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Artificial seed production in seedless plants

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Abstract
Artificial Seed Production in Seedless Plants has opened out entirely new approaches for plant improvement. Improving Plant Productivity is very important as several biotic and abiotic constraints affects its cultivation and Production. We can demarcate artificial seeds by way of encapsulation of somatic embryos, shoot bud, cell aggregates of any tissue (artificially) which has the competence to mature into a plant in *in vitro* or *in vivo* circumstances. They are also frequently called synthetic seeds or synseeds. In this Paper, how artificial seeds are produced, that is, the process for the making of artificial seeds in seedless plants is fully described. A review of the available research literature indicates that the performance of plants derived from artificial seeds has been encouraging with Increase in yield, Early Maturity and quality fruits.

Keywords: Seedless plants, embryos, early maturity and quality fruits

Introduction
Artificial seeds, as a new correspondent to the plant seeds were initially described by Murashige, in his definition of artificial seeds, he mentioned them as encapsulated single somatic embryo [1, 2]. For plants, which are not proficient to produce feasible seeds, the production of artificial seeds is extremely beneficial because with the help of artificial seeds, these plants can proliferate [3, 4]. Due to the small size of artificial seeds, they embrace several compensations in storage, conduct and transport [2, 3]. Hays and Garber in 1919 presented the practice of artificial seeds in commercial crops. They both used synthetic seeds in Maize.
There are two types of artificial seeds which are usually produced [4, 5, 6].

1. Dessicated synthetic seeds: Undissected artificial seeds, there is involvement of encapsulation of multiple somatic embryos and this step is followed by desiccation the material that is used for encapsulation during this case is Polyoxymethylene [4]. This material has various significant points, it prohibits the development of microorganisms which means it is disease resistant and also towards embryos, it does not have any toxic behaviour [7].
2. Hydrated synthetic seeds: The procedure for producing hydrated artificial seeds includes the encapsulation of the somatic embryos in hydrogels. The most frequently used hydrogels are: Potassium alginate, Carrageenan, Sodium alginate, Sodium pectate or Sodium alginate with gelatin [6, 8].

Now, if we compare these two categories of artificial/synthetic seeds, the one type that appears more likely to form synseeds is desiccated artificial seeds. The seeds that have more resemblance with true seeds are produced.

Advantages of producing artificial seeds
- They are easy to handle.
- Artificial seeds have the capability for short as well as long term storage [4, 11].
- Genetic homogeneity.
- The production of artificial seeds is not dependent on the environmental conditions. They can be produced at any time of the year or throughout the year.
- The production of artificial seeds is cost effective. For producing synthetic seeds cheap quality plants can be used.
- Germplasm preservation [12].

Material required for the production of artificial seeds
- The material that is primarily required for producing artificial seeds is the highly eminent dynamic somatic embryos.

Their function is to yield plants in more numbers as compared to natural seeds [4].

- With the synchronal maturation, better quality somatic embryos are formed at very low cost [13, 14].
- Now, for the transport of somatic embryos, better encapsulation and coating systems are very important and they do not stop the growth of artificial seeds.
- After the formation of the synthetic seeds, commercialization is very important.

What is the need for producing artificial seeds?

- Because of the technique of micropropagation, huge number of desired plant species can be formed.
- The technology of developing artificial seeds is presently considered as an operative and competent way of propagation in various significant crops [15].
- The production of synthetic seeds is also very important because they will be a canal in the direct transfer of novel plant lines that are produced through the developed biotechnology to a field or greenhouse.
- Somatic embryos are capable of producing large number of plants by the technique of micropropagation and artificial seeds can be stored for short and long term [16]. They also have less expensive transport. All these points open new approaches for clonal propagation in various commercially important crops of agronomy and horticulture [17].

Table 1: Comparison of natural and artificial seeds

Natural Seeds	Synthetic Seeds
Hard seed coat is present in natural seeds.	These seeds undergo encapsulation and have no hard coat.
Cotyledons or endosperm enclose the embryo.	There is no maternal tissue in synthetic seed for the protection of embryo.
Natural embryos undergo desiccation and a natural dormancy period.	Synthetic seeds do not undergo desiccation and a period of dormancy.
Endosperm or cotyledons are storage reserves of the natural seeds which provides food during the process of germination.	There is no tissue present in synthetic seeds for storage. But within the encapsulating material growth regulators can be supplied.

