Methods to Asses Self Incompatibility:

Pollination method- In cabbage it can be assessed by counting the seed after selfing. If more number of seeds is produced it is self compatible and if not reverse is true.

Cytological Method- Self incompatibility can be assessed by number of pollen grain penetrating the style. Stigma and style squashed on microscope after pollination stained with aniline blue which is accumulated in the pollen tube and can be observed in UV light as it fluoresces. So on the basis of extent of penetration of pollen tube in pistil tissue incompatibility can be measured accordingly.

Molecular Method- Validation of above method can be achieved by using marker associated with self incompatibility as done in apricot (Valanova et.al., 2003).

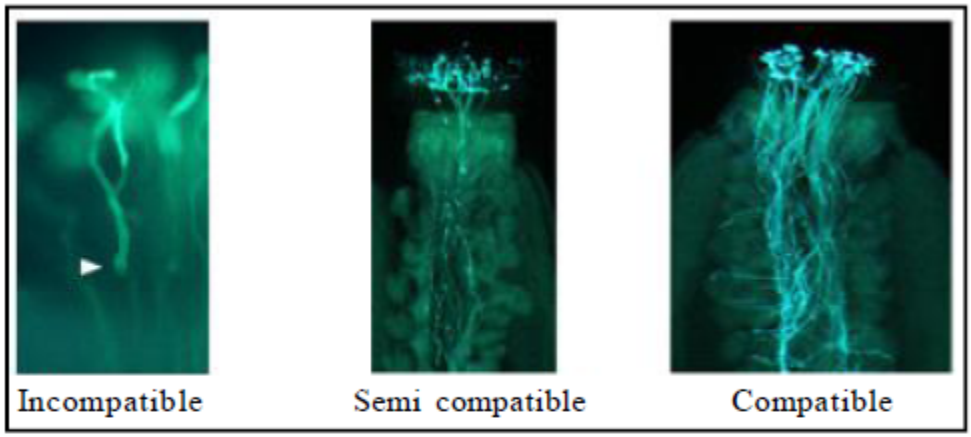
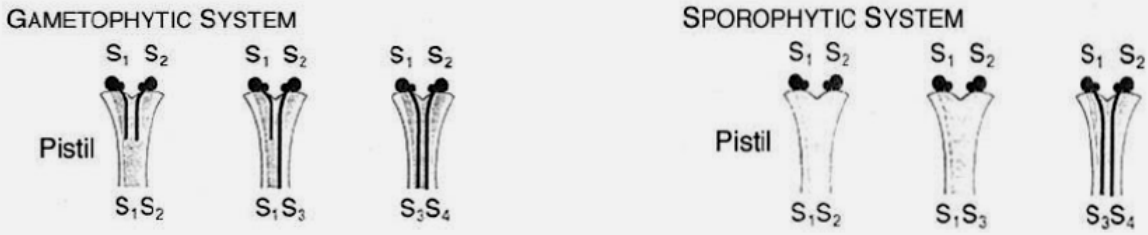


Plate 1 : Assessment of self-incompatibility in cabbage by cytological method

RNAase acts in the style in case of gametophytic selfincompatible system (GSI) and glycoproteins in stigma cause self-incompatibility reaction (SI) in sporophytic system (SSI). Further when a gametophytic locus is associated to the sporophytic system, as found in *Brassica*, development of pollen tube and fertilization seen even incompatible reaction. SSI system is predominant with glycoproteins causing incompatibility reactions on the stigmatic surface. Glyciproteies are presnt in higher concentration at anthesis.

