

Anther and pollen culture

HISTORY

- W.TULECKE(1953)

First observed that mature pollen grains of *Ginkgo biloba* (gymnosperm) can be induced to proliferate in culture to form haploid callus.

S.GUHA AND S.C MAHESWARI(1964)

- First reported the direct deveiopment of embryos from microspores of *Datura innoxia* by the culture of excised anther.

J.P. BOURGIN AND J.P.NITSCH(1967)

- Obtained complete haploid plantlets from anther culture of *Nicotiana tabacum*.

Anther culture

- Culturing of anther obtained from unopened flower bud in the nutrient medium under aseptic condition.
- callus tissue or embryoids that give rise to haploid plantlets either though **organogenesis** or **embryogenesis**.

Pollen culture

- Pollen or microspore culture is an *in vitro* technique by which the **pollen grains preferably at the uninucleated stage**, are squeezed out aseptically from the intact anther and then cultured on nutrient medium.
- the microspores develop into haploid embryoids or callus tissue that give rise to **haploid plantlets by embryogenesis or organogenesis**.

Androgenesis

- Androgenesis is the *in vitro* development of haploid plants originating from totipotent pollen grains through a series of cell division and differentiation.
- It is of two types.
- Direct androgenesis
- Indirect androgenesis

Androgenesis

1) Direct androgenesis:-

The microspores behaves like a zygote and undergoes chance to form embryoid which ultimately give rise to a plantlet.

2) Indirect Androgenesis:-

The microspores divide repeatedly to form a callus tissue which differentiates into haploid plantlets.

Principle of anther and pollen culture

- The production of haploid plants is to exploit the totipotency of microspore .
- In this process the normal development and function of the pollen cell to become a male gamete is stopped and is diverted forcibly to a new metabolic pathway for vegetative cell division .

Factors influencing anther culture

1) Genotype of donor plants:-

The genotype of the donor plant plays a significant role in **determining the frequency of pollen production.**

- Example :- *Horedum* of each genotype differs with respect to androgenic response in anther culture.

2) Anther wall factor:-

The anther wall provide the nourishment in the development of isolated pollen of a number of species.

- There are reports that glutamine alone or in combination with serine and myo inositol could replace the anther wall

Factors influencing anther culture

3) CULTURE MEDIUM:-

For anther culture, medium requirements vary with **genotype and the age** of the anther as well as condition under which donor plants are grown.

- Incorporation of **activated charcoal** into the medium has stimulated the induction of **androgenesis**.
- The **iron** in the medium plays a very important role for the induction of haploids .
- **Potato extracts, coconut milk and growth regulators like auxin and cytokinin** are used for anther and pollen culture.

Factors influencing anther culture

4) Stage of microspores:-

Anthers are most productive when cultured at the **uninucleate microspore stage**.

Example, **Barley, wheat, rice** etc.

- Anther of some species give the best response if pollen is cultured at first mitosis or later stage

Example:-*Datura ,tobacco*.

Factors influencing anther culture

Effect of temperature:-

Temperature enhance the induction frequency of microspore androgensis.

- The **low temperature** treatment to anther or flower bud enhance the **haploid** formation.
- The low temperature effects the number of factors such as dissolution of microtubules lowering of abscisic acid maintenance of higher ratio of viable pollen capable of embryogenesis.

