Artificial Seed or Synthetic Seed

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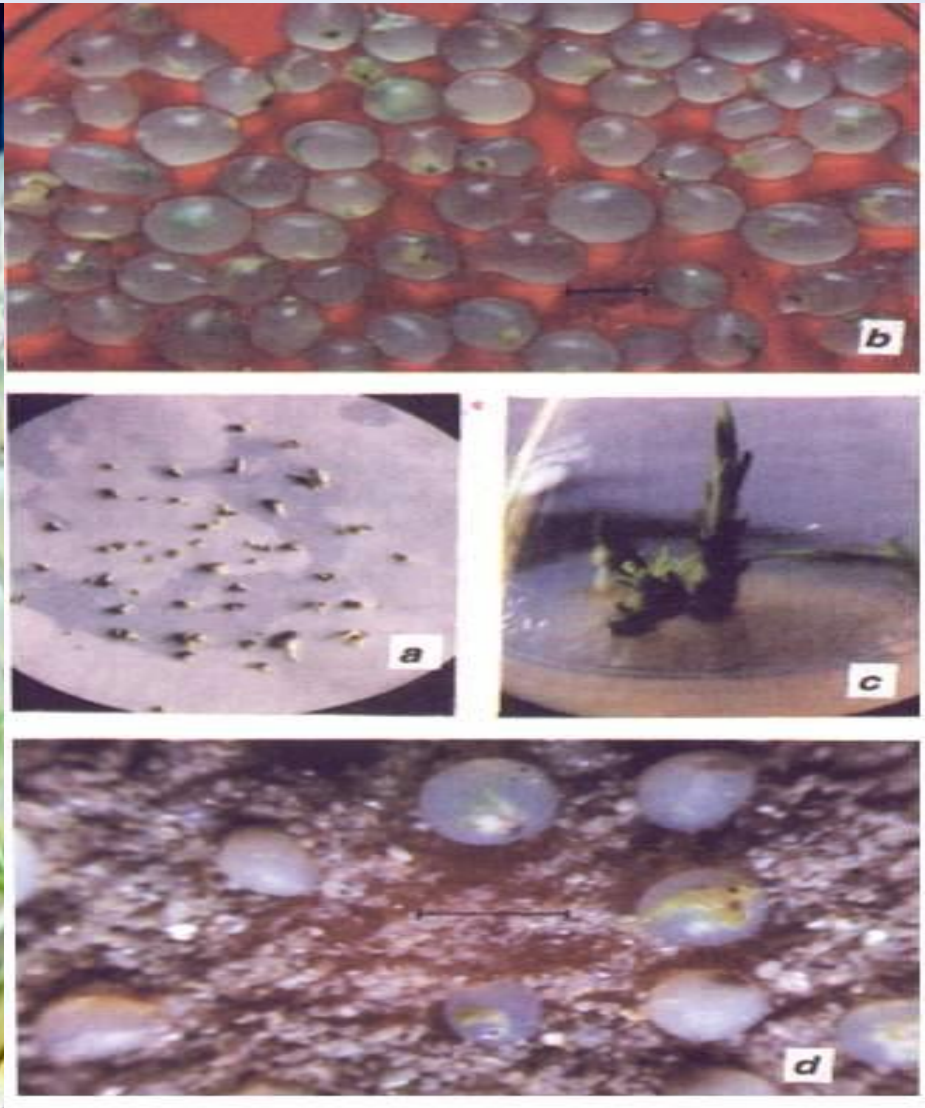
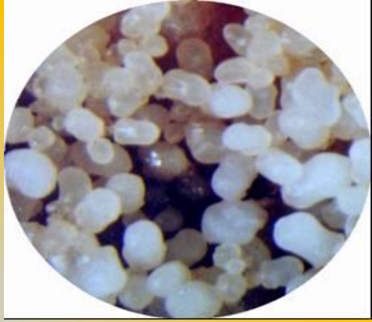
SYNTHETIC SEED

(A NOVEL CONCEPT IN SEED BIOTECHNOLOGY)



SEED

- Botanically-Seed is a ripened ovule
- Agriculturally-Any plant part with regeneration capacity
- Genetically-Connecting link between two generation for transfer of traits



SYNTHETIC SEED

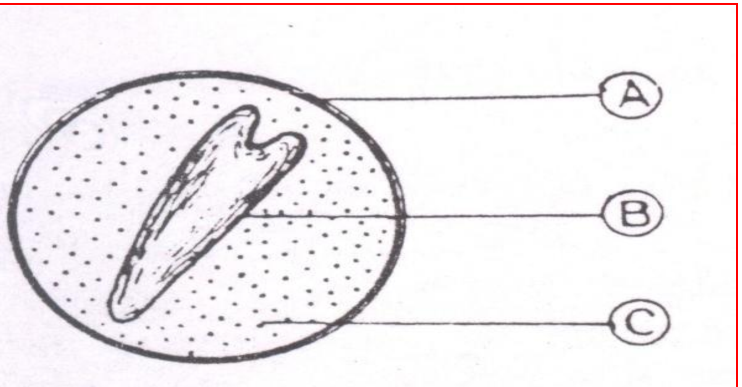
- Artificially encapsulated somatic embryo
- Shoot bud or any other meristatic tissue
- That can be used as functionally mimic seed
- Possesses the ability to convert into a plant
- Under *in vitro* or *ex vitro* conditions
- That can retain this potential even after storage.

