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Root Dynamics in *Bougainvillea glabra* Choisy Cuttings: Analyzing the Influence of Auxin Type, Concentration, and Cutting Type Over Time in Two Varieties

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Abstract

This study investigated the effects of three types of auxins (indolebutyric acid (IBA), naphthaleneacetic acid (NAA), and dichlorophenoxyacetic acid (2,4-D)) on the rooting of soft, semiwoody, and woody stem cuttings of bougainvillea (*Bougainvillea glabra* Choisy) during early winter and spring under greenhouse conditions. PGRs were prepared at concentrations of 250, 500, and 1000 mg L^{-1}. The stem cuttings were immersed in these solutions for 24 hours before being transferred to the growth media. The experiment was designed as a factorial experiment in a completely randomized design with 10 replications. The results indicated that timing (winter vs. spring) had no significant effect on the rooting percentage (p-value = 0.05). However, significant differences were observed in branching, with the shortest average branch length (2.0 cm) occurring in early winter and the longest (4.7 cm) occurring in early spring. Significant differences were also found between varieties, with the red variety outperforming the white variety (20.44% vs. 10.83%). The woody cuttings produced significantly better results than the soft and semiwoody cuttings did. Among the PGRs, those treated with 250 mg L^{-1} IBA (33.2%) or NAA (32.5%) presented the highest rooting percentages. Increasing concentrations of these auxins resulted in decreased rooting, with the lowest rooting percentage (0%) observed at 1000 mg L^{-1} 2,4-D. This study demonstrated that the type and concentration of auxins significantly affect the rooting and branching of bougainvillea stem cuttings, with optimal results achieved with 250 mg L^{-1} IBA and NAA. These findings suggest that careful selection of the auxin type and concentration, as well as consideration of the cutting type and timing, are crucial for the successful propagation of bougainvillea.

Keywords: Acetic acid, Greenhouse production, Propagation, Seasonal variation, Soft cutting.

INTRODUCTION

Bougainvillea, scientifically known as *Bougainvillea glabra* Choisy, is an ornamental shrub widely recognized across various regions of the world and belongs to the Nyctaginaceae family, which comprises 18 species of shrubs, bushes, and vines (Sattler and Perlin, 1982). In the tropical and subtropical regions of Iran, this plant is commonly used to cover walls, create arbors, and decorate fence walls in houses and parks (Danshur, 2013). Bougainvillea is notablele for its vibrant bracts of various colors and its resistance to salinity and drought, making it suitablele for tropical regions (Singh *et al*., 2011). Recent studies have highlighted the antioxidant and antidiabetic activities of *Bougainvillea glabra*, particularly focusing on the D-pinitol content in various cultivars (Abo-Elghiet *et al*., 2023).

The most prevalent method of propagating bougainvillea is through stem cuttings, although other asexual propagation methods, such as micropropagation via shoot tip cultivation, have also been reported (Lin *et al*., 2024). Propagation by stem cuttings is particularly advantageous because of its simplicity and several benefits, including the preservation of the plant's genetic characteristics, the requirement for fewer mother plants, cost-effectiveness, speed, and the lack of need for specialized techniques or equipment (Hartmann *et al*., 2002). Recent advancements in plant transformation techniques, including nanoparticle methods and protoplast-based approaches, have further expanded the possibilities for plant propagation (Levengood *et al*., 2024).

There is considerable variation in the rooting ability of stem cuttings among different plant species and cultivars. While some species easily root from stem cuttings, others require optimized conditions for successful rooting. The key factors influencing the success of rooting include careful selection of cuttings from the mother plant, proper management of cuttings, and control of environmental conditions during the rooting process (Hartmann *et al*., 2002). Essential considerations for enhancing the rooting of cuttings include selecting the appropriate type of cutting, preparing cuttings at the optimal time, maintaining suitablele temperature and humidity in the rooting environment, using an appropriate planting medium, performing pre- or postpreparation treatments, and applying PGRs at optimal concentrations (Hartmann *et al*., 2002). Recent research has shown that environmental stress conditions, such as drought and high salinity, significantly affect root development and plant survival (Vives-Peris *et al*., 2020). Given the challenges associated with rooting papaya cuttings, effective propagation is crucial for producers (Asl *et al*., 2012). Gehlot *et al*. (2014) reported that the application of IBA and NAA at specific concentrations improved the rooting percentage and survival of *Azadirachta indica* stem cuttings in an alternating mist system. Further research by Seyedi *et al.* (2014) revealed that the use of IBA in combination with talc powder and potassium nitrate solution increased the rooting percentage, weight, and length of roots. The results of the treatment with 3000–6000 mg L^{-1} IBA demonstrated that thicker cuttings with higher internal auxin concentrations produced more and better roots. Recent studies have also explored the role of phenolic compounds in enhancing the rooting ability of plant cuttings (Santos-Rufo *et al*., 2024). Additionally, some studies on bougainvillea have focused on optimizing callus induction and shoot formation through *in vitro* culture techniques (Aghaali, 2019).

