



Novel Approaches for Improving Root Development in *Bougainvillea Spectabilis* cv. Alexandra Stem Cuttings Using Hormonal and Physical Stimuli

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KEYWORDS

Bougainvillea spectabilis
Blue light
Bottom heat
IBA
NAA

ABSTRACT

This study aimed to enhance the propagation efficiency of *Bougainvillea spectabilis* Alexandra by evaluating the synergistic effects of auxins (NAA and IBA), bottom heat, and blue light durations on rooting performance of stem cuttings under controlled conditions. A full factorial experiment in a completely randomized design with three replications was conducted. Factors included auxin types (NAA, IBA) at 0, 5, and 10 mg/L, bottom heat ($\pm$), and blue light durations (0, 2, 4 h/day). Parameters assessed were callus formation, shoot survival, root fresh/dry weight, rooting percentage, and rooting time. The combination of IBA at 10 mg/L with bottom heat and 2 h/day blue light significantly improved rooting percentage, root fresh weight, root dry weight, and shoot survival rate, while reducing rooting time to 50 days. Correlation analysis showed strong positive relationships between rooting percentage, root biomass, and shoot survival, and negative correlations with rooting time. The study was limited to one specific cultivar ('Alexandra') and controlled greenhouse conditions; further validation under commercial production settings is needed. This research provides a cost-effective and sustainable protocol for improving the propagation of woody ornamentals by integrating low-dose IBA with environmentally friendly physical stimuli (bottom heat and blue light), enhancing commercial production efficiency.

ARTICLE

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1. Introduction

Bougainvillea (*Bougainvillea spectabilis*) is a popular ornamental plant valued for its vibrant bracts and adaptability to various climates. *Bougainvillea spectabilis* (commonly called "paper flower") is a popular ornamental plant prized for its vibrant, papery bracts (often mistaken for flowers). Native to South America, Brazil, Peru, Argentina (tropical) it was first documented in the 18th century by French botanist Philibert Commercon during explorations in Brazil (Almeida *et al.*, 2021).

Its drought tolerance and striking colors make it a favorite in tropical and subtropical landscapes. *Bougainvillea spectabilis* is a key ornamental plant, but its propagation via cuttings faces challenges like slow rooting and low success rates. Recent advances (2020-2025) integrate hot bottom heating (HBH), auxin application, and blue light exposure to enhance rooting efficiency.

Fresh flowers in Mexico are steeped in hot water and sound like tea. *Bougainvillea* is the symbol of peace, prosperity, and passion for relationships. For its paper-like bracts, this is called paper flowers. There are over 300 varieties of *Bougainvillea* in the world. Among the three species of this plant, *Bougainvillea spectabilis* is a greater plant, whereas *B. glabra* and *Bougainvillea* has 14 species, with three that are horticulturally important: *Bougainvillea spectabilis* (great bougainvillea), *B. glabra* (lesser bougainvillea), and *B. peruviana* (Peru bougainvillea).

This name comes from Louis Antonie de Bougainville, a French navigator and military commander. *Bougainvillea* has medicinal properties and can reduce gastrointestinal and respiratory ailments. The sap of this plant causes skin irritation and mild stomach upset of humans (Kabir, 2025).

Table 1. Scientific classification of *Bougainvillea spectabilis* (World Flora Online, 2023).

Kingdom	Plantae
Division	Magnoliophyta (Flowering plants)
Class	Magnoliopsida (Dicots)
Order	Caryophyllales
Family	Nyctaginaceae (Four-o'clock family)
Genus	<i>Bougainvillea</i>
Species	<i>B. spectabilis</i> (Shah <i>et al.</i> , 2022)

Hot bottom heating maintains root-zone temperatures at 25-30°C, promoting cell division and enzymatic activity for faster root initiation (Hartmann *et al.*, 2022). Studies show HBH increases rooting rates in woody cuttings by 30-50% (Gautam *et al.*, 2021). Exogenous auxins (IBA, NAA) remain critical, but recent work focuses on Nano-encapsulated auxins for sustained release (Sharma *et al.*, 2023).

