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# Rooting ability of *Rubia cordifolia* L. – a potential therapeutic plant species in the western ghats, Nilgiri biosphere reserve, India

**P. Senthilkumar, S. Paulsamy* and K.K. Vijayakumar**

PG and Research Department of Botany, Kongunadu Arts and Science College, Coimbatore- 641 029, Tamil Nadu, India.

## Abstract

A preliminary evaluation of the rooting ability of stem cuttings of the medicinal plant, *R. cordifolia* treated with various concentrations and combinations of auxins (IBA and NAA) revealed that stem cuttings treated with IBA and NAA at 2000 ppm each exhibited higher per cent (73) of adventitious root formation with the high number of roots per cutting (24.82/ cutting). Other rooting attributes like root length, fresh and dry weights of adventitious roots were more in the stem cuttings treated with IBA and NAA at 3000 ppm and 2000 ppm, respectively.

**Keywords :** medicinal plant, rooting ability, *Rubia cordifolia*, stem cutting, vegetative propagation

## INTRODUCTION

Many species of higher plants in natural habitats which failed to produce viable seeds are maintaining their population and genetic stock by adapting the vegetative propagation only. The goal of vegetative propagation is to reproduce progeny plants identical in genotype to a single source of plant. In the wild plants of economic importance, to meet the demand, in addition to multiplication through seeds, vegetative reproduction with particular reference to stem cutting according to species is most essential (Hartmann *et al*., 2004). From botanical point of view, for better distribution and survival, the propagation strategies adopted are important. However, the response of stem cutting with respect to rhizogenesis to the existing environmental conditions is varied according to species (Krishankumar *et al*., 2008). Earlier many researchers succeeded in the attempt to produce individuals from the stem cuttings after proper treatment with hormones (deAndres *et al*., 1999; Hartmann *et al*., 2004; Husen and Pal, 2007).

The shrub *Rubia cordifolia*, which is having many economic uses, is distributed in the shola forests at high altitudes of Nilgiris, South India. The root of the plant is commonly known as "*Manjistha*" and sold in the market under the commercial name "*Manjith*". The roots are sweet, bitter, acrid and used as anti-inflammatory, antidysentric, antipyretic, and to cure leucoderma, ulcers, urinary discharges, jaundice and piles (Sivarajan and Balachandran, 1994). The ruberytheric acid is one of the major constituents of the root and is widely used as phytotherapeutic in the treatment of calcium containing stones in the urinary tract (Laszlo *et al*., 1992). Earlier studies revealed that this species is present with weaker ecological perpetuation in its shola habitats owing to poor reproductive capacity (Paulsamy *et al*., 2006). The intensive exploitation by local public for its economic importance is also attributed for its less population size in the communities of shola understories (Paulsamy, 2005). Hence, to increase the population size and better conservation, the present study was carried out to know the rooting response of the cuttings of this species to various combinations and concentrations of growth hormones.

## MATERIALS AND METHODS

The species, *Rubia cordifolia* is having moderate size of woody stem. Stem cuttings were collected from their habitats of their occurrence in the shola forests of Nilgiris. Shoots were soaked in water immediately after cutting and brought to the nursery by wrapping with banana leaves. Semi-hardwood shoot cuttings of 25-30 cm length and 1.5-2.0 cm diameter of this species were prepared after removing leaves and terminal buds. The cuttings were surface sterilized with 0.1% mercuric chloride solution for 5 minutes followed by rinsing in water and bundled with fifty cuttings. The basal cut ends (up to 2.0 cm) of the cuttings were provided dipping treatment for 10 seconds with the growth regulators IBA and NAA in different combinations comprising 25 treatments (Table 1). Ten grams of talcum powder was used in all combinations to serve as binder for the growth hormones. The top cut ends of treated cuttings were sealed with inert paraffin wax to avoid desiccation through surface loss of water. These cuttings were then planted in polythene cups (15x23 cm) filled with formaldehyde fumigated coarse river sand and arranged in mist chamber. Each treatment had four replicates of 50 cuttings. The planted cuttings were irrigated and weeded as and when required, until the termination of the experiment.

