European Journal of Botany, Plant Sciences and Phytology
Vol.3, No.1, pp.61-69, May 2016
Published by European Centre for Research Training and Development UK (www.eajournals.org)

PLANT REGENERATION OF A LAKOOCHA FROM ENCAPSULATED NODAL EXPLANTS.

Shiv Verma[1*], Shweta Khosla[2], Dhiraj Kumar Choudhary[2], Anand Kumar[3] Moti Lal[3]

[1]School of Biochemical engineering IIT (BHU) Varanasi-221005
[2]Department of Biochemistry,CSK HPKV University, Palampur, HP India
[3]School of Biochemical engineering IIT (BHU) Varanasi-221005

ABSTRACT: *One of the alternative methods adopted in recent years is to use biotechnological approaches for improving the tree species. A proficient protocol for encapsulation of nodal segments of Artocarpus lakoocha Roxb has been developed for plant regeneration through non-embryogenic synthetic seeds. Concentrations of sodium alginate and calcium chloride greatly affected morphology and texture encapsulating gel. 3 % sodium alginate with 100 mM $CaCl_2$ has been found to be best possible concentration for the production of identical synthetic seeds. 25 days old in vivo plantlet of A. lakoocha was used for obtaining nodal segments, used in all experiments. Five experiments were performed using MS medium (agar solidified and liquid) supplemented with 1, 3 and 5 mg/l BAP. Concentration of 1 mg/l BAP was observed to be more effective for achieving highest regeneration frequency in comparison to other concentration of BAP (3 and 5 mg/l). In third experiment only different strength of MS medium was used , in last two experiments nodal segments prior encapsulation and encapsulated nodal segments were given pulse treatment with (IBA)for 48 hours and concentration of 3 mg/l BAP was observed to be most effective for synthetic seed regeneration . Data were recorded after 4 weeks of culture.*

KEYWORDS: Pulse treatment, MS medium, BAP, IBA, Acclimatization, synthetic seeds.

INTRODUCTION

There is worldwide susceptibility of plant genetic diversity due to unmatched perturbations, habitat loss and destruction rates. Various species are described as rare or endangered, and as a result integrated programs are required to defend and conserve biodiversity of currently available species (Dhir et al.2013). *Artocarpus lakoocha Roxb*, a species of family Moraceae, is an important tropical medicinal tree species native to India and used for various important purposes like;fruit, furniture, timber, and feed. Rip fruits of lakoocha are generally eaten fresh. Each fruit generally contains 20–30 seeds that are fleshy with thin seed coat. The edible fruit pulp is believed to contain essential ingredients which play key role in functioning if the liver. lkoocha seeds and milky latex are purgative in nature. Seeds contain artocarpins (ALA I and ALA II), the isolectins which exhibit high haemagglutination activity (Wongkham 1995). However, the agglutinin (ALA) from Artocarpus lakoocha is not organ specific in the plant. Moreover, the haemagglutination activity of ALA was demonstrated in various organs of the plant except fruit flesh (Joshee et al.2002) Traditionally the plant is propagated through seed but seed propagation is not rapid and season dependent. Extensive improvement has been made in the propagation of this plant through plant cell, tissue and organ culture (Bhatt et al., 1979; Shahzad et al., 1999; Hassanein and Soltan, 2000). However, in order to complete the demand of pharmaceutical industries, continuous supply of plant throughout year, there is need to develop a method of conservation and transport of healthy plant. In this context, for rapid

ISSN 2055-8139(Print), ISSN 2055-8147(Online)

