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Review Article

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Synthetic Seed Production as a Conservation Tool of Tinospora cordifolia: A Review

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ABSTRACT

In in-vitro plant propagation technology the processing of synthetic seed has established a broad range because it provides many valuable commercial benefits for the propagation of large number of crops. This technology promotes multiplication and conservation of best endangered medicinal plant species and agricultural, which is hard to accomplish with traditional method and natural seeds. This technology of synthetic seed is being used in multiple plants of economic importance like forage legumes, crops of vegetable, commercial important crops, spices, grains , crops of plantation, ornamental crops, fruit crops. This paper review studies on production of synthetic seed of Tinospora cordifolia, a medicinal plant used extensively for treatment against various diseases.

***Keywords:** Tinospora cordifolia, Synthetic seed, Somatic Embryogenesis, Zygotic seeds, Micropropagation, Encapsulation.*

Introduction

Tinospora cordifolia, especially in India, is one of the main medicinal plants commonly used for different medicinal purposes. It is deciduous climbing plant belongs to the family of Menispermaceae. *Tinospora cordifolia*, a medicinal plant known for its antiviral, antifungal, anti-inflammatory,antipyretic and antiulcer properties. This plant has been extensively used for commercial purpose because of its medicinal value. The plant is currently facing scarcity in India's natural ecosystems and thus need protection. Artificial seed technology is one such technology that promotes conservation, multiplication of germplasm by using micro propagation and plant tissue culture techniques. Synthetic seeds are commonly known as encapsulated somatic embryos. Other explant, aside from somatic embryos like shoot tips, axillary buds and any other meristematic tissue can also be used in preparation of artificial seeds and are being used for seed showing and having the capacity to transform under *in vitro* and in vivo conditions in whole plant and still retain its potential after storage [1]. The need for turning towards the technology of artificial seed is to facilitate the cost effectiveness of mass replication of genotype elite plants. New transgenic plants generated by plant tissue culture and biotechnological techniques will also have a channel to be transported directly to the greenhouse or field [2]. This review will provide all information of preparation of synthetic seed their need and objectives.

Review of Literature

Conventional Seeds, Disadvantages and need for Synthetic Seeds

Conventional seeds i.e Zygotic seeds are having nutritive tissues bound by many layers of protection. Because of defensive coat, seeds are considered to be desiccation resistant, quite and robust. Such properties of seeds are also used for germplasm preservation in seed storage. The outcome of sexual production, which is the progeny of two parents, is zygotic embryo seeds. Which has contributed to the formation of sometimes complex breedingprogram that establish inbred parental line. These inbred line give way, when crossed to uniform hybrid progeny. The challenge associated with zygotic seeds is that it is not possible to produce true breeding seed from two parents for several crops like nuts, fruits and ornamental plant because of genetic obstacles to self breeding. Another problem is, time is too long for certain crops such as forest trees to rationally accomplish an inbred breeding scheme. These are the major disadvantages of zygotic seeds and as many of these plants including Tinospora are facing rarity either due to commercial exploitation or due to expansion of human population i.e. deforestation there is need for their conservation. Synthetic seed technology is the technology that promotes rapid multiplication of rare and endangered plants *in vitro* conditions and later their transfer to in vivo conditions [3]. Thus synthetic seeds promote conservation, rapid multiplication, commercial use, clonal propagation and genetic uniformity. Other benefits are cost effective mass propagation, easy handling, storage and transportation [4].

Concept of artificial seed

One of the most significant methods for plant biologists dealing with *in vitro* cultures is artificial seed technology. When stock plants or *in vitro* colonies become contaminated with microorganisms, encapsulation may provide a supply of aseptic explant content. Furthermore, the use of artificial seeds allows for the preservation of gene collections that cannot be held in liquid nitrogen. Synthetic seeds were found to be ideal for medium-term storage under refrigeration conditions, but long storage periods often result in viability loss. The ideal gel matrix's composition is determined by the species and explant form. For *in vitro* germination, salts and sucrose should be added to the artificial endosperm. Benzyl adenine is useful in the recovery medium. Direct sowing of synseeds to *ex vitro* conditions is also possible, as long as the artificial endosperm is free of organic compounds. On the genomic, cytogenetic, biochemical, and phenotypic stages, the stability of plants recovered from artificial seeds was verified. More study is needed to standardize the best storage technique for improved performance and to extend the life of artificial seeds.

