OVARY CULTURE/GYNOGENESIS

Culture of unfertilized ovaries to obtain haploid plants from egg cell or other haploid cells of embryo sac is called ovary culture and the process is known as Gynogeneis

* The first report on gynogenesis was by San Noem in 1976 in case of barley

During induction of ovaries are floated on a liquid medium having low auxin and kept in dark while for regeneration they are transferred to an agar medium with higher auxin concentration and incubated in light.

In vitro parthenogenesis

Haploid plants generally originate from egg cell in most of species (rice)

In vitro apogamy

When haploid plants originate from other than egg cell called synergid (rice) or antipodal(Allium tuberosum)

Principle

The principle of gynogenesis is based on the Regeneration principle, where an ovary can regenerate into a fully differentiated plant. In the ovary culture, the flowers are excised from the plant either in pollinated or non-pollinated stage, and from that pistil containing ovary is removed. The ovary comprises of ovule which is a female reproductive part of a plant by the growth on nutrient medium and under controlled conditions.

Protocol

Gynogenesis includes the following steps:

* First, collect the polloinated/unpollinated flowers from a healthy plant, in a sterilized zip-lock bag.
* Wash the flowers with the distilled water.
* Dip the flowers into the 5% of Teepol solution for 10 minutes.
* Wash with the flowers with the distilled water.
* Wash the flowers again with the distilled water.

Bring the flowers to the laminar airflow chamber.

* Subject the flowers for surface-sterilization by immersing in a 5% of sodium hypochlorite solution for 5-7minutes. And, then wash it with the distilled water.
* To a sterile Petri-plate, transfer the surface sterilized flower and by using sharp scalpel dissect out the calyx, petal, anther filaments etc. to separate the ovary.
* Then, the ovary can culture either through induction or regeneration process. In induction, the ovaries float over the liquid medium. And, in regeneration culture the ovaries on the solid nutrient medium.

Incubate the cultures for 16hours at 25֯ C. For the regeneration of ovary, keep the culture plates in a daylight regime by using a fluorescent lamp. Place the culture tubes in a dark for the induction process.

After 2 weeks, haploid plantlets grow either through embryogenesis or through plant regeneration from callus.

Importance

Ovary culture helps in the study of the early stages of embryo development. Gynogenesis also helps in the study of fruit development and its physiology including maturation.

From the culture of the un-pollinated pistil, the effect of phytohormones on the parthenocarpic fruit can be studied. Role of floral organs can be studied which plays a significant role in fruit development.

Gynogenesis also helps in inducing Polyembryony where instead of single plantlet, many shoots also develop. Ovary culture also explains the*in-vitro* pollination and seed formation method.

The culture of an ovary from the apomictic plant can help us to understand the stimulus provided by pollination. As in apomistic plant, there is no fertilization, the only pollination occurs which alone stimulates the ovary growth and seed development.

Ovary culture also helps to understand the hybridization process to obtain hybrid varieties of seedlings by crossing over of interspecific and intergenic species.

Limitation

* The frequency of responding ovaries (1 – 5%) and the number of plants / ovary (1-2) is low
* It has been successful only in lesser number of plant species

Advantages of ovary culture

1. Ovary/ ovule culture may be useful when there is male sterility in crop species
2. For in vitro pollination and fertilization or for embryo rescue
3. To produce parthenogenetic haploids ( wheat, barley,)
4. To understand the physiology of fruit development
5. Reduction in the frequency of albino plants

Following pollination , whole flower buds are excised (2-15 days pollination) Calyx, corolla and stamen are removed. Ovaries are then surface sterilized and inoculated. To obtain unpollinated ovaries, flower buds are removed 24-48 hours prior to anthesis

Ovule culture

Flowers are sterilized inoculated on pre culture medium. After 10 to 14 days ovules are removed from flowers and plated on medium. Ovule requires exclusive skill . 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.