Research Article

# Impact of Different Lights on *in-vitro* Organogenesis from *Celastrus paniculatus*- A Threatened Medicinal Plant

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**ABSTRACT**

*Celastrus paniculatus* is an important medicinal plants belonging to the family celastraceae. Due to the important medicinal properties this species has been overexploited and now considered as a threatened species. Propagation through seed is very difficult because the viability and germination of seed is only 11.5% and vegetative propagation requires higher labor input. Hence in-vitro propagation offers an alternative tool for rapid multiplication of *celastrus paniculatus* in short span of time. The main objective of this study is to understand the effect of monochromatic lights (white, blue, yellow and red) and as well as different concentrations of auxins and cytokinins for better plantlet development. In our present study juvenile explants (leaves) were inoculated for callogenesis into MS medium fortified into different concentrations of auxins and cytokinins. Green compact nodular organogenic callus were transferred into MS medium fortified with (0.5, 1mg/lt) BAP and (0.5, 1mg/lt) TDZ shoot proliferation, then transferred into (0.5, 1mg/lt) NAA for rooting. Our studies conclude that the results are encouraging *in-vitro* organogenesis among all different monochromatic lights, blue and red light was found to be most suitable light for maximum shoot production and shoot length. The results obtained in this study showed that lower and higher wavelengths of the visible spectrum (Blue and Red light) influence shoot induction, proliferation and also increase shoot length.

**Keywords:** *Celastrus paniculatus*, Murashige and Skoog (MS), α-Naphthalene acetic acid (NAA), Thidiazuron (TDZ), 6-Benzylaminopurine (BAP), 6-Furfurylaminopurine (Kn).

## INTRODUCTION

*Celastrus paniculatus* Willd belonging to the family celastraceae commonly known as jyotishmati and malkangani. It is a large woody, unarmed climbing shrub occurring naturally in the northern region of India at an altitude of 1250 meters. The plant has also distributed in the range of sub-Himalayan region from Jhelum eastward upto 1875 meters throughout hilly parts of Bombay, South of Gujarat, Central India, Madras, Ceylon, Burma, Malay Peninsula and Archipelago, and is used primarily in the treatment of mental disorders.

Oil obtained from the seeds of this plant is a source of herbal medicine, which is used in the treatment of gout, leprosy, skin diseases, fever, rheumatism, beriberi, sores and neurological disorders[10,11]. The oil obtained from this seed has been found to be very beneficial in the treatment of pshyctratist patient and it also helps to improve memory. The powdered root is considered useful for the treatment of cancerous tumours[2] and leaves as antidote of opium poisoning and possess emmenagogue property. These important medicinal properties are due to the presence of secondary metabolites viz- alkaloids (celastrine, paniculatine) and saponin which are responsible for making this plant highly valuable.

Propagation of *Celastrus paniculatus* through seed is very difficult Poor seed viability and germination (11.5%) restricts the use of seeds in multiplication (4). Due to presence of important medicinal properties this species is being depleted fast and there is an urgent need to replenish the wild stock of this highly important medicinal plant. Tissue culture is the only technique being used globally for the conservation and utilization of genetic resources[12]. This article aim to study the effect of monochromatic lights (white, blue, yellow, red) and as well as different concentrations of auxins and cytokinins for better plantlet development.

## MATERIALS AND METHODS

### Plant material

Juvenile leave explants were collected from herbal garden of Rajmata Vijyarajae Scindia Krishi Vishwavidyalayay, Indore. The plant was diseases free and showed good biomass yield.

### Explants surface sterilization

For the surface sterilization of explants, the explants were washed thoroughly into running tap water about 20-30 min. Then after explants were washed with tween 20 (Himedia) for 5-10 minutes, after that explants were washed with bavistin (1%w/v) for 30 minutes with vigorous shaking. Explants were again rinsed with running tap water to remove the traces of bavistin and then washed with distill water. After these treatments explants were taken inside the laminar air flow. Explants firstly treated with (0.1%w/v) mercuric chloride (Himedia) for 3-4 minutes for the removal of bacterial flora. Then after


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explants rinsed with 70% ethyl alcohol, after that explants were thoroughly rinsed with sterile distill water for 4-5 times to remove the traces of mercuric chloride and 70% alcohol.

### Culture media and inoculation of explants

Murashige and skoog (MS, 1962) basal Medium with 3% sucrose (Himedia) and 0.6% agar-agar (Himedia) were used as a culture medium.