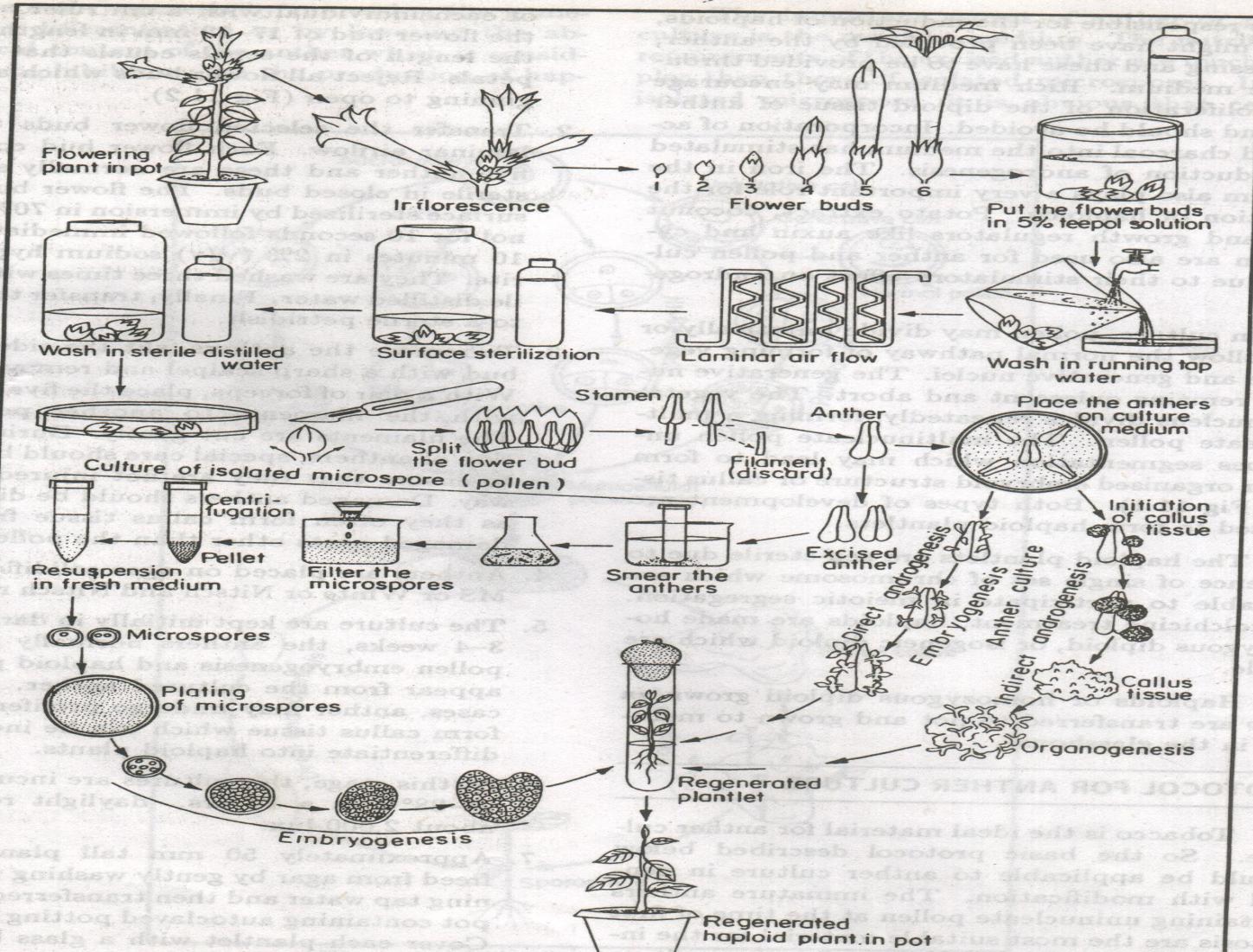
Factor influencing anther culture

Physiological status of donor plant

- Physiological status of donor plant such as **water stress** **nitrogen requirement** and **age** of donor plant highly affect the pollen embryogenesis.

- Plants **starved of nitrogen** may give more responsive anthers compared to those that are well fed with nitrogenous fertilizers.

Method of anther and pollen culture



Procedure for Anther culture

- 1.collection of unopened flower buds
- 2.surface sterilized with **tween 80** and **mercuric chloride**
- 3.Anthers excised from flower buds and kept seperately
- 4.Anthers in first meiotic division is selected by **acetocarmine test**
- 5.Inoculated in the medium containing **glutamine, L-serine and inositol**

- 6. Incubated the culture at 25 C for 15 days.
Here, anthers grow in to **embryoids**.
- **7. embryoids transfer to rooting medium under 3000 lux illumination after 4-5 weeks the embryoids became plantlets.**
- **8.After acclimatization, transfer to green house**

Procedure for pollen culture

- 1. Anther collected from flower buds and pollen grains are isolated and inoculated in the nutrients medium with the concentration of pollen 0.5ml.
- 2. Nitsh (1974) medium is used for pollen culture
- 3. Anthers are place on the medium
- some times nurse culture may used.
- 4. A paper disc is placed over anther or callus.
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- 0.5ml pollen suspension is poured on the disc
- Petridish covered with lid and incubated at 50 C for 4 weeks. Pollengrains grown in to individual clones with 60% efficacy.
- Clones are transferred to callus induction medium to produce calli.
- Calli transferred to shooting medium and then to rooting medium to produce ploatlets

Advantage of pollen culture over anther culture

- During **anther culture** there is always the possibility that somatic cells of the anther that are diploid will also respond to the culture condition and so produce **unwanted diploid calli or plantlets**.
- Sometimes the development of microspores inside the anther may be interrupted due to growth inhibiting substances leaking out of the anther wall in contact with nutrient medium.

Importance of pollen and anther culture

- Utility of anther and pollen culture for basic research
cytogenetic studies.
- Study of genetic recombination in higher plants.
- Study of mode of differentiation from single cell to whole organism.
- Study of factor controlling pollen embryogenesis of higher plants.
- Formation of double haploid that are homozygous and fertile.

- 2) for mutation study.
- 3) for plant breeding and crop improvement.
- 4) to obtain the secondary metabolites. Eg. *Hyoscyamus niger* obtain by anther culture having higher alkaloid content.
- 5) Haploids are used in molecular biology and genetic engineering. Example:- Haploid tissue of *Arabidopsis* and *Lycopersicon* have been used for the transfer and expression of three genes from *Escherichia coli*....