Research indicates that excessive use of PGRs during rooting can disrupt a plant's hormonal balance and increase costs. Therefore, determining the optimal concentration of PGRs, especially for woody species, is critical (Ersoy and Aydin, 2008). The rooting ability of cuttings also depends on their internal auxin content, phenolic compounds, and enzymes (Loreti and Morini, 1985). Additionally, the timing of cutting during the year significantly affects rooting outcomes, as it is more related to the plant's physiological conditions than to a specific calendar time, with genetic and anatomical factors playing a role (Selby *et al*., 1992). Recent findings have highlighted the importance of hormonal balance and the integration of hormonal signals in shaping root growth and development (Cheng *et al.*, 2023).

The aim of this research is to enhance the propagation of white and red varieties of bougainvillea, which have demonstrated resilience in the tropical and subtropical regions of Iran under adverse conditions. Despite their adaptablility, the current methods of asexual propagation remain suboptimal, leading to inconsistent results and limiting the potential for large-scale cultivation. This research seeks to address this gap by developing more reliable and efficient propagation techniques. Given the increasing demand for aesthetically appealing and environmentally resilient plants to expand green landscapes, it is essential to refine propagation methods, conduct comprehensive tests, and thoroughly study these species. By doing so, this study can contribute to the sustainable development of urban and rural landscapes while promoting biodiversity and ecological balance.

MATERIALS AND METHODS

This research was conducted in the Department of Horticultural Science at Agricultural Sciences and Natural Resources University of Khuzestan between 2019 and 2020.

The stem cuttings used in this experiment were sourced from the mother plants of bougainvillea, which are available at the University. The cuttings were collected twice: Once in the first week of winter (20 December) and once in the first week of spring (20 March). Each cutting was 20.0 cm in length and had a diameter between 0.5 and 1.0 cm. Both the lower and upper ends of the cuttings were cut diagonally, 2.0 cm away from the first bud, and the leaves from the lower two-thirds of the cuttings were removed.

Treatments: Initially, the cuttings were disinfected with a 2% benomyl fungicide solution for 10 min. Subsequently, the last 1.0 cm of the cuttings were immersed in solutions of IBA, NAA, and 2,4-D at concentrations of 0, 250, 500, and 1000 mg L^{-1} for 24 hours. Each experimental treatment consisted of 10 cuttings.

The cuttings were planted in a greenhouse in rooting media composed of equal volumes of washed sand-loam soil and peat. The greenhouse environment was maintained at an average temperature of 25-28 °C, with humidity levels regulated between 60-70 % to simulate optimal growth conditions. Supplemental lighting was provided for 12-14 hours daily to ensure sufficient photosynthesis, while ventilation systems were employed to maintain airflow and prevent fungal diseases. The cuttings were planted diagonally at a 45-degree angle to the horizontal direction and spaced 10.0 cm apart. The planting medium was irrigated daily via a hand-held sprinkler.

Three months after planting, the cuttings were carefully removed from the culture medium. The characteristics studied in this study included the rooting percentage, number of roots, average root length, longest root length and shoot length per cutting (Fig. 1). The rooting percentage was determined by calculating the proportion of cuttings that successfully developed roots out of the total number of cuttings planted. To assess the number of roots, all visible roots on each cutting were carefully counted after a designated growth period. The average root length was measured by summing the lengths of all roots per cutting and dividing the total by the number of roots. The longest root length was determined by identifying and measuring the single root with the greatest length for each cutting. Lastly, the shoot length per cutting was measured from the base of the cutting to the tip of the longest shoot using a ruler, ensuring consistency across all samples.

This study was designed as a factorial experiment with a completely randomized design. Mean comparisons were performed via Duncan's test with SAS software, and graphs were generated via Excel 2019 software.

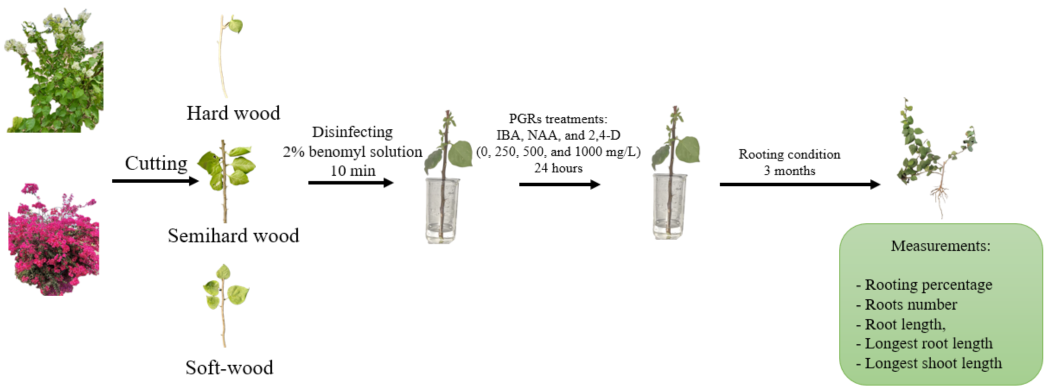


Fig. 1. Overview of the experimental design.

