further screened with
the synthetic oligodeoxyribonucleotide prepared based on the amino acid sequence of isolated S2 specific glycoprotein. The isolated cDNA encoding S2-glycoprotein was also tested in histochemical hybridization experiments using longitudinal sections of mature S2S3 and S1S3 styles. They reported that the cDNA probe binds to the cells of S2S3 transmitting tissue strongly but there was a weak binding to S1S3 transmitting tissue also. Later, Anderson et al. (1989) reported the cloning of two more S-alleles, i.e., S3 and S6 in N. alata and noted that the amino acid sequence of the three S-alleles (S2, S3, S6) show only 56% homology to each other, the homologous regions include the N-terminal sequence, most of the cysteine residues and the glycosylation sites.

Moore and Nasrallah (1990) successfully introduced the cloned Brassica oleracea SLSG gene into a self-compatible tobacco species (N. tabacum) to see its expression in the new host. They reported that the resulting transgenic plants showed tissue specific and developmentally regulated expression of the introduced gene. Immunolocalization experiments showed that the Brassica gene was expressed in the stylar transmitting tissue of the transgenic plants. The pattern of expression of the introduced gene showed greater similarity to that of the gametophytic system of N. alata than to the expression of the sporophytic system of Brassica. They reported however, that the introduced gene did not confer self-incompatibility in transgenic tobacco plants. The
authors suggested that the apparent lack of expression of the SLSG gene in the anther tissue offers a possible explanation for the absence of the self-incompatibility response in transgenic tobacco, or it may be possible that the molecular structure of *B. oleracea* SLSG, expressed at the dry stigma surface in *Brassica*, is not suitable for inhibition of pollen tubes at advanced stages of growth and/or can not inhibit pollen tube growth when expressed in the glandular matrix of the tobacco style.

McClure et al. (1989) studied the nature of the *S*-glycoproteins and reported that these are ribonucleases. They further reported that these stylar glycoproteins accounted for most of the ribonuclease activity recovered from the stylar extracts. They also reported that the ribonuclease specific activity of style extract of *SI* species of *N. alata* was 100 - 1000 fold higher than that of the related *SC* species *N. tabacum*. Further, they reported that these observations lead to the generalized hypothesis for inhibition of pollen tube growth based on uptake of a cytotoxic agent from the style by the gametophyte. In this model, the pollen *S*-gene product would enable non-self pollen to reject or inactivate the style cytotoxin, when a pollen lacking an *S*-allele or bearing an *S*-allele identical to one present in the style would be unable to perform this function. McClure et al. (1990) extended these studies to see the role of these ribonucleases in incompatibility. They produced labeled
pollen by growing plants in the presence of $^{32}$P and followed the fate of labeled RNA within the style after compatible and incompatible pollinations. They observed that the total amount of the labeled RNA recovered from the style after incompatible pollination was less than the amount recovered from the compatible pollination. Agarose-gel fractionation of the labeled RNAs recovered from styles after compatible and incompatible crosses showed that rRNAs were intact in the style of compatible crosses but degraded in the incompatible crosses. The authors suggested that expression of self-incompatibility is mediated by degradation of pollen rRNA in the incompatible pollen tubes during their growth in the style. It was already established (Mascarenhas, 1990) that the rRNAs and tRNAs are synthesized in the pollen grains before the anthesis and no rRNA or tRNA is synthesized during pollen germination or pollen tube growth. So the synthesis of proteins/enzymes required for pollen germination and pollen tube growth is mediated by this presynthesized store of rRNAs and tRNAs. If the style glycoproteins degrade the rRNAs in the pollen tubes, then the pollen tubes will not be able to synthesize the proteins/enzymes and their growth will be stopped, resulting in incompatibility. This suggestion of degradation of rRNAs by glycoproteins is supported by the $S$-allele specific pattern of rRNA degradation and the knowledge that the $S$-glycoproteins are RNases.
D. Morphology and Tissue Organization of Silk in Maize

The style or stigma of maize is commonly called silk. Raspail, 1824 cited in Heslop-Harrison et al. (1984a) reported that the silk in maize constitutes a greatly extended stigma being formed by the fusion of the two branches of the typical grass family stigma. Similarly, Weatherwax (1916) reported that if the term stigma is taken to connote the pollen-receptive part of the pistil, then the whole extended trichome-bearing silk must logically be regarded as stigma. Heslop-Harrison et al. (1984a) reported that the term silk is accepted as stigma in recent publication. They also reported that the silk is a flattened ribbon like structure with a bifurcated tip (Fig. 1A). Its length varies from 2 to 70 cm. The principal pollen receptive surface is constituted by two irregular marginal zones of multicellular silk hairs. The silk hairs (called trichomes) are present throughout the silk length with a naked zone of about 5 mm distal to the ovary. The density of the trichomes (counted on both sides) on the silk varies from 14 per mm in the forked tips to about 60 in the middle with slight decline towards the end of the stigmatic zone. The mean length of the trichomes in the central zone was 151.2 μm with a range of 28 to 280 μm.

Kroh et al. (1979) reported that most of the pollen grains grow on the silk hairs, although germination and penetration can also occur on the main body of the silk. Often pollen tubes grow first along the surface of the hair
cell and then enter the hair at different positions between
the hair cells, or the silk proper between the epidermal
cells. In the hair, the pollen tubes grow intercellularly to
the silk. The geometry of the basal cell of the trichomes
ensures that the pollen tube tip is directed towards the
ovary. The silk has two vascular bundles with phloem lying
external to the xylem, and two pollen tube transmitting tracts
adjacent to the vascular bundles towards the center of the
silk (Fig. 1B). The pollen tubes after penetrating the silk
grow intercellularly towards the transmitting tract and then
further down to the silk through the intercellular spaces of
the transmitting tissue towards the ovary.
Figure 1. Diagrammatic presentation of morphology and tissue organization of silk in maize. (A) Silk with ovary (B) Tissue organization in the silk
### III. MATERIALS AND METHODS

#### A. Designation of Symbols and Terms

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>$a_1$</td>
<td>A recessive allele of $A_1$, when present in homozygous recessive form, produces a colorless aleurone.</td>
</tr>
<tr>
<td>$am(pa-pu)$</td>
<td>An autonomous mutable allele of $A_1$, gives pale (pa) and purple (pu) sectors in a colorless background (Peterson, 1970).</td>
</tr>
<tr>
<td>$En$</td>
<td>Enhancer; an autonomous transposable element; acts in trans to induce mutability in reporter alleles (Peterson, 1960).</td>
</tr>
<tr>
<td>$sh_2$</td>
<td>A recessive allele of $Sh_2$, when homozygous recessive, produces shrunken kernels.</td>
</tr>
<tr>
<td>$et$</td>
<td>A recessive allele of $Et$; when homozygous recessive gives kernels with a scarred, pitted appearance; seedlings are virescent (Stadler, 1940).</td>
</tr>
<tr>
<td>RSS</td>
<td>A term applied to a situation in which crossing of two genotypes results in reduced seed setting (&lt;25 kernels per ear).</td>
</tr>
<tr>
<td>NSS</td>
<td>A term applied to a situation in which crossing of two genotypes results in normal seed setting.</td>
</tr>
</tbody>
</table>
RSS male  A line when used as a male parent in a particular cross results in RSS.

RSS female  A line when used as a female parent in a particular cross gives RSS.

Incompatibility  A term applied to a situation in which a viable pollen is unable to fertilize a viable female gamete.

Compatibility  A term applied to a situation in which pollination results in normal seed setting.

