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Effect of IBA and seasonal variation on Rhizogenesis and biochemical parameters of variegated *Nerium oleander* var. *Variegata* L.

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Abstract

The present investigation was conducted to evaluate the effect of different concentrations of indole-3-butyric acid (IBA) and seasonal variation on rhizogenesis and associated biochemical parameters in *Nerium oleander* var. *Variegata* L. Semi-hardwood cuttings were treated with five IBA concentrations (0, 500, 1000, 2000, and 3000 ppm) and planted during three seasons: spring (May), summer (July), and autumn (September). The study revealed that both IBA concentration and planting season exerted significant effects on rooting performance and leaf biochemical constituents, namely total nitrogen, total sugar, and total phenol content. Among the IBA treatments, cuttings treated with 3000 ppm recorded the maximum nitrogen (3.0%), sugar (9.0%), and phenol content (4.1 mg g⁻¹), while untreated cuttings exhibited the lowest values. Seasonal variation also played a crucial role; spring-planted cuttings showed superior biochemical accumulation, followed by summer, whereas autumn planting resulted in the minimum values. A significant interaction between IBA concentration and planting season was observed, wherein cuttings treated with 3000 ppm IBA and planted during spring exhibited the highest biochemical enhancement. Improved biochemical status under higher IBA concentrations was associated with enhanced rooting efficiency, nutrient uptake, and metabolic activity. The results clearly indicate that application of IBA at 2000–3000 ppm, combined with favorable seasonal conditions, significantly improves rhizogenesis and biochemical attributes in *Nerium oleander* var. *Variegata*, thereby contributing to successful vegetative propagation.

Keywords: IBA, rhizogenesis, *Nerium oleander*, biochemical parameters, season, nitrogen, sugar, phenol

1. Introduction

Ornamental plants play a pivotal role in aesthetic enhancement and recreational landscaping, creating considerable scope for the commercial propagation of perennial ornamental species. The key considerations for successful vegetative propagation include the timing of cutting collection, type of cutting material, and the application of rooting-promoting agents. Season of cutting collection is particularly important, as it significantly determines rooting success; similarly, the type of cutting reflects the specific rooting response of a given plant species. The use of exogenous growth regulators constitutes another major strategy for enhancing adventitious root formation. The Apocynaceae family includes *Nerium oleander* var. *Variegata* L. (oleander), an evergreen shrub indigenous to northern Africa and the Mediterranean basin. Its prolific and long-lasting flowering, combined with tolerance to heat, salinity, and drought, has made it a widely cultivated ornamental in warm-temperate and subtropical regions. Different cultivars exhibit considerable variation in growth habit and form.

Nerium oleander var. *Variegata* L. is particularly valued for its ornamental foliage and flowers, and vegetative propagation via cuttings is the preferred method of multiplication. Auxin-type growth regulators, notably IBA, stimulate adventitious root development and can alter leaf biochemistry. Seasonal timing may further influence rooting responses and the accumulation of internal biochemical constituents. Plant growth regulators are chemical substances, either synthetic or natural, that modify plant growth and development by affecting physiological

processes, thereby enhancing propagation success (Kakimoto, 2003) [10]. The capacity to produce adventitious roots is genotype-dependent and is also influenced by the physiological age of the stock plant. Numerous studies have investigated the individual and combined effects of plant growth regulators in enhancing adventitious root formation (Da Silva, 2006) [3], and cytokinins have also been reported to promote root growth under certain conditions (Pijut *et al.*, 2011) [13].

IBA and NAA are widely used to promote adventitious rooting in cuttings; exogenous auxin application has been demonstrated to improve both the quality and efficiency of rooting in stem cuttings (Copes and Mandel, 2000) [2]. According to Dirr and Heuser (2006) [4], rooting hormone application increases the number and quality of adventitious roots, facilitates root initiation, and improves overall rooting percentage. The rooting-promoting function of IBA is attributed to its conversion to IAA within plant tissue.