Preparation of Synthetic Seeds

Inducing somatic embryogenesis in vegetative cells

One requirement for synthetic seed production, through somatic embryo is the conversion of vegetative cells i.e somatic cells to somatic embryo and it is achieved by inductive treatment. Induction of somatic embryogenesis requires a change in polarity of somatic cell [5]. In order to induce cell division and to create a new polarity in the somatic cell, inductive therapy is primarily required. Using phytohormones, inductive therapy is obtained. Auxins are recognized to be necessary for the activation somatic embryogenesis among plant growth hormone. In some plants the most widely found auxin is 2, 4-dichlorophenoxyacetic acid (2, 4-D). For some other species, other auxin may be needed. Thus shaped embryogenic cells are distinctive, whereas superficially mimic meristematic cells are smaller, more isodiametric in shape, have large and tightly stained nuclei and denser cytoplasm. Essentially, these single cells are a condition for embryogenesis [6]. During this process, when auxin was eliminated from the medium, cell clustered acquired the ability to grow into embryos, contributing to the formation of state 1-cell cluster. When the embryogenic state is induced, globular, heart shaped and torpedo-shaped stage proceed through the mechanism of template forming that leads to the zygotic embryo [7].

Development of somatic embryo

In many plant species somatic embryogenesis has been recorded but the production of somatic embryos has been very low compared to their conversion into plantlets, because somatic embryos are usually not complete in their development. The somatic embryo does not proceed to the final stage of embryogenesis called embryo maturation, unlike zygotic embryos. In order to happen this i.e., somatic embryos maturation, it is important to move these somatic embryos to a medium containing a low concentration or absence of 2, 4 dichlorophenoxyacetic acid. By moving the embryos to a medium containing Abscisic Acid (ABA), is the final stage of maturation accomplished. By preventing secondary embryogenesis, ABA prevents germination and facilitates normal embryo growth and also recorded embryo maturation in many animals.

Encapsulation

The only commercially feasible strategy is cloning propagation by somatic embryogenesis, but somatic embryo needs mechanical power for planting and storage, so they need to be transformed into encapsulated units. Somatic embryos are often used for synthetic seed preparation because they have the ability of shooting and rooting in one stage, bur shoot tips in *M. Indica* [8], *Camellia sinensis*, bulbets in *Allium sativum* [9], cells aggregate originating from Horseradish hairy stem, sativum and protocorm like bodies were also used *Geodorum Densiflorum*. Additionally, to others meristematic tissues originating from *in vitro* , such as microtubers, rhizomes, and corm, synthetic seeds are often used for encapsulation [10]. Various chemical compounds are being tested for encapsulation sodium pectate, ethyl cellulose, nitrocellulose, polyox, water soluble resins, alginate, and polyacrylamide [11, 12]. However, sodium alginate (hydro gel) is most accepted for encapsulation and due to low toxicity, low cost, rapid gelling and bio compatibility, it is also used as matrix of synthetic seeds.

Different types of synthetic seeds

Two kinds of synthetic seeds, desiccated and hydrated synthetic seed, were produced as reported by literature. Desiccated synthetic seeds have been produced from nude and encapsulated somatic embryos, accompanied by desiccation [13]. Using chambers with declining relative humidity or quickly leaving the Petri dishes overnight on the laminar airflow chamber bench, desiccation was done either steadily or over a span of one or two weeks [14]. The technology of hydrated synthetic seed was first developed by encapsulating hydrated somatic embryos of *M. sativa*. This synthetic hydrated seed are used to develop plant species with recalcitrant and desiccation-sensitive somatic embryos. Artificial hydrated seeds typically developed by enfolding somatic embryos or by capsules of hydro gel to the plant tissue [15]. Various strategies have been investigated to develop artificial hydrated seeds, most of which have been used for encapsulation by calcium alginate.

Studies on synthetic seed production of Tinospora cordifolia

There are numerous studies on *Tinospora cordifolia in vitro* propagation. Various explants (nodal segments, leaf explants, shoot top, axillary bud and inter-node explants) were cultured on MS medium supplemented with different concentrations of plant growth hormone, for micropropagation of *Tinospora cordifolia.* In most research nodal explants and root explants are used to produce synthetic seeds and they are discussed below

