Synthetic Seed of Rice: An Emerging Avenue of Applied Biotechnology

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Abstract  Progress in plant biotechnological research has opened many avenues for basic and applied research in the field of crop plants. Plant tissue culture is an important component of biotechnology, involves in the improvement of crops. Plant tissue culture led to develop synthetic seed technology. Synthetic seed technology is an exciting and rapidly growing area of research in plant cell and tissue culture. This technology is currently considered as the most effective and efficient alternative technique for propagation. The base materials for production of synthetic seeds are somatic embryos or tissue culture derived materials. This technology also facilitates the way of handling cells and tissues, protecting them from external gradients, short-term and long-term storage under low temperature and ultra-low temperature, respectively and as an efficient system of delivery. The information in the areas of synthetic seed preparation technology, its implications, achievements and limitations are lying unorganized in different articles of journals and edited books and that information were presented in this article in organized way with up-to-date citations, which will provide comprehensive literatures of recent advances.

Keywords  Rice; Synthetic seed; Mass multiplication; Plant conservation

1 Introduction
Rice (Oryza sativa) is the major food crop in the world. Nearly 40% of the world population consumes rice as the major staple food. Most of the people, who depend on rice as primary food, live in the less developed countries. Since the dawn of civilization, rice has served humans as a life-giving cereal in the humid regions of Asia and, to a lesser extent, in West Africa. Introduction of rice into Europe and the America has led to its increased use in human diets. There are 42 rice producing countries throughout the world but China and India are major rice production centers. Rice provides fully 60% of the food intake in Europe and the America has led to its increased use in human diets. There are 42 rice producing countries throughout the world but China and India are major rice production centers. Rice provides fully 60% of the food intake in Southeast Asia and about 35% in East Asia and South Asia. The highest level of per capita rice consumption (130–180 kg per year, 55%–80% of total caloric source) takes place in Bangladesh, Cambodia, Indonesia, Laos, Myanmar (Burma), Thailand, and Vietnam (Kenneth and Kriemhild, 2000). In many cultures of the world rice is the central part of people’s life and culture. Rice is an excellent food and is an excellent source of carbohydrates and energy. In 2008, international rice price rose greatly due to general upward trend in grain prices caused by droughts, increased use of grains animal feed, and so forth, has led to worldwide food crisis. This caused the domestic rice price in Malaysia increase almost double. The only way to protect and stabilize local price is to increase local rice production.

Production of synthetic seeds endowed with high germination rate under in vitro and in vivo conditions bears immense potential as an alternative of true seeds. Encapsulation technique for producing synthetic seeds has become an important asset in micropropagation. Botanically seed is a mature ovule along with its food storage in the form of either endosperm or cotyledon. The essential part is the embryo contained within the integuments, but it may be used less critically to describe planting materials. In terms of seed science, seed can be described as any propagating material used for raising a crop. Whereas, synthetic seed could...
be defined as artificially encapsulated somatic embryos, shoot buds, cell aggregates or any other tissue that can be used for sowing as a seed and that possesses the ability to be converted into a plant under in vitro or ex-vitro conditions, and that retains this potential even after storage (Capurno et al., 1998).

Synthetic seed technology can also help in germplasm storage and transportation of elite genotypes. A strong potential exists for propagation of high yielding, individual hybrids through somatic embryogenesis and artificial seeds (Brar et al., 1994). For potential application of seed encapsulation, technology has been demonstrated for many crop plants (Bapat and Rao, 1988, Padmaja et al., 1995; Onay et al., 1996; Shigeta and Sato, 1994; Suprasanna et al., 1996). In this endeavour efforts have been made to aggregate research findings on synthetic seed technology with particular emphasis on rice.

2 Types of Synthetic Seed
Success in production of synthetic seeds mainly depends on how best callus development and plantlet regeneration are achieved. The primary goal of synthetic seed production is to produce somatic embryos that resemble more closely to the true seed embryo in storage and handling characteristics so that they can be utilized as a unit for clonal propagation and germplasm conservation. Synthetic seeds may or may not have a synthetic seed coat, may be hydrated or dehydrated and may be quiescent or not. Encapsulation of micropropagules enables to satisfy the requirements. The gelling agents used for encapsulation for production of synthetic seeds act as protective cover. The encapsulated synthetic seeds also contain growth nutrients, plant growth promoting microorganisms (mycorrhizah, rhizobium, etc.), and/or other biochemical constituents necessary for optimal embryo-to-plant development (Figure 1).

2.1 Desiccated synthetic seed
Kitto and Janick (1982) first time successfully formulated the plant synthetic seed production technology involving carrot somatic embryos. They used polyoxyethylene, which is readily soluble in water, dries to form a thin film, does not support the growth of microorganisms and is non-toxic to the embryo, leading to the production of desiccated synthetic seed. Desiccation can be achieved either slowly over a period of one or two weeks sequentially using chambers of decreasing relatively humidity, or rapidly by unsealing the Petri dishes and leaving them on the bench overnight to dry. Such types of synthetic seeds are produced only in plant species whose somatic embryos are desiccation-tolerant. Janick et al. (1989) have reported that coating a mixture of carrot somatic embryos and callus in polyoxyethylene glycol produced desiccated artificial seeds. The coating mixture was allowed to dry for several hours on a Teflon surface in a sterile hood. The dried mixture was then placed on a culture medium, allowed to rehydrate; and then scored for embryo survival. McKersie et al. (1989) induced somatic embryos to acquire desiccation tolerance by treatment with abscisic acid or any one of several environmental stresses, including water and nutrient stresses, applied to the embryoids at the cotyledonary stage of development. The embryoids were subsequently air dried slowly (over 7 days) or rapidly (over 1 day) to moisture contents of less than 15% and remained fully viable. Dry somatic embryos were stored with no loss of viability for 8 months at room temperature and humidity. Bornman et al. (2003) reported that the percentage germination of fresh or somatic embryos partially desiccated at relative humidities of 97% and 63% to moisture content approaching those of the seed was substantially lower. This sensitivity to drying suggests that the somatic embryo seed may behave either as an orthodox seed with limited ability to withstand desiccation or as a recalcitrant seed that cannot survive drying below a moisture content that is relatively high.

