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# DEVELOPMENT OF AN EFFECTIVE VEGETATIVE PROPAGATION PROTOCOL FOR *Catharanthus roseus*

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## ABSTRACT

*Catharanthus roseus, valued for both ornamental value and medicinal properties, shows substantial genetic variation when growing propagated from seeds. This leads to wide differences in alkaloid content and agronomic traits among plants. Vegetative propagation is important for transferring genetic characteristics to the next generation without inducing variability; therefore, it plays a key role in the plant breeding program of C. roseus. The aim of the present is to develop an effective vegetative propagation protocol for C. roseus. The experiment was conducted using a two-factor factorial completely randomized design incorporating three types of stem cuttings of C. roseus native plants (softwood, semi-hardwood, and hardwood) and five growing media (sand, coir dust, 1:1 volume basis mixture of sand:coir dust, sand:compost, and sand:topsoil). Prior to planting, stem cuttings were treated with Captan 50% w/w fungicide and 0.3% Indole Butyric Acid powder. All cuttings were maintained in a mist-propagator environment under 50% shade condition. Data were recorded in the fifth week after the propagator opened on survival rate, number and length of newly produced shoots, number of roots, and fresh root weight. The results indicated that there were an interaction effect of growing media and the cutting types on root number and the root fresh weight. Accordingly, tip cuttings of C. roseus grown in sand alone medium demonstrated superior rooting performance, producing the highest mean number of roots and the greatest fresh root biomass during the experimental period. In contrast, semi-hardwood cuttings exhibited significantly the highest numbers of newly produced shoots per cutting. Tip cuttings had a 100% survival rate, demonstrating significantly higher success. This study demonstrated the high potential of stem cutting propagation in C. roseus, enabling effective plant multiplication within a relatively short period*.

***Keywords: Catharanthus roseus, Vegetative propagation, Stem cuttings, Growing media, Propagator***

## INTRODUCTION

*Catharanthus roseus* (L.) G. Don (2n=16), commonly known as Madagascar periwinkle is one of the most beautiful flower species in the family Apocynaceae which has gained global recognition due to its high adaptability and exceptional pharmaceutical value. The genus Catharanthus includes seven species besides *C. roseus,* namely *C. coriaceus, C. lanceus, C. longifolius, C. ovalis, C. pusillus, C. scitulus* and *C.trichophyllus,* of which *C. pusillus* is endemic to India whereas others are endemic to Madagascar (Gupta et al., 2008). Besides its aesthetic charm, marked by beautiful five-lobed flowers in shades from white to dark pink, *C. roseus* is well recognized for its abundance of alkaloids, featuring over 125 indole and dihydroindole alkaloids (Mujib et al., 2022). Although, seed propagation of *C. roseus* is commonly favored due to its ease and high germination rates when conditions are optimal, its commercial cultivation is hindered by the genetic variability of such seed-propagated plants, leading, for example to inconsistent alkaloid yields (Rai et al., 2022), and heterogeneity in important agronomic and biochemical traits, such as flower color and plant vigour (Jaleel et al., 2009). This variability can limit the pharmaceutical industry's ability to obtain the uniformity and consistency in secondary metabolite content that is necessary.

As a result, vegetative propagation has become a reliable alternative for preserving true-to-type traits and ensuring genetic consistency. Stem cuttings, especially those from softwood (Sadowska et al.,

1983) and semi-hardwood are commonly employed to create uniform planting material, with success rates significantly affected by factors such as hormonal applications (Pandey, 2017), the physiological maturity of the cutting, environmental conditions, and the physical and chemical characteristics of the growing medium. Previous research has shown that auxin-based rooting enhancers and well-aerated substrates notably enhance rooting efficiency, shoot growth, and the overall survival rate of plants that are propagated vegetatively (Rout and Sahoo, 2015). Studies on related species within the Apocynaceae family further emphasize the necessity of choosing suitable rooting media and hormone treatments to optimize propagation results (Kentelky et al., 2023).