Combined auxin-light treatments to boost photomorphogenesis (Li *et al.*, 2021). Blue light (450-495 nm) enhances auxin transport via cryptochrome photoreceptors, improving root primordia formation (Zhang, 2020). A study found blue light + IBA increased *Bougainvillea* rooting by 70% vs. darkness (Patel and Lee, 2024).

The HBH-auxin-blue light triad synergistically and accelerates root emergence (5-7 days faster) (Kumar *et al.*, 2023) and reduces oxidative stress (Gupta and Agarwal, 2023). This review consolidates 2020-2025 findings on these innovations, highlighting mechanisms, protocols, and future directions. The vibrant colors of bracts are primarily regulated by anthocyanin biosynthesis genes. Key enzymes in this pathway include chalcone synthase (BsCHS), dihydroflavonol reductase (BsDFR), and anthocyanidin synthase (BsANS), which have been shown to be highly expressed during bract development.

Recent RNA-seq studies (Zhang *et al.*, 2021) revealed differential expression of these genes across different color cultivars, with purple varieties showing particularly high BsDFR expression. Some cultivars also express betacyanin-related genes like BsCYP76AD1, which contribute to unique red-violet pigmentation patterns.

Flowering time and floral development in *Bougainvillea* are controlled by conserved regulatory genes. The photoperiod-responsive BsFT gene shows seasonal expression patterns, peaking before

flowering initiation. This is accompanied by increased expression of floral meristem identity genes like BsLFY and BsSOC1 (Chen *et al.*, 2020). Interestingly, transcriptome studies have found that these flowering genes maintain relatively stable expression patterns even under varying environmental conditions, explaining Bougainvillea's ability to flower year-round in tropical climates.

Stress response genes play a crucial role in Bougainvillea's adaptability. Under drought conditions, there is significant upregulation of BsDREB2 and BsP5CS, which help maintain cellular osmotic balance (Wang *et al.*, 2023). Heat shock proteins (BsHSP70 and BsHSP90) show increased expression during temperature extremes, protecting cellular proteins from denaturation. These stress-responsive genes contribute to the plant's remarkable resilience in challenging environments.

Hormonal regulation in Bougainvillea involves complex interactions between auxin and gibberellin pathways. Auxin-responsive genes (BsAUX/IAA and BsARF) are highly expressed during root formation and vegetative growth, while gibberellin biosynthesis genes (BsGA20ox) peak during flowering stages (Gupta *et al.*, 2022). Current research is investigating how these hormonal pathways interact with environmental signals to regulate the plant's growth cycle and stress responses. This study aims to evaluate the effects of rooting hormones, light conditions, growing substrates and their temperatures on the rooting of *Bougainvillea spectabilis* (paper flower), providing practical strategies for improving propagation and promoting sustainable production of this ornamental plant.

Table 2. Classification of *Bougainvillea* cultivars into different groups based on bract color, growth habit, and morphological traits (a total of 106 varieties are listed) (Kabir, 2025).

Groups	Varieties	Number of varieties
Lilac	Formosa, Green apple	2
Orange	Hugh Evans, Sunset orange, Fire opal, Tanglong orange, Orange mashuri	5
Purple	Twilight delight, Purple star, Texas king, Glabra purple, Jewel vera purple, Royal madora	6
Red	Crimson, Mahara red, Thimma red, Duck feet red, Flame red, Chili red, Chili orange, Chili yellow, Chili ice cream; Chili pink, Silver red, Panas red, Monalisa red, Sweet heart, VF ruby	15
Violet	Sao Paulo, Refulgence	2
Yellow	California gold, Butterfly yellow, Jewel yellow, Tanglong yellow	4
Double	Bridal bouquet (white), Mahara beauty (purple).	2
Dwarf	Jewel pink, Kristina, Jewel peach, Thai queen, PV Sen, Jennah India purple, Jennah India pink	7
Fast grower	Pink, orange, white, yellow, purple, maroon, brown, and multi-colored types	31
Bi-color	Mahara roseville, Chitra batik, Adarna, Roseville delight	4
Variegated	Vicki thimma (lilac and white), Splash, Chitra, Dr Rao, Three stars, Miss Eva, Arjuna, Los banos variegata, Jakirana, Diamond orange, Royal dauphine, Sakura belacan, Golden summer purple	13
Thornless	Silhouette, Miss Alice	2
Hybrid	Chitra cream, Butterfly splash, Barbara karst, Vera purple, Magic sakura	5
Captive	Hawaii violet, Wajid Ali Shah, Thai delight	3
Grafted	Sufia Indiana, Multi-grafted shaft, Tanglong purple, Briza magenta, Maharani	5
Total		106



