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Research Article

IN VITRO PROPAGATION OF *CAPPARIS DECIDUA* (FORSK.) EDGEW.THROUGH NODAL EXPLANTS

Renu Sharma[1], Upma Bhatt[2], *Vineet Soni[2] and P.L. Swarnkar[3]
[1]School of Life Science, Jaipur National University, Jaipur-302017, Rajasthan, India
[2]Department of Botany, Mohanlal Sukhadia University, Udaipur-313001, Rajasthan, India
[3]Department of Botany, University of Rajasthan, Jaipur-302020, Rajasthan, India
**Author for Correspondence*

ABSTRACT
An efficient and reproducible protocol for *in vitro* regeneration of *Capparis decidua* (Forsk.) Edgew. was developed through nodal explants. Nodal explants exhibited high frequency shoot proliferation on Murashige and Skoog's (MS) basal medium supplemented with 3.0 mg l^{-1} BAP and 0.1 l^{-1} NAA. Microshoots were best rooted on ½ strength MS medium with 0.1 mg l^{-1} IAA and 1% (w/v) sucrose. The overall survival rate from *in vitro* growth to field transfer was 68 %. The developed micropropagation protocol can be used for large-scale multiplication and genetic modification of this high value medicinal plant species.

Keywords: *Capparis decidua, In vitro propagation, Nodal explants, Rhizogenesis*

INTRODUCTION
Capparis decidua (Forsk.) Edgew (Capparidaceae) a perennial woody plant, is naturally found in subtropical and tropical regions (Dhakad *et al.,* 2016). It is commonly known as Kair, Karyal, Karil, Kabra, etc. Traditionally the plant is used for the treatment of various ailments such as rheumatism, asthma, cough, toothache, pyorrhoea, dysentery, liver infections, diarrhoea, febrifuge, cardiac troubles, constipation, ulcer, piles, renal disorders, and skin diseases (Mann *et al.,* 2013). The plant contains anti-atherosclerotic (Purohit and Vyas, 2006), anti-microbial (Sharma and Kumar, 2008), antifungal (Keymanesh *et al.,* 2009), anti-inflammatory (Mohammed *et al.,* 2012), antibacterial (Gull *et al.,* 2015), anti-aging (Jadoon *et al.,* 2015), anthelmintic (Raza *et al.,* 2016), and antirheumetic (Kamal *et al.,* 2016). A significant amount of essential minerals, mainly, Fe and Zn is also present in the plant which advocates its potential use in recovering mineral deficiency in human diet (Gull *et al.,* 2015). Unfortunately, the plant is facing the threat of depletion in naturl habitats due to overexploitation, climate change, and urbanization (Khan *et al.,* 2003).
Conventionally *C. decidua* is mainly propagated through the seeds and stem cuttings. However, germination of seeds is poor in natural habitats and propagation through stem cuttings solely relies on season for multiplication, which makes it an inefficient way for the conservation of this medicinally important plant. So this plant species requires immediate attention for its large scale systematic cultivation and conservation. Plant tissue culture is an alternative to the conventional methods of propagation with the objective of conserving the threatened medicinal plant species. In the present investigation, we report a new plant *in vitro* regeneration method for *C. decidua* through nodal explants. The developed protocol can be used for large-scale multiplication and conservation of this high value medicinal plant species.

MATERIALS AND METHODS
Plant material and surface sterilization: Nodal explants were collected from healthy plants of *C. decidua* (Fig. 1 A) growing at natural habitats near Jaipur National University, Jaipur, (India) and University's College of Science, Mohanlal Sukhadia University, Udaipur, (India). The excised nodal explants were washed thoroughly under running tap water for 10 min to eliminate dust particles and then with 5 % teepol for 5–8 min and rinsed several times in sterile double distilled water (DDW). Then, the

nodal explants were treated with an antifungal agent (Bavistin) for 30 min and the again rinsed three times with sterile DDW. Thereafter, the explants were surface sterilized under a laminar flow chamber with aqueous solution of 0.1 % HgCl2 for 5 min and finally washed with sterile DDW for 5–6 times.

Culture media and growth conditions: The sterilized nodal explants were cultured on MS Medium (Murashige & Skoog 1962) supplemented with 3 % (w/v) sucrose and various combinations/concentrations of plant growth regulators. The pH of the media was adjusted 5.8 before autoclaving 121°C for 15 min. All the cultures were maintained at 25±2°C and 65–70 % relative humidity with photoperiod of 16-h using cool white fluorescent tubes (Philips, India).

***In vitro* rhizogenesis and hardening:** Elongated healthy shoots were excised and transferred carefully onto rooting medium supplemented with various auxins viz. IBA, IAA and NAA. Well rooted plantlets, derived from both nodal explants were transferred to plastic cups (10 × 8 cm) containing sterilized mixture of sterile soil, sand and coco peat (1:2:1). The plantlets covered in transparent polyethylene bags were kept for 4 weeks in growth chamber at 25 ± 2°C with 16 h photoperiod. The hardened plants were subsequently transferred to large pots containing normal garden soil.

