Scientific Papers. Series B, Horticulture. Vol. LXIV, No. 1, 2020
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE USE OF ARTIFICIAL SEED TECHNOLOGY IN THE PRODUCTION OF HORTICULTURAL PLANTS (REVIEW)

Hadi Hoobi SHALLAL[1, 2], Florin STĂNICĂ[1], Adrian George PETICILĂ[1], Ana Cornelia BUTCARU[1], Cătălina Ioana NICOLAE[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Middle Technical University, College of Applied Arts Iraq, Baghdad

Corresponding author email: hadi.hoobi@yahoo.com

Abstract

Artificial seeds technology allows to produce in large quantities, in vitro somatic embryos for seedless plant that share some similar properties of natural embryos, and can be used for in field propagation of selected plant species that have commercial value. Somatic embryos are the primary plant material for the production of synthetic seeds. This technology contributes to supply genetically homogeneous seeds, unlike traditional hybrid seeds, obtained from a gametic process which are known to produce plants that are different from the characteristics of the parent plants. They simply encapsulate the somatic embryos with a mixture of nutrient gel that contains carbon, organic and inorganic salts, vitamins, hormones, and antimicrobials to protect embryos from damage. It also allows the growth and germination to happen without unwanted differences. This study inquires the artificial seeds technology and their importance in facing the actual challenges of plant production. Previous studies have been reviewed for a better understanding of the field of artificial seed production.

Key words*: in vitro, sodium alginate, somatic embryos, tissue culture.*

INTRODUCTION

Three horticultural plant species: potato (*Solanum tuberosum* L.), fig (*Ficus carica* L.) and Chinese jujube (*Ziziphus jujuba* Mill.) have been nominated for this study due to their economic importance and high requirement by many countries.

One of the main objectives of the study is the use of somatic embryos by applying and adopting the method and mechanisms of accurate propagation, as well the analyse and assessment of the production of synthetic seeds - artificial seeds.

Schleiden (1838) and Schwann (1839) have proven that the basic building unit of living organisms is the cell, which has the ability to grow if the right environment is in contact with it so that a complete plant can form from it.

The process of tissue culture isolated single differentiated cells and planted them on a saline solution recommended by Knop (1865) and supported by glucose sugar. This process led to growth cells size and the accumulation of starch in them, but it failed to motivate them to divide. However, it set the ideas for the emergence of tissue culture science. Murashige and Skoog (1962) were able to prepare an integrated nutritional medium of salts, whose concentrations increased about 25 times the nutritional medium prepared by Knop.

This medium was called MS and it is still largely used to this day for the *in vitro* cultivation of plant tissues. Tissue culture is the basis for the development of somatic embryos and the production of artificial seeds.

Various plant techniques, have been used for the production of somatic embryos *in vitro*, which have shared some similar properties to natural zygotic embryos. Non-seed producing plants or propagation of elite plant species have commercial value (Saiprasad, 2001).

For imitating the natural seeds, somatic embryos are covered with nutrient gel consisted of basic organic or inorganic salts, compounds containing carbon, plant growth regulators and antimicrobials to protect the embryos from lesion while handling as well as allowing the growth and germination to take place. Some agents already been prepared for encapsulation such as SA complexed by $CaCl_2$ have been noticed and found to be the most convenient.

The production of artificial seeds depends on several factors such as selection of virus free seeds among other various diseases, in addition of seeds viability, high germination and moisture content.

Synthetic seeds have potential for a considerable level of cost lowering (Kok-Siong *et al.*, 2012; Roy and Mandal, 2008) with rapid multiplication of plant with genetic uniformity (Saiprasad, 2001). Artificial seeds are described as artificially encapsulated somatic embryos, which represent any vegetative part of plants (Rihan *et al.*, 2017). Because they are obtained from somatic cells, it is possible to be used for clonal propagation. Artificial seeds have different implementation in plant biotechnology - for instance in clonal propagation, germplasm conservation, plant breeding where propagation via normal seeds is not probable, easy storage, genetic uniformity etc. For some ornamental plants, propagation through somatic embryogenesis and synthetic seeds have been regarded as the only way out. Encapsulation technology is a fast-growing research domain in biotechnology and broadly studied. Artificial seeds are convenient for conservation and delivery of tissue-cultured plants, which is why many types of plants, fruits or cereal have been grown from artificial seeds.

The artificial or synthetic seeds are obtained mainly by deriving somatic embryos from plant tissue cultures and encapsulating them with the help of a hydrogel. Production technology of synthetic seeds in horticultural plants offered new techniques for preparing seed analogues from the micropropagules, like protocorm-like body (PLB) formation and plant regeneration (Bapat and Rao, 1988). Micropropagules are encased in productive coatings of gelling agents such as alginate, agar, carrageenan, gellan gum (gelrite), sodium pectate, and carboxyl methyl cellulose (Kok-Siong *et al.*, 2012).

