**In vitro Effect of Various Plant Growth Regulator on Micro Propagation of Celastrus paniculatus: An Important Medicinal Plant**

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**A B S T R A C T**

*In-vitro* effect of various growth regulators on propagation of *Celastrus paniculatus*, a medicinally herbs of India was observed in this study. *C. paniculatus* has been used in the various ancient traditional system of medicine and having a very poor seed germination percentage. Healthy nodal segments of the herb was used as explants with basic MS medium for shoot initiation and multiplication containing various combinations of different growth regulators. MS full and 1/2 MS were used for rooting of plantlets with 25 to 200 mg/l Activated Charcoal (AC). Maximum mean number of initiated plantlets 1.8 ± 0.42 with mean length 4.6 ± 1.34 were found in MS medium treated with lowest concentration of BA, while maximum mean number of multiplied plantlets 21.7 ± 1.25 with mean length 6.8 ± 0.91 were found in MS medium treated with 4.44 µM. Maximum mean number of roods 16.2 ± 0.78 with Mean length 9.19 ± 0.68 was observed on 1/2 MS medium with 100 mg/l activated charcoal. The rooted plantlets were successfully hardened in 1:1:1 ratio of sand: soil: vermicompost and successfully established in soil.

**Keywords**

*Celastrus paniculatus*, Micropropagation, Medicinal Plants, Plant Tissue Culture.

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**Introduction**

*Celastrus paniculatus* Willd.(family Celastraceae) also called ‘Jyotishmati’ in Sanskrit or ‘Malkangani’and ‘Duhudu’in Hindi improves memory and cognitive functions. It is widely used in ayurvedic system of medicine and beneficial in neurological diseases and pain disorders including muscle cramps, backache, sciatica, osteoarthritis, facial paralysis and paralysis. This plant is used in cure many diseases such as leprosy, leucoderma, skin diseases, paralysis, depression, arthritis, asthma and fever (Bhanumathy *et al.*, 2010; Kumar and Gupta, 2002; Godkar *et al.*, 2006; Godkar, *et al.*, 2003; Patil *et al.*, 2010; Sharma *et al.*, 2001). *C. paniculatus* Seeds contains around 30% oil content in which many alkaloids like Celapagin, Celapanigin, Celapanin, Celastrine, Paniculatine, etc. are present. Seeds and its oil are mainly used in ayurvedic medicines. Leaves are also used for de-addiction. Generally, leaf juice is beneficial for treating opium addiction. This species distributed in, India (widespread), Nepal, Sri Lanka, Myanmar, Bhutan, Thailand, Vietnam, Laos, Java, Malaysia, Sumatra, Philippines, Moluccas, China, Taiwan (Hassler, 2016).
Propagation of *C. paniculatus* by seeds is very poor owing to inhibitory compounds present in the seed coat, and the percentage seed germination is very low. Rooting of cuttings is also not successful (De Silva and Senarath). The theoretical basis for plant tissue culture was proposed by Gottlieb Haberlandt, German Academy of science in 1902 on his experiments on the culture of single cell. The first true cultures were obtained by Gautheret from cambial tissue of *Acer pseudoplatanus*. The term plant tissue culture (Micro propagation) is generally used for the aseptic culture of cells, tissues, organs and their components under defined chemical and physical conditions in vitro. Today Micropropagation method of plant tissue culture was widely used for conservation and mass production of endangered and economically important plant species. Some researchers work on the microporpagation of *C. Paniculatus* through callus (Sharada *et al.*, 2003) and through bud differentiation (Rao and Purohit, 2006) with limited success. Consequently, an attempt was made for propagate the *C. paniculatus* through micro propagation and to establish tissue cultured plants in the green house successfully.

**Materials and Methods**

**Collection of explant**

Branches of *C. Paniculatus* were collected from healthy growing plants from medicinal garden of Prof. T. S. Murthi Science and Technology Station Obedullaganj, Raisen (M.P.)

