Adv. Hort. Sci., 2026 40(2): 181-192
DOI: 10.36253/ahsc-18903
https://oaj.fupress.net/index.php/ahs

AHS
Advances in Horticultural Science

Influence of biostimulants and indole-3-butyric acid on seasonal rooting performance, growth, quality and biochemical dynamics of difficult-to-root *Bougainvillea* 'Thimma'

B. Kumar [1], S. Singh [1 (*)], P. Goyal [2], R.K. Dubey [1]

[1] *Department of Floriculture and Landscaping, College of Horticulture and Forestry, Punjab Agricultural University, Ludhiana-141004, Punjab, India.*

[2] *Department of Biochemistry, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana-141004, Punjab, India.*

(*) **Corresponding author:**
simratflori@pau.edu

Citation:
KUMAR B., SINGH S., GOYAL P., DUBEY R.K., 2026 - *Influence of biostimulants and indole-3-butyric acid on seasonal rooting performance, growth, quality and biochemical dynamics of difficult-to-root Bougainvillea 'Thimma'* - Adv. Hort. Sci., 40(2): 181-192.

ORCID:
KB: 0009-0006-9401-9723
SS: 0000-0001-9571-9955
GP: 0009-0000-4479-0479
DRK: 0000-0001-7433-3033

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no conflict of interests.

Received for publication 18 September 2025
Accepted for publication 2 February 2026

Key words: *Bougainvillea peruviana*, cutting, hardwood, indole-3-butyric acid, peroxidase, polyphenol oxidase.

Abstract: Achieving higher percentage and uniform rooting in difficult-to-root variegated *Bougainvillea* cultivars remain a challenge for nurserymen. In this study, 20 cm hardwood cuttings of *Bougainvillea peruviana* 'Thimma' were treated with different biostimulants including seaweed extract, coconut water, willow bark extract, Saridex powder and indole-3-butyric acid (IBA) at concentrations ranging from 500 to 6000 mg L^{-1} during rainy and spring seasons. The cuttings were placed into sand beds under partial shade in completely randomized design in three replications. Results indicated that cuttings treated with IBA at 4000 mg L^{-1} during the rainy season yielded promising outcomes, with earliest root initiation (33.8 days), highest survival rate (80.7%), and maximum rooting (70.0%). Coconut water-treated cuttings also showed comparable results, with a survival rate of 60.0% and a rooting percentage of 53.3% compared to control. Biochemical analysis further revealed seasonal variation across treatments, with total soluble sugars ranging from 60 to 220 mg g^{-1}, proteins from 20 to 45 mg g^{-1}, and polyphenol oxidase activity from 25 to 130 µ ml^{-1}. The findings indicate that treating *Bougainvillea* 'Thimma' cuttings with IBA at 4000 mg L^{-1} during the rainy season enhances rooting and survival percentage, improves plant quality, and increases propagation efficiency.

1. Introduction

Bougainvillea commonly known as 'Glory of the Tropics' is a popular hardy perennial, widely used in landscaping as a shrub, climber, ground cover, hedge and as a potted specimen (Shrestha *et al.*, 2023). The

Bougainvillea genus belongs to the *Nyctaginaceae* family comprising 14 species and is a native of South America (Datta *et al.,* 2021). The species of horticultural importance include *B. spectabilis* Willd., *B. glabra* Choisy, and *B. peruviana* Bonpl. (Datta, 2022). The shrub is appreciated for its vibrant and colourful bracts that add varying vibrant hues to the surroundings and is propagated vegetatively (Asif *et al.,* 2024).

Bougainvillea is multiplied through asexual means through hardwood cuttings, layering, and budding. Hardwood cuttings of *Bougainvillea* 'Sweet Heart', 'Dr. HB Singh' 'Red September', 'Thimma', 'Mahatma Gandhi' and 'Manila Red' are difficult-to-root and require optimal conditions for rooting (Kumar *et al.,* 2021). Stem cuttings are widely adopted for multiplication but often yield inconsistent rooting (authors personal observation). The rooting and survival percentage of cuttings is not high enough without use of rooting hormones (Singh *et al.,* 2021; Yoon *et al.,* 2021; Shrestha *et al.,* 2023). Successful outcome of rooting of cuttings depends on several endogenous factors (Hartmann *et al.,* 2013) such as variety, genetic traits, cutting type, anatomical structure, nutrient and physiological status (Khan *et al.,* 2022), along with exogenous factors including humidity, light, season, timing and growth substances such as biostimulants (Tripathi *et al.*, 2022). Auxin derivatives (Pereira *et al.,* 2021) and dilution carriers (Mofokeng *et al.,* 2020) have been known to significantly enhance root and shoot development.

The difficulty in propagation arises from the plant's limited ability to form adventitious roots (ARs), which is crucial for its perpetuation (Ma *et al.,* 2024). Variegated *Bougainvillea* cultivars are harder to root because they have reduced photosynthetic capacity due to leaf variegation, involve complex tissue patterns and are relatively sensitive to rooting environment. Further, complications related to root development in cuttings often lead to significant commercial losses (Schäfer *et al.,* 2022; El Malahi *et al.,* 2024). To enhance rooting, growers commonly use exogenous auxins of both organic and chemical origin (Gomes and Scortecci, 2021; Li and Yuan, 2025). The biostimulants of organic origin such as coconut water have emerged as viable options to improve rooting success in cuttings. Auxin (IBA) is well-known for enhancing rooting of cuttings (Yoon *et al.,* 2021) by enhancing carbohydrate (CHO) accumulation as energy source for ARs formation, while endogenous peroxidase activity may hinder root initiation (Lee *et al.,* 2025). Studies reveal that easy-to-root cultivars of *Bougainvillea* had higher contents of CHOs and lower Nitrogen (N) compared to those that are difficult-to-root (Huang *et al.,* 2022). In easy-to-root cultivars, the carbohydrate (CHO) content ranged from 24.0% to 40.6% and nitrogen (N) content varied between 2.2% and 2.5%, whereas in difficult-to-root cultivars such as 'Refulgens', 'Thimma' and 'Mahatma Gandhi', CHO and N contents ranged from 21% to 38.0% and 1.93% to 3.9%, respectively (Pratiksha *et al*., 2017).

