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AUXIN-MEDIATED ENHANCEMENT OF ADVENTITIOUS ROOT FORMATION AND MORPHO-ANATOMICAL CHANGES IN *GYPSOPHILA PANICULATA* STEM CUTTINGS

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ABSTRACT
Vegetative propagation of *Gypsophila paniculata* through stem cuttings is constrained by poor rooting and low survival, limiting large-scale commercial multiplication. This present study examined the effects of indole-3-butyric acid (IBA), applied alone or in combination with sulphuric acid (H_2SO_4), on rooting performance, root growth, biomass accumulation and anatomical characteristics. Among the treatments, IBA at 500 ppm gave the earliest root initiation (37.33 days), highest rooting percentage (44.66%) and maximum survival (88.33%). This treatment also produced 28.50 roots per cutting, with a root diameter of 1.02 mm, root volume of 0.95 cm^3, fresh weight of 1.77 g and dry weight of 0.28 g. Root anatomical observations showed the largest stele area (9884.2 μm^2) and a cortex-to-stele ratio of 0.27 under IBA at 500 ppm. Greater stele development was associated with improved rooting and root biomass accumulation, indicating enhanced vascular differentiation and root functionality. In contrast, the addition of H_2SO_4 did not consistently improve rooting and adversely affected rooting and survival when combined with IBA at 500 ppm. Among the treatments evaluated, IBA at 500 ppm was most effective in improving the rooting performance and establishment of *G. paniculata* stem cuttings.

KEYWORDS: Indole-3-butyric acid (IBA), Gypsophila, Rooting performance, Propagation

INTRODUCTION

Gypsophila paniculata L. (Baby's Breath), is a perennial ornamental species belonging to the family Caryophyllaceae, native to Eastern Europe and Central Asia. Its delicate flowers and highly branched inflorescences make it a popular filler flower in bouquets, floral arrangements and dried flower products. The species holds considerable importance in the cut-flower industry, particularly as a supplementary flower in floral designs (Isik *et al.,* 2015). Apart from its ornamental value, *G. paniculata* has industrial and pharmaceutical significance. Its roots are rich in triterpenoid saponins and other bioactive constituents used in pharmaceutical, cosmetic and detergent formulations (Hostettmann and Marston 1995).

Commercial multiplication of *G. paniculata* is often difficult because several cultivated varieties exhibit poor seed set or sterility. Stem cuttings are therefore commonly used to maintain varietal uniformity and produce planting material. However, poor rooting, low survival and irregular establishment can reduce multiplication efficiency and increase production costs (Rahman 2002). Improving adventitious root formation is consequently an important consideration in the commercial propagation of this crop.

Auxins play a major role in adventitious rooting by regulating cell division, differentiation and the formation of root primordia. Their external application has been used successfully to improve rooting in several ornamental and horticultural species (Balestri *et al.,* 2012). Although endogenous auxins participate in root initiation, their availability and distribution may not always favour satisfactory rooting in detached cuttings. Application of exogenous auxins can help overcome this limitation, depending on the species, concentration and propagation conditions (Kasim and Rayya 2009; Stefancic *et al.,* 2005). Among synthetic auxins, indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) are commonly used to stimulate adventitious root formation. However, IBA is generally regarded as the most effective rooting hormone because of its effectiveness in promoting root initiation and subsequent development (Stefancic *et al.,* 2005). Previous studies have demonstrated the positive effects of IBA on rooting, root growth, biomass accumulation and survival of vegetatively propagated ornamental species (Mao *et al.,* 2018; Li *et al.,* 2021; Govindan 2024). However, rooting responses vary with auxin concentration and plant species, emphasizing the need to identify suitable treatments for individual crops.

Chemical scarification using sulphuric acid (H_2SO_4) has also been reported to modify resistant seed coverings. Its application to stem cuttings may alter surface tissues and their permeability, although the consequences for root initiation are less clearly understood. Excessive acid exposure may also injure tissues and adversely affect cutting establishment. The response of *G. paniculata* cuttings to the combined application of IBA and H_2SO_4 has received limited attention.

