

## 19. Incompatibility and male sterility and their utilization in crop improvement

### Self-Incompatibility

Self-incompatibility and sterility are the two mechanisms, which encourage cross-pollination. More than 300 species belonging to 20 families of angiosperms show self-incompatibility.

### Definition

In self incompatibility plants, the flowers will produce functional or viable pollen grains which fail to fertilize the same flower or any other flower of the same plant.

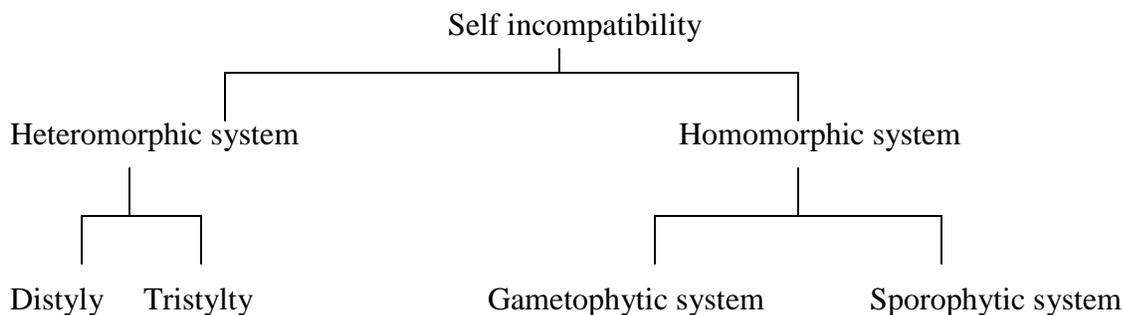
- Self incompatible pollen grain may fail to germinate on the stigmatic surface.
- Some may germinate but fails to penetrate the stigmatic surface.
- Some pollen grains may produce pollen tube, which enters through stigmatic surface, but its growth will be too slow. By the time the pollen tube enters the ovule the flower will drop.
- Some time fertilization is effected but embryo degenerates early.

### Reason

Self-incompatibility is appeared to be due to biochemical reaction, but precise nature of these is not clearly understood.

### Classification of self incompatibility

According to Lewis (1954) the self incompatibility is classified as follows:



### Heteromorphic system

In this case there will be difference in the morphology of the flowers. For example in *Primula sp* there are two types of flowers namely PIN and THRUM. PIN flowers have long style

and short stamens while THRUM flowers have short style and long stamens. This type of difference is known as Distyly. In case of distyly the only compatible mating is between PIN and THRUM. The relative position of anthers is determined by single gene S/s. The recessive *ss* produces PIN and heterozygotes *Ss* produces THRUM.

Homozygous dominant *SS* is lethal and do not exist. The incompatibility reaction of pollen is determined by the genotype of the plant producing them. Allele *S* is dominant over *s*. This system is also known as heteromorphic – sporophytic system. Pollen grains produced by PIN flowers will be all *s* in genotype as well as in incompatibility reaction: Whereas THRUM flowers will produce two types of gametes *S* and *s* but all of them would be *S* phenotypically. The mating between PIN and THRUM would produce *Ss* and *ss* progeny in equal frequencies. This system is of little importance in crop plants. It occurs in sweet potato and bulk wheat.



PIN FLOWER



THRUM FLOWER

Mating		Progeny	
Phenotype	Genotype	Genotype	Phenotype
Pin x Pin	<i>ss</i> x <i>ss</i>	Incom. Mating	-
Pin x Thrum	<i>SS</i> x <i>Ss</i>	1 <i>ss</i> : <i>Ss</i>	1 Thrum 1 Pin
Thrum x Pin	<i>Ss</i> x <i>ss</i>	1 <i>Ss</i> : <i>ss</i>	1 Thrum 1 Pin
Thrum x Thrum	<i>Ss</i> x <i>Ss</i>	Incom. Mating	-

TRISTYLY is known in some plants like *Lythrum salicaria*. In this case the style of the flower may be short, long or medium length

## Homomorphic System

Here the incompatibility is not associated with morphological difference among flower. The incompatibility reaction of pollen may be controlled by the genotype of the plant on which it is produced – (Sporophytic control) or by its own genotype – (Gametophytic control).