In artificial seeds, by the process of somatic embryogenesis, an embryo is produced. The artificial medium enclose this embryo. This medium is capable of supplying nutrients for germination and is wrapped in artificial seed layer. In the year 1982, Kitto and Janickformed the initialartificial seeds by the use of somatic embryos of carrot.

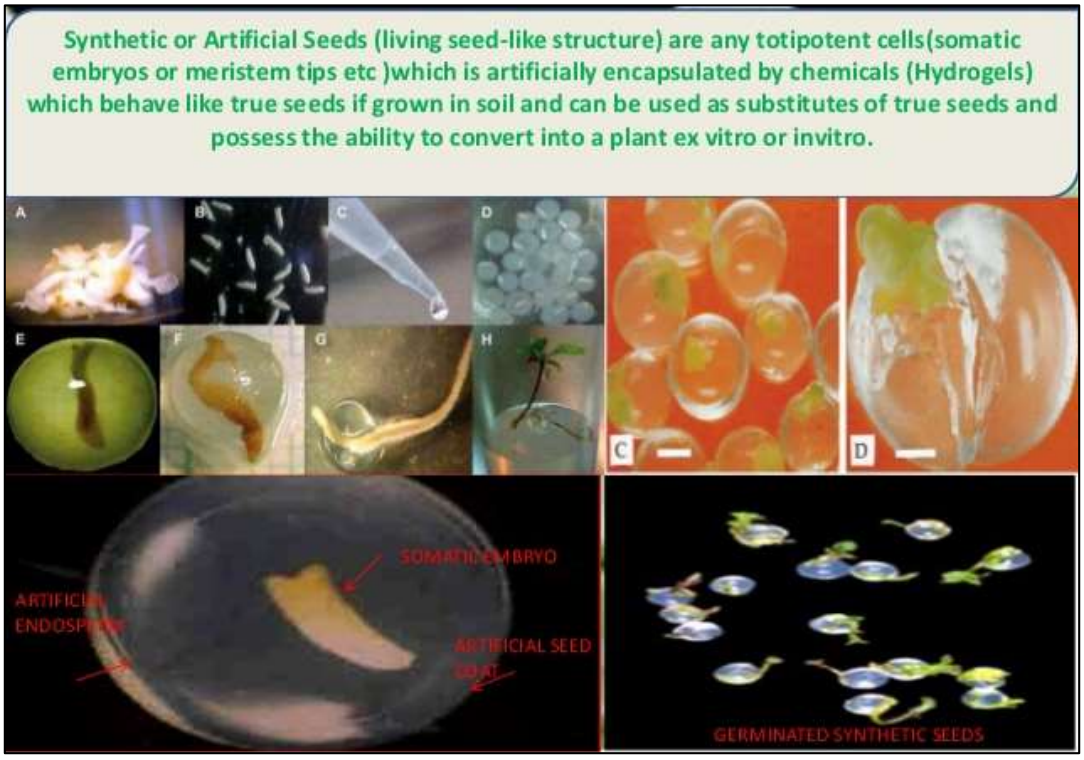

Fig 1: Artificial seed production

Methods of producing artificial seeds

Dropping method: In this method, hydrogels are used. Sodium alginate is the most common one. For the encapsulation of somatic embryos, they must be dipped in hydrogel.

Molding method: In molding method, a simple procedure is followed. In this, temperature dependent gels are used (such as agar) and embryos are mixed in these gels. At low temperature, coating of cells with gel takes place.

Table 2: In this table, materials required for producing artificial seeds and methods implied in this paper to explain the technique is mentioned:

Name of the plant species	Materials required	Methods
Banana (*Musa*) Family - Musaceae	Shoot of banana (extracted from shoot culture), sodium alginate solution, distilled water, MS medium, activated charcoal, antibiotic mixture, 1 mg/L NAA or 5 mg/L BA, salt solution, white's media.	As method, the literature review as a evaluating look at the available research that is operative to the field, was used.
Grapes (*Vitis vinifera*) Family – Vitaceae	Medium encompassing abscisic acid (ABA) for somatic embryo to mature, explant, sodium alginate (in most cases), synthetic endosperm containing Gibberellic acid.	As method, the literature review as a evaluating look at the available research that is operative to the field, was used.
Pineapple (*Ananas comosus)* Family - Bromeliaceae	Explant(small shoot), 3% sodium alginate, hormone-free Murashige basal medium of skoog, vitamins of murashige and skoog, 0.56 mm myoinositol, 0.06 M sucrose, medium with 0.8% of agar and different amounts of naphthalene acetic acid and indole-3 butyric acid.	As method, the literature review as a evaluating look at the available research that is operative to the field, was used.
Citrus jambhiri (Rough lemon) Family - Rutaceae	MS medium fortified with 1 and 2 mg/L of BAP (6-bezylamino purine), nodal segments are preferred over shoot tips, 2-5% sodium alginate, 3% calcium chloride solution, MS medium fortified with 1 mg/L of BAP, unipolar explants.	As method, the literature review as a evaluating look at the available research that is operative to the field, was used.
Rauvolfia tetraphylla Family – Apocynaceae	Nodal segments, aqueous solution(containing 5% labolen), sterile distilled water, Murashige and skoog medium with 10 micro metre NAA, 3% sucrose, 0.8% agar, sodium alginate and CaCl2.2H20 solutions are prepared by using MS liquid medium, petri dishes containing growth regulator free MS basal medium, 3% sucrose with suitable amounts of BA and NAA, 250 ml culture flask.	As method, the literature review as a evaluating look at the available research that is operative to the field, was used.
Carrot (*Daucus carota*) Family - Apiaceae	Cell suspension culture from which somatic embryos are extracted, alginic acid for encapsulation, ensuring rehydration is important for germination, abscisic acid(ABA).	As method, the literature review as a evaluating look at the available research that is operative to the field, was used.

Synthetic seeds in Banana (*Musa*)

Banana have more than 300 varieties. But out of these only limited are considered as commercially important kinds for humans, banana is most valued fruit crop. It is heavily used all over the world due to its nutritive worth [18]. Banana is rich in carbohydrates [22.2%], fibre, protein, water [75.7%] with a lesser amount of fat. About 95 million tons of banana is produced worldwide.

In our country also, banana is the most vital fruit crop. It is grown in 4.3 million hectare in India. The over-all production of banana in India is 13.9 million tons [18, 26].

Method of producing

In the initial phase, there must be establishment of shoot cultures in *In vitro* conditions. This is to extract numerous shoot tips [19]. The shoot tips, when placed in the liquid media can display excessive elongation. And when they are transferred to rooting media, under the span of 4 weeks complete plantlets will be formed. After another 4 weeks, the plantlets that are transferred to the greenhouse in polybags can show decent growth with their emerging leaves [18, 20].

First step for producing artificial seeds involves the encapsulation of tips of shoot which are extracted from the banana's shoot cultures [19, 20]. They should be encapsulated in sodium alginate solution. This solution can be prepared in distilled water or in MS medium with activated charcoal [0.1%] and a mixture which is antibiotic.

On MS or white's media, the shoot tips which are encapsulated should be placed with 1 mg/l NAA or 5 mg/l BA. For germination, they can also be placed on some other substrates such as soil, filter paper [they should be moistened with $1/4^{th}$ salt solution] [21]. In all these, white's medium is considered more effective because in this case, multiple plantlets are produced under one week. On white's media, the development of plantlets occur more rapidly as compared with MS media and other substrates [18, 21].

The conclusion suggests that for a single shoot to be encapsulated 1 ml of medium is adequate as compared to the requirement of 15 to 20 ml for the alteration of shoot tips into plantlets. So the shoot tips that are encapsulated can be held as a seed and are useful in lowering the production cost [22, 23]. When encapsulated shoot tips are directly sown in the soil, we can remove two steps of the process namely-rooting and hardening. For various important purposes such as exchange of germplasm, preservation and transference encapsulated shoot tips serves as the reasonable, easier and innocuous material.

Because banana is central fruit it has various research involvements in the field of molecular and cellular biology [26]. To get genetic variability, application of mutation by using several mutagens such as chemical and physical is greatly effective [24]. For the production of artificial seeds, somatic embryos, produced from somatic embryogenesis serves as an supreme arrangement [20]. To prohibit the expansion of fungal diseases in banana, use of fungal disease resistant genes will be effective and novellines of banana will be developed with the resistance of diseases [25]. The export of banana from developing countries like India can be increased by the use of antimicrobial peptides because by using them quality of fruit can be enhanced and also there is option of delayed ripening of fruit [18, 26]. Also, banana is impactful in the field of medicine, that is why it is an important factor in health-care for developing countries.

Artificial seeds in Grapes (Vitis vinifera)

Mainly grapes are of two types: table grapes and wine grapes. Table grapes are mainly used for eating purposes [27]. They are larger in size. The pulp of these grapes is thick and skin is thinner. In comparison with wine grapes, these grapes are less acidic and also a lesser amount of sugar. Table grapes are more physically tempting.