While the influence of auxins on adventitious rooting has been studied extensively, information on associated anatomical changes in *G. paniculata* remains limited. Root development involves changes in cortical and

vascular tissues that may influence root growth and establishment. Examining these anatomical characteristics alongside rooting and biomass traits could provide a better understanding of the responses induced by IBA. Therefore, the present study was undertaken to evaluate the effects of IBA applied alone and in combination with H_2SO_4 on rooting success, survival, root growth, biomass accumulation and root anatomical characteristics of *G. paniculata* stem cuttings. The study aimed to identify an efficient propagation protocol and elucidate the morpho-anatomical responses associated with auxin-mediated root development in this commercially important ornamental species.

MATERIAL AND METHODS

Experimental site

The experiment was conducted during 2024-25 at the Research Farm of the Department of Floriculture and Landscaping, Punjab Agricultural University (PAU), Ludhiana, Punjab, India (30°56′N, 75°52′E). The region has a subtropical climate characterized by hot and humid summers (May-September) and cool, dry winters (November-February). The average annual rainfall is approximately 760 mm, of which nearly 80% is received during the monsoon season (July-September).

Experimental design and treatments

Terminal shoot cuttings (7-8 cm long) were collected from healthy, disease-free mother plants of *Gypsophila paniculata* during July 2024. The basal leaves were removed before treatment application. The experiment was laid out in a Completely Randomized Design (CRD) comprising seven treatments: control (untreated), IBA at 200 ppm, IBA at 400 ppm, IBA at 500 ppm, IBA at 200 ppm + 1 N H_2SO_4, IBA at 400 ppm + 1 N H_2SO_4 and IBA at 500 ppm + 1 N H_2SO_4. The experiment consisted of three replications with 25 cuttings per replication. Cuttings receiving IBA-alone treatments were dipped directly in the corresponding IBA solution for 5 s, while control cuttings were dipped in distilled water. For combination treatments, the basal ends of the cuttings were dipped in 1 N H_2SO_4 for 5 s, rinsed with distilled water and subsequently dipped in the respective IBA solution for 5 s. The treated cuttings were subsequently planted in plug trays containing sterilized river sand as the rooting medium. The trays were maintained under open nursery conditions and irrigated as required.

Preparation of solutions: IBA solutions (200, 400 and 500 ppm) were prepared by dissolving the required amount of IBA in 95% ethanol and making up the volume with distilled water. Fresh solutions were prepared before use.

A 1 N H_2SO_4 solution was prepared by diluting concentrated sulphuric acid with distilled water.

Data recording

Root initiation was monitored from 30 days after planting (DAP) at three-day intervals and the number of days taken for root emergence was recorded. At 50 DAP, observations were recorded on rooting percentage, survival percentage, number of roots per cutting, root length (cm), root diameter (mm), root volume (cm^3), root fresh weight (g), root dry weight (g) and root density (g cm^{-3}).

Root volume was determined using the water displacement method and expressed in cubic centimetres (cm^3).

Root Density (g cm^{-3}) = Root Dry Weight (g) / Root Volume (cm^3)

Root anatomical analysis

Representative root samples were collected from each treatment at 50 days after planting and fixed in FAA solution (formalin: acetic acid: 70% ethanol; 5:5:90, v/v/v) for 48 h and subsequently dehydrated through a graded ethanol series. Transverse sections (10-15 µm thick) were prepared using a rotary microtome and stained with safranin and fast green following standard histological procedures. The stained sections were observed under a compound microscope and digital images were captured for analysis. Cortex area, stele area and cortex-to-stele ratio were quantified using Image J software.

Correlation analysis

Pearson's correlation analysis was performed using treatment mean values to determine the relationships among rooting, morphological, biomass and anatomical traits of Gypsophila paniculata cuttings. Correlation coefficients (r) were calculated between pairs of variables using the General R-based Analysis Platform Empowered by Statistics (Gopinath *et al.,* 2020). The strength and direction of associations were interpreted based on the magnitude and sign of the correlation coefficient, with positive values indicating direct relationships and negative values indicating inverse relationships.