Figure 1. Representative *Bougainvillea* cultivars showing variation in bract color and morphology: VF ruby, Monalisa red, Vicki thimma, Jewel pink, Kristina, Three stars, Tanglong orange, Butterfly yellow, Roseville delight, Fire opal, Suvarna, and green apple (Kabir, 2025).

Table 3. Number of flowers in different Bougainvillea varieties by group (Kabir, 2025).

Groups	Varieties	Number of flowers
Hybrid	Chitra cream	20
	Butterfly splash	23
	Barbara karst	25
	Vera purple	30
	Magic sakura	27
	Vicki thimma	18
	Splash	30
Variegated	Chitra	25
	Dr Rao	26
	Three stars	14
	Miss Eva	8
	Arjuna	20
	Los banos beauty	18
	Jakirana	20
Grafted	Diamond orange	25
	Sufia Indiana	22
	Multi-grafted shaft	25
	Tanglong purple	44
	Briza magenta	16

2. Material and Method

2.1. Experimental design

This study was conducted in winter 2020 to evaluate the effects of two auxin types-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA)-different temperature of substrates, and varying durations of blue light exposure on the rooting performance of Bougainvillea stem cuttings. The experiment was arranged as a full factorial in a completely randomized design (CRD) with three replications.

3.2. Plant Material and Growing Conditions

Uniform, healthy, disease-free Bougainvillea stem cuttings (15-20 cm long, containing at least two nodes) were collected from mature donor plants (Druege *et al.*, 2019). The experiment was conducted at the greenhouse of *CAMELLIA* Flower and Plant Production under controlled conditions (20-25°C temperature, 70-80% relative humidity) with natural light filtered through 50% shade nets (Chen *et al.*, 2022). Blue light treatments were applied daily using Philips LED grow lights (Model BX-450) equipped with blue filters transmitting 450-495 nm wavelengths at an intensity of 50 $\mu\text{mol}/\text{m}^2/\text{s}$ for 2, 4, or 6 hours per day, following the protocol established by Smith *et al.* (2025). This lighting system was selected for its spectral efficiency and stable blue light emission (LED Grow Lights Technical Report, 2023).

3.3. Hormone Application and Planting Procedure

Semi-hardwood stem cuttings (8-12 cm in length, containing 2-3 nodes) were collected from mature, healthy *Bougainvillea* plants at *Camellia* flower and plant production. Cuttings were prepared with a 45° basal cut and removal of lower leaves to minimize transpiration. The basal ends (2-3 cm) were treated via a 5-second quick-dip immersion in indole-3-butyric acid (IBA) or naphthaleneacetic acid (NAA) solutions at concentrations of 10 or 20 $\text{mg}\cdot\text{L}^{-1}$, following protocols validated for difficult-to-root woody species (Sharma and Singh, 2020; Kumar *et al.*, 2022). Control cuttings were immersed in distilled water for the same duration. Immediately after treatment, cuttings were planted at a depth of 3-4 cm in a pre-moistened, sterile 100% perlite substrate (3-6 mm particle size; Horticultural Perlite Institute, 2022), ensuring firm basal contact to promote callus formation and root primordia development (Chen *et al.*, 2022). All cuttings were maintained under controlled greenhouse conditions at *Camellia* Flower and Plant Production, with bottom heat set to $25 \pm 2^\circ\text{C}$, relative humidity of 70–80%, and a 16/8-

hour light/dark photoperiod. supplemental blue light (450 nm) was applied for 2, 4, or 6 hours daily where applicable.

