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# Impact of Monochromatic Lights Treatment on *In Vitro* Regeneration of *Celastrus paniculatus*



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## ABSTRACT

*Celastrus paniculatus*, belonging to the family Celastraceae, is an important medicinal plant of India. The species is being overexploited for its medicinal use. Poor seed viability coupled with low germination restricts its propagation through sexual means. Thus, alternative approaches such as *in vitro* techniques are highly desirable for large-scale propagation of this medicinally important plant. Celastrine and paniculatine are the alkaloids found in the seed oil which are responsible for making the plant medicinally highly potent (Parimala et al., 2009). Several studies have confirmed the memory and grasping-power-boosting properties of *C. paniculatus*. The Nodal section of *C. paniculatus* inoculated into Murashige and Skoog Medium supplemented with different concentrations and combination of BAP, Kn, TDZ, and NAA. Shoot multiplication was achieved by repeated transfer of mother explants and subculturing of *in vitro* produced shoot clumps on Murashige and Skoog's (MS) medium supplemented with various concentrations of 6-benzylaminopurine (BAP) alone or in combination with auxin  $\alpha$ -Naphthalene acetic acid (NAA). The maximum number of shoots was observed on MS medium supplemented with BAP (1 mgL<sup>-1</sup>) and NAA (0.5 mgL<sup>-1</sup>) kept under red light treatment. *In vitro* root induction were found on MS Medium supplemented with BAP and NAA after 15 days of growth period. We report for the first time the use of higher wavelength for an efficient rapid regeneration of this threatened medicinally important plant. This protocol will prove suitable in overcoming the demand and supply ratio of this plant for medicinal use. At the same time, it will help in conserving *C. paniculatus* in its natural habitat.

## INTRODUCTION

*Celastrus paniculatus* Willd (Celastraceae) commonly known as Malkangni in Hindi and Jyotishmati in Sanskrit, it is an important medicine in India. It is bittersweet in taste. It is distributed throughout India up to an altitude of 1250 meters mainly in deciduous forests and also distributed in sub-Himalayan region up to an altitude 1875 meter (M. Sharada *et al.*, 2003) Celapagin, celapanigin, celapanin, celastrine and paniculatine are the alkaloids found in the seed oil which are responsible for making the plant medicinally highly potent (Parimala *et al.* 2009). Several studies have confirmed the memory and grasping-power-boosting properties of *C. paniculatus* (Nair and Seenii 2001; Godkar *et al.* 2004).

Conventionally, *C. paniculatus* is propagated mainly through the seeds. However, the viability and germination (11.5%) of the seeds are poor (Rekha *et al.*, 2005). *C. paniculatus* has been prioritized by the National Medicinal Plant Board (Government of India) for conservation and cultivation emphasizing the need to develop agro-technology packages. Tissue culture techniques are being used globally for the conservation and utilization of genetic resources (Rao *et al.*, 1996). This leads to an urgent need for propagation and conservation of the plant species by plant tissue culture technology which offers a great potential for regeneration of these important medicinal plants.

## MATERIALS AND METHODS

### Plant material and surface sterilization

Nodal explants were collected from mature plant growing in the Herbal garden of Rajmata Vijayarajay Scindia College of Agriculture, Indore and then washed thoroughly in running tap water for 5-10 minutes, then washed with liquid detergent Tween20 (Himedia) and then again washed with tap water to remove the traces of detergent. They were surface sterilized in 0.1% HgCl<sub>2</sub> (Himedia) for 3-4 minutes aseptically under laminar air flow and then they were immersed in 70% ethanol for 30 seconds. Then explants were rinsed with sterile distilled water 3-4 times to remove the traces of mercuric chloride.