Wine grapes are the type of grapes which have seeds. They are smaller in size and are sweetest in taste [28]. Unlike table grapes, wine grapes have thick skin and high juice content. Due to their gentleness, they are hardto transport.

Method of producing

Highly eminent somatic embryos are must for the application

of artificial seed technology. Somatic embryogenesis has been reported in various important species but because somatic embryos do not undergo the phase of 'embryo maturation', the quality of these has not been excellent [29, 39]. Embryo maturation is the last phase of embryogenesis [30]. Now, for the somatic embryo to mature, following steps are followed:

Transfer to the media that contains less concentrated 2,4-D. This step is very crucial.

The maturation is achieved by the transferring the embryo to a medium encompassing Abscisic Acid (ABA). It is reported in several species that ABA promotes the maturation of embryo [31, 39].

After the formation of somatic embryos, encapsulation is another important step. Encapsulation is vital for the formation of synthetic seeds [32]. Basic requirements for encapsulation to take place: Explants, Encapsulating agents (Sodium alginate is the most accepted and most frequently used hydrogel), and Synthetic endosperm [34, 35]. The whole procedure of encapsulation must be done under aseptic conditions [39].

In recent times, many existing crops are seedless. Encapsulated somatic embryos are responsible for the propagation of seedless varieties of Vitis vinifera. The plantlets regenerate from the somatic embryo encapsulated in sodium alginate [34, 35, 39].

Antonietta et al., 1999 stated that those somatic embryos exhibit great capability of Plantlet conversion which are encapsulated with an artificial endosperm that contains Gibberellic acid [31].

It is also reported that frequent development of roots and plantlet conversion can be achieved by accumulation of thiophosphate-methyl in the artificial endosperm. This will also increase the level of sprouting [39].

The consequence of different storage circumstances on encapsulated somatic embryo and non- encapsulated somatic embryos was studied by the Antonietta et al. (20007b) and Singh et al. (2007) [33].

Thompson grapes (these are the seedless grapes, which are white in colour and mainly grown for raisins), Flame seedless, Concord and Ruby seedless are some important seedless varieties of grape [27, 28].

Grapes are enriched with Vitamin C, Vitamin K, Vitamin B6, fibres and proteins. They are very common and widely consumed all around the world in various different forms. They are advised for the patients of heart diseases, high blood pressure and chronic disorders [36, 37, 38]. They also contain important plant compound which are helpful in the prevention of different types of cancer.

Artificial seeds in Pineapple (*Ananas comosus*)

Pineapple (*Ananas comosus*) is also very old and vital plant for men, just like Banana. It is naturally occurring seedless fruit. Pineapple plant has a spike like inflorescence. It wears a crown of leaves on top. Pineapple is not a false fruit. The spike of pineapple have around 200 flowers. In normal conditions, pineapple takes around 2 years to grow [40].

Method of producing

In *In vitro* conditions, from latent, axillary buds of pineapple clumps of multiple shoots are produced. Now, these small shoot in approximately 2.5 mm in size should be encapsulated in 3% sodium alginate. These small shoots are extracted from the tuft of multiple shoots [41, 45]. The sodium alginate solution is prepared by using hormone-free Murashige basal medium of Skoog, and vitamins of Murashige and Skoog along with the 0.56 mm myoinositol and 0.06 M sucrose is used [43].

The shoot that are encapsulated represents artificial seeds and in *In vitro* conditions, they germinate and form roots [42, 50]. For this, they need to be subculture on any one of the following media with 0.8% of agar [41, 43]:

1. The first media that can be used is hormone free Murashige and basal medium of Skoog, Murashige and Skoog's vitamins can be used along with 0.56 mm myoinositol and 0.06 M sucrose [41, 50].
2. The second media that can be used for synthetic seeds to germinate is basal medium of Skoog and Murashige. The vitamins of Skoog and Murashige along with myoinositol (0.56mm), sucrose (0.06M), 1-nepthalene acetic acid, Indole-3-butyric acid and kinetin [50].
3. Another media that is used for the germination of artificial seeds is White's basal medium, White's vitamins, 0.56mm myo-inositol, 0.03 M sucrose and different amounts of napthelene acetic acid and indole-3 butyric acid [41, 50].

For the development of plantlets along with roots from encapsulated shoots, Pre-treatment of these shoots in third media is essential after culturing on either first, second or third media [46, 44].

If pre-treatment of shoots in third media is given for approx. 12 hours after the culturing of artificial seeds on second media, there are 100% chances of germination of artificial seeds to plantlets with roots [49, 47].