Unidirectional  When the cross is compatible in one direction but the reciprocal cross with the same parents is incompatible.

Male component  The male parent in the incompatible cross.

Female component  The female parent in the incompatible cross.

B. Genetic Stocks

The cross-incompatible female lines, first noticed in 1975 were the derivatives of a common source 1970: 1451. The pedigree of this source traces back to the pale green mutable stock (Sukhapinda and Peterson, 1983).

The cross-incompatible male lines originated from a separate source 1962: 792-25 and since then are being maintained by selfing or sibbing (Bdliya, 1984).
In the present study, the following genetic stocks were used.

1. RSS female
   a. 863505
   b. 863506
   c. 881331

2. RSS male
   881219-881226

All these lines have been developed and maintained by Dr. P. A. Peterson, Department of Agronomy, Iowa State University, Ames, Iowa.

C. Methods

1. Field experiments

   All experiments were grown at the Iowa State University Agronomy Research Center during the summer and in the Agronomy greenhouse during the winter for three years (1988, 1989, 1990). The crosses were made according to the usual corn genetics crossing procedure; protecting the silks with a shoot bag before their emergence, bagging tassels the day before use, shaking the pollen bag over the silks that had been cutback and recovered with shoot bag on the day previous to pollination.

2. Recording data for incompatibility

   Mature ears were harvested and data were recorded on seed setting. The ears with less than 25 kernels on a normal size cob were recorded as RSS (incompatible) and the rest as NSS (compatible).
3. Statistical analysis

The data for RSS or NSS from the crosses were analyzed to test the proposed hypotheses by Chi-square test (Steel and Torrie, 1980) using the following formula.

\[ \chi^2 = \sum \frac{(|\text{observed} - \text{expected}| - 0.5)^2}{\text{expected}} \]

4. In vivo pollen germination studies

Incompatible crosses (RSS female X RSS male) were made in the field during the 1990 summer and the silk samples were collected 6 hours after pollination. These silk samples were preserved and prepared for the scanning electron microscopy according to the following method described by Wagner (1984).

The silk samples were immediately placed in 2.5% glutaraldehyde-2% paraformaldehyde in sodium phosphate (\(KH_2PO_4\), \(NaHPO_4\)) buffer (0.15 M, pH 7.2) at room temperature. Then silks were placed in fresh buffered fixative overnight at 4°C. Fixation was followed by three buffer rinses, 10 minutes each, and then post-fixed in 1% osmium tetroxide (\(OSO_4\)) using the same buffer for two hours at 4°C. Then the silks were washed twice in double distilled water (dd \(H_2O\)) for 30 minutes followed by dehydration in a series of graded ethanol (Et.OH) to 100% Et.OH and then the specimens were critical-point dried using CO\(_2\).
The specimens were affixed to brass discs with silver cement and coated with gold-palladium target (40:60) in a polaron E5100 sputter coating unit.

Observations on pollen germination and pollen tube penetration in the silk were recorded and photographs were taken using JEOL JSM-35 Scanning Electron Microscope.

5. *In vivo* pollen tube growth studies

Silk samples were also collected from compatible (RSS male X RSS female) and incompatible (RSS female X RSS male) crosses 6, 12, 18 and 24 hours after pollination to study the pollen tube growth inside the silk *in vivo*. For this purpose, the silks were preserved and stained according to the following procedure proposed by Alexander, 1987.

a. **Preservation of specimens** Immediately after cutting the silks from the cob, they were submerged for 12 hours in a modified Carnoy’s fluid (absolute alcohol : chloroform : glacial acetic acid, 6:4:1) for preservation and fixation. After 12 hours, the samples were brought to water through descending alcohols (95%->75%-->55%-->35%-->5%-->water).

b. **Staining of specimens** The staining solution was prepared as given below:

i. **Stock solutions**

   - 1% Malachite green in distilled water
   - 1% Acid fuchsin in distilled water
   - 1% Aniline blue in distilled water
1% Orange G in 50% alcohol

ii. Staining solution

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid</td>
<td>78 ml</td>
</tr>
<tr>
<td>1% Malachite green solution</td>
<td>4 ml</td>
</tr>
<tr>
<td>1% Acid fuchsin solution</td>
<td>6 ml</td>
</tr>
<tr>
<td>1% Aniline blue solution</td>
<td>4 ml</td>
</tr>
<tr>
<td>1% Orange G solution</td>
<td>2 ml</td>
</tr>
<tr>
<td>Chloral hydrate</td>
<td>2 g</td>
</tr>
</tbody>
</table>

The constituents were added in the order given above and the staining solution was stored in amber bottles. The silk samples were incubated in this staining solution at 45±2° C for 12 hours.

c. Clearing and softening of specimens

The clearing and softening solution was prepared as given:

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid</td>
<td>78 ml</td>
</tr>
<tr>
<td>Phenol</td>
<td>10 g</td>
</tr>
<tr>
<td>Chloral hydrate</td>
<td>10 g</td>
</tr>
<tr>
<td>1% Orange G solution</td>
<td>2 ml</td>
</tr>
</tbody>
</table>

The silk samples were transferred to the clearing and softening solution from the staining solution and incubated at 45±2° C for 24 hours. After 24 hours of incubation, these samples were transferred to the fresh clearing and softening solution and hydrolyzed in hot air oven for 30 minutes at 58±1° C. Then these samples were washed twice in lactic acid and stored in fresh lactic acid for observations under light microscope.
d. **Microscopic observations** The light microscope model *Leitz Laborlux 12* fitted with 35mm camera was used to record observations on the extent of pollen tube growth 6, 12, 18 and 24 hours after pollination in compatible as well as incompatible crosses. The individual silks were placed on the slide and pressed lightly under the cover-slip. The observations on the pollen tube growth were recorded and photographs were taken using a *Kodak Ektachrome tungsten ASA 160* slide film.
IV. RESULTS

A. Genetic Analysis of the Unidirectional Cross-incompatibility

1. Role of the RSS female cytoplasm

The need to investigate the cytoplasmic role in the manifestation of the unidirectional cross-incompatibility arises from the observations that the crosses were incompatible only when the particular lines (RSS female) were used as a female parent whereas the reciprocal crosses between the same parents were compatible and resulted in normal seed setting (NSS). This unidirectional incompatibility makes a strong case for the involvement of cytoplasmic factors, therefore a crossing scheme was designed (Fig. 2) to test the hypothesis that the cytoplasmic factor(s) present in the RSS female lines \( am-(pa-pu) \) and \( a1 sh2 \) is (are) causing the incompatibility reaction. The rationale behind this scheme was that if this incompatibility is caused by cytoplasmic factor(s), then these cytoplasmic factors should not be transmitted to the progeny, if the RSS female lines are used in the crosses as male parent. The choice of the RSS male line as the female parent in the planned crossing scheme was based on the fact that this cross is compatible (Sukhapinda, 1981) and the RSS male lines' cytoplasm is different from the RSS female lines' cytoplasm. The cross (Fig. 2, line 1) was made during the 1988 summer and the F1s' were selfed in the
Figure 2. Crossing scheme used to study the role of the RSS female cytoplasm in the unidirectional cross-incompatibility. The numbers in parentheses indicate the row numbers used in this experiment.
Table 1. Results of the experiment conducted during 1990 to determine the role of cytoplasmic factors of the RSS female lines in the control of unidirectional cross-incompatibility

<table>
<thead>
<tr>
<th>F2 population Row #</th>
<th>Male tester</th>
<th>Number of ears with NSS</th>
<th>RSS</th>
<th>Total ears</th>
</tr>
</thead>
<tbody>
<tr>
<td>905858</td>
<td>RSS male</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>905859</td>
<td>&quot;</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>906401</td>
<td>&quot;</td>
<td>9</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>906402</td>
<td>&quot;</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

Total plants observed 20 6 26
Expected if cytoplasmic control 26 0 26
greenhouse during the winter to produce the F2 seed. During the 1990 summer, the F2 plants were grown and tested for the presence of the cross-incompatibility reaction by pollinating with the pollen from the RSS male lines. The expectation was that if this incompatibility is caused by cytoplasmic factor(s), then the RSS factor(s) will not be present in the F2 plants and their crosses with the RSS male lines will not show RSS ears. The results of this strategy are presented in Table 1 which show a segregation of RSS and NSS factors in the F2 population with approximately 23% of the F2 plants yielding RSS ears when crossed with the RSS male pollen. This means that the factors causing this unidirectional cross-incompatibility were transmitted to the progeny even when the RSS female line carrying these factors was used as a male parent. These results indicate that the factors causing RSS are not cytoplasmic but are segregating as nuclear factors.