Figure 1 Synthetic seed, gel encapsulated embryo with hydrophobic membrane
Note: A: Cross-section of a dicotyledonous true seed; B: Cross-section of a synthetic seed
Dry somatic embryos lack the vigour normally associated with seedling from normal seeds. The reason for this is not yet been specified, although there are several possibilities. The dry somatic embryos may lack storage proteins or some other critical components required after germination by the seedlings. The storage protein levels have been increased with some improvement in vigour (Lai et al., 1992; Lai and McKersie, 1993; Lai and McKersie, 1994a, Lai and McKersie, 1994b). Somatic embryos store starch and sucrose, whereas, seeds store a hemicellulose in the cell wall of the endosperm called galactomannan (McCleary and Matheson, 1974; McCleary and Matheson, 1976). The sucrose reserves in the dry somatic embryos are rapidly depleted after imbibitions (Lai et al., 1995). In some instances, there may be injury to the somatic embryos if the proper drying procedure is not followed (Lecouteux et al., 1993). In comparison to true seeds, water uptake during imbibitions of dry somatic embryos is quite rapid, because the somatic embryos lack a testa, there is no barrier to water uptake. Imbibition injury is, therefore, another possible cause of poor seedling vigour in synthetic seeds.

2.2 Hydrated synthetic seed

Hydrated seeds are produced in those plant species where the somatic embryos are recalcitrant and sensitive to desiccation. Encapsulation of somatic embryos in hydrogel capsules produces hydrated synthetic seeds. The most used method to induce artificial seed is isotropic gelation of sodium alginate by calcium ions. Redenbaugh et al. (1984) developed the technique of hydrogel encapsulation of individual somatic embryos of alfalfa. Since then encapsulation in hydrogel remains to be the most studied method of artificial seed production.

2.3 Double layered synthetic seed

Technology has been developed to prepare double-layered synthetic seeds (Kinoshita, 2002). For somatic embryo encapsulation, sodium alginate is largely used; however, this is excessively permeable with loss of the nutritive substances and/or dehydration risk during conservation and transport causing detrimental effects on the synthetic seed conversion and on the plantlet growth. In order to overcome these problems, Micheli et al. (2002) developed double coat encapsulation and encapsulation coating procedure in M.26 apple rootstock. The inner layer contained a large quantity of sucrose. To prevent the diffusion of sucrose from artificial seeds to non-sterilized substrate, artificial seeds were enveloped in a dialysis membrane. The enveloped artificial seeds germinated quickly in non-sterilized vermiculite.

3 Production Technologies

3.1 Gelling agent

A number of substances like, potassium alginate, sodium alginate, carrageenan, agar, gelrite, sodium pectate, etc. have been tested as hydrogels (Table 1). Sodium alginate has a low cost and good gelation and biocompatibility characteristics. Smidsrod and Skjak-Braek (1990) suggested alginate for preparation of artificial seeds. Though many coating materials have been tried for encapsulation of somatic embryos, sodium alginate obtained from brown algae has been considered as the best and is being popularly used at present. Alginate has been chosen for case of capsule formation as well as for its low toxicity to the embryo. Alginate capsulated embryos can resist unfavourable field conditions without desiccation. The rigidity of the gel beads protects the fragile embryo during handling.

Table 1 Gelling materials and complexing agent for encapsulation of plant propagules for production of synthetic seeds

| Gelling agent          | Concentration (%) | Complexing material | Substance       | Concentration (mM) |
|------------------------|-------------------|---------------------|-----------------|-------------------|
| 1. Sodium alginate     | 0.5~5.0           | Calcium salt        | Calcium chloride| 30~100            |
| 2. Sodium alginate with gelatin | 2.0               | Calcium chloride    | Potassium chloride| 500               |
| 3. Carrageenan         | 0.2~0.8           | Ammonium chloride   | -               | 500               |
| 4. Locust Beam Gum     | 0.4~1.0           | -                   | -               | -                 |
| 5. Gelrite*            | 0.25              | -                   | -               | -                 |
| 6. Agar*               | 0.8~2.0           | -                   | -               | -                 |

Note: *Temperature is lowered down for solidification
3.2 Encapsulation methods

3.2.1 Encapsulation by dropping of hydrogels into complexing agent

For encapsulation, embryos or buds are mixed with autoclaved sodium alginate (0.5%~5%, w/v), which is prepared in suitable tissue culture basal medium supplemented with sucrose. Embryos or buds are then picked up individually and dropped into sterilized aqueous solution of 2%~3% (w/v) calcium salt solution [CaCl$_2$ or (CaNO$_3$)$_2$] with occasional agitation. Calcium alginate beads were formed within 15~30 minutes. Here, ion-exchange reaction occurs and sodium ions are replaced by calcium ions forming calcium alginate beads or capsules surrounding the embryo/bud. The size of the bead depends upon the inner diameter of the pipette nozzle. Compactness and hardening of the encapsulated bead is modulated with concentration of sodium alginate and calcium chloride as well as duration of complexing. Optimum concentration of sodium alginate for production of synthetic seed ranges from 2%~3% with a complexing solution containing 75~100 mM calcium chloride (Ara et al., 1999; Bhattacharjee et al., 1998; Bekheet et al., 2002; Sparg et al., 2002; Priya et al., 2003, Roy and Mandal, 2008).

3.2.2 Mechanized encapsulation method

Automated encapsulation process is a quick method of artificial seed production. An encapsulation machine can be used successfully to encapsulate somatic embryo in order to achieve a time and hand labour saving and to increase the accuracy. Sicurani et al. (2001) developed mechanical excision of explants. This technique is useful for the production of synthetic seeds through encapsulation of differentiating propagules (tissue fragments with shoot primodia) in woody species. Brischia et al. (2002) also used mechanically manipulated explants of apple rootstock for encapsulation. They suggested that machine processed explants can be encapsulated for production of synthetic seeds.

The development of synthetic seed involving the production of artificial endosperm and self-breaking treatment for gel as well as equipment for encapsulation and sorting were described by Sakamoto et al. (1992). The development and preservation of embryogenic cell lines, somatic embryogenesis in bioreactors and embryo-to-plant conversion; preservation and coating were discussed by Petiard et al. (1993) with reference to carrots and Coffea camphora. About 55000 and 5000 embryos/litre can be produced daily for carrots and coffee, respectively.

Alternatively, the embryos could be mixed in a temperature depended gel such as, gelrite, agar, agarose, etc., placed in the well of a micro-titer plate, and gelled as the temperature was lowered. The judicious and intelligent coupling of artificial seed technology with that of microcomputer in achieving automated encapsulation and regeneration of plantlets would tremendously increase the efficiency of encapsulation and production of homogeneous and high quality seeds, and will thus revolutionize the current concept of commercial micropropagation method.