Artificial seeds can remain feasible without sprouting for about 40 days, if they are stored at 4 degree Celsius temperature [48, 44]. The plantlets that developed from artificial seeds in *In vitro* conditions can be efficaciously established in Soil [47].

In the field of medicine, Pineapple is amongst the most valuable and popular fruits. It is full of nutrients and various other plant compounds that are essential. It helps in the improvement of digestive system and also boosts our immunity.

Seedless Watermelon

Watermelon is very common and favoured fruit during summers. The vines of watermelon grow on the ground and tendrils are branched [51, 52]. Leaves are with deep cuts. On the leaf axil, flowers borne signally. This plant belongs to the family Cucurbitaceae. Watermelon is cultivated all around the world and mainly eaten in raw form.

Method of producing

About 50 years ago seedless watermelons were produced for the first time with no mature (black ones) seeds at all. Chromosomes are the ones that provide characters/traits to all living organisms. Same is the case with seedless watermelons. Seedless watermelons actually grow from a seed. Now, the question is how this seed is formed?

When a diploid plant (a plant with two set of chromosomes) is crossed with a tetraploid plant (a plant with four sets of chromosomes), a fruit will be formed that will produce a triploid seed (it will have 3 sets of chromosomes). Seedless watermelon is obtained from this triploid seed [53].

All this can be explain in other words also; seedless watermelon is obtained by the crossing of male pollen of a watermelon which contains 22 chromosomes in each cell with female flower of watermelon having 44 chromosomes in

every cell.
A seeded fruit is formed, and when this fruit matures, a small seed coat that is white in colour and contain 33 chromosomes make it sterile and incompetent to yield seeds.
Most important and interesting thing about seedless watermelon is that it is still needed to be pollinated by other seeded parents [53]. So, they are grown in the same field with other seeded plants more oftenly by the cultivators. Another important thing about seedless watermelon is that they are not made by genetic modifications.
Seedless watermelons hold great commercial value in comparison with the seeded watermelons, as 92% of all the sales are of seedless watermelon and remaining 8% is of seeded watermelons.
Like every other fruit, watermelon serves a lot of benefits. In this fruit, water content is 91%, sugar content is 6% and very low amount of fat [54]. Vitamin is also present in good amount [55]. The pulp of the watermelon contains carotenoids which also includes lycopene [56, 57]. Watermelon can also be eaten as a vegetable, stew and also as a pickle [58]. It is used in various different ways by the people of different regions [59, 60].

Seedless Breadfruit
Breadfruit is an important and very popular tropical fruit. There are both seeded and seedless varieties of breadfruit. It grows very fast and is capable of producing over 200 fruits in one particular season [62]. Scientific name of breadfruit is *Artocarpus altilis*. It belongs to the family Moraceae.
Breadfruit is native to the countries like Malaysia, Indonesia and Philippines. It is considered as a nutrition packed fruit in countries such as Australia, Hawaii, South America.
Seedless breadfruit usually have an oblong, hollow core. Hairs and tiny, flat, immature
Seeds measuring about 3mm in length are found in this core of the breadfruit. The seeds present here are sterile. Breadfruit reaches the height of 85ft (26m) and is 2 to 6 ft (0.6 to 1.8m) in width [64]. Multiple small flowers are also present on this plant.

Seedless varieties of Breadfruit
- Sici Ni Samoa: sicinisamoa is highly recommended variety of seedless breadfruit. It is oval in shape with the length of 12.5 to 15 cm and 9 cm wide. It is highly recommended Samoan variety.
- KuluDina: This seedless variety of breadfruit is round shape with 7.5 to 10 cm length. There is no need to peel this variety after boiling. It is highly recommended for its various benefits.
- Balekana Ni Vita: Balekana Ni Vita is the seedless variety with round shape. It is 9 to 10 cm long and does not decline quickly.
- KuluMabomabo: This is oval in shape with length of 15 to 20 cm and width of 10 to 14 cm. This variety is completely seedless.

The breadfruit is highly recommended because in many countries a decoction of the leaf of breadfruit is believed to be helpful in controlling blood pressure and also applicable in asthma. Also for the treatment of thrush, crushed leaves of breadfruit can be applied on the tongue. Adding to this, the juice of the breadfruit's leaf is used as ear drops in many regions of the world. Ashes remains after the burning of leaves of breadfruit can be used on various skin infections effectively. If you have tooth ache, toasted flowers of breadfruit can be very helpful. The pain will disappear if you rub them on your gums [63, 64]. The latex is also used on various skin related diseases and also as a bandage for the spine.