(Ara *et al*., 2000)

Landmarks in Synthetic Seed Production

year	Scientist	Events
1902	Haberlandt	Tissue culture technique
1919	Karl Ereky	Term “Biotechnology”
1941	Jost	Term “Genetic Engineering”
1958	Stewart	Somatic embryogenesis in carrot
1978	Murashige	Term “ Synthetic Seed”
1979	Drew	Somatic embryos as seed delivery system
1980	P.S.Rao	Synthetic seeds developed at BARC
1981	Lawrence	Encapsulation technique
1986	Redenbaugh	Hydrogel Encapsulation technique
1989	Fujii	Grow plants from synthetic seeds

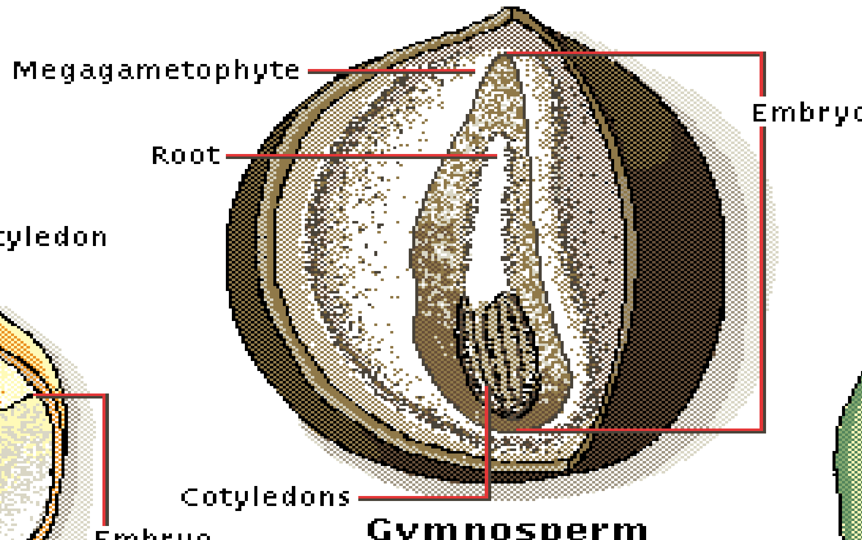
Synthetic and Natural seeds

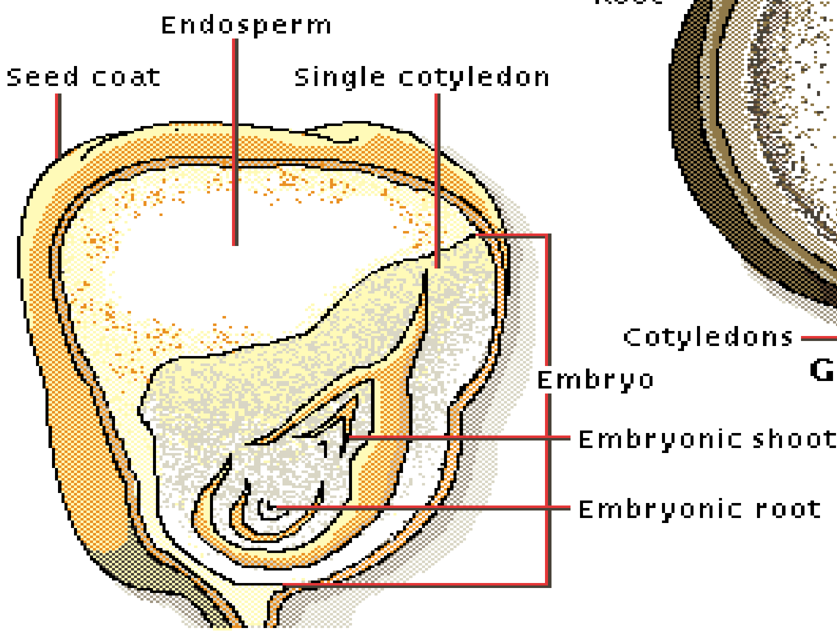


Artificial seed coat

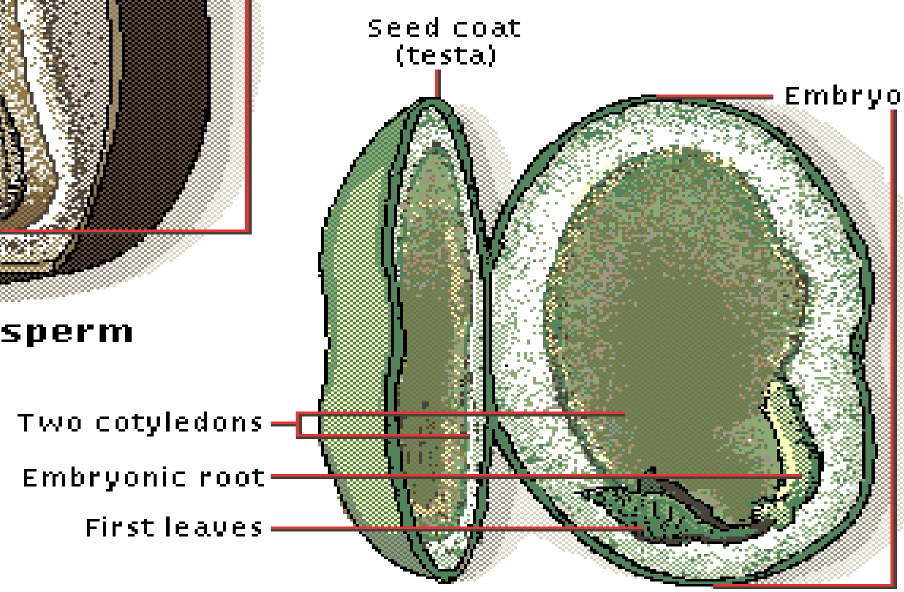
Somatic / asexual embryo

Artificial endosperm





Monocotyledon



Dicotyledon

Type of synthetic seeds

- Desiccated synthetic seed
- Hydrated synthetic seed

Steps of Synthetic Seed Production

Induction of somatic embryogenesis

↓

Maturation of somatic embryos

↓

Encapsulation of somatic embryos

↓

Evaluation of embryoid and conversion

↓

Planting in fields / green house

Somatic Embryo

- ✓ Asexual zygote
- ✓ Naked i.e. without seed coat and endosperm
- ✓ Haploid / diploid
- ✓ Larger than sexual embryo
- ✓ Bipolar structure
- ✓ Direct germination

Somatic Embryogenesis

- Somatic embryogenesis is the process by which the somatic cells or tissues develop into differentiated embryos and each fully developed embryo is capable of developing into plantlet
- *In vitro* process, by which somatic cells develop into somatic embryo without gametic fusion

Types of Somatic Embryogenesis (SE)