Micro shoots encapsulation and somatic embryos as well as retrieval of plantlets have been reported in a number of plant species, such as: cauliflower (Kok-Siong *et al.*, 2012), sandalwood (Bapat and Rao,1988), rosemary (Al Masoody and Stănică, 2015), apple (Piccioni *et al.*, 1996) (Micheli *et al.*, 2008), mulberry (Micheli *et al.*, 2017), banana (Ganapathi *et al.*, 1992) cardamom, sugar beet (Tsai and Saunders, 1999) rice and peer (Mower *et al.*, 2007) and other plants (Falcinelli *et al.*, 1997). Seeds production by utilizing somatic embryos and other kinds of explants is possibly fruitful for the huge scale propagation of superior hybrids of important species. The synthetic seed technology may only be successful with competent. Different micropropagules have been regarded for purpose of producing artificial seeds (Stănică, 1999); consequently, somatic embryos and axillary shoot buds have been favoured.

MATERIALS AND METHODS

As method the literature review as a critical look at the existing research that is significant to the field, was used.

RESULTS AND DISCUSSIONS

1.1. Importance of Artificial Seeds

The significance is based on the advantages that could be gained by producing artificial seeds which can bring good and higher percentage of outputs, specifically when seeds are implemented in shorter time, labour and lower cost as well as the possibility to be stored for longer time, since "seeds are desiccation tolerant, durable and quiescent due to protective coat. Such properties of seeds are also used for germplasm preservation in seed repositories" (Patricia *et al.*, 2004). "Many strategies can be used to evaluate plant genetic structure from *in vitro* derived plant clones, but most of them have limitations" (Patricia *et al.*, 2004). The researcher pointed out that such a topic may add a value to previous literature as well as contributing in bridging the gap in literature concerning production of synthetic seeds in horticultural plants using somatic embryos and other types of explants obtained *in vitro*. True seeds can be produced at the end of reproductive stage by the process of reproduction. A regular plant might take a long time to achieve reproduction stage, this matter implies that we are to wait to the end of reproduction stage of a plant to get seeds. But, artificial seeds can be available within one month or less. The majority of regular plants bear flower and produce their seeds at a

specified season. But, producing artificial seeds is not time dependent. It can be at any. The work on some kinds of plants is delayed because of the presence of long periods of dormancy, whereas growing artificial seeds, this period may be reduced where the life cycle of a plant can be shortened. It is probable to produce artificial seeds for any desired crops. Artificial seeds are possible to be applicable at large scale monocultures in addition to mixed genotype plantations. Artificial seed coating has the ability to deliver beneficial adjuvant like growth promoting plant nutrients as well as growth control agents Artificial seeds assist in studying the role of endosperm in addition to seed coat formation.

1.2. Is there a need for artificial seeds?

Many species are now considered to be on the verge of extension. Growing desertification and disappearing forests raises the chances of extinction for many plant native species, most of which cannot be propagated and which produce low quantities of seed. This is where synthetic seed play an important part as a substitutes (Kumar, 1998). By using synthetic seeds, the risks emerging from adjusted plants in reproduction might be prevented. Such risks include the possibility of gene introduction from different species during multiplication, which can become unstable. Artificial seeds can be used trough somatic hybrid propagation (Hwang *et al.*, 2005). Seeds are considered zygotic embryos with supported and improved nutritive tissues that provide protection. This layer makes them desiccation tolerant and durable, making the seeds useful for germplasm preserving in seed repositories. Zygotic embryos include progeny from two parents, which means that the growing or breeding process includes inbred lines that produce hybrid progeny when crossed. However, at crossing, genetic barriers occur for fruits and ornamental plants. Forest trees on the other hand have a too long generation time. In this cases propagation should be achieved in a vegetative manner, by using open pollinated seeds. Zygotic seeds have been replaced following the discovery of somatic embryogenesis, respectively in the 50s when they were created from somatic cells (Mohan, 2000; Jaiswal *et al.*, 2001). The somatic embryos emerging from shoots, roots, cotyledon leaves, epicotyls, hypocotyls, embryogenic calli, as well as protocorm or protocorm-like bodies, which can be perceived as clones originating from a single parent. They never become quiescent in the processes of in vitro culture. The synthetic seeds do not always need a synthetic protective layer. The use of somatic embryos as seeds with functional capacity is studies in the field of technology of artificial or synthetic seed. Their economic importance relies greatly in germplasm preservation and plant production for commercial purposes.

1.3. Types of artificial seeds

Synthetic seeds can be uncoated quiescent – somatic- embryos or uncoated non - q. s.e. The non-q. ones are used for crop production micro propagated trough tissue cultures, whereas the quiescent ones are useful for germplasm storage. The non-q. s.e. in a hydrated encapsulation provide a cost-effective seed. The dehydrated ones encapsulated in protective artificial layers are useful due to their handling qualities. The somatic embryos become quiescent and the protective layer hardens, making them be more resistant for a longer period of time: "the seed coat softens, allowing the somatic embryo to resume growth, enlarging and emerging from the encapsulation" (Grey, 2003).