Propagation
The seedless breadfruit is mainly propagated by the transplantation of suckers. These suckers grow naturally from the roots. By exposure or causing injury to a root, suckers can be induced [64]. The number of suckers can be increased by the pruning of the parent tree and before transplantation root pruning of each sucker multiple times for months will be very helpful in increasing its survival after transplantation. For multiplication to occur in quantity, preferred root cuttings are of 22 cm in length and 2.5 to 6.35 cm in thickness. For root cuttings to coagulate the latex, they must be dipped in a potassium permanganate solution. After this, the cuttings should be planted in horizontal lines, close to each other. The plantation must be in shady area and until it is possible to apply intermittent mist, they should be watered on daily basis [64].
Rooting time of breadfruit can vary from 2 to 5 months while calluses is formed in about 6 weeks. Now, the cuttings are transplanted to pots and until the plants are of 2 feet in height they must be watered 2 times a day. Another method of rapid propagation is also available. In this case, stem cuttings are taken from the root's shoot and are transplanted into plastic bags. The plastic bag is full of mixtures of soil, peat and sand [64]. This should be kept under mist for a week, after this under 65% shady conditions with proper liquid fertilizer and daily watering. After the complete development of root systems, they are allowed to full sun and after some time should be planted out in the field.

Citrus Species
Citrus plants includes various varieties of orange, lemon, lime, pomelons etc. These plants belong to the family Rutaceae. Their size can vary from very large too small. Their shoots are spiny and margined leaves are alternately arranged [67]. Corymb inflorescence is shown by the flowers. Essential oil glands are present in citrus fruits that's why they have strong scent [68]. For the production of high quality and healthy plants in citrus species, *In vitro* propagation by somatic embryogenesis is surely an efficient alternative method. The somatic embryos formed by the somatic embryogenesis need protection from any kind of mechanical damages.
The damage can be caused during their manipulation and transport. Also, after sowing, growth of plantlets is very important so for that, nutritive support should also be provided. In encapsulation technology, somatic embryos are covered with Calcium alginate. It is a gel, full of nutrients [74]. This technology allows the formation of artificial seeds.
The citrus plants are also used as ornamental plants. They are prone to various deficiency diseases. Chlorosis is the main deficiency condition developed by them. Chlorosis involves the yellowing of leaves [70]. If excessive amount of salt is present in the soil, citrus plants are sensitive to that. To diagnose nutrient deficiency in citrus fruits, soil testing is very important [71]. All important and common citrus fruits are eaten fresh [69]. Various types of flavours can be derived from their different partsand also by providing necessary treatment [69]. In some species of citrus, good amount of phytochemicals are also present [71, 72].

Citrus jambhiri

Common name of this citrus species is Rough lemon. It is also have various other names also- Jambiri, jambheri, jhamirdi, jatti-khatti, khatti. Several cultivars of rough lemon can be used as a citrus rootstock. This includes Florida [75], Schaub [76], and Vagassay rough lemon [77]. The plant is of medium size and produces globose rough fruits. *Citrus jambhiri* fruits have a tough peel and a thick rind that is somewhat adherent to the flesh and is moderately adherent in the segments that resemble lime segments. It is mainly used as citrus rootstock but in the NW India, where it is cultivated frequently, it is often made into pickels. It is adaptable to light sandy conditions and can also tolerate salty conditions. However, as a rootstock, it shows sensitivity to cold. The juice of rough lemon fruits is sharply acidic. Use of fresh, raw *Citrus jambhiri* is not good for health. Doctors approved the juice that is concentrated by heating.

Method of producing

For the propagation, tools of biotechnology are used. For the development of plant from the pre-existing meristemaic tissue, encapsulation of shoot tips is needed. For the mass multiplication of plantlets of *Citrus jambhiri* from nodal segments in *In vitro* conditions [79], MS medium fortified with 1 and 2 mg/L of BAP (6-bezylaminopurine) is considered suitable. For the multiplication of plantlets in *In vitro* conditions, nodal segments are considered more appropriate than shoot tips. 2-5% sodium alginate is dropped into 3% calcium chloride solution to prepare artificial seeds. By culturing beaded shoot tips on MS medium fortified with 1 mg/L of BAP, maximum germination can be obtained. However, as compared to the earlier findings, germination of artificial seeds is quite high. For the production of synthetic seeds, use of encapsulated unipolar explants is highly supported by the results.

