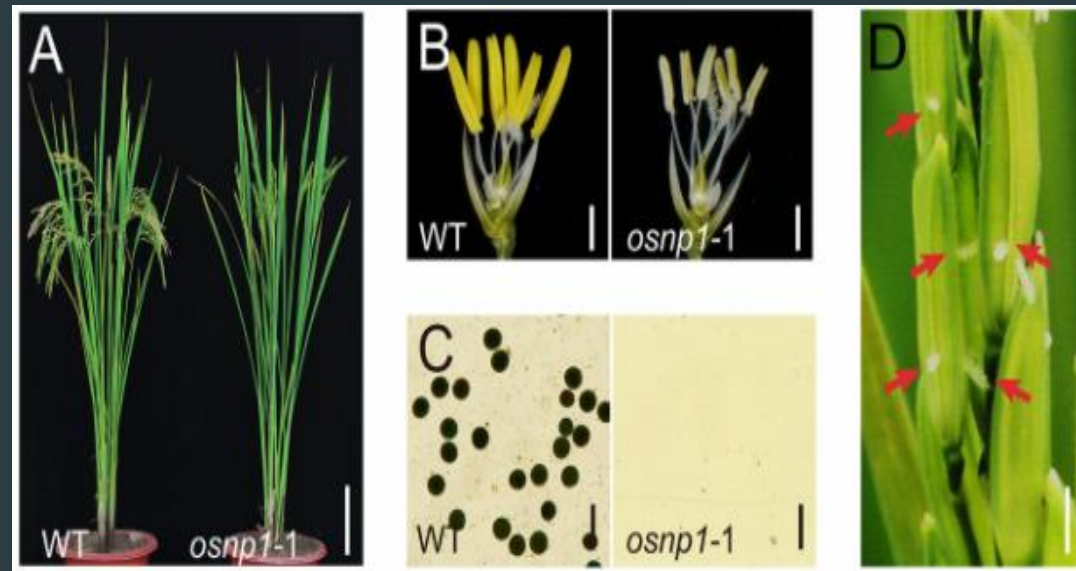


# Male sterility in plant breeding



By

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**Male sterility in plants** implies an inability to produce or to release functional pollen, and is the result of failure of formation or development of functional stamens, microspores or gametes.

Male sterility is usually classified into the following four groups:

- ▶ (1) Genetic Male sterility (GMS),
- ▶ (1a) Temperature-sensitive genetic Male sterility (TGMS),
- ▶ (1b) Photoperiod-sensitive genetic Male sterility (PGMS),
- ▶ (1c) Transgenic genetic Male sterility (TrGMS),
- ▶ (2) Cytoplasmic Male sterility (CMS),
- ▶ (3) Cytoplasmic-genetic Male sterility (CGMS),
- ▶ (4) Chemically-induced male sterility.



# Phenotypic classes of male sterility

- ▶ **Structural male sterility** (stamens absent, malformed or modified into other floral parts: a lack or malformation of microsporogenous tissue or absence of microsporogenesis).
- ▶ **Sporogenous male sterility** (apparently normal stamens, but abnormal microsporogenous tissue or PMC/microspore formation or anomalous meiosis).
- ▶ **Functional male sterility** (pollen development normal, but pollen function is impaired). It may, however, be reiterated that in these cases of male sterility, the development and function of the male reproductive organs is almost normal, and only pollen function is impaired.

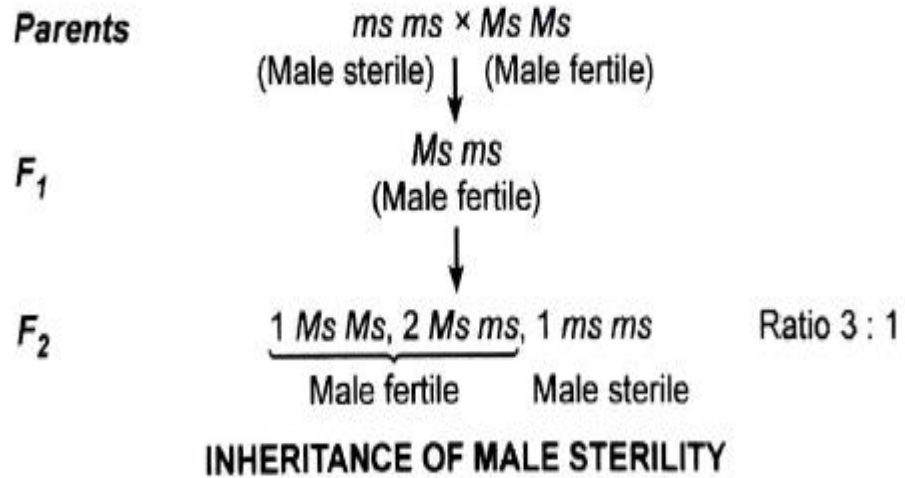
# Genetic Male Sterility (GMS)

- **MALE STERILITY** Caused by nuclear genes is called genetic male sterility. Reported in several plants like wheat, barley, cotton, pigeonpea, tomato, chilies etc.
- **Feature of GMS:-**
- GMS is mainly governed by a monogenic, recessive gene but rarely oligogenic, dominant. This system consists of two type line, viz. A line & B line.
- ✓ A line:- It is male sterile line which is used as female parent in hybrid seed production.
- ✓ B line:- It is a male fertile line which is used to maintain male sterility in A line. Also called maintainer line.
- ✓ Both A & B lines are isogenic lines with a differences only on fertility/sterility locus.