RESULTS

Root percentage

A comparative analysis of the average rooting percentage among cuttings revealed no statistically significant differences (Table 1). However, a significant variation was observed in the response to different plant growth regulators (PGRs), as determined by Duncan's multiple range test at a 5% probability level. Cuttings treated with NAA had the highest rooting percentage, while those treated with 2,4-D had the lowest. The interaction between cultivar and cutting type revealed that red woody cuttings achieved the highest rooting percentage (30.08 %), whereas soft cuttings of the red cultivar had the lowest (6.39 %), with no significant difference from semiwoody cuttings of the white cultivar (Fig. 2-left). Rooting percentages varied with PGR types and concentrations. The lowest rooting percentage occurred at 1000 mg L^{-1} for all PGRs, with no rooting in treatments involving 1000 mg L^{-1} 2,4-D. The highest rooting percentage (32.50%) was achieved using NAA at 250 mg L^{-1}, which was not significantly different from the IBA treatment at the same concentration (Fig. 2-right). The combined effects of time, cultivar, cutting type, PGR type, and concentration identified NAA (250 mg L^{-1}) in red woody cuttings during winter as achieving the highest rooting percentage (90.00%) (Table 1).

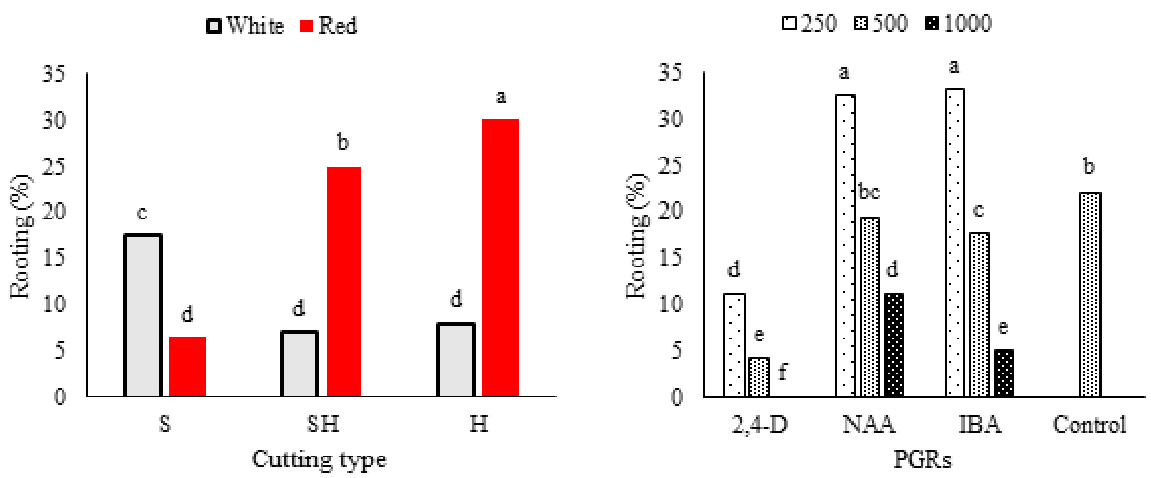


Fig. 2. Interaction effects of cultivar and cutting type (left); and PGRs and concentrations (right) on rooting percentage of bougainvillea.
*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the Duncan's test.

Root number

Cutting time in winter and spring had no significant effect on the number of roots produced, as determined by Duncan's multiple range test at a 5% probability level (Table 2). The use of NAA resulted in the greatest number of roots, while 2,4-D produced the lowest. Among PGR types and concentrations, the highest number of roots (4.25) was achieved with 250 mg L^{-1} NAA, whereas the lowest (0.00) was observed with 1000 mg L^{-1} 2,4-D. The 250 mg L^{-1} IBA treatment significantly increased the number of roots compared to the control (Fig. 3-right). The interaction of cultivar and cutting type showed that red woody cuttings had the highest number of roots (2.82 per cutting), while white semiwoody cuttings had the lowest (0.84 per cutting), with no significant difference from white woody cuttings (Fig. 3-left). The soft cuttings of the red cultivar treated with 250 mg L^{-1} NAA in spring produced the highest overall number of roots (20.00 per cutting) (Table 2).

Table 1. Effects of cultivar, cutting type, PGR type, and PGR concentration across two seasons on the rooting percentage of bougainvillea cuttings.

