Embryo culture and its application

In angiosperms, the embryo is the miniature sporophyte resulting from the fertilized egg or zygote. In seed-bearing plants, embryos are easily accessible as they can be separated with relative ease from the maternal tissues and cultures in vitro under aseptic conditions in media of known chemical composition.

Embryo culture involves isolating an immature or mature zygotic embryo aseptically and growing on an aseptic nutrient medium to obtain a viable plant.

For the in vitro culture of embryos generally, it is necessary to excise them from their surrounding tissues. The mature embryos can be isolated with relative ease by splitting open the seeds. Seeds with a hard seed coat are dissected after soaking them in water. For plants with minute seeds, the isolation of embryos can be done under the dissecting microscope on a sterilized slide. In plants like orchids, where the seeds are minute and lack functional endosperm, the entire ovules having embryos are cultured on the medium. There are two types of embryo culture: mature embryo culture and immature embryo culture (embryo rescue).

1. **Mature embryo Culture**

It is the culture of mature embryos derived from grape seeds. Mature embryos are isolated from ripe seeds and cultured in vitro. Mature embryo cultures are carried out when: the embryos remain dormant for long periods, embryos have low survival in vivo, to avoid inhibition in the seed for germination or to convert sterile seeds to viable seedlings. In some plants, seed dormancy may be due to chemical inhibitors or mechanical resistance exerted by structures covering the embryo. Seed dormancy can be successfully bypassed by culturing the embryos in vitro. Embryo culture is relatively easy as they can be grown on a simple inorganic medium supplemented with energy source (usually sucrose) to develop viable seedlings.

2. **Immature embryo culture (Embryo rescue)**

Embryo rescue involves the culture of immature embryos to rescue them from unripe or

hybrid seeds which fail to germinate. This approach is very useful to avoid embryo abortion and produce a viable plant. Wild hybridization involving crossing of two different species of plants from the same genus or different genera often results in failure. This is mainly because the normal development of zygote and seed is hindered due to genetic barriers. Consequently, hybrid endosperm fails to develop leading to the abortion of hybrid embryo. The endosperm may also produce toxins that ultimately kill the embryo. In normal circumstances, endosperm first develops and supports embryo development nutritionally. Thus, the majority of embryo abortions are due to failure in endosperm development. Embryo abortion can be avoided by isolating and culturing the hybrid embryos before abortion. The most important application of embryo rescue is the production of interspecific and inter-generic hybrids from wild plant species.

Steps of embryo culture:

1. Select the healthy and mature fruit and wash it thoroughly under running water for 10 min.
2. After the washing surface sterilize with 0.01% tween-20. After 15 minutes again wash with sterile distilled water.
3. Break down the seed aseptically and isolate the embryo with the help of forceps.
4. Culture the embryo in a callus proliferation medium.
5. After 4 to 5-week callus growth is observed.
6. Then callus is transferred to the shoot regeneration medium after some time shoot regenerates. After some time, the shoot is then transferred to a new fresh medium from which the complete plant develops.

