Role of Anther Culture in Haploid Production

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Anther culture is a type of tissue culture technique used to produce haploids and dihaploids. It is simpler than the pollen culture technique. It uses microspores or anthers for plant regeneration.The first successful report of anther culture was published in the 1970s by Guha and Maheshwari on pollen grains of Datura. Since then it has been used in numerous species (around , mainly rice (O. sativa) and tobacco (N. tabacum). During the process, the anthers are excised at a critical stage from an unopened flower bud aseptically. Then, they are cultured on a nutrient medium for the formation of callus tissue or embryoids that give rise to haploid plantlets through embryogenesis or organogenesis. In this article, we will cover more on the procedure of anther culture, the benefit of producing haploids, applications of anther culture, and its advantages and disadvantages.

Procedure of Haploid production

- Select unopened buds of about 17-22mm length, ensuring that the size of the sepal is equal to the petal size.
- Transfer the buds to sterilized airtight plastic bags.
- Move the selected buds to the laminar airflow chamber for surface sterilization.
- Surface sterilize the buds with 70% ethanol for 10 seconds and 20% sodium hypochlorite for 10 minutes.
- Wash the buds three times with distilled water.
- Transfer the buds to a sterilized Petri plate.
- Separate the stamen from the bud using a scalpel.
- Remove the filaments from an anther.
- Transfer the anther onto a solid or liquid nutrient medium and incubate it for 3-4 weeks at 24-28°C in the dark.
- Haploid plantlets will appear from the anther culture within 3-4 weeks through embryogenesis and organogenesis.
- Incubate the culture at 24-28°C for 12-18 hours in light and 6-12 hours in the dark.
- Once the plantlets reach about 50mm tall, transfer them to a pot containing bio compost followed by washing.
- Cover the pot with a sterilized glass beaker and remove it after some weeks to transfer the plant to a larger pot.

Factors Affecting The Anther Culture

- **Genotype of the donor plant:** Some plant species or varieties respond better than others. Thus, the genetic makeup of the plant matters a lot in how anthers respond to the in vitro environment.
- **Anther wall:** It provides nourishment during the developmental stages of the anther.
- **Anther stage:** Pre-mitotic, mitotic and post-mitotic stages are preferred stages for anther culture.
- **Physiological status of donor plant:** Buds from younger plants are preferable for anther culture.
- **Anther pretreatment:** Pretreatment of flower buds with high-temperature stimulate embryogenesis. For example, the bud of Nicotiana tabacum undergoes a pretreatment at a temperature of 5°C for a period of 72 hours.
- **Temperature and lights:** To stimulate embryogenesis, anther culture requires optimal conditions that involve culturing at high temperatures (35 °C) for up to 48 hours. However, the suitable temperature varies for each plant. For example, the ideal temperature for the production of embryoids in Datura stramonium is 20°C, while for Nicotiana tabacum it is 25°C.
- **Culture medium:** The induction of haploid plants in the culture medium is reliant on essential elements such as sucrose, iron, vitamins, coconut milk, and

hormones/growth regulators (such as auxins or cytokinins).

How Does Haploid Production Help Plants Growers

- Elimination of plants with lethal genes from the gene pool.
- Since haploids carry only one allele, any recessive mutations and characteristics are apparent in the process.
- Haploids allow the production of homozygous diploid plants.
- Haploid production shortens the time for inbreeding for superior hybrid genotypes.
- Breeding experiments typically require a lengthy period of time, ranging from 5 to 20 years, to develop new varieties or improve existing ones. In contrast, producing haploid plants can significantly reduce the time (by 2-6 years) required for various stages of crop improvement.

Applications Of Anther Culture

- Massive application in mutation studies and cryogenic studies.
- To study secondary metabolite content
- Extensive application in crop improvement and plant breeding.
- Enables the rapid development of homozygous inbred lines, without the need for traditional selfing methods such as bud pollination which can be time-consuming.
- Anther culture has application in transformation or transgenic plant formation, which can be done in lesser time compared to conventional approaches.
- Haploids can be used to produce monosomies, nullisomics, and other aneuploids.

Advantages

- Simple technique
- Less time consuming
- A high frequency of haploid plants, which is easily identified by their smaller sterile flowers.
- Easy to induce cell division in most species
- No requirement for a very high level of expertise.

Disadvantages

- Not all plants produced are haploid
- It is hard to remove the anthers in some species
- Moderate expertise is needed
- In cereals and other monocots, albinism is frequent.
- There's a risk of chimera and callus formation from the anther wall.

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