1.4. Advantages of artificial seeds

Throughout this study, the work will refer to three plants, namely, potato, figs and Chinese jujube and the explants will be chosen from these three plants are tubers, and buds respectively. The artificial seeds are somatic embryos and each plant uses a specific part for micropropagation and packaging to reach artificial seed production. "The propagation of plants by artificial seeds widens the horizon of plant biotechnology and agriculture" (Kinoshita, 1992), "the technology provides methods for preparation of seed analogues from the micropropagules such as axillary shoots, apical shoot tips, embryogenic calli, somatic embryos as well as protocorm or protocorm-like bodies" (Jaiswal *et al.*, 2001). Sodium is a chemical element of the Na symbol and its atomic number 11. It is a white coloured

substance, characterized by its great chemical activity. It reacts in the air and burns with yellow flame. In addition, it is famous for being highly reactive with water and air humidity, so it is stored in oils or oil derivatives, there is no free sodium in nature. There is a relatively large abundance of sodium, it is the sixth most abundant chemical element in the earth's crust, and it is found in many minerals such as Feldspar, Sudalite and Halalite. Sodium salts are highly soluble in water, most notably sodium chloride, which is the main cause of saline water. Sodium has one stable which is 23Na. Sodium has an important vital role; it is classified as a basic nutrient for humans, animals and some plants. Na + Na are the essential cations in extracellular fluid, and have a key role in controlling blood pressure and osmotic pressure in the body, in addition to the role in the transmission of nerve signals. Sodium is more chemically active than lithium, but less than potassium. Sodium can be dissolved in a completely oxygen-free atmosphere without any reaction. The interaction of sodium with oxygen is a special case, since the interaction between them depends on the presence of moisture, in the absence of water does not interact with sodium with oxygen. Alginic acid is also called alginate. It is a polycyclic polychromide widely spread in cellular walls of cellular algae. When mixed with water, it binds together and forms glue, Sodium alginate is a chemical compound with the formula NaC6H7O6. It is the sodium salt of alginic acid (algal acid), and it is in the form of gum extracted from the walls of algae cells. Sodium alginate is a good candidate for the removal of radioactive substances from the body, such as iodine-131 and strontium-90, which are produced by non-radioactive isotopes. The delivery of encapsulated material will save several subcultures for obtaining plants as well as eliminating the stage of acclimatization of in vitro plants. The uniform and simultaneous production of encapsulated propagules followed by uniform germination may remove many drawbacks connected to natural seeds. Many plant systems have been recognised to come up with a huge number of embryos in culture sharing many features similar to natural embryos consisting of germination, which lead to production. For imitating the natural seeds, embryos produced from cultures, are encapsulated in a nutrient gel consisting of basic salts either organic or inorganic, as well as carbon source, plant hormones and antimicrobial agents and coated totally for protecting the embryos from damages such as mechanical during handling, also for allowing developing and germination to take place without any undesirable variations. Several agents for encapsulation and sodium alginate complexing with calcium chloride have proved to be the most convenient. Through this method various types of synthetic seeds have been prepared: hydrated and desiccated. Hydrated synthetic seeds consist of embryos encapsulated individually in a hydrogel, while in desiccated kind, the coating mixture has been dried for several hours inside a sterile hood. The propagules (embryos/axillary bud's/shoot tips) are accurately separated from aseptic cultures and blot dried on filter paper, and are then mixed in sodium alginate prepared in nutrient medium. Then, and manually the propagules are picked up by forceps, and then dropped into a solution of calcium chloride for a period of 40 minutes. After the period of incubation, the artificial seeds were recovered by calcium chloride solution decanting then washing them in sterile water for 4 times before being cultured on nutrient medium or on a filter paper, cotton or soil for purpose of growth and plants conversion. There may be some possible artificial seed systems, relying on the type of artificial seed which is produced, the requirements for artificial seeds, the economic feasibility "will vary greatly among species" (Pond and Cameron, 2003). Production of artificial seeds depend on several steps, which can be summarized as follows:

- **First** comes crop selection, which relies upon commercial and tech potential, then the assembly of a somatic embryo system.
- **The second step** consists of clonal production, system optimization and embryo production and automation of embryo production;
- **The third step** consists of treatment of mature embryos that triggers quiescence;
- **The forth is** encapsulation development and coating system, optimization and automation.

- **The fifth step** consists of determination of economic practicableness of adopting the artificial seed delivery system for a specific crop compared with alternative propagation techniques (cost–benefit analysis regarding encapsulation). In general, some procedures apply to quite one species whereas alternative steps could also be species-specific (Pond and Cameron, 2003).

1.4.1. Encapsulation of somatic embryos

Isolated s. embryos were placed in a Na alginate-based solution. Based on the encapsulation adopted, and suctioned through a micropipette to supply protection. so as to seal the capsules, they're submerged in a $CaCl_2$ solution for a while then in sterile water for forty min. This method is enforced beneath antiseptic conditions. Then, the artificial seeds of potatoes, figs, Chinese jujube are cultivated through a germination medium in Petri dishes by the employment of macro and micro nutrients from the MS medium. It is further supplemented with thirty g/l of disaccharide and seven g/l of agar, then left within the culture chamber at 25ºC with no light.

