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In Vitro Multiplication and Conservation of the Medicinal Plant *Celastrus paniculatus* Willd

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Abstract

An *in vitro* method of obtaining plant from internodal explants of *Celastrus paniculatus* Willd., an under-exploited plant of medicinal value has been developed. Internodal explants grown on MS medium supplemented with 2,4-D (2.0 mg/liter) produced cent percent callusing. The cultured callus developed maximum number of shoots (11—13) in MS medium supplemented with BAP (2.0 mg/liter). Shoots (6.0 cm or longer) developed *in vitro* were rooted on MS medium containing NAA (1.0 mg/liter). The regenerated plantlets have been acclimatized and transferred to soil for normal growth under field condition with 70—80% success.

Key words : Malkangni, Internodal explants, *Celastrus paniculatus.*

C. paniculastus commonly known as Malkangni, is an important medicinal plant species belong to family Celastraceae. The plant is deciduous, woody climber reaching up to a height of 10 meters. The species is vulnerable in Western Ghat of South India (Rajesekharan and Ganeshan 2002). It has been reported between Pipli and Karnal in Haryana (Nair 1978). Once wide spread, that plants are very few now and there is a fast disappearance of all natural stands. Low success in conventional propagation by seeds and stem cuttings has led to gradual depletion of the species. For the conservation of these important and elite plant, which are threatened with extinction, micropropagation is of specials interest. Seeds of this plant are the source of an Ayurvedic drug Jyothismati used in treating rheumatism, gout and neurological disorder. *C. paniculatus* is well known for its ability to improve memory. Pharmacological studies suggest 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). More recently, rats treated with the seed oil of *C. paniculatus* for 15 days exhibited a significant decrease in the levels of norepinephrine, dopamine, serotonin, and their respective metabolites in both brain and urine (Nalini et al. 1995). The seed oil also reduces the cholesterol level by converting tryptophan into niacin, which is responsible for depression in blood cholesterol level. The seed oil is useful for treating abdominal disorders, beriberi and sores (Warrier et al. 1994). Chemical constituents of seeds as revealed by phytochemical analysis were sesquiterpene alkaloids like celapagine, celapanigine and celapanine (CSIR 1992). Leaf sap is an emmenagogue and antidote for opium poisoning (Warrier et al. 1994). Flower possesses analgesic and anti-inflammatory activities (Ahmad et al. 1994). Bark is reported to be abortifacient, depurative and a brain tonic and taken internally for snakebite (Govil 1993). Root-bark extract also shows antimalarial activity (Rastogi and Nehrotra 1998). The powdered root is considered useful for the treatment of cancerous tumours (Parotta 2001). Rekha et al. (2005) reported seed germination as low as 11.5%. The study provides a reliable method for *in vitro* regeneration of explants and acclimatization of *C. paniculatus* plants.

Methods

Plant Material

An adult plant of the *C. paniculatus* growing on

Table 1. *In vitro* response of internodal segments of *C. paniculatus* on MS media fortified with different growth regulator. – No callus induction, + Poor callus, ++ Moderate callus, +++ Good callus.

Medium composition (mg/l)		Percent explant callusing	Average no. of days required for callus induction	Color & Texture of callus	Visual growth of callus
MS basal medium		–	–	–	–
MS + 2,4-D	0.5	80	11.0	Light green, soft	++
	1.0	90	12.0	,,	++
	2.0	100	12.0	,,	+++
MS + NAA	0.5	70	13.0	Light green, soft	+
	1.0	80	14.0	,,	++
	2.0	90	14.0	,,	++
MS + IAA	0.5	65	14.8	Light green, soft	+
	1.0	70	14.5	,,	++
	2.0	80	14.3	,,	++
MS + BAP	0.5	30	16.0	Creamish yellow, fragile	+
	1.0	40	18.4	,,	+
	2.0	50	18.6	,,	++
MS + Kn	0.5	10	20.3	Creamish yellow, fragile	+
	1.0	20	25.7	,,	+
	2.0	30	30.5	,,	+

the campus of the Kurukshetra University, Kurukshetra was selected for present study. Young and small shoot segments were excised and washed thoroughly in running tap water to remove the superficial dust particles. Internodal explants excised from shoots were treated with 2% (vol/vol) teepol solution for ten minutes with constant stirring followed by washing in running tap water. The explants were surface sterilized with 0.1% (wt/vol) aqueous solution of mercuric chloride for 3—5 minutes and rinsed five times with sterile double distilled water. The surface sterilized internodal explants were trimmed at their edges aseptically and inoculated immediately to prevent the drying of the cut edges of the explants. Two to three explants were placed in each flask.