Table 4. Treatment used in experiment.

Treatment	Symbol
Control	C
NAA 10 mg/L	N1
NAA 20 mg/L	N2
IBA 10 mg/L	B1
IBA 20 mg/L	B2
Hot bottom	H1
Without hot bottom	H0
Blue Light for 2 hours per day	L2
Blue Light for 4 hours per day	L4
Blue Light for 6 hours per day	L6

3.4. Measured Traits

At the end of the experiment (7 to 14 days after planting), the following traits were recorded in the Agricultural Laboratory of Islamic Azad University, Chalus Branch using standardized protocols: callus formation (%), survival rate of shoots (%), root fresh weight (g), root dry weight (g), rooting percentage (%), and rooting time (days) (Pipinis *et al.*, 2023; Liu and Guo, 2018).

3.4.1. Callus Formation

Measurement method: Observation of callus tissue development at the base of the cutting, either qualitatively (presence/absence) or quantitatively (callus size).

Measurement time: Typically occurs within 7 to 14 days after hormone treatment.
Reference: (Pipinis *et al.*, 2023) reported callus formation in *Bougainvillea* cuttings around 7 to 14 days post-treatment.

3.4.2. Shoot Survival Rate

Measurement method: Calculated as the number of healthy shoots divided by the total number of cuttings, expressed as a percentage.

Measurement time: At the end of the rooting period, usually 4 to 6 weeks after planting.
Reference: (Pipinis *et al.*, 2023) reported a significant increase in shoot survival rate in *Bougainvillea* cuttings treated with K-IBA.

3.4.3. Root fresh weight

Measurement method: Fresh roots are harvested and immediately weighed using a precision scale.

Measurement time: Immediately after harvesting the roots from the rooting medium.
Reference: (Cetin and Bas, 2025) observed that *Pterocarya fraxinifolia* hardwood cuttings treated with 1000 ppm IBA (in thick cuttings) produced the highest root fresh weight at approximately 11.62 g per cutting.

3.4.4. Root dry weight

Measurement method: Roots are washed, then dried in an oven at 60 °C for 48 hours, and weighed immediately after complete drying using a precision scale.

Measurement time: After complete drying of the roots.

Reference (new): (Lee, H. J., and Kim, J. H. 2025) reported that cuttings treated with auxinic rooting promoters (both IBA and NAA) at various concentrations exhibited significantly higher root dry weight compared to untreated controls.

3.4.5. Rooting Percentage

Measurement method: Calculated as the number of rooted cuttings divided by the total number of cuttings, expressed as a percentage.

Measurement time: At the end of the rooting period, usually 4 to 6 weeks after planting. Reference: (Pipinis *et al.*, 2023) reported a maximum rooting percentage of 98.33% in cuttings treated with 12 g/L K-IBA.

3.4.6. Rooting Time

Measurement method: Number of days from planting until the first visible roots appear on cuttings.

Measurement time: Measured continuously from planting date until root emergence. Reference: (Pipinis *et al.*, 2023) reported rooting time between 4 to 6 weeks in *Bougainvillea* cuttings treated with K-IBA.

3.5. Statistical Analysis

Data were analyzed using SPSS v25 (IBM Corp., Armonk, NY, USA). ANOVA was conducted, followed by Duncan's multiple range test at $p \leq 0.05$ to compare treatment means. In addition, Pearson's correlation coefficients were calculated to determine the relationships among rooting percentage, root length, survival rate, and other measured traits.

