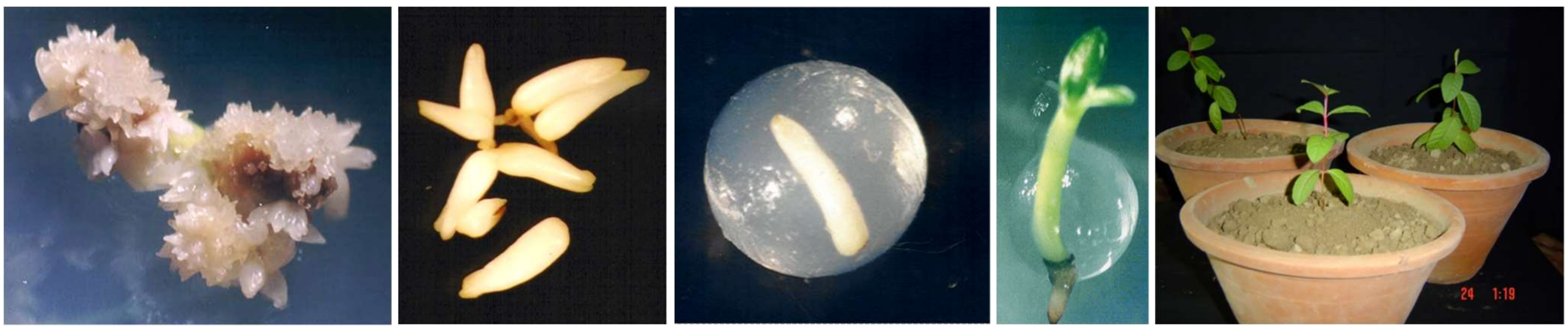
Somatic Embryogenesis: Concept, Process and Applications



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SOMATIC EMBRYOGENESIS

Somatic embryogenesis is the process by which somatic cells differentiate into somatic embryos.

Somatic embryogenesis has the following major advantage as compared to zygotic embryogenesis.

(1) The process of embryogenesis can be easily monitored
(2) The environment and the development phase of somatic embryo can be controlled, and
(3) Large number of embryos can be easily obtained

History

1958—F.C. Steward (USA) and Reinert (Germany), independently, reported the formation of embryos by the somatic cells of carrot (somatic embryogenesis). Since then somatic embryogenesis has been reported in more than 500 species of dicots and monocots

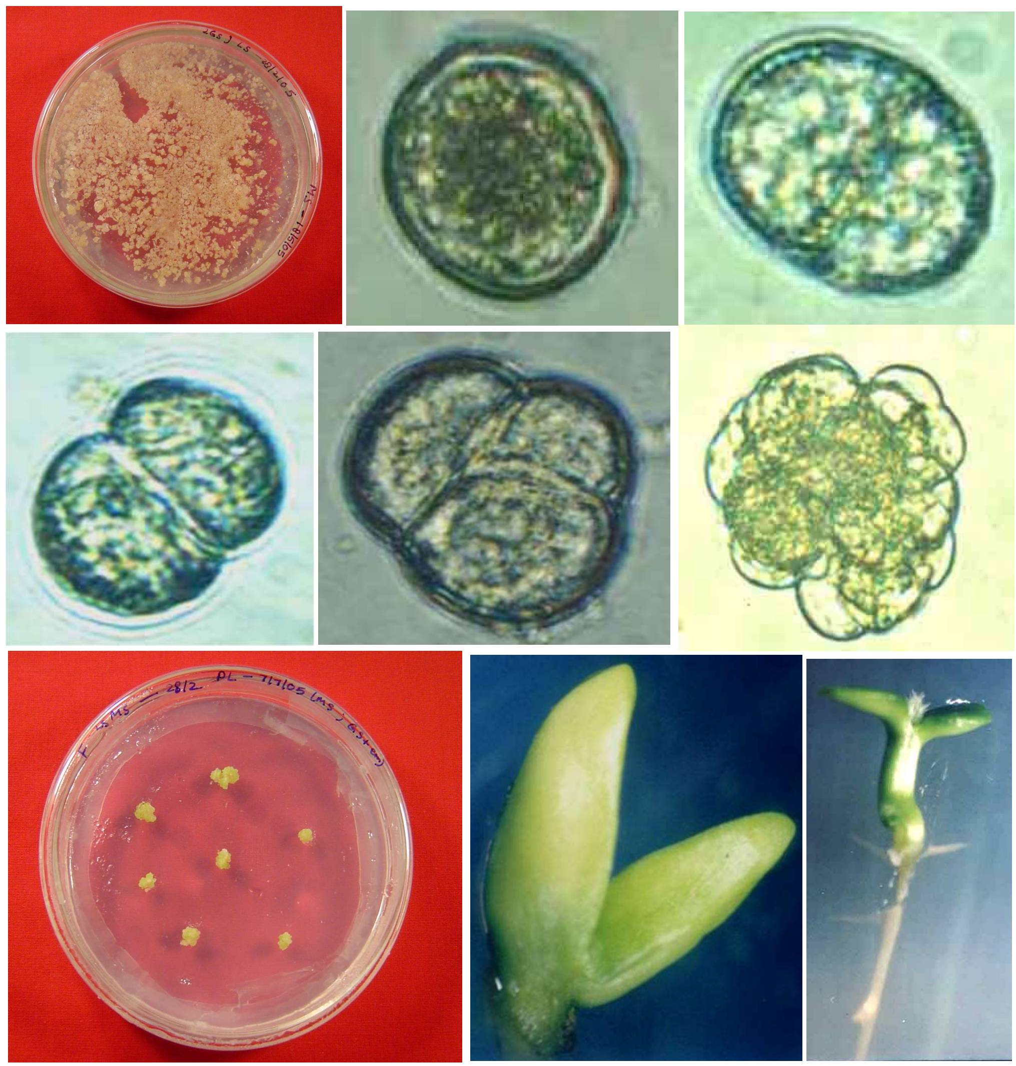
Single Cell Origin of Somatic Embryos

1965—Vasil and Hildebrand achieved regeneration of full plants starting from isolated single cells of tobacco.

1966—Kohlenbach succeeded in inducing divisions in isolated mature mesophyll cells of Macleaya cordata which later differentiated somatic embryos.

