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PRECULTURING EFFECT OF THIDIAZURON ON *IN VITRO* SHOOT MULTIPLICATION AND MICROPROPAGATION ROUND IN *CAPPARIS DECIDUA* (FORSK.) AN IMPORTANT MULTIPURPOSE PLANT

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An efficient protocol was developed for clonal multiplication of an important shrub: *Capparis decidua* (Forsk.) Edgew, through *in vitro* shoot induction and multiplication from nodal explants. Pretreatment of nodal explants in a liquid Murashige and Skoog (MS) medium augmented with various thidiazuron (TDZ) concentrations at relatively high levels (5–100 μM) for different time duration (4, 8, 12 and 16 d), proved a significant approach for *in vitro* shoot production. After an initial exposure time to TDZ, nodal explants were inoculated onto a MS basal medium devoid of TDZ for further induction and proliferation. The highest regeneration rate (85%), average number of shoots/explant (8.7 ± 0.22) and maximum shoot length (3.9 ± 0.33 cm) were obtained from the nodal explants exposed to 50 μM TDZ for 8 d. The nodal explants excised from the proliferated cultures of TDZ (50 μM) for 8 d were used as explants and showed an enhancement rate after next three round of *in vitro* propagation. Best results for rooting was obtained by *ex vitro* treatment of shoots with 200 μM indole-3-butyric acid (IBA) for 20 min. as it produced an average of 5.7 ± 0.41 roots per microshoot with 4.4 ± 0.39 cm root length in 84% shoots. Different planting substrates was tested for maximum survival of hardening off micropropagated plantlets and soilrite proved most effective than others as 97.1 ± 7.21 plantlets survived. All micropropagated plants grew well in natural conditions and showed similar morphology to the mother plant.

Keywords: Acclimatization – Clonal multiplication – *Ex vitro* – Murashige and Skoog medium – Planting substrate

INTRODUCTION

Capparis decidua (Forsk.) Edgew, a member of Capparidaceae family and known by its local name Kair, is an important indigenous shrub and found in the desert regions of Africa, Saudi Arabia and the Indian subcontinent [25]. It can grow under harsh climatic conditions because of its high tolerance capacity against drought and frost. It is an extremely hardy species and provides vegetative cover in hot, sandy desert areas where chances to grow for other plant species are very little. *Capparis* produces timbers that are hard, termite resistant and heavy [10]. The seeds contain 20% of high quality edible oil. Isocodonocarpine and other alkaloids present in the extracts of its root barks and stem are effective in the treatment of cough, asthma, inflamma-

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tion etc. [3]. The immature fruits are used to cure stomach problems. The extract prepared from this plant possesses sedative and anticonvulsant, antidiabetic and antistress, anthelmintic, anti-inflammatory, insecticidal, hypotensive and spasmolytic effects [22]. *Capparis* acts as a good bio-indicator and known as a good weather forecasting species because of its capacity to tolerate extremely high drought and heat conditions. It is also used as vegetable and pickle.

Due to rapid urbanization, change in climatic conditions, overexploitation and some agricultural reasons, *Capparis* is being depleted from natural populations [5, 12]. To maintain and sustain forest vegetation, conventional approaches like grafting, layering, and cutting have been used for propagation. Nonetheless, these conventional methods of plant propagation have limited applicability [28, 29]. Propagation through seed is not preferred for multiplication of this plant mainly for the reasons i.e. the low germination percentage, dormancy [16], and heterozygous nature of the seeds [19]. Therefore to overcome these hurdles in its large scale cultivation, there is a need to have a reliable, cost effective and rapid propagation method.

For *in vitro* propagation of aromatic, medicinal and multipurpose plants and for commercial exploitation of valuable plant-derived pharmaceuticals, tissue culture technology proved to be effective method [6, 9, 18, 20]. By *in vitro* technology, rapid and mass production of elite plant material is possible throughout the year, without seasonal limitation.