The surface sterilized explants were trimmed approximately into 1 cm length with help of sterile forcep and scalpel blade. Then explants were inoculated into sterile culture bottle containing MS Medium fortified with different concentrations of auxins (0.5, 1mg/lt) 2, 4-D, (0.5, 1mg/lt) NAA, and cytokinins (1mg/lt) BAP, (1mg/lt) TDZ. The pH of the medium was adjusted to 5.8 by using (1N NAOH and 1N HCL) and then 0.6% 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) (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. Every explant was sub-cultured on the fresh medium every 4 week.

### Callus induction and proliferation

Leaf explants were inoculated into MS Medium fortified containing 3% sucrose and 0.6% agar-agar and also fortified with different concentrations of plant growth regulators auxins (0.5, 1mg/lt) 2, 4-D, (0.5, 1mg/lt) NAA and cytokinins (0.5, 1mg/lt) BAP, (0.5, 1mg/lt) TDZ. Callus induction was observed after 12 days of growth period. Callus induction was observed from the cut ends of explants. The organogenic nature of callus was identified by its green color and compact texture of callus. The green compact organogenic callus was selected and subcultured every week for the induction of matured green organic calli.

### Shoot proliferation

After 8 weeks of growth culture organogenic callus were transferred on MS Medium fortified with BAP (0.5, 1 mg/lt) and TDZ (0.5, 1 mg/lt) for shoot multiplication medium. MS Medium also contains 3% sucrose and 0.6% agar-agar. Then these cultures were sub-cultured weekly for 8 weeks for the initiation and proliferation of shoots. These experiments were conducted with a minimum of 20 replicates per treatment.

### *In-vitro* Rooting

Single shoots were isolated from multiplied shoots and inoculated into MS Medium fortified with NAA (0.5, 1.0 mg $l^{-1}$) for initiation of root. No rooting was found on MS Medium without any growth regulators and NAA (0.5 $mgl^{-1}$) proved effective in root.

### Hardening and Acclimatization

After root induction plantlets were taken out from culture bottles with the help of forcep to prevent from any damage and then washed with distill water to remove agar-agar. The regenerated plants were transferred into plastic pots containing soil, sand and manure in (2:1:1) ratio for hardening of plants. These plantlets were irrigated with ½ MS Medium without any growth regulators and sucrose. 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 morphology of callus, 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 programme is presented in (Table 1, 2 and 3).

## RESULTS AND DISCUSSION

### Callus induction and proliferation

After 12 days of inoculation callus induction were observed from the cut ends of explants. Callusing was observed on MS Medium fortified with different concentrations of auxins and cytokinins along with 3% sucrose and 0.6% agar-agar. MS medium fortified with 2, 4-D+TDZ (1+0.5mg/lt) proved best concentration for callus. Green nodular oganogenic were observed after 25 days of growth period (Fig-1, 2, 3, and 4). This experiment was conducted three times with 10 replicates.

### Shoot initiation and proliferation

This study demonstrates the impact of higher and lower wave length on propagation of *Celastrus paniculatus* by using leave explants *in –vitro*. Plants have light receptors that detect visible light and generate a response. Through experimentation, scientists have concluded that red light and blue light have the greatest effects on plant growth. Organogenic callus starts to show sign of shoot induction after two weeks of subculture into shoot proliferation medium (Fig-1, 2, 3 and 4). After 14 days of growth period a new shoot bud observed on the axil part of callus and buds develop into shoots after 3 weeks of growth period. Completely formed shoots were excised individually from the proliferated explants and then transferred into the same culture medium to increase number of shoots.

After excising shoots they were transferred into MS medium fortified with BAP (1mg/lt) and TDZ (1mg/lt) for the multiplication of shoots. They proliferated for two more subculture but reduced then after. The lowest 2.6 average number of shoots per explants were observed in BAP (0.5mg/lt) under the influence of white light treatment. Whereas 3.6 average number of shoots per


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explants were observed in TDZ (0.5mg/lt) under the influence of white light treatment. There was least significant difference was found in BAP (0.5mg/lt) and TDZ (0.5mg/lt) under the influence of white light.

Maximum average numbers of 10.6 shoots per explants were observed in TDZ (1mg/lt) under the influence of blue light treatment, whereas 5.7 average number of shoots were observed in BAP(1mg/lt) under the influence of blue light treatment. Maximum average shoot length 3.2 cm per explants was observed in TDZ (1mg/lt) under the influence of red light treatment. There was significant difference in shoot length among all lights (Table-1). MS medium devoid of plants growth regulators failed induce any shoot induction.