2. Number of genes controlling the unidirectional cross-incompatibility reaction in the RSS female: Conversion of an RSS male into an RSS female line

Following this proof that this unidirectional cross-incompatibility is not controlled by cytoplasmic factors, the next question to be addressed is since it is controlled by nuclear genes, then how many genes are responsible for the incompatibility reaction of the female parent in this incompatible cross? To answer this question, a crossing
strategy (Fig. 3) was designed. The rationale behind this strategy was that if nuclear genes are controlling the incompatibility reaction of the RSS female lines, then it should be possible to introduce these genes into any line which is not showing incompatibility with the RSS male. For this purpose, the RSS male line ($A_1\text{ et}/A_1\text{ et}$) was selected for its transformation to an RSS female line because it was previously shown that the $A_1\text{ et}/A_1\text{ et}$ line does not have these genes as it gives normal seed setting when crossed with any other RSS male line (Bdliya, 1984). The crosses (Fig. 3, line 1) were made during the 1988 summer using the RSS male line ($A_1\text{ et}/A_1\text{ et}$) as female parent and the RSS female line ($a_1\text{ sh2}/a_1\text{ sh2}$) as the male parent. The F1s' were selfed in the greenhouse during the following winter to produce the F2 seed in order to determine the segregation ratios of the incompatibility factor(s). The F1 plants were also backcrossed (Fig. 3, line 4) using the RSS female line as recurrent pollen parent in the greenhouse.

The F2 and backcross plants (Fig. 3, line 7) were grown in the field during the 1989 summer and crossed with the RSS male pollen to test their incompatibility reaction. The data were recorded for seed setting (NSS or RSS) in these segregating populations and are presented in Table 2 and Table 3. The results in Table 2 show that the incompatibility factors were segregating in the F2 population. Six out of the 26 F2 plants showed RSS when crossed with the
Segregation of genes controlling cross-incompatibility in RSS female.

Figure 3. Crossing strategy used to determine the number of genes controlling the incompatibility reaction in the RSS female. Numbers in the parentheses indicate the row numbers used in this experiment.
Table 2. Results of the experiment conducted during 1990 to determine the number of genes controlling the incompatibility reaction of the RSS female lines

<table>
<thead>
<tr>
<th>F2 population row #</th>
<th>Male tester</th>
<th>Number of ears with NSS</th>
<th>Total ears</th>
</tr>
</thead>
<tbody>
<tr>
<td>905858</td>
<td>RSS male</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>905859</td>
<td>&quot;</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>906401</td>
<td>&quot;</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>906402</td>
<td>&quot;</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

Total plants observed

<table>
<thead>
<tr>
<th></th>
<th>Total ears</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

Expected if monogenic control

<table>
<thead>
<tr>
<th></th>
<th>Total ears</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.5</td>
<td>6.5</td>
</tr>
<tr>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

\[ \chi^2 = 0.0 \]
Table 3. Results of the experiment conducted during 1989 to determine the number of genes controlling the incompatibility reaction of the RSS female lines

<table>
<thead>
<tr>
<th>BCl plants row #</th>
<th>Male tester</th>
<th>Number of ears with NSS</th>
<th>RSS</th>
<th>Total ears</th>
</tr>
</thead>
<tbody>
<tr>
<td>890243 Y</td>
<td>RSS male</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>890243 Z</td>
<td>&quot;</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>890244 Y</td>
<td>&quot;</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>890244 Z</td>
<td>&quot;</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>890245 Y</td>
<td>&quot;</td>
<td>5</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>890245 Z</td>
<td>&quot;</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>890246 Y</td>
<td>&quot;</td>
<td>4</td>
<td>7</td>
<td>11</td>
</tr>
</tbody>
</table>

Total plants observed 26 28 54
Expected if monogenic control 27 27 54

\[ \chi^2 = 0.0185^{\text{ns}} \quad P > 0.90 \]
RSS male pollen which is approximately 23% of the total. If we assume that this trait is controlled by a single recessive gene, then 25% of the total plants are expected to give RSS ears when crossed with the RSS male pollen. The differences between the observed and the expected values were tested by a chi-square test which showed that the differences between the observed and the expected values were nonsignificant. These results indicate that the incompatibility reaction in the RSS female lines is controlled by a single recessive gene.

The data for the backcross population presented in Table 3 confirmed the monogenic inheritance of the cross-incompatibility in the RSS female lines. The expectation based upon monogenic recessive trait was 1 NSS : 1 RSS in the test cross and about the same ratio was observed, i.e., 26 NSS : 28 RSS. The chi-square test showed a nonsignificant difference between the observed and the expected values for NSS and RSS. Both of these strategies conclude that the unidirectional cross-incompatibility reaction in the RSS female lines is controlled by a single recessive gene and its presence in the homozygous recessive form in any line will make that line cross-incompatible as a female parent with the RSS male lines. This gene is now designated as cif (cross-incompatible female) with its wild type (normal) allele as $^+cif$. 
3. Number of genes controlling the unidirectional cross-incompatibility reaction in the RSS male lines

The unidirectional cross-incompatibility under investigation is always observed between specific female and specific male parents. It can be hypothesized that both of these parents must have genes which interact and cause incompatibility when brought together in a cross. The results in the previous section showed that the female component of this incompatible cross is controlled by a single recessive gene designated cif. In order to investigate how many genes are controlling the incompatibility reaction in the other component (RSS male) of the cross, a crossing scheme (Fig. 4) was designed. The rationale behind this strategy is that if the RSS male is crossed with any other line which does not show RSS when crossed with the RSS female lines, the segregation of these genes in the F2 or in the backcross populations will indicate the number of genes controlling this trait in the RSS male lines. The segregation ratios of the genes controlling the incompatibility reaction in the RSS male can be tested by pollinating the RSS female tester plants with the pollen of these F2 or BC1 plants. The crosses (Fig. 4, line 1) were made during the 1988 summer and the F1 plants were selfed in the greenhouse during the winter to produce the F2 seed. The F1 plants were also backcrossed (Fig. 4, line 4) with the RSS male line. The choice of RSS male as the recurrent parent was based on previous reports that the male
incompatibility reaction is recessive to the compatibility reaction (Sukhapinda, 1981). Subsequently, to get the test cross ratios in the backcross, the recessive parent (RSS male) was used as a recurrent parent. Both the F2 and the BC1 populations (Fig. 4, line 6) were grown in the field during the 1989 summer and the segregation ratios of the incompatibility factors were tested by pollinating the RSS female tester lines with the pollen from these F2 and the BC1 plants. The data recorded for RSS and NSS in these crosses are presented in Table 4 and Table 5.