Direct somatic embryogenesis

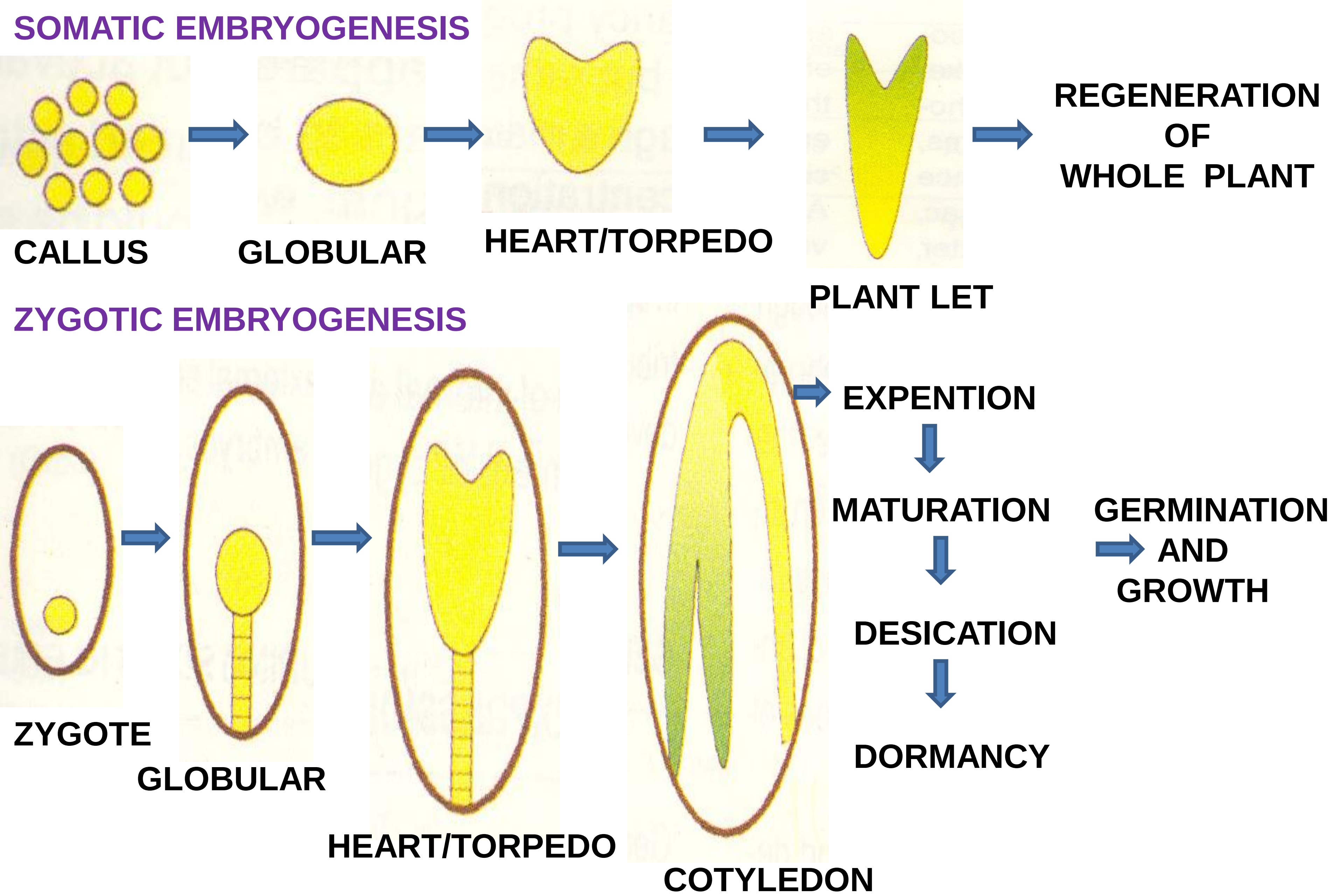
Explants → Somatic embryo → Plantlets