3.2.3 Semi-automated encapsulation

Somatic embryos are mixed in sodium alginate solution (2%~5%) prepared with the appropriate nutrient medium. Sodium alginate solution impregnated with somatic embryos is dropped into calcium chloride solution (Figure 2). Molle et al. (1993) have suggested the use of a dual nozzle pipette in which the embryos flow through the inner pipette and the alginate solution through the outer pipette. As a result, the embryos are positioned in the centre of the beads for better protection.

![Figure 2 Sodium alginate solution impregnated with somatic embryos is dropped into calcium chloride solution](image-url)
3.2.4 Plant growth regulators

To improve the storability and germination of synthetic seed, many workers used growth regulators. Encapsulation with GA3 was found to be useful for storage of somatic embryos of citrus at 4°C for 1 month (Mariani, 1992; Antonietta et al., 1998). Buds treated with 0.01~1.0 mM ABA either prior to encapsulation or even in the alginate, matrix to inhibit the precocious growth (Palmer and Jasrai, 1996). Synthetic seeds obtained from the excised embryos of intact seeds treated with higher (2~3 mg/L) concentrations of ABA showed tolerance to low temperature storage and retained higher germination percentage. ABA is implicated as a controlling factor for germination and dormancy in somatic embryos and seeds (Senaratna et al., 1995). Ruffoni et al. (1994) found that the addition of zeatin (0.5 mg/L) to the alginate encapsulation coating improved the shoot production and sucrose (40 mg/L) added to MS agar medium improves the percentage root emergence of Enstoma grandiflorum.

Desiccation tolerance is a characteristic of somatic embryos that must be induced and therefore, require a pretreatment with ABA or stress elicit the desired response. The importance of ABA application for imparting desiccation tolerance during storage of somatic embryos was well recognized (Senaratna et al., 1989; Senaratna et al., 1990; Lecouteux, 1993). Kitto and Janick (1985) reported that ABA effectively hardened carrot embryos, permitting them to survive desiccation. In a further study, Kim and Janic (1989) found that ABA at 10-6 M effectively increased embryo survival after desiccation suggesting that ABA hardens somatic embryos of celery. They also achieved desiccation tolerance by a combination of ABA and proline. ABA increased the accumulation and altered the distribution of fatty acids in somatic embryo. Takahata et al., (1992) reported that the desiccated embryos lost their viability if not treated with ABA. Dormancy and desiccation tolerance can be imparted to the embryos by treating them with appropriate concentration of ABA or high sucrose concentrations (Anandarajah and McKerrrie, 1990). The ability of somatic embryos to with stand to low moisture content is important for storage, and also plays a big role in developmental transition between maturation and germination. Phokajornyod et al. (2004) found that somatic embryos that treated with 0.5 mg/L of ABA for 20 days before encapsulation with sodium alginate, and dehydrated in laminar flow hood until 80% water loss still remained germinated to 58%. Furthermore, improvement of dry somatic embryos was accomplished by adding 60 g/L sucrose in the maturation medium which resulted 40% of plantlets conversion after four weeks of storage in ambient temperature.

The germination percentage of artificial seeds of Brassica oleracea var. botrytis was enhanced by the inclusion of 0.3 mg/L NAA and 3.0 mg/L BAP in the encapsulation matrix after 7 and 30 days of pregermination storage (Siong et al., 2012). The time taken for germination was also faster (5 days after 7 days of storage and 11 days after 30 days of storage) when MS fortified with 0.3 mg/L NAA and 3.0 mg/L BAP were used. Results Zhang et al. (2011) showed that maltose, active carbon and sodium alginate played the important role in germinate of D. Candidum artificial seeds. The optimal condition was maltose 4%, hormone rate between 6-BA and NAA 12:1, active carbon 0.3%, sodium alginate 4%, time of ion exchange is 5 min for artificial seeds procedure.

3.2.5 Use of protective chemicals and microorganisms

Protective chemicals may be used to protect the synthetic seed from fungal and/or bacterial infection. To avoid bacterial contamination, Ganapathi et al. (1992) added an antibiotic mixture (0.25 mg/L) containing rifampicin (60 mg), cefatoxime (250 mg) and tetracycline-HCl (25 mg) dissolved in 5 ml dimethyl sulphate to get matrix. Mycorhizas, pesticides and fertilizers can be incorporated into the capsules to enhance germination rates and seedling growth (Toruan and Sumaryono, 1994). Sharma et al. (2000) used mycorrhiza for preparation of synthetic seeds. Activated charcoal (0.1%) can also be added to the matrix to absorb the polyphenol exudates of the encapsulated shoots of banana (Ganapathi et al., 1992).

Zhang et al. (2001) prepared synthetic seeds of Dendrobium candidum using clay and vermiculate
powder as the encapsulating medium. Their results showed that the germination rate reached 56.8% when the proportion of clay: vermiculite: water was 2: 1: 2. When this system was added with only 1.0% activated charcoal, or 1.0% activated charcoal along with 0.5% starch, the corresponding average germination rates of the artificial seeds increased to 76.7% and 80.3%. The treatment enhanced the germination rate by 18.4% and 24.2%, respectively.

4 Encapsulating Materials

4.1 Embryogenic synthetic seeds

The process of somatic embryogenesis, in which the somatic cells or tissues develop into differentiated embryos, produces somatic embryos and each fully developed embryo is capable of developing into a plantlet. Embryos can be obtained either directly from cultured explants, anther or pollen, callus and isolated single cell in culture. For synthetic seed production, embryos may be obtained from the following sources.

4.1.2 Somatic embryos

The quality of the artificial seed depends on the temporal qualitative supply of growth regulators and nutrients along with an optional physical environment. The advantages of preparing synthetic seeds from somatic embryos have been discussed by many authors (Gray and Purohit, 1991; Flacinelli et al., 1993; Ara et al., 2000). The use of somatic embryos as artificial seeds is becoming more feasible as the advances in tissue culture technology define the conditions for induction and development of somatic embryos in an increasing number of plants species (Jain et al., 1995; Ipekci and Gozukirmizi, 2003). The sources of somatic embryos of rice are mature seed derived callus, root culture, immature inflorescence culture, immature embryo culture etc.

