Synthetic or artificial seeds have been defined as somatic embryos engineered for use in the commercial propagation of plants (Gray and Purohit, 1991; Redenbaugh, 1993). Synthetic seeds can also be defined in other ways, such as artificially encapsulated somatic embryos shoot buds, cell aggregates, or any other tissue that can be used for sowing as a seed and those possess the ability to convert into a plant under *in vitro* or *ex vitro* conditions and retain that potential even after storage (Capuano *et al.*, 1998).

In order to overcome low percent survival of *in vitro* plantlets during the *ex vitro* acclimatization synthetic seed technology holds much promise as this system enables the vegetative propagule to be stored for long periods of time in different matrices and attempting to grow these synthetic seeds (Synseeds) on different substrata (Aitken-Christie *et al.*, 1995; Hussain *et al.*, 2000) and at the same time ensures multiplication of the plant.

Various forms of synthetic seeds have been envisioned over time. The first were simply hydrated somatic embryos produced from vegetative cells in plant tissue culture. These had the particular advantage of enabling rapid clonal multiplication of some plants, but the labour and therefore, cost was high and the propagules were very delicate. This was partially overcome with the development of alginate capsules that encapsulated a single embryo in a protective coating enabling mechanised handling. These hydrated encapsulated embryos could only be stored using low temperatures for a few weeks (Redenbaugh *et al.*, 1986). The capability of prolonged storage was achieved when the somatic embryos could be dried to moisture contents less than 20% (McKersie *et al.*, 1993). This is the current state of the technology. The simplest definition of artificial seed can be such as: encapsulated somatic embryos which functionally mimic seeds and can develop into seedlings under sterile conditions.

PARTS OF A TYPICAL SYNTHETIC SEED

A typical synthetic seed has the following parts such as: (a) **Plant propagule** (somatic embryo or shoot bud) somatic embryos are bipolar structures with both apical and basal meristematic regions, which are capable of forming shoot and root, respectively. (b) **Matrix**,

is a gelling material encapsulating plant propagules which incorporate nutrients, biofertlizers, pesticides, nitrogen - fixing bacteria, antibiotics or other essential additives. (c) **Seed shell** these are the artificial seed coats prepared with complex mixture of alginate-gelatin which was used to develop the coat system for encapsulation. The concept of artificial seed technology has been applied, successfully in large numbers of plants (Table 1).

TECHNIQUE FOR PRODUCTION OF SYNTHETIC SEEDS

Basic hindrance to synthetic seed technology was primarily based on the fact that the somatic embryos lack important accessory tissues, i.e. *endosperm* and *protective coatings* that make them inconvenient to store and handle (Redenbaugh et al., 1993). Furthermore, they are generally regarded to lack a quiescent resting phase and to be incapable of undergoing dehydration. The primary goal of synthetic seed research was, therefore, to produce somatic embryos that resemble more closely the seed embryos in storage and handling characteristics so that they can be utilized as a unit for clonal plant propagation and germplasm conservation. In achieving such a goal the technology of encapsulation has evolved as the first major step for production of synthetic seeds. Later it was thought that the encapsulated synthetic seed should also contain growth nutrients, plant growth promoting microorganisms (e.g. mycorrhizae), and/or other biological components necessary for optimal embryo-to-plant development. A number of patents covering the development of seed analogues have been issued (Redenbaugh and Walker, 1990). However, success of the synthetic seed technology is constrained due to scarcity and undesirable qualities of somatic embryos making it difficult for their development into plants. The choice of coating material for making synseeds is also an important aspect for synseed production.

The propagules (embryos/ axillary buds/ shoot tips) are carefully isolated from aseptic *in vitro* cultures and blot dried on sterilized filter paper, and is then mixed in sodium alginate prepared in nutrient medium. The propagules are then picked up manually by forceps and dropped into a solution of calcium chloride for about 40 minutes. After the incubation period, the beads (synthetic seeds) are recovered by decanting the calcium chloride solution and washing them in sterile water for 3 to 4 times before culturing on nutrient medium or on different substrates such as filter paper, cotton or soil for their growth and conversion to plants.

Based on technology established so far, two types of synthetic seeds are known: *hydrated* and *desiccated*. Redenbaugh *et al.* (1984) developed a technique for hydrogel encapsulation of individual somatic embryos of alfalfa. Since then encapsulation in hydrogel remains to be the most studied method of artificial seed production (Mckersie *et al.*, 1993; Redenbaugh, 1990). Encapsulation is necessary to produce and to protect synthetic seeds. The encapsulation is done by various types of hydrogels which are awter soluble. The gel has a complexing agent which is used in varied concentrations (Table-1). A number of substances like potassium alginate, sodium alginate, carrageenan, agar, gelrite, sodium pectate, etc. have been tested as hydrogels but sodium alginate gel is the most popular (Redenbaugh, 1993).