## Gametophytic system

First discovered by East and Mangelsdorf in 1925 in *Nicotiana sanderae*. Here the incompatible reaction of pollen is determined by its own genotype and not by the genotype of the plant on which pollen is produced. Genetically the incompatibility reaction is determined by a single gene having multiple allele. Eg. *Trifolium Nicotiana, Lycopersicon, Solanum, & Petunia*. Here codominance is assumed.

Genotype of plant (Sporophyte)	$S_1S_2$		$S_3S_4$	
Genotype of gametes	$S_1$	$S_2$	$S_3$	$S_4$
	↓	↓	↓	↓
Incompatible reaction of pollen	$S_1$	$S_2$	$S_3$	$S_4$
Incompatible reaction of style	$S_1$	$S_2$	$S_3$	$S_4$
Mating	$S_1S_2 \times S_1S_2$ - Fully incompatible			
	$S_1S_2 \times S_1S_3$ - Partially compatible			
	$S_1S_2 \times S_3S_4$ - Fully compatible			

## **Sporophytic system**

Here also the self incompatibility is governed by a single gene *S* with multiple alleles. More than 30 alleles are known in *Brassica oleracea*. Here dominance is assumed.

The incompatibility reaction is determined by the genotype of the plant on which pollen grain is produced and not by the genotype of the pollen. This system is more complicated. The allele may exhibit dominance, co-dominance or competition. This system was first reported by Hugues and Babcock in 1950 in *Crepis foetida* and by Gerstel in *Parthenium argentatum*. The sporophytic system is found in radish, brassicas and spinach.

Lewis has summarized the characteristics of sporophytic system as follows :

1. There are frequent reciprocal differences
2. Incompatibility can occur with female parent
3. A family can consist of three incompatibility groups
4. Homozygotes are a normal part of the system
5. An incompatibility group may contain two genotypes.

## **Mechanism of Self Incompatibility**

This is quite complex and is poorly understood. The various phenomena observed in Self incompatibility is grouped into three categories.

1. Pollen – Stigma interaction
2. Pollen tube – Style interaction
3. Pollen tube – Ovule interaction

### **1. Pollen – Stigma interaction**

This occurs just after the pollen grains reach the stigma and generally prevents pollen from germination. Previously it was thought that binucleate condition of pollen in gametophytic system and trinucleate condition in sporophytic system was the reason for self incompatibility. But later on it was observed that they are not the reason for SI. Under homomorphic system of incompatibility there are differences in the stigmatic surface which prevents pollen germination. In gametophytic system the stigma surface is plumose having elongated receptive cells which is commonly known as wet stigma. The pollen grain germinates on reaching the stigma and incompatibility reaction occurs at a later stage.

In the sporophytic system the stigma is papillate and dry and covered with hydrated layer of protein known as pellicle. This pellicle is involved in incompatibility reaction. With in few

minutes of reaching the stigmatic surface the pollen releases an exine exudates which is either protein or glycerol protein. This reacts with pellicle and induces callose formation, which further prevents the growth of pollen tube.

<b>Gametophytic system</b>	<b>Sporophytic system</b>
Stigma surface Plumose Commonly known as wet Stigma	Stigma surface Papillate and dry. Covered with hydrated layers of protein known as pellicle which involves in incompatibility reaction
Pollen grain germinates and incompatibility reaction occurs at a later stage	Pollen grain releases exine exudates which is protein or Glyco-protein
	This protein reaction with pellicle and induces callose formation and arrests growth of pollen type.

## **2. Pollen Tube – Style interaction**

Pollen grains germinate and pollen tube penetrates the stigmatic surface. But in incompatible combinations the growth of pollen tube is retarded with in the style as in *Petunia*, *Lycopersicon*, & *Lilium*. The protein and poly saccharine synthesis in the pollen tube stops resulting in bursting up of pollen tube and leading to death of nuclei.

## **3. Pollen tube – Ovule interaction**

In *Theobroma cacao* pollen tube reaches the ovule and fertilization occurs but the embryo degenerates later due to some biochemical reaction.

### **Male Sterility**

Male sterility is characterized by nonfunctional pollen grains, while female gametes function normally. It occurs in nature sporadically.

### **Morphological features of male sterility**

The male sterility may be due to mutation, chromosomal aberrations, cytoplasmic factors or interaction of cytoplasmic and genetic factors. Because of any of the above reasons the following

morphological changes may occur in male sterile plants.