1.4.2 Applications of Artificial seeds

Synthetic seeds are used in biotechnology for cultivating various plant species. They are also used due to the potential for storing heterozygous plants genetically or with a single outstanding combination that could not be kept in regular methods of seed production "due to genetic recombination exists in every generation for seed multiplication" (Gray, 1997). The applications of artificial seeds is debated in various academic fields. It is important to note that somatic embryogenesis can be an alternative for the sterile species which produce no seeds. Tropical species also produce seeds that cannot be dired and long time storage in gene banks is not likely. "The artificial seeds can be an alternative as more is learned about the mechanism by which this type of seed has no tolerance to desiccation" (Leprince *et al.*, 1993).One limitation of micropropagation is the similarity of the physical tissue culture site, laboratories and greenhouses in order to synchronize the peak of demanded propagules period in the market (Gray and Purohit, 1991).The ornamental plants market is rising annually. The huge cost of production of such species is given by the efforts of the micro propagation and required manpower in the later propagation stages and production. "The use of somatic embryogenesis system in these species would significantly reduce labor costs" (Chee and Cantliffe, 1992) Coniferous forest species may be propagated cheaply, the regular breeding programs in such species are considered very time consuming because its life cycle of conifers is long. Coniferous forests are regarded as too heterogeneous. "Artificial seeds have the ability to clone those overhanging trees at reasonable cost and in minimum time" (Desai *et al.*, 1997). In the commercial field, it is not seen easy to come up with low-cost hybrid seed species like cotton and soybean because they contain cleistogamous flowers and abscission problems because the seed comes from self-pollinating species. Hybrid seed has been made and produced in small quantities in a very laborious by hand pollination. Such small volume of hybrid seed can be largely going up through synthetic seed technology. "the hybrid force would be used commercially to originate a significant reduction in costs" (Tian and Brown, 2000) . In some vegetable species, hybrid seeds are considered expensive, so the plant value is too high. For instance, tomatoes and seedless watermelon hybrid seeds are utilized in very high cost. The reason is that pollination is implemented manually, a matter that requires intensive labour. Whereas, in other species, vegetative reproduction is utilized, it takes much time, space and labor. "The use of artificial seed technology can significantly reduce costs by reducing the labor required, time and space in case of these plants"(Chee and Cantliffe, 1992).The majority of fruit species are propagated by vegetative means due to the fact that the presence of self-incompatibility and breeding cycles very long. "The use of synthetic seed facilitates its spread" (Towill, 1998).The most fruitful synthetic seed would be in the conservation of germplasm of the mentioned species. At the moment, seed banks have been dealt with as live plants in the field. Such method of conservation is too expensive and exposed to danger, as it is exposed to natural risks and disasters. The utility of artificial seeds would keep these

clones in a narrow space, under controlled conditions and without the risks or danger of natural disasters. Moreover, this system of germplasm conservation can be fruitful in tropical species where conservation methods are insufficient. (http://www.fao.org/3/v1430e/V1430E06.htm)

1.5. Previous studies

Literature review is important to access to the most accurate details and consequences. Another important thing to use the previous research is to give the researcher knowledge of the history of the evolution of the subject, and opens his eyes on points not to pay attention to and may be key to the solution. According to Ghanbarali *et al.* (2016), "Optimization of the conditions for production of synthetic seeds by encapsulation of axillary buds derived from minituber sprouts in potato (*Solanum tuberosum)*". The function of alginate encapsulation of axillary buds originated from potato minituber sprouts (PMSs) have been studied and optimized. It has been concluded that synthetic seeds have been obtained in potato using protocols on basis of other explants, normally nodal segments and single-node cuttings, as sources of encapsulable material. In terms of alginate encapsulation, the protocol described in this work is the same as these protocols. It has to be noted that these explants are obtained from in vitro propagated plantlets, a matter that implicates that the in vitro plantlets ought to be obtained. It is more time, labour and cost consuming than previous methods, "where the explants (PMSs) are obtained under *in vivo* conditions through minituber sprouting during 2 months (Ghanbarali *et al.*, 2016). Rosna *et al. (*2013), in their study “Synthetic Seeds Production and Regeneration of Oxalis triangularis for Mass Propagation and Conservation”, the potential of tissue culture technique has been discussed as an alternative method for mass propagation and conservation of this ornamental plant for future uses and exploitation. The key results were Synthetic seeds created by encapsulation of micro shoots of *Oxalis triangularis* in sodium alginate solution. The micro shoots have originated from stem explants of this species after being cultured for a period of 30 days on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BAP. After thirty days of storage, the ability of artificial seeds has been retained for germinating and provide high regeneration rate (90%) and the percentage of survival rate has also been high (77-86%) as compared to control. After 7 days of storage, the mean number of shoots formed was the highest in all treatments root formation was observed. It has been concluded that production of synthetic seeds was attempted from this species and the synthetic seeds survive after seven and thirty days after being stored at 4°C.The synthetic seeds conversion rate to complete plants after seven days was 96.67% with 4.57 mean shoots, whereas after thirty days of storage, the conversion rate goes down slightly to 90% with 3.97 shoots formation per bead (Rosna *et al.*, 2013). Shengrui (2013) has published an important study titled *Past, Present, and Future of Jujubes - Chinese Dates in the United States*, this study summarizes jujube importation, culture history and current jujube cultivars in the USA. It handles current issues with jujubes and probable solutions to them. Jujube adapts and grows well, in addition, it could become a valuable industry in the United States within 15 to 20 years. According to (Remya *et al.*, 2013), storage at 4°C is effective for long term preservation of artificial seeds. Determining the optimal temperature for the long-term storage of artificial seeds is imperative for the conservation and transport of useful germplasm resources; the basic part of biodiversity conservation. Maximum number of multiple shoots have been produced in MS medium supported by thidiazuron (TDZ) (3.0 µM) + naphthalene acetic acid (NAA) (1.0 µM). Thidiazuron has been reported as effective when combined with auxin (NAA) and cytokinin (BAP) in arousing morphogenic reactions. Moreover, it has been concluded that half strength liquid MS medium supplemented with 5 µM IBA was effective for root induction from the microshoots of *Solanum nigrum* with auxin. IBA has been reported to be beneficial for root induction during *in vitro* propagation of *S. nigrum* using nodal explants (Remya *et al.*, 2013).