Hydrated artificial seeds consist of somatic embryos individually encapsulated in a hydrogel. To produce hydrated synthetic seeds, the somatic embryos are mixed with sodium alginate gel (0.5–5.0% w/v) and dropped into a calcium salt solution [$CaCl_2$ (30–100 mM), Ca $(NO_3)_2$ (30–100 mM)] where ion-exchange reaction occurs and sodium ions are replaced by calcium ions forming calcium alginate beads or capsules surrounding the somatic embryos (Fig.1). The size of the capsule is controlled by varying the inner diameter of the pipette nozzle. Hardening of the calcium alginate is modulated with the concentrations of sodium alginate and calcium chloride as well as the duration of complexing. Usually 2% sodium alginate gel with a complexing solution containing 100 mM Ca^{2+} is used and is found to be satisfactory (Redenbaugh, 1990; Redenbaugh, 1993; Redenbaugh, 1984). However, Molle *et al.* (1993) found that for the production of synthetic seeds of carrot, 1% sodium alginate solution, 50 mM Ca^{2+} and 20--30 min time period were satisfactory for proper hardening of calcium alginate capsules. They have suggested the use of a dual nozzle pipette in which the embryos flow through the inner pipette and the alginate solution through the outer pipette. As a result, the embryos are positioned in the centre of the beads for better protection.

The desiccated synthetic seeds are produced from somatic embryos either naked or encapsulated in poly-oxyethylene glycol (Polyox') 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. On the contrary, 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. The production of synthetic seeds for the first time by Kitto and Janick (1982) involved encapsulation of carrot somatic embryos followed by their desiccation. Of the various compounds tested for encapsulation of celery embryos, Kitto and Janick (1982, 1985) selected polyoxyethylene which is readily soluble in water and dries to form a thin film, does not support the growth of micro-organisms and is non-toxic to the embryo. Janick *et al.* (1993) have reported that desiccated artificial seeds were produced by coating a mixture of carrot somatic embryos and callus in polyoxyethylene glycol. The coating mixture was allowed to dry for several hours on a Teflon surface in a sterile hood. The dried mixture was then placed on culture medium, allowed to rehydrate, and then scored for embryo survival.

Development of artificial seeds requires sufficient control of somatic embryogeny from the explants to embryo production, embryo development and their maturation as well. The mature somatic embryos must be capable of germinating out of the capsule or coating to form vigorous normal plants. A number of researchers have tried to improve the quality (Attree *et al.*, 1991; Attree *et al.*, 1993; Wetzstein and Baker, 1993; Attree *et al.*, 1995) and quantity (Parrot *et al.*,1988; Michler *et al.*, 1991; Ara, 1998; Burns *et al.*, 1997) of somatic embryos via modification of culture conditions, such as, medium composition, growth regulators (types and concentrations), physical state of the medium, as well as incubation conditions like temperature, illumination, etc. Although large quantities of somatic embryos can be rapidly

produced in many plant species, normal plants are difficult to obtain due to their improper or asynchronous maturation.