Time	Cultivar	Cutting	PGRs (mg L^{-1})										Season
			Control	IBA			NAA			2,4-D			
			0	250	500	1000	250	500	1000	250	500	1000	
Spring	W	H	30.0 ^{gh}	60.0^{d}	20.0^{i}	20.0^{i}	10.0^{j}	0.0^{m}	0.0^{m}	0.0 ^{m}	0.0^{m}	0.0^{m}	14.8^{a}
		SH	41.3^{f}	10.0^{j}	10.3^{j}	0.0^{m}	40.0^{f}	0.0^{m}	0.0^{m}	0.0 ^{m}	0.0^{m}	0.0^{m}	
		S	19.6^{i}	20.0^{i}	10.0^{j}	0.0^{m}	40.6^{f}	30.0^{gh}	20.0 ^{i}	10.0 ^{j}	0.0^{m}	0.0^{m}	
	R	H	6.0^{jk}	0.0^{m}	1.66^{km}	0.0^{m}	20.0^{i}	10.3^{j}	10.6 ^{j}	20.0 ^{i}	0.0^{m}	0.0^{m}	
		SH	40.0^{f}	10.0^{j}	40.0^{f}	0.0^{m}	70.0^{c}	40.0^{f}	0.0^{m}	24.0^{hi}	20.0^{i}	0.0^{m}	
		S	19.9^{i}	50.0^{e}	10.0^{j}	0.0^{m}	30.0^{gh}	40.0^{f}	41.3^{f}	0.0 ^{m}	0.0^{m}	0.0^{m}	
Winter	W	H	30.0^{gh}	60.0^{d}	20.3^{i}	20.0^{i}	40.0^{f}	40.0^{f}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	16.4^{a}
		SH	10.3^{j}	20.0^{i}	10.0^{j}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	
		S	10.0^{j}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	
	R	H	9.6^{j}	20.0^{i}	0.0^{m}	0.0^{m}	10.3^{j}	9.6^{j}	10.3^{j}	0.0^{m}	0.0^{m}	0.0^{m}	
		SH	30.0^{gh}	70.0^{c}	10.0^{j}	0.0^{m}	40.0^{f}	33.6^{g}	20.0^{i}	30.0^{gh}	20.0^{i}	0.0^{m}	
		S	20.0^{i}	78.6^{b}	80.0^{b}	20^{i}	90.0^{a}	30.0^{gh}	33.0^{g}	50.0^{e}	10.0^{j}	0.0^{m}	

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test.
The abbreviations W and R denote white and red cultivars, respectively.
The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

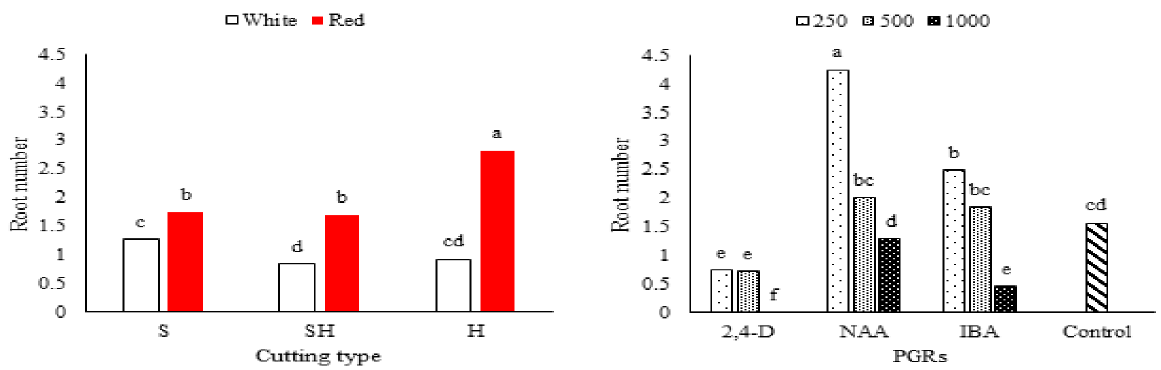


Fig. 3. Interaction effects of cultivar and cutting type (left); and PGRs and concentrations (right) on root number of bougainvillea.
*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test.
The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

Table 2. Effects of cultivar, cutting type, PGR type, and PGR concentration across two seasons on the number of roots of bougainvillea cuttings.

Time	Cultivar	Cutting	PGRs (mg L^{-1}) Control 0	IBA 250	IBA 500	IBA 1000	NAA 250	NAA 500	NAA 1000	2,4-D 250	2,4-D 500	2,4-D 1000	Season
Spring	W	H	0.5^{ij}	2.9^{c-f}	2.5^{d-g}	3.0^{cde}	3.7^{bcd}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	1.67^{a}
		SH	2.6^{d-g}	4.0^{bc}	2.5^{d-g}	0.0^{j}	2.2^{efg}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	
		S	1.7^{fgh}	2.7^{d-g}	2.5^{d-g}	0.0^{j}	4.0^{bc}	3.5^{cd}	3.0^{cde}	0.5^{ij}	0.0^{j}	0.0^{j}	
	R	H	0.5^{ij}	0.0^{j}	1.0^{hij}	0.0^{j}	20.1^{a}	0.5^{ij}	1.1^{hij}	3.0^{cde}	0.0^{j}	0.0^{j}	
		SH	0.5^{ij}	1.5^{ghi}	4.1^{bc}	0.0^{j}	3.7^{bcd}	3.1^{cde}	0.0^{j}	0.0^{j}	1.7^{fgh}	0.0^{j}	
		S	2.7^{d-g}	2.8^{def}	1.5^{ghi}	0.0^{j}	3.7^{bcd}	3.7^{bcd}	3.5^{cd}	0.0^{j}	0.0^{j}	0.0^{j}	
Winter	W	H	1.0^{hij}	1.0^{hij}	1.0^{hij}	1.5^{ghi}	2.0^{e-h}	3.0^{cde}	0.0^{j}	1.0^{hij}	1.0^{hij}	0.0^{j}	1.40^{a}
		SH	1.0^{hij}	4.0^{bc}	0.5^{ij}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	
		S	0.5^{ij}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	
	R	H	1.5^{ghi}	3.0^{cde}	0.0^{j}	0.0^{j}	1.5^{ij}	1.5^{ghi}	1.5^{ghi}	0.0^{j}	0.0^{j}	0.0^{j}	
		SH	1.5^{ghi}	3.0^{cde}	2.1^{e-h}	0.0^{j}	4.2^{b}	3.2^{cde}	3.0^{cde}	0.0^{j}	2.5^{d-g}	0.0^{j}	
		S	4.8^{b}	5.1^{b}	4.7^{b}	1.0^{hij}	5.9^{b}	5.5^{b}	3.5^{cd}	4.4^{b}	3.5^{cd}	0.0^{j}	