Indirect somatic embryogenesis

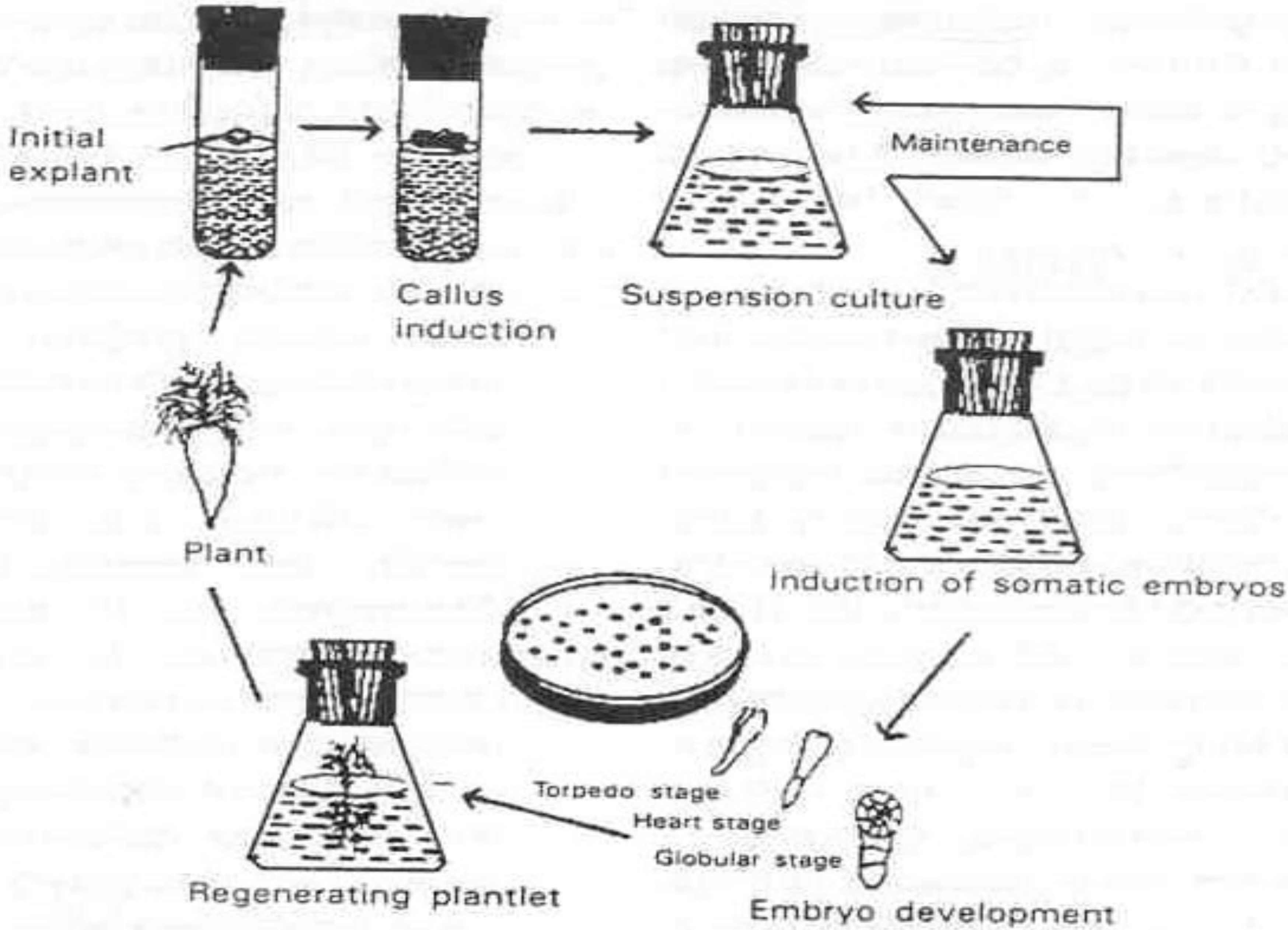
Explants → Callus → Somatic embryo → plantlets