### **Medium and Culture conditions**

The surface sterilized explants were inoculated on MS medium (Murashige and Skoog, 1962) containing 3% (w/v) sucrose fortified with various concentrations of BAP, TDZ, Kn and NAA for regeneration. The pH of the medium was adjusted to 5.8 by using (1N NaOH and 1N HCl) and then 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 incubated at 25±2°C and distributed under different monochromatic lights treatment (White-all lights) (Blue-495) (Yellow-580) (Red-750) with 16 hours in lights treatment and for 8 hours in dark cycle which is maintained by automatic timer.

### ***In-vitro* shoots multiplication**

Regenerated shoots were used to assess the effect of growth regulators on further *in vitro* shoot multiplication. Full strength MS medium fortified with varying concentrations of BAP (0.5, 1.0, 2.0 mg l<sup>-1</sup>) and NAA (0.5, 1.0 mg l<sup>-1</sup>) was tested for further multiplication of shoot. Single shoots of size which were up to 2-3 cm were used for this purpose. Observations were taken as average number of shoots and average shoot length after 4 and 8 weeks of growth period. Routine sub-culturing of multiple shoots was carried out at periodic interval of every 3-weeks.

### ***In-vitro* Rooting**

Single shoots were isolated from multiplied shoots and inoculated into MS Medium containing 3% sucrose and fortified with NAA (0.5, 1.0 mg l<sup>-1</sup>) and IAA (0.5, 1.0 mg l<sup>-1</sup>) for initiation of root. No rooting was found on MS Medium without any growth regulators and NAA (0.5 mg l<sup>-1</sup>) proved effective in root induction among all other treatments, root initiation was observed after 15 days of growth culture.

### **Hardening and Acclimatization**

*In vitro* rooted shoots were taken out from the culture bottle and washed several times with distilled water to remove the traces of agar-agar. The plantlets were transferred to pots containing autoclaved soil, sand and manure (3:1:1). These plantlets were irrigated with ½ MS Medium without any growth regulators and sucrose. The pots were covered with polythene bags to maintain shade. The plantlets were exposed to natural conditions daily for 2-3 hours for the

hardening of plantlets. Then after 30 days, plants were transferred to bigger pots and kept into polyhouse for the acclimatization of plants where temperature and humidity were maintained.

### **Data analysis**

The number of days required for shoots regeneration, proliferation, shoot length, root induction and root length were determined after 8 weeks of growth period. The standard deviation of the mean calculated in MS Excel program is presented in (Table 1, 2, 3, 4).

## **RESULTS AND DISCUSSION**

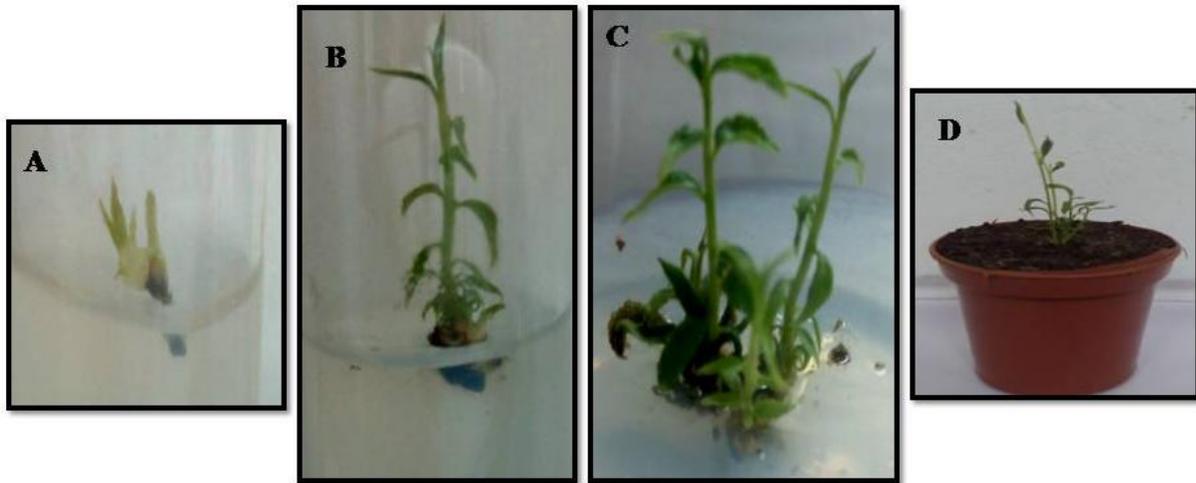
### **Shoot induction and multiplication**