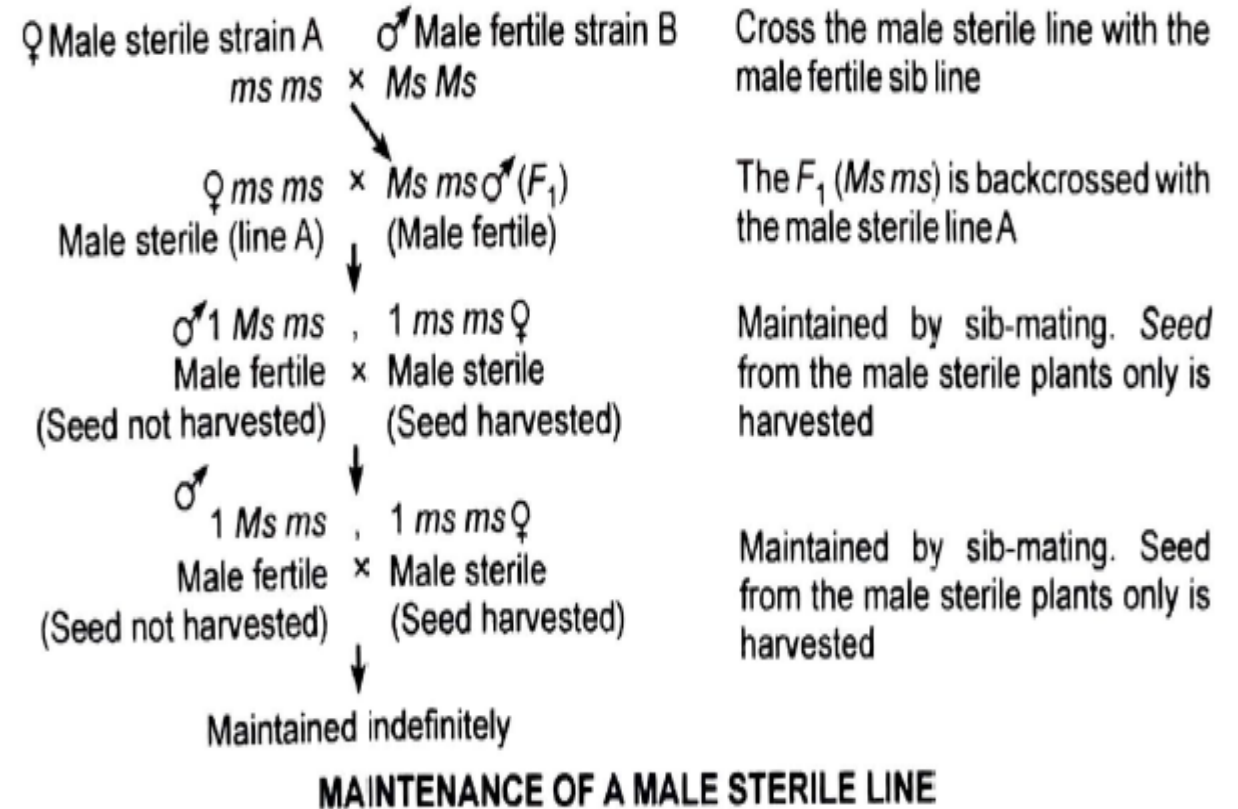
## Origin of ms Allele:

- ✓ The male sterility alleles may rise spontaneously or it can be induced artificially viz. Pigeon pea, castor, tomato, limabean, barley, cotton, etc.
- ✓ The most commonly used mutagens were gamma rays and EMS, but even colchicine (in jowar or sorghum), ethidium bromide (in groundnut, wheat and maize) and acetone (in barley) are reported to have induced mutation for male sterility.

# Inheritance of Male Sterility



# Maintenance of a Male Sterile Line



## Site of Action of *ms* Alleles:

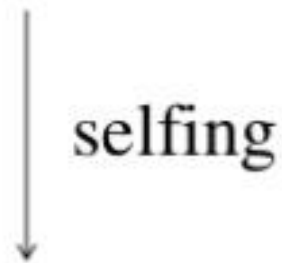
A majority of *ms* genes act either immediately prior to meiosis, during meiosis or at the end of meiosis.

# Temperature-Sensitive Genetic Male Sterility (TGMS)

TGMS line	Temperature	Expression
5460S	< 28 °C	Complete male fertility
	> 30°C	Complete male sterility

LOCATION A  
(< 28 °C Temperature)

**TGMS line (5460S.)**



TGMS line  
(Complete fertile)

LOCATION B  
(> 30°C Temperature)