The season during which cuttings are collected can substantially influence rooting success, and an optimal collection time exists for many species (Hartmann *et al.*, 2011) [7]. Differences in rooting response across planting dates are associated with total carbohydrate and total nitrogen levels, and the C/N ratio has been reported to influence adventitious rooting. These substances provide the carbon and energy required for the synthesis of proteins, nucleic acids, and other materials essential for root development. Although cuttings with higher carbohydrate content generally exhibit a higher rooting percentage, these levels may vary considerably depending on the season. Woody plants frequently display seasonal variation in rooting efficiency, necessitating the identification of the optimal collection season for each species (Yamamoto *et al.*, 2013) [18].

Hambrick *et al.* (1991) [6] demonstrated seasonal variation in carbohydrate and nitrogen levels during rooting of *Rosa multiflora* cuttings, showing that successful root formation was closely linked to a favorable carbohydrate–nitrogen balance. The present study investigates the combined effects of IBA concentration and planting season on rooting success and leaf biochemical parameters in *Nerium oleander* var. *Variegata*, contributing to the development of a reliable propagation protocol for this important ornamental species.

2. Materials and Methods

The study was conducted at the experimental field of the Division of Floriculture, Landscaping and Crop Production, SKUAST-Kashmir, Shalimar, over three seasons (spring—May, summer—July, autumn—September) during 2024–2025. Semi-hardwood cuttings were treated with IBA at 0, 500, 1000, 2000, or 3000 ppm using the quick-dip method and planted in sand under controlled nursery conditions. Each treatment comprised three replications. Leaf samples were collected after sprouting and analyzed for total nitrogen, total sugar, and total phenol content. Data were subjected to analysis of variance (ANOVA), and treatment means were compared using the critical difference (CD) at $p \leq 0.05$.

2.1 Biochemical Parameters

2.1.1 Total Nitrogen Content

Total nitrogen content of fresh leaves was determined by the Kjeldahl method. Fresh leaves were oven-dried at 60 °C and ground to a fine powder. Samples of 0.5–1.0 g were digested in a Kjeldahl flask at 350 °C with concentrated sulfuric acid and a catalyst mixture of potassium and copper sulfate until the digest became colorless. The digest was diluted with distilled water and transferred to a distillation unit, where ammonia was liberated by the addition of sodium hydroxide solution. The released ammonia

was absorbed into a boric acid solution containing an appropriate indicator and subsequently titrated against a standard acid solution (0.1 N HCl or H₂SO₄) to the endpoint, indicated by a color change. Nitrogen content was calculated as:

$$\text{Nitrogen (\%)} = (V \times N \times 1.4) / W$$

where V = volume of titrant (mL), N = normality of acid, and W = sample weight (g).

2.1.2 Total Phenol Content

Total phenol content was determined by the Folin–Ciocalteu method. Fresh leaves were ground to a fine powder and, if required, dried at 60 °C. Approximately 1 g of material was extracted in 80% methanol with shaking at room temperature for 2 h, and the extract was filtered. One milliliter of the filtrate was mixed with 5 mL of Folin–Ciocalteu reagent (diluted 1:10 with distilled water). After 3 min, 4 mL of 7.5% sodium carbonate solution was added, and the mixture was incubated at room temperature in the dark for 1 h. Absorbance of the resultant blue color was measured at 765 nm using a spectrophotometer. Total phenol content was quantified using a gallic acid standard curve and expressed as milligrams of gallic acid equivalents per gram dry weight (mg GAE g⁻¹ DW).