- Viable pollen grains are not formed. The sterile pollen grains will be transparent and rarely takes up stain faintly.
- Non-dehiscence of anthers, even though viable pollens are enclosed within. This may be due to hard outer layer, which restrict the release of pollen grains.
- Androecium may abort before the pollen grains are formed.
- Androecium may be malformed, thus there is no possibility of pollen grain formation.

### **Kinds of male sterility, maintenance and uses**

Male sterility may be conditioned due to cytoplasmic or genetic factors or due to interaction of both. Environment also induces male sterility. Depending on these factors male sterility can be classified in to

- Cytoplasmic male sterility (CMS)
- Cytoplasmic-genetic male sterility (CGMS)
- Genetic male sterility (GMS)

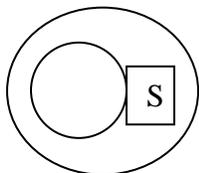
In this there are two categories.

- Environment insensitive genic male sterility- commonly referred as Genetic male sterility.
- Environment sensitive genic male sterility or Environmental induced sterility which is again sub divided in to
  - TGMS (Thermosensitive)
  - PGMS (Photo sensitive)
  - Photo thermo sensitive

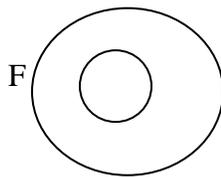
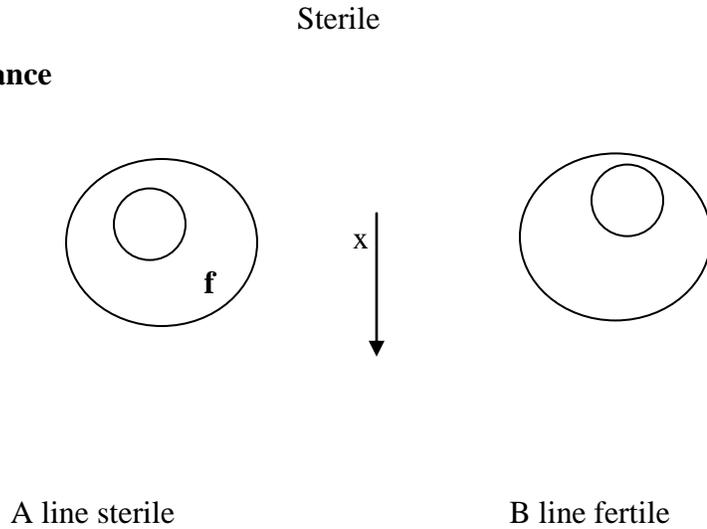
### **1. Cytoplasmic Male Sterility (CMS)**

It occurs due to the mutation of mitochondria or some other cytoplasmic factors outside the nucleus. Nuclear genes are not involved here. There is considerable evidence that gene or genes conditioning cytoplasmic male sterility. Particularly in maize DNA reside in mitochondria and may be located in a plasmid like element.