propagation, encapsulation technology may be an alternative method of conservation and germ plasm exchange of this medicinally important plant (Verma et al. 2010).In recent years, synthetic seed technology using encapsulation of in vitro-derived non-embryogenic propagules has become an important asset to micro propagation (Naik et al. 2006). In addition, synthetic seed technology could be useful in germplasm conservation of elite, endangered and commercially important plants by using appropriate storage technique as well as exchange of axenic plant material between laboratories and pharmaceutical industries (Verma et al.2010) that can be used if stock plants or proliferation cultures become infested with bacteria, fungi, or arthropods. Successful cases of synthetic seed production and plantlet regeneration have been reported for a wide range of plants including cereals, vegetables, fruits, ornamentals, medicinal plants and forest tree,(singh et.al.2009). Some other potential advantages of synthetic seed technology are ease in handling due to small size of capsules, reduction in costs, genetic uniformity of plants and direct delivery to the field (Maruyama et al.) . An artificial seed prepared by coating a somatic embryo using a polymer matrix is a true seed analog. An immobilized somatic embryo can germinate under suitable growth conditions and become a complete plant. Synthetic seed technology using encapsulation of vegetative propagules of woody plant species has become a potentially cost effective clonal propagation system. Successful plant regeneration from synthetic seeds has been reported in several plant species. However, in most of the cases, the embryogenic propagules such as somatic embryos were used for synthetic seeds production. There are only few reports on encapsulation of vegetative propagules (Mathur et al. 1989, Bapat and Rao 1990, Ganapathi et al. 1992, Sharma et al. 1992, Piccioni and Standardi 1995, Maruyama et al. 1997, Pattnaik and Chand 2000, Brischia et al. 2002, Danso and Ford-Lloyd 2003). Encapsulation of vegetative propagules could be used for mass clonal propagation at a reasonable cost (Chand et al. 2004). There has been no report on utilizing somatic embryos to produce synthetic seeds. The development of synthetic seeds from somatic embryos could offer a practical means for mass propagation of banana. In this study, encapsulation of somatic embryos developing from embryogenic suspension cultures in an important Indian cultivar of banana, Rasthali (AAB with two AA and one B genome), and conversion of the encapsulated embryos into plants are reported (Ganpati et al. 2001). Among several non-embryogenic propagules, shoot tip explants are more responsive than other explants because of greater mitotic activity in the meristem (Ballester et al., 1997). Although there are many reports on encapsulation of shoot tips obtained from *in vivo* raised plants (Singh et al., 2009; Ray et al., 2008). In many of the works, somatic embryos have already been used in the encapsulation process. However, encapsulation of somatic embryos were restricted mostly to plants in which somatic embryogenesis has been documented. But, in current time , use of non-embryogenic vegetative propagules like apical shoot buds, axillary buds, nodal segments, etc., for the encapsulation have also been extended as a suitable alternative to somatic embryos (Mathur et al., 1989; Ganapathi et al., 1992; Ara et al., 2000; Mandal et al., 2000; Chand and Singh, 2004; Singh et al., 2006a,b, 2009; Naik and Chand, 2006; Micheli et al., 2007; Rai et al., 2008a,b, 2009).

The present study was aimed to investigate encapsulation of somatic embryos of *Artocarpus lakooch* in sodium alginate beads and conversion of encapsulated somatic embryos into plantlets. Effect of different concentration of BAP, was also studied to explore the morphogenetic responses of encapsulated shoot tips. Validation of the possibility to store the encapsulated shoot tips for time period sufficient for exchanges and distribution of germplasm was discussed. In the present investigation, we describe r the encapsulation of shoot tips for the development of synthetic seed in *A. lakoocha*.

ISSN 2055-8139(Print), ISSN 2055-8147(Online)

