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Review Article

Synthetic Seeds: A Boon for Conservation and Exchange of Germplasm

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Abstract

Production of synthetic seeds is effective for conservation and propagation of rare, endangered, critically endangered and threatened plants which are difficult to regenerate through conventional methods and due to low seed set and poor seed germination. There are some factors which discourage commercial cultivation of plants like excessive human exploitation, low germination percentage, less viability of the seeds and long gestation periods. Unsystematic collection of plants for their medicinal, ornamental, perfumery etc. and habitat destruction are potential causes of threats to natural population. The synthetic seed technology provides alternative methods for production of seeds by using various plant parts viz. shoot apices, nodal explants, somatic embryos etc. The quick development in somatic embryogenesis methods permits the use of somatic embryos in plant micropropagation as synthetic seeds. The present review discusses the need for conservation and alternative methods for conservation and exchange of germplasm through the development of synthetic seeds.

Keywords: somatic embryogenesis, synthetic seed, encapsulation.

Introduction

Plants have been serving the mankind since the dawn of the civilization. Besides serving the basic needs, the medicinal plants posses enough therapeutic potential, being preferred by local people from ancient time for primary heatlh care. Plants propagate both through asexual i.e. vegetative means and sexually through seed production. Clonal propagation through the vegetative means concerns to the process of asexual methods of shoot multiplication where identical copies of individual plants are obtained. The *in vivo* clonal

propagation has advantages like multiplication of genetically uniform plantlets and preservation of plant characters. Plant tissue culture broadly concerns to the *in vitro* cultivation of plants, seeds, plant organs, embryos, tissues, single cells, protoplast under aseptic conditions in a culture medium. *In vitro* propagation or micropropagation offers production of [1] disease-free plants, identical and uniform plantlets, also producing genetically modified cells through prtoplast fusion and genetic transformation.

The concerns

In the present time, medicinal herbs are used in current therapeutics with great success in primary health care. Some ornamental and medicinal plants do not produce viable seeds and there is very low seed set. In Orchids, the very small seed size and the association with mycorrhizal fungi is urgently required for viable seed germination. Traditional *in vivo* propagation methods are more time consuming and very expensive [2]. For the above said reasons, the development of *in vitro* propagation methods, such as micropropagation and somatic embryogenesis studies represent a milestone for the production ofuniform plantlets, providing a powerful tool for continuous mass propagation in less time period and season independent production of identical plantlets. Futher, this method offers gene bank maintenance of elite material for conservation and exchange of germplasm to far off places or to academics and research institutions.

Somatic embryogenesis

Somatic embryogenesis (SE) is the process of induction of embryos from somatic cells, tissues, or organs. Somatic embryos are bipolar structures with both apical and basal meristematic regions, which are capable of forming shoot and root, respectively. They can be excised from the parent tissues and induced to germinate in tissue culture media under aseptic conditions provided all necessary physical conditions [3].

In vitro somatic embryo productions were carried out independently and this concept was firstly described by steward et al. 1958 [4]. SE helps in *in vitro* propagation and this has been reported in many medicinal plants [5, 6]. Raghavan (1986); Halperin (1995); Krikorian and Simola (1999) reviewed the early history of somatic embryogenesis [7, 8, 9]. SE can differentiate either directly from the excised plant without forming callus which is called as direct SE. Sometimes explants undergo callus formation and then somatic embryo induction; define as an indirect SE [10]. Direct and indirect SE is possible due to the presence of pre-embryonic determined cells (PEDC) and induced embryogenic determined cells (IEDC) respectively. Fig (1) gives schematic represention of micropropagation including embryogenesis. (A) Direct embryogenesis. (B) Indirect embryogenesis and synthetic seed production.

SE is a desirable method of plant regeneration [10]. First observation of *in vitro* somatic embryogenesis was made in *Daucus carota* [4, 11], after that Molle et al. (1993); reported various aspects of *in vitro* somatic embryogenesis in this plant [12]. Since then in many other plants somatic embryogenesis has been reported [10, 13]. SE studies further lead to the development of synthetic seed technology (fig 1) as an alternative method of conservation in several commercially important agronomic and horticultural crops [14].