They can be useful in exchange of sterile material between the laboratories, also for the Conservation of germplasm and direct propagation of the plant [78].

Rauvolfia tetraphylla

Plant *Rauvolfia tetraphylla* belong to the family Apocyanaceae. The tree is small in size. Be-still tree or devil pepper are common names of this plant. The fruits of *Rauvolfia tetraphylla* are called devil peppers [80]. In the traditional medicinal system they have a great importance. Many branches are present on the plant *Rauvolfia tetraphylla*. It has various medicinal uses and also is a source of dyestuff and ink. *Rauvolfia tetraphylla* was introduced to countries like India, China and Vietnam as the substitute of Rauvolfia serpentine in medicinal use. It was experimentally cultivated. In traditional medicine in South America, all parts of the plant such as latex, root, leaves, bark and fruit are used. In India, for medical purposes, roots of *Rauvolfia tetraphylla* are used frequently. The latex of the plant *Rauvolfia tetraphylla* has various benefits in medical field. It is cathartic, diuretic, emetic and expectorant. In the treatment of several disease such as dropsy it is very effective. *Rauvolfia tetraphylla* has many other important applications also. In the country Guatemala, it is very common to use this plant as a cure for malaria and also as an effective remedy for snake bites. Chal cupine A and B are two alkaloids which are present in *Rauvolfia tetraphylla*. In formation of Allopathic medicines, reserpine alkaloid is commonly used. This alkaloid is contained by the *Rauvolfia tetraphylla*. Due to this reason, *Rauvolfia tetraphylla* is an important commercial crop. Another alkaloid-deserpidine is contained by the roots of *Rauvolfia tetraphylla*. Apart from various medicinal uses, some other impacts of the plants are also here- From the fruit of *Rauvolfia tetraphylla*, black dye is obtained. Also, juice of the fruit of *Rauvolfia tetraphylla* is employed as as a substitute for ink [81].

Method of producing

In the field of micropropagation, making of artificial seeds by encapsulation technique is an important asset now [87, 88]. Vegetative propagules are encapsulated in *In vitro* conditions for the formation of synthetic seeds. For the initiation of *In vitro* culture, nodal segments of mature plants of *Rauvolfia tetraphylla* are used [82, 83, 84].

For the use, nodal segments needs to be washed with running tap water for the period of 1 hour [85, 86]. After this they should be immersed in aqueous solution (containing 5% labolen) for 5 minutes and then the last step includes rinsing with sterile distilled water. According to Faisal and Anis [84], on Murashige and Skoog [91] medium, sterile explants are cultured. The medium contains 10 micro metre NAA, 3% sucrose and 0.8% agar. Before autoclaving, for all the experiments, Ph of the medium should be 5.7. Autoclaving is done at 121 degrees for 20 minutes [89, 90].

Now, the shoots that are developed from the nodal segments are now transferred to the fresh medium for establishment of flourishing cultures of shoot. By using MS liquid medium sodium alginate is prepared in various different concentrations. In the MS liquid medium $CaCl_2.2H_2O$ solution is also prepared for complexation. After this, autoclaving of both the solutions-sodium alginate and $CaCl_2.2H_2O$ is done at 121 degrees for 20 minutes and Ph is adjusted to 5.7. By mixing the nodal segments into the alginate solution and then dropping it into the $CaCl_2.2H_2O$ solution, encapsulation is considered accomplished. They are held like this for about 30 minutes to achieve the polymerisation of sodium alginate. After that, beds of alginate are collected and for the removal of $CaCl_2.2H_2O$ they are washed with sterile distilled water. Now, they can be cultured on some different medium [94].

To increase sprouting in *In vitro* conditions, plantation of encapsulated nodal segments into petri-dishes is important. Petri dishes should contain the following media: Growth regulator free MS basal medium and 3% sucrose with suitable amounts of BA and NAA. Now, they are transferred to the 250ml culture flask after the development of shoot. The medium contained by flask should be same on which shoots are developed. After this, the same steps must be followed-gelling of the medium, adjustment of Ph to 5.7 and then autoclaving at 121 degree Celsius temperature [94]. When this step is completed, cultures are maintained at suitable conditions.

Before their transfer for regeneration, nodal segments (encapsulated) should be stored at 4 degree Celsius temperature. The time period of storage at this temperature, that is suitable to obtain maximum regeneration is of 6 weeks. In results, because of the different combinations of Sodium alginate and $CaCl_3.2H_20$, the encapsulated beds are morphologically different. They show differences in their texture, form and transparency. Another important thing is that for encapsulation, preparation of sodium alginate at high temperatures is not effective, because during the process of autoclaving it is exposed to very high temperature conditions which resulted in the reduction of its gelling [92, 93].