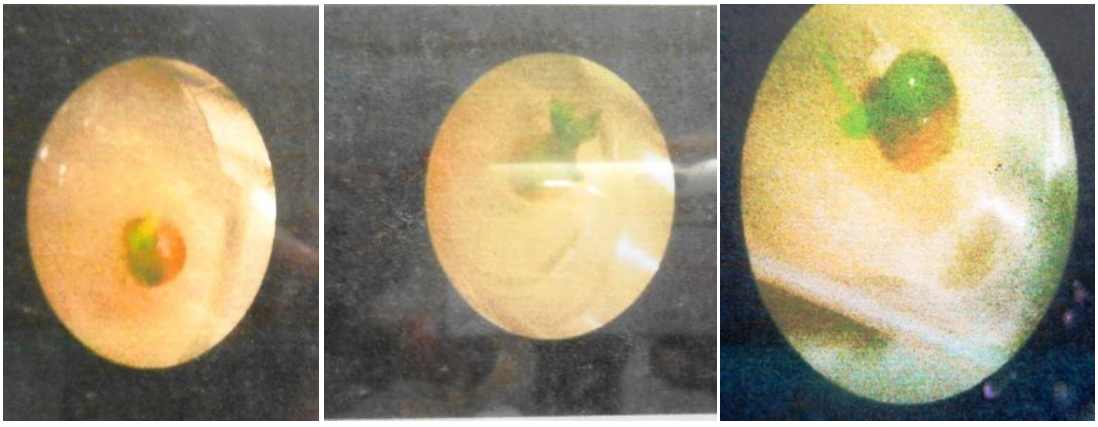
MATERIALS AND METHODS

Preparation of explants and culture conditions

Seeds of ripen fruits of A. lakoocha were obtained from agriculture field of Chandra Shekhar Ajad Agriculture University, Kanpur, India. Seeds were grown on agar solidified MS medium. 25 days old plantlets were used for making nodal segments. Nodal segments (0.5cm) of *A. lakoocha* were prepared. Initially the explants were washed in running tap water for 20–30 min to minimize microbial concentration on the surface of nodal segments. After that surface sterilized in laminar air hood with 70% ethanol for 30-40 s followed by 0.05% mercuric chloride (Hi-Media, India), for 3–4 min, and rinsed 4–5 times with sterile double distilled water [imp]. For shoot multiplication, nodal segments were cultured on MS (Murashige and Skoog, 1962) medium supplemented with sucrose (30 g /l). The media were solidified with 0.8% (w/v) agar (Hi-Media, India).The media were adjusted to pH 5.7using 1 N NaOH or 0.1 N HCl before autoclaving at 121 ^{0}C for 15 min. Cultures were maintained at 25 ± 2 ^{0}C with a 16 h photoperiod at a photon flux density of 50– 70 mmol m^{-2} s^{-1} from cool white fluorescent tubes (Philips, India). Nodal segments excised from in vitro proliferated plantlets were used as explants for the synthetic seed production.

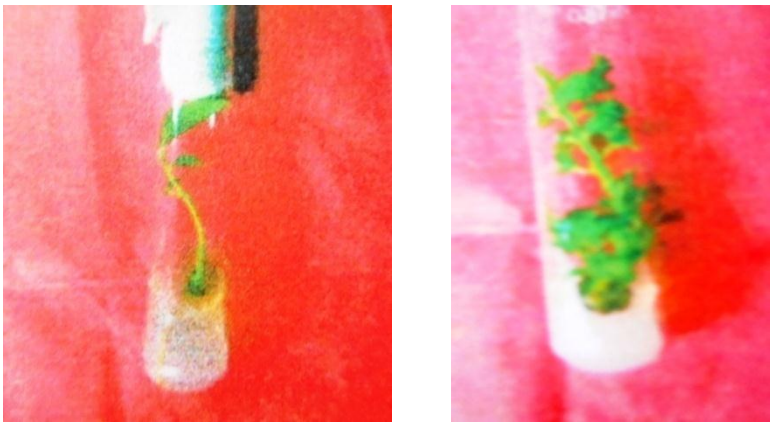
Encapsulation of nodal segments and preparation of sodium alginate beads

For encapsulation of nodal segments, sodium alginate (Hi media, india) was prepared in the range of 2.0, 3.0, 4.0, or 5.0% (w/v), whereas calcium chloride (CaCl2 .2H2O) solution was prepared in100, mM (w/v) in double-distilled water or liquid MS (Murashige and Skoog, 1962) medium without any plant growth regulator. Both the gel matrix and complexing agent were autoclaved at 121°C for 15 min. Encapsulation was accomplished by mixing the nodal explant into the sodium alginate solution and dropping these explants into the calcium chloride solution. The beads containing the somatic embryos were held for 20–30 min in the calcium chloride solution and after hardening of the beads, encapsulated somatic embryos were washed with sterilized distilled water two times to take away traces of calcium chloride.