Research on the rooting of hard-to-root *Bougainvillea peruviana* Bonpl. cv. Thimma is limited, especially regarding seasonal effects and the influence of biostimulants. This study hypothesized that treating cuttings of *Bougainvillea* cv. Thimma with biostimulants and IBA across different seasons would enhance rooting. Therefore the study was planned with an objective to evaluate the effects of biostimulants and IBA on rooting, growth, quality, and biochemical traits of hard-to-root cuttings of *Bougainvillea* during the rainy and spring seasons.

2. Material and Methods

Experimental site

The experiment was conducted at research farm of department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana, Punjab, India. The site is located at 30.9°N latitude, 75.85°E longitude, and elevated at 267 m a.s.l. The site experiences a humid subtropical to hot semi-arid climate with four distinct seasons. The soil is characterized as sandy loam, moderately fertile, slightly alkaline (pH 8.2), and had relatively low water-holding capacity.

Plant material

A 20 cm long approximately pencil-thick hardwood cuttings of *Bougainvillea* cv. Thimma comprising 6 to 7 nodes were excised from healthy stock plants during end-August 2023 (rainy season) and mid-March 2024 (spring season). A smooth slanting cut (45^o) was given one-cm above distal node and another circular cut was given one-cm below the node at proximal end (Fig. 1 a). The cuttings were immersed in sodium hypochlorite (0.5%) for surface sterilization for 5 min. Thereafter, the cuttings were washed in deionized water and were subjected to different rooting treatments.

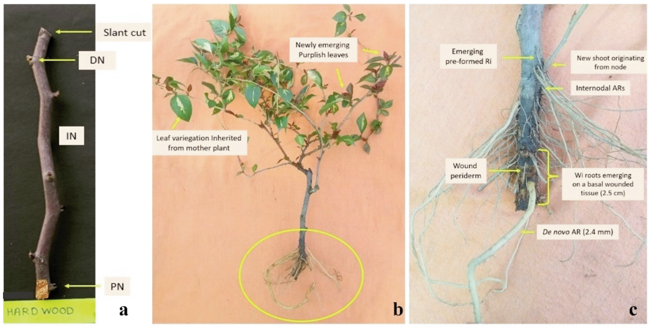

Fig. 1 - Hardwood cutting of bougainvillea (a); Rooted cutting of *Bougainvillea* var 'Thimma' 180 days after sticking of cuttings in rooting media (b); Emergence of multiple ARs originating in response to wounding (Wi roots) and from performed root initials (Ri) at basal stem tissue; mass of undifferentiated cells (callus) can also be seen over the wound tissue (c). DN= Distal node; PN= Proximal node; IN= Internode.

Treatment details

The treatments were broadly categorized under 'biostimulants', that contain a complex mixture of bioactive compounds, known to promote rooting indirectly by enhancing multiple physiological pathways. The auxin (IBA) treatments are hereby referred to as 'true PGR', directly influencing the plant's rooting hormonal pathways. Different treatments comprised 24h basal (1.5 cm) dip of cuttings in distilled water (dw; control), coconut water (100%), seaweed extract (100 ml L^{-1} water), willow bark extract (500 mL L^{-1} water) and Saridex powder (type hard-to-root; 60 g powder mixed in 50 ml water to make a thick paste). Immature green coconuts were drilled through the eye and water was collected in a clean container. Seaweed extract (28% w/w) was procured from Indian farmers fertiliser cooperative limited (IFFCO) traded as 'Sagarika' that contained a concentrated liquid extract from red and brown seaweed sap. A mature loose bark of willow tree (*Salix babylonica* L.) was extracted with knife. The bark was cleaned of any debris and dipped in once hot-boiled water and kept overnight. Next day, the decoction was sieved and collected in a clean container for treating the cuttings. The auxin treatments comprised Indole-3-butyric acid (IBA) i.e.: 500, 1000, 2000, 4000 and 6000 mg L^{-1} water for a holding period of 15, 7.5, 3.5, 1.5 and 0.5 min respectively. The laboratory grade active auxin-IBA (98% pure) with molecular weight 203.24 g mol^{-1} was procured from Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai, India. A stock solution of IBA (6000 mg L^{-1}) was prepared first by dissolving 6 g IBA in 20 ml ethanol (70%) ensuring the solution is clear of any precipitates. Thereafter, the volume was made to 1000 ml with DW and thereafter, this solution was preserved in an amber colored bottle labeled appropriately. The stock solution was further diluted with DW to make up the volume to 200 ml for formulating other concentrations (working solution) of IBA (i.e.: 500, 1000, 2000 and 4000 mg L^{-1}). The working solution for each of the IBA concentration was 200 ml.

Experimental procedure and growth measurements

Each treatment was replicated three times with 10 cuttings per replication, totaling 30 cuttings per treatment. Cuttings were planted in coarse sand (2 mm particle size), which was kept moist throughout the rooting phase. The phenological trait namely days to leaf sprouting was recorded with the emergence of first leaf from nodes from the date of sticking of cuttings. To assess root primordia initiation (2 mm outgrowth), six cuttings per treatment (two per replication) were examined bi-weekly, beginning 30 days after planting. Observations on survival percentage, rooting percentage, mean root count, and maximum root length were recorded after 180 days of sticking cuttings in rooting media. Growth traits including number of leaves, stem collar diameter, plant height and longest shoot length were measured. Quality attributes such as sturdiness quotient (SQ), Dickson's quality index (DQI) (Dickson *et al.,* 1960), and shoot-root ratio were also calculated to evaluate the vigour of rooted cuttings as per the following equation:

$$\text{Sturdiness Quotient (SQ)} = \frac{\text{Height of plant (cm)}}{\text{Collar diameter (mm)}}$$

$$\text{DQI} = \frac{\text{Total plant dry weight (g)}}{\left(\frac{\text{Height (cm)}}{\text{Collar diameter (mm)}}\right) + \left(\frac{\text{Shoot dry weight (g)}}{\text{Root dry weight (g)}}\right)}$$

The shoot:root ratio was derived from the respective dry weights of the aboveground and belowground biomass of *Bougainvillea*, expressed as a shoot to root (S:R) ratio.

