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METHODS TO OVERCOME INCOMPATIBILITY

1. MIXED POLLINATION

In this method the stigma is disguised from the incompatible pollen by pollinating it with a mixture of incompatible and compatible pollen (termed Mentor pollen or Recognition Pollen). To prevent fertilization by the mentor pollen they are either inactivated by irradiation or killing by treating them with chemicals eg. methanol or subjecting them to repeated freezing and thawing. These treatments do not disturb the wall bound proteins.

Stettler and Ager (1984) have listed several examples where the use of mentor pollen has facilitated overcoming interspecific, sporophytic-self incompatibility and gametophytic-self incompatibility.

The mechanisms implicated in overcoming incompatibility by the mentor pollen are summarised below—

Site of mentor effect	Proposed mechanism
1. At stigmatic surface	1. Mentor pollen provide recognition protein which permit incompatibility pollen to germinate.
	2. Mentor pollen provide P-factor which interact with S-factor from stigma to render it accessible to incompatible pollen.

2. In style	3. Mentor pollen provide a pollen growth promoting (PGS) or regulating substance which permits incompatible pollen to sustain tube growth.
3. In distal tissues of pistil	4. Mentor pollen after tube penetration provide substances critical for sustained growth of ovules, ovary and other fruit tissues.

2. BUD POLLINATION

In some plants pollination at an early bud stage has proved every effective in overcoming interspecific incompatibility. It is likely that in the style the factor complementary to the pollen factor for incompatibility reaction appears only at or just before anthesis. Therefore, if the stigma is self-pollinated at bud stage when this factor has not appeared the pollen tubes will grow normally and effect fertilization

3. STUB POLLINATION

Where the incompatibility reaction is restricted to the stigma or the length of the style is more than the maximum length attained by the pollen tubes of the male parent, removing the stigma and the part of the style have often proved helpful in overcoming incompatibility.

4. INTRA-OVARIAN POLLINATION

Where the zone of incompatibility lies on the stigma or in the style direct introduction of pollen suspension into the ovary would be helpful. In this technique, known as the intra-ovarian pollination, the ovary is surface sterilised with ethanol and two punctures are made in the wall - one for introducing the pollen suspension and other to permit the escape of air present in the ovarian cavity. A pollen suspension is prepared in distilled water and injected into the ovary with the help of a hypodermic syringe. Subsequently, both the holes are sealed with petroleum jelly. Pollen grains germinate inside the ovary and bring about fertilization.

5. TEST TUBE POLLINATION

The stigmatic, stylar and ovary wall tissues are completely removed from the path of pollen tube and the exposed ovules are directly dusted with pollen grains. The pollinated ovules are cultured on a suitable nutrient medium which favours pollen germination as well as the development of fertilized ovules into seeds. The technique is more effective if the ovules are cultured with the placental tissue intact.

6. HEAT TREATMENT OF STYLE

Moderately high temperature (up to 50°C) are also known to reduce self-incompatibility reaction in certain plants.

7. IRRADIATION

Irradiation has proved helpful in achieving compatibility in only those systems where the incompatibility process is ~~only~~ controlled gametophytically.

8. CHEMICAL TREATMENT

If the failure to set seeds following self or cross pollination is due to premature abscission of flowers, the application of certain chemicals may be helpful eg.
100 mg / l. of p-chlorophenoxyacetic acid

9. INCREASED CO_2 LEVEL

When the normal atmospheric level of CO_2 is raised 100 fold to 4-6% at high relative humidity for several hours after pollination, self-incompatible pollinations behave as compatible pollination in Brassica spp.

10. PARASEXUAL HYBRIDIZATION

In 1969 an altogether, new approach was proposed to raise such hybrids which could not be produced through the conventional methods of hybridization because of sexual incompatibility. This technique involves the fusion of isolated protoplast, since only somatic cell protoplasts are employed for this purpose, the technique is described as 'parasexual hybridization'.

In 1972 Bhojwani and Cocking demonstrated the possibility of isolating microspore protoplasts. Besides being haploid, the microspore protoplasts are richly cytoplasmic, which favours their ready fusion.

It involves three steps.

(a) Isolation of protoplasts

eg. Mesophyll cells of Tobacco.

Fully expanded leaves

↓

Peel off the lower epidermis

↓

Cut into pieces of $4 cm^2$

↓

Macerate for 2 hours at 25°C in the mixture containing

* [0.5% Macerozyme
0.8M Mannitol
0.3% Pot. Dextran Sulphate]

↓

Change the mixture * every 30 minutes

↓

Wash throughly the isolated mesophyll cells in 0.8M soln. of mannitol

↓

Degrade cell walls by incubating the free cell for 2 hours at 36°C in a mixture containing

[2% cellulose
0.8M Mannitol]

↓

Wash the released protoplasts with 0.8M Mannitol

↓

Culture the protoplast

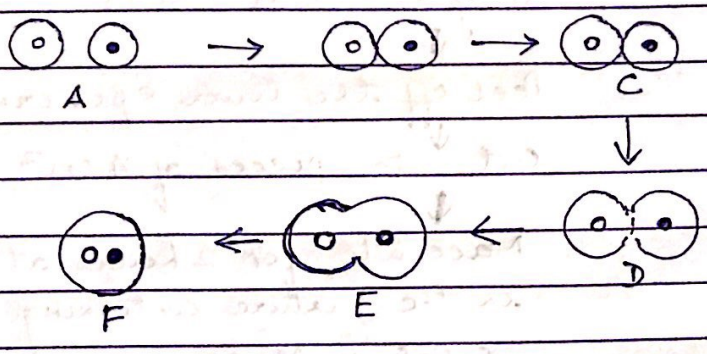
or

Fuse them for somatic hybridization

or

Use them for genetic transformation

(b) Fusion of isolated protoplasts
(c) Culture of hybrid protoplasts to regenerate whole plants.



Stages of protoplast fusion

A – Two seperate protoplasts
B – Agglutination of two protoplasts
C, D – Membrane fusion at localised sites
E, F – Formation of spherical heterokaryons.