**TGMS line (5460S.)** x **Male fertile line**

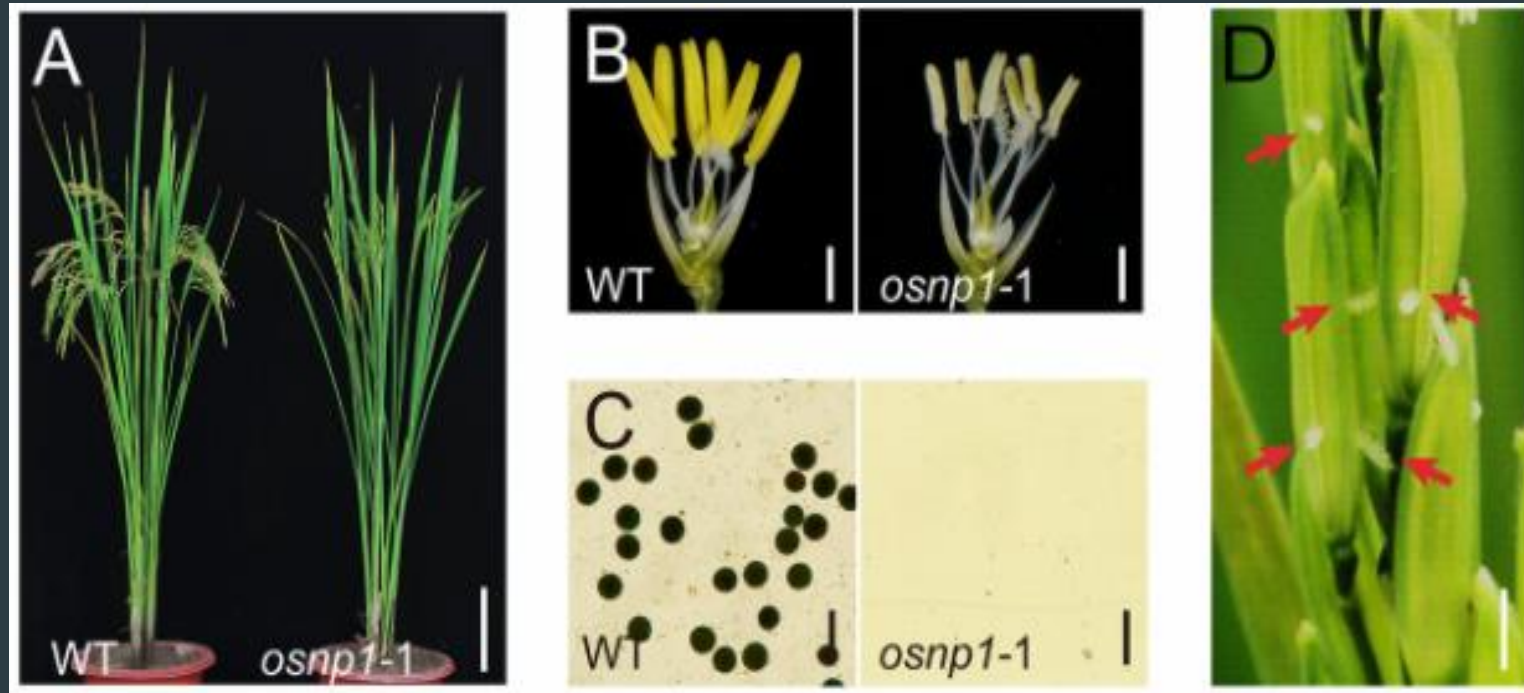


**Hybrid Seed**  
(Produced on TGMS line )



# Photoperiod-Sensitive Genetic Male Sterility (PGMS)

- ▶ In case of PGMS, Expression of ms gene is drastically affected by the prevailing photoperiod, provided the temperature is within a critical range (e.g., 23-29°C for rice PGMS) within this temperature range complete sterility obtained in rice plant grown under long day condition (Day length more than 13 hr 45 min).
- ▶ Generally short-day condition obtain normal fertility.



Phenotypes of *osnp1-1* mutant plant (A) A WT plant and *osnp1-1* mutant plant after bolting. (Scale bar, 10 cm.) (B) Spikelets of WT and *osnp1-1* with the palea and lemma removed. (Scale bar, 1 mm.) (C) I<sub>2</sub>-KI staining of the WT and *osnp1-1* pollen grains. (Scale bar, 100  $\mu$ m.) (D) Inflorescence of *osnp1-1*, showing stigma extrusion. (Scale bar, 2 mm.) Chang *et.al.*, 2016.



# Transgenic Genetic Male Sterility (TrGMS).

▶ Barnase (110 amino acids) is a secreted ribonuclease from *Bacillus amyloliquefaciens*.

▶ Barstar (89 amino acids) is a cytoplasmic barnase inhibitor.

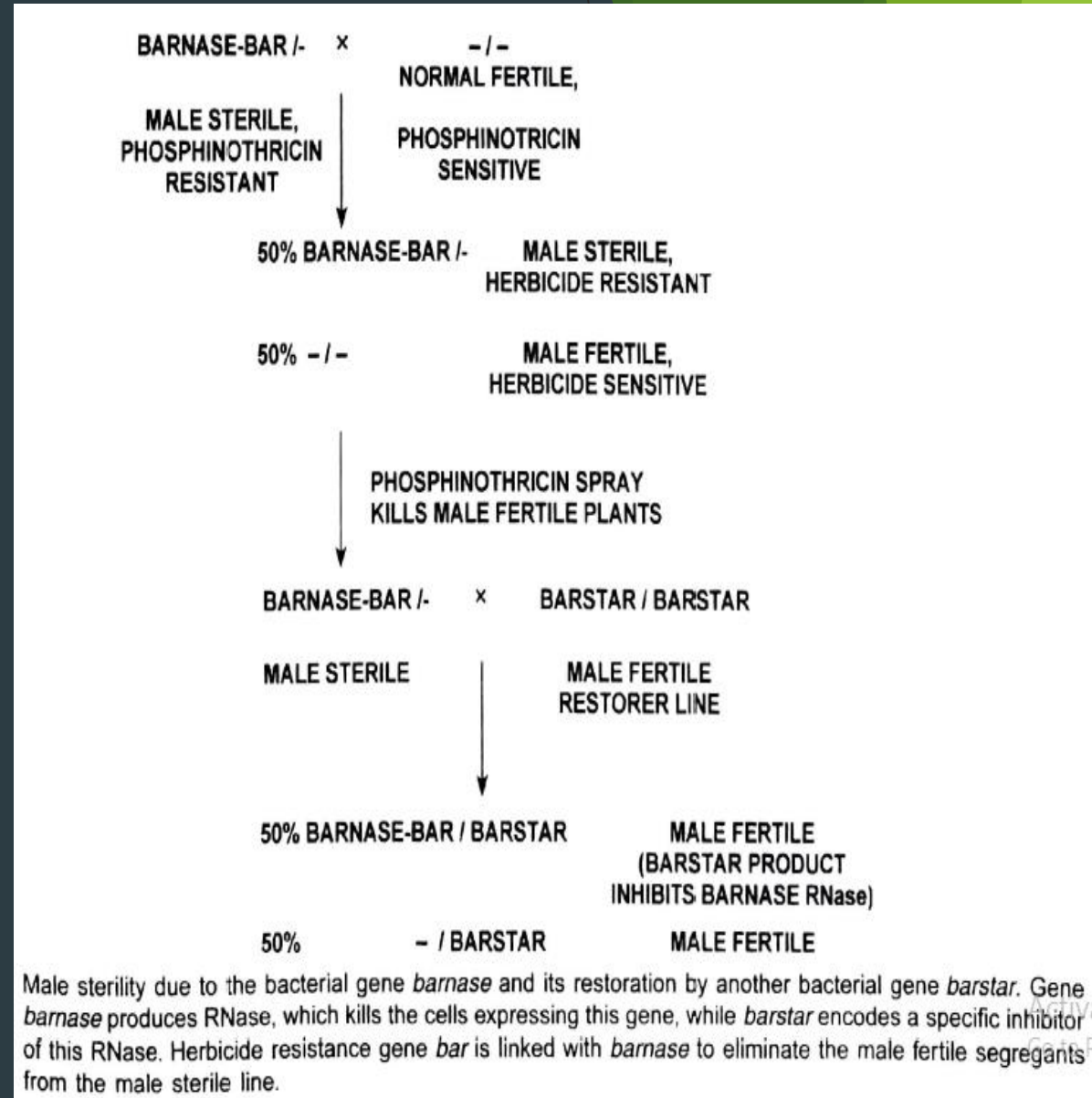
▶ Fuse the Barnase and Barstar genes to TA29 promoter (TA29 is a plant gene that has tapetum specific expression

▶ Plants containing the TA29-Barnase construct are male sterile. RNase is linked with bar gene (glufosinate tolerant), so glufosinate tolerant plant will be male sterile.