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test. The abbreviations W and R denote white and red cultivars, respectively. The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

Root length

Root length showed no significant differences between winter and spring cuttings, as determined by Duncan's multiple range test at a 5% probability level (Table 3). Cultivar type significantly affected root length, with the red cultivar having the highest average root length (Fig. 4-left). Cutting type also influenced root length, with the greatest length observed in hard woody cuttings and the lowest in semihard woody cuttings (Fig. 4-left). For PGR type, no significant differences were found between IBA and NAA, but both were significantly superior to 2,4-D. The interaction of PGR type and concentration indicated the highest root length with 250 mg L^{-1} NAA (Fig. 4-right). The overall greatest root length (13.8 cm) was achieved with hard woody cuttings taken in winter from the red cultivar treated with 250 mg L^{-1} NAA (Table 3).

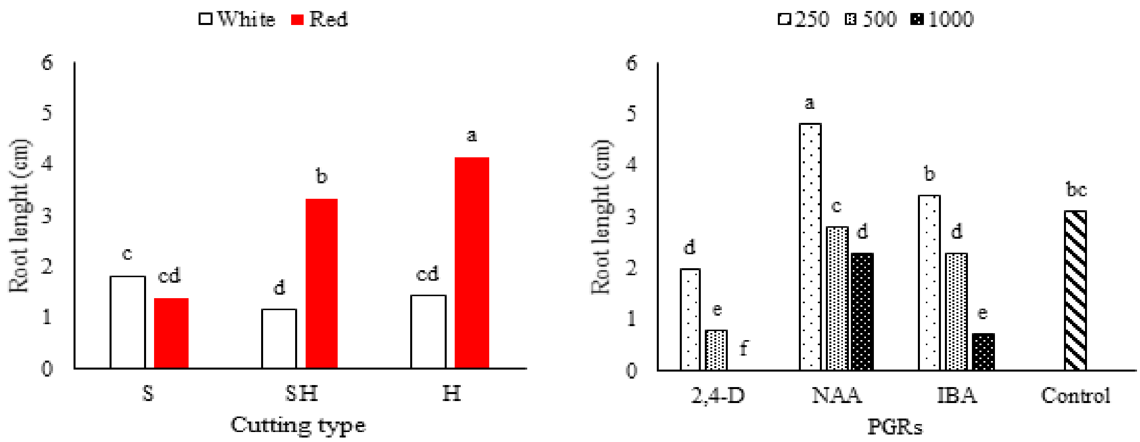


Fig. 4. Interaction effects of cultivar and cutting type (left); and PGRs and concentrations (right) on root lenght of bougainvillea.
*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test. The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

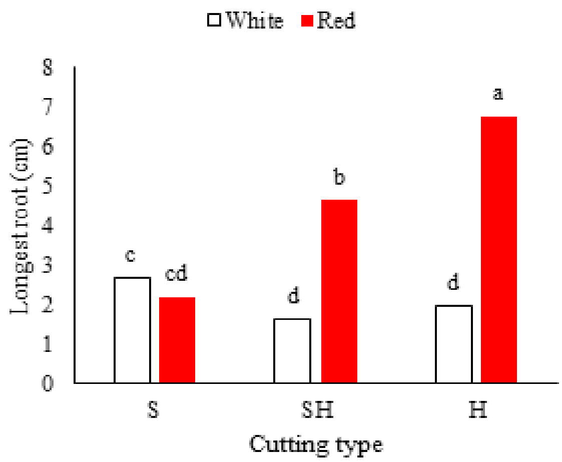
Table 3. Effects of cultivar, cutting type, PGR type, and PGR concentration across two seasons on the length of the roots of bougainvillea cuttings.

