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# Influence of growth hormones on adventitious root formation in semi-hardwood cuttings of *Celasturs paniculatus* Willd.: a contribution for rapid multiplication and conservation management

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**Abstract** *Celastrus paniculatus* Willd. (Celastraceae) is an important medicinal tree valued for its seeds. Indiscriminate collection of this plant from the habitat zones has posed a serious threat to its existence in the wild, results in globally a threatened plant species. Ex situ conservation is a prioritized agenda of research for redlisted plant species. Semi-hardwood stem cuttings collected from 10 to 15 year old trees were treated with different concentrations (0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g $l^{-1}$) of growth hormones; indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA) for positive effect on root induction. After 90 days of treatment, results were analyzed for percentage of rooting, root number and average root length per rooted cuttings. The highest rooting (57%) response with highest number of roots ($\sim$77.2) was obtained in indole-3-acetic acid at 3.0 g $l^{-1}$. Overall, high significant ($P \leq 0.05$) rooting response was noticed in IAA treatment comparative to hormones IBA and NAA. Rooted cuttings exhibited 100% survival in the experimental field. This study reveals the propagation of semi-hardwood cuttings derived from partially matured branches is possible and it provides a basis for conservation of this threatened plant species by reducing pressure on its natural populations.

**Keywords** Nearly threatened · Propagation · Semi-hardwood cuttings · Indole-3-acetic acid · Adventitious rhizogenesis

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## Introduction

*Celastrus paniculatus* Willd. (Celastraceae) is the most important medicinal tree found in dry deciduous to semi evergreen forests of India, South East Asia and European countries up to an altitude of 1,000–1,800 m. The bark, leaves, seeds and seed oil possess a number of medicinal properties and have been used in traditional medicine including high stimulancy in increasing intelligence and sharpen memory (Parrotta 2001; Martin et al. 2006). This has led to high commercial exploitation and extensive damage in the natural stands especially when the plants are harvested well before seed set. In the last decade, wild populations of *C. paniculatus* are severely depleted to meet the current demand for local usage and export trade. As a result, it is categorized under "Nearly threatened" (Balaguru et al. 2006).

In view of the increasing demand for *Celastrus*, there is a need to develop new approaches for efficient propagation. Moreover, propagation by seed is difficult in wild. To date, many in vitro protocols have been reported to conserve the species from extinction (Nair and Seeni 2001; Martin et al. 2006; Raju and Prasad 2007). However, information on the vegetative propagation of *C. paniculatus* is currently unavailable. Hence, the present study was undertaken to examine the feasibility of rapid vegetative multiplication using different hormones, affecting the rooting ability of *C. paniculatus*. This is the first report on adventitious rhizogenesis of *C. paniculatus* having potential for rapid vegetative multiplication and conservation.

## Materials and methods

Semi-hardwood cuttings of *Celastrus paniculatus* were collected from 10 to 15 year old trees of an average height of 6–8 m, from University of Hyderabad campus (Hyderabad, India) in the month of July. The cuttings were trimmed to 15 cm long terminal shoots with 3–4 nodes. Leaves were removed from the lower half of the cuttings, and were immersed in a solution of fungicide 0.5% Bavistin [Methyl *N*-(1H-benzoimidazol-2-yl) carbamate] for 15 min and thoroughly rinsed with distilled water. Finally the basal ends (2–3 cm) of each cutting were soaked in different concentrations (Control, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g $l^{-1}$) of hormones; indole 3-acetic acid (IAA), indole 3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA) for 12–14 h. The cuttings were then planted vertically in polythene bags containing soil, sand and farmyard manure (1:1:1). Rooting experiments were conducted in a green house with 80% relative humidity and 23°C temperature under diffused light. The experiment was arranged in a randomized block design with three replications (five cuttings) each repeating thrice. After a period of 90 days the cuttings were evaluated for various characteristics of adventitious rhizogenesis, viz., rooting percentage, number of roots and average root length per rooted cutting. To evaluate the significance of this experiment, the data was subjected to Analysis of Variance (ANOVA) and Duncun's multiple range test (DMRT) at a 5% probability level using MSTAT-C computer programme.