(A) (B) (C)

ISSN 2055-8139(Print), ISSN 2055-8147(Online)



(D) (E)

Figure 1-Synthetic seed prepared in different sodium alginate and calcium chloride composition

Plantlet regeneration in agar solidified MS medium (D) and MS medium supplemented with 1mg/l BAP (E)

EXPERIMENTS

Different factors affecting conversion were evaluated and the following experiments were performed. To maintain the culture, same light and temperature conditions were used in the growth room as described previously. Data for conversion frequency and shoot length was recorded after 3weeks of culture**.**

Effect of BAP in agar solidified MS full strength Medium

Synthetic seeds were prepared using in sodium alginate (3% w/v) and 100mM CaCl2, seeds were inoculated in solidified MS medium supplemented with BAP at the concentration of 1,3and 5 mgl^{-1}. After three weeks percent seed germination was recorded in all three supplement of BAP.

Effect of BAP in liquid MS full strength Medium

In this experiment same composition of synthetic seed and same concentration of BAP was used. Liquid MS medium was used. After three weeks inoculation percent seed germination was recorded in all three supplement of BAP.

Effect of Composition of Encapsulating Gel

Sodium alginate beads complexed with nodal explants were inoculated in MS full strength, 1/2MS and 1/4MS strength. Percent seed germination was recorded after three weeks of inoculation.

ISSN 2055-8139(Print), ISSN 2055-8147(Online)

Effect of Pulse treatment of IBA (Indole Butyric Acid) Before Encapsulation of nodal Explant

Nodal explants were pulse treated for 48 hours, with IBA (3mgl^{-1}), before preparation of synthetic seeds. After synthetic seed preparation, seeds were inoculated in full strength liquid MS medium supplemented with, 3 and 5 mgl^{-1} BAP.

Effect of Pulse treatment of IBA (Indole Butyric Acid) After Encapsulation of nodal Explant

In this experiment after encapsulation, nodal explants were subjected to 48 hours pulse treatment with same growth regulator and same concentration. Seeds were inoculated in full strength liquid MS medium supplemented with, 3and 5 mgl^{-1} BAP.

Acclimatization of plants in soil

Artificially grown plantlets regenerated from encapsulated nodal segments were transferred to plastic pots containig3:1(w/w) mixture of sterile sand and soil moistened with liquid MS medium. Plantlets were covered with polyethylene bags to maintain high humidity and irrigated with tap water. Pots with plantlets were kept under laboratories condition at 25 ^{0}C in artificial light (irradiance of 60 mmol m^{-2} s^{-1}) provided by cool white fluorescent tubes for 4 Weeks and then the pots were transferred to field levels.

RESULTS AND DISCUSSION

At present days new route in synthetic seed technology has been reported with the use of non embryogenic plant propagules viz; root stem leaves (Standardi and Piccioni, 1998). The most important benefit of using vegetative propagules for the preparation of synthetic seeds would be in those cases where somatic embryogenesis is not well established or somatic embryos do not germinate into complete plantlets (Rai et al., 2009). In such cases, synthetic seeds can be produced from shoot tips for cost-effective mass clonal propagation, potential long-term germplasm storage, and delivery of tissue-cultured plants. In the present exploration, nodal segments excised from *in vivo* proliferated samall plantlets were used as an explant for the development of synthetic seed in *A.lakoocha*. in many experimental studies Similar observations were also made in *Dalbergia sissoo* Roxb (Chand et al.2004), Ceropegia bulbosa var. bulbosa (Dhir et al.2013), *Vitex negundo* L.(Ahmad et al.2010) . Evaluation of concentration of sodium alginate and calcium chloride which are effective for the gelling properties of the matrix and eventually for the excellence of calcium alginate beads is an important aspect for the successful in vitro propagation of plants through encapsulation methods Concentration of sodium alginate and calcium chloride greatly affected the encapsulation of nodal segments differed qualitatively with respect to texture, shape, and transparency.