Data were recorded for rooting (%) and number, length, fresh weight and dry weight of the adventitious roots at

*Corresponding Author
email: *paulsami@yahoo.com*

**Table 1.** Scheme of the treatments for the stem cuttings of *Rubia cordifolia* in the present study

| IBA concentration (ppm) | NAA concentration (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 ($N_o$) | 1000 ($N_1$) | 2000 ($N_2$) | 3000 ($N_3$) | 4000 ($N_4$) |
| 0($I_0$) | $I_0 N_0$ | $I_0 N_1$ | $I_0 N_2$ | $I_0 N_3$ | $I_0 N_4$ |
| 1000 ($I_1$) | $I_1 N_0$ | $I_1 N_1$ | $I_1 N_2$ | $I_1 N_3$ | $I_1 N_4$ |
| 2000 ($I_2$) | $I_2 N_0$ | $I_2 N_1$ | $I_2 N_2$ | $I_2 N_3$ | $I_2 N_4$ |
| 3000 ($I_3$) | $I_3 N_0$ | $I_3 N_1$ | $I_3 N_2$ | $I_3 N_3$ | $I_3 N_4$ |
| 4000 ($I_4$) | $I_4 N_0$ | $I_4 N_1$ | $I_4 N_2$ | $I_4 N_3$ | $I_4 N_4$ |

the termination of the experiment in the month of January, 2007. For recording fresh and dry weights, 5 rooted cuttings per replicate were randomly selected from each treatment.

## RESULTS AND DISCUSSION

It was noted that root initiation started in the auxin treated cuttings, 46 days after treatment. However, in control it took 53 days for root initiation. It indicates that for early rhizogenesis the growth regulator, auxin played important functional role (Haissing and Davis, 1994; Singh and Chander, 2001). The rooting behaviours like rooting per cent, root number, root length and fresh and dry weights of adventitious roots of the study species, *R. cordifolia* are influenced by the growth regulators, IBA and NAA widely varied according to concentrations and combinations of growth hormones (Table 2). The composite auxin treatments consisting of different combinations of IBA and NAA significantly enhanced the induction and growth of adventitious roots. Blakesley *et al*. (1991) already reported that exogenous application of auxins like IBA and NAA stimulate adventitious rhizogenesis in shoot cuttings of many species. The shoot cuttings treated with IBA and NAA at the concentration of 2000 ppm each ($I_2N_2$) exhibited significantly higher adventitious rhizogenesis which was 13 times greater in comparison to that of control without hormonal treatment. The root number initiated was also higher (24.82/cutting) in the same combination of IBA and NAA at the same concentration. Many earlier reports also suggested that IBA and NAA combination in appropriate concentrations increased the rooting (Nautiyal *et al*., 1992; Thirunavoukkarasu and Brahmam, 1998; deAndres *et al*., 1999; Husen and Pal, 2007; Paulsamy *et al*., 2008). In addition, auxins are more essential for the induction of adventitious roots and the quantity required has been reported to be species specific (Ansari *et al*., 2002).

However, further growth of the roots of the study species, *Rubia cordifolia* was very effective in higher concentration of IBA at 3000ppm and NAA at 2000ppm. It is apparent that during the intriguing and complex process of adventitious root formation various auxins perform specific function at different levels (Singh *et al*., 2005). The fresh and dry weights of adventitious roots varied significantly with the influence of various combinations and concentrations of growth hormones, IBA and NAA and were higher 3.65mg and 0.97mg respectively, in the treatment, $I_3N_2$ (IBA – 3000 ppm; NAA – 2000 ppm) (Table 1). It shows that nitrogen source is essential for the continued growth of the roots which are supplied by these two auxins in a sustained manner. Similar results of the increase of root biomass after the treatment of stem cuttings with various auxins have already been documented (Lewu *et al*., 2006; Ehiabonare, 2007; Husen and Pal, 2007; Paulsamy *et al*., 2008).