In this context, creating an effective vegetative propagation protocol for *Catharanthus roseus* is crucial for both commercial farming and the production of medicinal compounds. Consequently, this study seeks to assess the impact of various stem cutting types and growing media on the successful propagation of *Catharanthus roseus*. By determining the most effective combinations for root and shoot growth, the research aims to establish optimized and consistent protocols that benefit horticultural management and the sustainable production of this important medicinal plant.

## MATERIALS AND METHOD

### *Preparation of growing media:*

Five types of growing media were evaluated to identify the most suitable medium for propagating each cutting type. The media included sand only (M1), coir dust only (M2), sand and coir dust at a 1:1 ratio (M3), sand and compost at a 1:1 ratio (M4), and sand and topsoil at a 1:1 ratio (M5). Sand, compost and topsoil were sieved using a 0.6 cm mesh. Coir dust was washed thoroughly before use. Sand and coir dust, sand and compost, sand and topsoil were mixed in a 1:1 ratio (v/v) to prepare medium three, four and five (M3, M4 and M5) respectively.

### *Preparation of cuttings*

Planting materials were collected from the mother plant stock maintained at UCIARS premises and coastal areas. According to the maturity stage of the stem, three different types of stem cuttings were tested. They were,

1. Softwood cutting (Tip cutting) – C1
2. Semi hardwood cutting – C2
3. Hardwood cutting - C3

Tip cutting | Semi hardwood (C2) | Hardwood (C3)

Plate 1. Effect of different cutting types on cutting survivability of *Catharanthus roseus*

All three types of cutting were tested such as 20cm soft wood cutting with a tip and 20cm semi hardwood and hardwood cuttings without leaves.

Cuttings were separated using sharp secateurs and they were prepared as 15cm length. Sharp cuts were made closer to a node at a 45° angle (Plate 1). Prepared cuttings were put into water and established on polybags after applying commercially available rooting hormone; 0.3% IBA to the lower end. All the planted pots were treated with Captan 50% (w/w) fungicide. The mist propagators were established and a Complete Randomized Design (CRD) with a two-factor factorial arrangement (3×5) was employed, including three types of cuttings (Factor 1) and five different growing media (Factor 2) as described above, with five replicates for each treatment.

After establishing the cuttings of *Catharanthus roseus* in poly bags, they were watered and kept in a sealed mist propagator under 50% shade condition for 5 weeks.

### *Data collection*

Propagator was opened and data was recorded 5 weeks after establishment. Data collection was done using a destructive sampling method. Each cutting was carefully extracted from its pot, and the rooting medium was gently washed away to expose the root system. Following parameters were recorded.

• Survival percentage (%)

$$Survival\ Percentage\% = \frac{No.of\ survived\ plants}{Total\ no.of\ plants\ established} \times 100$$

• Newly produced shoots per cutting (Nos)
• Shoot length (cm)
• Root number
• Root fresh weight (g)

Upon opening the propagator, all cuttings were thoroughly watered, and care was taken to uproot them gently. The soil particles were then removed, and data were systematically recorded. Cuttings were deemed to have survived if both the above-ground and below-ground portions remained alive. During the experimental period, the number of newly produced shoots was counted for each cutting and recorded accordingly. Shoot length was measured from the base of the shoot at the main stem to the apex of the leaves using a ruler. Roots that initiated from the main stem were counted to determine the total number of roots produced by each cutting. These roots were carefully separated from the cuttings using sharp secateurs and subsequently weighed using an analytical balance. The root fresh weight was recorded.

### *Data analysis*

Collected data were statistically analyzed through ANOVA procedures using SAS statistical software version 9.1.3. Treatment means were compared using Duncan's Multiple Range Test (DMRT) at 5% significance level.

## RESULTS AND DISCUSSION

### *Survivability*

No significant interaction was observed in 5$^{th}$ week between cutting type and potting media on survivability of *Catharanthus roseus* cuttings. Only the cutting type showed a significant effect on cutting survivability ($P \leq 0.05$), such as tip cuttings demonstrated 100% survival, semi-hardwood 76%, and hardwood with 36% survival. Only the tip cuttings were totally survived after 5 weeks from planting with zero mortality rate (Figure 1).

