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# **Production of artificial seeds of *Tinospora cordifolia*: an antidiabetic plant and their application.**

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## **Abstract**

Artificial seeds are most commonly described as encapsulated somatic embryos. They are product of somatic cells, so can be used for large scale clonal propagation. Apart from somatic embryos, other explants such as shoot tips, axillary buds have also been used in preparation of artificial seeds. Artificial seeds have a variety of applications in plant biotechnology such as large scale clonal propagation, germplasm conservation, breeding of plants in which propagation through normal seeds is not possible, genetic uniformity, easy storage and transportation etc. For some plants such as ornamental plants, propagation through somatic embryogenesis and artificial seeds is the only way out. In the present paper- the types, advantages, production of synthetic seed of *Tinospora cordifolia* and various applications of artificial seeds have been reviewed.

**Keywords:** Artificial seed, somatic embryogenesis, clonal propagation, *Tinospora cordifolia*, germplasm conservation.

## Introduction

Artificial seed technology is one of the most important tool for scientists of plant tissue culture. It has been offered powerful advantages for large scale mass propagation of important plant species. In general, Synthetic seed refers to encapsulated explants such as shoot tips, axillary buds and somatic embryos in cryoprotectant material like hydrogel, alginate gel, ethylene glycol, dimethylsulfoxide (DMSO) and others that can be developed into a plant. The coating protects the explants from mechanical damage during handling and allows germination and conversion to occur without inducing undesirable variations (Harikrishna and Ong 2002). They behave like true seeds and sprout into seedlings under suitable conditions. The somatic embryo can be encapsulated, handled and used like a natural seed was first suggested by Murashige (1977) and efforts to engineer them into synthetic seed have been ongoing ever since Kitto and Janick (1982), Gray (1987). Bapat *et al.* (1987) proposed the encapsulation of shoot tip in *Morus indica*; this application has made the concept of synthetic seed set free from its bonds to somatic embryos and broaden the technology to the encapsulation of various *in vitro* derived propagules. An implementation of artificial seed technology to somatic embryogenesis or the regeneration of embryos is based on the vegetative tissues as an efficient technique that allows for mass propagation in a large scale production of selected genotype (Ara *et al.*, 2000). The aim and scope for switching towards artificial seed technology was for the fact that the cost-effective mass propagation of elite plant genotypes will be promoted. There would also be a channel for new transgenic plants produced through biotechnological techniques to be transferred directly to the greenhouse or field. The artificial seed technology has been applied to a number of plant species belonging to angiosperms. Present review aimed to give a brief description of methodology involved in synseed preparation, types of synthetic seeds, species in which this technique has been developed successfully.

## **The need for artificial seed**

A seed is basically zygotic embryo with enhanced nutritive tissues and covered by several protective layers. Seeds are desiccation tolerant, durable and quiescent due to protective coat. Such properties of seeds are also used for germplasm preservation in seed repositories. Zygotic embryo seeds are the result of sexual reproduction that means the progeny of two parents. This has led to the development of often complex breeding programs from which inbred parental lines are developed. Such inbred lines are used to produce uniform hybrid progeny when crossed. Primary problem associated with such seeds is, on one hand for many crops, such as fruits, nuts, and certain ornamental plants; it is not possible to produce a true-breeding seed from two parents due to genetic barriers to selfing. On the other hand many crops, such as forest trees, the generation time is too long to achieve rationally an inbred breeding program. This is the major disadvantage of zygotic seeds. Therefore, for such crops, propagation is accomplished either vegetatively by cuttings or the use of relatively low quality open pollinated seed is tolerated. After the discovery of somatic embryogenesis in 1950 it was possible to have an alternative of conventional zygotic seeds. Somatic embryo arises from the somatic cells of a single parent. They differ from zygotic embryos since somatic embryos are produced through *in vitro* culture, without nutritive and protective seed coats and do not typically become quiescent. Somatic embryos are structurally equivalent to zygotic embryos, but are true clones, since they arise from the somatic cells of a single parent. The structural complexity of artificial seeds depends on requirements of the specific crop application. Therefore, a functional artificial seed may or may not require a synthetic seed coat, be hydrated or dehydrated, quiescent or non-quiescent, depending on its usage. The field that seeks to use somatic embryos as functional seed is termed “artificial or synthetic seed technology”. Thus, artificial seeds are defined from a practical

standpoint as somatic embryos engineered to be of use in commercial plant production and germplasm preservation.

### **Types of artificial seeds**

There are various types of artificial seeds; first two are essentially uncoated somatic embryos;

a.) Uncoated non quiescent somatic embryos, which could be used to produce those crops that are now laboriously micro propagated by tissue culture.

b.) Uncoated, quiescent somatic embryos would be useful for germplasm storage since they can be hand-stored in existing seed storage repositories.