Statistical Analysis

The experimental data were analyzed using the General R-based Analysis Platform Empowered by Statistics (GRAPES) (Gopinath *et al.,* 2020). Analysis of variance (ANOVA) was performed under the Completely Randomized Design to evaluate treatment effects. Treatment means were separated using Duncan's Multiple Range Test (DMRT) at the 5% probability level. Statistical significance was declared at $p \leq 0.05$.

RESULTS AND DISCUSSION

Rooting performance and survival of cutting

IBA and its combination with H_2SO_4 significantly influenced root initiation, rooting percentage and survival of *Gypsophila paniculata* cuttings (Table 1). The control recorded the longest time to root initiation (47.33 days) and the lowest rooting percentage (28.00%). Among the treatments, IBA at 500 ppm resulted in the earliest root initiation (37.33 days), highest rooting percentage (44.66%) and highest survival percentage (88.33%), which

represents an increase of approximately 59.5% over the control. The combination of IBA 400 ppm with H_2SO_4 also produced a high rooting percentage (43.33%), comparable to IBA 500 ppm. In contrast, IBA 500 ppm combined with H_2SO_4 adversely affected rooting and survival, recording the lowest values (25.33% and 68.33%, respectively).

Table 1 Effect of Indole Butyric Acid on rooting performance and survival of Gypsophila cuttings

Treatments	Days taken to rooting	Rooting percentage	Survival percentage
Control (no dip)	47.33 [a]	28.00 [c]	78.33 [ab]
IBA @200 ppm	43.66[b]	36.66 [b]	81.66 [a]
IBA @400 ppm	41.66[bc]	36.66 [b]	83.33 [a]
IBA @500 ppm	37.33[d]	44.66 [a]	88.33 [a]
IBA @200 ppm + H_2SO_4	43.66[b]	38.33 [b]	76.66 [ab]
IBA @400 ppm + H_2SO_4	39.00[cd]	43.33 [a]	80.00 [ab]
IBA @500 ppm + H_2SO_4	43.00[b]	25.33 [c]	68.33 [b]

Root morphological traits

Root morphological traits were significantly affected by the application of IBA and its combinations with H_2SO_4 (Table 2). Root number increased with increasing IBA concentration, with the highest number of roots per plant (28.50) recorded under IBA 500 ppm, representing an increase of 54.1% over the control (18.50). This treatment also produced comparatively longer roots (9.00 cm) and the greatest root diameter (1.02 mm). The addition of H_2SO_4 to IBA 400 and 500 ppm maintained relatively high root numbers (26.00 and 24.00 roots per plant, respectively) but did not significantly improve root length.

Table 2 Effect of Indole Butyric Acid on root morphological traits of Gypsophila cuttings

Treatments	No. of roots per plant	Root length (cm)	Root diameter (mm)
Control (no dip)	18.50[c]	9.50[a]	0.37[d]
IBA @200 ppm	12.00[d]	6.75[bc]	0.61[c]
IBA @400 ppm	17.50[c]	8.50[a]	0.81[b]
IBA @500 ppm	28.50[a]	9.00[a]	1.02[a]
IBA @200 ppm + H_2SO_4	19.50[c]	5.35[c]	0.81[b]
IBA @400 ppm + H_2SO_4	24.00[b]	8.15[ab]	0.93[a]
IBA @500 ppm + H_2SO_4	26.00[ab]	8.20[ab]	0.80[b]

Root growth and biomass traits

Significant differences among treatments were observed for root growth and biomass traits (Table 3). IBA at 500 ppm produced the highest root volume (0.95 cm^3), root fresh weight (1.77 g) and root dry weight (0.28 g), with 97.9%, 88.3% and 75.0% increase over the control. Similar improvements were observed with IBA 400 ppm combined with H_2SO_4, which recorded root volume, fresh weight and dry weight values of 0.84 cm^3, 1.52 g and 0.25 g, respectively. In contrast, IBA 200 ppm, either alone or in combination with H_2SO_4, resulted in comparatively lower values for these traits. Root density showed a different trend, with the highest value (0.34 g cm^{-3}) recorded under IBA 200 ppm, whereas IBA 500 ppm and its combinations with H_2SO_4 exhibited lower root density values (0.30 g cm^{-3}).