The F2 segregation results presented in Table 4 indicate that the incompatibility reaction in the RSS male component is apparently controlled by two independently segregating recessive genes as 16 plants out of the total 215 F2 plants showed the RSS trait when tested on the RSS female tester lines. This is about 1/14th of the total. If this trait is under the control of 2 independent recessive genes, then the pollen from 1/16th of the total F2 plants should have shown incompatibility (RSS) reaction with the RSS female testers. The differences between the observed and the expected values were tested by a chi-square test and found statistically non significant. The conclusion from this experiment is that the incompatibility reaction in the RSS male lines is controlled by 2 independently segregating recessive genes.

The data for the test cross (Table 5) show that 17 out of the 53 BC1 plants were incompatible as male parent when
Figure 4. Crossing scheme used to determine the number of genes controlling the incompatibility reaction in the RSS male lines. The numbers in the parentheses indicate the row numbers used in this experiment.
Table 4. Results of the experiment conducted during 1989 to determine the number of genes controlling the incompatibility reaction of the RSS male lines

<table>
<thead>
<tr>
<th>F2 plants row #</th>
<th>Female tester</th>
<th>Number of ears with NSS</th>
<th>RSS</th>
<th>Total ears</th>
</tr>
</thead>
<tbody>
<tr>
<td>890101 RSS female</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>890102 &quot;</td>
<td>7</td>
<td>2</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>890103 &quot;</td>
<td>8</td>
<td>1</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>890104 &quot;</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>890105 &quot;</td>
<td>9</td>
<td>1</td>
<td>10</td>
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<tr>
<td>890106 &quot;</td>
<td>11</td>
<td>1</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>890107 &quot;</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>890108 &quot;</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>890109 &quot;</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>890110 &quot;</td>
<td>7</td>
<td>2</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>890111 &quot;</td>
<td>10</td>
<td>1</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>890112 &quot;</td>
<td>10</td>
<td>1</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>890113 &quot;</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>890114 &quot;</td>
<td>12</td>
<td>0</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>890115 &quot;</td>
<td>12</td>
<td>0</td>
<td>12</td>
<td></td>
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<td>890116 &quot;</td>
<td>11</td>
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<td>12</td>
<td></td>
</tr>
<tr>
<td>890117 &quot;</td>
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<td>15</td>
<td></td>
</tr>
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<td>890118 &quot;</td>
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<td>9</td>
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<td>11</td>
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<tr>
<td>890120 &quot;</td>
<td>16</td>
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<td>16</td>
<td></td>
</tr>
<tr>
<td>890121 &quot;</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Total plants observed 199 16 215
Expected if controlled by two recessive genes. 201.56 13.44 215

\[ \chi^2 = 0.336^{\text{ns}} \quad P > 0.50 \]
Table 5. Results of the experiment conducted during 1989 to determine the number of genes controlling the incompatibility reaction of the RSS male lines

<table>
<thead>
<tr>
<th>BC1 plants row #</th>
<th>Female tester</th>
<th>Number of ears with NSS</th>
<th>Total ears</th>
<th>Number of ears with RSS</th>
<th>Total ears</th>
</tr>
</thead>
<tbody>
<tr>
<td>890146</td>
<td>RSS female</td>
<td>7</td>
<td>13</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>890147</td>
<td>&quot;</td>
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<td>14</td>
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<tr>
<td>890148</td>
<td>&quot;</td>
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<td>4</td>
<td>12</td>
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<tr>
<td>890149</td>
<td>&quot;</td>
<td>12</td>
<td>14</td>
<td>2</td>
<td>14</td>
</tr>
</tbody>
</table>

Total plants observed 36 17 53

Expected if controlled by two recessive genes. 39.75 13.25 53

\[ \chi^2 = 1.06^{ns} \quad P > 0.25 \]
crossed on the RSS female lines. This is about 32% of the total backcross plants tested. If we assume that the incompatibility reaction of the RSS male lines is controlled by two independent recessive genes, then 25% BC1 plants should have been incompatible as a male parent with the RSS female tester lines. The differences between the observed and the expected values were tested by a chi-square test and were found nonsignificant. These results of the F2 and the testcross prove that the incompatibility reaction of the RSS male parent is controlled by two independently segregating recessive genes which are not present in the other parent (RSS female) in this particular cross-incompatible combination. Any line in which these two genes are present in the homozygous recessive form will be incompatible as a male parent with the RSS female lines but will be compatible as female parent. These genes are designated as $cim1$ (cross-incompatible male) and $cim2$ whereas their wild type (normal) alleles as $^+cim1$ and $^+cim2$ respectively.

B. Factors Responsible for the Escape from the Incompatibility Mechanism

In this system of unidirectional cross-incompatibility, incompatibility is not always complete as it is evident that a few gametes escape the incompatibility mechanism and some seeds are set even in the incompatible crosses (Fig. 5). To investigate the reasons of this slippage in the
incompatibility mechanism, a crossing strategy was designed and is presented in Figure 6. The rationale behind this strategy was to test the hypothesis that this escape from incompatibility mechanism may be due to some mutations in the genes responsible for the incompatibility reaction in the RSS male or the RSS female lines. To test this hypothesis, "escape" seeds from the incompatible crosses were selfed to produce the F2 seed and the F2 populations were tested for the presence of the RSS female gene (cif) by crossing the F2 plants with pollen from the RSS male lines. Similarly, the presence of the RSS male genes (cim1 and cim2) was tested by pollinating the RSS female tester lines with the pollen from the F2 plants. The results from these studies are presented in Table 6. These results indicate that out of the six "escape" seeds tested, one (89g539-1) was the result of an accidental selfing which is evident from the fact that the genotype of all its F2 plants was the same as the female parent (RSS female), i.e., all the F2 plants of this seed were homozygous for the cif gene and none of them showed the presence of the RSS male genes (cim1 and cim2). The other five seeds tested showed the presence of both RSS male and RSS female genes in their F2 progenies. This indicates that neither RSS male nor RSS female genes were changed. These results (though from a small sample) indicate that the occasional seed setting in the incompatible crosses is not due to a change in the genetic nature of the incompatibility
Figure 5. Seed setting in the incompatible crosses
RSS female $\times$ RSS male
\[ \downarrow \]
F1
(Six "escape" seeds)
\[ \downarrow \]
RSS female $\times$ F2 $\times$ RSS male
\[ \downarrow \]

If the genes for the RSS male incompatibility reaction are not mutated, then there will be a segregation for RSS and NSS.

If the gene for the RSS female incompatibility reaction is not mutated, then there will be a segregation for RSS and NSS.

Figure 6. Crossing strategy to determine the factors responsible for the escape from the incompatibility mechanism of the incompatible crosses. Six "escape" seeds were tested.
Table 6. Results of the experiments conducted during 1989 and 1990 to determine the factors responsible for the escapes from the incompatibility mechanism of the incompatible crosses

<table>
<thead>
<tr>
<th>Seed from the incompatible cross</th>
<th>F2 population row #</th>
<th>Crossed by RSS male NSS RSS Total</th>
<th>Crossed on RSS female NSS RSS Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>881258Y-1</td>
<td>890214-0215</td>
<td>17 2 19</td>
<td>19 6 25</td>
</tr>
<tr>
<td>881258Z-12</td>
<td>890216-0218</td>
<td>27 1 28</td>
<td>29 0 29</td>
</tr>
<tr>
<td>881258Z-13</td>
<td>890219-0221</td>
<td>16 10 26</td>
<td>25 1 26</td>
</tr>
<tr>
<td>881258Z-14</td>
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<td>890250</td>
<td>26 13 39</td>
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<td>905829-5835</td>
<td>39 5 44</td>
<td>35 9 44</td>
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<td>89g539-1</td>
<td>905836-5843</td>
<td>0 40 40</td>
<td>39 0 39</td>
</tr>
</tbody>
</table>
mechanism but these may be due to the slippage of the incompatibility mechanism or other factors.