Tip cuttings are derived from actively growing apical regions that contain higher concentrations of endogenous auxins and meristematic cells. Auxins such as indole 3 butyric acid (IBA) and indole 3 acetic acid (IAA) play a central role in initiating adventitious root formation, a critical determinant of cutting survival and establishment. Auxin mediated regulation of cell dedifferentiation and root primordia initiation underpins successful vegetative propagation from cuttings, and this process is most effective in tissues with active growth potential (Blythe et al., 2007). *Catharanthus roseus* tip cuttings achieved 100 % survivability because they inherently possess physiological and anatomical attributes that enhance rooting competence and stress resilience compared to more mature stem segments. The higher endogenous auxin levels and meristematic activity at shoot tips accelerate adventitious root formation, while greater metabolic reserves and less lignification reduce physiological constraints that commonly lead to cutting failure in semi hardwood and hardwood types.

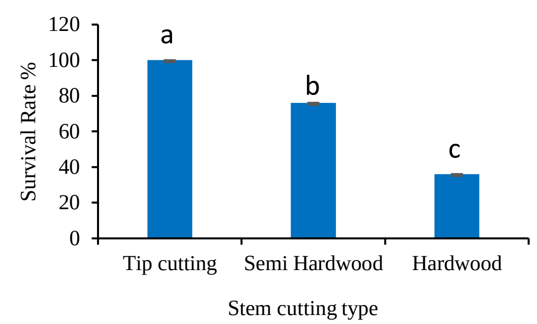


Figure 1. Effect of different cutting types on cutting survivability of *Catharanthus roseus*

*The values represent the mean ± standard error of 5 replicates. Different letters indicate significant differences at the 5% probability level.*

### *Number of new shoots emerged per cutting*

There was no significant interaction effect found between the cutting types and the medium ($P \geq 0.05$). Potting medium and cutting type showed a significant effect individually on number of new shoots emerged per cutting (Table 1).

The results demonstrated that the growing medium significantly influenced ($P \leq 0.05$) the number of newly produced shoots per cutting (Table 1). In the 5$^{th}$ week, sand (1.20) and the mixture of coir dust + sand (0.93) and topsoil + sand (0.73) yielded the significantly higher number of newly produced shoots when compared to other treatments. The coir dust alone media (0.60) and sand + compost (0.60) media produced significantly lower shoots per cutting. This suggests that certain growing media, particularly sand promote more vigorous shoot production compared to other media, possibly due to better drainage and aeration, which is required for root and shoot development.

Table 1: Effect of different cutting types and potting media on number of newly produced shoots per cutting

| Medium (M) | Mean value of newly produced shoots (Nos) |
|---|---|
| Sand | 1.20a |
| Coir dust | 0.60b |
| Coir dust + Sand | 0.93ab |
| Sand + Compost | 0.60b |
| Topsoil + Sand | 0.73ab |
| P value | 0.030 |
| **Cutting (C)** | **Mean value of newly produced shoots (Nos)** |
| Tip | 0.00c |
| Semi hardwood | 1.80a |
| Hardwood | 0.64b |
| P value | 0.000 |

*Means followed by the different letters in the same column are significantly different at 5% probability level.*

The type of cutting used also had a significant effect on shoot production. In the 5th week, semi-hardwood cuttings produced the highest number of shoots, with an average of 1.80 shoots per cutting. These results were significantly higher when compared to both hardwood and tip cuttings. Hardwood cuttings produced fewer shoots (0.64), while tip cuttings showed no new shoot production. Semi-hardwood cuttings consistently outperformed both hardwood and tip cuttings, which may be attributed to their intermediate lignification, allowing for better rooting and shoot formation compared to hardwood cuttings. Furthermore, tip cuttings being less mature probably faced challenges in forming new shoots due to insufficient physiological readiness. Further, Meerow, (2007) mentioned that the absence of shoot production in tip cuttings, may be due to apical dominance, where terminal auxins suppress lateral buds to prioritize root development overshoot multiplication.