Anatomical observation

To study the changes in cell de-differentiation and phases for root primordia formation, hand sectioning of basal 0.5 to 1 mm thick tissue of cut end was done with a sharp razor blade. The tissue was excised 30 days after cuttings were planted in coarse sand media. The freshly cut tissue sections (L.S.) were gently washed with dw to remove any dirt or debris. The cut sections were stained with a 1% Aniline blue dye to temporary stain the cells revealing division and differentiation during induction of root primordia. After staining, the sections were placed on clean glass slides over which a drop of glycerol was poured as a mounting medium to prevent the sample from drying prior to gently covering with a coverslip, avoiding air bubbles. The anatomical observations were viewed under AXL binocular compound microscope (Labovision) at 40X magnification to observe the cellular layers and root primordia formation. For scaling, the images were processed in ImageJ software (Schneider *et al.,* 2012) following calibration using line tool for measuring a known reference length. The scale bar was set by entering the known distance and units. The scale bars were added with custom adjustments for font size, position of bar and colour as needed. The images were then saved with the embedded scale bars for presentation.

Biochemical analysis

The biochemical analyses were performed on rooted cuttings (n=6) at 180 days post-planting of cuttings. From each replication per treatment, three healthy cuttings were gently removed from the rooting medium with their roots intact for biochemical determination as per established protocols. Total soluble sugars (TSS) were estimated by the phenol-sulphuric acid method (Nielsen, 2024). One gram of root tissue was extracted twice with 10 mL of 80% ethanol in a boiling water bath (20 min), then centrifuged at 5,000 rpm for 5 min. Lead ions were removed using lead acetate and sodium oxalate. A glucose standard curve (100 to 1,000 µg) was prepared. For each standard/sample, 5% phenol, dw (0.1-0.9 mL), and 5 mL concentrated H_2SO_4 were added, and absorbance was recorded at 490 nm and was expressed in mg g^{-1} of tissue.

Total protein content was determined using the Lowry method as described by Lowry *et al.* (1951). Root tissue (0.5 g) was homogenized in 5 mL phosphate buffer and the extract was centrifuged at 8,000 rpm for 20 min to clarify the solution. Protein was precipitated from the supernatant by adding 1 mL of 20 % trichloroacetic acid (TCA) and incubating for 30 min, followed by centrifugation. The resulting pellet was washed with acetone, re-dissolved in buffer, and the protein content was quantified colorimetrically. Absorbance was measured at 660 nm in spectrophotometer (Infitek SP IV722G 721G 100 Single beam Visible, 325-1100 nm), and concentrations were calculated against a standard curve prepared from known protein standards and expressed as mg L^{-1}.

Polyphenol oxidase (PPO) activity was determined following a standard protocol (Esterbauer *et al.,* 1977). One gram of root tissue was homogenized in 10 mL phosphate buffer, centrifuged at 10,000 rpm at 4°C for 20 min, and the crude extract was retained. Thereafter, 0.2 mL of extract was mixed with 2.5 mL of catechol substrate and incubated at 37°C for 30 min. PPO activity (Unit ml^{-1} or U ml^{-1}) was determined by measuring the absorbance change per minute at 410 nm wavelength in spectrophotometer.

Peroxidase activity was assayed using fresh root tissue (0.5 g), which was homogenized in 5 mL of chilled 0.1 M phosphate buffer (pH 6.8) and centrifuged at 15,000 rpm for 30 min at 4°C. The supernatant was used as the enzyme extract. POD activity was determined by the o-dianisidine method: the reaction mixture contained 2.4 mL phosphate buffer, 0.1 mL o-dianisidine (0.1 %), 0.1 mL hydrogen peroxide (0.2%), and 0.4 mL enzyme extract. The increase in absorbance at 460 nm was recorded over 2 to 3 min to monitor the oxidation of o-dianisidine by peroxidase. Enzyme activity was expressed (Unit g^{-1} Fresh weight or U g^{-1} FW) based on the rate of change in absorbance per unit time.

Statistical analysis

Descriptive statistics, including means ± standard error (SE), were calculated using Microsoft Excel (Office Home & Student, version 2501). Treatment effects were evaluated by two-way analysis of variance (ANOVA) using the general linear model in SPSS software (version 27, IBM). Homogeneity of variance and other ANOVA assumptions were verified prior to analysis. Significant effects were further examined by Tukey's honest significant difference (HSD) post-hoc test to compare treatment means at a significance threshold of p= 0.05.

3. Results

Leaf sprouting and rooting characteristics

The interaction between treatment and season for leaf sprouting was not significant (p= 0.236), indicating that the response to biostimulant and IBA treatments was consistent across seasons and therefore, treatment means can be interpreted independently of season (Table 1). Among treatments, IBA 1000 mg L^{-1} recorded significantly earlier (1.3 days) leaf sprouting relative to control. The biostimulant treatments and IBA above 1000 mg L^{-1} showed delayed sprouting and were found comparable to control. Rainy-season cuttings sprouted leaves approximately five days earlier compared to those planted in spring.

A highly significant T×S interaction ($p = 0.000$) was observed for days to root initiation, demonstrating that treatment effects depended strongly on season, where roots emerged relatively 6 days earlier during the rainy season than in spring. This seasonal advantage was more pronounced under IBA treatments, particularly IBA 2000 to 4000 mg L^{-1}, which showed relatively earlier root initiation, during the rainy season (Table 1). In contrast, biostimulant treatments (seaweed extract, coconut water, willow bark extract) including saridex powder and control exhibited higher seasonal responsiveness, with cuttings taking over 50 days to emerge roots during spring.