The other categories are;

c.) Non quiescent somatic embryos in a hydrated encapsulation constitute a type of artificial seed that may be cost effective for certain field crops that pass through a greenhouse transplant stage such as carrot, celery, seedless watermelon, and other vegetables.

d.) Dehydrated, quiescent somatic embryos encapsulated in artificial coatings are the form of artificial seed that most resembles conventional seed in storage and handling qualities. These consist of somatic embryos encased in artificial seed coat material, which then is dehydrated. Under these conditions, the somatic embryos become quiescent and the coating hardens. Theoretically, such artificial seeds are durable under common seed storage and handling conditions. Upon rehydration, the seed coat softens, allowing the somatic embryo to resume growth, enlarging and emerging from the encapsulation<sup>8</sup>. Many studies have been conducted on synthetic seed production in horticultural crops but the efforts in field grown crops are limited. So, there is a greater scope for synthetic seeds in commercial crops and ornamental plants (Birdar, 2008).

## **Advantages of artificial seeds**

There are various advantages of artificial seeds. One of the chief advantages is the possibility of large scale propagation and mixed genotype plantations – very much suitable for large scale monoculture. Another big advantage is the germplasm conservation of elite and endangered or extinct plant species. Other advantages are easy handling during storage; transportation and planting and inexpensive transport reason being their small size; storage life comparable to natural seeds; product uniformity – as somatic embryos used are genetically identical. In addition, other potential benefits can be direct field use, study of seed coat formation, fusion of endosperm in embryo development and seed germination; for production of hybrids in plants with unstable genotypes or show seed sterility. It can be used in combination with embryo rescue technique Pond *et al.*, 2003 and Bekheet., 2006.

## **MATERIAL AND METHODS**

### **Preparation of explants**

To obtain *in vitro* shoot tips, *in vitro* grown plantlets of *Tinospora cordifolia* were used as a source of experimental material. *In vivo* nodal segments were procured from the Botanical garden, University of Rajasthan, Jaipur. They were kept under running water for about 15-20 minutes and then rinsed with liquid detergent (Teepol 1% (v/v) for 2-4 minutes. They were then rinsed with sterile double distilled water at least thrice. Surface sterilized with 0.1% mercuric chloride solution (w/v HgCl<sub>2</sub>) for 2-3 minutes. These surface sterilized explants were then aseptically inoculated on sterile W.P.M. medium. The medium was supplemented with Kinetin 0.5 mg/l and pH of the medium was adjusted to 5.8±0.2 before autoclaving at 121°C for 15 minutes on 1.06Kg/cm<sup>-3</sup> pressure.

## **Encapsulation of shoots tips**

Nodal segments (0.6-0.7mm.) were isolated from mother elite. These were then blot dried on the filter paper for few second and these were enveloped by dropping sodium alginate solution through the pipette. However, calcium chloride solution was prepared in the range of 1.0, 1.5, 2.0, 2.5, 3.0% (w/v) in liquid W.P.M. and pH was adjusted at  $5.8 \pm 0.2$ .

The major principle involved in the alginate encapsulation process is that the sodium alginate droplets containing the shoot tips, when dropped into the  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  solution formed round and firm beads due to ion exchange between the  $\text{Na}^+$  in sodium alginate with  $\text{Ca}^{2+}$  in the  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  solution. The hardness or rigidity of the capsule mainly depends upon the number of sodium ions exchanged with calcium ions. Hence, the concentration of the two gelling agents i.e., sodium alginate and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , and the mixing time should be optimized for the formation of the capsule with optimum bead hardness and rigidity (Saiprasad, 2001).

Best encapsulation was accomplished by using 3% sodium alginate prepared in W.P.M. medium. Each nodal segment was coated with alginate and then picked up by a pair of forceps and gently dropped into  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  solution of different range in which they are allowed to stand for 30 minute for mixing.  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  at 2.5% showed better encapsulation. Bavistin (0.01%) (w/v) was also incorporated in W.P.M. medium to prevent the growth of microorganisms during the germination of encapsulated shoot tips.

## **Germination of encapsulated shoot tips into plantlets**

After 30 minute,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  was carefully decanted off and the encapsulated nodal segments were washed 3-4 times with sterile distilled water, blotted dry on sterilized filter paper and cultured on W.P.M. nutrient medium fortified with Kinetin (0.5 mg/l) and these initiated shoot tips were transferred to the BAP (2.5 mg/l) for multiplication. The germination response of the encapsulated shoot tips was scored after 28 days of culture.