***Length of newly produced shoots***

Results in table 2 on shoot length revealed that there was no significant interaction between medium and cutting type. Anyhow, significant differences were observed among only the cutting types on shoot length ($P \leq 0.05$).

Table 2: Effect of different cutting types and potting media on length of newly produced shoots of *Catharanthus roseus*

| Cutting (C) | Mean value of newly produced shoot length (cm) |
|---|---|
| Tip | 0.00b |
| Semi hardwood | 2.24a |
| Hardwood | 1.96a |
| P value | 0.000 |

*Means followed by the different letters in the same column are significantly different at 5% probability level*

Tip cuttings produced the lower number of new shoot growth throughout the entire observation period. Semi-hardwood cuttings and hardwood cuttings showed the significantly higher shoot elongation reached 2.24 cm and 1.96 cm respectively when compared to the tip cuttings.

These findings are aligned with established research highlighting the critical role of cutting characteristics and media composition in successful propagation. Hartmann and Kester (1975) reported that leaf presence on cuttings promotes root development, while Ismail (2011) confirmed that shoot tip cuttings of *Dieffenbachia* species achieved superior rooting percentages compared to alternative cutting types. Supporting evidence from Eed et al. (2015) in their Bougainvillea experiment showed that mixed media significantly outperformed single-component substrates, with soil and sand combinations (1:1 ratio) producing the greatest plant height, while soil-only media yielded the poorest growth results among all tested compositions.

***Number of roots produced per cutting***

Root number showed a significant interaction ($P \leq 0.05$) in between cutting types and the growing medium (Table 3).

Among the cutting types, tip cutting exhibited the highest growth when grown in sand, showing the best performance across all mediums. Root number showed a significant interaction ($P \leq 0.05$) where, tip cuttings in the sand medium produced the highest number of roots (30.4 roots) compared to all other treatment combinations (Table 3). Other media used for tip cuttings showed significantly lower performance when compared to the tip cutting in the sand media as sand + compost (13.2 roots), coir dust + sand (11.2 roots), coir dust (10.2 roots), and topsoil + sand (9.4 roots), but there was no significant difference among them. Semi-hardwood (0.6 roots) and hardwood cuttings (0.6 roots) in sand: compost medium demonstrated the significantly lowest root production when compared to tip cutting in sand media, while all treatments in semi hardwood and hardwood cuttings across all media were not significantly increased the root number (Plate 1 & 2).

Table 3: Interaction effect of different cutting types and potting media on number of roots of *Catharanthus roseus*

| Cutting (C) | Medium (M) | Mean number of roots (Nos) |
|---|---|---|
| Tip | Sand | 30.4a |
| | Coir dust | 10.2bc |
| | Coir dust + Sand | 11.2bc |
| | Sand + Compost | 13.2b |
| | Topsoil + Sand | 9.4bc |
| | Sand | 6.8bc |
| Semi hardwood | Coir dust | 8.2bc |
| | Coir dust + Sand | 8.0bc |
| | Sand + Compost | 0.6c |
| | Topsoil + Sand | 8.0bc |
| | Sand | 5.6bc |
| Hardwood | Coir dust | 8.2bc |
| | Coir dust + Sand | 2.2bc |
| | Sand + Compost | 0.6c |
| | Topsoil + Sand | 3.6bc |
| P value | C | 0.000 |
| | M | 0.000 |
| | C x M | 0.000 |

*Means followed by the different letters in the same column are significantly different at 5% probability level.*

Variability in plant propagation can be attributed to differences in the physical properties of growth media (Khayyat et al., 2007) and their capacity to supply optimal air and water ratios to develop plants (Baiyeri, 2003). Excessive water retention poses significant challenges, as water creates barriers to oxygen diffusion, potentially causing anoxic conditions at cutting bases that inhibit root development (Loach, 1985). Research comparing sawdust and sand as growth media revealed that sawdust required significantly less irrigation than sand or sand-sawdust mixtures throughout experimental periods, indicating superior water retention capacity. However, this enhanced water-holding ability can prove detrimental to plant development and survival, as demonstrated by Ofodile et al. (2013) and Caspa et al. (2014). These findings have important implications for the coir dust, particularly due to its high-water retention characteristics.