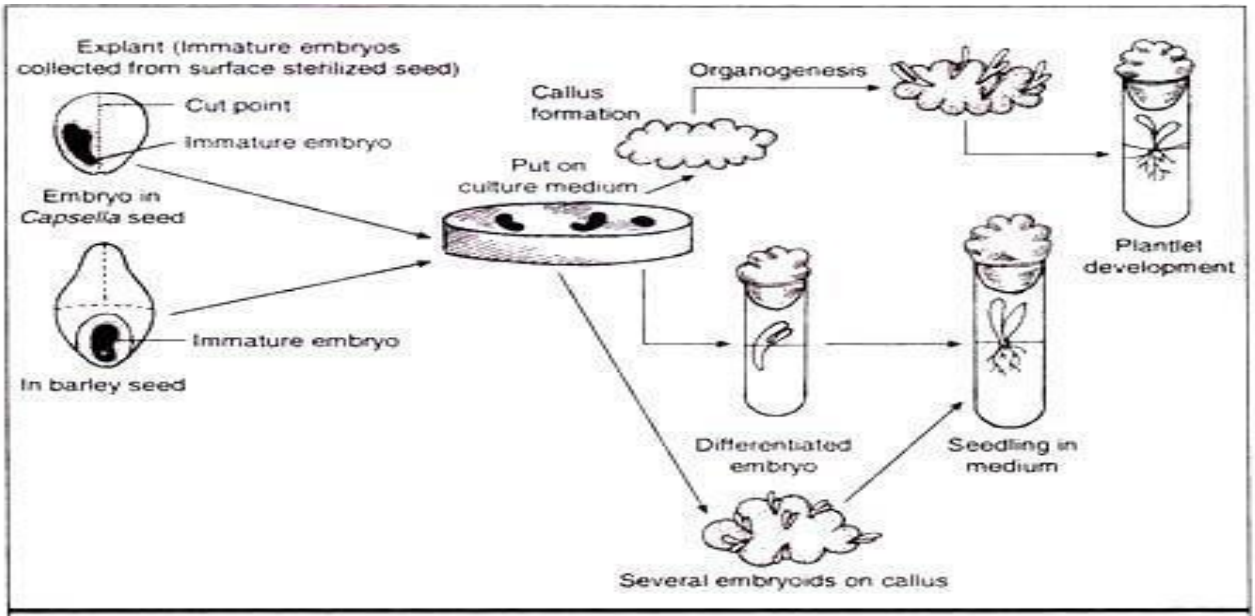

Fig. 19.1: Procedure of Embryo Culture

Culture Technique for Embryo Rescue:

The isolation of immature embryos often poses some difficulty. The aseptically isolated embryos can be grown in a suitable medium under optimal conditions. In general, a complex nutrient medium is required for culture methods involving embryo rescue. For adequate nutritional support of immature embryos, embryo-endosperm transplant is used.

Embryo-endosperm transplant: Steps of the endosperm transplant technique used for culturing immature embryos: The hybrid embryo from the ovule in which endosperm development has failed is taken out by excision. Another normally developed ovule with endosperm enclosing an embryo is chosen. This ovule is dissected and the normal embryo is pressed out. This leaves a normal endosperm with an exit hole. Now, the hybrid embryo can be inserted into the normal endosperm through exit hole. This results in embryo-endosperm transplant which can be cultured in a suitable medium. By using embryo-endosperm transplant, many interspecific and inter-generic plants have been raised e.g., hybrid plants of legumes.

Application of Embryo culture

1. Prevention of embryo abortion Incompatibility barriers in interspecific and inter-generic hybridization programs leading to embryo abortion can be successfully overcome by embryo rescue. Many distant hybrids have been obtained through embryo rescue techniques.
2. Overcoming seed dormancy: Seed dormancy is caused by several factors— endogenous inhibitors, embryo immaturity, specific light and temperature requirements, dry storage requirements, etc. Further, in some plants, the natural period of seed dormancy itself is too long. Embryo culture is successfully applied to overcome seed dormancy and to produce viable seedlings in these plant species.
3. Shortening of breeding cycle: Some of the plants in their natural state have long breeding cycles. This is mostly due to seed dormancy attributed to seed coat and/or endosperm. The embryos can be excised and cultured in vitro to develop into plants within a short period.
4. Overcoming seed sterility: Certain plant species produce sterile seeds that do not germinate e.g., early ripening varieties of cherry, apricot, and plum. Seed sterility is mostly associated with incomplete embryo development which leads to the death of the germinating embryo. Using embryo cultures, it is possible to raise seedlings from sterile seeds of early-ripening fruits e.g., apricots, and plum.
5. Clonal Propagation: Clonal propagation refers to the process of asexual reproduction by multiplication of genetically identical copies of individual plants. The term clone is used to represent a plant population derived from a single individual by asexual reproduction. Embryos are ideally suited for in vitro clonal propagation. This is because embryos are juvenile in nature with high regenerative potential.
6. Overcoming Self-sterility of Seeds: In some economically important plants like banana, and kachoo, seeds are produced, but such seeds are never known to germinate in nature. Such plants propagate very easily by vegetative means. This natural sterility barrier in the seed could be overcome by embryo culture.
7. Crop Plants: Embryo culture has been successfully employed in many unsuccessful interspecific crosses of crop plants. Tomato Cultivated tomato (Lycopersicon esculentum) is very susceptible to viruses, mold, and nematodes. However, the wild species *L. peruvianum* is relatively resistant. In a cross between the two species, the

fruit develops normally but the seeds containing underdeveloped embryos do not germinate. Hybrid plants have been raised from such seeds by embryo culture.

Factors affecting embryo culture:

1. Composition of the medium: Two main types of basal media are the most commonly used for embryo rescue studies, MS and B5 media. The composition of the media will vary in terms of the concentration of the media supplements required. This will generally depend on the stage of development of the embryo. Young embryos would require complex media with a high concentration of sucrose, while mature embryos can usually develop on a simple media on a low level of sucrose.
2. Temperature and light: The temperature and light requirement is generally specific and thus it is usually regulated to be within the same temperature requirement as that of its parent with embryos of cool season crops requiring cooler temperature than those of warm season crops. Embryos sometimes grow best when maintained in darkness for the first 1-2 weeks of culture.
3. Growth condition of mother plant: The growth conditions of the mother plant are also a consideration in embryo culture. The endosperm and the cotyledons will develop more if the mother plant is growing under well-controlled conditions, embryo growth consequently be promoted.