Kok-Siong *et al.*, 2012, in their study "Production of Artificial seeds derived from encapsulated *in vitro* microshoots of cauliflower, *Brassica oleracea* var. *Botrytis*", a

high number of micro shoots (21 ± 2.31) of cauliflower was obtained. The aim of this work has been to assess the effects of various plant growth regulators (PGRs) on the multiple shoots induction adopting hypocotyls as explants, *in vitro* micro shoots produced have been harvested and encapsulated in sodium alginate for creating artificial seeds. The ability of storage and *in vitro* germination rates of artificial seeds have also been assessed.

The artificial seeds lasted for twelve days (after seven days storage) and fourteen days (after thirty days storage) to germinate on MS basal medium. It has been observed through this work that PGR or hormones supplemented to MS medium. In the current study, 0.1 mg/L NAA and 5.0 mg/L BAP effectively induced a large number of double shoots on cauliflower hypocotyl explants for production of downstream artificial seeds. The germination percentage of encapsulated micro shoots has been influenced by encapsulation matrix composition and pre-germination storage duration. Isolated micro shoots encapsulated in MS supplemented with 0.3 mg/L NAA and 3.0 mg/L BAP presented high germination percentage for seven and thirty days of pre-germination storage period. (Kok-Siong *et al.*, 2012). Dhabhai and Prakash (2012), in their study "Production and Applications of Artificial seeds: A Review", the types, production methods, benefits and different applications of synthetic seeds have been reviewed. Shoot tips, axillary buds have been utilized in the preparation process of artificial seeds. Artificial seeds possess a variety of applications in plant biotechnology. For some plants, as for instance ornamental plants, propagation through s. e. and synthetic seeds have been the only way out. It has been seen that artificial seeds have a broad extended applicability in a huge scale plant propagation. In the case of ornamental and extinct plant species, it is the sole means of propagation. Except this, artificial seeds have been directed and adopted for commercial purpose production of autogamous plant species, genetically adjusted plants, conifers, algae etc. To conclude, "the technology of artificial seed has affected almost every aspect of plant biotechnology and has the potential to become the most promising and viable technology for large scale production of plants (Dhabhai and Prakash, 2012).

Hwang *et al.* (2011) studied the propagation of perennial brown alga *Sargassum fulvellum* by somatic embryogenesis and synthetic seeds production. *Sargassum fulvellum* is a brown alga introduced to the seaweed cultivation industry. Such species presents acceptable potential for diversifying seaweed cultivation. Hwang *et al.* (2011) investigated growth and maturation of this alga by somatic embryogenesis and artificial seeds**.**

Bradford and Still (2004), in their study "Applications of Hydro time Analysis in Seed Testing", aimed to show the hydro time in the process of describing the connection between water potential and rates of artificial seed germination.. The model depends on data derived from germination time courses, therefore it needs data for several time points at the time of germination at some water potentials. Experience with a broad array of priming and pelleting methods implicate that, relying on the cultivar, seed lot, and specific treatments adopted, influences on all three parameters of the hydro time model could be noticed. It has been concluded by this study that analysis of hydro time could offer several indices regarding seed quality in connection with stress tolerance, speed and uniformity of germination; in addition, the development of automated imaging for scoring germination can facilitate hydro time analysis applications (Bradford and Still, 2004).