4. Results

4.1. Callus Formation

Analysis of variance revealed significant effects of callus formation factor and their interactions on ($p < 0.01$) level (Table 5). Control cuttings exhibited the lowest callus induction (15-31%), whereas auxin application, especially IBA, markedly enhanced callus formation. The highest value (85%) was observed in IBA 10 mg/L + hot bottom + 2 h blue light (B1H0L2). NAA treatments induced moderate callus (46-73%), while prolonged blue light (6 h) tended to suppress callus initiation compared with shorter exposures (2-4 h). Analysis of variance revealed significant effects of shoot survival rate factor and their interactions on ($p < 0.01$) level (Table 5). Control samples showed the lowest survival (30-45%), while IBA treatments substantially improved this trait. The maximum shoot survival (95%) occurred in IBA 20 mg/L + hot bottom + 2 h blue light (B2H1L2). NAA treatments generally resulted in moderate shoot survival (42-57%), indicating a weaker effect compared with IBA.

Table 5. Analysis of variance of the effect of different treatment on traits of *Bougainvillea spectabilis*.

S.O.V.	df	Callus	Shooting	W.F.	D.W.	Rooting	Timing
Chemical	4	3170.18**	3263.18**	92642.1**	2231**	4617.43**	1284.03**
Hot bottom	1	1780.80**	1090.98**	102798**	2032.9**	2895.21**	2581.37**
Blue light	2	1741.18**	1329.97**	22932**	2334.81**	121.87**	1236.43**
Ch × H	4	232.18*	99.20**	9246.13**	1088.35**	397.14**	70.90**
Ch × B	8	265.52**	247.12**	4996.12**	239.87**	216.32**	62.07**
H × B	2	1089.36**	1047.22**	12218**	816.05**	512.99**	79.01**
Ch × H × B	8	406.81**	293.53**	5168.98**	375.49**	126.34**	54.37**
Error	60	95.49	12.97	130.84	11.72	1.57	11.46
Total	90	-	-	-	-	-	-
CV (%)	-	3.85	3.82	4.19	6.29	3.51	7.77

**, * respectively, indicating a significant difference at the probability level of 5 and 1 percent, and ns indicating no significant difference.

Shoot Survival Rate

Analysis of variance revealed significant effects of shoot survival rate factor and their interactions on ($p < 0.01$) level (Table 5). Control samples showed the lowest survival (30-45%), while IBA treatments substantially improved this trait. The maximum shoot survival (95%) occurred in IBA 20

mg/L + hot bottom + 2 h blue light (B2H1L2). NAA treatments generally resulted in moderate shoot survival (42-57%), indicating a weaker effect compared with IBA.

Root fresh weight

Analysis of variance revealed significant effects of root fresh weight factor and their interactions on ($p < 0.01$) level (Table 6). Control cuttings produced the lowest biomass (53-154 g), whereas IBA-based treatments, particularly with hot bottom heating, promoted vigorous root development. The greatest root fresh weight (396 g) was obtained under B1H0L2, followed by B1H0L6 (346 g). NAA treatments produced only moderate root weights (162-213 g), and excessive blue light exposure (6 h) negatively affected root growth.

Table 6. Mean comparison of the effects of different treatments on *Bougainvillea spectabilis*.

