Anther and Pollen Culture

What is Anther Culture?

Anther culture is a technique by which the developing anthers at a precise and critical stage are excised aseptically from unopened flower bud and are cultured on a nutrient medium where the microspores within the cultured anther develop into callus tissue or embryoids that give rise to haploid plantlets either through organogenesis or embryogenesis.

What is Pollen Culture?

Pollen or microspore culture is an in vitro technique by which the pollen grains, preferably at the uni-nucleated stage, are squeezed out aseptically from the intact anther and then cultured on nutrient medium where the microspores, without producing male gametes, develop into haploid embryoids or callus tissue that give rise to haploid plantlets by embryogenesis or organogenesis.

What is Androgenesis

Androgenesis is the in vitro development of haploid plants originating from totipotent pollen grains through a series of cell division and differentiation.

There are two modes of androgenesis:

(i) Direct androgenesis:

In this type, microspore behaves like a zygote and undergoes change to form embryoid which ultimately gives rise to a plantlet.

(ii) Indirect androgenesis:

In contrast to the direct androgenesis, the microspore, instead of undergoing embryogenesis, divide, repeatedly to form a callus tissue which differentiates into haploid plantlets.

Principle of Anther and Pollen Culture:

The basic principle of anther and pollen culture is the production of haploid plants exploiting the totipotency of microspore and the occurrence of single set of chromosome (n) in 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.

For this objective, microspores, either within intact anther or in isolated state, are grown aseptically on the nutrient medium where the developing pollen grain will form the callus tissue or embryoids that ultimately give rise to haploid plantlets.

In fact, anther culture is in essence the pollen culture. The principle behind the anther culture is that without disturbing the natural habitat and environment of the enclosed anther, pollen can be grown by culturing the intact anther. In culture condition, the diploid tissue of anther will remain living without proliferation at the selective medium and, at the same time, it will encourage the development of pollen by nursing and providing nutrient.

The haploid embryoids or the callus tissue can be seen as the

anther dehisces in culture. But there is always the possibility that the diploid somatic cells of the anther will also respond to culture condition and so produce unwanted diploid callus or plantlets.

In attempts to avoid this problem, free pollens isolated from the anther are grown in nutrient medium. The knowledge gained so far from anther and pollen culture has established that pollens at the uni-nucleate stage, just before the first mitosis, or during mitosis are most suitable for the induction of haploids.

Induction of haploids can be enhanced by keeping the anther or flower bud at low temperature. The low temperature has been ascribed to a number of factors such as dissolution of microtubules, alteration in the first mitosis or maintenance of higher ratio of viable pollen capable of embryogenesis. Cold treatment may also act to help the embryogenesis by repressing the gametophytic differentiation or by lowering the abscisic acid content of the anther which is considered to be inhibitory for the production of haploids.

The important aspect of anther- or pollen culture is the nutrient medium. The nutritional requirements of the excised anther are much simpler than those of isolated microspores. In the isolated microspore, it is obvious that certain factors responsible for the induction of haploids, which might have been provided by the anther, are missing and these have to be provided through the medium.
Rich medium may encourage the proliferation of the diploid tissue of anther wall and should be avoided. Incorporation of activated charcoal into the medium has stimulated the induction of androgenesis. The iron in the medium also plays a very important role for the induction of haploids. Potato extract, coconut milk and

growth regulators like auxin and cytokinin are also used for anther and pollen culture due to their stimulatory effect on androgenesis.

In culture, pollen may divide mitotically or can follow the normal pathway of forming vegetative and generative nuclei. The generative nucleus remains quiescent and abort. The vegetative nucleus divides repeatedly, forming a multinucleate pollen. The multinucleate pollen undergoes segmentation which may lead to form either organised embryoid structure or callus tissue (Fig 11.1). Both types of development are utilised to form haploid plantlets.

The haploid plantlets are self-sterile due to presence of single set of chromosome which are not able to participate in meiotic segregation. By colchicine treatment, haploids are made homozygous diploid, or isogeneic diploid which are fertile. Haploids or homozygous diploid grown in vitro are transferred to pot and grown to maturity in the glasshouse.
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