▶ Those with TA29-Barstar are not affected by the transgene barnase.

▶ Barstar is dominant over the Barnase

▶ GM canola containing barstar/ barnase system composes about 10% of commercially cultivated crops in Canada and is one of the few GMO cleared for agricultural use in Europe.



**Wild Type**



**Male Sterile Plants**



**Restored Male Fertile Plants**



**Normal Tapetum Development**



**Destruction of RNA in Tapetum**

Tapetal-specific Barnase

**Tapetum Destroyed**



Tapetal-specific Barnase



Tapetal-specific Barstar

+



Barnase/Barstar Complex

**Barnase INACTIVE**

**Normal Tapetum Development**

Active  
Go to P

## Advantages :-

- ▶ Fertility restoration in the hybrid and crossing plants are relatively easy.
- ▶ Use in both seed propagated and vegetatively propagated crops.
- ▶ Less area and labour because maintain only two lines.

## Disadvantage :-

- ▶ Less stable due to GMS affected by environmental factors like temperature and day length condition.
- ▶ Increases cost of production of hybrid seed because 50% fertile plants are removed every year.

## Utilization In Plant Breeding:-

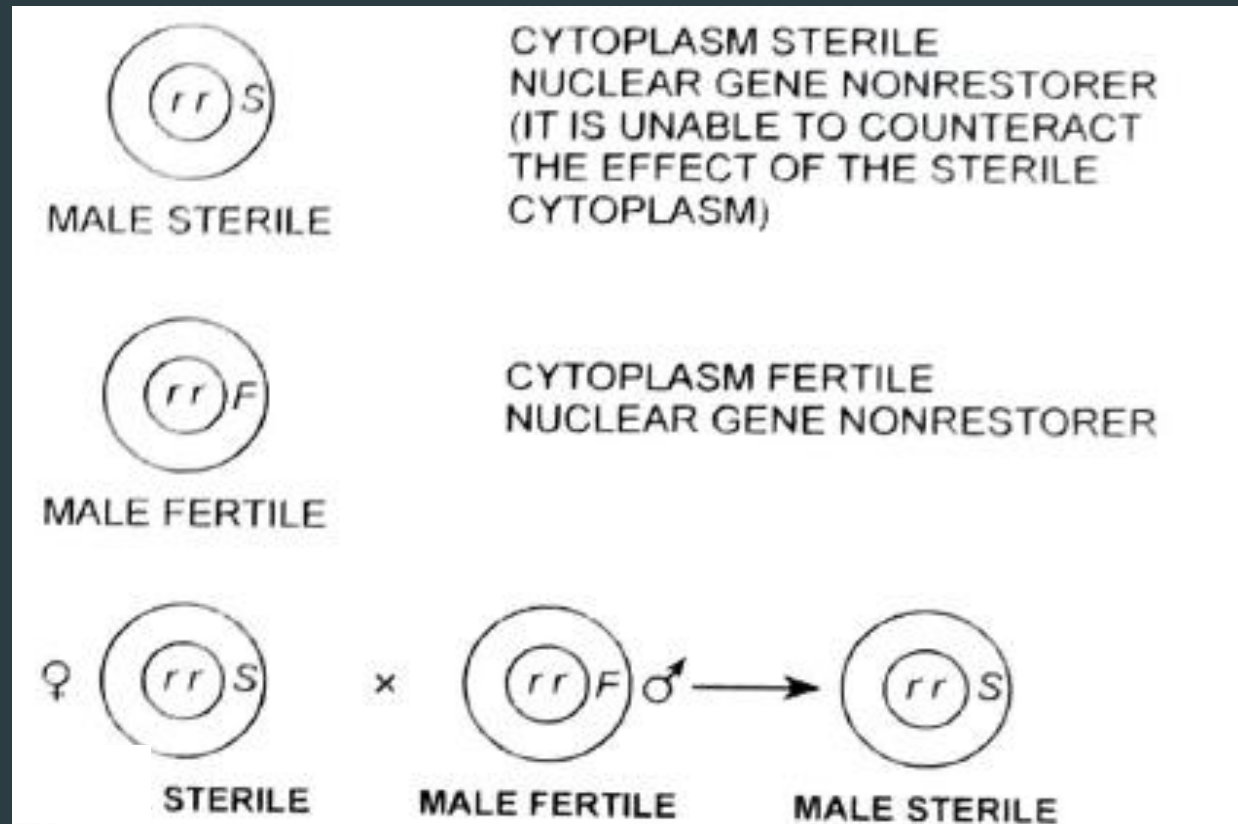
- ▶ GMS has been exploited commercially in few country, in U.S.A. successfully used in castor. In India, it was used for pigeon pea hybrid ICPH8 by ICRISAT, Hyderabad during 1991, and other public sector hybrids (ICPH4, CoH1, CoH2, AKPH4104, AKPH2022).
- ▶ It is used for seed and vegetatively propagated crops.

