J Plant Biotechnol (2021) 48:255–263
DOI:https://doi.org/10.5010/JPB.2021.48.4.255

ISSN 1229-2818 (Print)
ISSN 2384-1397 (Online)

Research Article

Regeneration of plants from alginate-encapsulated axenic nodal segments of *Paederia foetida* L. - A medicinally important and vulnerable plant species

Biswaranjan Behera · Shashikanta Behera · Shasmita · Debasish Mohapatra · Durga Prasad Barik · Soumendra Kumar Naik

Received: 22 September 2021 / Revised: 21 December 2021 / Accepted: 21 December 2021
ⓒ Korean Society for Plant Biotechnology

Abstract *Paederia foetida* L. is an important medicinal plant that has been used for the treatment of various gastrointestinal related ailments by different tribal communities in India. This plant is also known for its use as a food. Due to overexploitation, *P. foetida* has been classified as a vulnerable plant in some states of India. The propagation of *P. foetida* by conventional methods is easy but very slow. Synthetic seed technology offers incredible potential for *in vitro* propagation of threatened and commercially valuable plants, and can also facilitate the storage and exchange of axenic plant material between laboratories. However, synthetic seed production for *P. foetida* has not yet been reported. Thus, to the best of our knowledge, the present study is the first attempt to produce synthetic seeds of *P. foetida* by calcium alginate encapsulation of *in vitro* regenerated axenic nodal segments. Sodium alginate (3%) and $CaCl_2$ (100 mM) were found to be the optimal materials for the preparation of ideal synthetic seeds, both in terms of morphology and germination ability. The synthetic seeds showed the best germination (formation of both shoot as well as root; 83.3%) on ½ MS medium augmented with 0.5 mg/L indole-3-acetic acid. The plantlets obtained from these synthetic seeds could be successfully acclimatized under field conditions. We also studied the storage of these synthetic seeds at low temperature and their subsequent sprouting/germination. The seeds showed a germination rate of 63.3% even after 21 days of storage at 4 °C; thus, they could be useful for transfer and exchange of *P. foetida* germplasm.

Keywords Medicinal plant, micropropagation, *Paederia foetida*, synthetic seed

Introduction

Paederia foetida L. belonging to family Rubiaceae, is an important medicinal and perennial vine. It has a distinguishing foetid smell due to the presence of methyl mercaptan in its leaves and stems (Bose et al. 1953; Chopra et al. 1969). In India, different tribes use this plant for treating various ailments related to gastral intestine such as stomachache, gastromegaly, flatulence, ulcers, and piles (Chanda et al. 2013; Kumar et al. 2014). The plant was also reported to have anti-inflammatory, anti-cancer, anti-diarrheal, antioxidant, antibacterial and antifungal properties (Begum et al. 2007; Osman et al. 2009; Chanda et al. 2013; Kumar et al. 2014). The plant is component of a number of Ayurvedic products available in the Indian market. It is also widely used as a food in different states of India including Odisha, West Bengal and Arunachal Pradesh (Behera et al. 2017).

Unfortunately, *P. foetida* is enlisted as a vulnerable plant in some states of India including Odisha due to overexploitation by anthropogenic activities (e.g., deforestation, unsustainable harvesting by local baidyas, habitat fragmentation and urbanization) (Srivastava and Srivastava 2004; Ved et al. 2008). The proliferation rate of *P. foetida* by conventional methods of propagation (i.e., stem cuttings and seeds) is very slow and not enough to meet the com-

†These authors contributed equally to this study
B. Behera†
ICAR-National Rice Research Institute, Cuttack 753006, Odisha, India

S. Behera†
Centre of Excellence in Natural Products and Therapeutics, Department of Biotechnology and Bioinformatics, Sambalpur University, Sambalpur 768019, Odisha, India

Shasmita · D. Mohapatra · D. P. Barik · S. K. Naik (✉)
Department of Botany, Ravenshaw University, Cuttack 753003, Odisha, India
e-mail: sknuu@yahoo.com

This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

mercial demand (Aquilar 2001). The above-mentioned problems necessitate the development of *in vitro* propagation of *P. foetida*.

Production of synthetic (or artificial seeds) by encapsulation technology offers incredible potential for *in vitro* propagation of threatened and commercially valuable plants. Synthetic seed technology has, in fact, combined the advantages of clonal propagation and storage which helps in conservation of germplasm of elite plant species, short- and long-term storage. Besides, due to easy handling and small size of such seeds the exchange of axenic plant material between laboratories is always a possibility (Naik and Chand 2006; Gantait et al. 2015; Gantait et al. 2017a; Qahtan et al. 2019). During last two decades, development of synthetic seeds protocol has been intensified for the production of large number of fruit trees, medicinal and ornamental plant species such as *Punica granatum* (Naik and Chand 2006), *Drimiopsis kirkii* (Haque and Ghosh 2014), *Rauvolfia serpentina* (Gantait et al. 2017a), *Plumbago zeylanica* (Jain et al. 2018) and *Hedychium coronarium* (Behera et al. 2020) etc.

