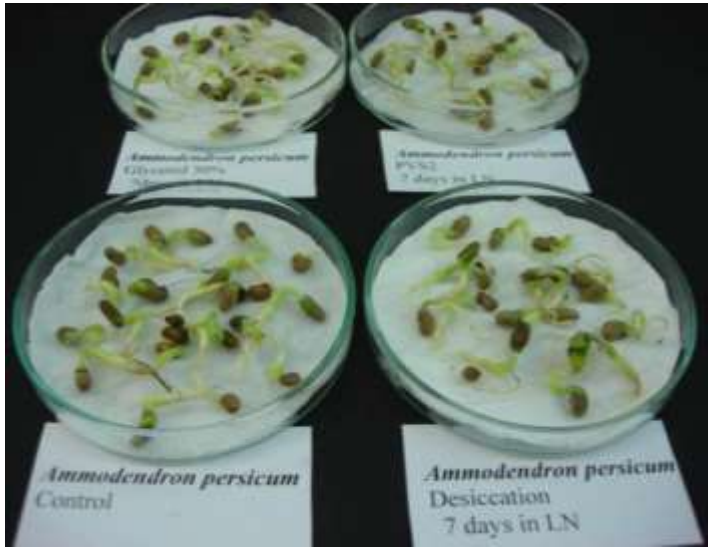
Session 59: Hardening of artificial seed, cryopreservation, storage of artificial seed, cryoplate technique



Introduction

Artificial/synthetic seeds, which are also known by other names such as "**synseeds**", are firstly described by Murashige .

According to him, the synthetic seed is as **"an encapsulated single somatic embryo"**

The definition of artificial seeds was then extended to be artificially coated somatic embryos (usually) or other vegetative parts such as shoot buds, cell aggregates, auxiliary buds, or any other micropropagules, provided that they have the capacity to be sown as a seed and converted into a plant under in vitro or ex vitro conditions

They should also be able to keep this ability for an extended period (storage ability).

Therefore, artificial seeds can eliminate the acclimation steps necessary in micropropagation and give breeders greater flexibility

Encapsulation

The **encapsulation technology** can be considered as a promising approach that can be used for the exchange of plant materials between public and private plant tissue culture laboratories, and also to achieve germplasm conservation and the propagules that are derived from in vitro or by micropropagation applied directly in nurseries or in a field

Coating substances and additives for somatic embryos

Hydrogels[a]	*Additives*	*Dry coatings (or coatings applied hydrated and then dried)*
Sodium alginate	Fertilizer	Polyethylene glycol 1000–4000
Potassium alginate	Nutrient salts (N, P, K)	Polyethylene oxide homopolymers
Guar gum	Cytokinins	Acrylic copolymer containing carboxyl group
Agar	Humic acids	Potassium propenoate–propenamide copolymers
Agarose	α-Keto acids	Carboxyl methylized cold soluble swelling starch
Amylase	Gibberellic $acid_3$	Synthetic trioctachedral smectite
Pectin	Gibberellic $acid_{4/7}$	Synthetic sodium-magnesium lithium silicate
Dextran	Pesticides (ethazol, thiophantenethyl, fenaminosulf, benomyl, chloroneb, captan, metalaxyl)	Starch + synthetic polymer of acrylamide
Gelatin	Biocontrol agents (*Trichoderma harsianum*, *Trichoderma kininoii*, *Rhizobium*)	Sodium acrylate
Starch	Sucrose	
Amylopectin	Activated charcoal	
Mannan		
Modified cellulose (e.g., carboxymethyl cellulose, polyacrylamide)		
Tragacanth gum		
Carrageenan with locust bean gum		
Polysaccharides and galactomannans + starch		
Sodium pectate		