General comparison between gametophytic and sporophytic self-incompatibility

Gametophytic SI	Sporophytic SI
– Stigma is smooth and wet	– Stigma is papillate and dry
– Genotype of the pollen (gamete)	– Genotype of the sporophyte (diploid tissue)
– S-locus products are synthesized after completion of meiosis	– S-locus products are synthesized before completion of meiosis
– Growth of the pollen tube arrests in the style	– Growth of the pollen tube arrests at the surface of the stigma



Male and female determinant genes reported in some families

Family	Types of SI	Male determinant	Female determinant
Brassicaceae	SSI	*SP11/SCR*	*SRK*
Solanaceae, Rosaceae Scrophulariaceae	GSI	*SLF/SFB*	S-RNase
Papaveraceae	GSI	Unknown	S-protein

SP11- S locus protein 11 *SCR*- S locus cysteine rich protein SRK- S locus receptor kinase
SLF- S locus F-box protein *SFB*-S-haplotype-specific F-box protein

Significance of Self incompatibility:

- Hybrid Production.
- Establishment of Clonal Garden.
- Evolutionary Significance by forcing out breeding in this way maintain heterozygosity.
- Production of seedless fruit in pine apple.

Self incompatibility and Male sterility:

If two parents are self incompatible hybrid seed can be produced from both the parents. A restorer is required to maintain self sterile line while self incompatible inbred can be easily maintained.

Methods to overcome self incompatibility

- **Mixed pollination** with the use of mentor pollen with incompatible pollen. Ex. *Nicotiana alata, Petunia hybrida, Cosmos bipinnata*.
- **Bud Pollination** effective as stigma used to contain substances taking part in recognition at the time of anthesis. Self-pollination at bud stage aims to enable self fertilization before the glycoprotein that reaches levels that prevent the development of the pollen tubes. Successive self-pollinations are able to generate self-fertilization probably because the first self-pollination neutralizes glycoprotein, so that subsequent pollinations may generate pollen tubes capable of fertilizing the embryo sac.
- **Stub Pollination** by cutting the stigma nd in some cases style up to certain length incompatible pollen grain germinate and fair growth of pollen tubes are grown for example *Ipomea trichocarpa*.
- **Ovarian Pollination**, ex. *Argemone mexicana, Papaver sominiferum*.
- ***In vitro* pollination and Fertilization** developed by Kanta et. al., in *Papaver somniferum*.