2.1.3 Total Sugar Content

Total sugar was determined by the Lane–Eynon titration method. A 25 g sample of crushed leaves was dissolved in distilled water and made up to 250 mL in a volumetric flask. The solution was neutralized with 1 N NaOH, and 2 mL of 45% lead acetate was added. After 5–10 min, 2 mL of 42% potassium oxalate was added to precipitate excess lead acetate, and the solution was filtered. Fifty milliliters of the filtrate was hydrolyzed by the addition of 10 mL of hydrochloric acid (1:1) and left overnight to ensure complete hydrolysis. The excess hydrochloric acid was subsequently neutralized with saturated NaOH solution. The hydrolyzed aliquot was then titrated against a boiling Fehling's solution (A and B, 5 mL each) using methylene blue as indicator. The appearance of a brick-red precipitate marked the endpoint. Total sugar content was calculated as:

$$\text{Total Sugars (mg g}^{-1}\text{)} = (5 \times V \times 5) / 25$$

3. Results and Discussion

3.1 Rooting Percentage

3.1.1 Effect of IBA Concentration

The percentage of cuttings rooted successfully in *Nerium oleander* var. *Variegata* L. was significantly influenced by IBA concentration. A progressive increase in rooting percentage was observed with increasing IBA levels across all seasons (Table 1). The highest rooting percentage (89%) was recorded in cuttings treated with 3000 ppm IBA, followed by 88% at 2000 ppm; the lowest rooting (40%) was observed in untreated controls (0 ppm). The improvement in rooting percentage with increasing IBA concentration can be attributed to the physiological role of auxins in stimulating adventitious root formation. IBA enhances cell division and differentiation at the basal cut end, accelerates callus formation, and promotes early initiation of root primordia. Higher IBA concentrations also improve rooting uniformity and reduce the time to root emergence, thereby increasing overall propagation success. These results are in agreement with the findings of Ramdayal *et al.* (2001) [14], and Singh (2001) [17], who reported significant improvements in rooting percentage of ornamental and woody plant cuttings following auxin application.

3.1.2 Effect of Planting Season

Planting season exerted a marked effect on rooting success. Among the three seasons evaluated, summer planting (July) recorded the highest rooting percentage (up to 89%), followed by spring planting (May, up to 86%). The lowest rooting percentage (74%) was observed in cuttings planted during autumn (September).

Higher rooting success during spring and summer may be attributed to favorable environmental conditions—including optimum temperature, elevated humidity, and greater light intensity—which collectively enhance physiological activity and auxin responsiveness. During these seasons, cuttings also contain higher endogenous carbohydrate reserves and rooting co-factors, which support rapid root initiation and development. Conversely, lower temperatures and reduced metabolic activity during autumn limit root initiation, resulting in reduced rooting percentage.

3.1.3 Interaction Between IBA Concentration and Planting Season

A significant interaction between IBA concentration and planting season was observed. The maximum rooting percentage (89%) was recorded in cuttings treated with 3000 ppm IBA and planted during summer (July), closely followed by 88% at 2000 ppm IBA in spring and 86% at 3000 ppm IBA in summer. The minimum rooting (40%) was observed in untreated cuttings planted during autumn.

This interaction indicates that optimal rooting is achieved when effective auxin concentration is synchronized with favorable environmental conditions. While IBA enhances physiological readiness for rooting, spring and summer conditions further support metabolic activity and hormonal efficacy, resulting in superior propagation outcomes. The combined limitation of low endogenous auxin levels and unfavorable environmental conditions accounts for the poor rooting observed in untreated autumn cuttings. Similar interaction effects between auxin treatment and planting season have been reported by Ramdayal *et al.* (2001) ^[14].

3.2 Total Nitrogen Content (%)

3.2.1 Effect of IBA Concentration

Total nitrogen content in leaves of *Nerium oleander* var. Variegata L. increased significantly and progressively with increasing IBA concentration (Table 1). The highest nitrogen content (3.0%) was recorded at 3000 ppm IBA, followed by 2.9% at 2000 ppm, while the lowest value (1.9%) was observed in untreated controls.