Table 3 Effect of Indole Butyric Acid on root growth and biomass traits of Gypsophila cuttings

Treatments	Root volume (cm^3)	Root density (g/cm^3)	Fresh weight of root (g)	Dry weight of root (g)
Control (no dip)	0.48[e]	0.32[ab]	0.94[d]	0.16[d]
IBA @200 ppm	0.42[f]	0.34[a]	0.82[e]	0.14[e]
IBA @400 ppm	0.67[d]	0.33[a]	1.23[c]	0.21[c]
IBA @500 ppm	0.95[a]	0.30[b]	1.77[a]	0.28[a]
IBA @200 ppm + H_2SO_4	0.37[g]	0.32 [ab]	0.71[f]	0.11[f]
IBA @400 ppm + H_2SO_4	0.84[b]	0.30[b]	1.52[b]	0.25[b]
IBA @500 ppm + H_2SO_4	0.78[c]	0.30[b]	1.46 [b]	0.23 [b]

Root anatomical traits

Root anatomical characteristics differed significantly among treatments (Table 4). The largest cortex area was observed under IBA 200 ppm (3148.4 μm^2), followed by IBA 500 ppm and IBA 500 ppm + H_2SO_4. The

maximum stele area was recorded with IBA 500 ppm (9884.2 μm^2), closely followed by IBA 500 ppm + H_2SO_4 (9789.4 μm^2). In contrast, IBA 200 ppm combined with H_2SO_4 produced the smallest stele area (2659.0 μm^2). The highest cortex-to-stele ratio (0.67) was recorded under IBA 200 ppm + H_2SO_4, whereas IBA 500 ppm and IBA 500 ppm + H_2SO_4 exhibited intermediate and statistically comparable ratios (0.27 and 0.28, respectively).

Table 4 Effect of Indole Butyric Acid on root anatomical traits of Gypsophila cuttings

Treatments	Cortex area (µm²)	Stele area(µm²)	Cortex :stele ratio
Control (no dip)	1645.3^{d}	6679.0^{d}	0.24^{cd}
IBA @200 ppm	3148.4^{a}	8379.9^{c}	0.37^{b}
IBA @400 ppm	2658.1^{b}	9689.4 ^{ab}	0.26^{c}
IBA @500 ppm	2714.0^{b}	9884.2^{a}	0.27^{c}
IBA @200 ppm + H_2SO_4	1759.2^{d}	2659.0^{e}	0.67^{a}
IBA @400 ppm + H_2SO_4	2140.6^{c}	9305.7^{b}	0.23^{d}
IBA @500 ppm + H_2SO_4	2660.3^{b}	9789.4^{a}	0.28^{c}

Correlation analysis

Correlation analysis revealed that stele area was strongly and positively associated with rooting percentage (r = 0.84), survival percentage (r = 0.71), root number (r = 0.61), root volume (r = 0.89), root fresh weight (r = 0.89) and root dry weight (r = 0.87) (Fig 1). In contrast, stele area exhibited a strong negative correlation with days to root initiation (r = −0.86), indicating that enhanced vascular development was associated with earlier rooting and greater root growth. Cortex area showed comparatively weaker relationships with rooting and biomass traits, suggesting that cortical expansion alone did not determine rooting success. The cortex-to-stele ratio was negatively correlated with rooting percentage (r = −0.73), root volume (r = −0.81), root fresh weight (r = −0.81) and root dry weight (r = −0.79), demonstrating that a disproportionately large cortex relative to the stele was associated with reduced rooting performance. These findings indicate that stele development is anatomical determinant of rooting efficiency and root system vigor in *Gypsophila paniculata* cuttings. These relationships suggest that stele development was more closely associated with rooting performance than cortical expansion. The strong positive correlations with rooting and biomass traits support the importance of vascular development during adventitious root establishment, although the associations do not establish a direct causal relationship.