C. Time and Place of the Incompatibility Reaction

The results in the previous sections showed that the incompatibility reaction occurs only when the RSS female lines are used as the female parent in the incompatible cross. The reciprocal crosses between the same parents result in normal seed setting. It is also known that the incompatible pollination does not adversely affect the pistil physiology and normal seed setting can be obtained with subsequent pollination with compatible pollen (Bdliya, 1984). This indicates that the incompatibility reaction occurs before fertilization of the ovules. These findings lead to the speculation that the incompatibility reaction might be due to the following reasons.

a. The incompatible pollen grains do not germinate on the incompatible silks.

b. The incompatible pollen germinate but the pollen tubes are unable to penetrate the incompatible silks.

c. The incompatible pollen tubes penetrate into the incompatible silks but their growth is arrested before they reach the ovules.

d. The pollen tubes reach the ovules but are unable to fertilize the ovules.
To investigate these possibilities, the following studies were made.

1. *In vivo germination of the incompatible pollen grains on the incompatible silks*

To investigate whether the incompatible pollen grains germinate on the incompatible silks *in vivo*, the incompatible crosses were made in the field during the 1990 summer and the silk samples were collected 6 hours after pollination. The silk samples were fixed and prepared for scanning electron microscopic examination as explained in the materials and methods section. The observations were made and photographs were taken using JEOL JSM-35 scanning electron microscope. It is clear from the observations that the incompatible pollen grains do germinate on the incompatible silks (Fig. 7). It was also observed that after germination, the pollen tubes grew toward the silk and did penetrate the silks through the silk hairs called trichomes (Fig. 8). It was also noted that sometimes the pollen tubes may penetrate directly into the silk body as seen in Fig. 9. All these observations lead to the conclusion that the incompatible pollen grains do germinate and the pollen tubes penetrate into the incompatible silks. The conclusion can be drawn that the incompatibility reaction occurs after the penetration of the pollen tubes in the silk body.
Figure 7. *In vivo* pollen germination in the silk samples collected 6 hours after *incompatible* pollination. The pollen tube can be seen near the silk (arrow)
Figure 8. *In vivo* pollen germination and pollen tube penetration in the silk hair (arrow) in sample collected 6 hours after *incompatible* pollination.
Figure 9. *In vivo* pollen germination and pollen tube penetration directly in the silk body (arrow) in sample collected 6 hours after *incompatible* pollination.
2. *In vivo* pollen tube growth studies inside the silk

The results of the *in vivo* pollen germination studies showed that the incompatible pollen grains do germinate and the pollen tubes penetrate into the incompatible silks. Now the question arises, what happens to the pollen tubes inside the silk body? To answer this question, the compatible (RSS male X RSS female) as well as incompatible (RSS female X RSS male) crosses were made in the field during the 1990 summer and the silk samples were collected 6, 12, 18 and 24 hours after pollination. These silk samples were fixed and stained according to the procedure described in the materials and methods section. When these stained silk samples are pressed lightly on a microscope slide under a cover-slip, the pollen tubes can be seen inside the silk body as these are stained in dark blue color while the surrounding pistil tissues are stained in the light greenish blue color.

The observations on the pollen tube growth inside the silks were recorded using a light microscope fitted with a 35mm camera. It was observed that in the silk samples collected 6 hours after pollination, the pollen tubes had already passed through the silk hairs (trichomes) both in the compatible as well as incompatible crosses and had entered the silk body but were still in the cortical tissues (Fig. 10 and Fig. 11). No difference in the pollen tube growth was observed between the compatible and the incompatible crosses.
The silk samples collected 12 hours after pollination showed that in the compatible crosses, the pollen tubes had reached into the transmitting tracts of the silk body and were growing towards the ovary (Fig. 12). In the incompatible crosses (RSS female X RSS male) also, the pollen tubes had reached into the transmitting tracts but their growing tips were swollen to a considerable extent (Fig. 13) compared with the tips of the pollen tubes in the compatible crosses (Fig. 12).

When the silks collected 18 hours after pollination were observed under the microscope, it was found that in the compatible crosses the pollen tubes were still growing in the transmitting tracts of the silk tissues (Fig. 14). In the incompatible crosses, the pollen tube growth seems to be arrested and the tips of the pollen tubes were still swollen (Fig. 15).

In the samples collected 24 hours after pollination, it appeared that the tips of the pollen tubes in the incompatible crosses had burst (Fig. 16) while no tube tip was seen in the silks with compatible pollinations but the pollen tubes can still be traced to the base of the silks (Fig. 17). Probably the tips of the pollen tubes in the compatible crosses had already entered the embryo sacs.

These observations indicate that in the compatible pollinations, the pollen tubes enter the silk body through the silk hairs within 6 hours of pollination. From the silk
Figure 10. *In vivo* pollen tube penetration in the maize silk samples collected 6 hours after *compatible* pollination. The pollen tube entered through the silk hair and is still in the cortical tissue of the silk (arrow)
Figure 11. In vivo pollen tube penetration in the maize silk samples collected 6 hours after incompatible pollination. The pollen tube entered the silk through the silk hair and is still in the cortical tissue of the silk (arrow)
Figure 12. *In vivo* pollen tube growth inside the maize silk samples collected 12 hours after *compatible* pollination. The pollen tube is growing in the transmitting tissue of the silk (arrow)
Figure 13. *In vivo* pollen tube growth in the maize silk samples collected 12 hours after *incompatible* pollination. The pollen tube is growing in the transmitting tissue and its tip is swollen (arrow).
Figure 14. *In vivo* pollen tube growth in the maize silk samples collected 18 hours after *compatible* pollination. The pollen tube is growing in the transmitting tract of the silk and the pollen tube tip is normal (arrow)
Figure 15. *In vivo* pollen tube growth in the maize silk samples collected 18 hours after *incompatible* pollination. The pollen tube is growing in the transmitting tract of the silk and its tip is swollen (arrow)
Figure 16. *In vivo* pollen tube growth in the maize silk samples collected 24 hours after *incompatible* pollination. The burst pollen tube tip can be seen in the transmitting tract of the silk (arrow).
Figure 17. In vivo pollen tube growth in the silk samples collected 24 hours after compatible pollination. No pollen tube tip was seen in the silk but pollen tube can be seen in the transmitting tract of the silk (arrow).
hairs, the pollen tube travels some distance in the cortical tissues (Fig. 10) and then enters the transmitting tract (Fig. 12) sometime between 6 to 12 hours of pollination. The fertilization process seems to be completed in about 24 hours after pollination. These results for the compatible pollinations agree with the earlier studies of Miller (1919), Weatherwax (1919), and Randolph (1936). In the incompatible pollinations, the pollen tube enters the silk body through the silk hair (Fig. 11) within 6 hours of pollination just like the compatible pollen tubes. After entry of the pollen tube into the transmitting tract, the incompatibility reaction occurs. The incompatible pollen tube tip becomes swollen (Fig. 13) within 12 hours of pollination. Sometime between 18 and 24 hours of pollination, the tips of the pollen tubes burst (Fig. 16) and the pollen tube growth is stopped.