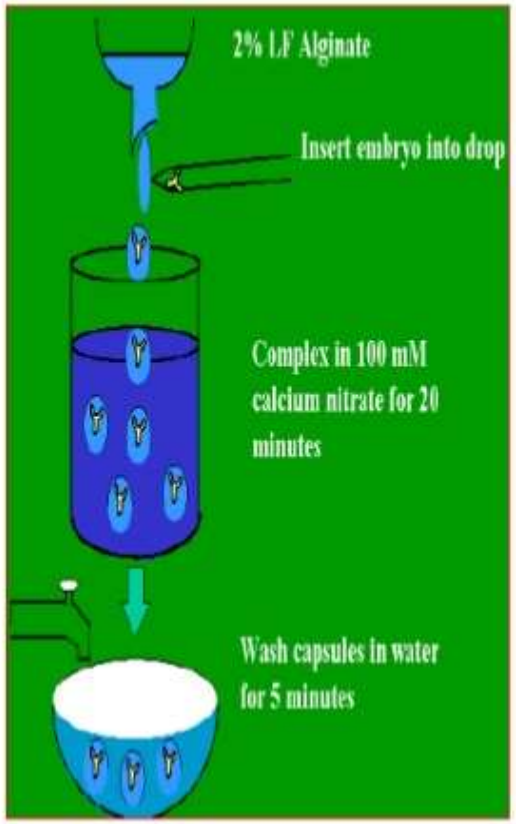
Methods of encapsulating synthetic seed

A) Dropping procedure

1) The most useful encapsulation system. Drip 2-3 % sodium alginate drops from at the tip of the funnel and the somatic embryos are inserted

2) Keep the encapsulated embryos complex in calcium salt for 20 min

3) Rinsed the capsules in water and then stored in a air tight container



B) Automate encapsulation process

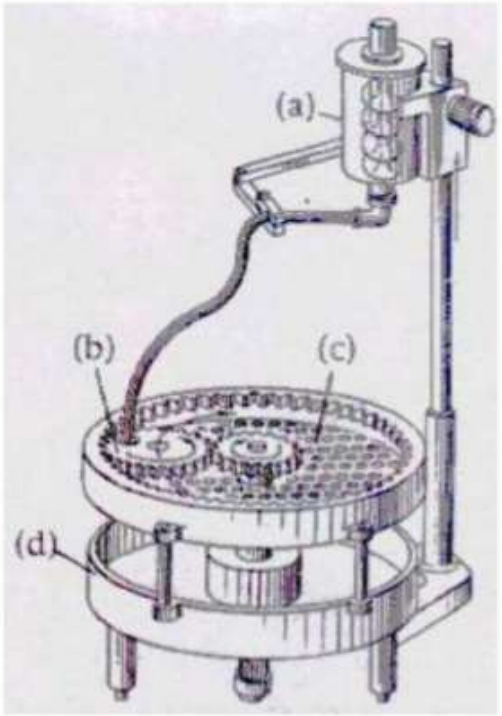
This is the quick method of artificial seed production

A) Alginate solution with embryo is feed from supply tank

B) Alginate capsules were planted in speeding trays using a vacuum seeder.

C) The capsules are planted in the field using a stanhay planter

D) A hydrophobic coating is required for mechanical handling



Hardening of synthetic seed

Hardening of tissue culture plantlets and delivering it into field incurred high labour, and other expenses

In this context, synthetic seed technology assumes greater importance of its low cost and high volume propagation system

Artificial seed prepared by encapsulating the somatic embryos or vegetative propagules can withstand external strains and stresses and thus can be used as an universal delivery system (Redenbough *et al*. 1988)

Encapsulated embryos could be useful delivery system for tissue cultured plants by eliminating the tissue hardening steps by directly sowing them in soil (Fizi *et al*. 1994)

Storage of Synthetic Seed

Gantait S. et al., 2017 developed synthetic seeds using in vitro shoot tips (3–4 mm long) polymerized with 3% (w/v) sodium alginate and 75 mM calcium chloride

Storage of synthetic seeds at 25 °C exhibited a high frequency of germination (82%) after 30-day storage. However, longer storage period (60 days) drastically reduced the germination frequency of synthetic seeds to 36%.