## Results and discussion

After 90 days of exogenous hormone treatment, semi-hardwood cuttings of *Celastrus paniculatus* were showed significant ($P \leq 0.05$) effect on adventitious rooting. The adventitious rhizogenous response was rapid in the cuttings treated with hormones than in control. However, the response was varied with different hormones and concentrations used. The rooting percentage, number of roots and length of root per cutting were significant in IAA treatments than either IBA or NAA (Table 1). The rooting percentage differed significantly from 23 to 57%. The greatest rooting percentage (57%) was noticed in 3.0 g $l^{-1}$ IAA and the lowest (23%) was in

**Table 1** The effect of different hormone treatments on rooting of *Celastrus paniculatus*

| Hormone treatments | % of cuttings rooted | Avg. no of roots per cutting | Avg. length of roots (cm) |
|---|---|---|---|
| Control | 24.84$^{hi}$* | 20.1$^{l}$ | 2.1$^{j}$ |
| IAA (g $l^{-1}$) | | | |
| 0.5 | 33.86$^{cde}$ | 32.7$^{h}$ | 3.6$^{gh}$ |
| 1 | 37.90$^{c}$ | 50.9$^{de}$ | 4.9$^{cde}$ |
| 2 | 49.48$^{b}$ | 65.7$^{b}$ | 5.9$^{b}$ |
| 3 | 57.38$^{a}$ | 77.2$^{a}$ | 8.0$^{a}$ |
| 4 | 48.18$^{b}$ | 65.0$^{b}$ | 6.0$^{b}$ |
| 5 | 33.86$^{cde}$ | 54.7$^{c}$ | 5.0$^{cd}$ |
| IBA (g $l^{-1}$) | | | |
| 0.5 | 26.56$^{ghi}$ | 24.0$^{k}$ | 2.7$^{i}$ |
| 1 | 31.11$^{ef}$ | 45.5$^{f}$ | 5.0$^{cd}$ |
| 2 | 36.57$^{cd}$ | 62.9$^{b}$ | 5.9$^{b}$ |
| 3 | 48.18$^{b}$ | 55.0$^{c}$ | 5.4$^{bc}$ |
| 4 | 32.49$^{def}$ | 49.5$^{de}$ | 4.7$^{de}$ |
| 5 | 29.59$^{efg}$ | 38.9$^{g}$ | 3.9$^{fg}$ |
| NAA (g $l^{-1}$) | | | |
| 0.5 | 28.08$^{fgh}$ | 28.8$^{ij}$ | 3.0$^{i}$ |
| 1 | 36.57$^{cd}$ | 47.4$^{ef}$ | 4.4$^{ef}$ |
| 2 | 29.59$^{efg}$ | 51.7$^{cd}$ | 5.0$^{cd}$ |
| 3 | 28.08$^{fgh}$ | 41.6$^{g}$ | 4.3$^{efg}$ |
| 4 | 24.84$^{hi}$ | 32.1$^{hi}$ | 3.9$^{fg}$ |
| 5 | 23.11$^{i}$ | 26.0$^{jk}$ | 3.1$^{hi}$ |

Data were recorded after 90 days of planting. Each mean is based on three replicates, each of which consisted of 15 cuttings

* Means followed by the same letters are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test