In the present experiment, we have studied the effect of different monochromatic light quality on *in vitro* regeneration of *Celastrus paniculatus*. MS medium devoid of any plant growth regulators failed to induce any shoot. MS medium fortified with cytokinins gives better results than MS medium fortified with auxins. Firstly shoot induction was observed after 10 days of inoculations under the influence of red light than after into white, yellow and blue light treatments. Among all lights, red light was found most favorable for shoot induction (Fig-1a and b, 2a and b, 3a and b, 4a and b).

Explants showed sign of proliferation after 2 weeks of growth period. After excising multiple shoots, they were transferred into fresh MS medium fortified with different concentration of cytokinins BAP (0.5, 1mg/l), Kn (0.5, 1mg/l), TDZ (0.5, 1mg/l) and auxins NAA (0.5mg/l) individually or in combinations for shoot proliferation (Fig-1c, 2c, 3c and 4c). MS medium fortified with BAP (1mg/l), and NAA (0.5mg/l) gives maximum 2.5 average number of shoots per explants under the influence of red light treatment. Whereas lowest 1.0 average number of shoots in MS medium fortified with TDZ (0.5mg/l) was observed under the influence of blue light treatment (Table no-2 and 4). There was a significant difference among white, blue, yellow and red light treatments (Table no-1, 2, 3 and 4).

Maximum average shoot length 1.9cm per shoot was observed under the influence of red light treatments. Whereas lowest average shoots length 0.5cm per shoot was observed under the influence of blue light treatments (Table no-2 and 4). There was a significant difference among

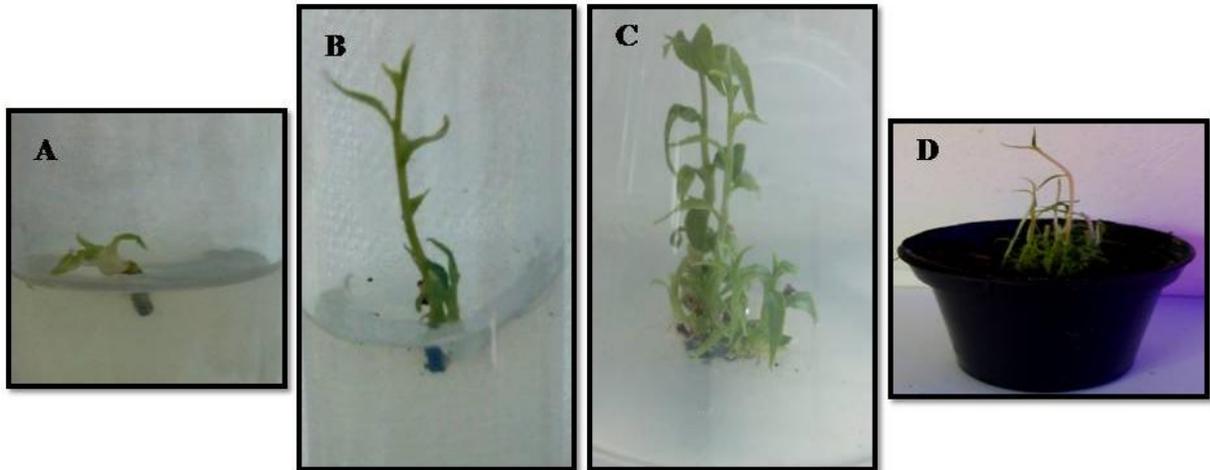
white, blue, yellow, red light treatments (Table no-1, 2, 3 and 4). Plants have light receptors that detect visible light and generate a response. Through experimentation, scientists have concluded that red light have the greatest effects on plant growth. Phytochrome absorbs mostly red light. Red light wavelengths set off a variety of responses in plants as well. They initiate seed germination, stem elongation (de-etiolation) and root development.