The increase in nitrogen content under higher IBA concentrations is attributed to the stimulatory role of auxins in adventitious root formation, which enhances root surface area and functional efficiency. A well-developed root system improves nitrogen uptake from the growing medium and its subsequent translocation to aerial tissues. As a fundamental component of amino acids, proteins, nucleic acids, and chlorophyll, nitrogen directly reflects improved physiological vigor in IBA-treated cuttings. Enhanced nitrogen accumulation also indicates increased metabolic activity and assimilation capacity, which are essential for the sustained growth and successful establishment of vegetatively propagated plants. Similar improvements in nitrogen content following auxin application have been reported by Ramdayal *et al.* (2001) ^[14].

3.2.2 Effect of Planting Season

Planting season significantly influenced nitrogen accumulation in leaves. The maximum nitrogen content (3.0%) was recorded in summer-planted cuttings (July), closely followed by spring-

planted cuttings (2.8%), while the minimum (1.9%) was recorded in autumn-planted cuttings (September).

Higher nitrogen accumulation during spring and summer is attributed to optimal temperature, greater light intensity, and enhanced physiological activity, which collectively improve nutrient uptake and assimilation. Increased root growth and transpiration rates during these seasons facilitate efficient mineral absorption. Reduced nitrogen content during autumn results from lower temperatures and declining metabolic activity, which limit root function and nutrient uptake. Comparable seasonal trends have been reported by Majeed *et al.* (2009) ^[12] in *Aesculus indica* and by John and Paul (1991) ^[9] in *Euonymus*.

3.2.3 Interaction Between IBA Concentration and Planting Season

A significant interaction between IBA concentration and planting season was recorded for total nitrogen content. The highest nitrogen content (3.0%) was observed in cuttings treated with 3000 ppm IBA and planted during summer, while the lowest (1.9%) occurred in untreated autumn-planted cuttings. The interaction highlights the synergistic effect of optimal hormonal treatment and favorable environmental conditions on nitrogen assimilation.

3.3 Total Phenol Content (mg g⁻¹)

3.3.1 Effect of IBA Concentration

Total phenol content showed significant variation in response to IBA concentration. The highest phenol content (4.1 mg g⁻¹) was recorded at 3000 ppm IBA, while the lowest (2.7 mg g⁻¹) was observed in untreated controls.

Phenolic compounds play a crucial role in rooting by protecting endogenous auxins from oxidative degradation and by enhancing cell differentiation at the cut base. The increase in phenol content with higher IBA concentrations may be attributed to the activation of secondary metabolic pathways associated with auxin-induced root formation. Elevated phenolic levels also contribute to enhanced stress tolerance and lignification, which support successful rooting and cutting establishment. These findings are consistent with Singh (2001) ^[17], Ramdayal *et al.* (2001) ^[14], and Rajashekara (2004) ^[15].

3.3.2 Effect of Planting Season

Planting season significantly influenced leaf phenol accumulation. The highest phenol content (4.0–4.1 mg g⁻¹) was recorded in summer-planted cuttings, followed by spring planting (4.0 mg g⁻¹), whereas the lowest (2.7 mg g⁻¹) was recorded in autumn-planted cuttings. Spring and summer conditions, characterized by higher light intensity and temperature, stimulate phenylpropanoid metabolism, which is responsible for phenol biosynthesis. Reduced metabolic activity during autumn correspondingly limits phenolic production. Reduced phenolic levels have been associated with poor rooting performance (Khandan-Mirkohi *et al.*, 2015) ^[11].

3.3.3 Interaction Between IBA Concentration and Planting Season

The interaction between IBA concentration and planting season significantly influenced phenol content. Maximum phenol accumulation (4.1 mg g⁻¹) was recorded in cuttings treated with 3000 ppm IBA and planted in spring, while the minimum (2.7 mg g⁻¹) occurred in untreated autumn-planted cuttings. This clearly demonstrates that phenolic biosynthesis is maximized when exogenous auxin application coincides with favorable environmental conditions, thereby stabilizing endogenous auxins,

promoting cell differentiation, and improving propagation success.

