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Influence of explanting season on *in vitro* multiplication of *Celastrus paniculatus* Willd. – An endangered medicinal herb

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An efficient and reproducible in vitro protocol for large scale multiplication of *Celastrus paniculatus* employing shoot tip explants has been described. Season of collection of explants showed direct influence on bud brek. Highest percentage of bud break (90%) with multiple shoot formation (4.3) was obtained on MS medium containing BAP (1.0 mg l^{-1}). Explants excised between December to March showed lowest (30%) percentage of bud break. With an increase in the concentration of growth regulators, the number of days required for bud break also increased. The regenerated shoots were further elongated on same medium. MS half strength medium supplemented with 0.5 mg l^{-1} NAA proved to be best with hundred per cent root formation. The regenerated plantlets were hardened in pots containing sterilized soil and sand mixture (3:1) and finally well established in the field; survival rate was seventy percent.

Key words: *Celastrus paniculatus*, regeneration, shoot tip, plant growth regulators.

Abbreviations: BAP-6-benzylamino purine, **Kn**-Kinetin, **IAA**-indole-3-acetic acid, **2,4-D**- 2,4-dichlorophenoxy acetic acid, **NAA**-α-naphthalene acetic acid, **IBA**-indolebutyric acid.

Introduction

Celastrus paniculatus Willd. (family Celastraceae) commonly known as Jyotishmati is an extremely important endangered medicinal plant believed to sharpen the memory and also used to cure a number of diseases. It is a large, woody, unarmed climbing shrub occurring naturally in hilly parts of India up to an altitude of 1200 m. This plant is widely used to cure depression, paralysis, leprosy, fever, abdominal disorders and cancerous tumors (Warrier *et al.,* 1994; Sharma *et al.,* 2001). The percentage of seed germination is low (11.5%) and other vegetative propagation methods also can't be used for cultivation (Rekha *et al.,* 2005). Due to excessive and destructive exploitation, it is getting fast

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depleted. Tissue culture techniques are being used globally for the conservation and utilization of genetic resources (Rao *et al.,* 1996).

The main objective of the present study was to select the best *in vitro* responding media for inducing morphogenesis in higher frequencies and optimization of conditions for complete plantlet development.

Materials and methods

Plant material and surface sterilization

Shoot tip bearing shoot segments were collected from mature plants growing in the Herbal Garden of Botany Department of Kurukshetra University, Kurukshetra. The collections were made in three seasons: April to July, August to November and December to March. Explants were initially washed under running water with teepol and sterilized with 0.1% (w/v) mercuric chloride for 3 - 5 min and then given a dip in ethanol (70%). Shoot tips were rinsed with sterile distilled water 4 - 5 times to remove traces of mercuric chloride.

Culture conditions

The surface sterilized explants (10 mm long) were inoculated on MS medium (Murashige and Skoog, 1962) containing 3% (w/v) sucrose supplemented with 0.5, 1.0 and 2.0 mg l^{-1} cytokinins (BAP and Kn) and auxins (IAA, NAA and 2, 4-D). The pH of the media was adjusted to 5.8 and 0.8% agar-agar was added before autoclaving at a pressure of 15 psi and 121°C temperature for 20 min. All the cultures were maintained at a temperature of 25 ± 2 °C under under a 16 hours photoperiod provided by cool white fluorescent tubes and 8 hours dark period.

The *in vitro* regenerated shoots were excised aseptically and implanted on half strength MS medium without or with growth regulators (IAA, NAA and IBA) at the concentrations of 0.5 and 1.0 mg l^{-1} for rhizogenesis.

The rooted plantlets were taken out from rooting medium and washed several times with sterile distilled water to remove the traces of agar-agar. The plantlets were then transferred to pots containing soil and sand mixture (3:1). The plantlets were initially irrigated with half strength (salts only) MS medium without sucrose on alternate days. The plantlets were exposed to the natural conditions for 3-4 hours daily after 10 days of transfer. After about 30 days the plants were transferred to bigger pots in greenhouse and were maintained under natural conditions of day

length, temperature and humidity. Finally the plants were transferred to the field conditions.

