Study Material

M.Sc. Ag. IV semester
Course: Principles & Practices of Seed Production
Chapter: 06 (Seed Testing)

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Topic: SYNTHETIC SEEDS

Synthetic seeds are defined as artificially encapsulated somatic embryos, shoot buds, cell aggregates, or any other tissue that can be used for sowing as a seed and that possess the ability to convert into a plant under in vitro or ex vitro conditions and that retain this potential also after storage. In simple words synthetic seed contains an embryo produced by somatic embryogenesis enclosed within an artificial medium that supplies nutrients and is encased in an artificial seed covering.
The technology designed to combine the advantages of clonal propagation with those of seed propagation and storage. The first synthetic seeds were produced by Kitto and Janick in 1982 using carrot

Why Synthetic Seeds?

In some of the horticultural crops seeds propagation is not successful due to;

- Heterozygosity of seeds particularly in cross pollinated crops
- Minute seed size eg; orchids
- Presence of reduced endosperm
- Some seeds require mycorrhizal fungi association for germination eg: orchids
- No seeds are formed

Characteristics of Synthetic Seeds

1. Large scale propagation method
2. Maintains genetic uniformity of plants
3. Direct delivery of propagules to the field, thus eliminating transplants
4. Lower cost per plantlet
5. Rapid multiplication of plants

Advantages of Synthetic Seeds Over Somatic Embryos For Propagation

1. Ease of handling while in storage
2. Easy to transport
3. Has potential for long term storage without losing viability
4. Maintains the clonal nature of the resulting plants
5. Serves as a channel for new plant lines produced through biotechnological advances to be delivered directly to the green house or field
6. Allows economical mass propagation of elite plant varieties

Types of synthetic seeds:

i) Desiccated: The desiccated synthetic seeds are produced from somatic embryos either naked or encapsulated in polyoxyethylene glycol (Polyoxr) followed by their desiccation. Desiccation can be achieved either slowly over a period of one or two weeks sequentially using chambers of decreasing relative humidity, or rapidly by unsealing the petri-dishes and leaving them on the bench overnight to dry. Such types of synseeds are produced only in plant species whose somatic embryos are desiccation tolerant.

ii) Hydrated: hydrated synthetic seeds are produced in those plant species where the somatic embryos are recalcitrant and sensitive to desiccation. Hydrated synthetic seeds are produced by encapsulating the somatic embryos in hydrogel capsules.

Procedure for Synthetic Seed Production:

The somatic embryos for synthetic seeds are produced in the lab through culturing of somatic cells and treating with different hormones to produce root and shoot. The following are the different steps involved in artificial seeds production;

1. Establish somatic embryogenesis
2. Mature somatic embryos
3. Synchronize and singulate somatic embryos
4. Mass production of embryos
5. Encapsulation of matured somatic embryos
6. Desiccation
7. Field planting

Somatic Embryos

Somatic embryos are bipolar structure with both apical and basal meristematic regions which are capable of forming shoot and root, respectively.
Somatic embryogenesis is the development of embryos form vegetative cells with in vitro systems. Specific tissues have a capacity for somatic embryogenesis in cultural systems. This allows the clonal propagation of normally seed-propagated crops analogous to the production of apomictic seedlings. Somatic embryos develop through stages similar to zygotic embryos, however, the final size for the cotyledons are usually reduced and there is no development of endosperm or seed coat.
After pollination, a zygotic embryo of a dicotyledonous species develops through a series of morphological stages termed globular, heart and torpedo. Cotyledons develop and expand as the storage reserves of protein, starch and/or oil are deposited. At some stages before the embryo achieves its maximum weight, it acquires the ability to tolerate drying. Then, the seed's vascular connections to the maternal plant are severed, it stops importing nutrients and it begins to lose water. Seeds of most crop plants can survive drying and can be stored for several years. Once they are hydrated, germination commences culminating in the emergence of a radicle and then the mobilization of the storage reserves by the seedling.