RESULTS AND DISCUSSION

***In vitro* multiplication:** Among the two different cytokinins (BAP and KIN) tested, BAP proved better than KIN for inducing the multiple shoots in nodal explants of *C. deciduas* (Table- 1). Development of one shoot per explant was noted on high concentration of KIN (Fig. 1 B)**.** BAP (3.0 mg l^{-1}) induced multiple shoot induction in nodal explants of *C. deciduas*. High frequency multiple shoot induction was obtained when low concentration of NAA (0.1 l^{-1}) was incorporated along with 3.0 mg l^{-1} BAP in nutrient medium (Fig. 1 C, D). Higher concentration of NAA along with 3.0 mg l^{-1} BAP could not evoke multiple shoot induction. Frequency of shoot induction was drastically decreased with decreasing concentration of BAP. Similarly, high concentration of BAP declined the frequency of shoot bud induction and induced callus formation at basal part of nodal explants (Fig. 1 D). Similar, inhibitory effect of higher concentrations of BAP on *in vitro* shoot proliferation has also been observed earlier in *Arachis hypogaea* (Banerjee *et al.*, 2007), *Doritis pulcherrima* (Mondal *et al.*, 2013) and *Salvia splendens* (Sharma *et al.*, 2014).

Table 1. Influence of BAP and NAA on multiple shoot induction in nodal explants of *C. decidua*

BAP + NAA concentration (mg l-1)	Explant response (%)	Mean number of shoots per node
0.5 + 0.1	07.05 ± 3.7	1.44 ± 0.52
1.0 + 0.1	21.54 ± 4.6	3.44± 0.61
2.0 + 0.1	71.74 ± 6.9	4.83 ± 0.53
3.0 + 0.1	86.68 ± 5.8	6.38 ± 0.68
4.0 + 0.1	50.72 ± 6.4	2.24 ± 0.51
5.0 + 0.1	21.67 ± 3.1	2.24 ± 0.41

***In vitro* rhizogenesis:** MS medium of ½ strength proved better for induction of *in vitro* rooting as compared to full strength. ½ strength MS medium supplemented with 0.1 mg l^{-1} IAA and 1% (w/v) sucrose IBA (3.0-5.0 mg l^{-1}) induced high frequency rhizogenesis *in vitro* in excised shoots of *C. decidua*. Similarly, IBA-induced rhizogenesis *in vitro* has been observed in *Cattleya* (Dewir *et al.*, 2015), and *Hemidesmus indicus* (Shekhawat and Manokari, 2016).

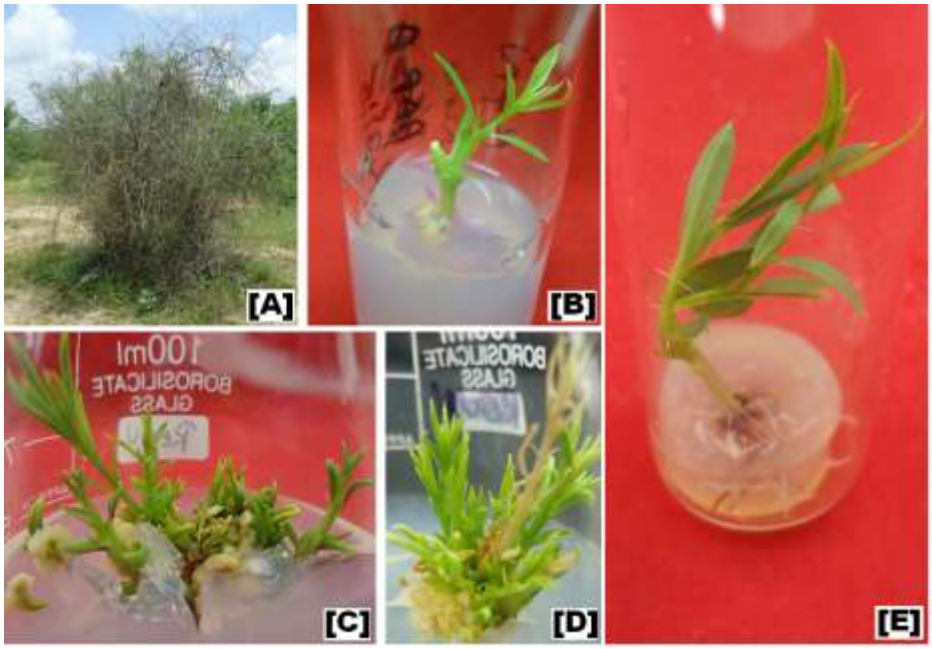

Figure 1 [A] Mature tree of *C. deciduas*, [B] development of one shoot per explants on KIN (5.0 mg l^{-1}), [C-D] multiple shoot induction on MS medium enriched with NAA (0.1 l^{-1}) and 3.0 mg l^{-1} BAP, [E] high frequency *in vitro* rhizogenesis on ½ strength MS medium augmented with 0.1 mg l^{-1} IAA and 1% (w/v) sucrose.

The quick and highly reproducible *in vitro* regeneration method standardized in the present investigation can be used to produce superior planting stock for various afforestation and reforestation programmes of *C. decidua*.

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