**Sterilization of Explant**

Nodal explants were cut and washed in running tap water to remove the superficial dust particles and mud adhering to its surface. Explants were washed with sevelon (3-4 drops/100ml) in a vial by gentle agitating conditions. The explants were thoroughly rinsed with distilled water for several times. Again these explants were dipped in to the 1% fungicide (Bavistin) treatment was given for 15 minutes and then washed with distilled water. For surface sterilization. Explants were transferred to sterile empty flasks under aseptic conditions and given a quick dip in 70% alcohol and subsequently they were washed in distilled water. After that, the explants were surface sterilized with different concentration of sterile (HgCl₂) for different duration as per the treatment to find out the best treatment for sterilization of explants. To remove the traces of sterile explants were washed in sterilized distilled water at least 5-6 times. The procedure was carried out in the inoculation chamber under laminar air flow hood.

**Preparation of MS Medium**

Culture media was prepared as per described method of Murashig and Skoog (1962) and different growth regulator was added as per requirement. For the initiation of ex-plant various concentration of BA (0.44 to 2.22 µM) alone and with IA (0.57 to 1.71µM) and NA (0.54 to 1.61µM) were used, while concentration of BA (2.22 to 11.1µM) alone and IA (0.57 to 1.71 µM) and NA (0.54 to 1.61 µM) with BA (2.22 to 6. 66 µM) were used for multiplication. MS full and 1/2MS were used for rooting of plantlets with 25 to 200 mg/l Activated Charcoal (AC) combination adding 30 g/l sucrose and 5.7% agar. The hormones used for experiment were taken from stock solutions, which were previously prepared and kept under cold condition in refrigerator (Doods and Roberts, 1985). The pH of the medium was adjusted to 5.7 with 0.1 NaOH before autoclaving at 15 lbs and 121°C for 18 min.
Aseptic Inoculation of Explant

Nodal segments about 0.5-0.8 cm were prepared aseptically and were implanted vertically on surface disinfected nodal explants were inoculated onto full strength MS medium (Murashige and Skoog, 1962) fortified with specific concentrations of growth regulators. The cultures were incubated at a constant temperature of 26±2°C with 16±1 h photoperiod (3000 lux).

Results and Discussion

Surface Sterilization and Induction of Axillary Shoots

Treatment of explants with 0.1% HgCl₂ for 3 minutes resulted 100% contamination-free viable cultures. Final observation after 3-4 weeks showed that MS media supplemented with 0.44 µM mg/l of BA proved to be most capability in shoot induction. On this medium an average of 1.8 ± 0.42 shoots with mean shoot length 4.6 ± 1.34 cm were obtained (table 1, figure 1A and 2).

Shoot Multiplication

Shoot multiplication is depend on different concentration of plant growth regulators. Sometimes BA increasing is best for shoot or just opposite. Activated auxiliary shoots from the nodal explants and transfer to fresh medium containing BA alone and combination of BA with NA and IA to establish a stock of shoots used for in vitro multiplication. When we look Results in the present study showed the essential of plant growth regulators for in vitro multiplication, as the shoots cultured on basal medium did not multiply and become dead. BA at a concentration of 4.44 µM just gave an average of 21.7 ± 1.25 shoots with mean shoot length 6.8 ± 0.91 cm after 3-4 weeks of culture (table 2, figure 1B and 3).

Increasing the concentration of BA to 4.44 µM, a decrease in shoot multiplication rate was observed. However, comparative number, length and health of shoots on media with BA + IA/NA were not good. De Silva and Senarath (2009) was observed that MS medium supplemented 10.0 µM BAP and 14.0 µM IAA produced maximum multiple shoots. They induced Multiple shoots in the MS media with 5.0 µM BAP and 0.5 µM IAA. Arya et al. (2002) reported multiplication in a medium containing NAA and BA. Martin et al., (2006) achieved Maximum shoot induction (five shoots per explant) in MS medium supplemented with 1.5 mg l−1 BA and 0.1 mg l−1 NAA. Lal and Singh (2010) found the maximum number of shoots (8.9 ± 0.5) along with hundred per cent bud break in the MS medium supplemented with 1.0 mg/l BAP. Senapati et al (2013) observed that the Murashige and Skoog (MS) basal medium supplemented with 0.5 mg/L 6-benzylaminopurine (BAP) and 0.1 mg/L naphthalene acetic acid (NAA) showed maximum percentage of shoot multiplication (83.4%) with 8.2 shoots/explants.