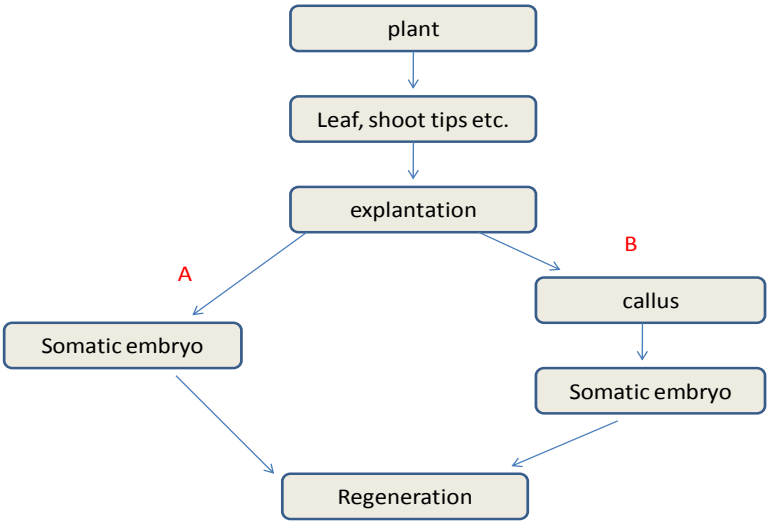

Figure1: Micropropagation of plants through embryogenesis. (A) Direct embryogenesis. (B) Indirect embryogenesis

Synthetic seeds

Synthetic seed technology is the most significant applications of plant tissue culture, have great potential for large scale production of different types of plants at low cost as an alternative to true seeds [15]. An artificial seed is often described as artificially encapsulated somatic embryos, shoot tips, shoot buds, cell aggregate or any other tissue that possess the ability to convert into a plant under *in vitro* or *ex vitro* condition, that can be used for sowing as a seed, that retain this potential also after storage [16], which is at most equivalent to an immature zygotic embryos, possibly at post- heart stage or early cotyledonary stage [17]. Development of synthetic seeds by the encapsulation of various explants (somatic embryos, axillary buds, nodal segment, protocorms and bulblets) from ornamental and medicinal species etc. have been tried in a number of plant species [18].There are several advantages of artificial seeds such as ease of handling, low production cost, ease of exchange of plant materials, genetic uniformity of plantlets, direct delivery to the soil, shorten the breeding cycle and reduction of the storage space [19]. In addition large scale propagation method, rapid multiplication of plants [17], used in advanced procedures of cryopreservation for the long term preservation of plant germplasm are other benefits. Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend in part on plants like fruit, root, leaves, bark, whole plant, tubers, dry tubers, rhizome, rasine, gum, stem for the production of pharmaceutical compounds [20] for example Gentianine and Xanthones which act as Anticarcinogenic, Antihepatotoxic; centellin, Podophyllotoxin.which act as Antimicrobial etc.[21, 22], there are various example of pharmaceutical compounds which are produces by medicinal plants. Due to the indiscriminate collection of huge amount of this plant by local herbalists, Ayurvedic and Unany

companies, this plant species is on the verge of extinction. To meet up the commercial need and also for protecting the genetic erosion, it is important to develop techniques for rapid mass propagation of these species. *In vitro* propagation through somatic embryogenesis has been reported in many medicinal plants for rapid mass propagation, to meet up the commercial need and also for protecting the genetic erosion [5, 6]. Preparation and germination of synthetic seeds from explants of medicinal plants is given in Table 1.

Table 1: Preparation and germination of synthetic seeds on storage at varied conditions and time period

Species	Explants	Encapsulation	Storage time	Storage temperature	Regeneration (%)	Reference
Cineraria maritime	microshoots	3%(w/v)sodium alginate,3%(w/v) $CaCl_2.2H_2O$	6 months	25±2°C	82.35%	[23]
Picrorhizakurrooa	Microshoots	3%(w/v)sodium alginate,3%(w/v) $CaCl_2.2H_2O$	3 months	25±2°C	89.33%	[24]
Khayasenegalensis	Somatic embryos	3%(w/v)sodium alginate, 100mM $CaCl_2.2H_2O$	8 weeks	25±2°C 4°C	73-88%	[25]
Clitoriaternatea L.	Somatic embryos	3%(w/v)sodium alginate, 100mM $CaCl_2.2H_2O$	5 months	25±2°C 4°C	92%	[26]
Rhinacanthusnasutus	Somatic embryos	4%(w/v)sodium alginate, 100mM $CaCl_2.2H_2O$	45 days	25±2°C	94%	[27]

Encapsulation methods

A technique that enhances transportation of *in vitro* derived plants to the field or to the greenhouse is an encapsulation [28]. Different types of explants used in encapsulation for synthetic seeds production are somatic embryos, nodal segments, zygotic embryos, axillary buds; shoot buds, protocorm like bodies. Among all these explants, somatic embryos were mostly used in an encapsulation for producing synthetic seed [29, 30]. Initially in 1982 Kitto et al. used somatic embryos for encapsulation in the case of *Daucus carota* [31]. In 1984 Redenbaugh et al. worked on alfalfa and used somatic embryos for encapsulation [32]. Different studies report production of synthetic seeds from shoot tips, somatic embryos, axillary buds etc.[28, 31-54] Table 2 describes the list of plant species in which encapsulation technology has been applied to produce synthetic seeds.