# Cytoplasmic Male Sterility(CMS)

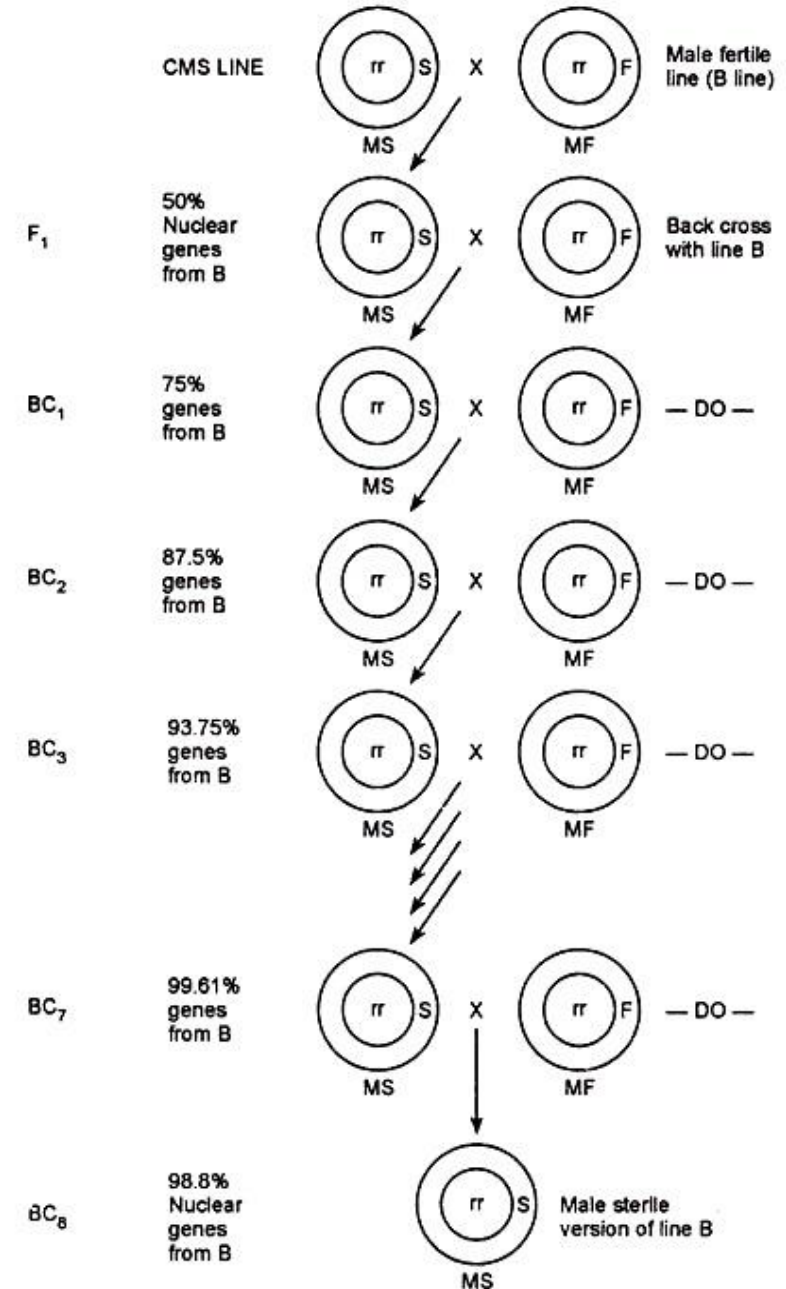
- ▶ CYTOPLASMIC MALE STERILITY (CMS) This type of male sterility controlled by cytoplasmic gene. Cytoplasm of a zygote comes from egg cell, the progeny of such plants would always be male sterile.
- ▶ This system consists of A line and B line.
- ▶ A line:- It is male sterile line which is used as female parent in hybrid seed production.
- ▶ B line:- It is a male fertile line which is used to maintain male sterile A line called maintainer line & used as male parent. Cytoplasmic male sterile line maintained by crossing of A line with B line.
- ▶ CMS cannot be utilized for hybrid seed production without use of restorer line, because F1 seed produce only male sterile plants.



# Cytoplasmic Male Sterility (CMS)



**FIG. 6.3.** Cytoplasmic male sterility. Male sterility is caused by the cytoplasm (depicted by S). The F cytoplasm is the normal cytoplasm. If a nuclear restorer gene (the dominant allele of r) becomes available, the male sterility will be included in the cytoplasmic-genetic group. This type of male sterility shows strict cytoplasmic inheritance.



Transfer of CMS from one line into another one (B) through repeated back crossing. After 6–8 back crosses, the CMS line will contain the same nuclear genes as the male fertile line B. The male fertile line B is used to maintain this new CMS line, therefore, it is called the maintainer line. (MS = male sterile; MF = male fertile).

► Advantages:-

- Highly stable because not influenced by environmental conditions such as temperature and day length.
- Less area requires, because the breeder has to maintains A & B line.

► Disadvantages:-

- Cannot be used for developments of hybrids in these crops where seed is economic product.
- It is impossible to restore fertility in hybrid due to governed by plasma genes.

► Utilization In Plant Breeding:-

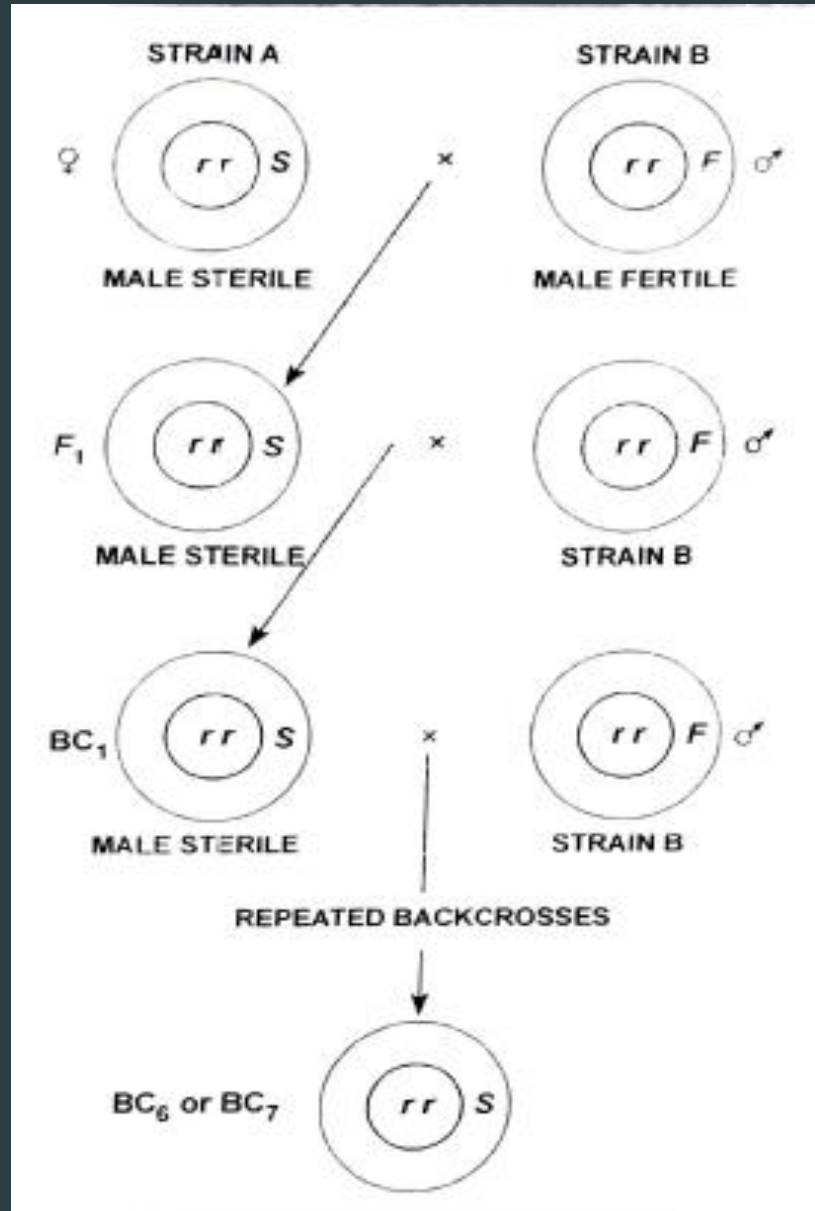
- CMS has limited application. It cannot be used for development of hybrid, where seed is the economic product.
- But it can be used for producing hybrid seed in certain ornamental species or asexually propagated species like sugarcane, potato, and forage crops.