Plant growth regulators are synthetically produced and may mimic hormones or have their own unique properties. Thidiazuron (N-phenyl-1,2,3-thidiazol-5yl urea) is a substitutive phenylurea derivative, and has now emerged as a highly effective bioregulant of morphogenesis in the tissue culture of many plant species. [9, 11, 13, 17]. Despite its importance, only few reports are available on *C. decidua* micropropagation [7, 8, 26, 27] and none of them tested the effect of TDZ on *in vitro* propagation. The aim of the present investigation was to determine the effectiveness of TDZ on nodal explants of *C. decidua* used at high levels for short time and then cultured on MS basal medium for shoot regeneration and proliferation and to check the efficiency of this protocol by successive micropropagation round.

MATERIALS AND METHODS

Plant material and explant source

Nodal segments measuring about 1.0–1.5 cm in length were excised from a 2-year-old mature plant grown in the Botanical garden of the University. Explants were washed thoroughly under running tap water for 30 min, and then a washing treatment with a liquid detergent, *Teepol* 5% (v/v) was given for 10 min. subsequently washed with sterile double distilled water for at least 3 times. Surface sterilization of the explants was performed by treatment with 0.1% (w/v) $HgCl_2$ solution for 4 min followed by repeated washing with sterile double distilled water.

Media type and growth conditions

For the present investigation, MS medium [14] fortified with 3% (w/v) sucrose and 0.8% (w/v) agar was used as culture medium. TDZ at different concentrations (5, 25, 50, 75 and 100 μM) was added in liquid MS media. The pH of the medium was adjusted to 5.8 with 1 N NaOH. After media preparation, it was autoclaved for 20 min. at 121 °C temperature and 1.06 kg cm^{-2} pressure. All cultures were kept in a culture room at temperature of 24 ± 2 °C, 16 h photoperiod with a photosynthetic photon flux density (PPFD) of 50 μmol m^{-2} s^{-1} provided by cool white fluorescent lamps and relative humidity of 60–65%.

Multiple shoot induction and proliferation

Liquid MS media amended with various levels of TDZ (5–100 μM) was prepared in conical flasks and nodal explants were inoculated onto them. The flasks were kept on a rotatory shaker at 100 rpm. Basal MS medium with no growth regulator was used as control. To optimize the time and level of TDZ, the explants after exposure to TDZ at various levels and time duration (4, 8, 12 and 16 d) were inoculated onto MS basal medium. After every 2 weeks, they were regularly transferred to a new basal media for their maximum growth. Data for regeneration rate, average number of shoot initiation and subsequent multiplication, and their length was noted after 8 weeks.

Successive micropropagation round

To check the reproducibility and effectiveness of the multiplication protocol, shoots developed in TDZ (50 μM) for 8 d were subcultured in MS basal medium. Counting of number of shoots produced and their measurement was done after 8 weeks of culture. Under the same culture environment, about 1.0 cm nodal segment were cut from the *in vitro* raised plants and served as new explants for another round of micropropagation. This step was repeated three times and after every round, yield was calculated.

Rooting and Acclimatization of plantlets

Direct indole-3-butyric acid (IBA) treatment at various concentrations (50, 100, 150, 200, 250 and 300 μM) was given to the fully developed shoots excised from proliferated cultures for *ex vitro* rooting. They were dipped in IBA solution for 20 min. and then transferred to plastic cups filled with different planting substrates i.e. sterile garden soil, soilrite or vermiculite (Keltech Energies Ltd., Bangalore, India) and kept in culture room under diffuse light (16:8 h photoperiod) conditions. High humidity conditions were maintained by covering them with a transparent polythene membrane

and watered every 3 d with half-strength MS salt solution for 2 weeks. Holes were made in the polythene membranes and opened gradually in order to acclimatize plants to field conditions. After 4 weeks, they were shifted to greenhouse under normal environmental conditions. Number of roots, root length, % rooting and survival rate was recorded after 4 weeks of experiment.

Statistical analysis

Each treatment consisted of twenty replicates and all experiments were repeated thrice. Statistical analysis of the data was done by one way analysis of variance (ANOVA) using SPSS software version 16 (SPSS Inc., Chicago, USA). Means were compared with Duncan's multiple range test at 5% level of significance and results were presented in the tables as the mean ± SE of three experiments.

