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Original paper

Germplasm conservation via encapsulating in vitro generated shoot tips and nodal segment of Capparis decidua (FORSK.) EDGEW and its regeneration

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Abstract A methodical procedure for the conservation of *Capparis decidua* explant and short term storage of its synthetic seed was established employing *in vitro* generated shoot tips and nodal segments for encapsulation and complexation in 3% alginate solution and 100 mM calcium chloride. Shoot tips and nodal segments were encapsulated and kept in 0.3M and 0.5M sucrose to provide nutritive media before storing the beads at 4°C and 8°C temperature for 20, 30 and 40 days. The maximum shoot length (3.5±0.1cm) with considerable viability was obtained with pretreatment with 0.5 M sucrose following storage at 4°C for 30 days. The conditions are therefore recommended for future conservation efforts. The seed viability or germination reached up to 80% in the seeds stored at 4°C and 8°C for 30 days on different MS media. The germination of the synthetic seeds in media MSA (containing1/2MS+0.1 mg/l BAP+0.7mg/l IBA+ 2.5gm phytagel) was advanced to 11 days when inoculated in MSA media against 19 days in media MSB (containing 1 mg/l BAP+1 mg/l NAA+ 0.2% charcoal). The synthetic seeds stored for 30 days at 4°C and 8°C had moisture content 69.7% and 50.9% respectively, which was coincident with the highest germination. The encapsulated shoot tips of *Capparis decidua* showed maximum regrowth frequency on media MSA and responded with almost 100% conversion to plantlets as compared to the nodal segment.

Keywords *Capparis decidua*, encapsulation, MS media, regeneration, synthetic seed.

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Introduction

The *Capparis decidua* is spread all over the Indian subcontinent and is confined in nature to the arid and semi-arid regions of North West India, mainly in the Indian desert of western Rajasthan. The wastelands of Haryana, Gujarat, and Punjab states of India are widely inhabited with this species. *Capparis decidua* (Forsk.) commonly known as Ker, a rangeland perennial bushy shrub with spines is an important medicinal plant of India. The Indian species is natural, wild and the propagation is through seeds or through root suckers (although at a very low rate). The percentage of seed germination is poor due to low seed viability. The species is facing the depletion of natural populations due to overexploitation, change in climatic conditions, habitat reduction for urbanization and agricultural reasons [1]. This risk of genetic erosion makes *Capparis decidua* an ideal entrant for the application of innovative germplasm conservation. The conventional methods involve continuous clonal cultivation in the field, which is a complicated task due to the exposure of pests, diseases, and soil or climatic stresses. Another cause of worry is the shorter storage duration as the seeds are either recalcitrant or are desiccation-sensitive. The pathogenic infestation further reduces the storage span [2]. Henceforth, the employment of synthetic seed via encapsulation technology presents an important alternative. The technique was initially reported by Kitto and Janick [3]. This technology is useful for multiplying and conserving the elite agricultural and endangered medicinal plant species, which are otherwise difficult to regenerate through conventional methods and natural seeds [4].

The production of synthetic seeds has unraveled new vistas in *in vitro* plant propagation technology, and the germplasm conservation. These tools provide methods for production of synthetic seeds for conversion into the plantlets under *in vitro* and *in vivo* conditions. Production of synthetic seed using shoot tips of *Salvia officinalis* (a medicinal plant) was reported by Izabela *et al* [5]. Shoot tips were productively used for synthetic seed production in several medicinal plant species, such as, *Adhatoda vasica* [6], *Simmondsia chinensis* [7], *Picrorhiza kurroa* [8], *Cucumis sativus* [9], and *Stevia rebaudiana* [10]. The use of vegetative propagules for the development of synthetic seeds has been reported in numerous medical plant species, such as *Valeriana wallichii* [11, *Rauvolfia serpentina* [12], *Cineraria maritima* [13]. Shoot tip and nodal segment explants are very useful in the plant species, where somatic embryogenesis is not well established or else good quality somatic embryos are not produced. To the best of our knowledge, there is no published data on the regeneration of plants from the synthetic seed of *Capparis decidua.*