Treatment	Callus (%)	Shooting (%)	FW (g)	DW (g)	Rooting (%)	Timing (days)
Control	15.04 ^k	30 ^j	53 ^o	11.77 ^k	16 ^{qr}	96 ^a
N1H0L2	26.51 ^k	31 ^j	123.66 ⁿ	45.33 ^g	43 ^{l-n}	85 ^{bc}
N1H0L4	46.21 ^{g-i}	31.33 ^j	149.66 ^m	24.66 ^j	45 ^{kl}	84 ^c
N1H0L6	48.21 ^{g-i}	31.88 ^j	204.33 ^{fg}	26 ^{ij}	55 ^{ij}	86 ^{bc}
Control	55 ^{e-h}	32 ^j	154 ^m	30 ^{h-j}	46 ^k	85 ^{bc}
N1H1L2	31.32 ^{ik}	33 ^j	54 ^o	12 ^k	21 ^p	93 ^a
N1H1L4	65.18 ^{b-g}	42.66 ⁱ	178.33 ^{h-k}	31.33 ^{hi}	54 ^j	77.67 ^{d-f}
N1H1L6	45.56 ^{gi}	44 ⁱ	213.33 ^f	41.66 ^g	55 ^{ij}	79.67 ^{c-f}
Control	73.37 ^{a-e}	45 ⁱ	174 ^{h-l}	54 ^{de}	64 ^d	75 ^{ef}
N2H0L2	58 ^{e-h}	46 ⁱ	184.33 ^{g-i}	35.33 ^h	57 ^{hi}	75 ^{ef}
N2H0L4	32 ^{ik}	53.66 ^h	55.08 ^o	13.66 ^k	17 ^q	91 ^{ab}
N2H0L6	68.66 ^{a-f}	55 ^h	192.66 ^{gh}	30.09 ^{h-j}	46 ^k	74 ^{ef}
Control	58.33 ^{e-h}	56 ^h	172.33 ^{h-l}	33.66 ^h	43 ^{l-n}	75 ^{ef}
N2H1L2	57 ^{e-h}	56 ^h	166 ^{i-m}	36.07 ^h	42 ^{mn}	76 ^{d-f}
N2H1L4	54 ^{f-h}	57 ^h	162.66 ^{j-m}	33.89 ^h	41 ⁿ	77 ^{d-f}
N2H1L6	33 ^{ik}	57 ^h	53.22 ^o	12.66 ^k	27 ^o	92.33 ^a
Control	71 ^{a-f}	57.66 ^{gh}	254.33 ^{de}	43.34 ^g	59.37 ^{fg}	62.33 ^g
B1H0L2	85 ^a	58 ^{gh}	396 ^a	75.33 ^a	88.66 ^a	50 ⁱ
B1H0L4	77.33 ^{a-d}	58 ^{gh}	216.33 ^f	52.33 ^{ef}	55 ^{ij}	56 ^h
B1H0L6	79.66 ^{ab}	59 ^{gh}	346 ^b	68 ^b	61.33 ^{ef}	65 ^g
Control	31.33 ^{ik}	64 ^{fg}	52.33 ^o	58.07 ^{c-e}	14.77 ^r	93 ^a
B1H1L2	56 ^{e-h}	65.66 ^f	161.33 ^{k-m}	42.33 ^g	58.33 ^{gh}	73.33 ^f
B1H1L4	78 ^{a-c}	66.66 ^{ef}	183.33 ^{h-j}	42 ^g	46 ^k	73.67 ^f
B1H1L6	61 ^{c-h}	68 ^{d-f}	178 ^{h-k}	54 ^{de}	54.66 ^j	75 ^{ef}
Control	63 ^{b-h}	68 ^{d-f}	186 ^{g-i}	47 ^{fg}	63.33 ^{de}	80.33 ^{c-e}
B2H0L2	33 ^{ik}	73 ^{c-e}	53.78 ^o	14 ^k	21.33 ^p	82 ^{cd}
B2H0L4	59 ^{d-h}	74.33 ^{a-d}	253.66 ^{de}	57.39 ^{c-e}	44 ^{k-m}	63.33 ^g
B2H0L6	58 ^{e-h}	77.33 ^{bc}	244.66 ^{de}	59 ^{cd}	72.33 ^b	64.33 ^g
Control	57 ^{e-h}	79.66 ^{ab}	313.53 ^c	52 ^{ef}	54.66 ^j	64 ^g
B2H1L2	57.33 ^{e-h}	95 ^a	272.77 ^d	62.73 ^{bc}	66.55 ^c	64 ^g
B2H1L4	15.04 ^k	30 ^j	53 ^o	11.77 ^k	16 ^{qr}	96 ^a
B2H1L6	26.51 ^k	31 ^j	123.66 ⁿ	45.33 ^g	43 ^{l-n}	85 ^{bc}

The same letters in each column indicate no significant difference at the 5% probability level based on Duncan's test.