Backs-Hüsemann and Reinert (1970) achieved embryo formation from an isolated single cell of carrot.

Single cell to plant



Zygotic embryos vs Somatic embryos

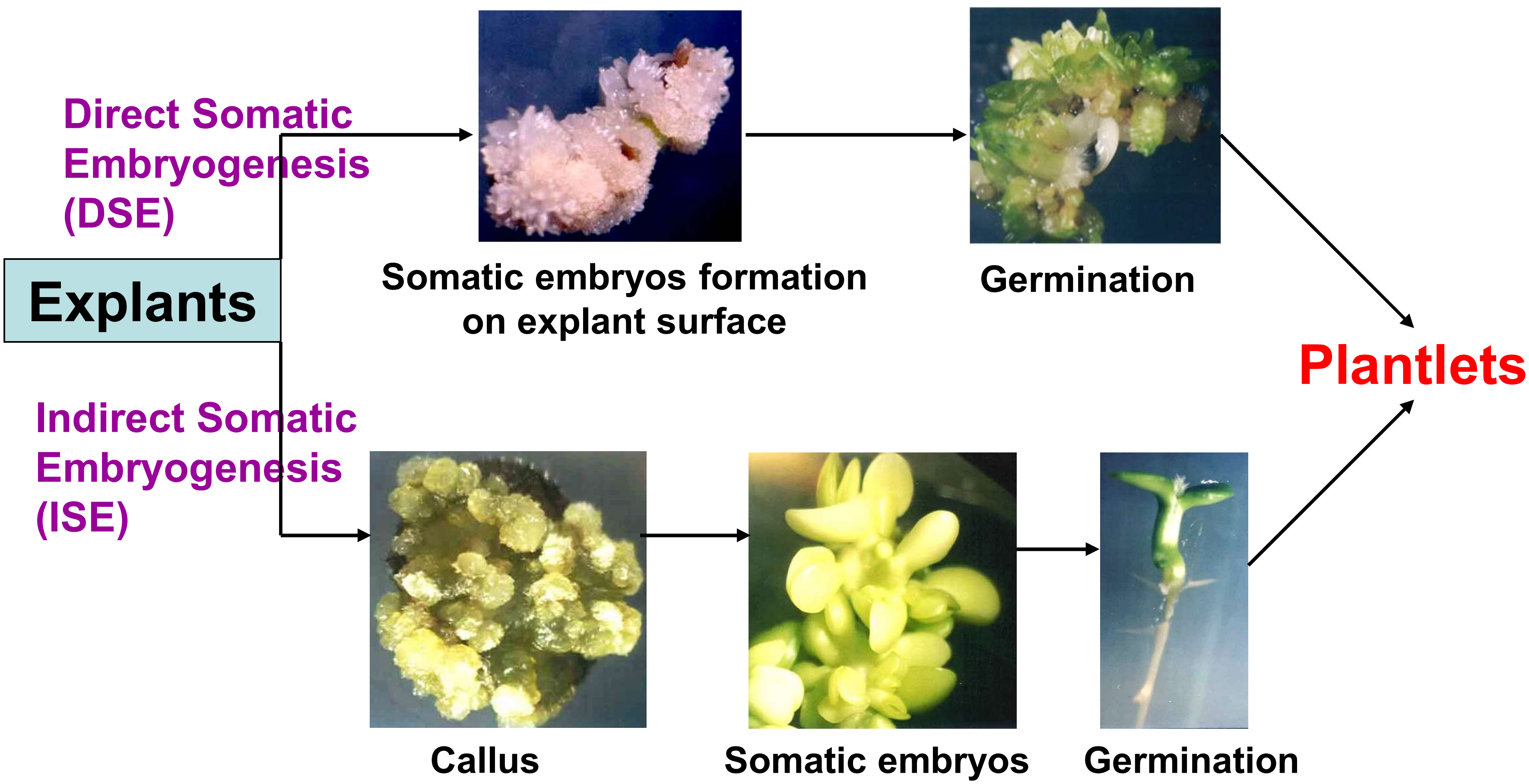
1. Somatic embryogenesis is an inductive process and embryogenesis can take place without the involvement of fertilization or gametic fusion.
2. Early divisions do not follow sequence in somatic embryos
3. Suspensor is absent in somatic embryos
4. Somatic embryos do not become dormant
5. Lack of endosperm differentiation in somatic embryos
6. No vascular connection in somatic embryo
7. Unlike the zygotic embryos, SEs, often show secondary embryogenesis and their development is asynchronous.
8. Zygotic embryos are usually diploid (sometimes may be polyploidy) but somatic embryos may be haploid, diploid or triploid

Zygotic embryos and SEs share similar gross ontogenesis, with both typically passing through globular, heart, torpedo and cotyledonary (in Dicot) and globular, scutellar, and coleoptilar stages (in monocot).
The SEs also accumulate seed-specific storage reserves and proteins characteristic of that species, although in lesser amounts.

Somatic Embryogenesis

Somatic embryogenesis can be induced in two different ways:

Shorp et al. (1980) termed PEDCs (Preinduced Embryogenic determined Cells) and IEDCs (Induced Embryogenic Determined Cells)