Sarkar (1998), in his study "Synseeds in potato: An investigation using nutrient-encapsulated *in vitro* nodal segments", investigated the production of synthetic seeds for potato propagation by the use of alginate-mediated, nutrient-encapsulated, *in vitro* nodal shoot segments. Micropropagated single node cuttings 5 mm long were encapsulated in calcium-free 3% sodium alginate–MS solution and they have been incubated under light (ca. 60 μE m^{-2} s^{-1} light intensity) at 24 ± 1°C for 3, 6 or 9 days. The encapsulated segments have been treated with rooting hormone powder before and after light incubation, then they have been planted by using plastic trays, they consist of 1:1 mixture of soil and FYM. A decline in survival of alginate capsules took place when they have been incubated under

light for 3 days or more. Rooting hormone powder application at the time of planting has been effective for establishment of soil for alginate capsules. 57% encapsulated segments have survived in the soil when they have been incubated under light for three days and, they have been treated with rooting hormone powder during the period of planting. The study has shown that micropropagated nodal segments encapsulated in alginate–MS solution may be utilized for purpose of producing potato propagule (Sarkar, 1998).

Simmonds (1997), in the study "A review of potato propagation by means of seed, as distinct from clonal propagation by tubers" has concentrated on the majority of cultivated potatoes are vegetative propagated, outbred auto tetraploids. Disease problems have been shown and the maintenance of vegetative stocks. There have been suggestions for propagating the crop by sexual seed so as to evade disease problems. A success has been fulfilled but control is necessary for avoiding inbreeding depression as well as avoiding seed propagation which is not as cheap or simple as hoped. The idea has raised a broad interest in the tropics and has growing practical impact on China, India and Vietnam. There has been a considerable tendency for using tuberlets borne on crowded nursery plants. There has been emerging recognition that seed propagation are complementary rather than competitive and that good breeding programmes will serve both (Simmonds, 1997)

Kariuki (1991), in his research paper "Production of synthetic seeds from nodal segments of *Solanum nigrum*", presented an efficient protocol for the production of synthetic seeds in *Solanum nigrum*, a medicinal plant. Artificial seeds were made by encapsulating nodal segments of *S. nigrum* in calcium alginate gel. 3% (w/v) sodium alginate and 100 mM CaCl $2H_2O$ were seen most convenient for encapsulation of nodal segments. 22 maximum number of multiple shoots have been made in Murashige and Skoog medium supplemented with thidiazuron (3.0 μM) + naphthalene acetic acid (1.0 μM). The encapsulated nodal segments could regenerate after sixty days when they are stored at 4°C. The shoots have been routed by the use of a half strength liquid MS medium supplemented with 5 μM Indole butyric acid and have been acclimated to greenhouse circumstances. This work shows and describes an efficient protocol for the producing synthetic seeds. The protocol can be adopted as an alternative technique for germplasm conservation of this valuable medicinal plant (Kariuki, 1991). By adopting artificial seeds process, the tissue culture raised plants may be regenerated on a simplified medium eliminating subcultures, it may reduce the cost of process and functions. Developing protocols for recovery of plants from artificial seeds under non-sterile circumstances might be of a larger influence. Despite the fact that a large number of plants may be made in tissue cultures through multiple shoot cultures, their delivery is cumbersome. Embryos or shoots are to be isolated and transferred for rooting to conduct root shoot balance, and the plants ought to be hardened in the green house before field planting. Direct sowing regarding artificial seeds in the soil does not require acclimatization usually needed for the tissue cultured plants. It offers an ideal delivery system enabling an easy flexibility in handling as compared to big parcels of plants. in synthetic seeds technology, for big scale commercialization, improved product of propagules is important. Present tissue culture techniques have not generated sufficient propagules and are not enough to meet the requirements of commercial use of artificial seeds technology methods. Standardization for synchronization of developed propagules followed by automation of all operation of sorting, encapsulation and germination of the coated propagules may improve the pace in producing synthetic seeds.

1.6. Summary on previous studies

According to the literature review, the benefits of this study can be summarized as follows:

- The problem of the research and the determination of its dimensions and areas: by looking at what has been written studies and research on the problem
- To enrich the problem of this study with knowledge, studies, hypotheses, and the results reached by others

The study included the identification of the tools used in this study as well as the ideas and procedures that have been used.

Previous studies on the topics of production and applications of artificial seeds in horticultural plants using somatic embryos and other types of explants as well as the studies regarding producing artificial seeds of the three plants namely, potato, fig and Chinese jujube and the explants will be chosen from these three plants are tubers, and buds respectively. At the conclusion of research, the researcher is going to link the results obtained throughout this study with those mentioned in previous studies so that he could make a comparison and contrast process for showing aspects of similarity, and contrast points. So, such previous studies and the present research show that the scientific gap in research is incomplete and more new research study shall be required.