Nodal Segments as Explants

Nodal explants were used in MS basal media with different combinations and concentrations of plant growth hormones in the majority of the studies [16]. *Tinospora cordifolia* nodal explant culture has produced several shoots with a high frequency in recent experiments [17]. Latest studies have mostly used MS basal medium with various variations and concentrations of Benzyl Amino Purine (BAP), Kinetin, and Thidiazuron (TDZ). Within 30 days of inoculation, a mixture of BAP (2 mg/l), Kinetin (4 mg/l), and TDZ (0.20 mg/l) in MS medium produced a maximum average of 10.29 shoots per explant. In half strength MS medium combined with IBA (2 mg/l), root initiation was observed after 5-6 days and became profuse after 5 weeks. The rooted plantlets were transplanted ex vitro and grew in pots for a month under greenhouse conditions before being transferred to the field. Various explants (shoot top, axillary bud, and cotyledonary node) were cultured on MS medium supplemented with different concentrations of plant growth hormone, according to a micropropagation protocol for *Tinospora cordifolia* [18]. Kinetin at 3.0 mg/l was found to be the most effective at inducing shoots in the literature. When opposed to shot tip explants, axillary bud and cotyledonary node explants responded well. Kinetin (3.0mg/l) and Gibberellic Acid (0.5mg/l) were found to be the most effective in elongating shoots in MS medium. Polyvinylpyrrolidone (PVP) was also used to refine the method for regulating phenol exudation in the culture. Another protocol was created for rapid Tinospora clonal propagation using mature nodal explants cultured *in vitro* [19]. MS medium and Woody Plant Medium (WPM) supplemented with 2.32 µm Kinetin were used to start the shoots. WPM was found to be superior to MS medium for the induction of several shoots of the two basal media studied. For axillary shoot proliferation, Benzyl Adenine (BA) was more successful than Kinetin among the cytokinins studied. Explants from nodes were found to be the safest for *in vitro* regeneration of *Tinospora* [20]. A mixture of 11.38µM Triacontanol [CH_3 (CH_2)**2**8CH_2OH] and 13.94µM Kinetin has been identified as the strongest shot inducing hormone combination in different studies. However, the effects of various auxins and their combinations with cytokinin on shoot proliferation from nodal explants were ineffective. When nodal fragments, leaf, and inter-node explants were planted on various hormone combinations in MS Medium, callus formation was observed in *Tinospora* tissue culture. In MS medium containing kinetin (1.5 mg/l), only nodal explants showed improved shoot growth. Roots were grown in a medium containing 1.0 mg/l BAP (1.0 mg) and 2.5 mg/l Naphthaleneacetic Acid [21]. Nodal explants were cultured in MS medium supplemented with kinetin (8 µM) or a mixture of kinetin and BAP (12 µM and 2 µM, respectively) to induce direct shoot induction. In

medium enriched with NAA (8 µM), the microshoots formed roots [22]. *Tinospora* nodal segments were also used to regenerate various shoots in MS basal medium combined with a mixture of BA (0.5 mg/l) and NAA (0.2 mg/l). Half-strength MS basal medium containing both BA (1.0 mg/l) and IAA (0.2 mg/l) was used to root regenerated shoots. For three weeks, rooted plantlets were moved to pots containing soil for acclimatization and were successfully formed in soil [23]. In MS medium containing BA and kinetin, shoot proliferation was also observed. The microshoots are rooted in a half strength MS medium combined with 0.4 mg/l NAA [24]. The scientific community is also interested in the production of active principles by *in vitro* culture.

Leaf Segments as Explants

Tinospora cordifolia leaf explants (taken from 15 days old plant) grown on MS medium supplemented with 2, 4-D (0.5mg/l) and glutamine (20mg/l) created viable somatic embryos *in vitro* via direct somatic embryogenesis [25]. Leaf explants were also used to induce callus when cultured in MS medium with 2, 4-D alone or in conjunction with kinetin. Such a callus, on the other hand, did not differentiate. Berberine, an isoquinolene alkaloid, was found in cell suspension cultures extracted from *Tinospora cordifolia* leaf explants, along with its associated analogues protoberberine and palmatine. Berberine was generated in a modified Linsmaier and Skoog's medium containing specific pH, plant growth regulators, and carbon sources. Berberine yield was stated to be 5-14 times higher in *Tinospora* cell suspensions than in whole plants [26]. Four week old *Tinospora* leaf, petiole, and stem derived calli were sub-cultured on MS medium, supplemented with various growth regulators, in an effort to up-grade the quality of berberine in *Tinospora* through biotechnological interventions. The basal development medium for *in vitro* berberine production was established as MS medium with NAA (2 mg/l) supplemented with BA or kinetin, each at 2 mg/l, yielding 7.55 µg and 7.36 µg berberine per gram of calli, respectively. In comparison to leaf and petiole-derived callus cultures, stem segment calli had the highest level of berberine [27]. *Tinospora cordifolia* hairy root cultures transformed with *Agrobacterium rhizogens* yielded protoberberine alkaloids. *Tinospora cordifolia* hairy roots were induced from shoot cultures using *Agrobacterium rhizogenes* on a strong YMB medium. Under optimal growth conditions, roots were subcultured on liquid MS medium containing B5 vitamins and 3% sucrose without hormones. The cultures treated with 500 mg/L of L-Tyrosine as a precursor produced a higher volume of berberine (0.034%) than the control cultures [28]. It's worth noting that *Tinospora cordifolia* has been the subject of intensive chemical research, with a large number of constituents isolated so far. Alkaloids, diterpenoid lactones, glycosides, hormones, sesquiterpenoid, phenolics, aliphatic compounds, and polysaccharides are among the most commonly isolated constituents [29].

Conclusion

Synthetic seed technology is the most important technology with widespread applications as it promotes large scale clonal propagation, preservation and multiplication. It is helping in cultivation of various ornamental plants and forest trees in which conventional techniques are not so helpful. Various rare and endangered plants including *Tinospora* are being conserved by synthetic seeds that cannot be conserved by conventional methods, due to inadequate presence of plant tissue and time.

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