Procedure of Somatic Embryogenesis:

1. Petiole explants plants are surface sterilized and cultured on SH medium (Schenk and Hildebrandt, 1972) containing 2,4-D, kinetin and many other nutrients. 2,4-D activates the cell cycle of many cells in the petiole - those in the vascular cambium develop into a callus, whereas some sub-epidermal cells develop into a somatic embryo.
2. The initial somatic embryos, which are only small dense cell clusters at this stage, are embedded in a callus mass of non-differentiated cells.
3. To liberate these pro-embryonic structures, and to stimulate the formation of more embryos, the callus is dispersed in a liquid medium to form a suspension culture containing 2,4-D but not kinetin
4. After 7 days, the suspension is sieved and transferred to solid medium lacking 2,4-D On this medium the embryos develop through morphological stages that appear to be globular, heart and torpedo.
5. Once the majority of embryos reach the torpedo stage (7-10 days after sieving) they are transferred to an enriched medium containing a high level of sucrose, nitrogen and sulphur to prevent precocious germination and to enable deposition of storage

reserves. The embryos rapidly accumulate fresh and dry weight, reaching 1-2 mg dry weight .

6. To induce the acquisition of desiccation tolerance, the somatic embryos are placed on a modified medium containing abscisic acid (ABA) for 3 days. Then they are removed from the medium, washed to remove sugar and other nutrients, and dried.
7. The standard method of drying is to place the somatic embryos in a sealed chamber over a saturated salt solution designed to give specific relative humidifies. Daily for one week, the embryos are transferred to a progressively lower relative humidity chamber and finally are dried at ambient conditions. At this stage, the embryos have reached approximately 15% moisture and can be stored for a year or more with good viability.

Encapsulation of Matured Somatic Embryos

Somatic embryos produced naked embryos without storage materials and protective layer (seed coat). This is very difficult for handling so this demand the encapsulation and coating. The somatic embryos produced are encapsulated using gel agents like agar, alginate, polyco, carboxy methyl cellulose, guar gum, sodium pectate etc. Among these alginate encapsulation was found to be more suitable and practicable. Alginate hydrogel is frequently selected as a matrix for synthetic seed because of its moderate viscosity and low spinnability of solution, low toxicity for somatic embryos and quick gellation, low cost and bio-compatibility characteristics. The use of agar as gel matrix was deliberately avoided as it is considered inferior to alginate with respect to long term storage. Alginate was chosen because it enhances capsule formation and also the rigidity of alginate beads provides better protection to the encased somatic embryos against mechanical injury.

The somatic embryos are mixed with sodium alginate (2 %) and the suspension is dropped into the calcium salts solution (200mM). The principle involved is when sodium alginate dropped into the calcium salt solutions it from round firm beads due to the ion exchange between Na+ in sodium alginate and Ca2+ in calcium slat solutions and sodium alginate form calcium alginate in 20-30 minutes. Since somatic embryos lack seed coat and endosperm the matrix of encapsulation can be added with nutrients and growth regulator, which will serves as an artificial endosperm. This will increase the efficiency of germination and viability of seeds. Addition to these nutrients other useful materials fungicides, pesticides, antibiotics and micro organism can also be incorporated.

Application of Synthetic Seeds

By combining the benefits of a vegetative propagation system with the capability of long-term storage and with the clonal multiplication, synthetic seeds have many diverse applications

1. Multiplication of non-seed producing plants, ornamental hybrids or polyploids plant
2. Propagation of male or female sterile plants for hybrid seed production
3. Germplasm conservation of recalcitrant species
4. Multiplication of transgenic

Limitations

1. Limited production of viable micro-propagules that are useful in synthetic seed producer
2. Asynchrous development of somatic embryos
3. Improper maturation of somatic embryos that makes them inefficient for germination and conversion in to normal plants
4. Lack of dormancy and stress tolerance in somatic embryos that limit the storage of synthetic seeds
5. Somo clonal variations which may alter the genetic constituent of the embryos

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