In *P. foetida*, synthetic seed production has not yet been reported. Hence, to the best of our knowledge, the present study is the first report on production of synthetic seed of *P. foetida* by using *in vitro* regenerated nodal segments. Low temperature storage and subsequent plant retrieval from the encapsulated nodal segments were also studied so that the techniques will be useful for short term storage and transfer of *P. foetida* germplasm to different places as per the requirement.

Materials and Methods

Explant source

The axenic nodal segments (approximately 0.8 ~ 1.0 cm) excised from *in vitro* established shoot cultures of *P. foetida* on Murashige and Skoog's (1962) (MS) medium + 3.0 mg/l N^6-benzylaminopurine (BAP) using nodal explants (Behera et al. 2018) were used as explants for encapsulation.

Encapsulation matrix

Sodium alginate (Hi-media, India) solutions were prepared in five different concentrations i.e., 1, 2, 3, 4 and 5% using liquid MS medium supplemented with 3.0 mg/l BAP (Hi-media, India). For complexation calcium chloride ($CaCl_2$) at three different concentrations i.e., 75, 100, 125 mM was prepared in liquid MS medium supplemented with 3.0 mg/l BAP. The pH of both sodium alginate and $CaCl_2$ was adjusted to 5.8 ± 0.1 prior to autoclave at 121°C and 15 pounds per square inch pressure for 17 min.

Encapsulation of axenic nodal segments and effect of encapsulation matrix on sprouting of synthetic seeds

Axenic nodal segments were immersed in sodium alginate solution (1 ~ 5%). Drops of sodium alginate solution containing the nodal segment was allowed to fall on $CaCl_2$ (75 ~ 125 mM) solution using a glass pipette and kept for 20 min for ion exchange. The beads, each with a single axenic nodal segment, were collected by pouring out the $CaCl_2$ solution. The beads were washed three times with autoclaved double distilled water and placed on sterilized tissue paper to remove excess water. The encapsulated nodes are now called as synthetic seeds and ready for further experiments. To examine the sprouting frequency of synthetic seeds, they were cultured/ inoculated on plant growth regulator free ½ MS medium. Data were recorded regarding the percentage of sprouting at day 14 of culture.

Evaluation of culture media and auxins on sprouting and/or germination of synthetic seeds

For experimentation on sprouting [production of only shoot(s)] and germination [production of both shoot(s) and root(s) on the same medium], the axenic nodal segments were encapsulated with best encapsulation matrix i.e., sodium alginate (3%) + $CaCl_2$ (100 mM) with a complexation time of 20 min. To adjust the optimum strength of MS basal media as substrate for germination, the synthetic seeds were inoculated on various strengths of MS media (i.e., MS, ½ MS, ¼ MS, 1/8 MS, and 1/16 MS) devoid of any plant growth regulators. Percentage of sprouting and germination was recorded after 14 and 30 days of culture, respectively.

Among the above mentioned five different media substrates tested, the best one (i.e., ½MS; on which maximum response was recorded) was fortified with different auxins at two different concentrations such as indole-3-acetic acid (IAA; 0.5 and 1.0 mg/l) or indole-3-butyric acid (IBA; 0.5 and 1.0 mg/l) or naphthaleneacetic acid (NAA; 0.5 and 1.0 mg/l), to check the influence of auxins for germination of synthetic seeds. All the media used for sprouting and germination were fortified with 30 g/l of sucrose as carbon source with 0.7% agar for solidification.

Influence of low temperature storage on germination frequency of synthetic seeds

For the study on the effect of low temperature storage on

germination frequency of synthetic seeds, encapsulation of the axenic nodal explants was done with best combination of sodium alginate (3%) and $CaCl_2$ (100 mM), with a complexation time of 20 min. Synthetic seeds were then kept in petri-dishes, wrapped with parafilm (Bemis, USA) and stored at 4°C in a refrigerator for different time duration i.e., 7, 15, 21 and 30 days. After each storage period the synthetic seeds were transferred to culture vessels containing ½ MS + 0.5 mg/l IAA and were evaluated for their germination frequency after 30 days.

Rooting of shoots sprouted from synthetic seeds

For those synthetic seeds which failed to germinate into plantlets, another set of experiment was carried out for root induction from the shoots obtained from the sprouted synthetic seeds. The regenerated shoots obtained from such synthetic seeds were transferred to the plant growth regulator free half-strength MS medium for rooting.

Culture conditions

All the cultures for synthetic seed experiments were maintained in a culture room at 25 ± 1°C under 16 h of photoperiod. The light was provided by cool white fluorescent tubes (50 μmol m^{-2} s^{-1} photon flux density).

Acclimatization and transfer of plants to soil

Well developed plantlets derived from germinated synthetic seeds as well as from rooting experiment were removed from culture medium, washed in tap water to remove traces of calcium alginate and/or agar, and transferred to Styrofoam cups (7.5 cm dia.) having autoclaved soil and sand in the ratio of 1:1 (w/v) as potting mixture. The plantlets were acclimatized following the procedure as described by Behera et al. (2018).

Statistical analysis

For synthetic seed protocol, five conical flasks each containing four synthetic seeds were used. For rooting, ten tubes containing one explant each were taken into consideration. All these experiments were repeated thrice. Observations were recorded at regular interval. The data analysis was carried out using ANOVA. Means with significant effect at $P \leq 0.05$ were designated by using DMRT (Duncan's New Multiple Range Test; Gomez and Gomez 1984).