Table 2: List of plant species in which encapsulation technology has been applied to produce synthetic seeds

No.	Plant	Propagule used for encapsulation	Reference
1	*Actinidia deliciasa*	Shoot buds	[28]
2	*Arachis hypogaea*	Somatic embryos	[33]
3	*Asparagus cooperi*	Somatic embryos	[34]
4	*Betula pendula*	Shoot buds	[28]
5	*Brassica campestris*	Somatic buds	[35]
6	*Camellia japonica L.*	Somatic embryos	[36]
7	*Cannabis sativa*	Axillary buds	[37]
8	*Catharanthus roseus*	Somatic embryos	[38]
9	*Crataegus axyacantha*	Somatic buds	[28]

10	*Cymbidium giganteum*	Protocorm-like bodies	[39]
11	*Daucus carota*	Somatic embryos	[31]
12	*Dioscorea bulbifera*	Shoot tips	[40]
13	*Eleusine coracana Gaertn*	Somatic embryos	[41]
14	*Eucalyptus citriodra*	Somatic embryos	[42]
15	*Flickinq erianodosa*	Protocorm like bodies	[43]
16	*Medicago sativa*	Somatic embryos	[44]
17	*Morus indica*	Somatic buds	[45]
18	*Paulownia elongata*	Somatic embryos	[46]
19	*Pseudostellaria heterophylla*	Micro-tubers	[47]
20	*Rubus*	Somatic buds	[28]
21	*Rubusidaeus L.*	Somatic buds	[28]
22	*Siberian ginseng*	Somatic embryos	[48]
23	*Solanum nigram*	Shoot tips	[49]
24	*Stevia rebaudiana*	Shoot tips	[50]
25	*Stevia rebaudiana Bertoni*	Shoot tips, axillary buds	[51]
26	*Swertia chirayita*	Somatic embryos	[52]
26	*Tylophora indica*	Somatic embryos	[53]
27	*Tylophora indica*	Shoot tips	[54]

Several gelling agents like potassium alginate, carrageenan, agar, sodium alginate, sodium pectate, gelrite, guargum, carboxymethyal cellulose, polyco 2133, tragacanth gum etc. have been tested as hydrogels for the hydrated artificial seed production, but sodium alginate gel has been most widely used [3]. In general, during encapsulation, explants are mixed with sodium alginate solution and dropped, using a pipette, into a $CaCl_2.2H_2O$ solution, form round and firm beads due to an ion exchang reaction, sodium ions are replaced by calcium ions forming Calcium-alginate. The hardness or rigidity of the capsule depends open the number of sodium ions exchanged with calcium ions. The size of the beads could be controlled by varying the inside diameter of the pipette [14].

The main advantages of sodium alginate are its moderate viscosity, non-toxicity, low cost, the long term storability, quick gellation properties, and hardening of beads at room temperature. Calcium–alginate capsules are difficult to handle due to the tendency to stick together slightly because they are very wet but other side these capsules lose water fastly and convert into the hard capsules within a few hours when exposed to the atmosphere. These problems can be solved by coating the capsules with Elvax 4260 (ethylene vinyl acetate acrylic acid terpolymer [44]).

Case study:

In *Swertia chirayita* plant**,** large scale production of somatic embryos (fig 2) could be applied for the production of synthetic seeds [55]. Kumar and Chandra, (2014) reported SE in *S. chirayita* [52]. Various concentrations of sodium alginate (2, 3, 4 and 5 %) and $CaCl_2.2H_2O$ (100 mM) have been used for synthetic seed production [52]. However, somatic embryos encapsulated with 4 % of sodium alginate and submerged for 30 min in 100 mM calcium chloride were the best for uniform synthetic beads (Fig.3), with smooth texture and easy handling. Similar results have been found for sodium alginate percentage by other researchers also [56, 57]. Somatic embryogenesis in *S. chirayita* from leaf explants is shown in fig (2) and synthetic seed production, germination and field transfer of synthetic seeds of *S. chirayita* is shown in fig (3).