Time	Cultivar	Cutting	PGRs (mg L^{-1})										Season
			Control	IBA			NAA			2,4-D			
			0	250	500	1000	250	500	1000	250	500	1000	
Spring	W	H	0.5^{no}	4.6^{fg}	5^{fg}	5.5^{e}	5.2^{f}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	2.1^{a}
		SH	3.3^{hi}	4.2^{g}	2.5^{j}	0.0^{o}	5.0^{fg}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	
		S	3.0^{ij}	2.8^{ij}	3.5^{hi}	0.0^{o}	6.0^{de}	3.5^{hi}	4.5^{fg}	3.0^{ij}	0.0^{o}	0.0^{o}	
	R	H	6.0^{de}	0.0^{o}	1.7^{l}	0.0^{o}	2.7^{ij}	0.5^{no}	1.5^{lm}	4.$^{5\ gh}$	0.0^{o}	0.0^{o}	
		SH	2$^{.8\ ij}$	1.0^{m}	4.0^{h}	0.0^{o}	4.8^{fg}	5.0^{fg}	0.0^{o}	4.1^{gh}	1.5^{lm}	0.0^{o}	
		S	3.5^{hi}	4.7^{fgh}	3.0^{ij}	0.0^{o}	5.7^{e}	5.5^{e}	5.5^{e}	0.0^{o}	0.0^{o}	0.0^{o}	
Winter	W	H	1.3^{lm}	1.3^{lm}	1.6^{lm}	2.0^{kl}	4.0^{h}	4.5^{gh}	0.0^{o}	0.0^{o}	1.0^{m}	0.0^{o}	2.2^{a}
		SH	1.5^{lm}	6.0^{de}	0.5^{no}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	
		S	2.5^{jk}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	
	R	H	2.0^{kl}	1.5^{lm}	0.0^{o}	0.0^{o}	3.5^{hi}	2.5^{j}	1.5^{lm}	0.0^{o}	0.0^{o}	0.0^{o}	
		SH	6.5^{de}	5.3^{ef}	4.0^{h}	0.0^{o}	7.0^{c}	4.0^{h}	8.0^{c}	5.3^{ef}	3.5^{hi}	0.0^{o}	
		S	4.4^{gh}	9.5^{b}	1.6^{lm}	1.0^{m}	13.8^{a}	8.2^{bc}	6.5^{d}	6.8^{cd}	3.5^{hi}	0.0^{o}	

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test.
The abbreviations W and R denote white and red cultivars, respectively.
The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

Longest root length

The season of cutting preparation significantly influenced the longest root length, with winter cuttings yielding longer roots than spring cuttings (Table 4). Hard wood cuttings of the red variety produced the longest roots, while semihard wood cuttings of the white variety had the shortest (Fig. 5-left). No significant difference was observed between IBA and NAA in terms of the longest root length, but both were significantly superior to 2,4-D. The interaction between PGR type and concentration revealed that 250 mg L^{-1} NAA produced the longest roots (Fig. 5-right). The overall longest root length (20.5 cm) was achieved from soft wood cuttings of the red variety taken in winter and treated with 250 mg L^{-1} NAA (Table 4).



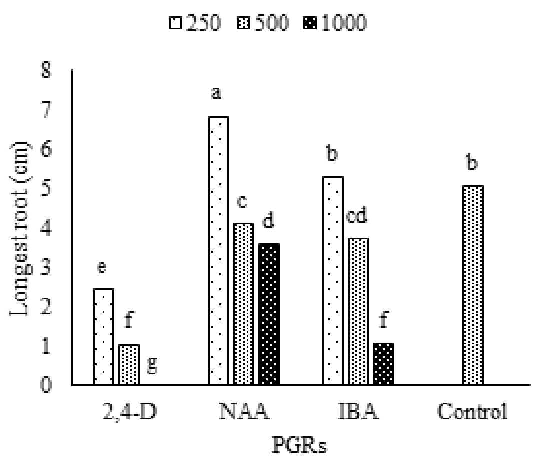


Fig. 5. Interaction effects of cultivar and cutting type (left); and PGRs and concentrations (right) on longest root lenght of bougainvillea.
*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test. The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

Table 4. Effects of cultivar, cutting type, PGR type, and PGR concentration across two seasons on the longest root length of bougainvillea cuttings.

Time	Cultivar	Cutting	PGRs (mg L^{-1})										Season
			Control	IBA			NAA			2,4-D			
			0	250	500	1000	250	500	1000	250	500	1000	
Spring	W	H	3.5^{op}	5.8^{lm}	7.5^{h-k}	9.0^{ef}	6.0^{lm}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	3.2^{b}
		SH	4.5^{no}	5.2^{mn}	3.0^{pq}	0.0^{t}	7.2^{ij}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	
		S	3.7^{op}	4.5^{no}	4.5^{no}	0.0^{t}	8.7^{efg}	5.8^{lm}	6.5^{kl}	3.1^{pq}	0.0^{t}	0.0^{t}	
	R	H	8.5^{fgh}	0.0^{t}	2.3^{q}	0.0^{t}	5.3^{mn}	0.5^{s}	5.2^{mn}	6.5^{kl}	0.0^{t}	0.0^{t}	
		SH	4.6^{mno}	3.0^{pq}	6.0^{lm}	0.0^{t}	6.45^{kl}	9.0^{ef}	0.0^{t}	0.0^{t}	2.2^{q}	0.0^{t}	
		S	8.7^{efg}	7.8^{ghi}	3.5^{op}	0.0^{t}	8.0^{f-i}	8.2^{f-i}	8.0^{f-i}	0.0^{t}	0.0^{t}	0.0^{t}	
Winter	W	H	1.8^{r}	2.6^{q}	3.0^{pq}	2.5^{q}	5.5^{m}	6.5^{l}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	3.4^{a}
		SH	2.5^{q}	9.5^{de}	0.5^{s}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	
		S	2.5^{q}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	
	R	H	2.5^{q}	2.0^{r}	0.0^{t}	0.0^{t}	4.5^{no}	3.8^{op}	2.5^{q}	0.0^{t}	0.0^{t}	0.0^{t}	
		SH	8.5^{fgh}	8.5^{fgh}	5.0^{mn}	0.0^{t}	10.0^{d}	5.0^{mn}	11.5^{c}	7.9^{ghi}	5.1^{mn}	0.0^{t}	
		S	9.5^{de}	14.6^{b}	9.3^{e}	1.0^{s}	20.5^{a}	10.5^{c}	9.0^{ef}	11.6^{c}	5.0^{mn}	0.0^{t}	