Results

Effect of encapsulation matrix on sprouting of synthetic seeds

The sprouting ability of the synthetic seeds (Fig. 1 A, B) produced by using different combinations of sodium alginate (1, 2, 3, 4 and 5%) and $CaCl_2$ (75, 100 and 125 mM) was assessed by culturing them on ½ MS medium.

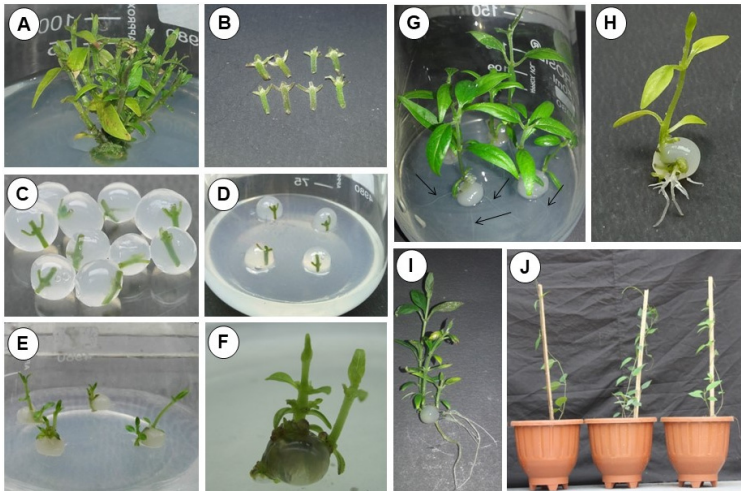

Fig. 1 (A) *In vitro* multiple shoots (on MS + 3.0 mg/L BAP) that were the source of axenic nodal segments for encapsulation and preparation of synthetic seeds. (B) Excised axenic nodal segments from the primary shoots ready for encapsulation. (C) Production of synthetic seeds by encapsulation of axenic nodal segments using sodium alginate (3%) and $CaCl_2$ (100 mM) with 20 min of ion exchange. (D) Synthetic seeds cultured on ½ MS medium. (E) Sprouting of the synthetic seeds on ½ MS medium. (F) Enlarged view of a sprouted synthetic seed. (G) Germination of synthetic seeds on ½ MS medium (roots indicated by arrow). (H) Magnified view of a single germinated synthetic seed after 14 days of culture. (I) Germinated synthetic seed showing emergence of roots and shoots on ½ MS + 0.5 mg/L IAA after 30 days of culture. (J) Acclimatized plants in pots containing garden soil

Table 1 Effects of sodium alginate and $CaCl_2$ concentrations (with a 20-min complexation time) on the sprouting of synthetic seeds (encapsulated axenic nodal segments) of *Paederia foetida* on ½ MS after 14 days of culture

$CaCl_2$ (mM)	Sodium alginate (%; w/v)	Sprouting of synthetic seeds (%)
75	1	21.7^{jk}
	2	46.7^{gh}
	3	68.3^{de}
	4	66.7^{def}
	5	58.3^{efg}
100	1	43.3^{hi}
	2	86.7^{abc}
	3	100^{a}
	4	88.3^{ab}
	5	66.7^{def}
125	1	33.3^{hij}
	2	58.3^{efg}
	3	75.0^{bcd}
	4	46.7^{gh}
	5	16.7^{kl}

Values within a column followed by same letters are not significantly different ($P \leq 0.05$; DMRT)

The combination of 3% sodium alginate and 100 mM $CaCl_2$ produced ideal synthetic seeds and showed best results with a maximum sprouting frequency of 100% on ½ MS, after 14 days of culture (Table 1; Fig. 1C-F). When 2% and 4% sodium alginate were used with optimum concentrations of $CaCl_2$ (100 mM) the rate of sprouting of synthetic seed was 86.7% and 88.3% respectively, which was less than 3% sodium alginate.

Higher concentration(s) of sodium alginate and $CaCl_2$ combinations i.e., 4% sodium alginate with 125 mM $CaCl_2$ and 5% sodium alginate with 125 mM $CaCl_2$ were not found to be suitable for production of synthetic seeds as the sprouting ability of these combinations were quite low i.e., 46.7% and 16.7%, respectively. Synthetic seeds prepared using lower concentration of sodium alginate i.e., 1% or 2% and $CaCl_2$ (75 mM) failed to show good sprouting on ½ MS medium (Table 1). Similarly, combination of 1% or 2% sodium alginate and 125 mM $CaCl_2$, produced synthetic seeds which exhibited poor sprouting response i.e., 33.3% and 58.3%, respectively. Lower concentration of sodium alginate (1%) in combination with 100 mM $CaCl_2$ was also unsuitable and only 43.3% sprouting of synthetic seeds was observed (Table 1). Among all the combinations of sodium alginate and $CaCl_2$ tested for sprouting of synthetic seeds, least percentage (16.7%) of sprouting was recorded when they were prepared using highest percent of sodium alginate (5%) and $CaCl_2$ (125 mM).