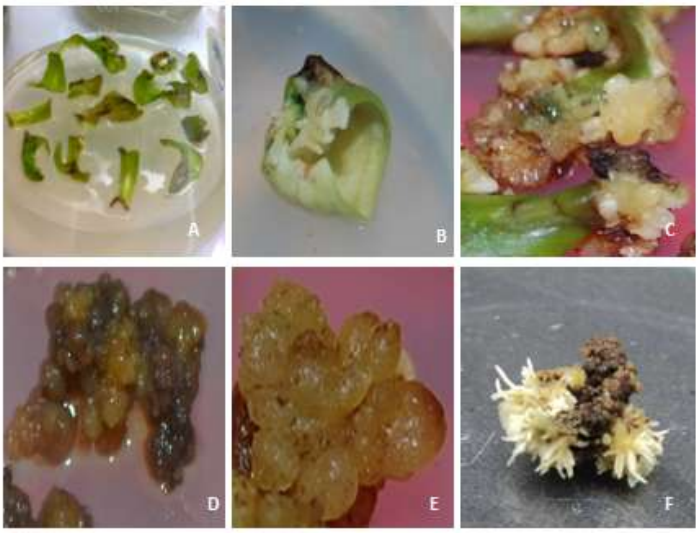

Fig: 2 Somatic embryogenesis in *S. chirayita* from leaf explants

A) Cultured explants; B) white coloured emryogenic calli developed on to the MS medium supplemented with (0.5 mg/L) 2,4-D and (0.5 mg/L) Kn from *in vitro* leaf explants after 4 weeks of culture; C) Yellow coloured emryogenic calli developed on to the MS medium supplemented with plant growth regulators; D) Globular somatic embryo development from embryogenic callus on to the same medium after another 2 weeks; E) heart shaped somatic embryo development from embryogenic callus on to the same medium after another 4 weeks; F) The somatic embryos were further developed into torpedo shaped structures after 3 weeks of culture.

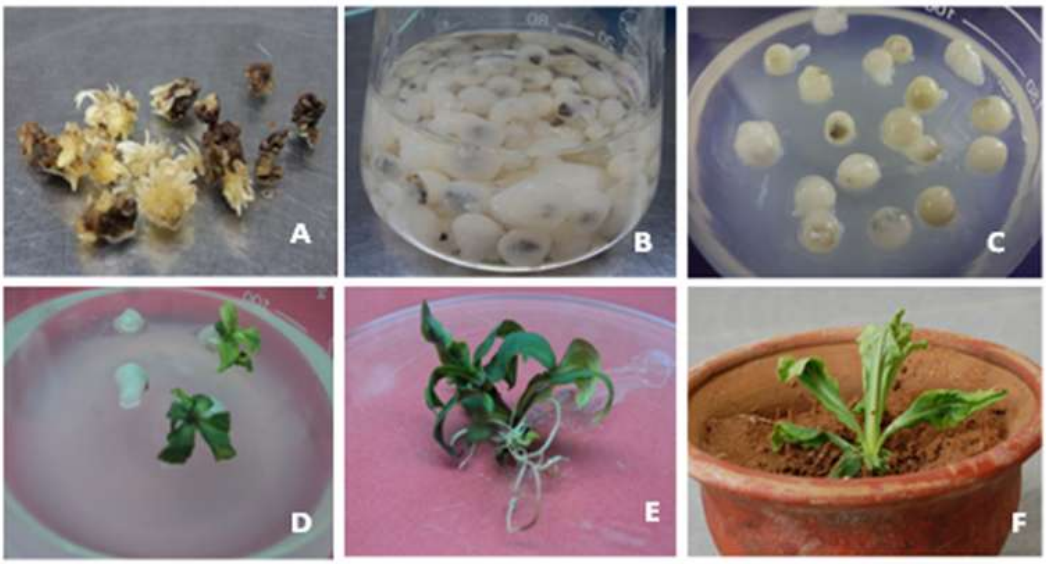

Fig 3: Synthetic seed production, germination and field transfer of synthetic seeds of *S. chirayita*.

A) Torpedo shaped somatic embryos; B) Encapsulated torpedo shaped somatic embryos in $CaCl_2$ (100mM) solution; C) Synthetic seeds on MS medium; D) Germination of a synthetic seed on MS medium after 30 days of culture; E) Complete rooted plant from synthetic seed; F) Acclimatized plant after transfer to soil in *ex vitro* conditions

Conclusion and future prospects

Synthetic seeds have wide range applications in large scale plant multiplication, conservation and production of uniform plantlets. For critically endangered plant species, it is the rapid means of conservation and multiplication. Further studies involve genetic fidelity testing in order to study the variation in germplasm during storage condition for different time period and at varied temperature.

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