The interaction between treatment and season was also found significant ($p = 0.007$), for root elongation, indicating a differential response to treatments across seasons. IBA (4000 and 6000 mg L^{-1}) produced substantially longer roots (over 20.0 cm) during the rainy season, which got reduced (over 24% to 30.0%) during spring (Table 1). Biostimulant treatments showed moderate root elongation with limited seasonal variation, except for cuttings treated with willow bark extract that had almost 5-times shorter root length in spring relative to rainy season. Higher IBA dose (up to 4000 mg L^{-1}) were more effective in root elongation in rainy season.

A highly significant T×S interaction was also recorded for root number ($p = 0.000$). The mean root count for IBA-treated cuttings was higher compared to those treated with biostimulants during rainy season.

The biostimulant treatments including control showed minimal seasonal plasticity, yielding consistently lower mean root count during both the seasons, except for coconut water treated cuttings that had 1.5-times higher root count in rainy season cuttings relative to spring (Table 1).

Survival and rooting percentage

The TxS interaction for mean survival and rooting percentage was found non-significant ($p=0.214$), indicating treatment means can be interpreted

Table 1 - Effect of biostimulants and Indole-3-butyric acid on days to leaf sprout and rooting characteristics of *Bougainvillea* cuttings planted during rainy and spring season

Treatment (T)	Leaf sprout (days)			Root initiation * (days)			Maximum root length (cm)			Root count (No.)		
	Season (S)		Mean	Season (S)		Mean	Season (S)		Mean	Season (S)		Mean
	Rainy	Spring		Rainy	Spring		Rainy	Spring		Rainy	Spring	
Control (dw)	8.7	14.3	11.5 a	47.7	53.7	50.7	4.3	0.2	2.3	2.7	0.3	1.5
Seaweed extract	8.3	13.7	11.0 abc	44.7	51.7	48.2	7.0	7.0	7.0	2.7	6.3	4.5
Coconut water	8.7	14.3	11.5 a	44.7	49.7	47.2	12.2	9.7	10.9	10.0	6.3	8.2
Willow bark extract	8.3	13.0	10.7 abc	46.0	51.7	48.8	8.5	1.7	5.1	2.7	2.7	2.7
Saridex powder	9.0	13.7	11.4 ab	45.7	52.3	49.0	7.8	4.3	6.0	7.0	2.3	4.7
IBA 500 mg L^{-1}	8.3	12.3	10.3 bc	40.3	47.3	43.8	11.7	8.0	9.8	8.3	5.0	6.7
IBA 1000 mg L^{-1}	7.7	12.7	10.2 c	41.3	45.3	43.3	13.7	11.0	12.4	11.7	3.7	7.7
IBA 2000 mg L^{-1}	8.7	13.7	11.2 abc	38.7	43.7	41.2	16.1	12.0	14.1	16.3	7.7	12.0
IBA 4000 mg L^{-1}	8.7	13.7	11.2 abc	37.3	46.7	40.0	21.0	16.0	18.5	22.3	14.0	18.2
IBA 6000 mg L^{-1}	8.7	14.0	11.4 ab	38.3	46.7	42.5	20.4	14.0	17.2	21.3	9.0	15.2
Mean	8.5	13.5		42.5	48.5		12.3	8.4		10.5	5.7	
Sig. of fixed factors	T (0.000); S (0.000)			T (0.000); S (0.000)			T (0.000); S (0.000)			T (0.000); S (0.000)		
	TXS (0.236)			TXS (0.000)			TXS (0.007)			TXS (0.000)		

Significant difference ($p \leq 0.05$) between rooting treatment and seasonal mean values were computed using Tukey's mean separation test and are indicated by different alphabetical letters for varying treatments for days to leaf sprout. Post-hoc multiple comparison tests were not conducted for season because there were fewer than three groups in this factor. The means for above observations were computed from n= 9 cuttings; except for days to root initiation (n=6).

* Any root initials that were >2 mm in length and easily visible with a naked eye.

independently of seasons. The average data of rainy and spring seasons across different biostimulant and IBA treatments revealed significant differences (Fig. 2). The highest mean per cent survival was recorded in cuttings that were treated with IBA (4000 mg L^{-1}), which was over 1.5-fold higher compared to the untreated cuttings (control). At the same IBA level, the cuttings recorded highest rooting percentage, which was nearly 2.5-fold higher compared to the untreated lot. The percentage of rooting declined at highest IBA level, however the reduction was found non-significant compared to IBA (4000 mg L^{-1}). The rooting percentage of coconut water treated cuttings was also found comparable to that observed in IBA treated cuttings at any level, but differed significantly compared to other biostimulant treatments (Fig. 2 a). The per cent survival and rooting of cuttings was significantly higher (1.5 and 0.5-times respectively) in rainy season, compared to those planted during spring (Fig. 2 b).

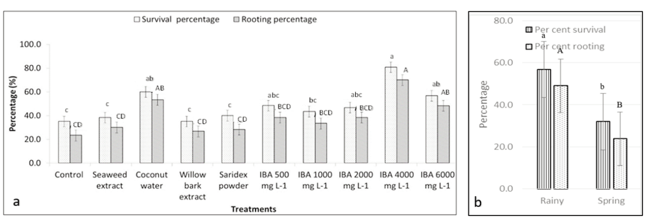

Fig. 2 - Effect of different rooting treatments (a) and seasons (b) on survival and rooting percentage of hardwood cuttings of *Bougainvillea* cv. Thimma. The mean values in column graph (a) represent average of rainy and spring seasons. Columns designated with same case alphabets did not reveal significant differences among the treatment means computed at p≤0.05 level of significance using Tukey's post hoc test.