Culture Medium and Culture Conditions

Internodal segments were cultured on MS (Murashige and Skoog 1962) medium (containing analytical grade chemicals and 3% wt/vol sucrose and 0.8 wt/vol agar) of pH 5.8 (adjusted with IN NaOH or IN HCl), supplemented with various concentration of cytokinins, viz. BAP or Kn, auxins, viz. 2,4-D, IAA and NAA. The culture medium was prepared and 30—40 ml dispensed into each 150 ml/200 ml flask or glass bottles and autoclaved at 121 C, 1.2 kg/cm^2 pressure for 15 minutes. The effect of different treatments was quantified on the basis of the percentage of explants showing response. Callus obtained on culture initiation medium was subcultured after four weeks in to culture flask on the same medium. Each treatment consisted of ten flasks/culture bottles containing 2—3 explants each. All the cultured were incubated at 25 ± 2 C under white fluorescent light with a photoperiod of 16 h loght (intensity of 2000 lux) and 8 h of darkness.

Rooting and Acclimatization

In vitro raised shoots (5.0—6.0 cm long) were excised from the callus and cultured on MS basal and MS medium supplemented with various auxins, viz. IAA, IBA and NAA to induced rooting. Plantlets with well-developed roots were taken out from the culture

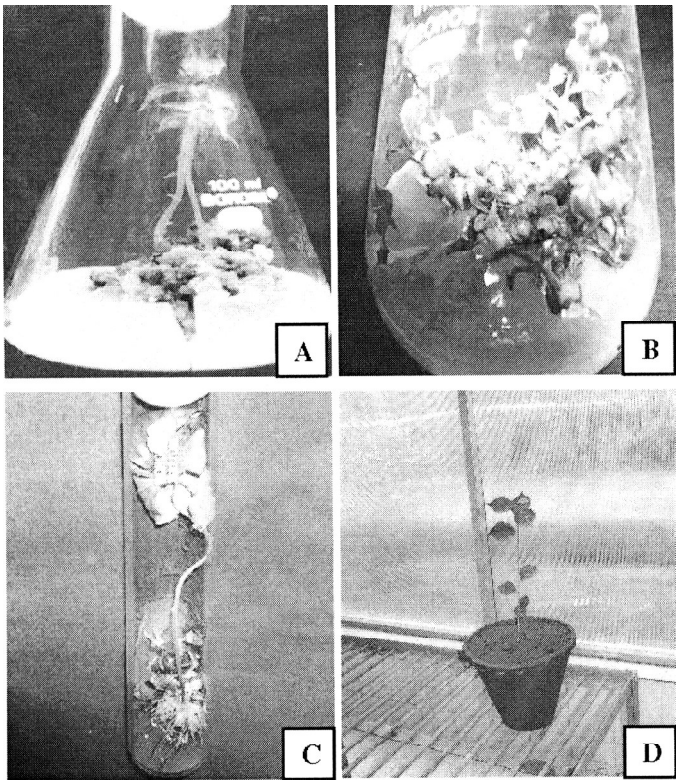

Figure 1. A. Callus formation from internodal explant on MS medium supplemented with 2.0 mg/l 2,4-D; B. Regeneration of plantlets from callus in MS medium fortified with 2.0 mg/l BAP; C. Elongated shoots with rooting on MS medium supplemented with 1.0 mg/l NAA; D. Plants in the pots during acclimatization in greenhouse.

vessels and the roots were thoroughly washed in running tap water by using fine brush, they were transferred to small thermocol pots containing sterilized sand. These pots were supplied with half strength MS salt solution and covered with polythene bags to maintain high relative humidity. These were placed in growth chamber at 25 ± 2 C under 16 h photoperiod. After two weeks, plastic bags were removed gradually (3—4 h daily) for their acclimatization to natural humidity and sun light. Plantlets were transferred along with sand to the pots filled with garden soil and kept in greenhouse for the normal growth.