### *In-vitro* root induction

Minimum average number of 1.3 roots per shoot was observed in NAA (0.5mg/lt) under the influence of white light treatment. Whereas maximum average number of roots 5.1 per shoot was observed in NAA (1mg/lt) under the influence of white light treatment. Minimum average root length 0.9 cm per shoot was observed under the influence of red light treatment. Maximum average root length 3.1 cm per shoot was observed under the influence of white light treatment (Table-2).

**Fig. 1 A- Green organogenic callus B- Shoot induction, C- Shoot proliferation under the influence of white light treatment**

**Fig. 2 A- Green organogenic callus B- Shoot induction, C- Shoot proliferation under the influence of blue light treatment**

**Table 1:** Effect of different monochromatic lights and different concentrations of BAP on shoot proliferation and shoot length

| Light treatments | MS medium+ BAP (mg/lt ) | Average number of shoots | Average shoot length in cm |
|---|---|---|---|
| | 0 | 0 | 0 |
| White | 0.5mg/lt | 2.6±1.1 | 1.1±0.2 |
| | 1mg/lt | 4.5±0.5 | 1.5±0.5 |
| Blue | 0.5mg/lt | 5.0±1.4 | 1.0±0.1 |
| | 1mg/lt | 5.7±0.7 | 1.2±0.0 |
| Yellow | 0.5mg/lt | 3.6±1.6 | 1.9±0.6 |
| | 1mg/lt | 4.3±1.7 | 2.3±0.0 |
| Red | 0.5mg/lt | 2.3±0.5 | 1.7±0.5 |
| | 1mg/lt | 4.0±2.0 | 2.9±1.1 |


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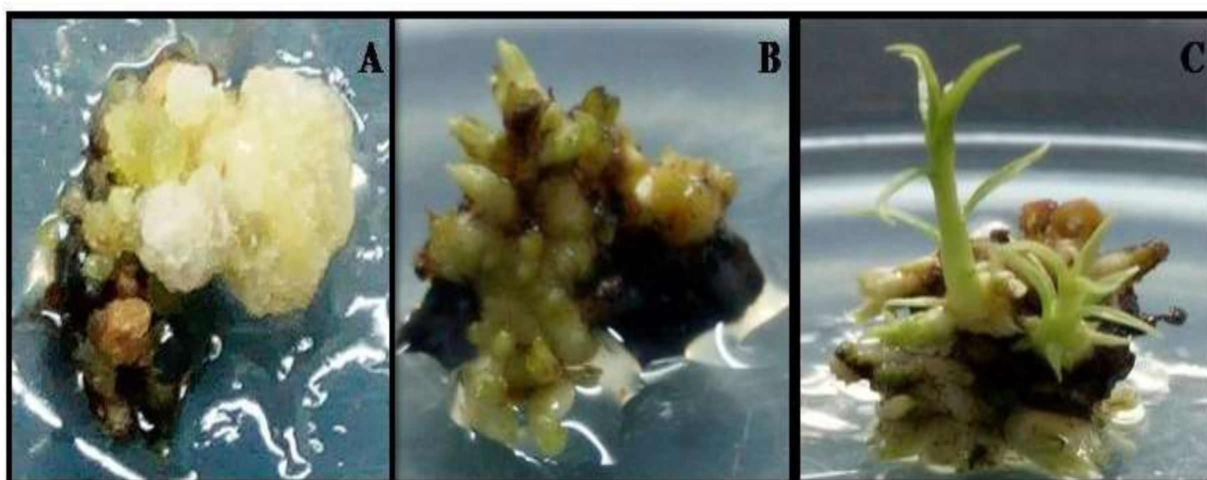


**Fig. 3 A- Green organogenic callus B- Shoot induction, C- Shoot proliferation under the influence of red light treatment**