D. Prevalence of the RSS Genes in the Commercial Inbred Lines: a Test for the cif Gene in Inbreds

During the 1990 summer, 23 inbred lines released by different universities and Research organizations (Table 7) were planted to check the presence of the unidirectional cross-incompatibility genes. The seed was obtained with the courtesy of Dr. A. R. Hallauer, Professor of Agronomy, Iowa State University. Due to poor germination, only the preliminary observations were recorded for 9 inbred lines by
crossing with pollen from the RSS male lines to check the presence of the RSS female gene (cif) only. The results obtained from these crosses are presented in Table 7 which indicate that none of these lines has the RSS female incompatibility gene. Probably during the breeding process, these incompatibility genes have been eliminated or these genes have originated de novo in the cytogenetics nursery material Ames, Iowa. This study needs to be repeated with a greater number of inbred lines and with more plants per inbred to detect the presence of RSS genes even if these are present at very low frequencies.
Table 7. Results of the experiment conducted during 1990 to check the presence of the RSS female gene (cif) in the commercial inbred lines

<table>
<thead>
<tr>
<th>Inbred line</th>
<th>Institution</th>
<th>Total plants tested</th>
<th>Seed setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>B76</td>
<td>Iowa State Univ.</td>
<td>12</td>
<td>12 RSS 0</td>
</tr>
<tr>
<td>B84</td>
<td>Iowa State Univ.</td>
<td>12</td>
<td>12 RSS 0</td>
</tr>
<tr>
<td>CI31A</td>
<td>USDA</td>
<td>8</td>
<td>8 RSS 0</td>
</tr>
<tr>
<td>DE811</td>
<td>Univ. of Delaware</td>
<td>11</td>
<td>11 RSS 0</td>
</tr>
<tr>
<td>H99</td>
<td>Purdue Univ.</td>
<td>10</td>
<td>10 RSS 0</td>
</tr>
<tr>
<td>NC264</td>
<td>North Carolina State Univ.</td>
<td>8</td>
<td>8 RSS 0</td>
</tr>
<tr>
<td>R225</td>
<td>Univ. of Illinois</td>
<td>7</td>
<td>7 RSS 0</td>
</tr>
<tr>
<td>R226</td>
<td>Univ. of Illinois</td>
<td>7</td>
<td>7 RSS 0</td>
</tr>
<tr>
<td>R227</td>
<td>Univ. of Illinois</td>
<td>7</td>
<td>7 RSS 0</td>
</tr>
</tbody>
</table>
V. DISCUSSION

A. Genetics of the Unidirectional Cross-Incompatibility

Many cases of unidirectional cross-incompatibility have been reported in the literature both for inter as well as intraspecific crosses (Anderson and de Winton, 1931; Harrison and Darby, 1955; Lewis and Crowe, 1958; Grun and Aubertin, 1966; Martin, 1961b, 1964, 1967; Pandey, 1968; Keulemans, 1984). In most of these cases it has been reported that the unidirectional cross-incompatibility reaction occurs whenever the self-incompatible species/line is used as female parent and self-compatible species/line as a male parent, though some exceptions to this rule are also reported (Harrison and Darby, 1955; Lewis and Crowe, 1958; Pandey, 1962). However, what is controversial is whether the unidirectional cross-incompatibility is controlled by the same genes which control the self-incompatibility or by different genes. Some authors such as Lewis and Crowe (1958), Keulemans (1984) and Boyel and Stimart (1986) reported that the unidirectional cross-incompatibility is controlled by the same S-locus which controls the self-incompatibility. But other workers like Grun and Aubertin (1966) and Pandey (1968) are of the opinion that the SI and UI are controlled by different genes. Still others like Pushkarnath (1953) and Pandey (1962) reported that the UI is controlled by an interaction of the S-locus and other modifying loci. Pushkarnath (1953) observed that
whenever a dominant modifying factor $R$ is present in the style in homozygous or heterozygous condition, it will prevent pollen tubes carrying any $S$-allele to affect fertilization. However, it is generally known that the unidirectional cross-incompatibility is noticed only in the species which have self-incompatibility systems. The cases of unidirectional cross-incompatibility in maize are different from all these species because self-incompatibility has never been reported in maize (Heslop-Harrison and Heslop-Harrison, 1985). The detailed studies at genetic and molecular level of these cases in maize may be helpful in resolving this question.

Unlike the previously described unidirectional cross-incompatibility systems in maize (Nelson, 1952; Ashman, 1975; Kermicle and Allen, 1990), the system investigated in this study is more closely related to the general incompatibility systems found in the Gramineae family in which generally more than one locus control the incompatibility reaction (Lundqvist, 1954; Murray, 1974; Larsen, 1977a).

In the present study it was found that the unidirectional cross-incompatibility reaction is controlled by three independent recessive loci. One locus (cif) controls the incompatibility reaction in the female and the other two, cim1 and cim2, control the incompatibility reaction in the male component. The cross is incompatible only when the female parent has the cif locus in the homozygous recessive form and the male parent has the cim1 as well as the cim2 locus also in
homozygous recessive form. Sukhapinda (1981) studied the possibility of gal factor involvement in this cross-incompatibility system and reported that gal factor did not play any role in its control. This current study provides additional evidence in this direction. The first evidence is that the incompatibility is dominant over compatibility in the gal system while in the RSS system, it is recessive. The other difference between these two systems is that the gal system is controlled by a single locus while this new system is controlled by the three interacting loci. Similarly, this system is also different from the TIC-CP incompatibility system described by Kermicle and Allen (1990) who transferred the incompatibility factors from teosinte to dent corn. In the TIC-CP system also, the incompatibility is dominant over the compatibility and is controlled by a single locus.

The other interesting aspect of the present study is that the factors controlling the incompatibility reaction in the male and female are non-allelic and segregate independently which has never been reported in any other species (Haring et al., 1990). This separation of male and female incompatibility factors may be important for the determination of some gene products of the incompatibility genes from the male gamete also. As of now, the S-gene products such as S-glycoproteins have been isolated only from the pistil tissue (Nasrallah et al., 1987; Anderson et al., 1989) and their counterpart from the pollen grains has never been isolated.
Among the reasons given for not finding the \(S\)-gene product from the pollen may be the tight linkage between pollen and style functional parts of the complex \(S\)-locus which has never been separated by conventional breeding (Haring et al., 1990). In this new system (RSS system), the genes controlling the incompatibility reaction in the male are different from the genes controlling the incompatibility reaction in the female, and therefore the chances of distinguishing both biochemical components of the incompatibility mechanism are likely more accessible in this system.

B. Factors Responsible for the Escape from the Incompatibility Mechanism

In the RSS system, the incompatibility is not always complete because a few gametes escape the incompatibility mechanism. The reasons for this escape were investigated and it was found that the escape from the incompatibility mechanism is not due to a change in the genetic nature of the incompatibility factors because the genes controlling the incompatibility reactions in the RSS male and the RSS female were recovered unchanged in the F2 progenies of the escaped seeds. This implies that it may be due to some other genetic modifiers or it may be due to some environmental factors. This experiment needs to be repeated on a larger scale before reaching a conclusion. However, the escape from the incompatibility mechanism is not unique to this system because
almost every incompatibility system described has this kind of slippage and is known as pseudocompatibility. Nelson (1952) classified the crosses as incompatible with seed setting less than 10% of the normal. Kermicle and Allen (1990) reported about 2% seed setting in the incompatible cross Gal-s/Gal-s X gal/gal. Martin (1964) reported that the environmental conditions particularly, temperature, may cause some variation in the pollen-style interaction and may thus lead to the pseudocompatibility in *Lycopersicon hirsutum*.