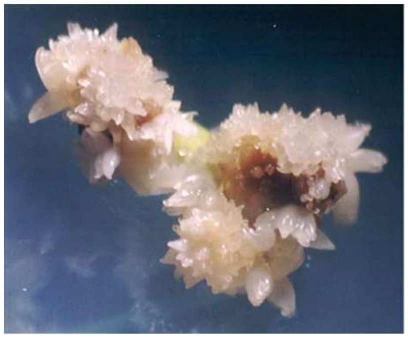


Developmental process of somatic embryogenesis

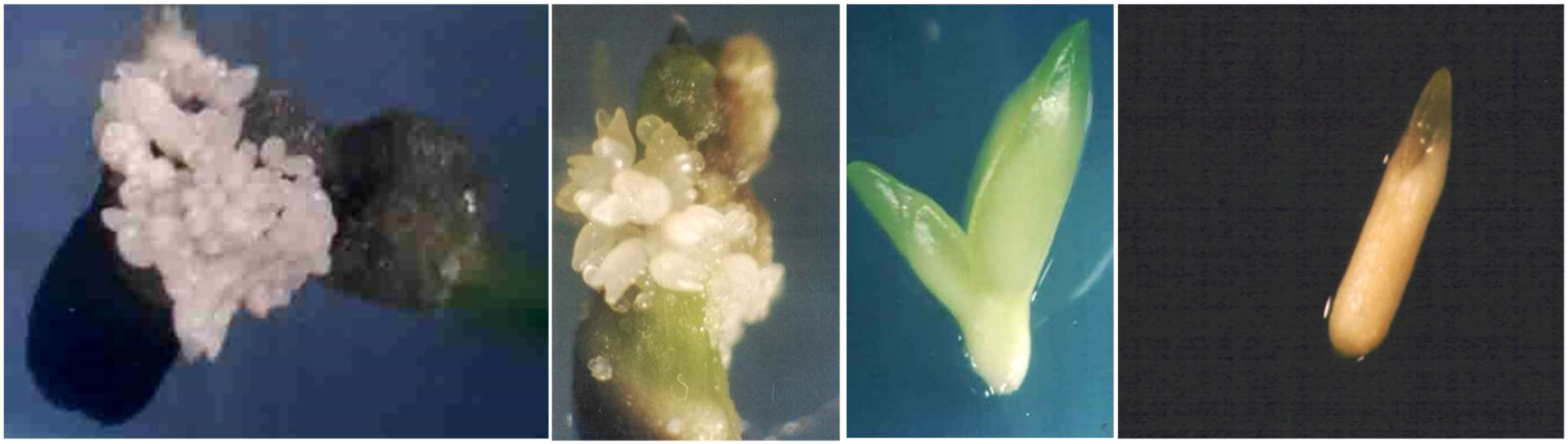
Four phases can be recognized during somatic embryogenesis

Phase ‘0’	**Cells become competent in the presence of auxins**
Phase ‘1’	**Competent cells have the ability to develop somatic embryos when auxin is removed from medium**
Phase ‘2’	**Rapid cell division occurs in cell clusters leading to the formation of globular stage somatic embryos**
Phase ‘3’	**Plantlets develop from globular embryos via heart, cotyledonary and torpedo shaped somatic embryos**