Recurrent SE/ Secondary SE

Explants → Callus → Primary SE → Secondary SE → Plantlets



COMPARISION OF SOMATIC AND ZYGOTIC EMBRYOGENESIS



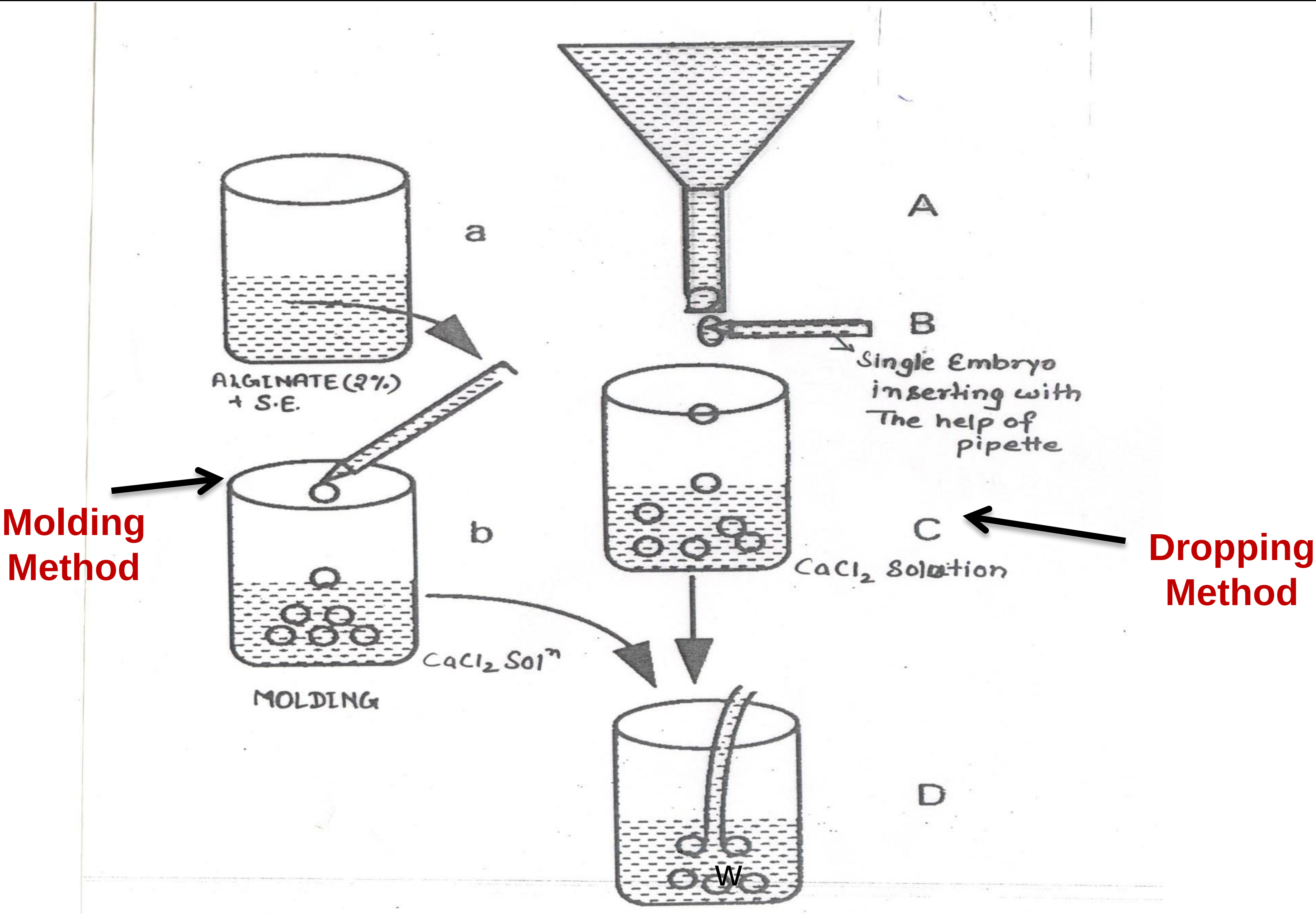
Protocol for somatic embryogenesis in carrot