### **Genetic structure**



**Maintenance**

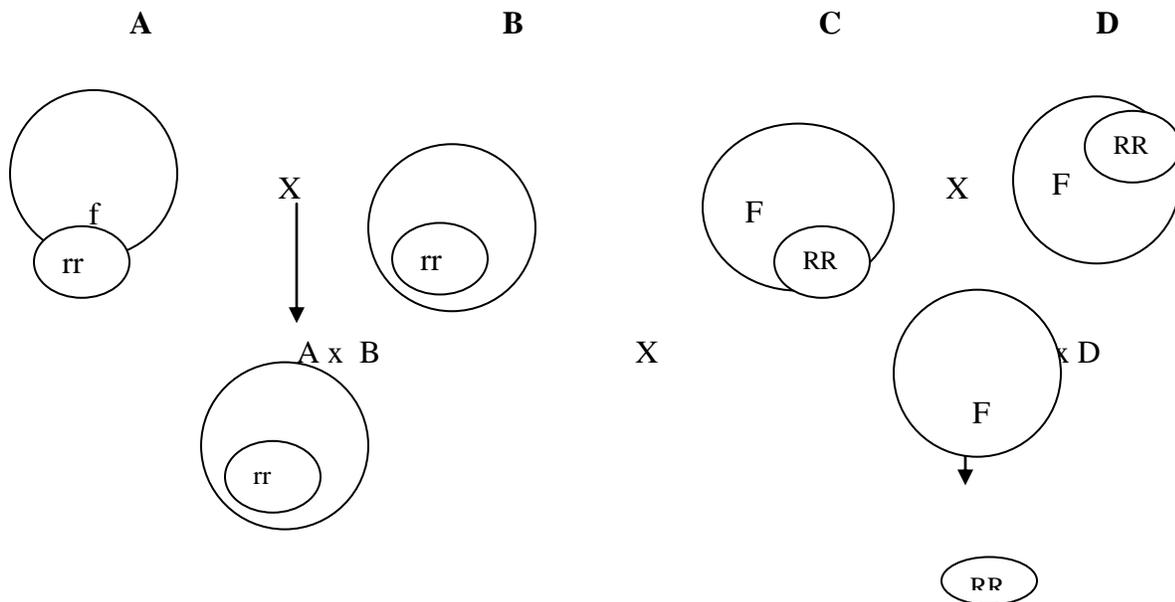


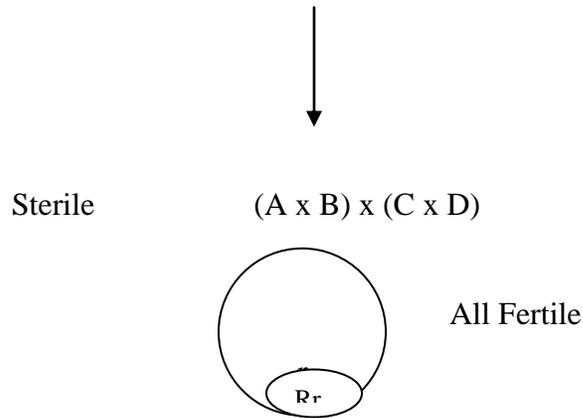
Sterile

Since mother contributes the cytoplasm to the offspring, the sterility is transferred to the  $F_1$ .

**Uses**

Since there are no R lines available, this type of sterility is useful only in crops where seed is not the end product. For example in onion and many ornamental plants the hybrids developed exhibit maximum hybrid vigour with respect to longer vegetative duration and larger flower size and larger bulb size. Cytoplasmic male sterility has successfully been exploited in maize for producing double cross hybrids.

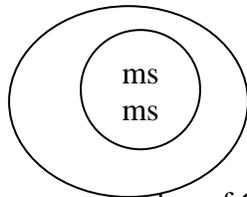




**Genetic Male Sterility (Gms)**

Genetic male sterility is normally governed by nuclear recessive genes  $ms\ ms$ . Exception to this is safflower where male sterility is governed by dominant gene  $Ms\ Ms$ . This type of male sterility is used in Redgram and Castor for production of hybrids.

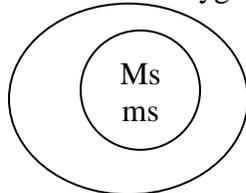
**Genetic structure**



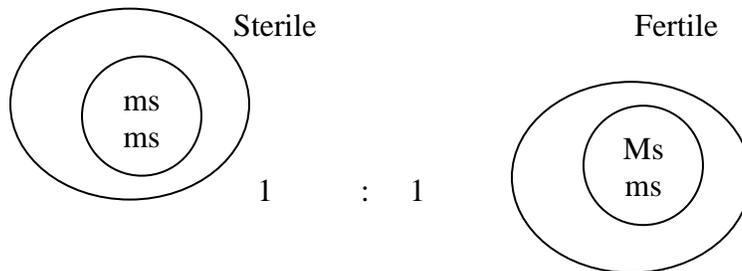
In red gram there are number of GMS lines are available. E.g.  $Ms\ Co\ 5$ ,  $Ms\ T21$

**Maintenance**

In genetic male sterility, the sterile line will be maintained from heterozygous condition. The genetic structure of heterozygous line will be.