Plant regeneration through somatic embryogenesis

Induction	Development	Maturation	Germination
			

Stages of somatic embryos

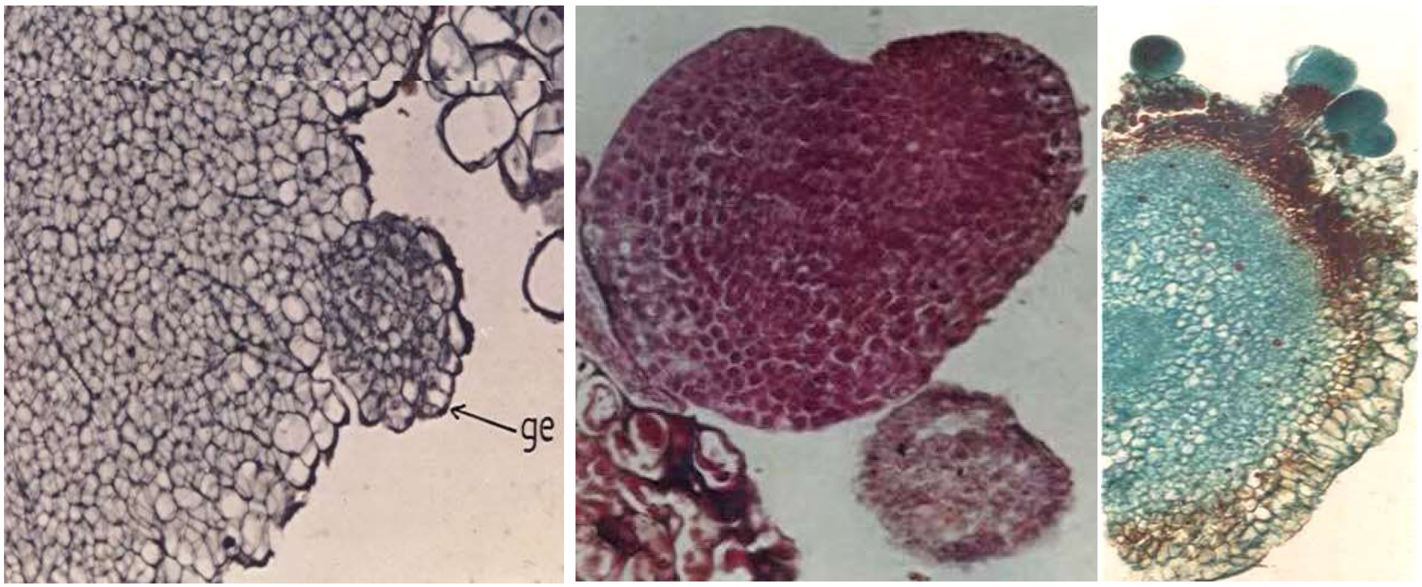


Globular | Heart | Cotyledonary | Torpedo

Lower or immature stage

Mature stage

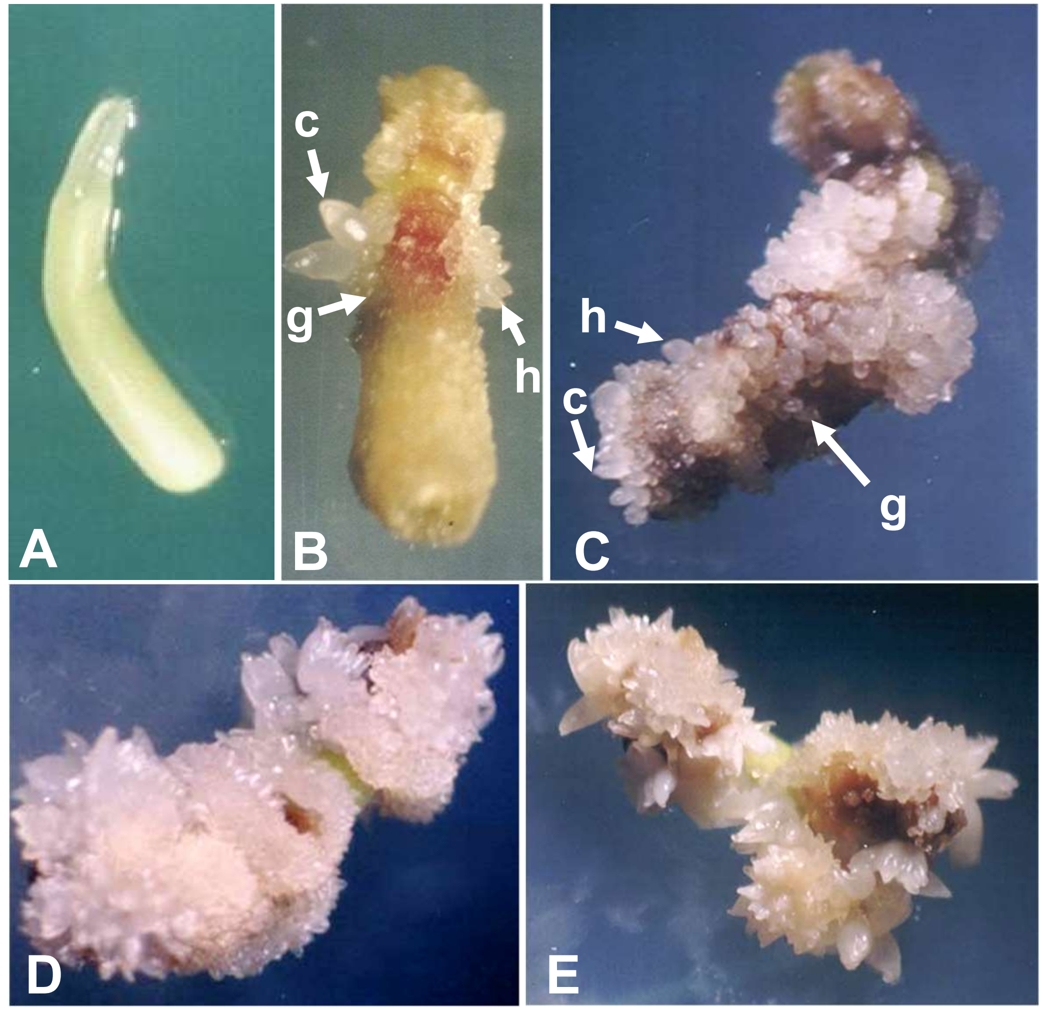
Histology: globular structure formation and somatic embryo differentiation



Induction of somatic embryogenesis

Induction medium:
0.8% agar-solidified full-strength MS medium + 1 mg l^{-1} 2, 4-D + 5% sucrose

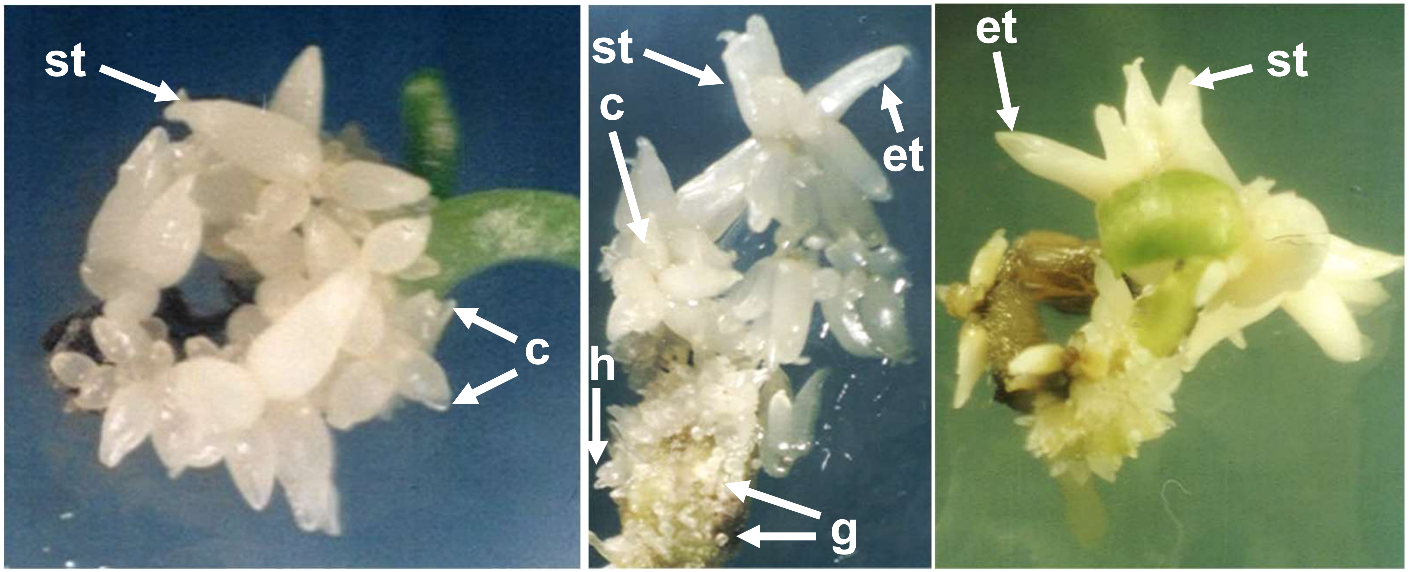
Development medium:
0.8% agar-solidified growth regulator free full-strength MS medium + 5% sucrose



c- cotyledonary stage somatic embryo , g- globular stage somatic embryo, h- heart stage somatic embryo

Rai et al. (2007) Sci. Hort. 113:129-133

Development of somatic embryos



Earlier formed younger stage somatic embryos proceeded towards mature stage of development in 4-7 week old cultures
The development of somatic embryos was asynchronous and somatic embryos at different stages of development could be seen on the same explant at the same time

c- cotyledonary stage somatic embryo , et- elongated torpedo stage somatic embryo, g- globular stage somatic embryo, h- heart stage somatic embryo, st- short torpedo stage somatic embryo