Dry weight and shoot-root ratios

The interaction among rooting treatments and seasons for root and shoot dry weight, including S:R ratio was found non-significant. The root dry weight revealed mean variation of 0.68 to 2.12 g across the treatments (Fig. 3 a). The IBA-treated cuttings revealed higher mean root dry weight compared to other treatments, and was found comparable to coconut water, except for IBA at 4000 and 6000 mg L^{-1}. The cuttings treated with IBA at 6000 mg L^{-1} had highest root dry weight, however was found non-significant compared to IBA at 2000 and 4000 mg L^{-1}. Coconut water treated cuttings also had nearly twice the dry weight of roots weighed compared to control and variation was found non-significant for IBA-treated cuttings up to 2000 mg L^{-1}. The dry weight of shoots was found highest in cuttings dip treated with IBA at 4000 mg L^{-1} and at higher dose (6000 mg L^{-1}), the dry weight of shoots decreased (12.4%) with non-significant variation (Fig. 3 a). Non-significant variation was observed in dry weight of shoots in cuttings that were dip treated with IBA (500 to 1000 mg L^{-1}) and were found comparable with cuttings treated with coconut water. Seasons had a significant effect on dry weight of shoot and roots. The shoot and root dry weight was measured 1.87-times greater in rainy-season cuttings compared to spring (Fig. 3 b).

The S:R increased with increase in concentration of IBA, however, the ratio decreased significantly (16.3%) at highest IBA dose compared to the S:R ratio of cuttings that were treated with IBA 4000 mg L^{-1} (Fig. 3 a). The coconut water treated cuttings had a comparable S:R ratio with seaweed extract and IBA treated cuttings (500 to 2000 mg L^{-1}), whereas non-significant variation was observed for lineale ratio of cuttings across the seasons (Fig. 3 b).

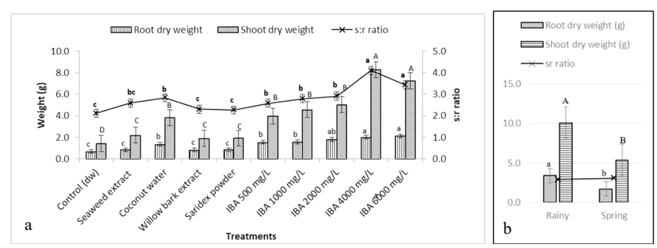

Fig. 3 - Effect of different rooting treatments (a) and seasons (b) on root dry weight (g), shoot dry weight (g) and shoot-root ratio of hardwood cuttings of Bougainvillea cv. Thimma. The mean values in column graph (a) represent average of rainy and spring seasons. Destructive analysis of cuttings (n=9) was done to assess the dry weight of shoots and roots. Columns and line markers designated with same case alphabets are not significantly different as computed by Tukey means separation test at the 0.05 level of confidence. Error bars represent ± SE.

Shoot growth characteristics

The effect of seasons and interaction effects among TxS for mean leaf number was found non-significant, indicating observed variation in leaf number was due to rooting treatments. The cuttings treated with IBA at 4000 mg L^{-1} produced nearly 5-times more leaf count and 4-times longer shoots than the cuttings raised under control (Table 2). The mean leaf count among other IBA concentrations didn't differ significantly, except 500 mg L^{-1}, which had notably fewer leaves. IBA at 500 mg L^{-1} had significantly lower number of leaves compared to cuttings treated with the coconut water. No significant differences in collar diameter were found due to treatment, season, or their interaction (Table 2).

A significant treatment × season interaction ($p \leq 0.05$) was observed for both plant height (p=0.000) and shoot length (p=0.002), highlighting the inconsistency of applied rooting treatments for these traits across the seasons. The height of plants was nearly one-third greater during the rainy season planted cuttings compared to those planted during spring, however, the magnitude of seasonal differences varied among treatments. All the treatments revealed taller plants during rainy season, except for the cuttings that were treated with willow bark extract, where the spring planted cuttings had taller plants (Table 2). The shoots length in rainy season planted cuttings was nearly 20.0% longer, compared to those planted during spring season. Except for the cuttings treated with seaweed extract and coconut water, the length of shoots was found higher for the cuttings planted during rainy season (Table 2).

Quality characteristics

Non-significant TxS interaction was observed for the quality characteristics of cuttings, for SQ and DQI indicating the observed variation was due to rooting media treatments and that was found consistent across the seasons. The SQ of rooted cuttings ranged 3.85 to 1.53 with the highest SQ computed in cuttings that were dip-treated with IBA at 2000 mg L^{-1}, that was found non-significant compared to cuttings dip treated with IBA 4000 mg L^{-1} (Fig. 4 a). Non-significant differences in mean SQ values were found in organic-based biostimulants including saridex powder, however, coconut water treated cuttings had significantly highest SQ value, that was comparable to IBA at 4000 mg L^{-1} and to other biostimulants, including control (Fig. 4 a).

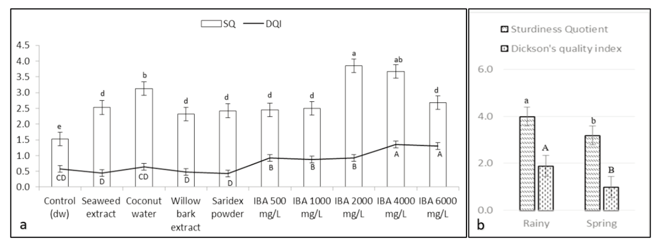

Fig. 4 - Effect of different rooting treatments (a) and seasons (b) for Sturdiness Quotient and Dickson quality index of hardwood cuttings of *Bougainvillea* cv. Thimma. Columns and line markers designated with same case alphabets are not significantly different as computed by Tukey means separation test at the 0.05 level of confidence. Data refer to mean values (n = 10), error bars represent ±SE.