Conclusions of such mentioned previous studies have been as follows:

- synthetic seeds have been obtained in potato using protocols on grounds of other explants, normally nodal segments and single-node cuttings, like sources of encapsulable material
- the synthetic seeds survive after seven and thirty days after being stored at 4°C
- jujube taxonomy, biology, adaptation, propagation, and research conducted have been described.
- storage at 4°C is effective for long term preservation of artificial seeds. Determining the optimal temperature for the long-term storage of artificial seeds is imperative for the conservation and transport of useful germplasm resources
- assessment of the effects of various plant growing regulators on the multiple shoots induction following hypocotyls as explants, *in vitro* micro shoots produced have been encapsulated in sodium alginate for making artificial seeds.
- artificial seeds have a broad extended applicability in a huge scale plant propagation.
- some studies present the properties and features regarding different bioactive substances in this different plants and seeds.
- applying artificial seeds process, the tissue culture raised plants may be regenerated on a simplified medium eliminating subcultures.

CONCLUSIONS

- The tissue culture raised plants and artificial seeds process can be regenerated on a simplified medium eliminating subcultures, such processes may lower the cost of process and functions.
- Synthetic seeds were made by encapsulating the micro shoots derived from multiple shoot induction, and for the aim of creating artificial seeds, an effective technique of multiple shoots induction ought to be developed so that a large number of micro shoots for encapsulation could be provided.
- Several agents have been made for encapsulation and sodium alginate complexing with calcium chloride has been discovered to be the most convenient.
- Applying artificial seeds production process, may be regenerated on a simplified medium eliminating subcultures, it may reduce the cost, time, limitations as well as some functions.
- Artificial seeds provide applications in the field of plant biotech
- Furthermore, Clinical and Pathological studies should be conducted to isolate and characterize the bioactive components present in the selected plants.
- The technology of artificial seed influences plant biotechnology and is considered in important technique for big scale plant production.
- Artificial seeds offer an ideal delivery system enabling easy flexibility in handling and transport as compared to large parcels of seedlings or plants.
- Development of protocols for direct recovery of plants from synthetic seeds under non sterile conditions may have a greater impact
- There is emerging recognition that vegetative and seed propagation are complementary rather than competitive and that good breeding programmes will therefore serve both.
- It may be probable to produce artificial seeds in any desired crops.

REFERENCES

Al Masoody, M.M.M. and Stănică, F. (2015). The effect of growth regulators on callus colors and production of somatic embryos *in vitro* of Rosemary Plant (*Rosmarinus officinalis* L.), IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS), 8 (9). 96-100.

Bapat, B.A. and Rao, P.S. (1998). Sandalwood plantlets from synthetic seeds. Plant Cell Reports. 7. 434-436.

Bradford, K.J. and Still, D. W. (2004). Applications of Hydro time Analysis in Seed Testing. An International Journal Serving Seed Scientists and Technologists, 26(1). 75-85.

Chee, R.P. and Cantliffe D.J. (1992). Improved Procedures for production of sweet potato somatic Embryos for a synthetic seed system. HortScience, 27. 1314-1316.

Desai, B.B., Kotecha, P.M. and Salunkhe, D.K. (1997). Seeds handbook: biology, production, processing, and storage. New York, NY: M. Dekker.

Dhabhai, R. and Prakash, A. (2012). Production and Applications of Artificial seeds. International Research Journal of Biological Sciences, 1(5). 74-78.

Falcinelli, M., Standardi, A. and Piccioni, E. (1997). Il seme sintetico nelle pianbte agrarie stato attuale della ricercar. Riv. Sementi elette. XLII (1), pp. 9-17.

Ganapathi, T.R., Suprasanna, P., Bapat, V.A. and Rao, P.S. (1992). Propagation of banana through encapsulated shoot tips. PlantCell Rep., 11. 571–575.

Ghanbarali, S., Abdollahi, M.R., Zolnorian, H., Moosavi, S.S., Segui – Simarro, J.M. (2016). Optimization of the conditions for production of synthetic seeds by encapsulation of axillary buds derived from minituber sprouts in potato (*Solanum tuberosum*). Springer Science+Business Media Dordrecht. Innovare Journal Of Agri. Sci, Vol 4, Issue 4, 13-16 .

Gray, D.J. and Purohit, A. (1991). Somatic embryogenesis and development of synthetic seed technology. Crit. Rev. Plant Sci., 10. 33-61.

Gray, D.J. (1997). Synthetic for Clonal Seed Production of Crop Plants. In: R.B. Taylorson (ed.) Recent Advances in the Development and Germination of Seeds, 29-45.

Grey, D.J. (2003). Artificial Seed, In: Seed Development/ Artificial Seed, Elsevier Ltd.

Kariuki, T.K. (1991). Production of synthetic seeds from nodal segments of *Solanum nigrum.* Department of Biotechnology, K.S. Rangasamy college of Arts and Science, Tiruchengode, Tamil Nadu, IndiaPARIPEX-INDIAN JOURNAL OF RESEARCH volume 5 Issue : 12. 79-96

Kinoshita, I. (1992). The Production and Use of Artificial Seed, Research Journal of Food and Agriculture, 15(3). 6-11.

Kok-Siong, P., Sadegh, M. and Rosna, M. T. (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

Hwang, E.K., Gong, Y.G. and Park C.S. (2011). Cultivation of a brown alga, *Undariopsis peterseniana* (Kjellman) Miyabe and Okamura, as a warm-temperature species by artificial seed production in Korea. J Appl Phycol., 23:449–455.