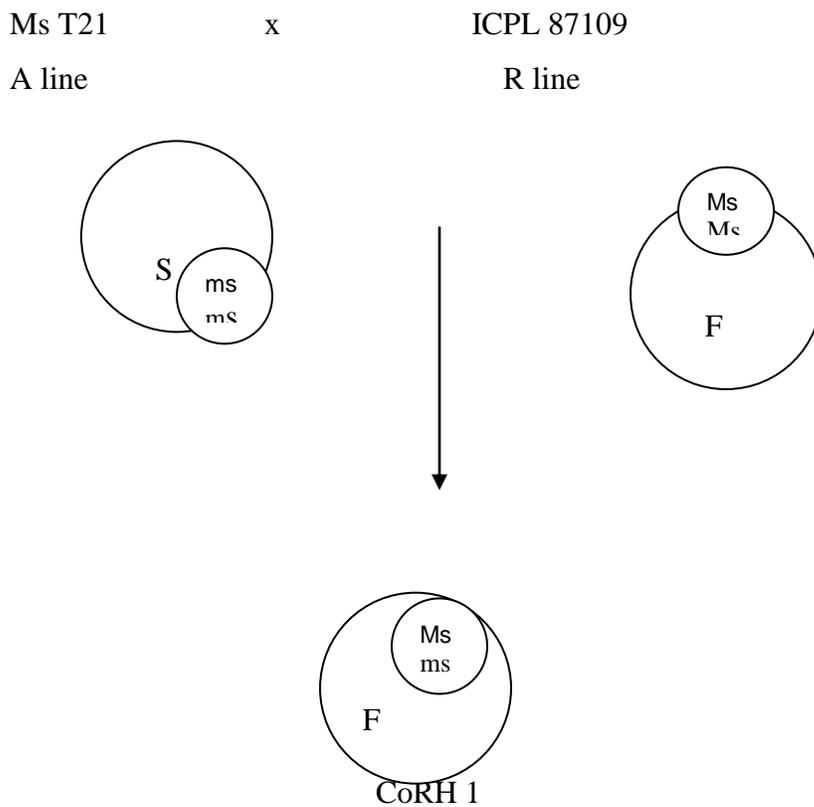
When this heterozygous line is grown in the field it will segregate in the ratio of 1 Fertile : 1 sterile.



The pollen from the Fertile line will pollinate the sterile line and as a result seed set will be there in the sterile line. These seeds are to be harvested and used for hybrid seed production.

For hybrid seed production, the seeds collected from sterile plants will be grown using double the seed rate since it will segregate in the ratio of 1 fertile : 1 sterile line. At the time of flowering, the fertile line will be identified by yellow plumpy anthers and removed from the field. Only the sterile line will remain in field. These will be pollinated by the R line and the R1 obtained will be hybrid redgram.

**Utilisation: Hybrid development. Eg: Redgram**



**Difficulties in use of Gms**

1. Maintenance of GMS requires skilled labour to identify fertile and sterile line. Labelling is time consuming and costly.
2. In hybrid seed production plot identification of fertile line and removing them is costly.
3. Use of double the seed rate of GMS line is costly
4. In crops like castor high temperature leads to break down of male sterility.

**Transgenic Genetic Male Sterility**

A gene introduced into the genome of an organism by recombinant DNA technology or genetic engineering is called transgene. Many transgenes have been shown to produce genetic male sterility, which is dominant to fertility. Consequently, it is essential to develop effective fertility restoration system if these are to be utilized for hybrid seed production. An effective restoration system is available in at least one case called Barnase or Barstar system.

The Barnase gene of *Bacillus amyloliquefaciens* encodes an RNAs. When Barnase gene is driven by TA 29 promoter, it is expressed only in tapetum cells causing their degeneration. Transgenic tobacco and *Brassica napus* plants expressing Barnase were completely male sterile. Another gene, Barstar, from the same bacterium encodes a protein, which is a highly specific inhibitor of Barnase RNase. Therefore, transgenic plants expressing both Barstar and Barnase are fully male fertile.

The Barnase gene has been tagged with bar gene, which specifies resistance to the herbicide phosphinothricin. This male sterile line is maintained by crossing with a male fertile line. The progeny so obtained contain 1 male sterile : 1 male fertile plants; the latter are easily eliminated at seedling stage by a phosphinothricin spray. The male sterile plants are crossed with the Barstar line to obtain male fertile hybrid progeny. This system of male sterility is yet to be commercially used.