*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the Duncan's test.
The abbreviations W and R denote white and red cultivars, respectively.
The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

Shoot length

A statistically significant difference, as determined by Duncan's multiple range test at a 5% probability level, was observed in average branch lengths based on cutting timing, with spring cuttings yielding the longest shoots (Table 5). Hard wood cuttings of the red variety had the longest branch length, whereas soft wood cuttings of the same variety had the shortest. The shortest branch length was not significantly different from that of semihard wood cuttings in the white variety (Fig. 6-left). Significant differences in branch length were noted between the control group and PGR-treated groups. The control group exhibited the longest branch length (46.0 cm), while the shortest branch length (0.0 cm) was recorded for 1000 mg L^{-1} 2,4-D (Fig. 6-right). The longest branch length (46.0 cm) was achieved in soft wood cuttings of the red variety taken in winter under the control treatment (Table 5).

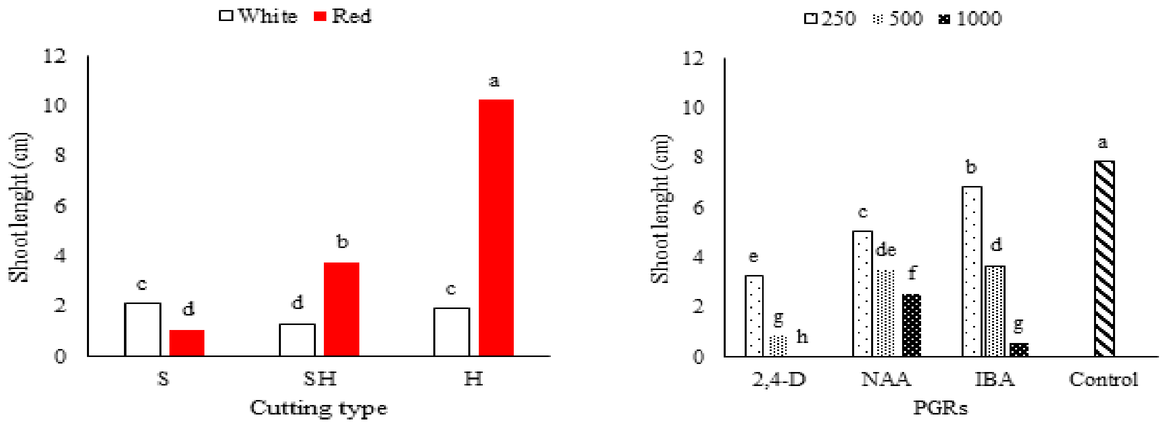


Fig. 6. Interaction effects of cultivar and cutting type (left); and PGRs and concentrations (Right) on shoot lenght of bougainvillea.
*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the Duncan's test.
The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

Table 5. Effects of cultivar, cutting type, PGR type, and PGR concentration across two seasons on the longest shoot length of bougainvillea cuttings.

Time	Cultivar	Cutting	PGRs (mg L^{-1})										Season
			Control	IBA			NAA			2,4-D			
			0	250	500	1000	250	500	1000	250	500	1000	
Spring	W	H	3.5^{no}	3.2^{op}	4.5^{lmn}	4.0^{mno}	2.0^{pqr}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	2.0^{b}
		SH	4.6^{lm}	4.2^{mno}	1.5^{qrs}	0.0^{t}	1.0^{rs}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	
		S	5.5^{kl}	5.0^{lm}	2.0^{pqr}	0.0^{t}	5.7^{kl}	5.0^{lm}	6.0^{kl}	6.5^{jk}	0.0^{t}	0.0^{t}	
	R	H	2.7^{p}	0.0^{t}	0.8^{rst}	0.0^{t}	1.0^{rs}	0.5^{st}	0.5^{st}	2.0^{pqr}	0.0^{t}	0.0^{t}	
		SH	3.8^{no}	1.5^{qrs}	3.5^{no}	0.0^{t}	2.0^{pqr}	1.0^{rs}	0.0^{t}	1.3^{qrs}	2.2^{pq}	0.0^{t}	
		S	4.0^{mno}	7.4^{hij}	2.0^{pqr}	0.0^{t}	7.5^{hij}	6.5^{jk}	6.2^{jkl}	0.0^{t}	0.0^{t}	0.0^{t}	
Winter	W	H	4.0^{mno}	7.8^{hi}	3.0^{op}	1.5^{qrs}	1.5^{qrs}	7.5^{hij}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	4.7^{a}
		SH	3.0^{op}	9.0^{gh}	3.0^{op}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	
		S	2.5^{p}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	
	R	H	2.5^{p}	10.1^{g}	0.0^{t}	0.0^{t}	0.5^{st}	0.5^{st}	0.5^{st}	0.0^{t}	0.0^{t}	0.0^{t}	
		SH	12.0^{f}	8.2^{hi}	8.2^{hi}	0.0^{t}	12.0^{f}	4.0^{mno}	2.0^{pqr}	12.0^{f}	1.0^{rs}	0.0^{t}	
		S	46.0^{a}	25.5^{c}	15.6^{de}	1.0^{rs}	27.2^{b}	17.0^{d}	15.0^{e}	17.0^{d}	7.0^{ijk}	0.0^{t}	