Materials and Methods

The *in vitro* regenerated explants from 8-weeks-old culture of *Capparis decidua* were used for encapsulation. The shoot tips, nodal segment were excised into sections of approximately 4 mm in length. The alginate solution low viscosity-usually Sigma A 2158, 3% alginate, low viscosity in 0.4 M sucrose calcium free MS [14] medium) was used for encapsulation with 100 mM calcium chloride solution as a complexing agent. The encapsulation was attained by tenderly putting the micro shoots, nodal segments in the sodium alginate solution, where it allows the alginate to polymerize into calcium alginate to form beads in which apices are enclosed. The sterile cut pipette tips were used to make beads (Tip capacity 1000 µl, approximate cut diameter of the tips: 4 mm depending on the size of tissues), the excised explants in the sodium alginate were pipetted out and dropped into the $CaCl_2$ for hardening. After 20 minutes beads were drawn up from the calcium chloride solution and were submerged in sterile distilled water for 2 minutes to remove calcium chloride remnants. The beads were stored at 4°C and 8°C for 20, 30 and 40 days. Whereas immediately cultured synthetic beads on MS media were taken as a control. The beads were weighed before and after storage to estimate the moisture content.

The encapsulated shoot tips and nodal segments were precultured on MS medium enriched with 0.3 M and 0.5 M sucrose concentration for 11 hours. The beads were then stored in cryovials in a refrigerator set at 4°C and 8°C for 20, 30 and 40 days, respectively. The above-mentioned temperature (4°C and 8°C) and the storage periods were evaluated for their effect on regeneration and related moisture content was also noted. The complete process was carried out in a laminar air flow to maintain the aseptic conditions.

For culturing on the regrowth medium, the synthetic beads were inoculated on MS basal media for *in vitro* germination after each period of storage at 4°C and 8°C. The MS basal media with 3% sucrose and 0.8% agar supplemented with different combinations of plant growth regulators. The beads were cultured on MS Media – MS1-5 mg/l BAP, 50 mg/l ascorbic acid, 25 mg/l adenine sulphate and 25 mg/l arginine; MS2-0.1 mg/l NAA, 5 mg/l TDZ, 25 mg/l ascorbic acid, 25 mg/l adenine sulphate and, 25 mg/l arginine; MSA- Half MS+0.1 mg/l BAP, 0.7 mg/l IBA and 2.5 gm phytagel; and Media MSB-1 mg/l BAP, 1 mg/l NAA and 0.2% activated charcoal. The viability (germination percentage) and incubation period required for germination of artificial seed were recorded. The response to the media was evaluated to recommend the best media for the conservation attempts.

Each experiment was conducted in 10 replicates and was repeated thrice. The data was tested for the difference in the significance level using Analysis of variance (ANOVA). Further, the significance among the means was calculated by HSD Tukey test. Student's T-test was applied to the data of regrowth frequency of encapsulated shoot tips and nodal segments. P value less than 0.05 is considered significant to reject the Null's hypothesis.

Result

Encapsulation of shoot tips and nodal segments

The shoot tips and nodal segments were encapsulated and stored at 4°C and 8°C temperature whereas immediately cultured synthetic beads served as a control. Seed longevity was studied by storing the synthetic seeds at 4°C and 8°C for 20, 30 and 40 days. The viability/ germination of encapsulated shoot tip and nodal segment bead was upto 80% in plantlet conversion after storing at 4°C and 8°C for 30 days (Table 1). However, no significant change was observed after 30 days at 4°C w.r.t the control, which may be indicative of the conditions as the best method of storage. Based on the results of the moisture content percentage, synthetic seeds stored for 30 days at 4°C and 8°C manifested moisture content of 69.7% and 50.9% respectively, which was coincident with the highest germination.