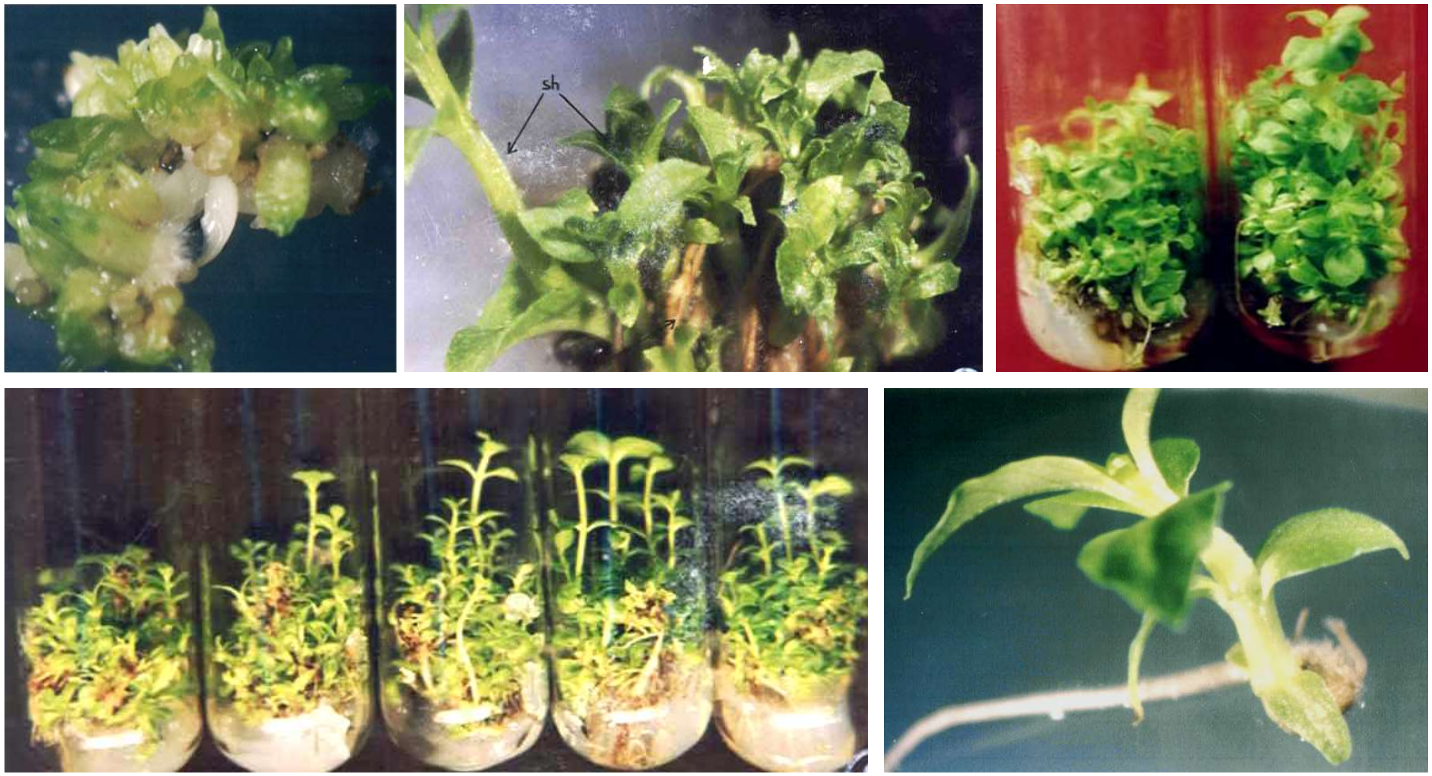
Maturation of somatic embryos

Maturation is a key phase between embryo development and germination. During maturation, several storage proteins synthesized which is necessary for the germination of somatic embryos.

st – short torpedo
et – elongated torpedo

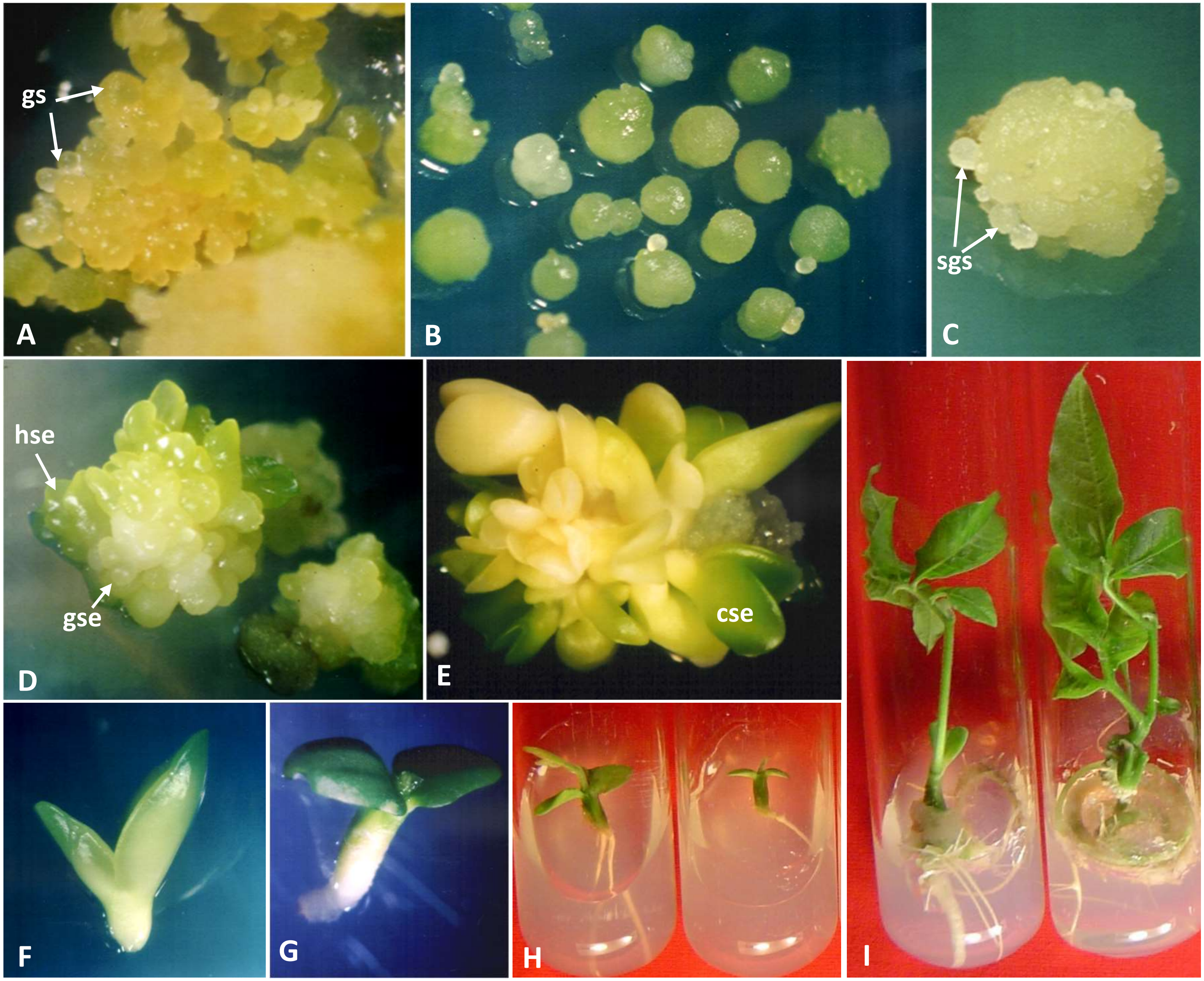
Rai et al. (2009) Sci. Hort. 121: 233-236