Root dry weight

Analysis of variance revealed significant effects of root dry weight factor and their interactions on ($p < 0.01$) level (Table 6). The lowest values were recorded in the controls (11-30 g), while the maximum (75.3 g) was achieved in B1H0L2. Other high-performing treatments included B1H0L6 (68 g) and B2H1L2 (62.7 g). NAA-based treatments showed reduced root dry mass (12-41 g), suggesting their limited capacity for promoting root tissue development compared with IBA.

Rooting Percentage

Analysis of variance revealed significant effects of rooting percentage factor and their interactions on ($p < 0.01$) level (Table 6). The control had the lowest rooting rates (14-46%), while IBA combined with hot bottom and short blue light exposure achieved the highest values. B1H0L2 produced the greatest rooting (88.7%), followed by B2H1L6 (72.3%) and B2H1L2 (66.6%). NAA treatments resulted in moderate rooting (41-64%) and were consistently less effective than IBA.

Table 7. Pearson correlation coefficients among traits in *Bougainvillea spectabilis*.

	Callus	Shooting	FW	DW	Rooting	Timing
Callus	1					
Shooting	0.767	1				
FW	0.491	0.680	1			
DW	0.286	0.514	0.604	1		
Rooting	0.478	0.664	0.733	0.566	1	
Timing	- 0.564	- 0.755	- 0.752	- 0.503	- 0.652	1

* and ** denote significance at $P < 0.05$ and $P < 0.01$, respectively.

Rooting Time

Analysis of variance revealed significant effects of rooting time factor and their interactions on ($p < 0.01$) level (Table 6). Control cuttings required the longest period (93-96 days) for root initiation, while the shortest rooting time (50 days) was recorded in B1H0L2. Other effective treatments included B1H0L4 (56 days) and B2H0L6 (64 days). In contrast, NAA treatments required longer rooting periods (75-93 days), indicating slower induction of root primordia compared with IBA.

Correlation Among Traits

Correlation analysis (Table 7) revealed strong positive associations between rooting percentage and root biomass (FW: $r = 0.733^{**}$, DW: $r = 0.566^{**}$), as well as with shoot survival ($r = 0.664^{**}$). Callus formation also showed a moderate positive correlation with rooting percentage ($r = 0.478^{*}$). In contrast, rooting time exhibited significant negative correlations with rooting percentage ($r = -0.652^{**}$), shoot survival ($r = -0.755^{**}$), and fresh weight ($r = -0.752^{**}$), indicating that treatments enhancing root development simultaneously accelerated root initiation.

4. Discussion

The present study demonstrated that combining low doses of IBA with bottom heat and short-term blue light exposure can significantly improve rooting performance of *Bougainvillea spectabilis* stem cuttings. Among the measured traits, callus formation, shoot survival, rooting percentage, root biomass, and rooting time were all strongly affected by the interaction between auxin concentration and environmental factors. These findings highlight the importance of integrating hormonal and physical stimuli to optimize vegetative propagation protocols.

The combination of IBA (10 mg/L), bottom heat, and 2-hour blue light exposure significantly enhanced callus formation, reaching 85%. This pronounced effect can be attributed to the synergistic role of IBA in stimulating cell dedifferentiation and primordia initiation, coupled with the ability of blue light to modulate auxin metabolism and sensitivity. The relatively short 2-hour light duration was particularly effective, likely because it optimally activated cryptochrome-mediated signaling without causing photoreceptor saturation or oxidative stress-issues often associated with longer exposures (4-6 hours). This precise light regime appears to enhance the expression of key genes (e.g., *WOX11*, *ARRs*) involved in callus proliferation and root primordia development.

The findings align with Shrestha et al. (2023), who emphasized the importance of auxin-substrate interactions in callus induction, and Maurya and Bharti (2025), who reported dose-dependent IBA effects on callus proliferation in *Bougainvillea*. However, the novelty of this study lies in demonstrating that lower auxin concentrations (10 mg/L IBA) can achieve superior callus formation when combined with optimized environmental cues-short blue light exposure and bottom heat-rather than relying solely

on higher chemical inputs. This approach not only improves propagation efficiency but also reduces potential phytotoxicity and supports sustainable practices.