Table 2 - Effect of biostimulants and Indole-3-butyric acid on vegetative characteristics of *Bougainvillea* cuttings planted during rainy and spring season

Treatment (T)	Leaves (No.)			Collar diameter (mm)			Height (cm)			Shoot length (cm)		
	Season (S)			Season (S)			Season (S)			Season (S)		
	Rainy	Spring	Mean	Rainy	Spring	Mean	Rainy	Spring	Mean	Rainy	Spring	Mean
Control	5.3	1.0	3.2 e	9.3	8.0	8.7	20.7	8.7	14.7	9.2	1.3	5.2
Seaweed extract	8.0	7.0	7.5 de	9.2	8.5	8.9	36.7	21.7	29.2	8.8	12.8	10.8
Coconut water	11.0	13.0	12.0 bcd	8.9	8.6	8.8	43.3	33.7	38.5	9.3	15.1	12.2
Willow bark extract	5.0	5.0	5.0 e	8.3	8.7	8.5	21.3	24.7	23.0	6.4	5.0	5.7
Saridex powder	7.7	4.0	5.9 e	9.0	8.4	8.7	26.0	22.0	24.0	8.8	4.2	6.5
IBA 500 mg L^{-1}	8.3	5.0	6.7 e	8.0	8.5	8.3	34.0	15.0	24.5	7.8	3.6	5.7
IBA 1000 mg L^{-1}	9.7	8.7	9.2 cde	8.9	8.1	8.5	32.0	27.7	29.8	12.2	9.2	10.7
IBA 2000 mg L^{-1}	12.3	14.3	13.3 bc	8.1	8.6	8.4	39.0	32.2	35.6	13.9	11.4	12.6
IBA 4000 mg L^{-1}	17.7	20.0	18.9 a	8.6	8.7	8.7	51.2	41.0	46.1	22.7	20.7	21.7
IBA 6000 mg L^{-1}	17.0	15.7	16.3 ab	9.8	8.5	9.2	49.3	40.3	44.8	22.3	17.1	19.7
Mean	10.2	9.4		8.8	8.5		35.3	26.7		12.1	10.0	
Sig. of fixed factors	T (0.000); S (0.473)			T (0.899); S (0.157)			T (0.000); S (0.000)			T (0.000); S (0.005);		
	TXS (0.465)			TXS (0.640)			TXS (0.000)			TXS (0.002)		

Significant difference ($p \leq 0.05$) between rooting treatment and seasonal mean values were computed using Tukey's mean separation test and are indicated by different alphabetical letters for varying treatments for leaf number. Post-hoc multiple comparison tests were not conducted for Season because there were fewer than three groups in this factor. The means for above observations were computed from n = 9 cuttings.

The DQI ranged 1.35 to 0.43 with significantly higher value recorded in cuttings dip treated with IBA at 4000 mg L^{-1} which was found comparable with IBA at 6000 mg L^{-1}. The DQI of IBA 4000 mg L^{-1} treated cuttings was 3.13 times higher compared to control. Non-significant differences were observed for DQI values for cuttings treated with biostimulants of organic origin, that were relatively on the lower side compared to those cuttings dip-treated with IBA (Fig. 4 a). Quality indices were also elevated in rainy-season cuttings, with significantly higher SQ and DQI during rainy season, relative to spring (Fig. 4b).

Histology of root primordia development during AR formation

Histological analysis of lateral section of basal stem tissue of hardwood cuttings showed a periderm layer followed by the cortex, comprising 2 to 6 layers of collenchyma cells (Fig. 5). The collateral vascular bundles were found circular in arrangement. The central portion of stem contained a parenchymatous pith and secondary xylem. Study identified four stages in AR formation in *Bougainvillea* stem cuttings. In essence, the IBA treatment had a marked effect on cellular activity in the cambial and phloem regions of the stem. The IBA treated tissues had a more condensed active cell growth during AR formation, while the untreated stem tissues didn't reveal profusely dividing cells. The formation of root primordia (Rp) occurred 25^{th} DAP (Fig. 5) with IBA treatments 4000mg L^{-1}. These primordia grew perpendicularly to the cutting's main axis, displacing the phloem tissue. The Rp expanded owing to rapid cell division along with the development of tracheal elements (newly formed vascular tissue).

Biochemical modulation during AR formation

The biochemical assessment showed pronounced seasonal and treatment effects on TSS in *Bougainvillea* cuttings. The TSS increased from 60 mg g^{-1} in spring (control group) to 220 mg g^{-1} in rainy-season (6000 mg L^{-1} IBA), with mild increase through untreated control to saridex powder and sharp rises at IBA treatments i.e. 500 mg L^{-1} to IBA 6000mg L^{-1}. The highest TSS accumulation appeared in IBA at 4000 mg L^{-1} followed by 6000 mg L^{-1} (Fig. 6 A). The protein content ranged from 20 mg g^{-1} in the control lot to 45 mg g^{-1} in IBA treated cuttings at 6000 mg L^{-1}. While protein levels remained relatively stable across control to IBA treatments up to 2000 mg L^{-1}, thereafter a substantial increase was observed in cuttings treated with higher IBA levels, indicating that these biostimulants applications may have stimulated greater synthesis of structural or enzymatic proteins. Notably, the rainy season consistently outperformed the spring season for both TSS and protein content (Fig. 6 B).