Effect of 0.3 Mand 0.5 M sucrose concentration on plant development from encapsulated beads

Sucrose treatment serves as an artificial endosperm therefore providing nutrients to the encapsulated explants for short time storage and for good plant regrowth on MS Media [15, 16]. The best sucrose concentration is 0.5 M with respect to shoot length and viability and shows 3.5 cm (±0.1) shoot length from 30 days stored synthetic beads (Figure 1).

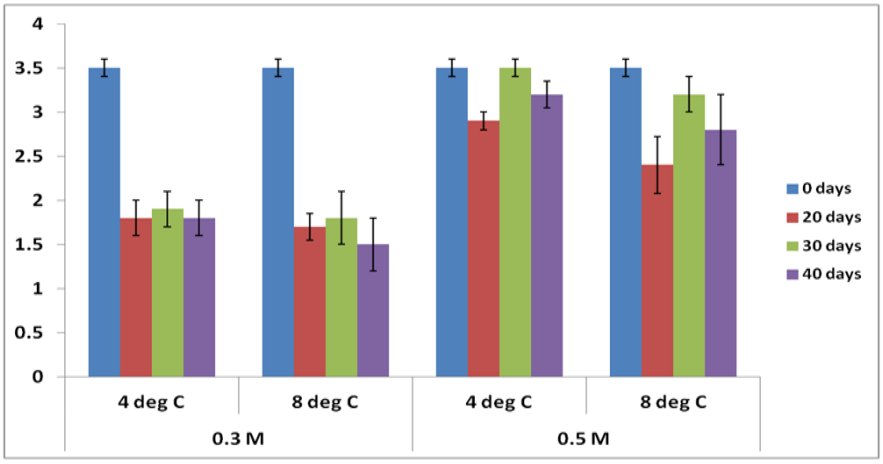

Figure 1. Effect on shoot length of encapsulated beads exposed to preculture treatment of 0.3 M and 0.5 M sucrose.

In 0.5 M sucrose treated beads stored for 30 days at 4°C, no significant change was observed w.r.t the respective control. The finding is further supported when comparisons were carried out between the treatments that revealed significant difference between encapsulated beads stored for 30 days and that stored for 20 or 40 days [HSD Tukey, P value<0.01 & 0.05 respectively]. P value 0.01 indicates that shoot length decreased significantly, so these storage conditions may not be recommended for future such work. The conclusion is further supported by the observations at 8°C. Moreover, the shoot length was non-significantly different from the control even upto 40 days. However, we recommend the storage up to 30 days in 0.5 M sucrose since the shoot length assumed a shortening trend.

Table 1. Effect of different storage temperatures on moisture content percentage and viability percentage of the encapsulated *in vitro* synthetic propagules of *Capparis decidua*.

Storage Duration (days)	MC%*		Viability/ germination	
	4°C	8°C	4°C	8°C
20	76.8(±4.08)	65.7(±4.26)	50.0(±1.66)[b]	61.1(±0.96)[b]
30	69.7(±1.08)	50.9(±4.26)	80.5(±1.73)[a]	77.7(±3.80)[a]
40	49.6(±3.89)	39.4(±3.48)	19.4(±1.73)[c]	30.5(±3.36)[c]

*where, mc% is calculated using formula-mc% = $M2 - \frac{M3}{M2} - M1 \times 10$

Plantlet development from encapsulated beads and their storage behaviour

Three percent of sodium alginate solution and 100 mM calcium chloride solution plays a vital role in the formation of iso-diametric beads and proper softness, so that ion exchange reaction can occur appropriately for synthetic beads to show bud outbreak at a right time. We found that at 4°C, the synthetic beads of shoot tips after 30 days storage period showed regrowth frequency of 91.6 (±1.44).