ISSN 2055-8139(Print), ISSN 2055-8147(Online)

Table1.Effect of BAP in agar solidified MS full strength Medium

Concentration of BAP(mgl^{-1}) + MS (S)	Per cent response of synthetic Seed Germination	Days
1+ MS (S)	91	21
3+ MS (S)	81	21
5+ MS (S)	73	21

In our study 3.0% sodium alginate and 100 mM CaCl2.2H2O was found most suitable for formation of ideal calcium alginate beads. Concentration of BAP along with MS (Murashig and Skoog 1962.) medium greatly affected the percent conversion frequency of encapsulated nodal segments of A.lakoocha, highest conversion frequency (91%) was observed on solid MS medium supplemented with 1 mg/l BAP(6-Benzyle Amino Purine) followed by 3 and 5 mg/l BAP with percent synthetic seed germination of 89% and 72% respectively (Table 1).

Table2.Effect of BAP in liquid MS full strength Medium

Concentration of BAP(mgl^{-1}) + MS (L)	Per cent response of synthetic Seed Germination	Days
1+ MS (L)	96	21
3+ MS (L)	84	21
5+ MS (L)	78	21

We have observed in our experiment that when same experiment was performed with liquid MS medium with same concentration of BAP, percent conversion frequency was observed higher than the earlier experiment. It was 96% on liquid MS medium supplemented with 1 mg/l BAP. Liquid MS medium supplemented with 3 and 5 mg/l BAP showed conversion frequency of 84% and 78% respectively (Table2).this difference of conversion frequency in two conditions of medium may be due to fact that in liquid MS medium nutrient components are more easly and rapidly absorbed through the surface of sodium alginate beads while in solid MS medium nutrient components are slowly absorbed which is responsible for slow regeneration of nodal segments.

Table3. Effect of Composition of Encapsulating Gel

Strenth of MS (L)	Per cent response of synthetic See Germination	Days
MS (FS)	87	21
MS(1/2S)	54	21
MS(1/4S)	48	21

When only different strength of liquid MS medium (MS FS, MS1/2 S and MS1/4S) was used it was observed that synthetic seed regeneration frequency of full strength liquid MS was highest (87) among three different strength of liquid MS medium. Possible reason of this difference of regeneration frequency may be due to insufficient concentration of all nutrient components, because inside a plant cell for a biochemical reaction particular concentration of

ISSN 2055-8139(Print), ISSN 2055-8147(Online)

nutrient component (metal ions etc.) is needed. Due to this fact in MS1/2 S and MS1/4S) regeneration frequency was observed to be 54% and 48% respectively (Table3).

Table4. Effect of Pulse treatment of IBA (Indole Butyric Acid) Before Encapsulation of nodal Explants

Concentration of BAP(mgl^{-1}) + MS(L)	Per cent response of synthetic See Germination	Days
1+ MS(L)	81	21
3+ MS(L)	87	21
5+ MS(L)	78	21

When, before encapsulation, nodal segments were pulse treated with IBA (Indole Butyric Acid) for 48 hrs and then encapsulated in sodium alginate beads and inoculated in medium composition as described in table 2 synthetic seed regeneration frequency observed was different. In this experiment was observed that liquid MS medium supplemented with 3 mg/l BAP showed highest regeneration frequency of 87% in comparison to MS medium supplemented with 1 and 5 mg/l BAP which showed regeneration frequency 81% and 78% respectively (Table 4). In our last experiment after encapsulation, beads were pulse treated with IBA for same time period as in experiment

Table5.Effect of Pulse treatment of IBA (Indole Butyric Acid) After Encapsulation of nodal Explants

Concentration of BAP(mgl^{-1}) + MS (L)	Per cent response of synthetic Seed Germination	Days
1+ MS (L)	81	21
3+ MS (L)	87	21
5+ MS (L)	68	21

Same pattern of results were obtained with difference in regeneration frequency value. These values were 81%, 87% and 68% on MS medium supplemented with 1,3and 5 mg/l BAP respectively (Table 5). Possible reason of effect of

Pulse treatment to affect regeneration frequency is not known. All experiments were performed in triplicate to minimize error during data calculation.