Jaiswal, V.S., Hussain A. and Jaiwal, U. (2001). Synthetic seed: Prospects and limitations. Current Science, 78(12). 1438-1444

Kumar, U. (1998). Synthetic Seeds for Commercial Crop Production. New Delhi, India. Vedams Books (P) Ltd.

Leprince, O., Hendry, G.A.F. and McKersie B.D. (1993).The Mechanisms of desiccation tolerance in Developing seeds. Seed Sci. Res., 3. 231-246.

Micheli, M., Standardi, A. and Stănică, F. (2008). The technology of *in vitro* encapsulation for propagation, exchange, and storage of plant genotypes. Proceedings International Scientific Symposium "Modern agriculture achievements and prospectives." UASM Chişinău.

Micheli, M., Bececco, V., Gardi, T., Martorana, L., Chiancone, B. and Germanà, M.A. (2017). Encapsulation of black mulberry microcuttings: studies on capsules and synthetic seeds. Acta horticulturae. 65-70.

Mohan S. J. (2000). Somatic Embryogenesis in Woody Plants, Springer-Science Business Media, B.V., 6

Mower, J.P., Touzet, P., Gummow, J.S., Delph, L.F. and Palmer, J.D. (2007). Extensive variation in synonymous substitution rates in mitochondrial genes of seed plants. BMC Evol. Biol., 7. 135.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol Plant 15(3). 473-497.

Patricia, N. Silva, Derly H., Maria, José, Cruz, Cosme D., & Fontes, Elizabeth P. (2004). Somaclonal variation on in vitro callus culture potato cultivars. Plant Transformation Facility,.Horticultura Brasileira, *22*(2), 300-304.

Piccioni, E., Standardi, A. and Ţuţuianu, V.C. (1996). Storage of M.26 apple rootstock encapsulated microcuttings. Adv. HortScience, 10. 185-190.

Pond, S. and Cameron, S. (2003). Artificial Seeds. Tissue Culture, Elsevier Ltd., 1379-1388

Remya, M., Bai, V.N. and Mutharaian, V.N. (2013). *In vitro* regeneration of *Aristolochia tagala* and production of artificial seeds. Biologia Plantarum, 57(2). 210-218.

Rihan, H.Z., Fakhriya, K., Mohammed, E.E.M. and Fuller, M.P. (2017). Artificial Seeds (Principle, Aspects and Applications), Agronomy 2017, doi:10.3390/agronomy7040071.

Rosna, M. T., Noraini, M., Jamilah, S. Y., Noorlidah, A. and Sadegh, M. (2013). Synthetic Seeds Production and Regeneration of *Oxalis triangularis* for Mass Propagation and Conservation. International Journal of Environmental Science and Development, 4(5), 461-464 .

Roy, A. and Mandal, B. (2008). Development of synthetic seeds involving androgenic and pro-embryos in elite indica. Indian Journal of Biotechnology, 7(4). 515-519.

Saiprasad, G.V.S. (2001) Artificial Seeds and their applications. Resonance, 6. 39-47. http://dx.doi.org/10.1007/BF02839082

Sarkar, D. (1998). Synseeds in potato: An investigation using nutrient-encapsulated in vitro nodal segments. ICAR-Central Research Institute.scientia horticult 73, 299-315.

Schleiden, M. J. (1838). Beiträge zur Phytogenesis. Arch. Anat Physiol. Wiss. Med. J. Müller.

Schwann, T. (1839). *Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur und dem Wachstume der Tiere und Pflanzen*. Leipzig: W. Engelmann, Nr. 176, Ostwalds Klassiker der exakten Wissenschaften, 1910.

Shengrui, Y. (2013). Past, Present, and Future of Jujubes Chinese Dates in the United States. Department of Plant and Environmental Sciences, Sustainable Agriculture Science Center at Alcalde, New Mexico State University, HORTSCIENCE, 48(6): p 672-680.

Simmonds, N.W. (1997). A review of potato propagation by means of seed, as distinct from clonal propagation by tubers. Potato Research 40(2). 191–214.

Stănică, F. (1999). Microînmulţirea plantelor horticole şi alte tehnici de cultură *in vitro* [Micropropagation, of horticultural plants and other *in vitro* culture techniques]. Bucharest, RO: Grand Publishing House.

Tian, I. and Brown, D.C. (2000). Improvement of soybean somatic embryo development and maturation by abscisic acid steam treatment. Can. J. Plant Sci., 80. 721-726.

Towill, L.E. (1988). Genetic Considerations for clonal germplasm preservation of materials. HortScience, 23. 91-93.

Tsai, C.J. and Saunders, J.W. (1999). Evaluation of sole nitrogen sources for shoot and leaf disc cultures of sugarbeet. Plant Cell Tiss Organ. Cult., 59. 47-56.

http://www.fao.org/3/v1430e/V1430E06.htm