**Ovule Culture: Meaning, Principle, Protocol, Importance and Applications | Plant Tissue Culture**

### What is the Meaning of Ovule Culture?

Ovule culture is an elegant experimental system by which ovules are aseptically isolated from the ovary and are grown aseptically on che­mically defined nutrient medium under controll­ed conditions.

### Principle:

An ovule is a mega sporangium covered by integument. Ovules are attached with placenta inside the ovary by means of its funiculus. An ovule contains a megaspore or an egg cell. After fertilization, a single cell zygote is formed which ultimately leads to form a mature embryo pos­sessing shoot and root primordia.

Ovules can be isolated and cultured in nu­trient medium. In vitro ovule culture helps to understand the factors that regulate the devel­opment of a zygote through organised stages to a mature embryo. Alternatively, it may be possible to germi­nate pollen in the same culture as the excised ovule and to induce in vitro fertilisation and sub­sequently embryo production.

### Protocol:

(1) Collect the open flower (unfertilized ovules). If fertilized ovules are desired, col­lect the open flower where the anthers are dehisced and pollination has taken place. To ensure the fertilization, collect the flower after 48 hrs. of anther dehiscence.

(2) Remove sepals, petals, androecium etc. from the ovaries containing either fertilized or unfertilized ovules.

(3) Soak the ovaries in 6% NaOCl solution.

(4) Rinse the ovaries 3-4 times with sterile dis­tilled water.

(5) Using sterile techniques, ovules are gently prodded with the help of spoon shaped spa­tula by breaking the funicles at its junc­tion with placental tissue.

(6) The spatula with ovules is gently lowered into the sterile solid or liquid medium as the culture vial is slanted about 45°.

(7) Damaged or undersized ovules are rejected when possible, during transfer.

(8) Incubate the ovule culture in either dark or light (16 hrs. 3,000 lux) at 25°.C

### Importance of Ovule Culture:

Isolated ovule culture as early as the zygote or two to four celled pro-embryo stage is of con­siderable importance. Ovule culture is a boon for the plant breeders in obtaining seedlings from crosses which are normally unsuccessful because of abortive embryos.

**Importance and applica­tion of in vitro ovule culture are discussed below on different specific aspects:**

#### Test Tube Pollination and Fertilization:

An important achievement of research on ovule culture has been the development of the technique of test tube pollination and fertiliza­tion. By this technique, it may be possible to germinate pollen in the same culture as the ex­cised ovule and to induce in vitro fertilization.

Excised unfertilized ovules of Argemone mexicana, Eschscholtzia califormca, Papaver sonniferum, Nxcotxana tabacum, N. rustica have been cultured along with their respective pollen gra­ms. All the stages of development starting from the germination of pollen to double fertilization have been observed and the mature seeds con­taining viable embryos have been obtained by the above experiments.

Using the same method, it has been possible to fertilize the ovules of Melandrium album with pollen grains from other species of caryophyllaceae and subsequently even with pollen of Datura stramonium. Employing ovule culture technique, the incompatibility bar­rier in Petunia axillaris has been overcome.

### Application of Ovule Culture in Hybridization:

In many interspecific and inter-generic cross­es, the F1 hybrid embryos frequently become abortive in the developing seeds or the F1 seeds are not capable to support the development of embryos. Ovule culture has been successfully em­ployed to obtain hybrid seedlings. It has been observed that in several inter specific crosses; the hybrid embryo of Abelmoschus fails to de­velop beyond the heart or torpedo-shaped em­bryo.

By ovule culture, viable hybrids have been obtained in three out of five interspecific crosses attempted, namely, A esculentus x A ficuneus A esculentus x A moschatus and A tuberculatus x A moschatus. Similarly, a true hybrid be­tween Brassica chinensis and B pekinensis has been obtained by culturing the fertilized ovule in vitro. A hybrid between Lolium perenne and Festuca rubra has also been obtained successfully by means of ovule culture.

Several attempts have been made to hy­bridize between different species of the New World and Old World cotton. Although successful crosses between differ­ent species of cotton have been achieved, hybrid plants have not been obtained through fertilized ovule culture. But the seed development and the production of fibre from the cultured ovule have been demonstrated.

The in vitro growth ovule and the development of fibre from the develop- mg seed can also be regulated by exogenous hor­mones and in this respect ovule culture of cotton offers an unique method for the studies on the effect of phytohormones on fibre and seed devel­opment.

#### Production of Haploid Callus through Ovule Culture:

Uchimiya et al. (1971) attempted culturing unfertilized ovules of Solanum melongena and obtained vigorous callus formation on a medium supplemented with IAA and kinetin. Although the origin of the callus tissue was not known, a cytological assay revealed it to be haploid in na­ture. So it is an important attempt to obtain a haploid cell line or plant from an alternative source rather than anther or pollen culture.

#### Ovule Culture and Angiospermic Parasites:

It is generally believed that in obligate root parasites such as Striga or Orobanke the forma­tion of seedlings is dependent on some stimu­lus from the host root. Studies on ovule culture of Orobanche aegyptica and Cistanche tubulosa have demonstrated that the formation of shoots in vitro can be induced in any absence of any stimulus from the host.

#### Ovule Culture of Orchid Plants:

In nature, the seeds of orchid germinate only in association with a proper fungus. As a re­sult numerous seeds are lost due to unavailability of proper fungus. Beside this, the seed capsule of many orchid takes a long time to mature. To overcome such problems, several attempts have been made to culture the fertilized ovule of or­chid in vitro. Poddubnaya-Arnoldi (1959, ’60) successfully grew the fertilized ovule of Calanthe veitchn, Cypripedium insigne, Dendrobium no- bile and Phalaenopsis schilleriana.

#### Induction of Poly-embryo by Ovule Culture:

In horticultural practices, the artificial in­duction of poly-embryo holds a great potential. It has been observed that the nucellus of mono-embryonic ovule of citrus can be induced to form adventive embryos in culture. Therefore, such achievement is very significant.

#### Virus Irradiation through Ovule Culture:

In the varieties of Citrus which are impossi­ble to free of virus by other means, the ovule cul­ture has proved decisively advantageous to make them virus free.