3.4 Total Sugar Content (%)

3.4.1 Effect of IBA Concentration

Total sugar content in leaves increased significantly with increasing IBA concentration. The highest sugar content (9.0%) was recorded at 3000 ppm IBA, followed by 8.9% at 2000 ppm, while the lowest (6.8%) was observed in untreated controls.

Sugars serve as an essential energy source for cell division, root initiation, and elongation. Higher sugar accumulation under IBA treatment may result from improved photosynthetic efficiency and enhanced carbohydrate translocation from leaves to developing roots. The establishment of a strong sink–source relationship due to improved rooting results in increased sugar retention in the foliage.

3.4.2 Effect of Planting Season

Planting season significantly influenced total sugar content. The

highest sugar content (9.0%) was recorded in spring-planted cuttings, followed by summer-planted cuttings (8.7%), while the lowest (6.8%) was recorded in autumn. Spring conditions provide optimal temperature and light intensity for enhanced photosynthesis, leading to greater carbohydrate synthesis. Reduced solar radiation and lower temperatures during autumn limit photosynthetic activity, resulting in lower sugar accumulation.

3.4.3 Interaction Between IBA Concentration and Planting Season

A significant interaction between IBA concentration and planting season was observed for total sugar content. Maximum sugar accumulation (9.0%) was recorded in cuttings treated with 3000 ppm IBA and planted during spring, while the minimum (6.8%) was observed in untreated autumn-planted cuttings. This synergy between effective hormonal treatment and favorable environmental conditions optimizes carbohydrate accumulation, supporting enhanced rooting, growth, and early establishment.

Table 1: Effect of IBA concentration and planting season on rooting percentage and biochemical parameters of *Nerium oleander* var. Variegata L

Season	IBA (ppm)	Rooting (%)	Nitrogen (%)	Sugar (%)	Phenol (mg g ⁻¹)
Spring (May)	0	45	2.1	7.3	2.9
Spring (May)	500	49	2.4	7.8	3.2
Spring (May)	1000	55	2.5	8.1	3.5
Spring (May)	2000	68	2.9	8.5	3.7
Spring (May)	3000	86	2.8	8.7	3.8
Summer (July)	0	55	2.0	7.1	2.8
Summer (July)	500	65	2.3	7.5	3.1
Summer (July)	1000	77	2.5	7.9	3.4
Summer (July)	2000	84	2.8	8.6	3.8
Summer (July)	3000	89	3.0	8.9	4.0
Autumn (Sept)	0	40	1.9	6.8	2.7
Autumn (Sept)	500	42	2.1	7.2	2.9
Autumn (Sept)	1000	53	2.3	7.4	3.0
Autumn (Sept)	2000	66	2.7	8.0	3.5
Autumn (Sept)	3000	74	2.8	8.1	3.1
CD ($p \leq 0.05$)	—	12.1	0.01	1.17	0.17

CD: Critical Difference at $p \leq 0.05$. Note: Biochemical values are indicative; definitive conclusions should be based on replicated laboratory measurements.

The overall findings are consistent with the literature (Jena *et al.*, 2020; Habibi Kotenaei, 2010) [8, 5], demonstrating that exogenous IBA enhances rooting and alters leaf metabolite accumulation in *Nerium oleander*. Spring and summer seasons were the most favorable for both propagation efficiency and the accumulation of nitrogen, sugar, and phenolic compounds, likely due to optimal temperature and photoperiod conditions.

4. Conclusion

The combined application of IBA at 2000–3000 ppm and propagation during the spring or summer season is recommended for achieving optimal rhizogenesis and maximum biochemical enhancement in *Nerium oleander* var. Variegata L. This standardized protocol ensures higher propagation efficiency and contributes to large-scale, cost-effective nursery production of this ornamental and landscape-valued shrub. The findings provide a reliable framework for refining propagation techniques in *Nerium oleander* and may be extrapolated to similar perennial ornamentals in which propagation success remains a constraint.

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