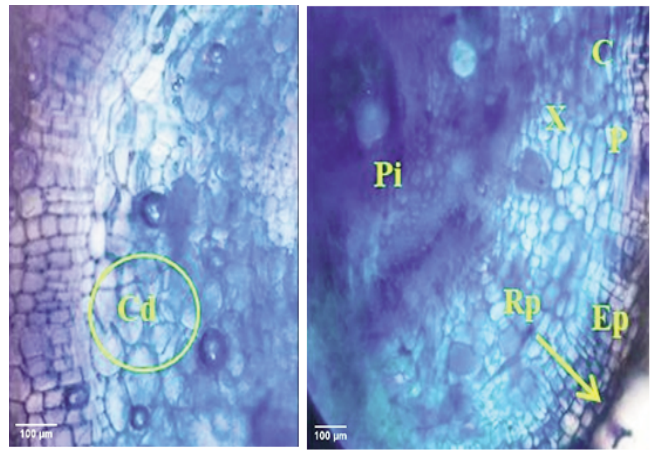

Fig. 5 - The anatomical features of lateral section of basal 0.5 to 1 mm of tissue of Bougainvillea cutting treated with IBA 4000 mgL^{-1}. The sections were excised 30 days after the hard-to-root cuttings were sticked in media for rooting. The observations were made under AXL binocular compound microscope, (Labovision) at 40X magnification. Cd= cell division (encircled). The picture on left shows the advent of induction phase of AR formation with the increase of cell number and cell size in cambial zone. The LS section (on right) reveals different cellular layers and initiation of root primordia. Rp= root primordia, Ep= Epidermis, Pi= pith, X= xylem, Ph= phloem, C= cortex.

The PPO activity ranged from 25 to 130 U ml^{-1}, with significantly higher values recorded during the rainy season compared to the spring season across all treatments. Among the treatments, IBA at 4000 mg L^{-1} exhibited the highest PPO activity (130 U ml^{-1} in the rainy season), closely followed by IBA at 6000 mg L^{-1}. In contrast, the lowest activity (25 to 30 U ml^{-1}) was observed in cuttings under control and seaweed extract, particularly during the spring season in all biostimulant treatments, except coconut water which differed significantly from other biostimulants. IBA at 4000 mg L^{-1} and IBA at 6000 mg L^{-1} were found most effective in enhancing PPO enzyme levels (Fig. 6 C).

POD activity in *Bougainvillea* cuttings ranged between 0.180 and 0.455 U g^{-1} FW. The highest activity (0.455 U g^{-1} FW) occurred in spring under control conditions, closely followed by the rainy season. Similarly, cuttings treated with Saridex powder exhibited elevated POD activity. Conversely, the lowest activity was observed with IBA treatments, particularly at 6000 mg L^{-1}, followed by 4000 mg L^{-1} during the rainy season (0.180 U g^{-1} FW). Overall, seasonal variation was evident, with spring cuttings generally recording higher POD activity than those grown during the rainy season (Fig. 6 D).

4. Discussion and Conclusions

The results demonstrate that both biostimulants and varying concentrations of IBA influenced rooting success, survival, vegetative growth, and biochemical responses in the hard-to-root *Bougainvillea* var. 'Thimma'. Significant treatment × season interactions were observed for several traits, including days to root initiation, root length, root count, plant height, and shoot length. Consequently, the effectiveness of specific rooting treatments and optimal seasons for these traits should be interpreted in a season-specific context. Our findings suggest that application of coconut water and auxin (IBA) treatments at suitable concentration can enhance overall cutting performance of hard-to-root *Bougainvillea*. Coconut water is a rich source of endogenous growth regulators (auxins, cytokinins, gibberellins) and supportive nutrients that promote cell division, callus formation, and AR initiation and growth (Yong *et al.*, 2009; Druege *et al.*, 2019). These bioactive compounds are known to elicit stress-like signalling and metabolic changes, including up-regulation of antioxidant and defense pathways that involve PPO and POD enzymes (Ali *et al.*, 2021; Hasanuzzaman *et al.*, 2021).

In our study, the IBA effectively promotes rooting in hard-to-root *Bougainvillea* cv. Thimma. The IBA concentrations between 3000 to 4000 mg L^{-1} have been reported to induce early root initiation, enhance rooting percentage, root number, and root length in *Bougainvillea* (Maurya and Bharti, 2025). These effects resulted from a stimulated callus formation and improved cambial differentiation (Ma *et al.,* 2024). The application of IBA also boosts CHO hydrolysis and metabolite build up, aiding root growth (Zhang *et al.,* 2024; Zhou *et al.,* 2025). Higher IBA levels correlate with increased root fresh and dry weight, indicating enhanced biomass (Singh *et al.,* 2023). At optimum dose, IBA accelerates earlier leaf sprouting, higher leaf count, and mobilizes stored carbohydrates resulting in greater shoot growth in cuttings. Studies reveal that IBA treated cuttings develop more roots that increase surface area for nutrient and moisture absorption. Increased leaf area supports metabolism, while auxin-driven secondary growth promotes cell division in lateral meristems, improving plant vigor. IBA, particularly at higher concentrations (4000 mg L^{-1}), is most effective for enhancing rooting of *Bougainvillea* 'Thimma'

