Mass Multiplication of *Celastrus paniculatus* Willd - An Important Medicinal Plant Under *In Vitro* Conditions using Nodal Segments

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Abstract: A rapid clonal propagation system has been developed for *Celastrus paniculatus* (Celastraceae) an important medicinal plant under *in vitro* conditions. Nodal explants from mature plant of this species were collected and cultured on MS medium supplemented with various concentrations (0.5, 1.0 and 2.0 mg l^{-1}) of cytokinins (BAP and Kn) and auxins (IAA, NAA and 2, 4-D) alone and in various combinations under controlled condition of 16 hours of photoperiod and 8 hours dark period at a temperature of 25±2ºC. The maximum number of shoots (8.9±0.5) along with hundred per cent bud break was recorded in the MS medium supplemented with 1.0 mg l^{-1} BAP. Most of the combinations of cytokinins with IAA induced the formation of less number of shoots. The *in vitro* regenerated shoots were excised aseptically and implanted on full and 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 rooting. MS half strength medium supplemented with 0.5 mg l^{-1} NAA proved best with hundred per cent rooting. The regenerated plantlets were successfully acclimatized in pots containing sterilized soil and sand mixture (3:1). The plantlets were then transferred to the field conditions. Seventy per cent of the regenerants survived well. [Journal of American Science 2010;6(7):55-61]. (ISSN: 1545-1003).

Key words*:* Micropropagation, nodal segments, multiple shoots, *Celastrus paniculatus.*
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. (Celastraceae) commonly known as Malkangni, Jyotishmati, Bitter sweet is a rare and endangered important medicinal plant with vine like habit reaching up to a height of 10 meters. It is distributed through out India up to an altitude of 1200 meters, mainly in deciduous forests. The species is vulnerable in Western Ghat of South India (Rajesekharan and Ganeshan, 2002). Seeds of this plant are the source of an Ayurvedic drug Jyothismati used in treating rheumatism, gout and neurological disorder. *Celastrus paniculatus* is well known for its ability to improve memory (Nadkarni, 1976). Pharmacological studies suggested that the oil obtained from the seeds possess sedative and anticonvulsant properties (Gatinode et al., 1957). Seed oil has also been found to be beneficial to psychiatric patients (Hakim, 1964) and increased the intelligence quotient of mentally retarded children (Nalini et al., 1986). The seed oil is useful for treating abdominal disorders, beriberi and sores. Leaf sap is an emmenagogue and antidote for opium poisoning (Warrier et al., 1994). Bark is reported to be abortifacient, depurative and a brain tonic and taken internally for snake bite (Govil, 1993). Root-bark extract also shows antimalarial activity (Rastogi and Mehrotra, 1998). The powdered root is considered useful for the treatment of cancerous tumors (Parotta, 2001). Chemical constituents of seeds as revealed by phytochemical analysis were sesquiterpene alkaloids like celapagine, celapanigine and celapanine (CSIR, 1992).

The conventional method of propagation of this medicinally important plant is through seeds. Poor seed viability and germination (11.5%) restricts the use of seeds in multiplication (Rekha *et al*., 2005). Indiscriminate over exploitation from natural sources to meet the growing demand by pharmaceutical industry coupled with low seed viability, lack of vegetative propagation methods and insufficient attempts for replenishment of wild stock of this medicinally important plant species have contributed to its threatened status. So realizing the threat of extinction and to meet the growing need of pharmaceutical industry, a mass multiplication protocol was developed for its better future supply.