Plate 2: Vegetatively propagated *C. roseus* tip cuttings with the highest root performances


Plate 3: a) Higher number of shoots produced by hard wood cuttings; b) Poor root performances of hard wood cuttings; c) High shoot and root performances of semi hardwood of *C. roseus*

Adventitious rooting is a complex developmental process that requires competent cells to undergo reprogramming and subsequent differentiation into root initials. Young, pliable tissues at shoot tips have a higher density of responsive cell types capable of dedifferentiation, which directly enhances rooting success. This enhanced rooting competence contributes to rapid root development, improving water uptake and reducing desiccation stress (Blythe et al., 2007). Tip cuttings, being taken from actively growing parts of the plant, tend to have higher concentrations of hormones, such as auxins, that promote root formation. In contrast, hardwood cuttings, taken from mature, woody parts of the plant, often show slower or reduced root development due to lower auxin levels and reduced cell activity (Zandalinas et al., 2021). The results reinforce the finding that *Catharanthus roseus* cuttings taken from young, actively growing shoots (tip cuttings) are the most suitable for propagation, especially when rapid and successful rooting is desired. Semi-hardwood and hardwood cuttings, while still capable of rooting under appropriate conditions, generally require more time and precise environmental control to achieve the same success (Zandalinas et al., 2021).

***Root fresh weight per cutting***

Root fresh weight showed a significant interaction (P ≤ 0.05) between the cutting type and medium (Table 4).

Table 4: Effect of different cutting types and potting media on fresh weight of *Catharanthus roseus* roots per cutting

| Cutting (C) | Medium (M) | Mean root fresh weight (g) |
|---|---|---|
| Tip | Sand | 2.08a |
| | Coir dust | 0.16b |
| | Coir dust + Sand | 0.36b |
| | Sand + Compost | 0.12b |
| | Topsoil + Sand | 0.29b |
| Semi hardwood | Sand | 0.10b |
| | Coir dust | 0.27b |
| | Coir dust + Sand | 0.12b |
| | Sand + Compost | 0.01b |
| | Topsoil + Sand | 0.08b |
| Hardwood | Sand | 0.51b |
| | Coir dust | 0.06b |
| | Coir dust + Sand | 0.01b |
| | Sand + Compost | 0.01b |
| | Topsoil + Sand | 0.01b |
| P value | C | 0.002 |
| | M | 0.000 |
| | C x M | 0.032 |

*Means followed by the different letters in the same column are significantly different at 5% probability level.*

Only tip cuttings in sand medium demonstrated significantly better root development when compared to all other treatments (Table 4). Root fresh weight of tip cuttings planted in sand media was recorded as the significantly highest fresh root weight as 2.08g. Tip cuttings planted in all media except sand alone, as well as semi-hardwood and hardwood cuttings planted across all media, did not show significantly different root fresh weights and were statistically similar to one another.

These findings were aligned with several previous studies. Studies demonstrate that improved aeration in rooting substrates stimulates metabolic processes and accelerates root initiation (Yeboah and Amoah, 2009). Research on *Vitellaria paradoxa* rooting performance revealed that media selection significantly influences cutting success rates. Multiple studies confirm the substantial impact of growing medium on root biomass production. Similarly, Haile (2017) found that rooting media substantially affected root fresh weight, with agricultural soil producing the lowest root mass in stem cuttings. These findings align with earlier research by Shah et al. (2021), which also identified significant relationships between rooting material type and root fresh weight in rooted cuttings.

## CONCLUSIONS

This research indicates that the effectiveness of vegetative propagation for *Catharanthus roseus* is significantly affected by the type of stem cutting used and the growing medium. Tip cuttings have great survivability and tip cuttings planted in sand medium show the best results in terms of root formation, whereas semi-hardwood cuttings are more effective for shoot development. This protocol offers a practical strategy for generating uniform, true-to-type plants, which benefits both commercial horticulture and the reliable production of valuable alkaloids.

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