Table 2 Effect of media substrates on the sprouting and germination of synthetic seeds (prepared by encapsulating an axenic nodal segment with 3% sodium alginate and 100 mM $CaCl_2$) of *Paederia foetida* after 30 days of culture

Media substrate	Sprouting (%)	Germination (%)
MS	83.3^{b}	46.7^{ab}
½ MS	100^{a}	53.3^{a}
¼ MS	75.0^{bc}	40.0^{bc}
⅛ MS	58.3^{d}	31.7^{d}
1/16 MS	38.3^{e}	0.0^{e}

Values within a column followed by same letters are not significantly different ($P \leq 0.05$; DMRT)

Evaluation of culture media and auxins on sprouting and/or germination of synthetic seeds

The sprouting and germination frequency of the synthetic seeds, prepared in sodium alginate (3%) and $CaCl_2$ (100 mM), varied with different strengths (MS, ½ MS, ¼ MS, ⅛ MS, 1/16 MS) of media used for culture (Table 2). Out of different strengths of MS media tested, ½ MS was found to be the most suitable medium for sprouting as well as germination of synthetic seeds, where 100% sprouting and 53.3% germination was recorded in 14 days and 30 days, respectively (Table 2; Fig. 1G-H). On this medium the sprouting of synthetic seeds started within 3 ~ 4 days of culture. Further reduction of strength of medium beyond ½ MS reduced the frequency of sprouting and germination of synthetic seeds. Among all the media tested, least percentage of sprouting (38.3%) was recorded on 1/16 MS. On this medium the synthetic seeds also failed to show any germination (Table 2).

The germination frequency of synthetic seeds was quite low (53.3%) on ½ MS as all the seeds unable to produce roots on the above medium at day 30 of culture. Further to test the influence of auxins on germination if any, synthetic seeds were inoculated on ½ MS augmented with various concentrations (0.5 ~ 1.0 mg/l) of auxins (IAA, IBA or NAA). The percentage of germination varied with various types and concentrations of auxins tested (Table 3). After 30 days of culture, ½ MS medium fortified with IAA (0.5 mg/l) was found to be best, with highest germination percentage (83.3%) of the synthetic seeds. The average number of shoots and roots per synthetic seed were 3 and 9, respectively (Fig. 1 I). The average shoot and root length was recorded to be 4.5 cm and 3.8 cm, respectively.

Table 3 Influence of auxins on the sprouting and germination of synthetic seeds of *Paederia foetida* after 30 days of culture

Media substrate	Sprouting (%)	Germination (%)	Shoot/ synthetic seed	Shoot length (cm)	Root/ synthetic seed	Root length (cm)
½ MS	100[a]	53.3[c]	3.0[a]	3.3[abc]	7.0[bc]	2.5[bc]
½ MS + IBA 0.5 mg/l	25.0[c*]	25.0[d]*	2.0[ab]	2.8[bcd]	**	**
½ MS + IBA 1.0 mg/l	25.0[c*]	25.0[d*]	1.0[bc]	1.0[e]	**	**
½ MS + NAA 0.5 mg/l	C	C	-	-	-	-
½ MS + NAA 1.0 mg/l	C	C	-	-	-	-
½ MS + IAA 0.5 mg/l	100[a]	83.3[a]	3.0[a]	4.5[a]	9.0[a]	3.8[a]
½ MS + IAA 1.0 mg/l	86.7[ab]	68.3[b]	1.0[bc]	4.0[ab]	7.9[ab]	2.8[ab]

C, Callusing; *, sprouting/conversion with callus; **, abnormal root with callus. Values within a column followed by same letters are not significantly different ($P \leq 0.05$; DMRT).

Table 4 Germination of synthetic seeds of *Paederia foetida* after storage at 4°C for different durations

Storage (days)	Germination (%)	Shoots/ synthetic seed	Shoot length (cm)	Roots/ synthetic seed	Root length (cm)
0	83.3[a]	3.0[a]	4.5[a]	9.0[a]	3.8[ab]
7	83.3[a]	2.8[ab]	4.3[ab]	9.0[a]	3.9[a]
14	73.3[b]	2.0[c]	4.2[abc]	8.9[ab]	3.8[ab]
21	63.3[c]	2.0[c]	3.9[abcd]	7.6[c]	3.2[c]
30	40.0[d]	1.3[cd]	3.5[e]	5.2[d]	3.0[d]

Values within a column followed by same letters are not significantly different ($P \leq 0.05$; DMRT)

Half-strength MS medium when supplemented with IBA (0.5 mg/l or 1.0 mg/l) showed 25% germination of synthetic seeds, after 30 days of culture. The synthetic seeds cultured on these media showed shoot regeneration with abnormal rooting accompanied by callus formation (Table 3). However, the number of shoots produced was comparatively more in lower concentration (0.5 mg/l) of IBA. The synthetic seeds failed to germinate or even sprout on ½ MS medium supplemented with NAA, as callusing was seen on the explants within synthetic seeds (Table 3).

Effect of low temperature storage on germination frequency of synthetic seeds

Variation in the duration (7, 15, 21 and 30 days) of storage of synthetic seeds (at 4°C) influences both germination and regeneration frequency (Table 4). The germination frequency of the synthetic seeds after 7 days of storage was found to be at par with the freshly prepared synthetic seeds on ½ MS supplemented with 0.5 mg/l IAA. The synthetic seeds were stored for a period of 14 days without significant decrease in their germination efficiency (Table 4). It was observed that with an increase in storage time, the percentage of germination of synthetic seeds gradually goes on declining. Thus, the percentage of germination of synthetic seeds declined gradually with the increase in storage time. After 21 and 30 days of storage the germination rate of the synthetic seeds was about 63.3% and 40.0%, respectively.