The Media MS-A (1/2 MS+0.1 mg/L BAP+0.7 mg/L IBA+2.5 gm phytagel) seems to be the best media for quick bud outbreak, multiple shoot proliferation, and root emergence. The shoot outgrowth was observed just after 11 days of inoculation in the Media MS-A, when the seeds were stored at 4°C after preculturing in 0.5 M sucrose as compared with that in media MS-B where seed germination happened after 19 days under similar storage conditions (Table 2) & (Figure 2).

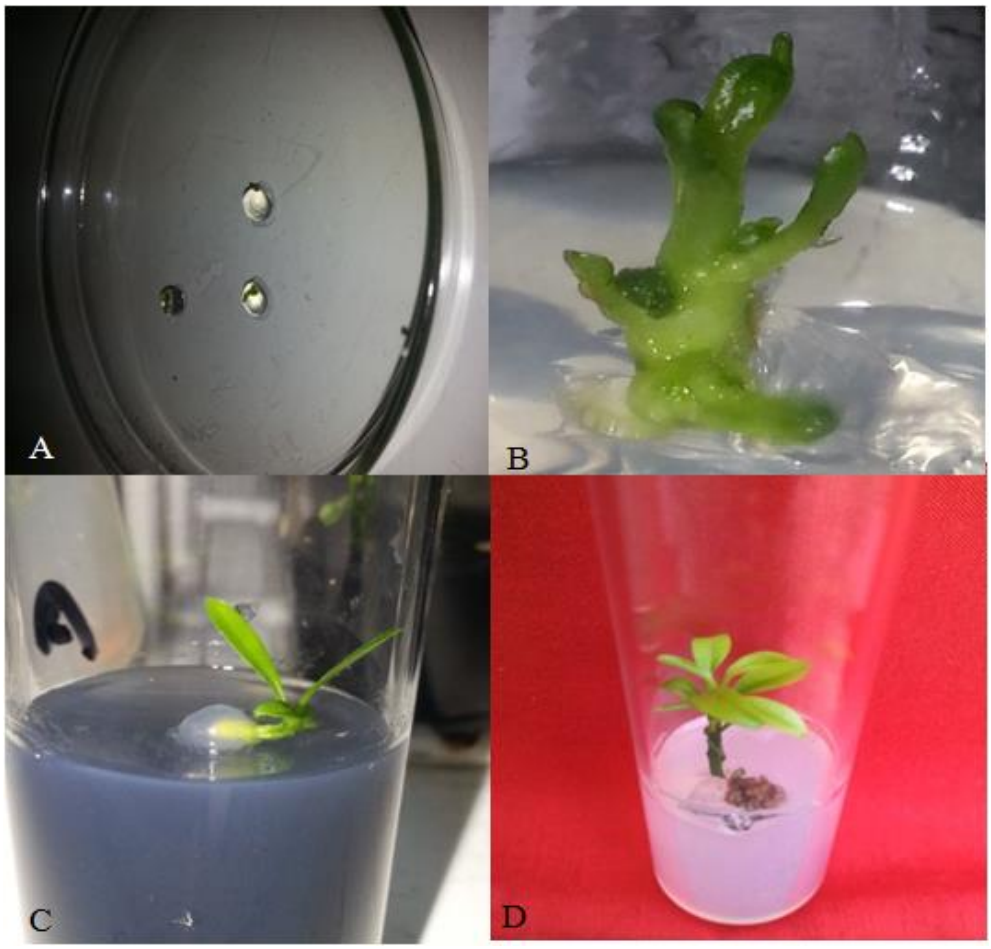

Figure 2. A) Synthetic beads of *in vitro* generated explants (shoot tip and nodal segment); **B)** Regrowth of explant from synthetic bead; **C)** Shoot formation from encapsulated shoot tips of *Capparis decidua* on MS +1 mg/l BAP+ 1 mg/l NAA+0.2% charcoal; **D)** Shoot formation from encapsulated shoot tips of *Capparis decidua* on ½ MS+0.1 mg/l BAP+ 0.7 mg/l IBA+ 2.5 gm phytagel.