4.2 Gametic Embryos

4.2.1 Androgenic embryos

In several plants regeneration of artificial seeds into plantlets has been reported. However, information about production of artificial seeds from androgenic embryos derived from androgenic callus is scanty. Haploid plant breeding has been found to be well established in many crops (Aljera et al., 1995; Plamer et al., 1996; Roy and Mandal, 2004a; Roy and Mandal, 2004b). The induction of pollen embryogenesis, which genetically differs from zygotic embryogenesis, may be used for synthetic seed production. In rice, Roy (2006) developed protocol for rapid and recurrent mass multiplication of androgenic embryos and embryo-like-structure of IR 72 an elite indica cultivar. These embryos and embryo-like-structures can be used as source of synthetic seed production.

4.2.2 Microspore culture

Haploid plants can also be regenerated from microspore culture. Anther culture may associate with production of diploid plantlets from anther wall or from other parts of anther other than pollen. Thus anther derived plantlets are with various ploidy levels. This can be avoided by culturing isolated pollen. Microspore culture usually produces homogenous population, whereas anther culture could constitute a heterogenous population.

4.2.3 Ovule culture

Haploid plants have been successfully developed from culture of female gametophytic cells, that is, the egg nucleus or ovum. It was considered as an alternative means of haploidy as well as the expression of totipotency of female gametophytic cells in angiospermic plants. The first gynogenic haploid was obtained in barley (San Noeum, 1976) culturing ovaries. Subsequently, Chinese scientists could regenerate haploid plants in rice, wheat, sunflower, sugar beet and onion by culturing female gametophytic cells.

The appropriate time of embryo sac for its culture is uninucleate to mature stage. But it may differ species to species, such as nearly mature (1~2 days before anthesis), embryo sac mother cell to megaspore tetrad stages are also suitable for culture. The explant for development of gametophytic haploid may be the ovary, isolated ovule or even unhusked flower.

4.2.4 Recurrent embryogenesis

The genetic improvement of crop plants en route biotechnological approaches largely depends upon the maintenance of differentiated cultures of callus or redifferentiated embryos. Success in production of synthetic seed depends on how best callus develop
and plantlet regeneration achieved. Long-term maintenance of embryogenic masses in culture tubes or mechanically stirred bioreactors requires frequent transfer of tissue to fresh media, which is both labour intensive and costly. However, over time the morphogenic competence of differentiated cultures declines (Lynch and Benson, 1991). Therefore, new culture has to be regularly initiated and characterized in order to maintain a constant supply of embryogenic callus. This approach is highly cumbersome. To cope with these difficulties, the embryo masses of Pistacia vera have been encapsulated in sodium alginate gel and stored at 4 °C after treatment with BAP. The maintenance of recurrent cycle of somatic embryogeneis can be spontaneous as is the case with alfalfa, Medicago sativa (Lupotto, 1983). The cycles were maintained without growth regulators (Lupotto, 1986), and with specific growth regulator at specific concentration (Roy, 2006; Roy and Mandal, 2011).

The initiation of recurrent culture requires that the developing embryos be locked into a developmental stage beyond which they cannot proceed, thereby repeating a cycle. This can be achieved through its initial exposure to a very high auxin concentration such as 40 mg/L 2,4-D followed by maintenance of the recurrent system using a lower level of auxin, such as 5 mg/L of 2,4-D (Finer and Nagasawa, 1988), which prevent the transition from pro-embryogenic to embryogenic development. Onay et al. (1996) have reported that the encapsulated embryogenic masses recovered their original proliferate capacity after two month storage following two subcultures. Petiard et al. (1993) elaborated tool for large-scale propagation of coffee (Coffee canephora) and carrot from mass-somatic embryogenesis in bioreactors. Subsequently they have stressed upon the economics of mass somatic embryogenesis.

4.3 non-embryogenic synthetic seeds- microtillers

Information about production of artificial seeds from microshoots/microtillers in rice is extremely limited. Roy and Mandal (2006) developed a protocol for rapid and recurrent mass-multiplication of androgenic embryos and microtillers of indica rice var. IR 72. Those embryo masses and microtillers were used to prepare synthetic seeds (Roy and Mandal, 2008) and germinated in vitro and in vivo on vermiculite.

Taha et al. (2012) induced microshoots from stem explants on MS medium supplemented with 1.5 mg/L BAP. The microshoots were encapsulated in 3% (w/v) sodium alginate, 3% sucrose, 0.1 mg/L BAP, and 0.1 mg/L α-Naphthalene acetic acid (NAA). Germination and plantlet regeneration of the encapsulated seeds were tested by culturing them on various germination media. They also investigated the effect of storage period (15–30 days) was also. Maximum germination and plantlet regeneration (100.0%) were recorded on MS media containing 3% sucrose and 0.8% agar with and without 0.1mg/l BAP. However, a low germination rate (6.67%) was obtained using top soil as a sowing substrate. The germination rate of the encapsulated microshoots decreased from 93.33% to 3.33% after 30 days of storage at 4°C in the dark.

5 Implications

By combining the benefits of vegetative propagation system with the capability of long-term storage and with clonal multiplication, synthetic seeds have many diverse applications in agriculture (Gray and Purohit, 1991; Redenbaugh et al., 1991; Redenbaugh, 1993). The exact application of synthetic seeds will vary from species to species. The possible implications of rice synthetic seed technology have been detailed hereunder.

5.1 Clonal propagation

The potential application of synthetic seed technology is to produce true-to-type propagules of different crop plants. The sexually reproduced of seed in cross-pollinated crops is undesirable because it assures that the seeds are not alike genetically following meiotic recombination. Synthetic seed technology is an alternative to traditional micro-propagation for production and delivery of cloned plants. It offers the possibility of low cost, high-volume propagation system that will compete with true seeds and transplants. The explants used in synthetic technology are somatic in origin, which satisfy the asexual means of reproduction. There are two distinct routes for clonal propagation through synthetic seeds- somatic embryo and microtillers.

5.2 Mass multiplication of transgenic and elite plants

This newly emerging technology would also be useful
for multiplication of genetically engineered plants (transgenic plants). Transgenic plants require separate growth facilities to maintain original genotypes may also be preserved using somatic embryos followed by preparation of synthetic seeds for multiplication or further propagation. Multiplication of elite plants selected in plant breeding programmes through somatic embryos avoids the genetic recombination, and therefore, does not warrant continued selection inherent in conventional breeding, saving considerable time and resources.

The putative transformed tissue may be regenerated into plantlets. Regeneration of large numbers of transgenic plants is quite difficult or sometime impossible. The transformed plant can be used for clonal propagation through somatic embryogenesis followed by preparation of artificial seeds.

5.3 Germplasm conservation
Besides rapid and mass propagation of plants, the artificial seed technology has added new dimensions not only to handling and transplantations but also for conservation of endangered and precious plant propagules.