Table 1

Optimization of various TDZ levels and exposure time followed by their transfer to MS basal semisolid medium on shoot formation from nodal explants of *Capparis decidua* after 8 weeks of culture

TDZ (µM)	Culture duration (d)	% Regeneration	Mean number of shoots/explant	Mean shoot length (cm)
0.0	0	0.0	0.0^{k}	0.0^{h}
5.0	4	45	2.0 ± 0.40^{i}	1.3 ± 0.21^{ef}
	8	54	3.9 ± 0.25^{f}	2.0 ± 0.30^{d}
	12	50	3.3 ± 0.40^{g}	1.5 ± 0.24^{e}
	16	46	3.0 ± 0.22^{g}	1.1 ± 0.10^{g}
25	4	50	3.1 ± 0.75^{g}	2.0 ± 0.20^{d}
	8	58	4.5 ± 0.89^{e}	2.6 ± 0.29^{c}
	12	54	3.8 ± 0.69^{f}	2.0 ± 0.33^{d}
	16	49	2.9 ± 0.40^{gh}	1.6 ± 0.26^{e}
50	4	71	5.9 ± 0.12^{cd}	3.3 ± 0.36^{b}
	8	85	8.7 ± 0.22^{a}	3.9 ± 0.33^{a}
	12	80	7.6 ± 0.36^{b}	3.4 ± 0.26^{b}
	16	74	5.8 ± 0.49^{cd}	3.0 ± 0.27^{bc}
75	4	66	5.1 ± 0.61^{de}	3.0 ± 0.32^{bc}
	8	79	7.0 ± 0.48^{bc}	3.3 ± 0.53^{b}
	12	74	6.6 ± 0.33^{c}	2.4 ± 0.46^{cd}
	16	60	5.5 ± 0.30^{d}	2.1 ± 0.23^{d}
100	4	60	4.0 ± 0.60^{e}	3.0 ± 0.14^{bc}
	8	72	3.6 ± 0.40^{fg}	2.4 ± 0.37^{cd}
	12	67	2.8 ± 0.21^{gh}	1.9 ± 0.43^{d}
	16	57	1.1 ± 0.13^{j}	1.7 ± 0.20^{e}

Values represent means ± SE. Means followed by the same letter within columns are not significantly different ($P = 0.05$) using Duncan's multiple range test.

RESULTS AND DISCUSSION

A two step culture method was adopted during the present study. In the first step, explants were exposed to TDZ at relatively high concentrations added in liquid MS media for short time duration subsequently inoculated to MS semisolid basal medium in the second step. To optimize the response of nodal explants to TDZ, the nodal segments were inoculated in liquid MS medium supplemented with TDZ at different concentrations (5.0, 25, 50, 75 and 100 μM) on a rotary shaker at 100 rpm for 4, 8, 12 and 16 d (Table 1), after that inoculated on MS basal medium for further induction and regeneration. Shoot induction was noticed in all the TDZ treatment with different regeneration frequencies and number of shoots. Basal MS medium without growth regulator were not found supportive for any shoot induction. After one week, initial bud break was noticed from all the explants. A remarkable difference was observed in all the parameters evaluated after exposure to different concentrations and duration to TDZ. Among different concentrations and culture durations tested, highest shoot regeneration frequency (85%), mean number of shoots (8.7±0.22) and shoot length (3.9±0.33 cm) were recorded after 8 weeks at 50 μM TDZ for 8 d culture duration. The reduction in number of shoots and shoot regeneration frequency were noticed when explants were kept for long time on the TDZ augmented medium beyond the optimal duration i.e. 12 and 16 d and the shoots regenerated were deformed in morphology. Similar results were observed in *Hypericum perforatum* [15] *Cassia angustifolia* [24] and *Rauvolfia serpentina* [4].