Results and discussion

Supplementation of cytokinins gave better results than auxins in present investigation. Of the two cytokinins (BAP and Kn), BAP was found to be more suitable than Kn for initiation and proliferation of multiple shoot buds. In case of BAP, maximum number of shoots (4.3) per explant was recorded in the medium fortified with 1.0 $mg1^{-1}$ (Table 1, Fig. 2b) and it decreased with any decrease from this concentration. The highest per cent bud break (90%) was found on the above mentioned concentration of BAP. In case of Kn, maximum numbers of shoots (1) per explant was recorded in all the concentrations (Fig. 2c). The numbers of days taken for bud break increases with the increase in the concentrations of cytokinins.

Table 1. Effect of cytokinins and auxins on shoot induction in shoot apex explants.

Growth regulators ($mg\ l^{-1}$)	Bud break (%)	Average No. of days required for bud break	No. of shoots per explants (Mean±SE)
MS control	–	–	–
BAP			
0.5	80	12.4	2.9±0.34
1.0	90	14.6	4.3±0.05
2.0	70	15.3	2.2±0.37
Kn			
0.5	60	18.2	1.0±0.0
1.0	70	19.8	1.0±0.0
2.0	40	20.5	1.0±0.0
IAA			
0.5	40	24.1	1.0±0.0
1.0	30	28.3	1.0±0.0
2.0	20	30.6	1.0±0.0
NAA			
0.5	–	–	–
1.0	–	–	–
2.0	–	–	–
2,4-D			
0.5	–	–	–
1.0	–	–	–
2.0	–	–	–

*Data based on 20 explants per treatment and taken after 28 days of culture, - No bud break

Earlier reports about the effectiveness of BAP has been reported by Purohit *et al.* (2004) in *Achras sapota,* Thind *et al.* (2008) in *Aloe vera,* Kumar and Singh (2009) in *Prosopis cineraria,* Goel *et al.* (2009) in *Peganum harmala,* Yadav and Singh (2011a,b) in *Aegle marmelos* and in

Spilanthes acmella. The presence of BAP alone in the medium appeared to be optimal for shoot induction. This may suggest that bud formation required cytokinin.

The increased multiple shoot formation in excised shoot tip explants may be due to rapid division of cells in the excised shoot tip and production of several primordial out growth, which eventually develop into shoots (Niranjan *et al.,* 2010).

The percent bud break is greatly influenced by different explanting seasons (Rani and Rana, 2010). Significant effect of explanting season on percent bud break was also noticed in *C. paniculatus* (Fig.1). Highest percentage of bud break (90%) was recorded with the explants cultured between April to July. While a decline (70%) was observed during August to November. Explants established during December to March exhibited poor bud break (30%). Similar observation was also obtained by Rani and Rana (2010), Yadav and Singh (2011a).

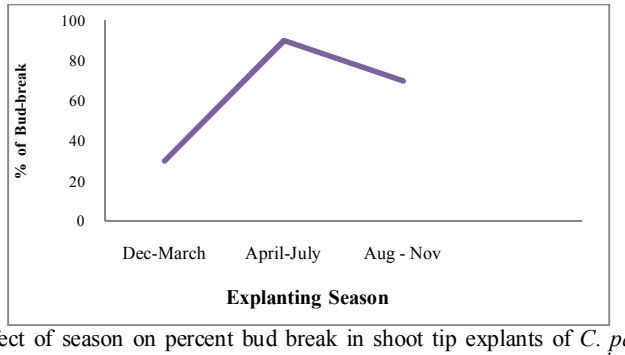

Fig. 1. Effect of season on percent bud break in shoot tip explants of *C. paniculatus* recorded after 4 weeks of culture on MS medium containing BAP (1.0 mg l^{-1}).