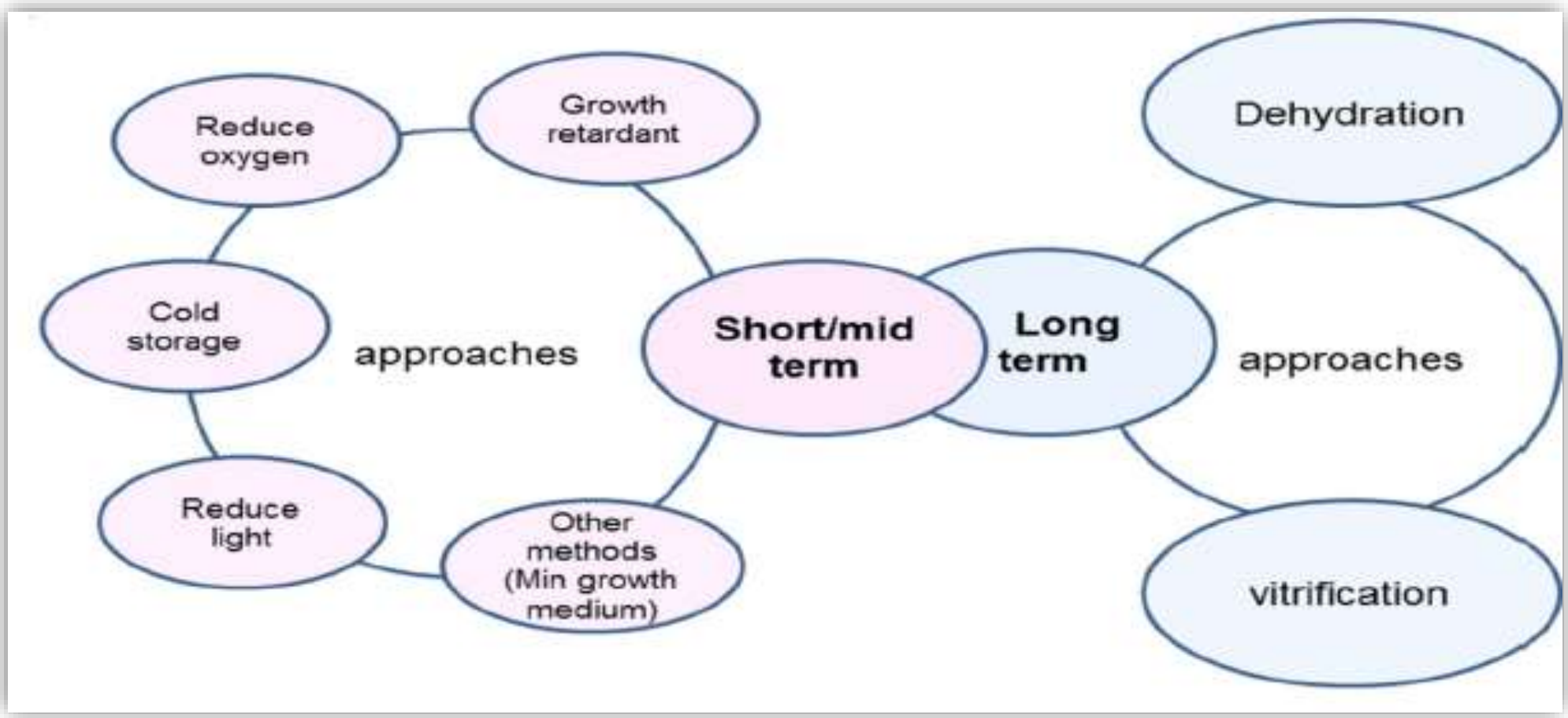
Alternatively, in case of 8 °C storage temperature, 60% synthetic seeds were germinated after 30-day storage with a minor decline to 52% following 60-day storage.

Moreover, synthetic seed-germinated plantlets from 25 °C storage condition showed a regression in reserpine content (203.38 ± 0.21 µg gm^{-1} of dried sample) than that of the plantlets regenerated from 8 °C storage (249.37 ± 0.21 µg gm^{-1} of dried sample).

Their result clearly infers that a lower temperature condition (above freezing; 8 °C) is appropriate for storage, post-storage germination, and upholding the reserpine content of ***R. serpentina*** synthetic seeds.

Rai *et al.* 2008, reported that a high concentration of sucrose or ABA could be useful for short-term conservation of guava (*Psidium guajava* L.) because of their temporary inhibition in encapsulated somatic embryos germination

Synthetic seed storage approaches



Using suitable temperature (usually 4 C), suitable capsulation materials, and optimal storage conditions (reduced heat, light, oxygen, etc.), long storage can be achieved using dehydration and/or cryopreservation techniques

Cryopreservation of synthetic seed

Cryopreservation is a long-term preservation method used for the safe storage of living cells and tissues with the aid of ultra-low temperatures. It's the most common technique for long-term storage of plant genetic resources (Tsai and Lin, 2012; Yi et al., 2013).

Long-term preservation at ultra-low temperatures is provided by liquid nitrogen in its liquid (-196°C) or vapour phase (-130 to -180°C).

Cryoprotectants provide chemical dehydration and water properties alteration.

Cryoprotectants enlarge the steadiness of the plasma lemma and decrease the freezing temperature and also protect the cells from the freezing of the water inside and between the cells

There are two different type of cryoprotectans in function of their capacity to non-penetrate into the cell (e.g., **carbohydrates**) of their capacity to penetrate into cells (e.g., **DMSO, glycerol, ethylene glycol**).