Use of TGMS or PGMS eliminates this problem. These male sterile lines are maintained by growing them in a locality where the temperatures and photoperiods during the sensitive developmental stages are such that they exhibit complete male fertility (phenotypically).

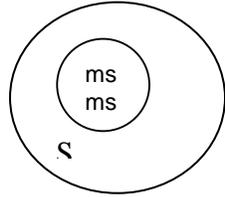
The selfed seeds from such lines are then grown for hybrid seed production in those locations where their ms genes produce complete male sterility due to the prevailing temperature and photoperiod regimes. Clearly, all the plants in the male sterile line will be sterile and no rouging will be required. TGMS and PGMS are being used for hybrid rice development in China.

### **Cytoplasmic Geneic Male Sterility**

This is a case of cytoplasmic male sterility where dominant nuclear gene restores fertility. This system is utilized for the production of hybrids in bajra, jowar, maize, rice, wheat and many other crops.

## Genetic structure

A line



Male sterile

**A line or ms line:** This term represents a male sterile line belonging to any one of the above categories. The A line is always used as a female parent in hybrid seed production.

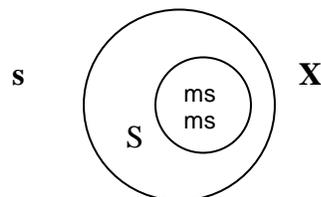
**B line or maintainer line:** This line is used to maintain the sterility of A line. The B line is an isogenic line which is identical for all traits except for fertility status.

**R line and restoration of fertility:** It is otherwise known as Restorer line which restores fertility in the A line. The crossing between A x R lines results in F<sub>1</sub> fertile hybrid seeds which is of commercial value.

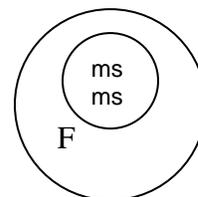
## Maintenance

A line

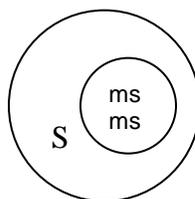
B line



Sterile



Fertile

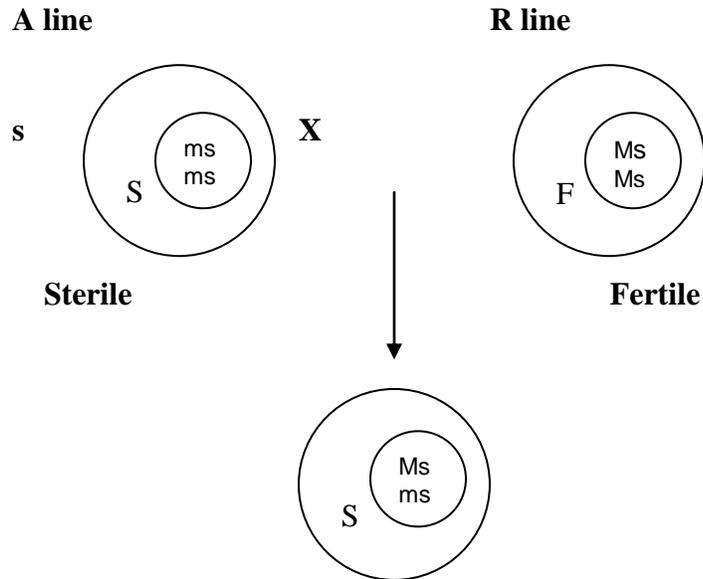


Male sterile line

The A line which is male sterile is maintained by crossing it with isogenic B line which is also known as maintainer line. The B line is similar to that of A line in all characters (isogenic) except fertile cytoplasm.

## Utilisation

The male sterile. A line is crossed with R line (Restorer) which restores fertility in  $F_1$ .



### Hybrid Fertile

#### Limitations of CGMS lines

1. Fertility restoration is a problem. E.g. Rice.
2. Seed set will be low in crops like rice where special techniques are to be adopted to increase seed set.
3. Break down of male sterility at higher temperature
4. In crops like wheat having a polyploidy series it is difficult to develop effective R line.
5. Undesirable effect of cytoplasm. Eg. Texas cytoplasm in maize became susceptible to Helminthosporium. In bajra Tift 23 A cytoplasm became susceptible to downy mildew.
6. Modifier genes may reduce effectiveness of cytoplasmic male sterility.