Germination of somatic embryos and plantlet development



Germination medium: 0.8% agar-solidified half-strength MS medium + 3% sucrose

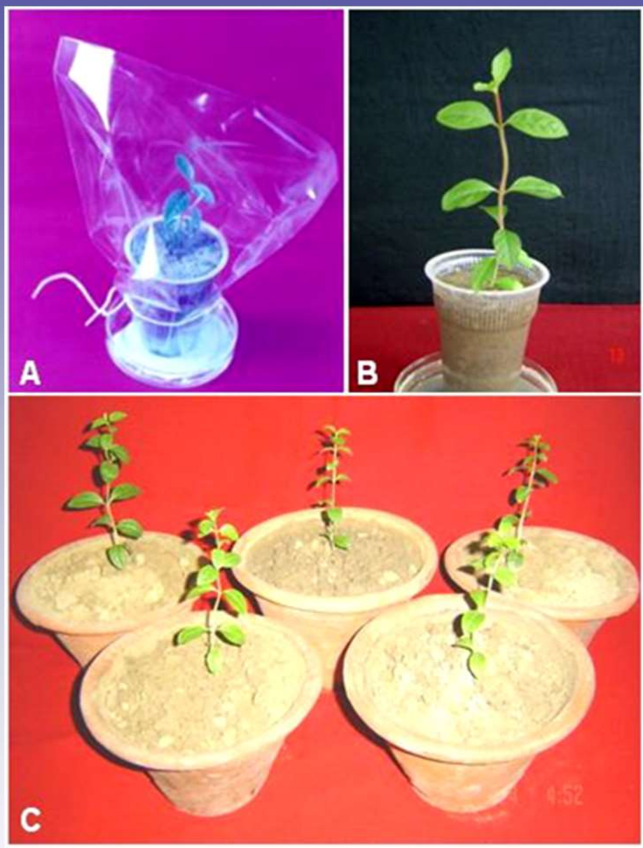
Indirect Somatic embryogenesis


gs
A
B
sgs
C
hse
gse
D
cse
E
F
G
H
I

Factors affecting somatic embryogenesis

1. Explants (PEDCs or IEDCs, Physiological age of explants)
2. Genotypes
3. Culture medium
4. PGRs
5. Sucrose concentrations in medium