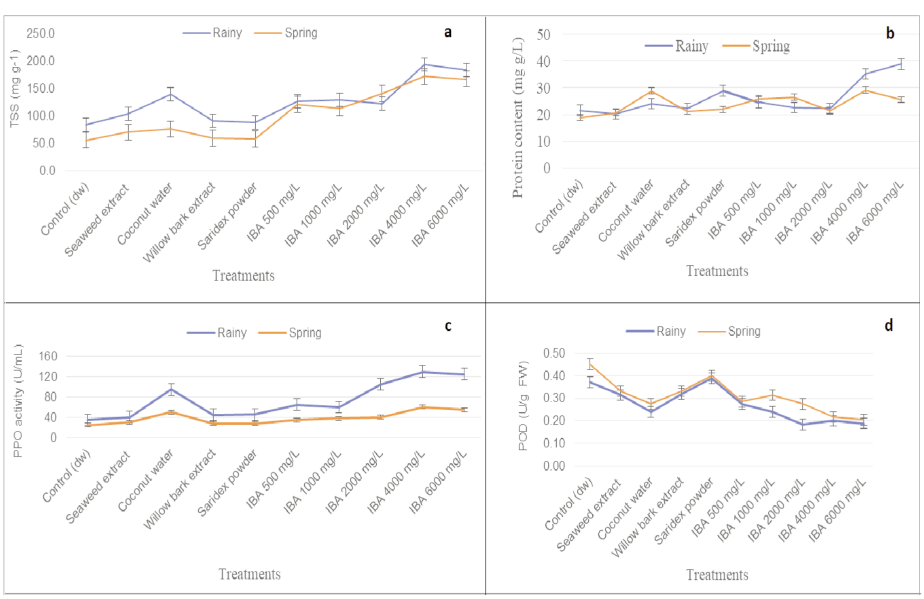

Fig. 6 - Seasonal variation in the TSS (mg g^{-1}) content (a); protein (mg g^{-1}) content (b); PPO (U ml^{-1}) (c); POD (U g^{-1} FW) (d) in response to different biostimulant treatments and Indole-3-butyric acid concentration at basal stem tissue of hardwood cuttings of *B. peruviana* cv. Thimma during adventitious root formation. Line markers designated with dissimilar capital and small letter alphabets represents significant differences to separate means across treatments in rainy and spring season, respectively. The observations for cuttings (n=6) were recorded 180 days after sticking of cuttings in media. Error bars represent ±SE.

cuttings raised during the rainy season (Zhou *et al.,* 2025).

The histology of rooting also presented an interesting variation in terms of the origin of ARs. The ARs may arise either directly from pericycle, parenchyma, or cambium cells, or indirectly via wound-induced callus formation (Huang *et al.,* 2022; Singh *et al.,* 2023). However, sometimes excessive callus can sometimes impede proper root development by creating a barrier between the developing root and the vascular system of the cutting (Irvani *et al*., 2010; Joel *et al.,* 2023). In *Bougainvillea* AR induction was clearly associated with callus formation where parenchyma cells regained meristematic potential and differentiated into root primordia, thereby classifying it as a callus-type rooting process (Huang *et al*., 2022). The AR formation is believed to be induced by activation of dormant pre-formed root initials (Mhimdi and Pérez-Pérez, 2020), suggesting auxin as a core regulator of AR formation by modulating physiological and molecular processes during rooting (Liu *et al.*, 2025).

The quality indices, SQ and DQI of rooted cuttings are critical metrics for evaluating the potential for survival and subsequent growth of cuttings post-transplanting (Wilson and Jacobs, 2006). High SQ values (more than 6.0) indicate tall, slender seedlings, while favorable ratios (less than 6.0) reflect stress tolerance and resource efficiency. The DQI value nearly to 2.0 or higher signifies superior seedling quality and establishment potential (Dickson *et al.,* 1960). An optimum shoot-to-root ratio in hardwood seedlings indicate sturdier growth, with ARs arising from diverse tissues (Warrier *et al.,* 2022).

Antioxidant enzymes like POD and PPO are vital not only for plant defence but also for regulating AR initiation and development (Sato *et al.,* 1993; Meng *et al.,* 2019). The PPO, in particular, plays a crucial role in cell division, tissue differentiation, and the formation of root primordia. It also supports the synthesis of IAA-phenolic complexes, which enhance AR formation (El Malahi *et al.,* 2024; Lee *et al.,* 2025). In our study, PPO activity steadily increased with auxin treatment indicating its continuous involvement throughout the rooting process (Warrier *et al.,* 2022). On the other hand, POD is important for lignin production; however, excessive lignin can restrict root formation by reducing cell wall flexibility, limiting callus formation, and hindering root primordia development (Meng *et al.,* 2019; Wang *et al.,* 2022). Higher IBA concentrations can suppress POD activity during rooting, lowering lignin buildup and enhancing cell wall plasticity, thus facilitating better root development (Pütter and Becker, 1983). Soluble proteins in cuttings are vital for enzyme activity, cell wall formation, and cell signalling during AR development. A brief increase in protein content during root initiation is likely due to active protein synthesis driven by auxin signalling (Wang *et al.,* 2022). Later, during root elongation, protein levels drop again as resources shift to root development. Similar findings were revealed in *Altingia gracilipes*, suggesting that IBA promoted rooting by enhancing protein synthesis and also highlighting the synergistic effect of seasonal conditions and hormonal treatments in enhancing protein content.

Rainy season substantially influenced PPO activity relative to spring season, and higher IBA concentrations had minimal impact on enhancing POD activity. Humidity and water availability during the rainy season can reduce accumulation and oxidation of phenolic compounds usually produced in response to water deficit (Cho *et al.,* 2011). In addition, studies have shown that higher PPO activity in rainy season rapidly oxidise phenols or convert these to relatively bound forms during humid weather, resulting in better rooting compared to spring planted cuttings that may reveal relatively have higher phenolic content that interfere with rooting and stability of auxin (Thakur and Kapila, 2017).

Hardwood cuttings of *Bougainvillea peruviana* Bonpl. cv. Thimma treated with 4,000 mg L^{-1} IBA exhibited markedly enhanced rooting, growth, and quality characteristics compared with untreated material, achieving 80.7% survival and 70.0% rooting along with favorable sturdiness quotient (SQ), Dickson quality index (DQI), and shoot:root ratios. These findings corroborate earlier reports that appropriate IBA concentrations substantially improve rooting performance in *Bougainvillea* cuttings. Cuttings treated with coconut water similarly demonstrated improved survival and rooting, suggesting that coconut water may be a viable, low-cost alternative for the small-scale propagation of difficult-to-root cultivars. Additionally, the rainy season provided the most conducive environmental conditions for successful establishment of hardwood cuttings, indicating the importance of seasonal timing on rooting response. Collectively, these results offer practical insights for nursery practitioners aiming to improve rooting efficiency and survival in the challenging propagation of *Bougainvillea* cv. 'Thimma', especially where large-scale multiplication is desired.

Acknowledgements

The authors thank Head of the Department of Floriculture and Landscaping, PAU, Ludhiana for his constructive suggestions in formulating this need-based research work. Also, due thanks to the Head, Department of Biochemistry, PAU, Ludhiana for the facilities provided to undertake the laboratory work.

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