Rooting of shoots sprouted from synthetic seeds

Half-strength MS medium was observed to be best for rooting (90%) of shoots excised from sprouted synthetic seeds, which failed to germinate into plantlets. From each shoot an average of 17.8 roots with 2.6 cm length were obtained.

Acclimatization of plantlets

The plantlets obtained from germinated synthetic seeds, after removing traces of agar and alginate were transferred to small Styrofoam cups containing equal proportion (1:1) of autoclaved garden soil and sand where about 86.7% of plantlets were acclimatized. Zero percent mortality was recorded when they were subsequently transferred to larger pots containing soil and then to field conditions. The plantlets regenerated from rooting of sprouted shoots also showed similar trend in acclimatization and successfully transferred to field.

Discussion

Production of synthetic seeds by alginate encapsulation of somatic embryos or vegetative propagules such as nodal segment or shoot bud provides combined advantages of rapid multiplication of plants with that of storage for germplasm conservation (Standardi and Piccioni 1998, Ara et al. 2000; Srivastava et al. 2009). Ease in handling and transportation of germplasm to other laboratory due to small size of capsules, cost reduction and genetic uniformity of plants are some of the other advantages of synthetic seed technology (Ara et al. 2000; Naik and Chand 2006; Rai et al. 2009; Verma et al. 2015).

Synthetic seeds do not contain nutritive tissue like endosperm, thus preparation of sodium alginate and $CaCl_2$ without nutrients and/or plant growth regulators may cause a relatively low rate of sprouting and/ or germination (Verma et al. 2010). Amalgamation of nutrients as well as plant growth regulators in the alginate beads can fulfil the role of endosperm for encapsulated explant (Verma et al. 2010). Accessibility of nutrients to the encapsulated explants plays a key factor for germination of synthetic seeds (Fujii et al. 1987; Gantait et al. 2017a) and exogenous application of essential nutrients to serve the role of endosperm is necessary to improve the frequency of germination (Gantait et al. 2017a). Thus, in the present study sodium alginate and $CaCl_2$ were prepared using MS medium supplemented with 3.0 mg/l BAP. MS + 3.0 mg/l BAP has already been found as optimal medium for axillary shoot proliferation of axenic nodal segments and was the basis of opting the same for preparation of synthetic seed. Preparation of sodium alginate along with appropriate basal medium with or without plant growth regulator (to act as artificial endosperm) has also been reported in different plants such as *Bacopa monnieri* (Rency et al. 2017), and *Salvia officinalis* (Grzegorczyk and Wysokiska 2011).

In recent years, the use of non-embryogenic vegetative propagules like nodal segments, axillary buds, apical shoot buds, protocorm like bodies etc., as an alternative to somatic embryos for encapsulation and production of synthetic seeds has also been reported in a number of plant species (Gantait et al. 2015). Plants in which either somatic embryogenesis is not reported or production of uniform quality of embryos is lacking, the only alternative is to use the non-embryogenic vegetative propagules (Rai et al. 2009). Incidentally, somatic embryogenesis is yet to be reported in *P. foetida*. At the same time use of nodal segments for axillary shoot proliferation has been by far the safest method for micropropagation, as it does not usually induce somaclonal variation. For the reasons mentioned above, in this study the axenic nodal segments obtained from shoots culture established on MS + 3.0 mg/l BAP were selected as the explants for synthetic seed production. Use of axenic nodal segments for successful production of synthetic seeds was well documented in a number of medicinal plant species like *Eclipta alba* (Singh et al. 2010), *Ruta graveolens* (Ahmad et al. 2012), *Tylophora indica* (Gantait et al. 2017b), *Vitex trifolia* (Alatar et al. 2017) and *Withania somnifera* (Fatima et al. 2013).

The concentration of sodium alginate and $CaCl_2$ affect the sprouting as well as germination ability of the synthetic seed. In the present report, highest percentage of sprouting (100%) was reported in the synthetic seeds which were produced using sodium alginate (3%) and $CaCl_2$ (100 mM). Similar reports of highest frequency of sprouting and/ or germination of synthetic seed using 3% sodium alginate and 100 mM $CaCl_2$ has also been reported in many medicinal plants including *Artocarpus lakoocha* (Verma et al. 2015), *Vitex trifolia* (Alatar et al. 2017). Synthetic seeds prepared with higher concentrations of sodium alginate i.e., 4% or 5% as well as $CaCl_2$ (125 mM) resulted in suppression of sprouting ability of the synthetic seeds which may be either due to the hardness of the bead which inhibit the emergence of the shoots (Khan et al. 2013) or may be due to $CaCl_2$ toxicity (Gantait et al. 2015). As discussed earlier, optimal ion exchange of Na^+ and Ca^{2+} controls the hardness of the beads of the synthetic seeds which in turns influence its sprouting and/ or germination. The optimum concentrations of sodium alginate and $CaCl_2$ for maximum sprouting and/ or germinations of synthetic seeds vary from propagules type and plant species (Perveen and Anis 2014). Contrary to our finding 4% sodium alginate with 100 mM $CaCl_2$ was reported as the best combination for preparation and utilization of synthetic seeds in medicinal plants including

Clitoria ternatea (Kumar and Thomas 2012), *Ficus carica* (Sharma et al. 2015), and *Zingiber officinale* (Sundararaj et al. 2010). Such difference in the required concentrations of sodium alginate for production and utilization of synthetic seed was may be due to variation in purity level of commercial source from which the chemicals were procured (Redenbaugh et al. 1986; Pattnaik and Chand 2000; Sharma et al. 2015; Rathore and Kheni 2017).