5.3.1 Conservation at low temperature
Germplasm conservation in clonal crops, particularly rooted crops, tuber crops and trees associated with many problems. Conservation through tissue culture, after a period of time, it becomes necessary to transfer to fresh media and the sub-culture is a repeated and continuous process. Repeated sub-culture overtime may reduce the morphogenic competence of differentiated cultures (Lynch and Benson, 1991). Therefore, new culture has to be regularly initiated and characterized in order to maintain a regular supply. This approach is highly tedious and costly. The technology of synthetic seed production may help in this regard. Storage of encapsulated embryos for a considerable time allows preservation of valuable, elite germplasm. Many authors successfully stored synthetic seeds at low temperature (4°C) for varying periods (Datta et al., 1999; Madhav et al., 2002; Ipekci and Gozukirmizi, 2003; Ahmed and Talukdar, 2005). Pintos et al. (2008) also successfully stored synthetic seeds of cork oak at 4°C for two months without significant loss in conversion capacity. The hydrated synthetic seeds could be stored using low temperature for a few weeks (Redebaugh et al., 1986; Fujii et al., 1989; Fujii et al., 1992). The capability of prolonged storage was achieved when somatic embryos could be dried to moisture content less than 20% (McKersei et al., 1989).

5.3.2 Conservation in plant tissue culture room
The alginate coated beads, made by encapsulating small propagules are excellent stable germplasm units. Bhattacharyya et al. (2007) successfully stored synthetic seed of Plumbago indica, a medicinal plant at culture room conditions (22–24°C temperature and in dark) for 3 months without significant reduction of conversion ability.

5.3.3 Cryopreservation
Cryopreservation is commonly used technique for long-term preservation of biological material. This can be defined to the stepwise viable freezing of biological materials (seed, planting materials, plant callus, somatic embryos, synthetic seeds etc.) followed by storage at ultra-low temperature, preferably at that of liquid nitrogen (196 °C). This process preserves growth and biosynthetic potencies of biological materials. It arrests all metabolic activities and biological deterioration of cells, thus the material can be preserved for longer period of time. Practically, it can be stored on solid CO2 (-79 °C), in deep freezers (-80°C or above), in vapour phase of nitrogen (-150°C) or in liquid nitrogen (-196 °C). Cryopreservation is considered as an ideal means of avoiding loss of embryogenic potential during repeated subcultures and as a means of preventing the occurrence of somaclonal variation during long-term maintenance of embryogenic culture.

5.3.4 Encapsulation-vitrification
Encapsulation-vitrification is a new technique of preservation of plant materials, which combines the advantages of vitrification (rapidity of implementation) and of emasculation-dehydration (ease of manipulation of encapsulated explants) has been established (Matsumoto et al., 1995). This method is user-friendly and greatly reduce the time requires for dehydration. Thus, the method is frequently is being
used for long term preservation of plant materials.

5.3.5 Encapsulation-dehydration
Encapsulation-dehydration is a valuable procedure for various plant materials, including in vitro-grown shoot tips and somatic embryos. In this method, the synthetic seeds are treated with a high sucrose concentration, dried down to moisture content 20%~30% (under airflow or using silica gel) and subsequently frozen in liquid nitrogen (Fabre and Dereuddre, 1990). This technique may prove interesting in two situations- (1) for the materials which are recalcitrant to standard freezing techniques, (2) the seeds that the protection conferred by the beads allows submitting the embedded material to pretreatment conditions which would otherwise be detrimental. It may be beneficial also if the encapsulation allows to carryout rapid freezing, thus avoiding the use of a programmable freezing apparatus and simplifying the process. The drawback of this procedure is that it is rather lengthy and labour-intensive.

6 Achievements
Research on artificial seeds in field crops, especially cereals is still in infancy. However, many workers successfully encapsulating somatic and androgenic embryos of cereal crops (Datta and Potrykus, 1989; Suprasanna et al., 1996; Roy and Mandal, 2008). Brar et al. (1994) emphasized the need for research on artificial seeds in rice through embryogenesis and outlined its impact on mass propagation of true-breeding hybrids. Somatic embryogenesis can also be used in the regeneration of genetically transformed plants (Vicient and Martinez, 1998). Subsequently those somatic embryos could be economically and successfully propagated though synthetic seed technology.

Rice is the world’s most important food crop and a primary food source for more than one third of the world’s population. This crop has received considerable attention in biotechnology research programmes. Research in artificial seeds in rice scantly and this technology through somatic embryogenesis would offer a great scope for large-scale propagation of superior elite hybrids (Brar and Khush, 1994). Somatic embryos of rice have been encapsulated to produce synthetic seeds (Suprasanna et al., 1996). Suprasanna et al. (2002) studied the viability of encapsulated embryos derived from five year old long term culture of Oryza sativa cv. basmati 370. The encapsulated embryos showed better conversion into plantlets than non-encapsulated embryos. Xing et al. (1995) prepared artificial seed from hybrid embryos of japonica and javanica rice. The germination rate of artificial seeds in sterile conditions was 15%~60% on vermiculite and agar media. The induction of pollen embryogenesis genetically differs from zygotic embryogenesis, and the androgenic embryos, may be used for synthetic seed production. Datta and Potrykus (1989) encapsulated microspore-derived embryos of barely and germination response was found to be high (80%) and seedlings were more vigourous than that of non-encapsulated embryos. Arunkumar et al. (2005) reporptd the addition of protectants, bavistin and streptomycin as constituents of synthetic endosperm and found that there was no negative effect on germination and conversion. They also studied the conversion of synthetic seeds into seedlings in hybrid rice and reported that the application of self-breaking gel beads technology increased the germination (52%) and conversion (47%) of synthetic seeds. Kumar et al. (2005) reported that the synthetic seeds of rice with artificial endosperm constituents of MS nutrient, sucrose (3% w/v) 0.5 mg/L IAA, 0.5 mg/L NAA, 0.5mg/L BAP and activated charcoal (1.25% w/v) gave maximum germination rate of 30% by using somatic embryos at globular stage as propagules. They also reported that the inclusion of activated charcoal had enhanced the germination to the maximum extent by increasing the diffusion of gases, nutrients and respiration of embryoids.

Roy and Mandal (2008) prepared synthetic seeds from androgenic embryos, embryo-like-structures and microtillers of indica rice var. IR 72 (Figure 3). The results indicated higher germination in the beaded embryos than the unbeaded embryos. The reduced rate of germination of artificial seeds may be attributed to the damage incurred while separating the embryos from clusters and/or owing to adverse affects of
chemicals used for encapsulation. However, further research is needed to optimize protocols for production of androgenic viable synthetic seeds of rice.