In vitro Rooting

After 30 Days of growth, rooting growth is rarely increase day by day in best culture. The multiple-shoot clumps produced on this medium were transferred to solidified MS growth-regulator-free medium for shoot elongation and rooting. On opposite, shoots were also observed for rooting on full or half-strength MS medium with Activated Charcol ensuing excellent response for root induction. Maximum rooting was recorded in medium containing 100 mg/l Activated Charcol Supplemented with ½ MS medium (figure 1C). On this medium an average of 16.2 ± 0.78 roots with average root length 9.19 ± 0.68 cm was observed after 3-4 weeks (table 3, figure 4).
**Table.1** Effect of plant growth regulators on in vitro auxiliary shoot induction in *Celastrus paniculatus*

| SL | MS + PGR (µM) | Observations after 25 days |
|----|----------------|---------------------------|
|    | BAP | IAA | NAA | Mean shoot number | Mean shoot length (cm) |
| 1. | 0   | 0   | 0   | 0.2 ± 0.42        | 0.7 ±1.49               |
| 2. | 0.44 µM | 0   | 0   | 1.8 ± 0.42        | 4.6 ± 1.34              |
| 3. | 0.88 µM | 0   | 0   | 0.8 ± 0.78        | 2 ± 1.76                |
| 4. | 1.33 µM | 0   | 0   | 0.7 ± 0.70        | 1.8 ±1.68               |
| 5. | 1.77 µM | 0   | 0   | 0.5 ± 0.70        | 1.3 ±1.76               |
| 6. | 2.22 µM | 0   | 0   | 0.4 ± 0.69        | 1.1 ±1.79               |
| 7. | 0.44 µM | 0.57 µM | 0   | 0.4 ± 0.51        | 1.5 ±1.95               |
| 8. | 0.88 µM | 1.14 µM | 0   | 0.7 ± 0.82        | 1.4 ±1.50               |
| 9. | 1.33 µM | 1.71 µM | 0   | 0.8 ± 0.91        | 1.3 ±1.41               |
| 10. | 0.44 µM | 0   | 0.54 µM | 1 ± 0.94        | 2.1 ± 1.91              |
| 11. | 0.88 µM | 0   | 1.07 µM | 0.8 ±0.91         | 1.7 ±1.88               |
| 12. | 1.33 µM | 0   | 1.61 µM | 0.9 ±0.87        | 1.2 ± 1.03              |

**Note:** Each treatment consisted of 10 replications. Data (Mean ±SE) were recorded after 20 days of culture.

**Table.2** Effect of plant growth regulators on in vitro axillary shoot multiplication in *Celastrus paniculatus*

| SL | MS + PGR (µM) | Observations after 25 days |
|----|----------------|---------------------------|
|    | BAP | IAA | NAA | Mean shoot number | Mean shoot length (cm) |
| 1. | 0   | 0   | 0   | 3.7 ± 0.67        | 3.6±0.69               |
| 2. | 2.22 µM | 0   | 0   | 17.6 ± 1.50       | 4.7 ± 0.82              |
| 3. | 4.44 µM | 0   | 0   | 21.7 ± 1.25       | 6.8 ± 0.91              |
| 4. | 6.66 µM | 0   | 0   | 19.1 ± 1.66       | 4.7±0.94                |
| 5. | 8.88 µM | 0   | 0   | 15.3 ± 1.56       | 3.7 ±0.63               |
| 6. | 11.1 µM | 0   | 0   | 14.2 ± 1.39       | 3.4±0.51                |
| 7. | 2.22 µM | 0.57 µM | 0   | 11.9 ± 1.28       | 4.1±0.73                |
| 8. | 4.44 µM | 1.14 µM | 0   | 11.1 ± 1.19       | 3.9±0.56                |
| 9. | 6.66 µM | 1.71 µM | 0   | 10.5 ± 1.26       | 3.3±0.67                |
| 10. | 2.22 µM | 0   | 0.54 µM | 12.3 ± 1.33       | 4.6±0.84                |
| 11. | 4.44 µM | 0   | 1.07 µM | 11.2 ± 0.91       | 4.4±0.84                |
| 12. | 6.66 µM | 0   | 1.61 µM | 10.7 ± 0.94       | 3.6±0.699               |