Carrot (*Daucus carota*)

Carrot belongs to the family Apiaceae. In Afghanistan, around 900 AD, carrots were first grown. They are root vegetables. Best known colour of carrot is orange but along with orange, there are many other colours in which they come, such as-yellow, white, red and purple. Depending upon their colour, size and the area in which they are grown, their taste differs. Carrots contain sugar, due to this they have slightly sweet taste. They have a great nutrition value, half cup of carrot contains carbohydrates (6 grams), 25 calories, fibre (2 grams), sugar (3 grams), and protein (0.5 grams).

Along with this, they are also rich in Vitamin A, Vitamin K and Vitamin C. Potassium, Calcium, Iron is also present in them in good amount. Because of this, various health benefits also come with carrots-they are effective in controlling diabities, because of the presence of vitamin K and calcium, bone health can be improved by having them in your meal. As vitamin A is present, so have good effects on eyes. Carrots also contain potassium, which keeps blood pressure in check [95]. Carrots are one of the most effective, important and desirable vegetable.

Method of producing

Carrots do not have true seeds, they have dry fruits called Schizocarps. So artificial seed production is carried out: For somatic embryos to be used as artificial seeds, they are extracted from cell-suspension cultures of carrot and then encapsulated with alginic acid. Germination of freshly encapsulated somatic embryos can be seen when they are stored at 4 degree Celsius temperature. For the germination of encapsulated embryo, sufficient water is retained by the hydrated capsule. But when air-blowing dehydration at 80% water loss is provided to hydrated capsule, germination does not take place. By providing ensuing rehydration, 97% of the capsules which are dehydrated by 88% water loss, are capable of germination. Capsules that are dehydrated by 95% water loss, their germination can also be increased. This can be done by treating somatic embryos with abscisic acid (ABA), 10 days before the encapsulation. This step should be followed by the rehydration. Germination of hydrated capsules is also increased by 31% when concentration of ABA is increased to 0.13 mg L. After being stored for 10 days at 4 degree Celsius temperature, on suitable rehydration, germination of 68% of the capsules dehydrated by 92% of the water loss can be seen [96].

From these results, indicated thing is that as compared to hydrated seed, dehydrated capsule in which somatic embryo is present, has more resemblance towards natural seed in terms of form and function.

Various applications and benefits of artificial seeds are mentioned earlier in this paper, but there are some drawbacks also:

- In several crops which are propagated by means of artificial seed technology, high levels of soma-clonal variations are reported, and there is no understanding of any method or process for the reduction in variations [98].
- Along with vigor, quality of embryo is the biggest concern in this technology.
- In various important commercial plants, controlled, well defined embryogenesis is missing.
- Another important challenge is to store and maintain viability of somatic embryos for long period of time [97]. It is due to their improper maturation.
- In synthetic seeds, period of dormancy is absent or very less, which results in the improper maturation of somatic embryos and also several storage difficulties are also originated. Synthetic seeds are less tolerant to the adverse conditions.
- The value of artificial seeds and the technology of production of artificial seeds is significantly affected by the low conversion rates of normal somatic embryos to plantlets.
- In the practical use of artificial seed technology main disadvantage is that when synthetic seeds are sown directly in soil or other commercial substrates (compost, vermiculture etc.,) in non - sterile conditions, various difficulties are present [99, 100].

Conclusion

Surely, artificial seeds create rising propagation system. Technique of artificial seeds have a lot of benefits. Delivery system in this technique is economical, plantlets are inexpensive, and in micropropagation this technique proposes marvellous potential. Direct use of artificial seeds in in-vivo circumstances, that is, conservation of germplasm through cryopreservation is promised by this technique. There are various aspects of artificial seed technology by which crop worth can be increased, this comprises cloning of elite genotypes, such as genetically engineered varieties that can-not produce true seeds. The technique of production of artificial seeds is very advance but in the first step, it is always dependent on the plant species.

However, in non-embryogenic artificial seeds, for the improvement of root formation, further research is required. Artificial seed's cryopreservation capacity has scope for improvement. In some plant species, for this further detailed research is desirable. Additional studies are looked-for the enhancement of artificial seeds to be cultivated on commercial substrates and in the occurrence of non-sterilized surroundings.

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