ENCAPSULATION / COATING METHODS

Molding method

Dropping method

ENCAPSULATION METHOD



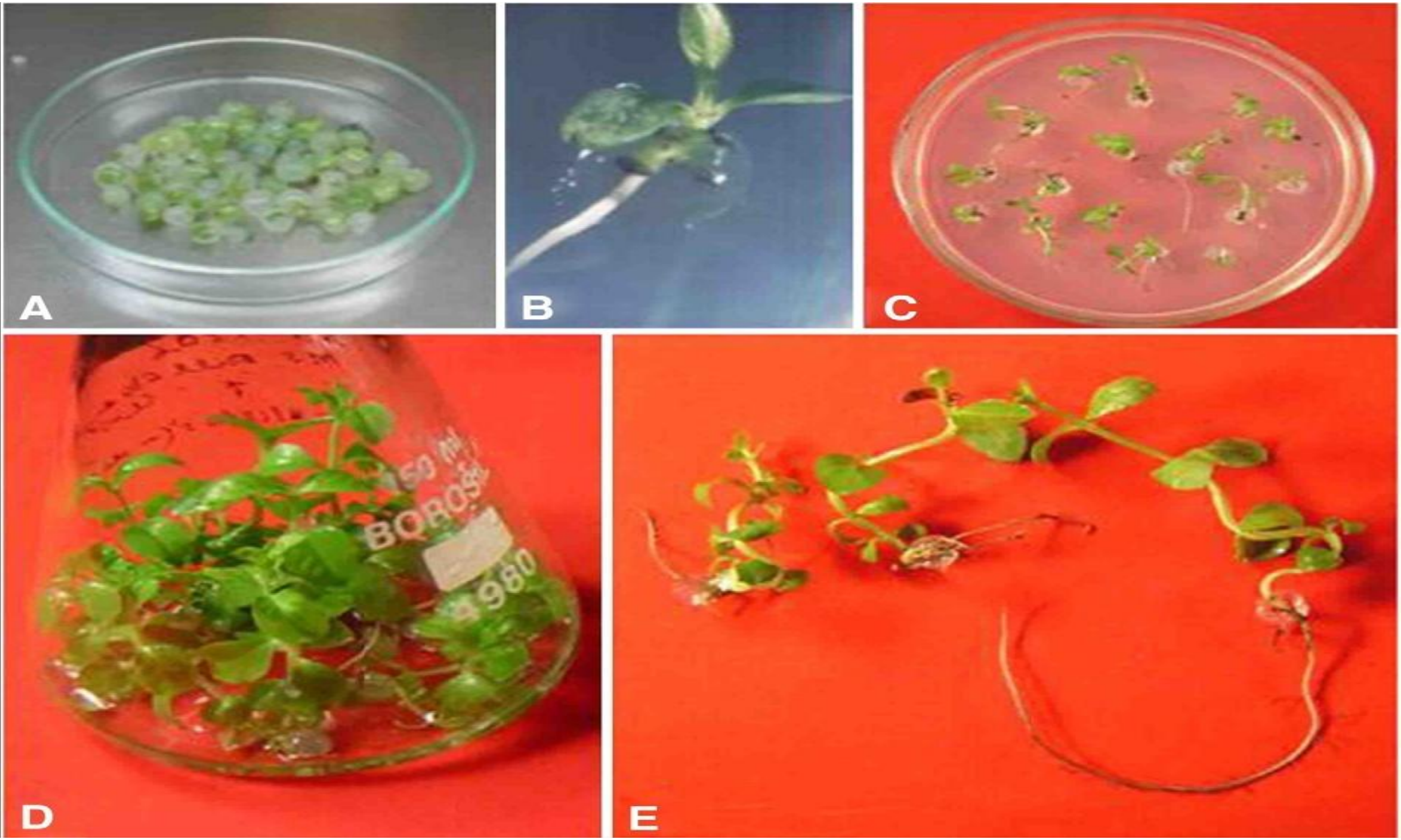
Commonly Used Hydrogel For Production of Synthetic Seeds

Gel	Source	Capsule formed
Agar	Sea weed extract	Yes
Alginate	Sea weed extract	yes
Agarose	Sea weed extract	No
Guar gum	Seed gum	Yes
Locust bean gum (Carrageenan)	Seed gum	Yes
Gum Arabic	Plant exudates	No
Dextran	Microbial	No

Redenbaugh *et al.*, 1987

Alternative to Somatic Embryos

- Axillary shoot buds
- Apical shoot tips
- Embryogenic masses
- Protocorms or protocorm-like bodies



Plant regeneration from encapsulated shoot tips of guava. (A) Shoot tips encapsulated in Ca-alginate beads. (B) Shoot and root emergence from alginate-encapsulated shoot tip. (C) Plantlet regeneration on agar-solidified MS medium. (D) Plantlet regeneration in full-strength liquid MS medium. (E)Well-developed plantlets regenerated from encapsulated shoot tips.