Figure 3 Pictorial depictions of synthetic seed production and plantlet regeneration in rice (Roy, 2002)
Note: a: Stereomicroscopic view of germinating microtillers; b: Encapsulated microtiller in sodium alginate (4%) beads; c: Mass germination of beaded microtillers on MS medium with no hormones; d: Seedling elongation from germinating synthetic seeds; e: growing plantlets under in vitro culture condition on MS basal medium

7 Limitations
Although results of intensive researches in the field of synthetic seed technology seem promising for propagation of crop plants. Several aspects of the techniques are still underdeveloped and hinder its commercial application:

1. High hand labour requirements and costly procedures for the production of encapsulated propagules.

2. Strain in recurrent production of somatic embryos essential for synthetic seed production: Although large quantities of somatic embryos can be rapidly produced in many plant species, continuous supply is difficult as the totipotency decrease with age of culture.

3. Improper maturation of the somatic embryos and asynchronous development: Development of artificial seeds requires sufficient control of somatic embryogenesis from the explants to embryo production, embryos development and their maturation. Hence, mature somatic embryos must be capable of germinating out of the capsule or coating to form vigorous normal plants. In some cases, somatic embryos often develop extra cotyledons or poorly developed apical meristems. This asynchronous embryo development makes harvest difficult. Uniformly mature somatic embryo development have included physical separation of proembryonal cultures to assure uniform callus size and physiological synchronisation by adding abscisic acid appears to cause cell water (turgor) content to decrease, thereby slowing embryo growth which inhibit germination of embryos that would tend to germinate precociously.

4. Multiple somatic embryos development: Multiple somatic embryos are often found on a single callus, in which multiple stages of embryo development are observed. This causes the non-uniform embryos to be subjected to change nutrient conditions since the nutrients are depleted by the developing tissues and the replenished. Consequently many somatic embryos have organs developing at different rates, which contribute to asynchronous embryo development. In some cases, this leads to precocious germination, while in others the prevailing nutrient environment may be conducive to shoot or root development but not both.

5. Poor conversion of micropropagules into plantlets: For commercial applications, somatic embryos must germinate rapidly and should be able to develop into plants at least at a rate and frequencies more or less similar, if not superior to true seeds. To achieve conversion of somatic embryos into plantlets and to overcome deleterious effects of recurrent somatic embryogenesis as well as anomalous development of somatic embryos on their conversion, it is necessary to provide optimum nutritive and environmental conditions (Roy and Mandal, 2008).

6. Lack of dormancy and stress tolerance: Usually somatic embryos continue to grow into seedling or they revert back into disorganized callus tissue. This inability to produce a resting phase where all embryos are at the same arrested physiological and morphological state also is a challenge to synthetic seed development. Without this arrested growth stage, synthetic seed cannot be successfully stored or treated
using traditional seed technology practices.

7. Genetic variability: Genetic variability for regeneration via somatic embryogenesis has been documented. Genetic control of regeneration capacity is largely additive and highly heritable.

8. Coating material may limit synthetic seed production: The coating materials may also limit success of synthetic seed technology. The hydrated capsules are more difficult to store because of the requirement of embryo respiration. A second problem is that capsules dry out quickly unless kept in a humid environment or coated with a hydrophobic membrane. Calcium alginate capsules are also difficult to handle, because they are very wet and tend to stick together slightly. In addition, calcium alginate capsules lose water rapidly and dry down to a hard pellet within a few hours when exposed to the ambient atmosphere. The coating material should be non-damaging to the embryo, mild enough to protocol the embryo and allow germination and be sufficiently durable for rough handling during manufacture, storage, transportation and planting.

8 Conclusion
The synthetic seed production technology is constrained due to several major problems, which hinder its commercialization. The first requirement for the practical application of synthetic seed technology is large-scale production of high quality micropropagules, which is at present a limiting factor. The second limiting factor is the survival and germination of the encapsulated somatic embryos are low. Poor germination of synthetic seeds may be due to lack of nutrient and/or oxygen supply, microbial infection and mechanical damage of the embryo. The conversion of desiccated somatic embryos to plantlets is low. The desiccation process, which damages the embryos, and other problems associated with desiccated synthetic seeds need regulation. Third major obstacle in synthetic seed technology is reduction in viability on storage. Most of the research efforts reflect on short-term storage only. Further research is needed for prolong maintenance viability somatic embryos. Either hydrated calcium alginate based or desiccated polyoxyethylene glycol based artificial seeds might be used, but it is likely that some degree of drying before cryopreservation would be beneficial. Occurrence of somaclonal variation in tissue culture is another aspect to be considered which recommending the use of synthetic seeds for clonal propagation. In nutshell, somatic seed remains a poor analog of natural seed in terms of viability, handling and storage. More basic comparative biochemical and physiological research is needed to understand the differences in response between zygotic and somatic seed, so as to determine whether or not somatic embryo is behaving like recalcitrant seed and/or like an isolated zygotic embryo in vitro. Synthetic seed technology provides a rich analog of natural seed in terms of clonal propagation, handling in national and international exchange of seeds for genetic improvement and germplasm conservation.

Author contributions
The first author developed the concept, guided the second author for collection of referenced articles and both have contribution in compilation and correction of this review article in the present shape. Both authors read and approved the final manuscript.

References
Ahmed H., and Talukdar M.C., 2005, In vitro propagation and artificial seed production of Arundina bambusiflolia, J. Ornamental Hort New-Series., 8(4): 281-283
Alijera., M.S., Zapata D., Khush G.S., and Datta S.K., 1995, Utilization of anther culture as a breeding tool in rice improvement, In: Current issues in plant molecular and cellular biology, M. Terzi et al. (eds.), Kluwer Academic Publishers, The Netherlands., pp: 137-142
Anandarajah K., and McKersie B.D., 1990, Manipulating the desiccation tolerance and vigour of dry somatic embryos of Medicago sativa L. with sucrose, heat shock and abscisic acid, Plant Cell Rep., 9: 451-455 http://dx.doi.org/10.1007/BF00232271
Antonietta G.M., Emanuele P., and Alvaro S., 1998, Effects of encapsulation on Citrus reticulata Blanco somatic embryo conversion, Plant Cell Tiss. Org. Cult., 55(3): 235-237 http://dx.doi.org/10.1023/A:1006250707040
Ara H., Jaiswal U., and Jaiswal V.S., 1999, Manipulating the desiccation tolerance and vigour of dry somatic embryos of mango (Mangifera indica L.), Plant Cell Rep., 19: 166-170 http://dx.doi.org/10.1007/s002990050728
Ara H., Jaiswal U., and Jaiswal V.S., 2000, Synthetic seed: Prospectus and limitations, Current Sci, 78(12): 1438-1444