CONCLUSIONS

The artificial seed technology provides an optional method of micro propagation for a wide array of medicinal plants, especially desirable elite genotypes. However, successful plant retrieval from encapsulated vegetative micropropagules following short-term storage is mostly depends on plant species, matrix composition and period of storage. Trees generally have a long generation time and are mostly heterozygous. Due to these reasons, genetic development

ISSN 2055-8139(Print), ISSN 2055-8147(Online)

in these species has been a most important hindrance. Large scale clonal propagation of superior clones along with accelerated tree improvement programs is necessary for successful and rapid exploration of tree plant. It is possible to use easy-to-obtain somatic fragments of the plants by nodal segment cuttings by applying artificial seed technology in *A.lakoocha*. One of the advantages of synthetic seed technology is that the risk of somaclonal variation would also be reduced because mutations are known to occur more frequently when the de- and re-differentiation processes occur during initial growth. However, the re-growth ability of the unipolar propagules after encapsulation hindered by factors such as precocious growth and lack of a tap root. Encapsulated nodal segments or apical/ auxiliary shoot buds are considered to be more effective come into view if we make the relative ease and extensibility of the technique, as well as the possibility of undamaged and uninfected moving small-sized micro propagated plant material between laboratories and countries, while reducing phytosanitary and quarantine problems (Bapat, 1993., Mathur et al., 1989; Hasan and Takagi, 1995; Maruyama et al., 1997b,. Piccioni and Standardi, 1995).

The results presented in this paper support the feasibility of this cost-effective method, for a large scale propagation of this ecologically and medicinally potent plant species. One of the drawback of synthetic seed technology is that on storage of seeds, percent regeneration frequency is greatly reduced Therefore, for large-scale application of this synthetic seed technology, further experiments are necessary; to accomplish a higher percentage of conversion make them viable even after long time storage of encapsulated nodal segments of *A.lakoocha.* Further, encapsulated somatic embryos could offer an important system for the transportation and exchange of germplasm in a safe and economical manner.

REFERENCES

Ahmad N., Anis M., , 2010. Direct plant regeneration from encapsulated nodal segments of *Vitex negundo* Biologia Plantarum 54 : 748-752

Ara H., Jaiswal U., Jaiswal VS., 2000. Synthetic seed: prospects and limitations. Current Science 78:1438-1444

Ballester A., Janeiro L.V., Vietez A.M., 1997. Cold storage of shoot cultures and alginate encapsulation of shoot tips of *Camellia japonica* L. and *Camellia reticulata* Lindley. Scientia Horticulturae. 71: 67-78

Bapat V.A., Studies on synthetic seeds of sandalwood (*Santalum album* L.) and mulberry (*Morus indica* L) - In: Redenbough, K. (ed.), CRC Press, Boca Raton, Synseeds: Applications of Synthetic Seeds to Crop Improvement. 381-407.

Chand S., Singh A. K., 2004. Plant regeneration from encapsulated nodal segments of Dalbergia sissoo Roxb., a timber-yielding leguminous tree species Journal of Plant Physiology161. 237–243

Chandramu C., Rao D.M., Reddy V.D., 2003. High frequency induction of multiple shoots from nodal explants of *Vitex negundo* L. using sodium sulphate. – Journal of Plant Biotechnology. **5**: 107-113,

Ganapathi T. R., Srinivas L., Suprasanna P., Bapat V. A., 2001. Regeneration Of Plants From Alginate-Encapsulated Somatic Embryos Of Banana Cv. Rasthali (Musa Spp. Aab Group). In Vitro Cell Development Biology 37:178-181,

Ganapathi, T.R., Suprasanna, P., Bapat, V.A., Rao, P.S., 1992 .Propagation of banana through encapsulated shoot tips. Plant Cell Reprduction. 11: 571-575,

ISSN 2055-8139(Print), ISSN 2055-8147(Online)

Hasan S.M.Z., Takagi H., 1995.Alginate coated nodal segments of yam (*Dioscorea* spp.) for germplasm exchange and distribution. - Plant genetics. Resource. Newsletters. 103: 32-35,

Joshee N., Bastola D.R., Agrawal V.P. Yadav A.K., 2002. Lakoocha: A Multipurpose Tree of Warm Climate Trends in new crops and new uses.. J. Janick and A. Whipkey (eds.). ASHS Press, Alexandria, VA.