Materials and methods

Nodal explants from mature plants growing in wild near Kurukshetra city were collected. The explants were initially washed with teepol under running tap water. Finally these were surface sterilized under aseptic conditions with freshly prepared 0.1% (w/v) mercuric chloride solution for 3-5 minutes and then given a dip in absolute alcohol. After this, these explants were washed with sterilized double distilled water 4-5 times. The surface sterilized explants (10 mm long) were inoculated on MS medium (Murashige and Skoog, 1962) containing 3% (w/v) sucrose and 0.8% (w/v) agar-agar supplemented with 0.5, 1.0 and 2.0 mg l^{-1} cytokinins (BAP and Kn) and auxins (IAA, NAA and 2, 4-D) individually as well as in various combinations. The cultures were incubated in culture room under controlled condition of 16 hours of photoperiod and 8 hours dark period at a temperature of 25 ± 2ºC. The intensity of light was approximately 2000 lux. The *in vitro* regenerated shoots were excised aseptically and implanted on full and 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. Statistical analysis was done by using the formula:

$$SE = \pm \sqrt{\frac{(X^2)}{n(n-1)}}$$

SE = Standard error

X = deviation of mean

n = number of replicates (Snedecor, 1956)

Results and discussion

The medium devoid of growth regulators failed to induce the formation of shoot buds. Similarly, no shoot buds developed in *Peganum harmala* (Saini and Jaiwal, 2000) and *Crataeva nurvala* (Walia et al., 2003) on MS basal medium. It has been suggested that the growth regulators applied externally during *in vitro* studies might disturb the internal polarity and change the genetically programmed physiology of explants resulting in organogenesis.

Figure 1.Callus induction from nodal explant on MS medium with 0.5 mg l^{-1} NAA.

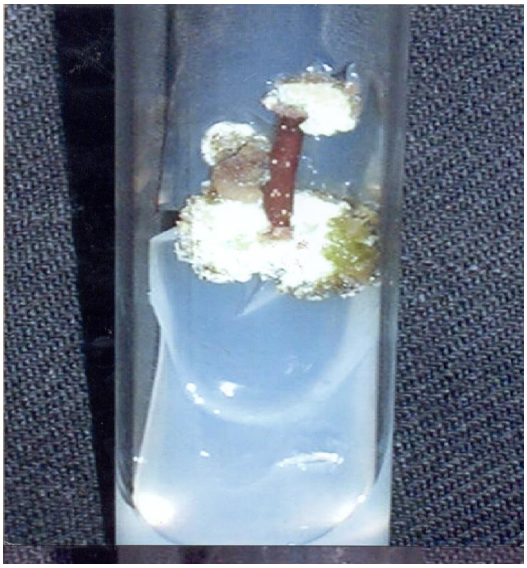

Figure 2.Callus induction from nodal explant on MS medium with 1.0 mg l^{-1}2,4-D

Table 1. Effect of cytokinins and auxins supplemented individually on nodal explants.

Growth regulators (mg l^{-1})	Bud break (%)	Average No. of days required for bud break	No. of shoots (Mean±SE)
MS control	–	–	–
BAP			
0.5	90	14.0	6.5±0.3
1.0	100	14.0	8.9±0.5
2.0	70	30.4	5.4±0.2
Kn			
0.5	70	18.6	1.0±0.0
1.0	100	25.0	1.0±0.0
2.0	100	25.0	3.1±0.2
IAA			
0.5	50	35.3	1.0±0.0
1.0	30	43.7	1.0±0.0
2.0	20	45.5	1.0±0.0
NAA			
0.5	–	–	–
1.0	–	–	–
2.0	–	–	–
2,4-D			
0.5	–	–	–
1.0	–	–	–
2.0	–	–	–

– **No Bud break**

In the present investigation, bud break was observed in MS medium supplemented with 0.5 and 1.0 mg l^{-1} BAP after 14 days of inoculation while it was delayed at higher concentration of BAP (2.0 mg l^{-1}). In the MS medium fortified with 0.5 mg l^{-1} Kn, bud break was noticed after 18.6 days of inoculation whereas, it occurred after 25 days of inoculation in the media with 1.0 and 2.0 mg l^{-1} Kn. The supplementation of auxins in place of cytokinins further delayed the process. No bud break was observed in MS medium supplemented with 0.5, 1.0 and 2.0 mg l^{-1} NAA and 2, 4-D but only callus formation took place (Table-1) (Figure 1&2). In most of the combinations of IAA with BAP and Kn, the number of days required for bud break increased with increase in concentration of IAA (Table-2&3). The development of axillary shoots was accompanied by basal callusing of the explants. However, this remains undifferentiated. Same type of observations were made by Dhawan and Bhojwani (1985), Kackar *et al.* (1991) and Nandwani and Ramawat (1991) working with *Leucaena leucocephala, Prosopis cineraria* and *P. juliflora*, respectively.