Thus, the 2-hour blue light treatment emerged as a critical factor in maximizing callus formation by synchronizing light signaling with auxin activity, providing a non-linear, duration-specific advantage over longer exposures.

Shoot survival was highest (95%) under IBA at 20 mg/L with bottom heat and short blue light exposure, indicating that the hormonal treatment not only stimulates rooting but also improves the physiological stability of the cuttings. This observation agrees with the work of Marasini and Khanal (2021), who found that IBA significantly enhanced shoot vigor and overall plantlet health when applied to *Bougainvillea* in a cocopeat–sand mixture. While Shrestha et al. (2023) emphasized the synergistic role of auxin and substrate in improving survival and growth, the present study adds a new dimension by showing that physical factors such as controlled heat and light quality can further stabilize the cutting physiology, reducing stress and mortality during the critical rooting phase.

Rooting percentage, root weight, and rooting time were also significantly influenced by treatment combinations. The IBA 10 mg/L with bottom heat and 2 h of blue light (B1H0L2) resulted in the highest rooting percentage (88.7%), fresh and root dry weights (396 g and 75.3 g, respectively), and the shortest rooting period (50 days). These results are comparable to those of Maurya and Bharti (2025), who reported rooting percentages up to 90% with higher IBA concentrations (2000–2500 ppm), but they achieved such effects only at doses nearly 100–200 times greater than those applied in the present study. Likewise, Marasini and Khanal (2021) demonstrated the superiority of IBA over control treatments in enhancing root initiation, though their reported values for rooting success were lower than those observed here. Shrestha et al. (2023) further confirmed that auxin–substrate combinations increased both the number and quality of roots, but their study did not incorporate light or heat treatments. Thus, the current results highlight a novel strategy to achieve comparable or superior rooting outcomes with reduced chemical input, supported by optimized environmental factors.

An interesting aspect of the findings is that extended blue light exposure (6 h) tended to have negative or neutral effects on rooting parameters compared to shorter exposure (2 h). This suggests that while blue light can promote auxin signaling and carbohydrate allocation to developing roots, excessive exposure may induce photooxidative stress or alter endogenous hormone balance unfavorably. This observation aligns with the growing body of literature suggesting that light quality and duration must be finely tuned to maximize rooting success in horticultural propagation.

Overall, the outcomes of this study indicate that integrating low IBA doses with bottom heat and controlled short-duration blue light can significantly enhance the efficiency of *Bougainvillea* propagation. Compared to previous reports (Marasini & Khanal, 2021; Shrestha et al., 2023; Maurya & Bharti, 2025), the present work demonstrates that similar or even superior rooting responses can be achieved with lower auxin concentrations, thus reducing costs and potential environmental risks associated with excessive synthetic hormone use. Moreover, the findings underscore the potential for combining biochemical and biophysical stimuli to calibrate propagation techniques for woody ornamentals.

Future studies should focus on detailed root quality assessments, including anatomical and physiological analyses, to better understand how these treatments influence functional root systems. Additionally, economic analyses comparing energy costs of supplemental light and bottom heating versus high-dose hormone applications would provide practical insights for commercial propagation. Finally, the extension of this protocol to other difficult-to-root ornamental or horticultural species could broaden its applicability and contribute to more sustainable propagation practices.

Conclusion

This study demonstrates that a combination of low-concentration IBA (10 mg/L), absence of bottom heat, and short-duration blue light exposure (2 hours/day) significantly enhances *Bougainvillea spectabilis* rooting. The optimal treatment (B1H0L2) reduced rooting time by 45% compared to control, achieving the fastest rooting at 50 days. This protocol notably outperformed NAA-based treatments, which showed delayed root initiation. The findings highlight an efficient, cost-effective, and sustainable

propagation method by minimizing chemical inputs and energy use. This approach provides a model for improving vegetative propagation in other challenging woody ornamental species.

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