NAA at 5.0 g $l^{-1}$. This indicates that the hormone IAA was most effective in promoting rooting in semi-hardwood cuttings of this species. Regarding the number of roots per cutting, different hormones had different effects. The number of adventitious roots increased from 20.1 to 77.2 (Table 1). Best results were achieved in 3.0 g $l^{-1}$ IAA, after a period of 90 days treatment. The adventitious root production increased rapidly at lower concentrations of IAA and began to level off between 2 and 4 g $l^{-1}$. However, with increase in hormone concentration (>3.0 g $l^{-1}$) levels, the number of roots was decreased. Similar rooting response was observed with IBA and NAA hormonal treatments. The mean rate of root length was changed significantly with various concentrations of IAA, IBA and NAA as shown in Table 1. The highest root length (8.0 cm) densities were observed in the cuttings treated with IAA (3.0 g $l^{-1}$) and the least response was noticed with IBA and NAA treatments. This showed that exogenously applied hormones play a significant role in adventitious rhizogenesis. Overall, IAA treatment significantly affected the rooting percentage, root number and mean root length per rooted cutting in *Celastrus paniculatus*. Therefore, the best concentration for rapid rhizogenesis is 3.0 g $l^{-1}$ of IAA. After 90 days of hormonal treatment, the rooted stem cuttings were successfully transplanted to experimental field and 100% survival rate was noticed.

Among the various methods of asexual reproduction, vegetative propagation inducing rhizogenesis on stem cuttings is the most feasible method for tree improvement and establishment of clonal plantations (Sharma et al. 2006). The data presented in our study suggests that for successful formation of adventitious roots, factors like origin of cutting, type of hormone, age of the mother plant and the season of collection play a significant role (Kibbler et al. 2004). Softwood cuttings are too juvenile and hardwood cuttings reduce the ability of rhizogenesis for the above experiment, hence semi-hardwood cuttings were selected due to the material abundance and successful propagation.

Considering the above results, rooting was significantly ($P \leq 0.05$) greater in the cuttings treated with 3.0 g $l^{-1}$ of IAA. This positive response in rooting of stem cuttings with the application of hormones was also reported by Saranga and Cameron (2007). The mean number of roots as well as root length was also high in 3.0 g $l^{-1}$ of IAA. Thus, IAA greatly influenced the rhizogenesis in semi-hardwood cuttings of *C. paniculatus* than IBA and NAA. When treated with concentrations >3 g $l^{-1}$, percentage of rooted cuttings, number of roots and root lengths began to decrease rapidly in all hormone treated cuttings, especially in IAA and IBA. This shows that the rooting percentage changed significantly depending on the type and concentration of hormone used. It is also important to note that, the dosage of hormone is a significant factor in the induction of rooting as found in the study. Similar observations have also been reported for stem cuttings of medicinal plant species *Podophyllum hexandrum* (Nadeem et al. 2000) and *Pausinystalia johimbe* (Tchoundjeu et al. 2004). However, the response to hormones varies with species and the percentage of response varies with hormone treatments. The successful application of IAA on rooting response was also reported by Agnihotri and Ansari (2000) and Danthu et al. (2008). Davies and Hartmann (1988) stated that though auxins promote rooting, adventitious root formation is a synchronized developmental process involving various biochemical, physiological and histological events in the induction, initiation, development and elongation of root primordial.

Our results showed that vegetative propagation of *Celastrus paniculatus* by stem cuttings is possible, but the response and level of rooting varied accordingly with respect to cuttings and hormone treatment. This has also been suggested by Leakey (2004) where genetic potential, propagation environment, cutting origin, stock plant physiology and management practices might influence on rooting. Semi-hardwood cuttings treated with 3 g $l^{-1}$ IAA have given the highest rooting response. Hence, the current experiment could be an alternative resource for low-cost large-scale propagation of *Celastrus paniculatus*, which involves significant time saving to get superior phenotypes in conserving this rare species.

## Conclusion

In conclusion, *Celastrus paniculatus* needs special attention for its conservation in the very sense of its existence and genetic diversity. Though it is possible to conserve the species by in situ conservation in protected forest areas, conservation by germplasm collection and ex situ measures often increases the efficiency of survival and multiplication. The above results highlighted that adventitious rhizogenesis in semi-hardwood cuttings of *C. paniculatus* in response to exogenous application of growth hormones provides a new tool that improves vegetative propagation. The standardized protocol with 3 g $l^{-1}$ IAA described here can be successfully employed for large-scale multiplication in plant propagation industry and ensure good prospects for pharmacological or phytomedicinal screening and applications.

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