Table 2.-Effect of BAP and IAA supplemented in combinations on nodal explants.

Growth regulators	Concentration (mg l^{-1})	Bud break (%)	Average No. of days required for bud break	No. of shoots per explant (mean±SE)
BAP+IAA	0.5+0.5	100	25.4	5.3±0.17
	0.5+1.0	100	30.6	3.8±0.20
	0.5+2.0	60	32.3	2.6±0.13
BAP+IAA	1.0+0.5	100	12.7	6.9±0.28
	1.0+1.0	80	14.0	4.3±0.08
	1.0+2.0	70	14.0	3.1±0.12
BAP+IAA	2.0+0.5	100	10.0	7.8±0.07
	2.0+1.0	90	10.0	3.4±0.14
	2.0+2.0	70	10.0	2.3±0.04

Table 3. Effect of Kn and IAA supplemented in combinations on nodal explants.

Growth regulators	Concentration (mg l^{-1})	Bud break (%)	Average No. of days required for bud break	No. of shoots per explant (Mean±SE)
Kn+IAA	0.5+0.5	80	20.0	1.0±0.0
	0.5+1.0	70	25.7	2.9±0.15
	0.5+2.0	60	32.8	2.2±0.11
Kn +IAA	1.0+0.5	90	18.2	1±0.0
	1.0+1.0	70	22.3	1±0.0
	1.0+2.0	60	30.7	1±0.0
Kn +IAA	2.0+0.5	100	20.0	1±0.0
	2.0+1.0	80	24.5	1±0.0
	2.0+2.0	60	26.8	1±0.0

Figure 3.Shoot formation from nodal explant on MS medium with 1.0 mg l^{-1} BAP

When the growth regulators were applied individually, hundred per cent bud break was observed in MS medium supplemented with BAP (1.0 mg l^{-1}) and 1.0 and 2.0 mg l^{-1}Kn. Only fifty per cent bud break was noticed in the medium fortified with 0.5 mg l^{-1} IAA. The increase in concentration of IAA resulted in the decrease in per cent bud break (Table-1). Among the various combinations of BAP and Kn with IAA, hundred per cent bud break was noticed in 0.5 mg l^{-1} BAP + 0.5 mg l^{-1} IAA, 0.5 mg l^{-1} BAP + 1.0 mg l^{-1} IAA, 1.0 mg l^{-1} BAP + 0.5 mg l^{-1} IAA and 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} IAA. It has been found that increase in concentration of IAA resulted in reduced percent bud break (Table-2&3).

Figure 4. Shoot formation from nodal explant on MS medium with 1.0 mg l^{-1} Kn

Figure 5.Development of roots on half strength MS medium with + 0.5 mg l^{-1}NAA

Multiple shoot formation was recorded in all concentrations of BAP while in case of Kn, it occurred only in 2.0 mg l^{-1} treated MS medium. BAP was found more efficient than Kn with respect to initiation and subsequent proliferation of axillary buds. Similar observations have been made in many species like *Rotula aquatica* (Sebastian et al., 2002), *Cinnamomum camphora* (Babu et al., 2003) and *Mamordica charantina* (Agarwal and Kamal, 2004). BAP was also an efficient growth regulator for shoot multiplication in *Chlorophytum borivilianum* (Sharma and Mohan, 2006) and *Cyphomandra betacea* (Chakraborty and Roy, 2006). Medium supplemented with 1.0 mg l^{-1} BAP induced maximum shoots (8.9) per nodal explant while medium supplemented with Kn resulted in a reduced number of shoots albeit with longer internodes (Figure 3&4). Similar results were also reported in *Rotula aquatica* (Martin, 2002). No multiple shoot formation was observed in IAA treated explants (Table-1). Most of the combinations of BAP and Kn with IAA tested in nodal explant culture, induced the formation of less number of shoots (Table-2&3). Higher concentrations (>0.05 mg l^{-1}) of IAA and NAA induced callusing and inhibited shoot formation. These results presumably indicated a threshold level of endogenous auxin in the explants (Julliard et al., 1992).