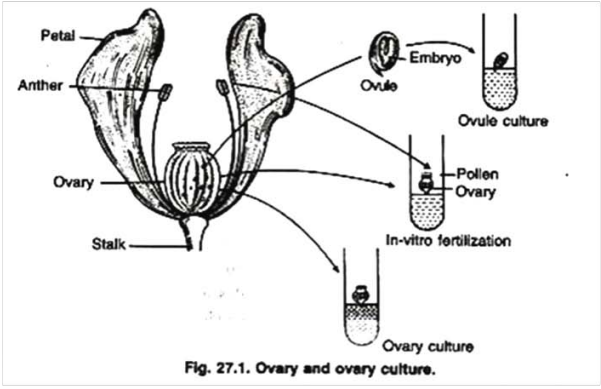


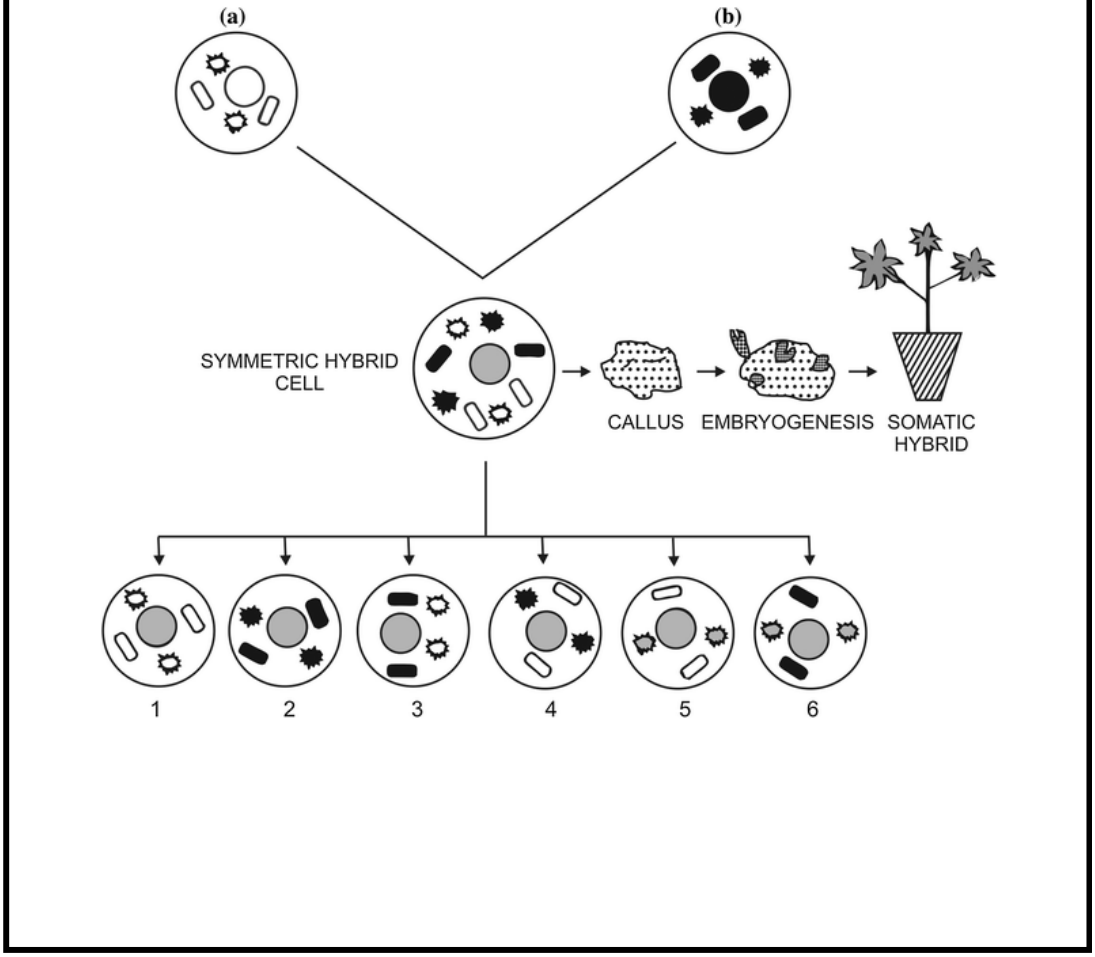
Fig. 27.1. Ovary and ovary culture.

- Modification of Stigmatic surface, pretreatment of stigma with **Triton X-100** and hexane is effective to modify stigmatic surface by disturbing pellicle layer for ex. in *Brassica*. Other treatment such as heat and irradiation of has been proven to overcome incompatibility such as in *Linum longiflorum*, *Lycopersicon peruvianum* (gamma radiation).
- **Parasexual Hybridization:** First parasexual hybrid was produced by Carlson et.al, 1972 from *Nicotiana glauca* and *N. longsdorffii*.

  i) Isolation of protoplast.

  ii) Fusion of isolated protoplast.

  iii) Culture of protoplast.



Questionnaire

1. What is “opposition S allele” theory and how it is applied to explain incompatibility in SSI and GSI.
2. Explain the cytological method to assess the mechanism of self incompatibility.
3. What do you understand by mentor pollen?
4. Explain the significance of self incompatibility in production of clonatl garden.
5. How self incompatibility can be compared with male sterility and explain the benefit of self incompatibility over male sterility.
6. Define the morphological barriers occurring within the species to impose self incompatibility
7. What factors are responsible for inhibition of growth of pollen tube in style in case of GSI.
8. What is the origin of exine and intine held proteins in pollen grain.
9. Write short note on the following

    i) Stub pollination
    ii) In vitro pollination and fertilization
    iii) Microgametogenesis

10. Give a detailed account on Parasexual Hybridization.