Next part of experimentation on development of synthetic seed technology was evaluation of different culture media substrate (i.e., various concentrations of MS basal media like 1/16 MS, 1/8 MS, ¼ MS, ½ MS and MS) for sprouting and germination of synthetic seeds. Different strengths of MS basal media showed varied responses to sprouting and/ or germination of synthetic seeds. Half-strength MS media was recorded to have better results (sprouting, 100% and subsequent germination, 53.33%) in comparison to other strengths of MS media tested. The reason for less responsiveness of other strengths of MS was probably due to their nutrient toxicity or deficiency (Gantait et al. 2017a). Germination of synthetic seeds were enhanced and best conversion rate (83.3%) was recorded when ½ MS was augmented with 0.5 mg/l IAA. Addition of auxin to the sprouting/ germination medium was also found necessary in some medicinal plant species including *Glycyrrhiza glabra* (IAA; Mehrotra et al. 2012) and *Salvia officinalis* (IAA; Grzegorczyk and Wysokiska 2011) for better germination of synthetic seeds prepared from different vegetative propagules. In this study supplementation of IBA and NAA with ½ MS failed to enhance the germination frequency, rather detrimental, indicating the use of auxin for such response is species specific.

One of the desirable characters of synthetic seed is their ability to retain viability (i.e., the sprouting and germination potential) even after a considerable period of storage. This feature is necessary for their use in germplasm distribution and exchange (Ahmad et al. 2012; Jahan and Anis 2015). Low temperature and proper moisture were necessary during storage for maintenance of viability of synthetic seeds (Hegazi 2011). In this study, storage duration (7, 15, 21 and 30 days) was found to influence the frequency of germination of synthetic seeds stored at 4°C. The viability of synthetic seeds goes on decreasing with increased duration of storage of synthetic seeds. This type of decreased viability in stored synthetic seeds was might be because of inhibition of tissue respiration due to the presence of alginate (Redenbaugh et al. 1987; Naik and Chand 2006; Hegazi 2011) or loss of moisture during storage (Danso and Ford-Lloyd 2003; Fatima et al. 2013). Similar type of observation of decrease in viability after storage of synthetic seeds was also recorded in various medicinal plants like *Capparis orientalis* (Hegazi 2011), *Eclipta alba* (Singh et al. 2010) and *Ficus carica* (Sharma et al. 2015).

In the present study, the shoots generated from sprouted synthetic seeds (those who failed to germinate) were rooted successfully on plant growth regulator free ½ MS. Similar observation of rooting were also reported in *Albizia lebbeck* (Perveen and Anis 2014), *Bacopa monnieri* (Rency et al. 2017), *Cassia angustifolia* (Bukhari et al. 2014), *Cineraria maritime* (Srivastava et al. 2009), *Nyctanthes arbortrisits* (Jahan and Anis 2015), *Ruta graveolens* (Ahmad et al. 2012) and *Withania somnifera* (Fatima et al. 2013). Acclimatization of *in vitro* raised plants is always considered as a critical stage due to possibilities of high percentages of plant loss during transplantation (Pospóšilová et al. 1999; Ahmad et al. 2012). The plantlets obtained after germination of synthetic seeds have been successfully acclimatized in field conditions.

The synthetic seed system using encapsulation of axenic nodal segments of *P. foetida* has been reported here. In recent years, such synthetic seeds system has been employed in a number of medicinal plant species and established as a fitting alternative for clonal propagation, conservation and germplasm distribution and exchange (Ahmad et al. 2012, Fatima et al. 2013, Gantait et al. 2015). *P. foetida* plants regenerated through axillary shoot proliferation of axenic nodal segments usually produced clonal plant. Since in this study axenic nodal segments have been used for encapsulation, the plants produced from this system are expected to be clonal. Retrieval of *P. foetida* plants following storage at low temperature (4°C) implies the potential of the synthetic seed system to be used for germplasm distribution and exchange without losing its viability during transportation. At the same time the developed synthetic system could be useful for short term storage of this threatened plant species and form the basis of further research for developing long-term conservation methods using cryopreservation.

Acknowledgements

BB gratefully acknowledge the RGNF funding of UGC. The grant of DST, Government of India under DST-FIST programme to the Department of Botany is gratefully acknowledged.