Arunkumar M.B., Vakeswaran V., and Krishnasamy V., 2005, Enhancement of synthetic seed conversion to seedlings in hybrid rice, Plant Cell Tiss. Org. Cult. 8: 97-100

Bapat V. A., and Rao P.S., 1988, Sandalwood plantlets from synthetic seeds, Plant Cell Rep., 7: 434-436

Bekheet S.A., Taha H.S., Saker M.M., and Moursy H.A., 2002, A synthetic seed system of date palm through somatic embryos encapsulation, Annals Agril. Sci. Cairo., 47(1): 325-337

Bhattacharjee S., Khan H.A., Reddy P.V., and Bhattacharjee S., 1998, In vitro seed germination, production of synthetic seeds and regeneration of plantlets of Phalaenopsis hybrid, Annals. Agril. Sci. Cairo., 43(2): 539-543

Bhattacharyya R., Ray A., Gangopadhyay M., and Bhattacharya S., 2007, In vitro conservation of Plumbago indica - a rare medicinal plant, Plant Cell Biotechnol Mol. Biol., 8(1/2): 39-46

Bornman C.H., Dickens O.S.P., Merwe C.F.-Van-der., Coetzee J., Botha A.M., der-Merwe C.F.-van., and Van-der-Merwe C.F., 2003. Somatic embryo of Picea abier behave like isolated zygotic embryos in vitro but with greatly reduced physiological vigour, South African J. Bot., 69(2): 176-185

Brar D.S., and Khush G.S., 1994, Cell and tissue culture in plant improvement, In: Mechanisms in Plant Growth and Improved Productivity, Basra A (ed.), Mercel Dekker Inc., New York, USA., pp. 229-278

Brar D.S., Fujimura T., McCouch S., and Zapata F.J., 1994, Application of biotechnology in hybrid rice, In: Hybrid Rice Technology : New Developments and Future Prospects (Virmani, S.S. Ed.), International Rice Research Institute, Manila, Philippines, pp. 51-62

Brischia R., Piccioni E., and Standardi A., 2002, Micropropagation and synthetic seed in M.26 application root stock (II): A new protocol for production of encapsulated differentiating propagules, Cell Tiss. Org. Cult., 68(2): 137-141

Capuano G., Piccioni E., and Standardi A., 1998, J. Hortic. Sci. Biotechnol., 73: 299-305

Datta K.B., Kanjilal B., Sarker, D-de., and De-Sarkar D., 1999, Artificial seed technology, Development of a protocol in Geodorum densiflorum (Lam) Schltr. – an endangered orchid. Current Sci., 76(8): 1142-1145

Datta S.K., and Potrykus I., 1989, Artificial seeds in barley: encapsulation of microspore derived embryos, Theor Appl Genet., 77: 820-824

http://dx.doi.org/10.1007/BF00268333

Fabre J., and Dereuddre J., 1990, Encapsulation-dehydration: A new approach to conservation of Solanum shoot-tips, Cryo-Letters., 11: 413-426

Falcinelli M., Piccioni E., and Standardi A., 1993, Synthetic seed in crop plants: problems and prospects., Sementi Elette., 39(2): 3-13

Finner J.J., and Nagasawa A., 1988, Development of an embryogenic suspension culture of soybean (Glycine max Merrill.). Plnat Cell Tiss. Org. Cult., 15: 125-136

http://dx.doi.org/10.1007/BF00035754

Fujii J.A., Slade D., Aguirre Rascon J., and Redenbaugh K., 1992, Field planting of alfalfa artificial seeds, In Vitro Cell Dev. Biol., 28P: 73-80

Fujii J.A., Slade D., and Redenbaugh K., 1989, Maturation and green house planting of alfalfa artificial seeds, In Vitro Cell Dev. Biol., 25: 1179-1182

http://dx.doi.org/10.1007/BF02621271

Micheli M., Standardi A., Dell Orco P., and Mencuccini M., 2002, Preliminary studies on the synthetic seed and encapsulation technologies of in vitro derived olive explants, Acta Horticulutecae., 586: 911-914

Molle F., Dupius J.M., Ducos J.P., Anselm A., Cerolus S.I., Petiard V., and Freyssinet G., 1993, In: Synseeds, Redenbaugh, K. (ed.), CRC Press, Boca Raton., pp. 257-270

Onay A., Jeffree C.E., and Yeoman, M.M., 1996, Plant regeneration from encapsulated embryos and an embryogenic mass of pistachio (Pistaci vera L.), Plant Cell Rep., 15: 723-726

http://dx.doi.org/10.1007/BF00231933

Padmaja G., Reddy L.R., and Reddy G.M., 1995, Plant regeneration from synthetic seeds of groundnut (Arachis hypogaea L.), Indian J. Expt. Biol., 33: 967-971

Palmer C.E., Keller W.A., and Armison P.G., 1996, Utilization of Brassica haploids. In : In vitro haploid production in higher plants, Vol. 3: Important selected plants (Jain, S.M., Sopory, Veilleux R.E., (eds.), pp. 173-192

Palmer J.P., and Jasraie Y.T., 1996, Precocious growth and effect
of ABA: encapsulated buds of Kalanchoe tubiflora, J. Pl. Bioche. Biotechnol., 5(2): 103-104

Petiard V., Ducos J.P., Florin B., Lecouteux C., Tessereau H., and Zamarripa A., 1993, Mass somatic embryogenesis: a possible tool for large-scale propagation of selected plants, In: Proceedings of the Fourth International Workshop on Seeds: basic and applied aspects of seed biology, Agers, France, 20-24 July, 1992, pp. 175-191

Phnkajornyod P., Pawezik E., and Vearsaip S., 2004, Dry synthetic seed production and desiccation tolerance induction in somatic embryos of sweet papaya, Deutscher Tropentag, Berlin, October, 2004, pp. 5-7

Pintos B., Bueno M.A., Cuenca B., and Manzanera J.A., 2008, Synthetic seed production from encapsulated somatic embryos of cork oak (Quercus suber L.), and automated growth monitoring, Plant Cell Tiss. Organ Cult., 95(2): 217-225