Among the auxins tested, no bud-break was observed on the media supplemented with NAA and 2, 4-D (Table. 1). In case of IAA only one shoot per explant with forty per cent bud break was recorded after 24.1 days in the medium fortified with 0.5 $mg1^{-1}$(Fig. 2a). Root formation is an energy demanding process and thus, exogenous supply of carbohydrates is required. However, this being the last stage of *in vitro* culture, it is important to transform the plant from heterotrophic to autotrophic mode of nutrition. Thus, the supply of exogenous sugars should be reduced at this time. The rooting response differed according to different concentrations and combinations of auxins used (Gaspar and Coumans, 1987). In the present study, elongated and well developed regenerated shoots were aseptically excised and implanted on half strength MS medium supplemented with different auxins (IAA, NAA and IBA) for rhizogenesis. Excised shoots failed to

develop roots on both full and half strength MS medium without growth regulators. Root formation with callusing at the shoot base occurred in all concentrations of NAA, IAA and IBA. Moreover, this callus formation increased with increase in concentration of auxins. MS half strength medium supplemented with 0.5 mg l^{-1} NAA proved best with hundred per cent rooting and very less callusing at the base (Fig. 2d).The effectiveness of NAA in rooting has also been reported in other plants like *Santolina canescens* (Casado *et al.,* 2002), *Leucaena leucocephala* (Singh and Lal, 2007) and *Portulaca grandiflora* (Jain and Bashir, 2010).

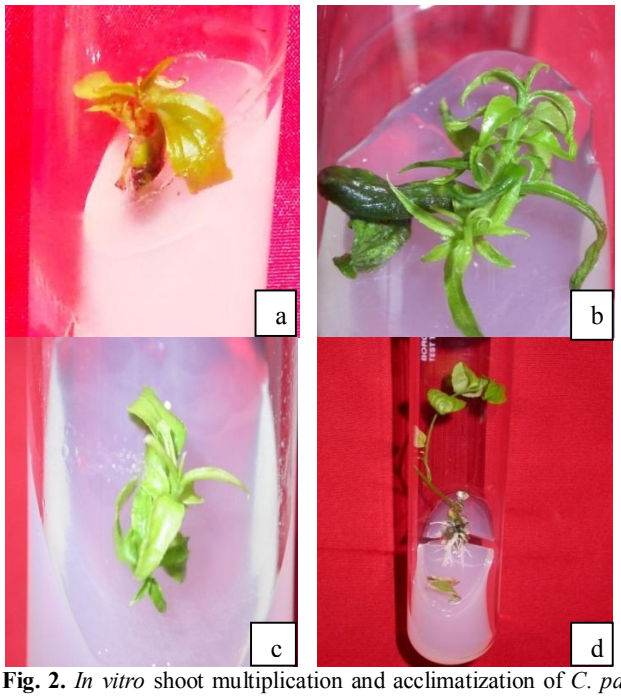

Fig. 2. *In vitro* shoot multiplication and acclimatization of *C. paniculatus* through shoot tip culture.
a. Shoot tip explant on MS medium; **b.** Multiple shoot induction on MS medium supplemented with BAP (2.0 mgl^{-1}); **c.** Elongated shoots on MS medium supplemented with Kn (0.5 mgl^{-1}); **d.** Rooting of regenerated shoots on half strength MS medium supplemented with 0.5 mgl^{-1} NAA.

Plantlets with 6-7 leaves and well developed root system were removed and transferred to pot containing sterilized soil and sand mixture (3:1). These pots were kept in growth chamber for 15 days at 26 ± 2°C and 2000 lux intensity for acclimatization. In order to maintain high humidity, the pots were covered with inverted glass beaker. After thirty days these plantlets were taken outside the growth chambers and kept in shady place under normal temperature and light. A seventy per cent survival rate was obtained when acclimatized

plantlets were transferred to green house. From our experimental data, it is evident that BAP are best suited for inducing multiple shoots and NAA for rooting. In conclusion, this communication describes an efficient rapid propagation system of *C. paniculatus*, an endangered medicinal plant.

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