C. Time and Place of the Incompatibility Reaction

In the species with incompatibility systems, the incompatibility reaction occurs at the stigma surface in the sporophytic system (Sarker et al., 1988) and inside the style in the species with the gametophytic system (Bernatzky et al., 1988), with some exceptions in both the systems. The general features of the incompatibility reaction in the gametophytic system are the swelling and bursting of the tips of the pollen tubes inside the incompatible styles which results in arresting of the pollen tube growth.

The results of the present study are not different from the general features of the gametophytic system of incompatibility. Bdliya (1984) suggested the occurrence of the incompatibility reaction before fertilization in this RSS system of unidirectional cross-incompatibility. The results of the present investigations revealed that there is no
difference in the compatible and the incompatible pollen tube growth during first 6 hours of pollination as in both cases, the pollen grains germinated and the pollen tubes entered the silk body through the silk hairs (Fig. 10 and Fig. 11). The incompatibility reaction seems to occur between 6 and 12 hours after pollination because the tips of the pollen tubes in the incompatible pollinations appeared swollen (Fig. 13) compared with the tips of the pollen tubes in the compatible pollinations (Fig. 12). It was also observed that sometime between 18 and 24 hours after pollination, these pollen tube tips burst (Fig. 16) and the pollen tube growth stopped. A similar mechanism of incompatibility has been reported by Clarke et al. (1985) in another family (Solanaceae) with the gametophytic system of incompatibility.

D. Prevalence of the RSS Genes in the Commercial Inbred Lines

There is no history of self-incompatibility in maize but few cases of cross-incompatibility have been reported in this species (Nelson, 1952; Ashman, 1975; Kermicle and Allen, 1990). The system reported here (RSS system) was studied by Sukhapinda (1981), Sukhapinda and Peterson (1983) and Bdliya (1984). This RSS system is different from the other reported unidirectional cross-incompatibility systems in maize as discussed earlier. The question that remains is whether this system originated de novo in the cytogenetics nursery Ames, Iowa or is it also present in the breeding material used by
maize breeders at different research organizations? Based upon the limited testing, it can be said that at least the RSS female gene \((cif)\) is not present in the limited number of inbred lines tested. It seems that this gene may has been eliminated from the inbred lines due to selection against reduced seed setting or it originated \textit{de novo} in the maize cytogenetics nursery Ames, Iowa. However, this experiment needs to be repeated before reaching any final conclusion with more inbred lines/populations and with a greater number of plants per line/population so that the RSS genes may be detected even if they may be present at very low frequencies.

E. Molecular Model for the Incompatibility Mechanism

Most of the previous models for the molecular mechanism of self-incompatibility postulate that the products of a single locus \((S\)-locus\) interact with each other and cause incompatibility. This locus is considered to be comprised of a pollen and a pistil part and the proteins encoded by these parts are expressed in the pollen and pistil. On self-pollination, these products form dimers and give rise to the incompatibility reaction (de Nettancourt, 1977). The occurrence of unilateral incompatibility is also believed to be caused when mutations change only the stylar or the pollen part of the \(S\)-locus (Lewis, 1979).

The present studies do not agree with this single locus hypothesis as it is shown that the incompatibility reaction in
the pistil and in the pollen is controlled by different genes and these genes segregate independently from each other. These results indicate that the products of the non-allelic incompatibility genes interact and cause incompatibility. This hypothesis that interaction of the products of the non-allelic genes cause incompatibility is also supported by the following reports.

1. The most prominent $S$-locus products are the glycoproteins which cosegregate with the specific $S$-alleles and their appearance also matches with the onset of the incompatibility reaction in the pistil during its maturation (Bernatzky et al., 1988). It has also been shown that these glycoproteins have ribonuclease activity (McClure et al., 1989) and cause $S$-allele specific degradation of rRNAs in the incompatible crosses in vivo (McClure et al., 1990). However, as of now, these glycoproteins are found only in the pistil extracts and not from the pollen (Haring et al., 1990). This indicates that if this locus controls the incompatibility reaction, it controls only the pistil part of the incompatibility reaction and not of the pollen.

2. Clark et al. (1990) reported that a pseudo-self-compatible Petunia line MSU1093 produced the same kind and quantity of stylar proteins as the self-incompatible line and the level of the ribonuclease activity in the stylar extract of this pseudo-self-compatible variety MSU1093 was also identical to that found in the self-incompatible line. They
demonstrated by transient expression assays of the reporter gene β-glucuronidase that the 5' flanking sequences of the S1 allele directs the tissue specific expression of the S-locus only in the style and not in the pollen. From these results they suggested that the S-locus is expressed only in the style and not in the pollen.

3. Similarly, Xu et al. (1990) reported a mutant HAG-5 in *Solanum chacoense* which was self-compatible. This mutant was cross-compatible with self-incompatible HAG-6 plant as a female parent but not as a male parent. The analysis of the stylar glycoproteins from these two plants (HAG-5 and HAG-6) showed no difference from each other. From these results they concluded that the mutation which made the HAG-5 plant self-compatible did not occur in the S-locus.

4. Another proof that the incompatibility reaction in the pollen and the pistil is not controlled by the same locus was provided by Moor and Nasrallah (1990) when they transformed the self-compatible *N. tabacum* plants with genomic clones of *Brassica oleracea* self-incompatibility alleles S13 and S22. They noted the expression of these foreign genes in the stylar transmitting tract and their expression in the style was also developmentally regulated as was expected but the resultant transgenic plants were not self-incompatible. These results also indicate that the genes controlling the incompatibility reaction in the pollen and the pistil are not the same.
All the above discussions lead to the hypothesis that the incompatibility reaction is not controlled by one locus (S-locus) but it is the result of an interaction of more than one gene product. Based upon these reports and the findings of the present studies, a molecular model for the incompatibility mechanism is suggested as:

The genes responsible for the incompatibility reaction in the female encode glycoproteins which have ribonuclease activity (McClure et al., 1989). These glycoproteins can degrade the rRNAs (McClure et al., 1990) in the pollen or pollen tubes but these glycoproteins are separated from the rRNAs of the pollen or pollen tubes by the pollen or pollen tube walls, so the rRNAs are safe from the ribonuclease activity unless the glycoproteins enter in the pollen or the pollen tubes.

The genes responsible for the incompatibility reaction in the pollen encode the transmembrane receptor proteins which are located in the pollen or pollen tube walls and these receptor proteins have binding specificities for the glycoproteins. After pollination, when pollen or pollen tubes come in contact with these glycoproteins, the transmembrane receptor proteins regulate the intake of these glycoproteins into the pollen or the pollen tubes and thus the incompatibility reaction becomes allele specific. This type of mechanism is also present in animals and some viruses enter the cells via the receptor proteins and this process is called
receptor mediated internalization (Maddon et al., 1986).

In the self-incompatible pollinations, the glycoproteins present in the stigma/style can bind to the receptors proteins which mediate the intake of these glycoproteins into the pollen or the pollen tubes and as a result the rRNAs of the pollen or pollen tubes are degraded and the pollen cannot germinate or the pollen tube growth is stopped. In case of self-compatible pollinations, the glycoproteins can not bind to the receptor proteins, therefore can not enter into the pollen or the pollen tubes and thus do not show incompatibility reaction.