References

Ahmad N, Faisal M, Fatima N, Anis M (2012) Encapsulation of microcuttings for propagation and short-term preservation in *Ruta graveolens* L.: a plant with high medicinal value. Acta Physiol Plant. 34(6):2303-2310

Alatar AA, Ahmad, Javed SB, Abdel-Salam EM, Basahi R, Faisal M (2017) Two-way germination system of encapsulated clonal propagules of *Vitex trifolia* L.: an important medicinal plant. J Hortic Sci Biotechnol. 92(2):175-182

Aquilar NO (2001) *Paederia foetida* L. In: Van Valkenburg JL, Bunyapraphatsara N (Eds) Plant Resource of South-East Asia. Medicinal and Poisonous Plants. Ledien, The Netherlands, Blackhuys Publisher, 2001, pp 396-400

Ara H, Jaiswal U, Jaiswal VS (2000) Synthetic seed: prospects and limitations. Curr Sci. 78(12):1438-1444

Begum J, Yusuf M, Chowdhury JU, Khan S, Anwar MN (2007) Antifungal activity of forty higher plants against phytopathogenic fungi. Bangladesh J Microbiol. 24:76-78

Behera B, Behera S, Jena PK, Barik DP, Naik SK (2017) Adventitious shoot organogenesis and plant regeneration from internode explants of *Paederia Foetida* L.: a valuable medicinal plant. Biosci Biotech Res Asia. 14(3):893-900

Behera B, Sinha P, Gouda S, Rath SK, Barik DP, Jena PK, Panda PC, Naik SK (2018) *In vitro* propagation by axillary shoot proliferation, assessment of antioxidant activity, and genetic fidelity of micropropagated *Paederia foetida* L. J App Biol Biotech. 6(2):41-49

Behera S, Rout, KK, Panda, PC, Naik SK (2020). Production of non-embryogenic synthetic seeds for propagation and germplasm transfer of *Hedychium coronarium* J. Koenig. J Appl Res Med Arom Plant, 19, 100271

Bose PK, Banerjee AK, Ghosh C (1953) Chemical investigation of *Paederia foetida* Linn. Transaction of Bose Research Institute. 19:77-78

Bukhari N, Siddique I, Perveen K, Siddiqui I, Alwahibi M (2014) Synthetic seed production and physio-biochemical studies in *Cassia angustifolia* Vahl. - a medicinal plant. Acta Biol Hung. 65(3):355-367

Chanda S, Sarethy IP, De B, Singh K (2013) *Paederia foetida* - a promising ethno-medicinal tribal plant of north-eastern India. J Forestry Res. 24:801-808

Chopra RN, Chopra IC, Verma BS (1969) Supplement to glossary of Indian medicinal plants. New Delhi: CSIR

Danso KE, Ford-Lloyd BV (2003) Encapsulation of nodal cuttings and shoot tips for storage and exchange of *Cassava* germplasm. Plant Cell Rep. 21(8):718-725

Fatima N, Ahmad N, Anis M, Ahmad I (2013) An improved *in vitro* encapsulation protocol, biochemical analysis and genetic integrity using DNA based molecular markers in regenerated plants of *Withania somnifera* L. Ind Crops Prod. 50:468-477

Fujii JA, Slade D, Redenbaugh K, Walker KA (1987) Artificial seeds for plant propagation. Trends Biotechnol. 5:335-339

Gantait S, Kundu S, Ali N, Sahu NC (2015) Synthetic seed production of medicinal plants: a review on influence of explants, encapsulation agent and matrix. Acta Physiol Plant. 37:98

Gantait S, Kundu S, Yeasmin L, Ali MN (2017a) Impact of differential levels of sodium alginate, calcium chloride and basal media on germination frequency of genetically true artificial seeds of *Rauvolfia serpentina* (L.) Benth. ex Kurz. J Appl Res Med Aromat Plant. 4:75-81

Gantait S, Vijayan J, Majee A (2017b) Artificial seed production of *Tylophora indica* for interim storing and swapping of germplasm. Horticult Plant J. 3(1): 41-46

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research. John Wiley & Sons

Grzegorczyk I, Wysokińska H (2011) A protocol for synthetic seeds from *Salvia officinalis* L. shoot tips. Acta Biol Crac Ser Bot. 53(1):80-85

Haque SM, Ghosh B (2014) Somatic embryogenesis and synthetic seed production—a biotechnological approach for true-to-type propagation and *in vitro* conservation of an ornamental bulbaceous plant *Drimiopsis kirkii* Baker. Appl Biochem Biotechnol. 172(8):4013-4024

Hegazi GA (2011) Viability of encapsulated shoot tips of *Capparis orientalis* Duh. J Nat Sci. 9(8):223-228

Jahan AA, Anis M (2015) Retrieval of plantlets from cryopreserved alginate buds of *Nyctanthes arbortristis* L. - an effectual approach for germplasm conservation. Int J Dev Res. 5(5): 4397-4402

Jain P, Danwra K, Sharma HP, Mahato D (2018) *In vitro* tissue culture studies and synthetic seed formation from *Plumbago zeylanica* L. Indian J Exp Biol. 56:769-773

Khan MK, Sharma T, Misra P, Shukla PK, Singh Y, Ramteke PW (2013) Production of plantlets on different substrate from encapsulated *in vitro* nodal explants of *Stevia rebaudiana*. Int J Recent Sci Res. 4(3):211-215

Kumar GK, Thomas TD (2012) High frequency somatic embryogenesis and synthetic seed production in *Clitoria ternatea* Linn. Plant Cell Tiss Org Cult. 110(1):141-151