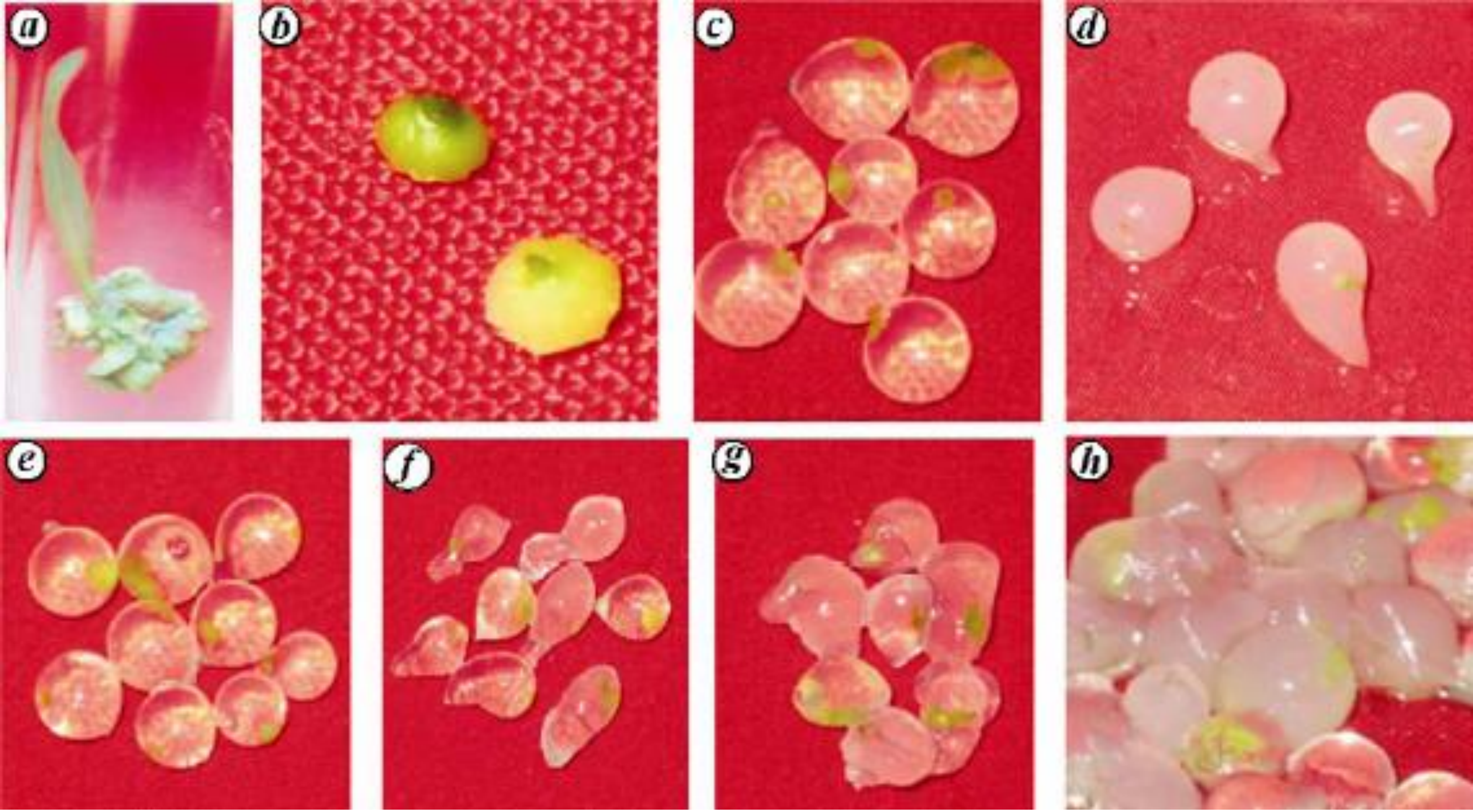
Rai *et al., 2009*

Effect of Different Concentrations of Sodium Alginate and $CaCl_2 \cdot 2H_2O$ on Formation of Encapsulated Beads in *Vanda coerulea*- An Endangered Orchid.

Concentration of sodium alginate (%)	Concentration of $CaCl_2 \cdot 2H_2O$ (mM)	Nature of bead formation	Remarks
1	50	+	Fail to coat protocorm-like bodies
1	75	++	Too soft and very fragile
1	100	++	Poor bead formation
2	50	+	Malformed beads, very fragile and soft to handle
2	75	++	Solid texture and formed clusters
2	100	++	Rigid, solid texture and form cluster
3	50	+++	Uniform size, solid and isodiametric
3	75	+++	Uniform size, solid and sort tail at the surface
3	100	+++++	Clear, firm, round and uniform size
4	50	++++	Uniform size and isodiametric
4	75	++++	Uniform size, isodiametric and quite rigid
4	100	+++++	Rigid, firm, clear and isodiametric

+, Poor quality; ++, Poor quality; +++, Slight better; ++++, Good but solid; +++++, Best quality.