This model can explain both gametophytic and sporophytic incompatibility systems. The gametophytic system is one glycoprotein - one receptor system, so any pollen having a gene encoding the receptor protein specific to the glycoprotein encoded by the female genes will not be able to fertilize the gamete but the pollen grains with any other genotype will affect the fertilization. In the sporophytic system, the dominance/recessive phenomenon can be explained by the expression of only the dominant gene. These kinds of examples are available in animals as allele exclusion in antibodies producing cells (Early and Hood, 1981) or the expression of genes of only one X chromosome out of the two X chromosomes in the female.

The unilateral incompatibility can also be explained by this model as if only the genes responsible for male or female
incompatibility reaction is/are mutated. The unidirectional cross-incompatibility case investigated in this study is explained by this model as:

The female locus cif produces a glycoprotein in the silk and the male loci cim1 and cim2 are expressed in the pollen. Each of the two male genes encode a protein and the two products of these genes form a dimer and become an active receptor. The glycoproteins encoded by the cif allele can bind to the receptor protein dimer encoded by the male alleles cim1 and cim2 but can not bind to the hybrid protein or proteins encoded by +cim1 and +cim2, so the former situation gives rise to RSS and the latter to NSS. The F1 between the compatible RSS female and the incompatible RSS female is compatible because compatibility is dominant over incompatibility. So in this case only the +cif allele is expressed in the heterozygote and this may be due to the allele exclusion phenomenon (Early and Hood, 1981).

This model will explain the existence of other complicated or multigenic systems of incompatibility by assuming that during the evolutionary process, the incompatibility genes get duplicated and independent chance mutations altered their sequences and as a result their products also, thus the incompatibility can only be caused if the specific receptors of all these genes are present in the pollen or pollen tubes. The hypothesis that the self-compatible species originated from the self-incompatible
species (Stebbins, 1957) seems to be logical because mutations in only one of the incompatibility genes will result in the loss of incompatibility reaction. The efforts to obtain new incompatibility alleles failed (Lewis, 1979) because it is very unlikely to get mutations in all the incompatibility genes perfect enough so that their products match their moieties and give rise to the incompatibility reaction.

The discussion in the preceding pages leads to some general conclusions about the incompatibility systems described in different plant species and these general conclusions are summarized in Table 8.
Table 8. General features of different incompatibility systems described in plant species

<table>
<thead>
<tr>
<th>Name of the system</th>
<th># of genes controlling the system</th>
<th>Site of the incompatibility reaction</th>
<th>Dominance relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Self-incompatibility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Sporophytic</td>
<td>1</td>
<td>Stigma</td>
<td>Dominance or codominance in style only</td>
</tr>
<tr>
<td>2. Gametophytic</td>
<td>1-4</td>
<td>Style</td>
<td></td>
</tr>
<tr>
<td>B. Cross-incompatibility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Gal-s</td>
<td>1</td>
<td>-a</td>
<td>Incompatibility is dominant</td>
</tr>
<tr>
<td>2. TIC-CP</td>
<td>1</td>
<td>-a</td>
<td></td>
</tr>
<tr>
<td>3. RSS</td>
<td>3</td>
<td>Style</td>
<td>Compatibility is dominant</td>
</tr>
</tbody>
</table>

* Not reported.
VI. SUMMARY AND CONCLUSIONS

The RSS system of unidirectional cross-incompatibility was first discovered in 1975 in the maize cytogenetics nursery Ames, Iowa when the derivatives of a particular line (am(pa-pu)/al sh2) failed to set seed when pollinated with a particular male, namely al et/al et (Sukhapinda, 1981). The reciprocal crosses with the same parents gave normal seed setting. It was also reported that the incompatibility reaction in the RSS male was recessive to the compatibility reaction. Subsequently Sukhapinda and Peterson (1983) and Bdliya (1984) reported that some other lines were also behaving in the same fashion and were showing cross-incompatibility. The lines am(pa-pu)/al sh2 along with its derivatives and al sh2/al sh2 were behaving alike as incompatible females. These lines are now called RSS females. The other two lines called RSS male lines (al et/al et and Al et/Al et) were behaving as incompatible males. All these lines were self-compatible and the crosses between lines within groups (RSS male or RSS female group) were compatible in both directions. It was also found that this cross-incompatibility was neither caused by the gal factor nor controlled by the am(pa-pu) allele or the al locus.

The present investigations were made to study the genetics of this unidirectional cross-incompatibility and the mechanism involved in the rejection of the RSS male pollen.
It was found that this system is not controlled by cytoplasmic factors but nuclear genes are involved in the incompatibility reaction. The results showed that this system is controlled by three recessive loci. One locus named \textit{cif} (cross-incompatible female) controls the incompatibility reaction in the RSS female lines and the other two, \textit{cim1} and \textit{cim2} (cross-incompatible male), are controlling the incompatibility reaction in the RSS male lines. The cross will be incompatible only when the female parent has the \textit{cif} locus in homozygous recessive form and the male parent is also homozygous recessive for the \textit{cim1} as well as \textit{cim2} locus. The reciprocal cross between the same parents will be compatible.

Regarding the incompatibility mechanism, Bdliya (1984) suggested that the incompatibility reaction occurs before the fertilization of ovules. The present studies on the \textit{in vivo} pollen germination and the pollen tube growth revealed that there was no difference between the compatible and the incompatible pollen tube growth during first 6 hours of pollination as in both cases the pollen grains germinated and the pollen tubes entered the silk body through the silk hairs. The incompatibility reaction occurs between 6 and 12 hours of pollination as the tips of the pollen tubes in the incompatible pollinations appeared swollen to a considerable extent compared with the tips of the pollen tubes in the compatible pollination in the silk samples collected 12 hours after pollination. It was also observed that sometime between
18 and 24 hours after pollination, the swollen tips of the pollen tubes burst and the pollen tube growth is stopped.

The reasons for some "escape" from the incompatibility mechanism were also investigated and it was found that the slippage from the incompatibility mechanism is not due to any genetic change in the factors controlling the incompatibility reaction of the RSS male or RSS female lines. It may be due to some modifiers or due to some environmental factors.

Efforts were also made to determine the presence of the genes controlling the RSS system of unidirectional cross-incompatibility in the commercial inbred lines released by different research organizations. The results indicate that at least the RSS female gene cif is not present in the inbred lines tested. It may mean that this gene has been eliminated from the inbred lines due to selection against reduced seed setting during the breeding process or this system originated de novo in the maize cytogenetics nursery Ames, Iowa. However, this experiment needs to be repeated with a greater number of inbred lines/populations and additional plants per inbred line/population so that the RSS genes may be detected even if these may be present at very low frequencies.
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VIII. ACKNOWLEDGEMENTS

First of all, I thank God almighty who gave me strength and endurance to complete my studies. I also extend my thanks to my mother for her prayers for my success. My thanks are also due to Govt. of Pakistan who supported my studies in the United States.

I would also like to express my heartiest sense of gratitude to Dr. Peter A. Peterson, my major professor, for his encouragement, patience and guidance throughout my graduate program. I also wish my gratitude to Drs. Arden R. Campbell, Kendall R. Lamkey, Irvin C. Anderson, Donald K. Hotchkiss and Jonathan F. Wendel for serving on my graduate committee. A special thanks is given to Mr. Bruce L. Wagner who helped me in electron microscopy.

I am also extremely grateful to Ch. Nazir Ahmed to look after my official business during my absence from Pakistan.

Last but not least, I am very grateful to my wife for her love, consideration and patience during a very stressful period of my life.