Kumar V, Anwar F, Ahmed D, Verma A, Ahmed A, Damanhouri ZA, Mishra V, Ramteke PW, Bhatt PC, Mujeeb M (2014) *Paederia foetida* Linn. Leaf extract: an antihyperlipidemic, antihyperglycaemic and antioxidant activity. BMC Complement Altern Med. 14:76

Mehrotra S, Khwaja O, Kukreja AK, Rahman L (2012) ISSR and RAPD based evaluation of genetic stability of encapsulated micro shoots of *Glycyrrhiza glabra* following 6 months of storage. Mol Biotechnol. 52(3):262-268

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tabacco tissue cultures. Physiol Plant. 15:473-497

Naik SK, Chand PK (2006) Nutrient-alginate encapsulation of *in vitro* nodal segments of pomegranate (*Punica granatum* L.) for germplasm distribution and exchange. Sci Hortic. 108: 247-252

Osman H, Rahim AA, Isa NM, Bakhir NM (2009) Antioxidant activity and phenolic content of *Paederia foetida* and *Syzygium*

aqueum. Molecules. 14:970-978

Pattnaik S, Chand PK (2000) Morphogenic response of the alginate-encapsulated axillary buds from *in vitro* shoot cultures of six mulberries. Plant Cell Tiss Org Cult. 60(3):177-185

Perveen S, Anis M (2014) Encapsulation of internode regenerated adventitious shoot buds of Indian Siris in alginate beads for temporary storage and two-fold clonal plant production. Acta Physiol Plant. 36(8):2067-2077

Pospóšilová J, Tichá I, Kadleček P, Haisel D, Plzáková Š (1999) Acclimatization of micropropagated plants to *ex vitro* conditions. Biol Plant. 42(4):481-497

Qahtan AA, Abdel-Salam EM, Alatar AA, Wang QC, Faisal M (2019) An introduction to synthetic seeds: production, techniques, and applications. In: Faisal M, Alatar A (Eds) Synthetic Seeds. Springer, Cham, pp 1-20

Rai MK, Asthana P, Singh SK, Jaiswal VS, Jaiswal U (2009) The encapsulation technology in fruit plants-a review. Biotechnol Adv. 27(6):671-679

Rathore MS, Kheni J (2017) Alginate encapsulation and *in vitro* plantlet regeneration in critically endangered medicinal plant, *Withania coagulans* (Stocks) Dunal. Proc Nat Acad Sci, India, Sec Biol Sci. 87(1):129-134

Redenbaugh K, Paasch BD, Nichol JW, Kossler ME, Viss PR, Walker KA (1986) Somatic seeds: encapsulation of asexual plant embryos. Nat Biotechnol. 4(9):797-801

Redenbaugh K, Slade D, Viss PR, Fujii J (1987) Encapsulation of somatic embryos in synthetic seed coats. Hort Sci. 22:803-809

Rency AS, Satish L, Pandian S, Rathinapriya P, Ramesh M (2017) *In vitro* propagation and genetic fidelity analysis of alginate-encapsulated *Bacopa monnieri* shoot tips using *Gracilaria salicornia* extracts. J Appl Phycol. 29(1):481-494

Sharma S, Shahzad A, Mahmood S, Saeed T (2015) High-frequency clonal propagation, encapsulation of nodal segments for short-term storage and germplasm exchange of *Ficus carica* L. Trees. 29(2):345-353

Singh SK, Rai MK, Asthana P, Sahoo L (2010) Alginate-encapsulation of nodal segments for propagation, short-term conservation and germplasm exchange and distribution of *Eclipta alba* (L.). Acta Physiol Plant. 32:607-610

Srivastava SK, Srivastava N (2004) *In vitro* multiplication of *Paedaria foetida* L.- a rare medicinal plant. J Plant Biochem Biotechnol. 13:89-91

Srivastava V, Khan SA, Banerjee S (2009) An evaluation of genetic fidelity of encapsulated microshoots of the medicinal plant: *Cineraria maritima* following six months of storage. Plant Cell Tiss Org Cult. 99(2):193-198

Standardi A, Piccioni E (1998) Recent perspectives on synthetic seed technology using nonembryogenic *in vitro* - derived explants. Int J Plant Sci. 159(6):968-978

Sundararaj SG, Agrawal A, Tyagi RK (2010) Encapsulation for *in vitro* short-term storage and exchange of ginger (*Zingiber officinale* Rosc.) germplasm. Sci Hort. 125(4):761-766

Ved DK, Kinhal GA, Ravi Kumar K, Vijaya Shankar R, Sumathi R, Mahapatra AK and Panda PC (Eds.) (2008) Conservation assessment and management prioritization for medicinal plants of Orissa. Regional Plant Research Centre and Foundation for Revitalization of Local Health Traditions, Bhubaneswar and Bangalore

Verma SK, Choudhary DK, Kumar AA, Lal M (2015) Plant regeneration of *A. lakoocha* from encapsulated nodal explants. Arch Appl Sci Res. 7(1):22-27

Verma SK, Rai MK, Asthana P, Jaiswal VS, Jaiswal U (2010) *In vitro* plantlets from alginate-encapsulated shoot tips of *Solanum nigrum* L. Sci Hort. 124(4):517-521