Results and Discussion

In vitro Culture Establishment and Shoot Regeneration

To achieve microprogagation through callus, it requires the induction and formation of the callus as an initial step. MS medium without growth regulator failed to induce callus from tested explants (Table 1). Among the auxins, 2,4-D showed the cent percent callus induction after 12.0 days of inoculation (Fig. 1A). The medium supplemented with the IAA resulted in lesser percent of callus induction. Besides low response to callus induction, the time taken for callus induction was more in IAA. The medium supplemented with cytokinins failed to initiate higher percent of callus even after 28 days of inoculation at higher concentration (2.0 mg/liter) of BAP and Kn (Table 1). However, the percent callus induction was low in these media. The color of the callus in cytokinin were creamish yellow and fragile in texture. The callus was soft and light green in color in auxin fortified medium. The callus initiated from the cut ends of the explant and later on, it covers the whole explant. Similar observations have been made in hypocotyls and cotyledon explants of *Sesbania grandiflora*

Table 2. Regeneration potential of callus derived from internodal explant of *C. paniculatus* (after 45 days). – No regeneration.

Medium composition (mg/l)		Average no. of days required for shoot induction	Average no. of shoots	Length of shoots (cm)
MS + BAP	0.5	42.0	11.3	3-4
	1.0	44.0	13.2	5-7
	2.0	45.0	13.6	6-7
MS + Kn	0.5	–	–	–
	1.0	–	–	–
	2.0	–	–	–
MS + IAA	0.5	–	–	–
	1.0	–	–	–
	2.0	–	–	–

Table 3. Effect of different concentration of growth regulators on rooting of *in vitro* regenerated shoots of *C. paniculatus.* – No root formation.

Medium composition (mg/l)		Days required for root induction	No of roots per shoots (after 28 days)
MS basal medium		–	–
MS + IAA	0.5	18	1—2
	1.0	20	3—4
	2.0	16	2—4
MS +IBA	0.5	14	2—3
	1.0	17	2—3
	2.0	15	3—4
MS + NAA	0.5	14	3—4
	1.0	12	5—8
	2.0	12	5—6

(Khattar and Zohan Ram 1993) and *Leucaena leucocephala* (Singh and Lal 2007). This may be due to the production of endogenous auxins from the damaged cell of cut surface which trigerred the cell division as found in *Ornithogallum* (Hussey 1976). However, in the present studies, the auxins released by the damaged cells were not sufficient to trigger the cell division and it required the exogenous supply of auxins. It has been noticed that with the increased in the concentration of auxins and cytokinins, percent callus induction increased. The role of auxins and cytokinins in callus induction was also advocated by Rao and De (1987) in *Albizzia*, Yasmeen and Rao (2005) in *Vigna radiate*, Latto et al. (2006) in *Chlorophytum arundinacem*, Singh et al. (2006) in *Chlorophytum borivilianum*. The cultured callus developed maximum number of shoots (11—13) in MS medium supplemented with BAP (2.0 mg/liter) (Table 2, Fig. 1B). The role of BAP in shoots regeneration also reported in *Punica granatum* (Sgaron and Sinha 2000), *Salvadora persica* (Batra et al. 2001), *Bacopa monnieri* (Mohapatra and Roth 2005) and *Peristrophe bicalyculata* (Sharma and Devi 2006).

Rooting and Acclimatization of Plant

Shoots developed *in vitro* were excised (5—6 cm long) and subjected to rooting on MS basal medium supplemented with various auxins (Table 3). Amongst all the cultured media tried for rooting, the most responsive medium that induced 5—8 roots per shoot was MS medium with NAA (1.0 mg/liter) which resulted in 80—90% rooting (Table 3, Fig. 1C). Addition of the external auxin in the medium for the rooting was also reported by Gulati and Jaiwal (1996), Rajendra and D'Souza (1998), Batra et al. (2001), Shahzad and Siddiqui (2001), Arya et al. (2006), Chennaveeraiah et al. (2006), and Chavan et al. (2007) in different plant species. The rooted shoots after hardening acclimatization were transferred to the field under natural conditions with 70—80% success (Fig. 1D).

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