Acclimatization of regenerated plantlets and field transfer



After transfer to field

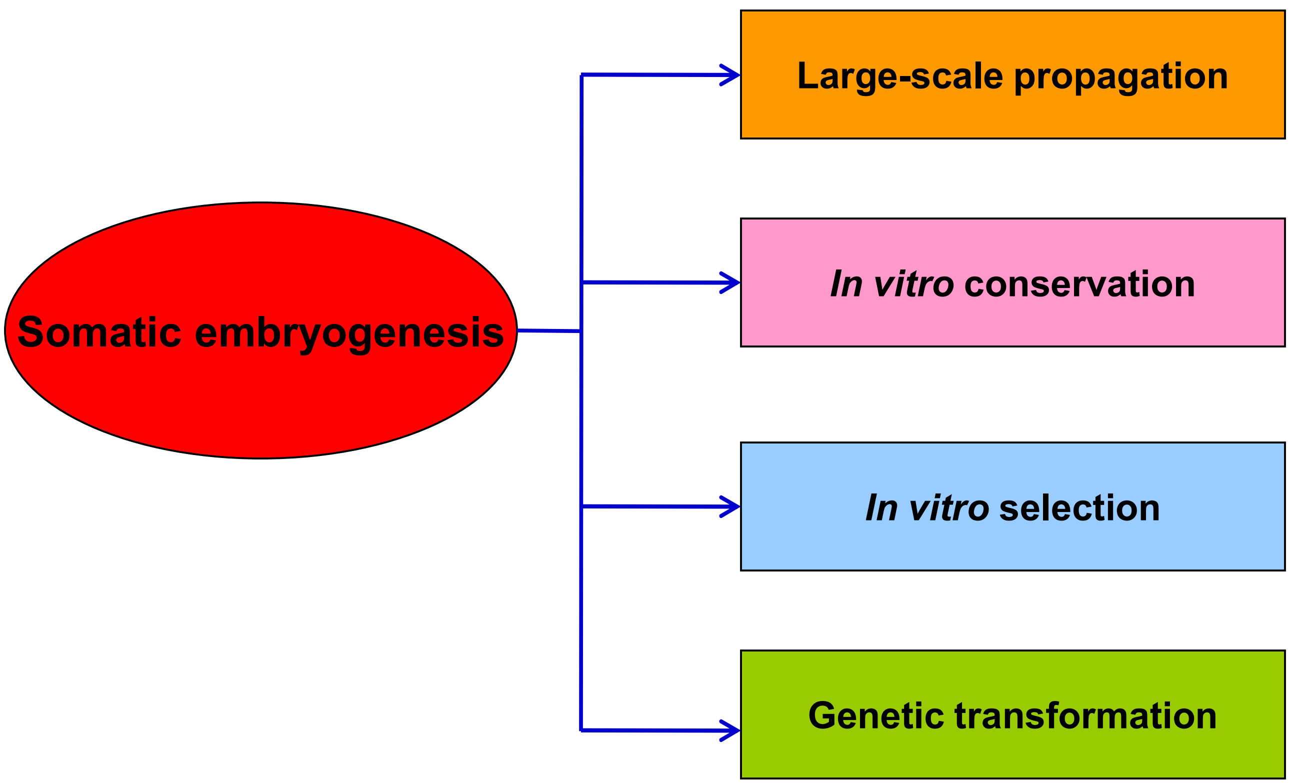
After six month of transfer

After one year of transfer

Under laboratory condition

Under field condition

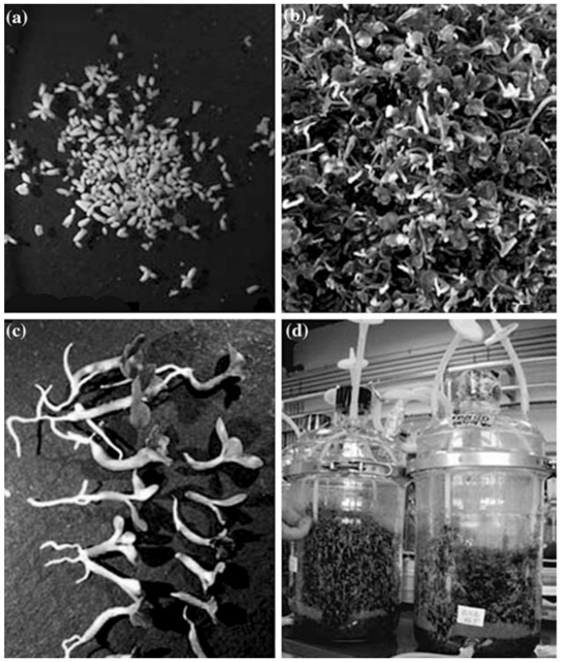
Applications of somatic embryogenesis



Large-scale propagation

Depending on the plant species, virtually unlimited numbers of embryos can be generated from a single explant.

Drew (1980) estimated that one liter of a carrot suspension culture contained 1.35 million somatic embryos. Thus, in comparison to conventional propagation methods (e.g., rooted cutting, grafting) and other *in vitro* regeneration method (e.g., organogenesis), somatic embryos offer better potential for large-scale propagation systems. Even greater economies of scale may be possible if bioreactor and continuous culture technologies can be applied to embryogenic systems.



Encapsulation technology

Production of synthetic seeds by encapsulating somatic embryos, shoot buds or any other meristmatic tissue

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Research review paper

The encapsulation technology in fruit plants—A review

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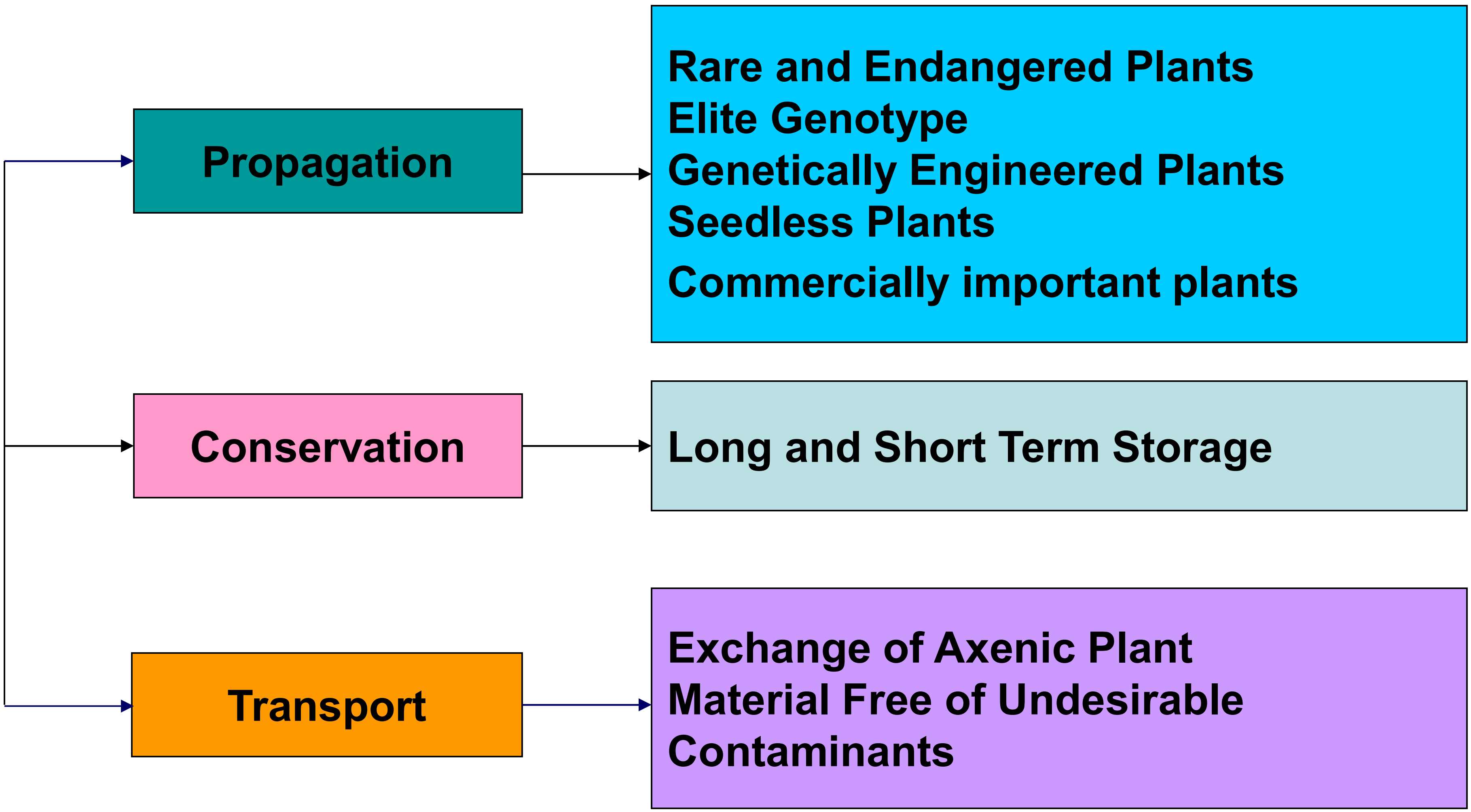
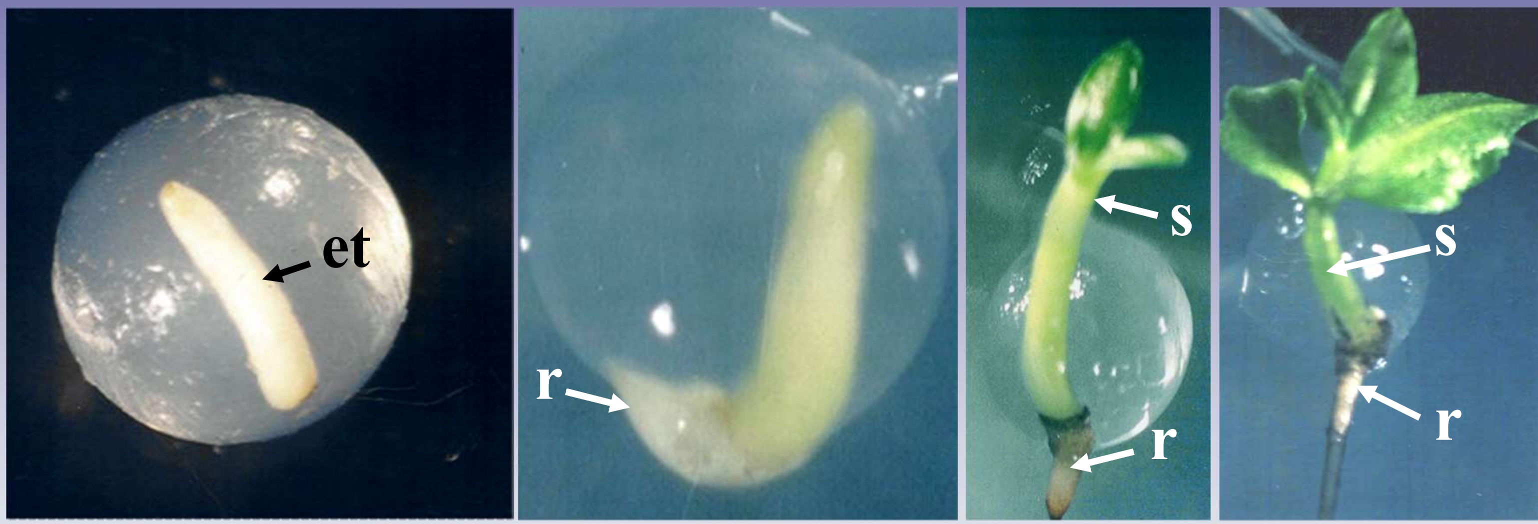
A B S T R A C T