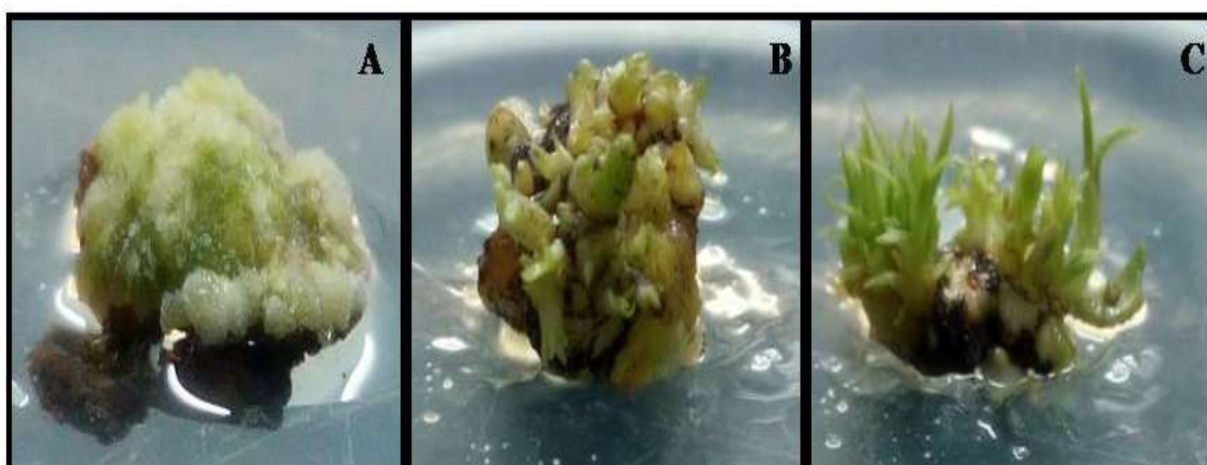


**Fig. 4 A- Green organogenic callus B- Shoot induction, C- Shoot proliferation under the influence of yellow light treatment**

**Table 2:** Effect of different monochromatic lights and different concentrations of TDZ on shoot proliferation and shoot length

| Light treatments | MS medium +TDZ (mg/lt) | Average number of shoots | Average shoot length in cm |
|---|---|---|---|
| | 0 | 0 | 0 |
| White | 0.5mg/lt | 3.6±1.1 | 1.3±0.5 |
| | 1mg/lt | 9.6±1.5 | 1.7±0.6 |
| Blue | 0.5mg/lt | 4.6±2.0 | 1.0±0.0 |
| | 1mg/lt | 10.6±1.5 | 1.5±0.4 |
| Yellow | 0.5mg/lt | 5.1±2.0 | 1.2±0.2 |
| | 1mg/lt | 8.0±1.0 | 2.6±1.2 |
| Red | 0.5mg/lt | 3.3±0.5 | 1.9±0.5 |
| | 1mg/lt | 5.3±2.0 | 3.2±2.0 |


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**Fig. 5 A- Root induction, B- Root proliferation**

**Table 3:** Effect different monochromatic light treatment and different concentrations of NAA on root induction and root length

| MS medium +PGR mg/lt | Light treatments | Average number of roots | Average root length in cm |
|---|---|---|---|
| **MS0** | 0 | 0 | 0 |
| **MS+NAA 0.5mg/lt** | White | 2.0±0.5 | 1.5±0.7 |
| | Blue | 1.7±0.6 | 1.1±0.1 |
| | Yellow | 1.5±0.5 | 1.0±0.1 |
| | Red | 1.3±0.5 | 0.9±0.3 |
| **MS+NAA 1mg/lt** | White | 5.1±0.9 | 3.1±1.1 |
| | Blue | 4.5±1.3 | 2.4±1.1 |
| | Yellow | 3.6±0.5 | 1.7±0.3 |
| | Red | 2.3±1.5 | 1.4±0.3 |

## CONCLUSION

Our studies conclude that among all different monochromatic lights, blue and red light was found to be most suitable light for maximum shoot production and shoot length of *Celastrus paniculatus*. The results obtained in this study showed that lower and higher wavelengths of the visible spectrum (Blue and Red light) influence shoot induction, proliferation and also increase shoot length. In addition, the results of this study suggest that the light quality emitted by red and blue lights were both beneficial for vegetative propagation of *Celastrus paniculatus*. Similar findings on effect of light were also reported in *Gerbera* under red LEDs and blue LEDs (Wang)[17]. The Blue and Red light receptors cryptochromes, phytochrome A and phytochrome B appears to regulate growth of *Celastrus paniculatus* cultures. Blue-light photoreceptors absorb wavelengths of blue light and trigger a number of reactions in plants.

Blue wavelengths affect phototropism, the opening of stomata (which regulates a plant's retention of water), and chlorophyll production. Phytochrome absorbs mostly red light.

Red wavelengths set off a variety of responses in plants as well. They initiate seed germination and root development.

Hence a strong possibility of combinatorial effect of Blue and Red light treatment for high frequency regeneration can be explored. This protocol will help in regeneration and conservation and also bears the potential to accomplish the demand and supply ratio for pharmaceutical industry.


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