**Note:** Each Treatment consisted of 10 replications. Data (MEAN±SE) were recorded after 20 days of culture.
Table 3 Rooting response of in vitro regeneration excised shoots (10 repeats).

| SL | Medium | Activated charcoal (mg/l) | No. of roots/Plantlets (Mean ±SE) |
|----|--------|----------------------------|---------------------------------|
| 1. | --     | --                         | 4.2 ± 0.42                      |
| 2. | MS     | --                         | 6.3 ± 0.94                      |
| 3. | 1/2MS  | --                         | 6.7 ± 0.67                      |
| 4. | 1/2MS  | 25                         | 7.7 ± 0.82                      |
| 5. | 1/2MS  | 50                         | 9.1 ± 0.87                      |
| 6. | 1/2MS  | 100                        | 16.2 ± 0.78                     |
| 7. | 1/2MS  | 125                        | 12.7 ± 0.82                     |
| 8. | 1/2MS  | 150                        | 9.2 ± 0.0.78                    |
| 9. | 1/2MS  | 200                        | 7.1 ± 0.87                      |

Note: Each treatment consisted of 10 replications. Data (Mean ±SE) were recorded after 20 days of culture.

Fig.1 Micropropagation of *Celastrus paniculatus.*
A. Initiation  B. Multiplication  C. Rooting
Fig. 2 Effect of plant growth regulators on in vitro auxiliary shoot induction in *Celastrus paniculatus*

Fig. 3 Effect of plant growth regulators on in vitro axillary shoot multiplication in *Celastrus paniculatus*
De Silva and Senarath (2009) found good rooting on elongated shoots and the highest rooting percentage (73.3%) in the MS medium supplemented with 5.6 μM IAA and 9.6 μM Indole-3-butyric acid (IBA). Martin et al., (2006) reported that the shoots that were pulse-treated in a solution of 100 mg l−1 each of IBA and NOA for 2 h and then for 3 min in 10 mg l−1 chlorogenic acid gave 90% rooting Two to three roots of 2–4 cm in length were produced within a period of 5 weeks. Lal and Singh (2010) proved that the MS half strength medium supplemented with 0.5 mg/L NAA best with hundred per cent rooting. Senapati et al., (2013) was achieved Maximum rooting of 73.3% with 4.8 roots/shoot on half-strength MS media supplemented with 0.5 mg/L indole-3-acetic acid (IAA) and the percentage of survival was 91% after acclimatization.

Various studies showed that the positive effect of NAA at lower concentrations during rooting in various medicinal plants like Verbascum thapsus (Turker et al., 2001) and Santolina canescens (Casado et al., 2002). Peeters et al. (1991) reported that NAA was taken up six times faster than IAA and Van der Krieken et al., (1993) confirmed that IBA was taken up four times faster than IAA.

In conclusion, C. Paniculatus has always been a topic of interest of researchers. From tissue culture point of view several studies have been performed to propagate the plant in vitro. The highlights of the study are Use of low concentration of plant growth regulators and minimization of time required for field transfer of tissue culture raised plantlets. Free plants produced open the scope for utilization of plant material for antimicrobial testing and suitable pharmaceutical preparations. Apart from this in vitro propagation of C. paniculatus showed a highest rate of multiplication which cannot be seen in naturally found species of C. paniculatus. The Malkangani research will give a new insight of research...
in medicinal components of plants through various advance techniques.

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