Encapsulation technology is an exciting and rapidly growing area of biotechnological research. This has drawn tremendous attention in recent years because of its wide use in conservation and delivery of tissue cultured plants of commercial and economic importance. Production of synthetic seeds by encapsulating

Scope of Synthetic Seeds



Plantlet conversion from encapsulated somatic embryos



et – elongated torpedo stage somatic embryo
s – shoot
r – root

Rai et al. (2008) Sci. Hort. 117:302-305

In vitro selection

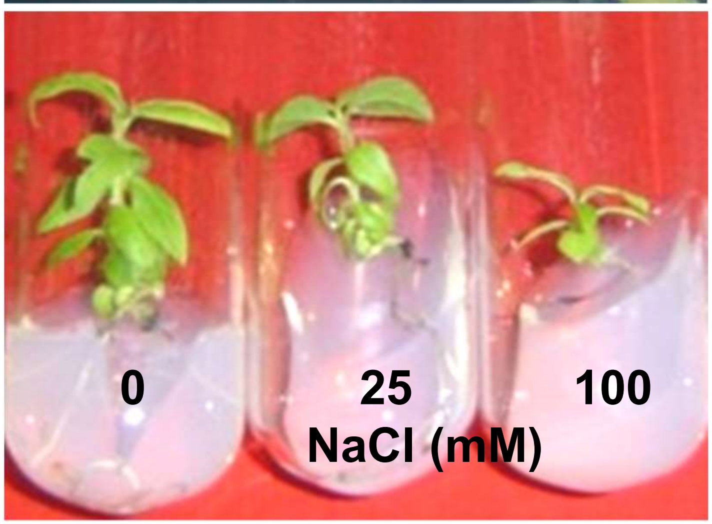
In vitro **culture of plant cells, tissues or organs on a medium containing selective agents offers the opportunity to select and regenerate plants with desirable characteristics. The technique has also been effectively utilized to induce tolerance which includes the use of some selective agents that permit the preferential survival and growth of desired phenotypes**

The presence of a selective agent in the embryogenesis induction or callus initiation medium could increase the probability of recovering stress tolerant plants through somatic embryogenesis.

NaCl	**Salt tolerant**
PEG or mannitol	**Drought tolerant**
Phytotoxin i.e. fusaric acid or culture filtrate or Pathogen it self	**Disease resistant**

In vitro screening of salt-tolerant plant of guava

Salt-tolerant somatic embryos



Germination of somatic embryos and plantlet growth on different concentrations of NaCl

Rai et al. (2010) Acta Physiol Plant

Genetic transformation

Due to its potentially high multiplication rates and potential for scale-up via bioreactor, somatic embryogenesis has been emphasized as a suitable target for gene transfer

Conclusion

Somatic embryogenesis has offered the opportunity to develop an efficient propagation system in a large no. of angiospermic and gymnospermic plants.

Micropropagation through somatic embryogenesis has many advantages over conventional vegetative propagation and its commercial use in horticulture, agriculture and forestry is currently expanding worldwide.

Somatic embryogenesis could also applied in a variety of applications like *in vitro* conservation, genetic transformation, development of stress-tolerant plants etc.

THANK YOU !