The *in vitro* regenerated shoots were excised aseptically and implanted on full and half strength MS medium without or with growth regulators (0.5 and 1.0 mg l^{-1} NAA, IAA and IBA) for rhizogenesis. Excised shoots failed to develop roots on both full and half strength MS medium without growth regulators. Although root formation with callusing at the shoot base occurred in all concentrations of NAA, IAA and IBA, but more callus formation was observed in MS full strength medium. Moreover, this callus formation increased with increase in concentration of auxins. Induction of roots was noticed after 14.2 days of implantation in the medium with 0.5 mg l^{-1} NAA. It got delayed in other media. 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 (Figure 5) followed by IBA and IAA (Table-4). The efficacy of NAA at lower concentrations under *in vitro* rooting of shoots has been reported in various medicinal plants like *Verbascum thapsus* (Turker et al., 2001) and *Santolina canescens* (Casado et al., 2002). Peeters et al. (1991) found that NAA was taken up six times faster than IAA, and Van der Krieken et al. (1993) reported that IBA was taken up four times faster than IAA. Consequently, the efficacy of rooting in the presence of NAA may be due to its faster uptake. This may be due to the variation in the route of auxin uptake (De-Klerk et al., 1997). Similarly, Singh and Lal (2007) also achieved root induction on MS medium fortified with 0.5 or 1.0 mg l^{-1} of NAA in *Leucaena leucocephala*.

Figure 6. Plantlet transferred to pot

Figure 7.Plantlet transferred to field conditions

Table 4. Effect of various auxins on root formation.

Growth regulators (mg l^{-1})	Root induction (%)	Avg. No. of days required for root induction	Remarks
MS full strength without growth regulators	–	–	–
MS half strength without growth regulators	–	–	–
MS full strength+ NAA 0.5	90	14.2	C+++, stout, white
MS full strength+ NAA 1.0	80	15.3	C+++, stout, white
MS full strength+ IAA 0.5	70	16.5	C+++, thin
MS full strength+ IAA 1.0	60	17.6	C+++, thin
MS full strength +IBA 0.5	80	17.2	C++, thin
MS full strength +IBA 1.0	60	17.4	C++, thin
MS half strength+ NAA 0.5	100	12.2	C+, stout, white
MS half strength+ NAA 1.0	80	14.8	C++, stout white
MS half strength+ IAA 0.5	80	16.3	C++, thin, white
MS half strength+ IAA 1.0	70	17.4	C++, thin, white
MS half strength+ IBA 0.5	90	16.8	C++, thin, white
MS half strength+ IBA 1.0	70	17.2	C+,thin, white

C+= normal callus formation, C++ = moderate callus formation, C+++ = vigorous callus formation
- No root formation

The regenerants with well developed roots were transferred to sterilized soil and sand mixture (3:1) in earthen pots placed in growth chamber at 25 ± 2ºC. The roots were gently pulled out of the medium and immersed gently in running tap water. Medium particles sticking to the root system were carefully removed with fine brush. High humidity was maintained for the initial 20 days by using beakers. The plantlets were initially irrigated with half strength MS medium(salts only) without sucrose on alternate days. The plantlets were exposed to 3-4 hours daily to the conditions for natural humidity 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. Seventy per cent of the regenerants survived well (Figure 6&7). Therefore, the protocol could be used for the mass multiplication of this highly important medicinal plant species in a short duration. It will cater to the growing needs of pharmaceutical industry and also a mean for germplasm conservation and large scale plantation.

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