DISCUSSION

The enhanced rooting performance, root growth, biomass accumulation and survival observed in *Gypsophila paniculata* cuttings following IBA application highlight the central role of auxin in adventitious root development. Among the treatments, IBA at 500 ppm was most effective, indicating that a relatively higher auxin concentration was required to stimulate root initiation and subsequent root system establishment in this species. Auxin promotes root formation through the induction of cell dedifferentiation, division and differentiation in tissues adjacent to the vascular cambium, leading to the development of root primordia and adventitious roots (Hartmann et al. 1983; Davies 2004). In addition, auxin-mediated activation of hydrolytic enzymes and enhanced translocation of carbohydrates towards the basal region of cuttings provide the metabolic resources necessary for rapid root development and biomass accumulation (Hartmann et al. 1983).

The increase in root number, root volume and root biomass under higher IBA concentrations suggests improved rhizogenic activity and a more vigorous root system capable of supporting greater water and nutrient acquisition. Consequently, improved root development likely contributed to the higher survival observed in IBA-treated cuttings (Singh 2012; Galavi et al. 2013). Similar promotive effects of IBA on rooting and root growth have been reported in rose (Yeshiwas et al. 2015; Susaj et al. 2012), bougainvillea (Parmar et al. 2010; Mehraj et al. 2013), hibiscus (Shadparavar et al. 2011), carnation (Gowda et al. 2017), croton (El-Gedawey 2021) and *Jasminum sambac* (Netam 2018).

The anatomical observations further support the physiological responses induced by IBA. Enhanced cortex development suggests increased parenchymatous cell division and expansion, which may improve storage capacity and radial transport within the root system (Esau 1977). More importantly, the substantial increase in stele area under IBA 500 ppm indicates enhanced vascular differentiation, a process known to be regulated by auxin through its influence on cambial activity and xylem-phloem development (Aloni 2013). The larger stele area likely improved the transport of water, minerals and assimilates, thereby contributing to superior root functionality and establishment. Furthermore, the intermediate cortex-to-stele ratio observed under IBA 500 ppm suggests a balanced anatomical organization that supports both resource acquisition and transport, which may explain the superior rooting performance of this treatment. A strong positive correlation (r = 0.84) was observed, indicating that increased stele development was associated with improved rooting performance

(Fig.1). This suggests that enhanced vascular differentiation contributes to successful adventitious root formation and establishment of cuttings.

In contrast, the combined application of H_2SO_4 with higher IBA concentrations did not consistently improve rooting and, in some cases, adversely affected rooting and survival. This response may be attributed to localized tissue injury or physiological stress caused by acid exposure, which could have limited the beneficial effects of auxin on root initiation and development. Overall, the findings indicate that the improved propagation success of *G. paniculata* under IBA treatment is associated not only with enhanced rhizogenesis and biomass accumulation but also with auxin-mediated modifications in root anatomical structure, particularly vascular development.