Mandal J., Pattnaik S., Chand P.K., 2000. Alginate encapsulation of axillary buds of *Ocimum americanum* L.(hoary basil), *O. basilicum* L. (Sweet basil), *O. gratissimum* L. (shrubby basil), and *O. sanctum* (sacred basil). In Vitro Cell Development Biology Plant 36:287-292

Maruyama E., Kinoshita I., Ishi K., Shigenaga H., Ohba K., Saito A.,1997b. Germplasm conservation of the tropical forest trees, Cedrela odorata C. Plant Cell Reproduction. 16:393–396

Micheli M.,Hafiz I.A., Standard A., 2007. Encapsulation of in vitro derived explants of olive (Olea europaea L. cv. Moraiolo). II. Effects of storage on capsule and derived shoots performance. Scientia Horticulturae .113:286–292

Min A. K., Park J. K., 2002.High Frequency Plant Regeneration of Garlic (*Allium sativum* L.) Calli Immobilized in Calcium Alginate Gel. *Biotechnology. Bioprocess Engineering*. 7: 206-211

Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. – Physiology of Plant 15: 473-494

Naik S.K., . Chand P. K., Nutrient-alginate encapsulation of in vitro nodal segments of pomegranate (*Punica granatum* L.) for germplasm distribution and exchange

Piccioni, E., Standardi, A., 1995. Encapsulation of micropropagated buds of six woody species. Plant Cell Tissue Organ. Culture. 42, 221–226

Rai M. K. , Jaiswal V.S., Jaiswal U. , 2008. Encapsulation of shoot tips of guava (Psidium guajava L.) for short-term storage and germplasm exchange. Scientia Horticulturae 118: 33–38

Ray A., Bhattacharya S., 2008. Storage and plant regeneration fromencapsulated shoot tips of Rauvolfia serpentina—an effective wayof conservation and mass propagation. *South African* Journal of Botany 74:776–779

Singh A.K., Sharma M., Varshney R., Agarwal S.S., Bansal K.C., 2006a. Plant regeneration from alginate-encapsulated shoot tips of *Phyllanthus amarus* Schum and Thonn, a medicinally important plant species. In Vitro *Cellular* & *Developmental Biology* 42:109-113

Singh A.K., Varshney R., Sharma M., Agarwal S.S., Bansal K.C., 2006b. Regeneration of plants from alginate-encapsulated shoot tips of *Withania somnifera* (L.) Dunal, a medicinally important plant species. Journal of Plant Physiology 163:220-223

Singh, S.K., Rai, M.K., Asthana, P., Pandey, S., Jaiswal, V.S., Jaiswal, U., 2009. Plant regeneration from alginate-encapsulated shoot tips of Spilanthes acmella (L.) Murr., amedicinally important and herbal pesticidal plant species. *Acta* Physiologiae Plantarum 31: 649–653.

Thiruvengadam M., Nagella P., Chung I.M ., 2012 Plant regeneration from alginate-encapsulated shoot tips of *Momordica dioica* for short term storage and germplasm exchange and distribution Acta Biologica Hungarica5:266-270

Verma, S.K ., Rai K.M., Asthana p., Jaiswal V.S., Jaiswal U., 2010. In vitro plantlets from alginate-encapsulated shoot tips of Solanum nigrum L. Scientia Horticulturae 124:517–521

ISSN 2055-8139(Print), ISSN 2055-8147(Online)

Zeynali M., zanjani B. M., Jalal S., Mohsen N., Mostafa G. , Alireza E., Seyyed H., Mousavi-A.,2013: *In vitro* plant regeneration from alginate-encapsulated somatic embryos of rapeseed (*Brassica napus* cv. Tallayeh) International Journal of Traditional and Herbal Medicine. Vol., 1:13-18,

ISSN 2055-8139(Print), ISSN 2055-8147(Online)