Propagation of medicinal plants through vegetative methods may be helpful to overcome the constrains in seed germination and seedling establishment. The protocol standardized from the present study is easy and reproducible. It is suggested that the clones of this species produced by this protocol can be introduced into shola habitats of Nilgiris after identifying the suitable micro-sites for its increase in population. It could serve as a viable option for the conservation and bioprospecting of this valuable genetic stock.

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**Table 2.** Effect of the growth regulators IBA and NAA interaction on characteristics of adventitious rooting in the species, *Rubia cordifolia*.

| S. No. | Treatment | Rooting (%) | Root number | Root length (cm) | Fresh weight (mg) | Dry weight (mg) |
|---|---|---|---|---|---|---|
| 1 | $I_0N_0$ | $6.42^{a} \pm 0.02$ | $3.66^{a} \pm 0.01$ | $6.55^{c} \pm 0.02$ | $1.95^{c} \pm 0.01$ | $0.23^{a} \pm 0.02$ |
| 2 | $I_0N_1$ | $8.89^{c} \pm 0.02$ | $6.89^{c} \pm 0.02$ | $8.12^{no} \pm 0.02$ | $2.66^{jk} \pm 0.02$ | $0.96^{m} \pm 0.02$ |
| 3 | $I_0N_2$ | $12.75^{d} \pm 0.04$ | $9.66^{g} \pm 0.04$ | $7.67^{l} \pm 0.02$ | $2.85^{m} \pm 0.03$ | $0.47^{c} \pm 0.02$ |
| 4 | $I_0N_3$ | $7.81^{b} \pm 0.02$ | $5.36^{b} \pm 0.02$ | $7.12^{h} \pm 0.02$ | $2.27^{f} \pm 0.05$ | $0.60^{d} \pm 0.01$ |
| 5 | $I_0N_4$ | $28.62^{j} \pm 0.02$ | $16.74^{p} \pm 0.03$ | $8.26^{p} \pm 0.04$ | $2.36^{g} \pm 0.01$ | $0.83^{jk} \pm 0.02$ |
| 6 | $I_1N_0$ | $8.80^{c} \pm 0.02$ | $7.33^{d} \pm 0.02$ | $6.86^{e} \pm 0.03$ | $2.53^{i} \pm 0.02$ | $0.67^{ef} \pm 0.02$ |
| 7 | $I_1N_1$ | $24.21^{h} \pm 0.02$ | $11.67^{k} \pm 0.02$ | $7.59^{j} \pm 0.01$ | $3.43^{p} \pm 0.02$ | $0.76^{ghi} \pm 0.03$ |
| 8 | $I_1N_2$ | $29.42^{k} \pm 0.01$ | $18.50^{q} \pm 0.03$ | $7.81^{m} \pm 0.02$ | $2.68^{k} \pm 0.02$ | $0.95^{m} \pm 0.01$ |
| 9 | $I_1N_3$ | $34.20^{m} \pm 0.01$ | $19.33^{s} \pm 0.02$ | $8.37^{r} \pm 0.02$ | $2.46^{h} \pm 0.03$ | $0.67^{ef} \pm 0.01$ |
| 10 | $I_1N_4$ | $16.55^{f} \pm 0.01$ | $10.74^{j} \pm 0.03$ | $8.10^{n} \pm 0.01$ | $2.65^{jk} \pm 0.04$ | $0.75^{ghi} \pm 0.01$ |