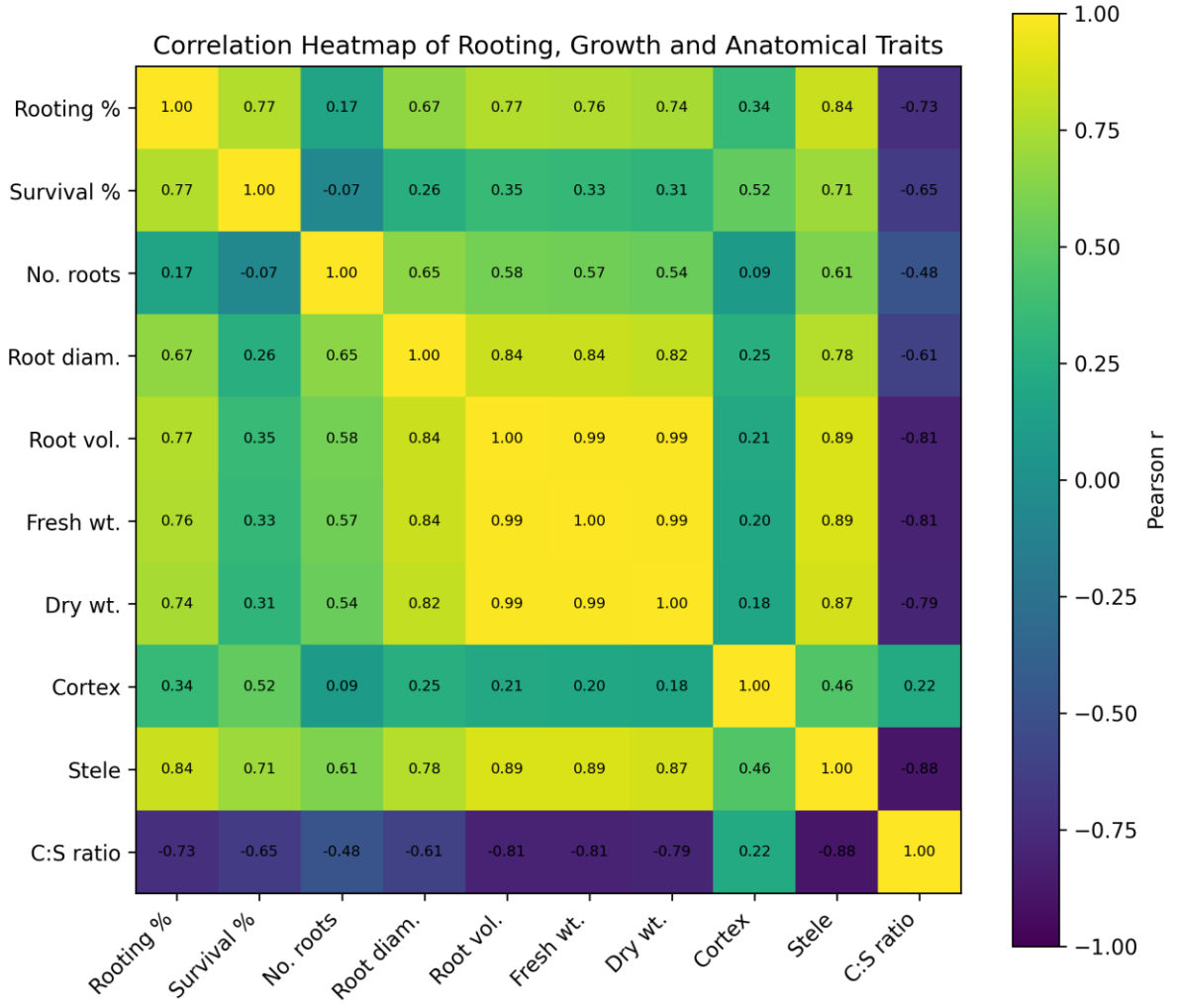

Fig 1. Pearson correlation heatmap illustrating the relationships among rooting, morphological, biomass and anatomical traits of *Gypsophila paniculata* cuttings under different IBA and H_2SO_4 treatments. Positive correlations indicate direct relationships, whereas negative correlations indicate inverse relationships.

CONCLUSION

Application of indole-3-butyric acid (IBA) significantly improved rooting, survival, root growth, biomass accumulation and root anatomical development in *Gypsophila paniculata* stem cuttings. Among the treatments, IBA at 500 ppm proved most effective, producing superior rooting performance, enhanced root biomass and greater stele development, indicating improved vascular differentiation and root functionality. In contrast, the addition of H_2SO_4 failed to provide consistent benefits and adversely affected propagation success at higher concentrations. The results demonstrate that successful rooting in *G. paniculata* is closely associated with auxin-mediated modifications in root morphology and anatomy. Therefore, IBA at 500 ppm can be recommended as an efficient treatment for commercial vegetative propagation of gypsophila and provides a physiological basis for optimizing propagation protocols in this species.

Credit authorship contribution statement

Tanya Thakur and Prinka Goyal- Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization.

Aradhna and Manvir Singh Sidhu - Investigation, Formal analysis, Data curation.

Tanya Thakur and Aradhna- Writing - review & editing, Writing - original draft.

Conflict of interest

The authors declare no competing interests

Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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