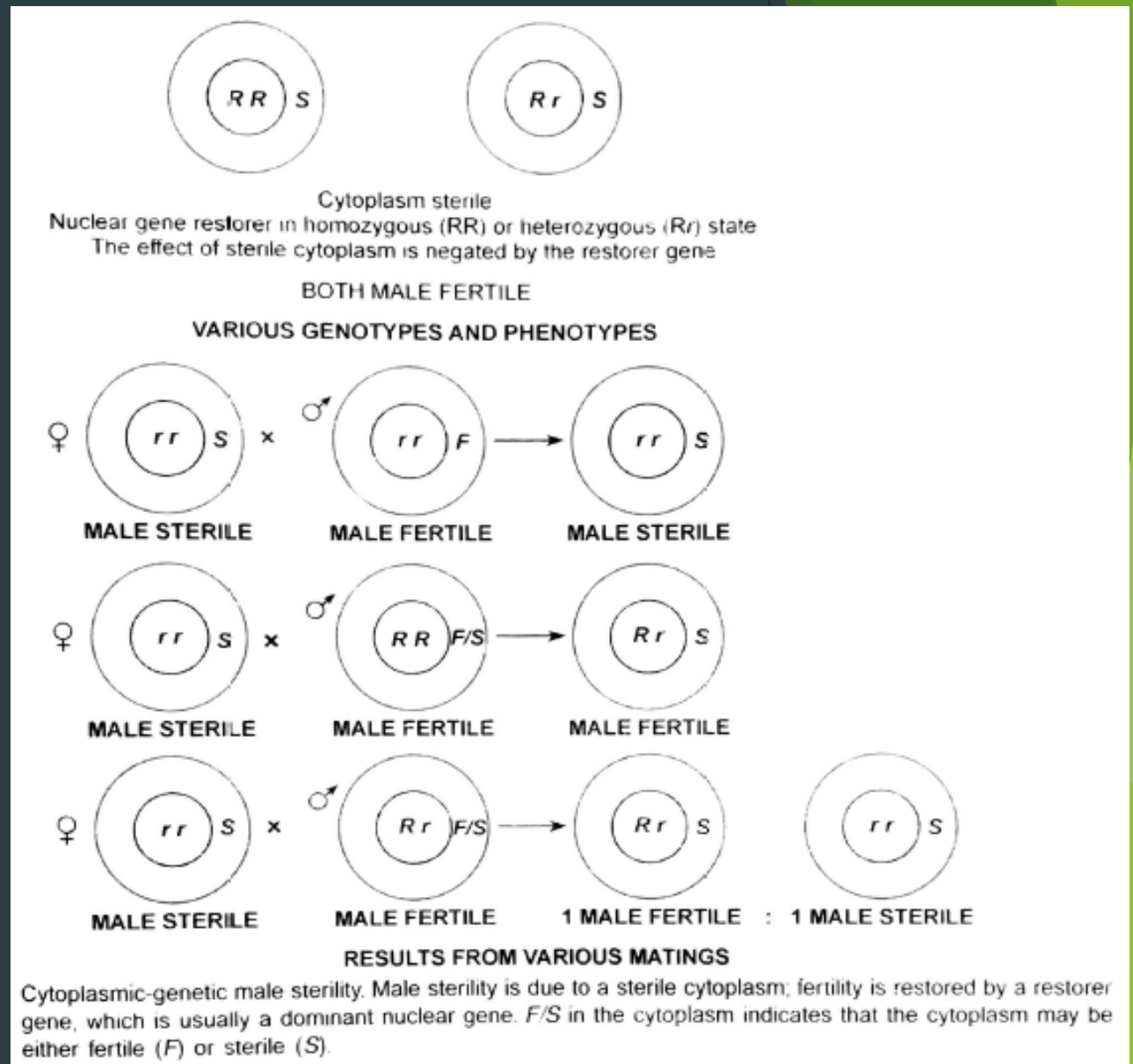
# Cytoplasmic-genetic Male Sterility(CGMS)