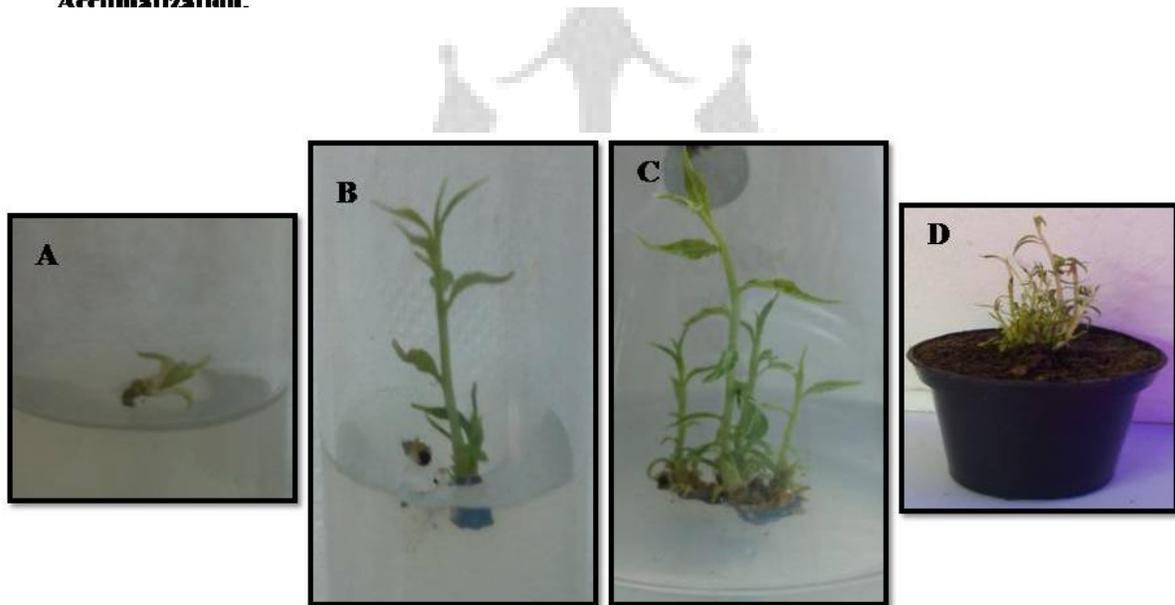
**Fig 1- A- Shoot induction from nodal section, B&C- initiation & Proliferation of multiple shoots of *Celastrus paniculatus* under the influence of white light treatment, D- Acclimatization.**



**Fig 2- A- Shoot induction from nodal section , B&C- Initiation & Proliferation of multiple shoots of *Celastrus paniculatus* under the influence of blue light treatment, D- Acclimatization.**



**Fig 3- A-Shoots induction from nodal section, B&C- Initiation & Proliferation of multiple shoots of *Celastrus paniculatus* under the influence of yellow light treatment, D- Acclimatization.**



**Fig 4- A- Shoot induction from nodal section, B&C Initiation & Proliferation of multiple shoots of *Celastrus paniculatus*, under the influence of red light treatment D- Acclimatization.**

Treatments	MS Medium + PGR concentrations				Average number of shoots	Average number of shoot length in cm
	BAP	Kn	TDZ	NAA		
White light	0	0	0	0.5	0	0
	0.5			0.5	1.6±0.36	1.3±0.36
	1			0.5	2.2±0.1	1.5±0.25
		0.5		0.5	1.5±0.98	1.0±0.30
		1		0.5	2.0±0.50	1.1±0.20
			0.5	0.5	1.3±0.20	0.9±0.36
			1	0.5	1.8±0.51	1.2±0.15

**Table 1-Effect of white light and different concentrations of BAP, Kn, TDZ and NAA on shoot proliferation and shoot length**