Sarmah *et al*.2010



a, PLB formation in cultures of leaf base of Vanda coerulea. b, Isolated PLB from leaf base (enlarged). Encapsulated seeds in ***c, 4% and 100 mM; d, 4% and 75/50 mM; e, 3% and 100 mM; f, 3% and 75/50 mM; g, 2% and 100 mM; h, 2% and*** 75/50 mM sodium alginate and CaCl2·2H2O solution respectively.

Sarmah *et al*., 2010

Effect of different concentrations of sodium alginate and (%) and $CaCl_2 \cdot 2H_2O$ (mM) on germination percentage of encapsulated PLBs on Ichihashi and Yamashita basal medium without storage in *Vanda coerulea*.

Sodium alginate (%)	CaCL2 2H2O (mM)	Days for germination	Percentage of germination
3	75	8	56.5
3	100	7	94.9
4	75	9	36.5
4	100	14	23.5

Sarmah *et al*., 2010

Synthetic Seeds in Agro / Horticultural Crops

Crop	(Botanical name)	Reference
Rice	(*Oryza sativa)*	Fujii *et al*., (1995)
Soybean	*(Glycine max)*	Grey and Purohit (1991)
Sugarcane	(*Saccharum officinarum)*	Paulet *et al*., (1993)
Citrus	*(Citrus aurantifolia)*	Antonietla (1998)
Mango	(*Mangifera indica*)	Litzres *et al*., (1993)
Cofee	(Coffee arabica)	Dereuddre *et al*., (1994)
Ginger	(*Zingiber officinale*)	Sharma and Singh (1994)
Potato	*(Solanum tuberosum*)	Fiegert *et al*., (1998)

Plant Species	Plant Material	Conservation method	Rference
Morus indica (Mulberry)	ABs	Low temp. storage	Bapat *et al* .,1987
Malus domestica	ST	Encapsulation dehydration	Nino 1992
Pyrus communis (pear)	NS	Encapsulation dehydration	Nino and sakai 1992
Actinidia deliciosa (Kiwifruit)	ST	Encapsulation dehydration	Suzuky *et al*., 2001
Ananas comosus (pine apple)	SBs	Low temp. storage	Soneji *et al*., 2002
Mangifera indica (Mango)	SEs	Encapsulation dehydration	Wu *et al*., 2003
Vitis vinifera (grape)	ST	Encapsulation dehydration	Wang *et al* ., 2004
Fragaria ananassa (strawberry)	ST	Low temp. storage	Lsek and orlicovasca 2004
Punica granatum (pomegranate)	NS	Low temp. storage	Naik and chand 2006
Citrus species	SEs	Low temp. storage	Antonita *et al* ., 2007
Psidium guajava (guava)	SEs	Low temp. storage	Rai *et al*., 2008
ABs = Axilary buds, ST = Shoot tips, NS = Nodal segment, Ses = somatic embryos, SBs = shoot buds			

Rai *et al*., 2009

Advantages of Synthetic Seed

- Easy handling
- Inexpensive transport
- Storage life
- Production uniformity
- To avoid extinction of endangered species
- Large scale propagation
- Germplasm conservation
- Hybrid production

Scope of Synthetic Seeds.

Synthetic Seed

Propagation

- Rare and endangered plants
- Elite genotype
- Genetically engineered plants
- Seedless plants
- Commercially important plants

Conservation

Short to Medium Term Storage

Slow growth conservation

- Maintenance under reduced temperature and/or reduced light intensity
- Use of growth retardants such as ABA
- Use of minimal growth medium
- Reduction in oxygen concentration

Long-Term Storage

Cryopreservation

- Encapsulation-dehydration
- Encapsulation-vitrification

(synthetic seed based cryopreservation)

Transport

- Exchange of axenic plant material free of undesirable contaminants globaly

LIMITATION OF SYNTHETIC SEED

- Limited production of viable micropropagules
- Asynchronous development of somatic embryos
- Improper maturation of the somatic embryos
- Lack of dormancy and stress tolerance in somatic embryos
- Tissue culture dependent
- Poor conversion of even apparently normally matured somatic embryos and other micropropagules into plantlets

Future Thrust

- **Optimization of *in vitro* culture systems**
- **Incorporation of beneficial microorganisms like *Trichoderma* against seed borne diseases.**
- **Encapsulation methods**
- ***Ex- vitro* and soil conversion**
- **Benefit : Cost analysis and marketing**
- **Standardized sowing techniques without significant modification of existing planting equipments**


Thank You