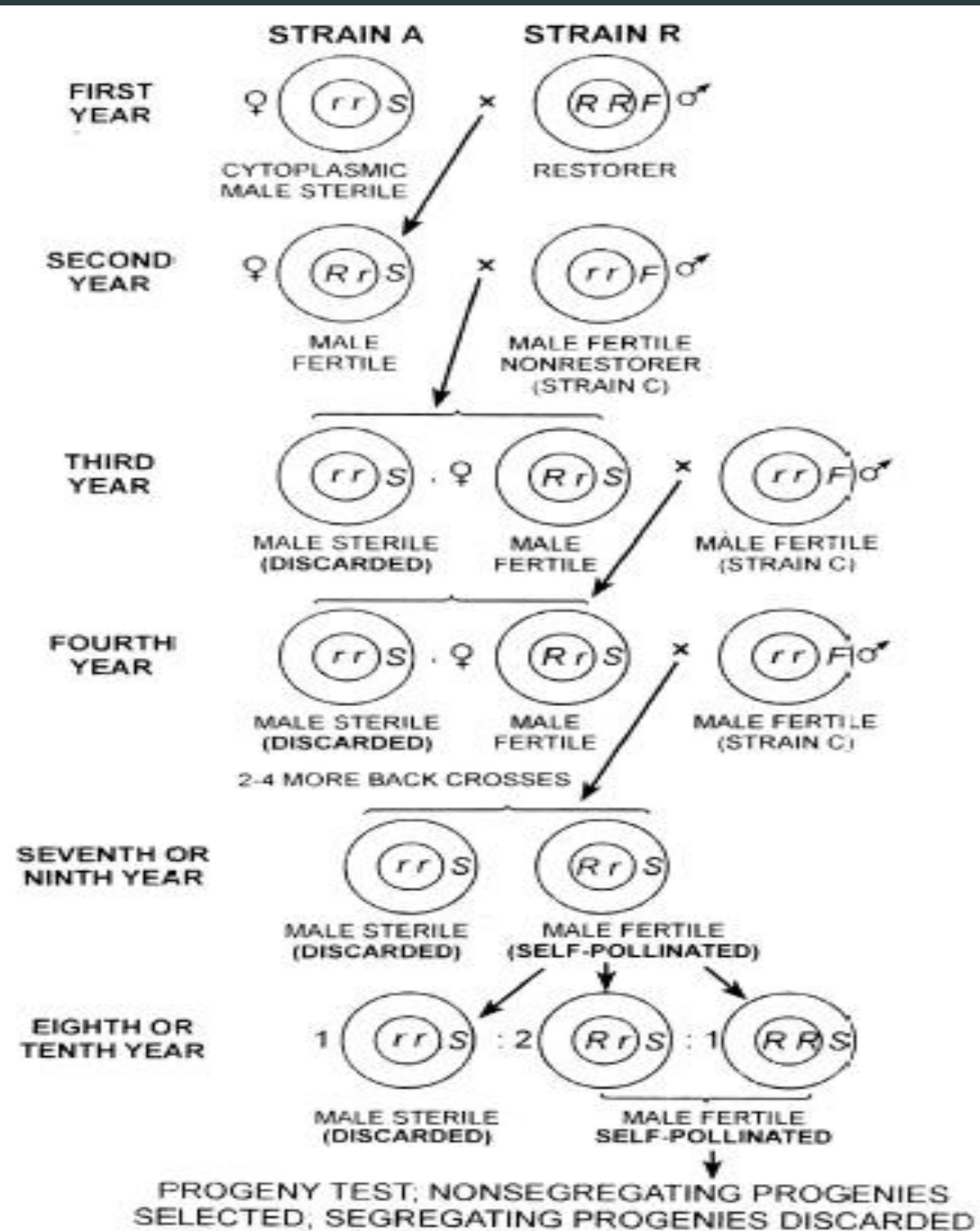
- ▶ CYTOPLASMIC GENETIC MALE STERILITY (CGMS) First time reported by Jones and Davis in 1944 in onion. Controlled by both plasma genes and nuclear genes, called cytoplasmic genic male sterility.
- ▶ Reported in both sexually propagated and asexually propagated species such as pearl millet, sorghum, cotton, maize. This system includes A, B & R lines.
- ▶ A line:- It is male sterile line which is used as female parent in hybrid seed production.
- ▶ B line:- It is a male fertile line which is used to maintain male sterility in A line called maintainer line.
- ▶ R line:- Which is used to restore male fertility in male sterile plant, used as male parent in hybrid seed production.

# Cytoplasmic-genetic Male Sterility (CGMS)



Transfer of Cytoplasmic Male Sterility to a New Strain





### Origin of Male Sterile Cytoplasm:

1. Spontaneous Mutation.
2. Interspecific Hybridization.
3. Mutation Induction through Ethidium Bromide.

► **Advantages :-**

- Widely used for hybrid seed production in both seed propagated & vegetatively propagated species.
- Highly stable and reliable.
- Not affected by environmental factors.

► **Disadvantages:-**

- Its requires more area and labour, because the breeder has three types of material, viz., A, B & R line.
- Moreover, sometimes CGMS line has inferior performance.

► **Utilization In Plant Breeding :-**

CGMS can be successfully utilized for the development of commercial hybrids in both seed propagated and vegetatively Propagated crops. CGMS has been effectively used for commercial production of hybrids in maize, sorghum, onion, sugarbeet, pearlmillet and other crops.



# CHEMICALLY INDUCED MALE STERILITY

- ▶ A chemical hybridizing agent (CHA) is a chemical that induces artificial, nongenetic male sterility in plants so that they can be effectively used as female parents in hybrid seed production.

<i>Chemical hybridizing agent (CHA)</i>	<i>Critical stage for application</i>	<i>Effective in crop</i>	<i>Remarks</i>
<b>Arsenicals</b>			
Zinc methyl arsenate (MG1) Sodium methyl arsenate (MG2)	5 days before heading	Rice	MG2 is extensively used in China in rice hybrid seed production
DPX3778	Late tillering/early boot + late boot stages	Wheat and triticale	Very high application rates required
Ethephon or ethrel	Depends on crop	Barley, oats, pearl millet, rice, etc.	Variable male sterility in different tillers
Mendok (FW450)	In cotton, complete sterility for 35 days; application every 35 days in sugarbeet	Cotton, pearl millet, chillies, sesamum, sugarbeet	Female fertility affected; phytotoxic effects
<b>Gibberellic acid</b>			
LY195259	1-3 days prior to onset of meiosis Soil surface treatment or foliar spray when spike of main shoot is 5 mm	Maize, barley, wheat, rice, sunflower Wheat	Excessive internode elongation; in sunflower, reduced female fertility Commercially promising; no known side effects
<b>Maleic hydrazide</b>			
RH0007 (Hybrex)	Early microsporogenesis	Maize, wheat, cotton, onion, etc. Wheat	Phytotoxicity, flower abscission, etc. No effect on female fertility; used commercially in wheat
WL84811 (Shell)	Booting stage	Wheat, oat, barley, maize	Hybrid purity 88%; may reduce female fertility