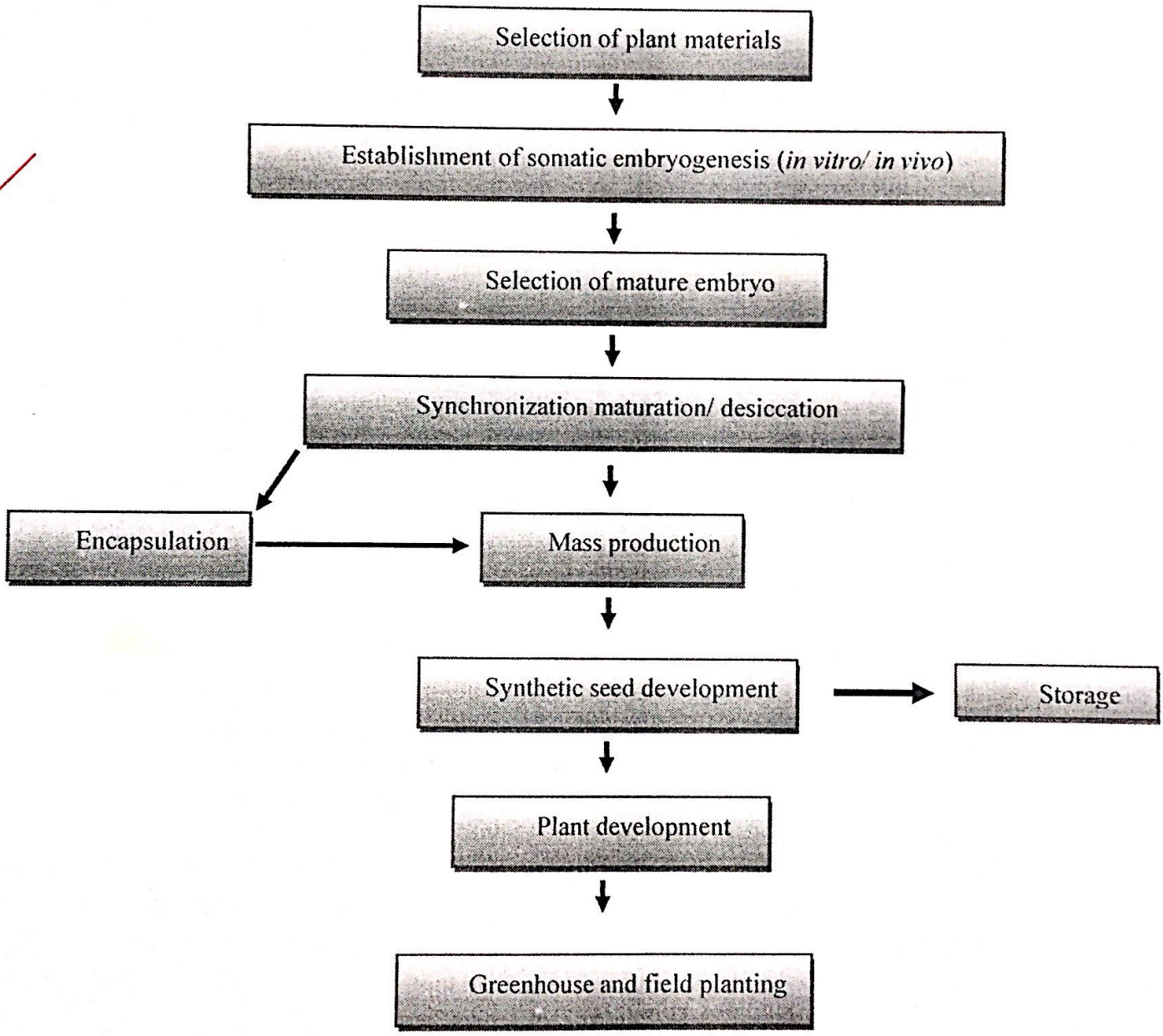

Fig 1: Schematic diagram for production of synthetic seed

Hence, maturation of somatic embryos, which eventually controls germination and conversion rate, is one of the major bottlenecks for synthetic seed production. While studying the effects of different types of osmotica on maturation of somatic embryos of spruce, Attree and Fowke (1993) and Fowke and Attree (1996) have described that inclusion of high levels

Advantages

Merits of artificial or synthetic seed are listed below.

1. Easy handling and Inexpensive transport: As the synthetic seeds are small in size hence it is easier to store, transport and planting.
2. Storage life: Synthetic seeds posses long storage life and also the seed viability remains good for longer period of time.
3. Product uniformity: As somatic embryos are used for the production of artificial seeds hence most of the seeds are identical in there uniformity.
4. To avoid extinction of endangered species and seed less plants: The most important advantages of synthetic seed are such as it helps in conservation of endangered species e. g. in hedgehog cacti (*Echinocereus* sp.) and seedless varieties e.g. grapes.
5. Large scale propagation: After the standardization of protocol it is very much suitable for large scale monoculture.
6. Germplasm conservation: A synthetic seed plays an important role in germplasm conservation.
7. Elite plant genotypes: artificial seed technology preserves, protects and permits economical mass propagation of elite plant genotypes such as orchids.
8. Independent of environmental conditions: The technologies of synthetic seeds production are not a season dependent as these are prepared inside the laboratory.
9. Permits direct field use: For tissue culture raised plants rooting, hardening is necessary but in case of synthetic seeds direct field sowing can give good yield.

10. Facilitates study: basic steps involved in the synthetic seed technology involves seed coat formation, function of endosperm in embryo development and seed germination, somaclonal variation provides wide open facility for study.
11. Supply of beneficial adjuvants: beneficial adjuvants like plant nutrients, plant growth regulators, microorganisms, fungicides, mycorrhizae, antibiotics can be made available to the developing plant embryo as per the requirement as these can be added in to the matrix.
12. Hybrid production: synthetic seed production technology can be used for production of hybrids which have unstable genotypes or show seed sterility such as not susceptible towards infection. It can be used in combination with embryo rescue technique. The rescued embryo can be encapsulated with this technique to form synthetic seeds.
13. In self-pollinated crops that currently have good seed production systems, synthetic seeds will not have any practical applications, but in cross pollinating species, especially those where seed production is difficult and expensive, synthetic seeds offer many advantages and opportunities.

Disadvantages

Several intensive researches in the field of synthetic seed technology were applied in propagating and conserving a number of plant species, but practical implementation of the technology is limited due to the following main reasons.

1. Production and storage of synthetic seed is cost effective hence the production technique itself becomes costlier.
2. Production of viable micropopagules useful in synthetic seed production is less.
3. Anomalous and asynchronous development of somatic embryos.
4. All the embryos cannot mature at a time hence that makes them inefficient for germination and conversion in to normal plants.
5. Seed dormancy is considered as a problem but due to lack of dormancy and stress tolerance in somatic embryos the storage of synthetic seeds are limited.
6. The technology of synthetic seed is limited due to poor conversion of even apparently normally matured somatic embryos and other micropopagules into plantlets (Ara *et al.*, 2000).