## RESULTS

In the present research study, characteristics of sodium alginate beads and the effect of encapsulating matrix on bead formation was cultivated. Sodium alginate beads with entrapped nodal segments differed morphologically in respect to texture, shape, and transparency with different concentrations of sodium alginate and calcium chloride. Nodal segments derived from mother elite plant (Figure: A) shoots were encapsulated into synthetic seeds. Encapsulation of nodal segments was affected by the concentration of sodium alginate and calcium chloride. The presence of 3% sodium alginate and 2.5% calcium chloride was found to be optimum for synthetic seed production or proliferation phase of nodal segment (Figure: B). Subsequently, conversion of encapsulated shoot tips into multiples was successfully done (Figure: C). During the present studies, however higher concentrations of sodium alginate (4–5%) inhibited the conversion of encapsulated shoot tips into shoots, whereas lower concentrations of sodium alginate (1–2%) resulted in the formation of fragile sodium alginate beads that were difficult to handle. Even, higher concentrations of calcium chloride (100–200mM) also inhibited the conversion of encapsulated shoot tips into plantlets, whereas, lower concentrations (25–50mM) not only prolonged the ion exchange (polymerization) duration but also resulted in fragile beads, which were difficult to handle. Plantlet development was obtained from sodium alginate-encapsulated beads when cultured on W.P.M. medium supplemented with Kinetin (0.5 mg/l). Encapsulated nodal explants, when cultured in *in vitro*, showed shoot initiation after 2 weeks but on their subsequent subculturing, multiplication comes after 4 weeks. Retrieval of plantlets from stored encapsulated shoot tips was feasible only when gelling matrix was prepared in W.P.M. salts. The use of distilled water for preparing gelling matrix failed to support sprouting due to poor nutrition after 45 and 60 days of storage at 48°C in the dark. After 30 days of storage, the percentage responses for conversion of encapsulated nodal segments were 70%.



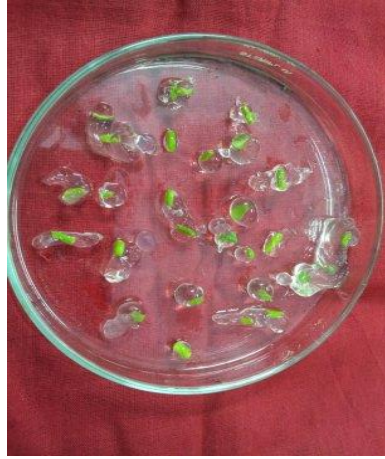


Fig A: Petri Plate with nodal segments  
encapsulated in synthetic seeds



Fig B: Proliferation phase of nodal segment



Fig C: Different proliferation stages of nodal segments

**Table 1 Concentration of Sodium Alginate on conversion of encapsulated shoot tips of *Tinospora cordifolia* into multiple shoots.**

<b>Sodium alginate (%)</b>	<b>% Conversion into multiples shoots</b>
<b>1.0</b>	Fragile beads difficult to handle
<b>2.0</b>	Fragile beads difficult to handle
<b>3.0</b>	<b>70</b>
<b>4.0</b>	<b>35</b>
<b>5.0</b>	<b>20</b>

**Table 2 Effect of calcium chloride concentration on conversion of encapsulated shoot tips of *Tinospora cordifolia* into shoots.**

<b>Calcium chloride (%)</b>	<b>% Conversion into plantlets</b>
<b>1.0</b>	Fragile beads difficult to handle
<b>1.5</b>	Fragile beads difficult to handle
<b>2.0</b>	<b>45</b>
<b>2.5</b>	<b>70</b>
<b>3.0</b>	<b>15</b>

**Table 3 Plantlet development (%) from encapsulated shoot tips of *Tinospora cordifolia* on different media.**

<b>Encapsulation matrix (3% sodium alginate)</b>	<b>% response of growth on hormones free W.P.M. medium</b>	<b>% response of growth on W.P.M. + Kinetin (0.5 mg/l)</b>	<b>% response of growth on M.S. medium</b>
Distil water (H <sub>2</sub> O)	32.66	55.23	25.33
MS Salts	40.55	70.00	30.76

Each treatment consisted of 28 replicates and the experiment was repeated at least thrice.

### **Conclusion**

Artificial seeds have wide spread applicability in large scale plant propagation. For some ornamental and extinct plant species, it is the only means of propagation. Apart from this, they have been used in commercial production of autogamous plant species, genetically modified plants, conifers, algae etc. In sum, artificial seed technology has influenced almost every aspect of plant biotechnology and has the potential to become the most promising and viable technology for large scale production of plants.

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