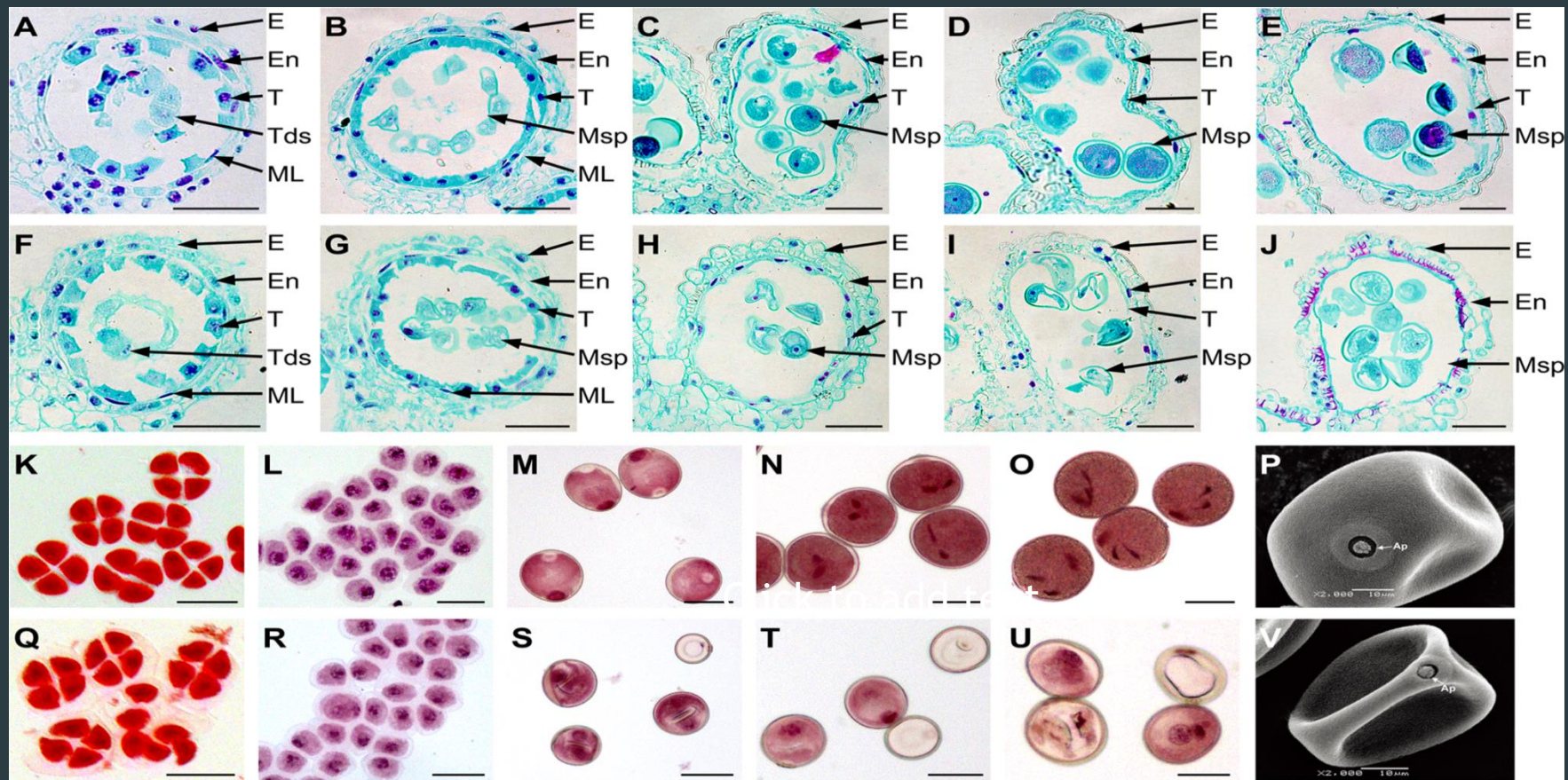
# Features of an ideal CHA

- ▶ Highly male and female selective.
- ▶ Economical.
- ▶ Flexible time.
- ▶ Should not be mutagenic.
- ▶ Should not be carried to F1.
- ▶ Should cause 0% reduction in seed set.
- ▶ Eco friendly.

## Hybrid Seed Production based on CHAs

- 1) Proper Environmental Condition.
- 2) Synchronization of Flowering of Male and Female parents.
- 3) Effective Chemical Emasculation and Cross-Pollination.





**Development of anthers and microspores in untreated (A to E and K to P) and chemical hybridization agent (CHA)-SQ-1-treated wheat plants (F to J and Q to V).**

(A to J) safranin O/fast green-stained transverse sections. (K to O and Q to U) 1% acetocarmine-stained microspores. (P and V) a scanning electron micrograph was used to analyse the mature pollen grains. (A, F, K and Q) the tetrad stage. (B, G, L and R) the early-uninucleate stage. (C, H, M and S) the later-uninucleate stage. (D, I, N and T) the binucleate stage. (E, J, O and U) the trinucleate stage. E, En, ML, T, Tds, Msp and Ap indicate the epidermis, the endothecium, the middle layer, the tapetum, the tetrads, the microspore and the germination aperture, respectively. Scale bars are 50  $\mu\text{m}$  in A to O and Q to U.

# Advantages of CHAs

- ▶ **Any line can be used as the female parent.** Further, the lengthy and cumbersome production of CMS or GMS lines for hybrid seed production becomes unnecessary.
- ▶ **Any line can be used as the male parent** of hybrid, and restorers, etc. are not required.
- ▶ The hybrid seed production is based on only two lines.
- ▶ Maintenance of the parental lines of hybrids is readily achieved by self-pollination.
- ▶ **CHA-based F2 hybrids are fully fertile**, while some plants in such hybrids based on CMS or GMS will be sterile. This would allow commercial cultivation of the F2 generations of such hybrids whose F2 generation shows higher vigour and yield; this would reduce the Cost of hybrid seed substantially.

# Limitations of CHAs

- ▶ The expression and duration of CHA-induced male sterility is very **stage-specific**.
- ▶ CHA-induced male sterility is very **vulnerable to the prevailing environmental conditions**
- ▶ **Incomplete male sterility might lead to the production of selfed seed** on the female parent; this would reduce hybrid seed purity and may cause problems in seed certification
- ▶ **Many CHAs are toxic to plants and animals.**
- ▶ Some CHAs e.g., arsenicals and WL84811, may produce **carryover residue effect** in F1 seeds
- ▶ Certain CHAs, eg. RH531 and RH532, **interfere with cell division**.
- ▶ Some CHAs stimulate neoplastic growth and affect human growth. Efforts are being made to develop a new generation of toxicity-free, effective and low-cost CHAs.
- ▶ CHAs are generally genotype-, dose- and application stage-specific.