| 11 | $I_2N_0$ | $51.41^{p} \pm 0.02$ | $19.77^{t} \pm 0.01$ | $6.82^{d} \pm 0.02$ | $3.13^{n} \pm 0.02$ | $0.78^{hij} \pm 0.01$ |
| 12 | $I_2N_1$ | $24.22^{h} \pm 0.02$ | $11.66^{k} \pm 0.01$ | $7.57^{j} \pm 0.03$ | $3.41^{p} \pm 0.01$ | $0.96^{m} \pm 0.01$ |
| 13 | $I_2N_2$ | $73.27^{q} \pm 0.01$ | $24.82^{u} \pm 0.01$ | $8.33^{q} \pm 0.03$ | $3.48^{q} \pm 0.01$ | $0.77^{g\text{-}j} \pm 0.01$ |
| 14 | $I_2N_3$ | $17.80^{g} \pm 0.02$ | $12.51^{l} \pm 0.02$ | $7.05^{g} \pm 0.02$ | $2.75^{l} \pm 0.04$ | $0.80^{ij} \pm 0.01$ |
| 15 | $I_2N_4$ | $36.89^{n} \pm 0.02$ | $18.72^{r} \pm 0.02$ | $8.14^{o} \pm 0.03$ | $3.23^{o} \pm 0.02$ | $0.73^{gh} \pm 0.03$ |
| 16 | $I_3N_0$ | $31.40^{l} \pm 1.21$ | $13.43^{n} \pm 0.02$ | $7.57^{j} \pm 0.02$ | $3.13^{n} \pm 0.01$ | $0.87^{kl} \pm 0.01$ |
| 17 | $I_3N_1$ | $17.88^{g} \pm 1.38$ | $9.37^{f} \pm 0.02$ | $6.23^{a} \pm 0.02$ | $2.16^{e} \pm 0.03$ | $0.90^{l} \pm 0.01$ |
| 18 | $I_3N_2$ | $47.69^{o} \pm 0.03$ | $18.75^{r} \pm 0.03$ | $9.15^{s} \pm 0.02$ | $3.65^{r} \pm 0.02$ | $0.97^{m} \pm 0.05$ |
| 19 | $I_3N_3$ | $14.19^{e} \pm 0.03$ | $9.40^{f} \pm 0.03$ | $7.48^{i} \pm 0.01$ | $2.62^{j} \pm 0.03$ | $0.45^{c} \pm 0.01$ |
| 20 | $I_3N_4$ | $37.43^{n} \pm 0.02$ | $12.57^{l} \pm 0.02$ | $8.12^{no} \pm 0.01$ | $3.15^{n} \pm 0.02$ | $0.63^{de} \pm 0.02$ |
| 21 | $I_4N_0$ | $16.50^{f} \pm 0.60$ | $8.47^{e} \pm 0.02$ | $6.98^{f} \pm 0.01$ | $1.69^{a} \pm 0.02$ | $0.50^{c} \pm 0.02$ |
| 22 | $I_4N_1$ | $27.41^{i} \pm 0.02$ | $14.63^{o} \pm 0.02$ | $8.14^{o} \pm 0.03$ | $2.68^{k} \pm 0.02$ | $0.36^{b} \pm 0.02$ |
| 23 | $I_4N_2$ | $16.70^{f} \pm 0.01$ | $10.56^{i} \pm 0.02$ | $7.62^{k} \pm 0.02$ | $1.86^{b} \pm 0.02$ | $0.38^{b} \pm 0.01$ |
| 24 | $I_4N_3$ | $24.83^{h} \pm 0.03$ | $13.23^{m} \pm 0.02$ | $8.12^{no} \pm 0.02$ | $2.67^{k} \pm 0.02$ | $0.72^{fg} \pm 0.01$ |
| 25 | $I_4N_4$ | $17.63^{g} \pm 0.02$ | $10.44^{h} \pm 0.03$ | $6.46^{b} \pm 0.02$ | $2.11^{d} \pm 0.02$ | $0.45^{c} \pm 0.01$ |

Means followed by common letter (s) in columns are not significantly different at 5% level by DMRT.

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