Treatments	MS Medium + PGR concentrations				Average number of shoots	Average number of shoot length in cm
	BAP	Kn	TDZ	NAA		
Blue light	0	0	0	0.5	0	0
	0.5			0.5	1.1±0.15	0.6±0.86
	1			0.5	1.3±0.15	0.9±0.17
		0.5		0.5	1.3±0.43	0.5±0.20
		1		0.5	1.6±0.25	0.6±0.15
			0.5	0.5	1.0±0.05	0.7±0.30
			1	0.5	1.2±0.25	0.8±0.25

**Table 2-Effect of blue light and different concentrations of BAP, Kn, TDZ and NAA on shoot proliferation and shoot length**

Treatments	MS Medium + PGR concentrations				Average number of shoots	Average number of shoot length in cm
	BAP	Kn	TDZ	NAA		
Yellow light	0	0	0	0.5	0	0
	0.5			0.5	1.5±0.30	1.1±0.23
	1			0.5	2.0±0.92	1.4±0.32
		0.5		0.5	1.4±0.25	0.7±0.25
		1		0.5	1.8±0.70	1.0±0.05
			0.5	0.5	1.1±0.1	0.8±0.30
			1	0.5	1.6±0.51	1.0±0.32

**Table 3-Effect of yellow light and different concentrations of BAP, Kn, TDZ and NAA on shoot proliferation and shoot length**

Treatments	MS Medium+ PGR concentrations				Average number of shoots	Average number of shoot length in cm
	BA P	Kn	TDZ	NAA		
Red light	0	0	0	0.5	0	0
	0.5			0.5	1.7±0.60	1.5±0.26
	1			0.5	2.5±0.43	1.9±0.20
		0.5		0.5	1.5±0.28	1.1±0.05
		1		0.5	2.1±0.30	1.2±0.20
			0.5	0.5	1.4±0.35	1.0±0.15
			1	0.5	2.0±0.70	1.6±0.26

**Table 4-Effect of red light and different concentrations of BAP, Kn, TDZ and NAA on shoot proliferation and shoot length**

*In-vitro* rooting

Minimum average number of 1.1 roots per shoot was observed in NAA (0.5mg/lt) under the influence of blue light treatment. There were least significant differences were observed in number of roots between yellow and white light treatments. Whereas maximum average 2.3 number of roots per shoot was observed in NAA (1mg/lt) under the influence of red light treatment (Table-5). Minimum average root length 0.7cm per shoot was observed under the influence of blue light treatment. Whereas Maximum average roots length 1.8cm per shoot was observed under the influence of red light treatment (Table-5).

MS medium + PGR mg/lt	Light treatments	Average number of roots	Average root length in cm
MS0	0	0	0
NAA 0.5mg/lt	White	1.3±0.6	1.0±0.3
	Blue	1.1±0.3	0.7±0.2
	Yellow	1.4±0.6	1.1±0.1
	Red	1.6±1.5	1.2±0.2
NAA 1mg/lt	White	2.0±0.4	1.7±0.1
	Blue	1.5±0.2	1.4±0.2
	Yellow	1.9±0.3	1.6±0.3
	Red	2.3±1.5	1.8±0.7

**Table 5- Effect of different monochromatic lights and different concentrations of NAA on *in-vitro* rooting.**

## CONCLUSION

The main object of this project is to develop a cost effective, high frequency reproducible *in vitro* regenerative protocol for replenishment of natural and depleting population of *C. paniculatus* by producing their quality clones in short duration through tissue culture.

From the above studies conducted this year, it can be concluded that light significantly affected regeneration of *Celastrus paniculatus*. Among all light treatments, red light was found suitable for multiple shoot induction and proliferation from Nodal explants of *Celastrus paniculatus*. Shoot length and proliferation was influenced by red light treatment. Hence, light exhibit to be an effective factor which can be utilized for high quality, rapid multiple plant formation to meet up the demand and supply ratio of this plant for its medicinal importance.

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