Student T-test was employed to diagnose the significance of the difference between the regrowth frequencies at in different media at different storage conditions. A significant difference (T test; p value<0.01) was noticed between the regrowth frequencies of shoot tip explants and nodal segment explant stored at 4°C and grown in the media MS-A. The observations were supported by the experiment where the storage condition was 8°C. The experiment also supported our previous conclusion that the media MS-A is better media than media MS-B.

Discussion

The 3% alginate solution was found to be the most appropriate concentration for encapsulation of explants and shoot and root emergence from the beads as was reported earlier by Ballester et al, [17] in the encapsulated shoot tips of *Camelia japonica.* No significant change was observed after 30 days at 4°C with respect to the control, which may be indicative of the conditions as the best method of

storage. The encapsulated microshoots of *C. maritima* showed similar regrowth frequency of 82% at 25 degree stored for 6 months [13]. The 30 days storage period at 4°C is known to show maximum regrowth frequency with respect to the shoot emergence and maximum number of shoots in *Brassica oleracea* [18].

The best sucrose concentration is 0.5M with respect to shoot length and viability and shows 3.5 cm±0.1 shoot length from 30 days stored synthetic beads. It may be worth mentioning over here that as per our results, the 0.5 M sucrose treatment plays an important role with respect to regrowth frequency, which is 90% in encapsulated shoot tip of *Capparis decidua* at 4°C. In contrast, the fresh encapsulated beads showed good shooting but poor rooting efficiency when not given a prior sucrose treatment. Similar findings were also reported in the synthetic seeds of *Salvia officinalis* [5]. Preculture with 0.5 M sucrose maintained the viability values about 70% or 90% in zygotic embryos [19] or shoot primordia [20], respectively. Storage at was always found reassuring for low temperature storage in various plants [21-26]. Shoot tips as explant in synthetic seed yields better response with respect to the germination than the nodal segment, which may be attributed to the greater mitotic activity reported in shoot tips as compared with the lateral growth which are subjected to apical dominance [27]. The Media MS-A (1/2MS+0.1 mg/L BAP+0.7 mg/L IBA+ 2.5 gm phytagel) seems to be the best media for quick bud outbreak, multiple shoot proliferation, and root emergence.

Table 2. Explants showing maximum Regrowth frequency and the best media response.

Explant Used	**Regrowth frequency (±S.D.)**		**Media**
	4^0 C	8^0 C	
Shoot Tip	91.6 (±1.44)a	80.0 (±1.73)a	A
	80.5(±1.73)b	77.7(±3.8)b	B
Nodal Segment	61.1 (±0.9)d	52.7(±2.4)c	A/B

Conclusion

We have established a methodical procedure for conservation and short term storage synthetic seed of *Capparis decidua* by employing *in vitro* generated shoot tips and nodal segments for encapsulation and complexation in 3% alginate solution and 100 mM calcium chloride. The results recommend storage of synthetic seeds upon preculture treatment of 0.5 M sucrose at 4°C or 8°C for 30 days. The results also support Media MS-A (containing 1/2 MS+0.1 mg/l BAP+0.7 mg/l IBA+ 2.5 gm phytagel) for shoot tip explants regeneration. This study explores the possibility of preserving the germplasm and bestows an operable/workable system for storage and clonal multiplication of the encapsulated beads. This work further could effectively be used for establishing a certain system for conservation considering the influence of sucrose concentration during preculturing, short term storage, and production of synthetic seed of *Capparis decidua* to be released for commercial purposes. We, hereby, report the first successful attempt of the utilization of synthetic seed technology for *Capparis deciduas* using the shoot tips and nodal segments from *in vitro* cultures for encapsulation.

Conflict of Interest Statement

The Authors have NO conflict of interest.

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