Cryopreservation methods are divided into two groups as basic and newest and diverse modifications on these techniques provide a wide usage area (Kulus and Zalewska, 2014)

Cryopreservation of synthetic seed

Fabre and Dereuddre (1990) working on *Solanum* shoot tips and aiming to increase the tolerance of plant tissue to dehydration–cryopreservation storage conditions, reported a full protocol for encapsulation-dehydration and storage.

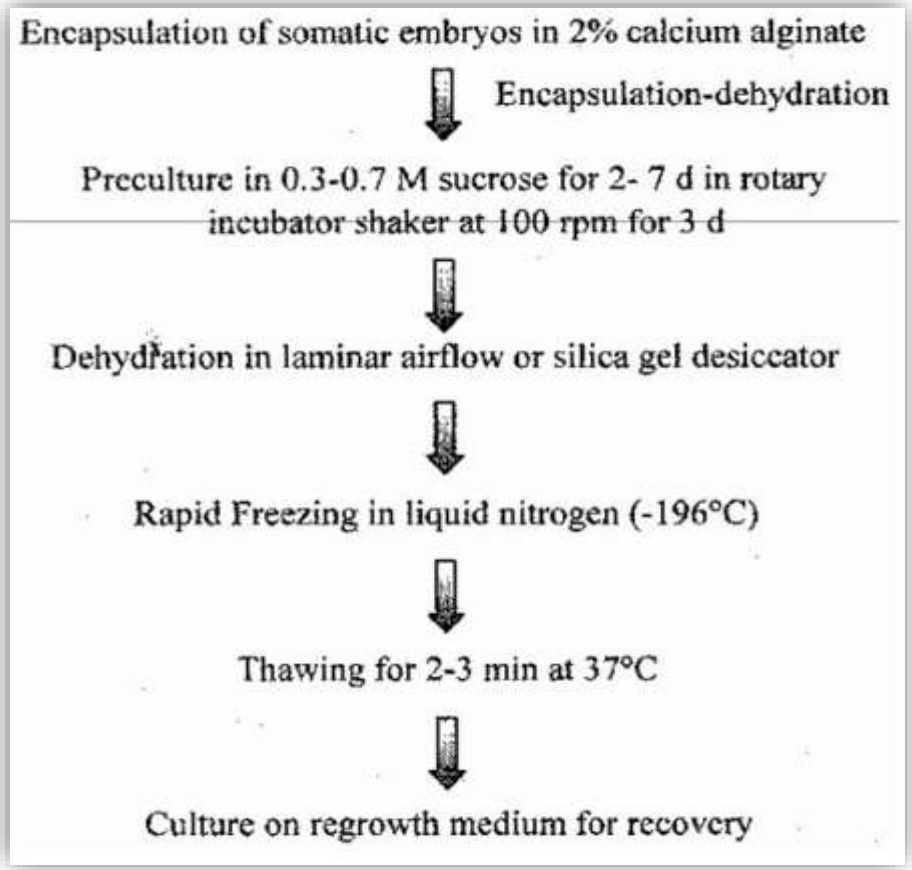
The protocol consists of three procedures:

(a) Pre culturing encapsulated explants in a medium containing high concentrations of sucrose;

(b) drying the encapsulated micro-organism; and

(c) direct plunging into liquid nitrogen.

Cryopreservation of somatic embryos using encapsulation-dehydration technique



Unfortunately, few research projects have investigated in depth the artificial seed preservation (dehydration cryopreservation), and this technique still needs more studies in view of the great value of artificial seeds as an easy and cost-efficient method of germplasm preservation

CRYO-PLATE TECHNIQUE

The cryo-plate is a newly developed technique that includes two different versions:

The V cryo-plate and
The D cryo-plate

The D cryo-plate technique is an air dehydration method and the V cryo-plate is originated from PVS 2 vitrification dehydration (Yamamoto et al., 2011).

In these techniques explants are dehydrated in PVS 2 solution for V cryo-plate, on the other explants dehydrated in laminar flow air cabinets for D cryo-plate technique after loading solution treatment.

Cryo-plate method has many advantages:

1) low level of injury or loss of shoot tips during handling,

2) shoot tips do not float or adhere to the cryotubes during treatments with LS and PVS2 and

3) only dipping of the cryoplate in LN and in the unloading solution for cooling and warming of shoot tips are required, respectively (Yamamoto et al., 2011)

In comparison with D cryo-plate and V cryo-plate techniques, D cryo-plate technique prevented the explants from PVS solutions damage (Niino et al., 2013)

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