

Anther Culture Techniques

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SUMMARY

Anthers in controlled aseptic condition. This techniques used in most of crops from different family such as, Grammineae, Crucifera, Ranunculaceae and Solanaceae. Aim of this process is production of haploid androgenic plants by using regeneration capacity.

INTRODUCTION

A tissue culture technique, in which a part of a plant (anther) gives rise to an androgenic haploid plant when cultured on a nutrient medium under optimal conditions, is defined as anther culture. The nutrient media provide all the necessary factors that are required for the growth of the cell. This technique has been implanted among most common species from Grammineae, Solanaceae, Ranunculaceae, Cruciferae etc. This technique is method of Androgenesis, i.e. production of haploid plantlets by the in-vitro condition, in which the anther is separated from the bud aseptically and cultured on the nutrient media to give rise to the haploid plantlets. Production of haploid androgenic plants by using the regeneration capacity or the totipotency of the anther is an aim of this process. Developing anther from the bud is used as explant for the anther culture. The microspores of the anthers turn into the callus tissue, which in turn produce haploid plants.

History:

Anther culture was first reported in the 1970s through *in vitro* methods by Guha and Maheshwari . It has been used in numerous species, mainly rice (*O. sativa*) and tobacco (*N. tabacum*). Several discoveries have led to the discovery of producing haploid plantlets by the anther culture. Many scientists have contributed to know more of this technique by their experiments. Bergner in 1921, introduced the existence of haploids in *Datura stramonium*. Whereas, introduction of the proliferation of haploid callus from the mature pollen grains of *Ginkgo biloba* discovered by W.Tulecke (1953). After that in 1966, Guha and S.C. Maheshwari, confirmed the origin of haploid plantlets from the anther. Bourgin and Hitsch (1967), discovered the full-fledged haploid plants from the *Nicotiana tabacum*.

Methods of Anther culture:

Direct or indirect methods are involved in anther culture. This culture accomplished by using either of both methods.

- The direct method of an anther culture involves Embryogenesis. In this method, the anther behaves as a zygote and forms embryoid that eventually develops haploid plantlets.
- The indirect method of an anther culture involves Organogenesis. In this method, the anther undergoes cell division repeatedly to form the callus tissue, which later gives rise to the haploid plantlets.

Factors affecting on Anther culture:

Stage of Anthers: A pre-mitotic, mitotic and post-mitotic stage of an anther is mostly preferred for the anther culture. Pre-mitotic is the stage, in which the first meiotic division occurs in the anther and the pollens are at an immature stage. In the mitotic stage of an anther, there is the division of the pollen. The bi-cellular stage i.e. the post-mitotic stage in which, the development in pollen grains form embryoids.

Bud size: Its preferable size of bud is up to the length of 17-22 mm.

Age of plant: The anther culture prefers the buds from the younger plants.

Culture medium: In the culture medium sucrose, iron, vitamins, coconut milk, hormones and growth regulators mostly auxins or cytokinins play an essential role in the induction of haploid plant.

Temperature: It varies with the different plant species. Example: In *Datura stramonium*, the optimum temperature is 20° C for the formation of embryoids. In *Nicotiana tabacum*, the optimum temperature is 25° C for the formation of embryoids. In *Brassica campentris*, the optimum temperature is much higher, i.e. 35° C for the formation of embryoids.

Role of activated charcoal: Activated charcoal plays an important role in the removal of inhibitory substances like both exogenous and endogenous growth hormones from the culture medium by stimulating anther growth.

Pre-treatment method: The pretreatment of bud is necessary for the production of embryoids. Example: In *Nicotiana tabacum*, the bud is pretreated at a temperature of 5° C for 72 hours

Advantages of Anther culture:

- The anther culture technique used to developed haploid plants.
- The advantages of this include high frequency of haploid plants, easy to induce cell division in most species.
- No requirements of a high level of expertise to work out this process.
- It involves selecting suitable parents in a breeding program depending on the goals and conducting a cross.
- Suitable parents are those that carry excellent agronomic traits, the trait of interest, and culture easily.
- The F₁ is used as the source of immature pollen grains located within the anthers.
- The donor parents' growing environment must be controlled for everything including light, nutrients, humidity, and temperature.
- It is also important to note that the developmental stage of the plant is very important upon selection.
- Flowers must be selected at the correct stage and this varies among species.
- The flowers or buds may also need to be sterilized depending on the species and the media requirements may differ.
- After the flower or bud is removed, it is kept on ice and the anthers are dissected via microscopy and sterilized forceps.
- The anthers are then transferred to solid nutrient-rich media and a callus is formed. Breeders should try culturing before conducting large-scale production as some genotypes within a species are better suited for haploid production than others.
- Afterward, a callus is formed and transferred to another media to produce embryos where the haploid plant is generated.
- After sufficient growth, the haploid plant is placed in soil or rooting media and then is subjected to artificial chromosome doubling.
- That means chromosome doubling is also an important goal of anther culture.

Disadvantages of anther culture:

- This include that not all plants produced are haploid, it is hard to remove the anthers in some species, moderate expertise is needed, and in cereals and other monocots albinism is frequent.
- In rice cultivars, it was discovered that species and genotype are largely responsible for the success of callus formation and haploid production more so than growing condition and media types.

Important of Anther culture:

Anther culture was one of the first DH techniques discovered and has played an important role in many plant-breeding programs. As like this technique improves, is the hope of many plant breeders and researchers that this technique becomes more efficient and will be successfully transferable to many other plant species. Production of haploids through anther culture can be used for inducing mutations, for transformation, for cell line selection, and in the production of homozygous lines for further breeding programs.

CONCLUSION

Anther culture technique as a efficient alternative to the conventional inbreed line development which is usually achieved thought a number of inbreeding cycles. Different critical factor which affecting on this

technique has been noted here for its optimum condition since it can be potentially developed as a supplementary breeding tool for crop improvement.

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