Redenbaugh K., Nichol J., Kosseler M.E., and Paasch B.D., 1984, Encapsulation of somatic embryos for artificial seed production, In Vitro., 20: 256-257

Redenbaugh K., Paasch B.D., Nichol J.W., Kossler M.E., Viss P.R., and Walker K.A., 1986, Somatic seeds: Encapsulation of asexual plant embryos, Bio/technology., 4: 797-801

Roy B., 2002, Genetic studies on seed components and standardization of genetic transformation in rice, Ph.D. thesis submitted to Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India., pp. 235-246

Roy B., and Mandal A.B., 2002, Microtillering of androgenic plantlets in an elite indica rice variety IR 72, In : International Rice Congress, Beijing, China., pp. 293

PMid:12235263

Roy B., and Mandal A.B., 2004a, Anther culture in indica rice and variation in major agronomic characters in androclones of Karnal local. African J. Biotechnol., 4(3): 235-240

Roy B., and Mandal A.B., 2004b, Toward development of mapping population through anther culture and conventional recombination breeding for molecular tagging of salt-tolerant gene/s involving IR 28 and Pokkali, In: 9th National Rice Biotechnology Network Meeting, New Delhi, India., pp. 183-185

Roy B., and Mandal A.B., 2004c, Encapsulation of endrogenic embryos and pro-embryos for production of synthetic seeds in elite indica rice var. IR72, In: Abstracts, ISTA Congress Seed Symposium, Budapest, Hungary, May 17-19, 2004., pp. 38

PMid:14719653

Roy B., and Mandal A.B., 2006, Rapid and recurrent mass-multiplication of androgenic embryos in indica rice, Indian J. Biotechnol., 5(2): 239-242

Roy B., and Mandal A.B., 2008, Development of synthetic seeds involving androgenic embryos and pro-embryos in an elite indica rice, Indian J. Biotechnol., 7(4): 515-519

Ruffoni B., Massabo F., and Giovannini A., 1994, Artificial seed technology in ornamental species Lisianthus and Genista, Acta Horticulturae., 362: 277-304

Sakamoto Y., Mashiko T., Suzuki A., Kawata H., Iwasaki A., Hayashi M., Kano A., and Goto E., 1992, Development of encapsulation technology for synthetic seeds, Acta Horticulturae., 319: 71-76

Senaratna T., McKersie B.D., and Bowley S.R., 1989, Desiccation tolerance of alfalfa (Medicago sativa L.), somatic embryos. Influence of abscisic acid, stress pretreatments and drying rate, Plant Sci. Limerick., 65(2): 253-259

http://dx.doi.org/10.1016/0168-9452(89)90072-1

Senaratna T., Saxena P.K., Rao M.V., John Afele., 1995, Significance of Medicago sativa L. somatic embryos, Plant Cell Rep., 14: 375-379

http://dx.doi.org/10.1007/BF02624160

Sharma S., Kashyap S., and Vasudevan P., 2000, Development of clones and somaclones involving tissue culture, mycorrhiza and synthetic seed technology, J. Scientific Industrial Res., 59(7): 531-540

Shigeta J., and Sato K., 1994, Plant regeneration and encapsulation of somatic embryos of horse radish, Plant Science., 102: 109

http://dx.doi.org/10.1016/0168-9452(94)90024-8

Sicurani M., Piccioni E., and Standardi A., 2001, Micro-propagation preparation of synthetic seed in M.26
apple root stock I: Attempts towards saving labour in the production of adventitious shoot tips suitable for encapsulation, Plant Cell. Tiss. Org. Cult., 66(3): 207-216. 
http://dx.doi.org/10.1023/A:1010627720368

Siong P.K., Mohajer S., and Taha R.M., 2012, Production of Artificial seeds derived from encapsulated in vitro micro shoots of cauliflower, Brassica oleracea var. botrytis, Romanian Biotechnological Letters., 17(4): 7549-7556

Smidsrod O., and Skjak-Braek G. 1990, Alginate as immobilization matrix for cells, Trend Biotechnol., 8(3): 71-78. 
http://dx.doi.org/10.1016/0167-7799(90)90139-O

Sparg S.G., Jones N.B., Staden J-van., and Van-Staden J., 2002, Artificial seed from Pinus patula somatic embryos, South African J. Bot., 68(2): 234-238

Suprasanna P., Bharati G., Ganapathi T.R., Bapat V.A., 2002, In vitro development of encapsulated somatic embryos in rice, Trop. Agric. Res. Extension., 5:76-78

Suprasanna P., Ganapathi T.R., and Rao P.S., 1996, Artificial seed in rice (Oryza sativa L.): Encapsulation of somatic embryos from mature embryo callus culture, Asia Pacific J. Mol. Biotechnol., 4(2): 90-93

Taha R.M., Saleh A., Mahmad N., Hasbullah N.A., and Mohajer S., 2012, Germination and plantlet regeneration of encapsulated microshoots of aromatic rice (Oryza sativa L. Cv.MRQ 74), The Scientific World Journal, 2012: 6

http://dx.doi.org/10.1100/2012/578020 
PMId:22919338 PMCid:PMC3419424

Takahata Y., Wakui K., Kaizuma N., and Brown D.C.W., 1992, A dry artificial seed system for brassica crops, Acta Horticulturae., 319: 317-322

Toruan Mathius N., and Sumaryno., 1994, Application of synthetic seed technology for mass clonal propagation of crop plants, Bulletin Bioteknologi Perkebunan., 1(1): 10-16

Vicent C.M., and Martinez F.X., 1998, The potential uses of somatic embryogenesis in agroforestry are not limited to synthetic seed technology, Revista Brasileira de Fisiologia Vegetal., 10(1) : 1-12

Xing X.H., Shen Y.W., Gao M.W., Yin D.C., Xing X.H., Shen V.W., Gao M.W., and Yin D.C., 1995, Studies on production of artificial seeds of rice hybrid between indica and japonica, Acta Agronomica Sinica., 21(1): 45-48

Zhang M., Wei Xiao Y., Huang H.R., Zhang M., Wei X.Y., and Huang H.R., 2001, A study on the solid emasculation system of the artifical seed of Dendrobium candidum, Acta Horticulturae Sinica., 28(5): 435-439

Zhang Y.F., Yan S., and Zhang Y., 2011, Factors affecting germination and propagators of artificial seeds of dendrobium candidum. International Conference on Agricultural and Biosystems Engineering, Advances in Biomedical Engineering Vols., 1-2, pp.404-410