Ovule culture

Culturing of immature ovules is resorted to in cases, where pollination is successful but for certain reason of incompatibility, the seeds do not develop beyond a certain stage. The procedure can succeed only if at least a few days have elapsed after fertilization.

Depending upon, when the embryo aborts, the ovules have to be excised any time from soon after fertilization to almost developed fruits, which may sometimes be lost due to premature abscission.

For a variety of difficult interspecific/intergeneric crosses involving members of the families Malvaceae, Fabaceae, Cruciferae, Solanaceae, etc., ovules after fertilization have been successfully cultured to obtain mature embryos/seeds.

However, ovule culture is mainly tried only in those cases, where embryo aborts very early, and embryo culture is not possible due to difficulty of its excision at a very early stage. In some cases, the medium may need to be supplemented with some fruit/vegetable juice to accelerate initial growth.

Ovule culture in orchids

Nimoto and Sagawa (1961) were the first to attempt this in orchids. According to them the seeds could be taken out for culturing only about 55 days after pollination. But Israel (1963) reports having taken seeds out of the ovaries seven days after pollination and cultured them to seedling stage.

There are some genera like Cypripedium and Paphiopedilum whose seeds are especially difficult to germinate. In such cases the seeds coat is said to contain certain substances which retard or even prevent the process of germination (Northern, 1970). Culturing of ovules before seed coat is developed fully will give a higher percentage of germination in such cases. The immature pod is sterilised, cut open with a sterile knife and the seeds scooped out and put in a vial containing distilled water. It is shaken well and the seeds sown to come in direct contact with the seeds. Other special techniques have also been suggested to tackle such hard to germinate seeds. Kano (1968) reported that immersing of seeds in sterilised water for 5 hours prior to flasking and sealing the flasks entirely quickened the process of germinate of the seeds

of *Cymbidium virescens* and *C. gyrokuchin*. Similarly soaking of seeds of *Cypripedium acaule* (Which is very difficult to germinate under normal conditions) in a sterile nutrient solution for 15-45 days and putting them in unaerated flasks, is reported to hasten germination. Flasks should preferably be kept at 25^0C and under diffuse light, in an incubator or in a green house under glass.

The process of ovule culture can be divided into two parts: first is the preparation of the medium where the ovule will be cultured and the second is the actual culture of the ovule.

The components of the culture medium include inorganic nutrients that are essential for the plant to complete its life cycle, such as sugar, vitamins, amino acid, organic supplement such coconut water, growth regulators, agar as a gelling agent, and other supplements that are deemed necessary. In the case of orchids, the Knudson medium is being utilized as it is specially formulated for orchids.

The actual culture proceeds after the preparation of the medium. To achieve an aseptic condition, the inoculation chamber is disinfected by spraying 80 percent ethyl alcohol on the surface where the whole process will be performed.

An orchid pod which contains the seeds of the orchid is secured and rubbed with 95 percent ethyl alcohol for preliminary sterilization. Inside the chamber, the whole pod will be dipped in a bottle 1/3 full of 95 percent ethyl alcohol for 3 to 5 seconds with the aid of a scalpel and forceps. The pod, after being dipped in an ethyl alcohol, will be flamed at least thrice until the alcohol on the surface has evaporated. Such series of steps are performed to ensure that the surface of the pod is free from contaminants.

After the surface sterilization, the pod is sectioned on a sterile petri dish with the aid of sterile forceps. Once the pod has been opened, thousands of orchid ovule will be revealed. The ovules will be carefully scraped off from the pod with the use of scalpel and will be carefully dropped into the bottle of the culture medium. Once the ovules have settled inside, the bottle will be covered tightly with cotton plugs and will be placed in a cool and well-lighted place. .
Signs of successful germination in the culture of orchid are when the orchid seeds start to swell and turn green. Sooner, the embryo becomes bigger and assumes the shape of a top. At this point, the structure is no longer an embryo, but a protocorm. At this stage, the protocorms are

ready for reflasking. The protcorms will be transferred from one culture bottle to another with the use of a spatula. Reflasking is necessary since this will provide room for further growth and development for the protocorms. Four to eight months after reflasking, the protocorms will become bigger and ready to be planted out of the culture bottle for potting.

Just like any process, this technique requires skills in performing the media preparation and culture, and knowledge, especially on stages of development of embryo.

Questions

1. Ovule culture is practiced in the members of the families like ……

a). Malvaceae b). Fabaceae c). Cruciferae **d). All the above**

2. Ovule culture is mainly tried in …….. case(s).

a). embryo aborts very early b). embryo culture is not possible due to difficulty of its excision at a very early stage

c). **Both a & b** d). None of the above

3. Ovule culture is first attempted in …….

a). Orchidaceae b). Fabaceae c). Malvaceae d). Cruciferae

4. Ovule culture is first attempted by ……

a). Northern **b). Nimoto and Sagawa** c). Kano d). None of the above