To see the potential of the plants produced from the preculturing experiments and its reproducibility, shoots induced from 50 μM TDZ concentration and 8 d duration were again used for three extra rounds of propagation. About 1.0 cm long nodal explants were excised and inoculated on new MS liquid medium supplemented with 50 μM TDZ for 8 d followed by their transfer to MS semisolid basal media (Table 2). Enhancement in shoot multiplication rate was recorded from one micropropagation round to next round with an average of 4–5 shoots from each cultured nodal segment. Similar pattern of reproducibility of shoots from pre-developed plantlets has also been recorded [2, 21, 24].

Table 2

Effect of treatment of TDZ (50 μM) for 8 d on newly excised explants from the micropropagated plants and on their yield of *C. decidua*. These were again served as explants for next round of propagation

Micro-propagation round	Initial shoots	Number of explants	Shoot length (cm)	New explants/ shoot explants	Yield
First	140	8.7 ± 0.22^{b}	3.9 ± 0.33^{bc}	6.3 ± 0.34^{b}	12.1±0.76^{b}
Second	180	10.2 ± 0.54^{a}	4.4 ± 0.21^{b}	7.4 ± 0.27^{a}	16.5± 0.58^{a}
Third	180	11.4 ± 0.71^{a}	5.0 ± 0.31^{a}	8.7 ± 0.51^{a}	19.3± 0.67^{a}

Values represent mean ± SE. Means followed by the same letter within columns are not significantly different ($P = 0.05$) using Duncan's multiple range test.

Table 3
Ex vitro rooting of *C. decidua* shoots dipped in IBA solution for 20 min.
Data were recorded after 4 weeks of treatment

IBA (μM)	% Rooting	Mean number of roots/shoot	Mean root length (cm)
50	59	2.2 ± 0.16^{e}	2.0 ± 0.13^{e}
100	65	4.2 ± 0.20^{c}	2.6 ± 0.24^{d}
150	78	4.6 ± 0.31^{b}	3.9 ± 0.33^{ab}
200	84	5.7 ± 0.41^{a}	4.4 ± 0.39^{a}
250	72	4.0 ± 0.30^{cd}	3.3 ± 0.33^{bc}
300	60	2.6 ± 0.18^{e}	2.8 ± 0.20^{d}

Values represent means ± SE. Means followed by the same letter within columns are not significantly different ($P = 0.05$) using Duncan's multiple range test.

Table 4
Optimization of planting substrates types for hardening of *micropropagated* plantlets of *C. decidua*.
Data observation was done after 4 weeks of transfer to greenhouse

Planting substrate	Number of plants transferred	Number of survived plants	% Survival
Garden soil	70	55	78.5 ± 6.40^{c}
Soilrite	70	68	97.1 ± 7.21^{a}
Vermiculite	70	62	88.5 ± 5.13^{b}

Values represent means ± SE. Means followed by the same letter within columns are not significantly different ($P = 0.05$) using Duncan's multiple range test.

In the present experiment, *Ex vitro* method was used for rooting. The basal part of regenerated microshoots were dipped in various concentrations of IBA (50–300 μM) for 20 min and subsequently planted in plastic pots containing sterile planting substrate. Of the different concentrations of IBA tested, the maximum frequency of root formation (84%), number of roots (5.7±0.41) and root length (4.4±0.39 cm) was achieved in 200 μM IBA (Table 3). *Ex vitro* rooting was also found to be effective in *Tylophora indica* [9], *Cyamopsis tetragonoloba* [1], *Cassia angustifolia* [24], *Passiflora edulis* [23]. The advantage of this method is that rooting and acclimatization was done simultaneously.

The well developed plantlets were transferred to earthen pots containing sterile garden soil, soilrite and vermiculite and kept in a growth room. Later on, they were transferred to a greenhouse. Among the different planting substrates examined, soilrite was most effective where 97.1±7.21 plantlets survived (Table 4). The micropropagated plants grew well and didn't show any variation when compared to the source plant morphologically.

CONCLUSIONS

The results from this study demonstrated an efficient and rapid protocol for large scale production of this important multipurpose plant and has advantage over the previous studies. The developed protocol could be used for *ex situ* conservation of this multipurpose plant.

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