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test.
The abbreviations W and R denote white and red cultivars, respectively.
The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

DISCUSSION

This study revealed no significant difference in the average rooting percentage, number of roots, or root length between cuttings taken in winter and those taken in spring. However, winter cuttings exhibited significantly longer root lengths compared to spring cuttings. Additionally, the timing of cuttings significantly affected branch length, with the longest shoot lengths observed in spring cuttings. These findings highlight the complex interplay of factors influencing rhizogenesis in woody plants, including species, cutting type, environmental conditions, and plant physiological state (Zhao *et al*., 2022). Below, we discuss these results in detail, their implications, and potential influencing factors.

Seasonal effects on rooting success: Seasonality plays a critical role in the rooting success of cuttings. During dormancy (late fall to early spring), hardwood cuttings benefit from high carbohydrate reserves stored in stems and roots, providing energy for root initiation (Zheng *et al*., 2020). However, endogenous auxin levels are low, and growth inhibitors are high, preventing premature growth (Hartmann *et al.*, 2002). This may explain why winter cuttings, despite their longer root lengths, did not show significant differences in rooting percentage compared to spring cuttings. The lack of significant differences in rooting parameters could also be attributed to the genetic variability of the plant material, environmental conditions during the experiment, or the specific hormonal treatments applied. For instance, variations in endogenous hormone levels among cuttings may have influenced rooting responses, masking potential differences (Druege *et al*., 2016). Future studies should explore these factors in greater detail to better understand their impact on rooting success.

In contrast, during active growth (spring and summer), softwood cuttings have high endogenous auxin levels, promoting rapid cell division and growth, but lower carbohydrate reserves, as they are used for shoot growth (OuYang *et al*., 2015). Softwood cuttings can root quickly but are susceptible to desiccation and fungal diseases, requiring high humidity and careful management (Saradha and Samydurai, 2015). Semihardwood cuttings, taken from midsummer to early fall, strike a balance with moderate auxin and carbohydrate levels, offering moderate rooting speed and success. These seasonal variations underscore the importance of selecting the appropriate cutting type and timing based on the species and propagation goals.

Advantages of hardwood cuttings: Hardwood cuttings, taken from mature, fully lignified stems, offer several advantages over semihardwood or softwood cuttings, particularly for deciduous trees and shrubs. One key benefit is the greater carbohydrate reserves found in hardwood cuttings. During dormancy, stems accumulate high levels of stored carbohydrates, providing the energy needed to support early callus formation and subsequent root growth (Ashok and Ravivarman, 2021). Additionally, hardwood stems have tougher bark and thicker tissues, making them more robust and better able to tolerate stress. This reduces the likelihood of desiccation, fungal entry, or mechanical injury, allowing the plant tissue to focus more energy on producing roots instead of repairing damage (Mumtaz *et al*., 2022).

Hardwood cuttings also face reduced disease pressure. Taken during dormancy, they are less likely to carry active fungal or insect pests compared to softwood material, which can reduce disease and pest problems and thereby aid healthy root development (Kaushik and Shukla, 2020). Furthermore, hardwood cuttings benefit from reduced water loss through transpiration. Lacking leaves or having very few, small, dormant buds, hardwood cuttings significantly reduce water loss compared to softwood and semihardwood cuttings, which lose water much more rapidly (Dalbro, 1975). This makes hardwood cuttings more tolerant of drier conditions and less reliant on constant, high humidity, reducing the risk of fungal diseases that thrive in overly humid environments (Sytar *et al*., 2019). Despite being slower to root than softwood or semihardwood cuttings, hardwood cuttings often exhibit stronger, more vigorous growth and greater long-term health, particularly for woody species (Mumtaz *et al*., 2022). These attributes make hardwood cuttings a favorable choice for establelishing strong root systems in many woody plants.

Role of PGRs: The type and concentration of PGRs significantly influenced rooting success in this study. NAA-treated cuttings exhibited the highest rooting percentage, consistent with findings by Tripathi *et al*. (2022), who reported that auxins such as NAA are effective at promoting root initiation and development. NAA is often more effective than 2,4-D and IBA in stimulating root initiation, especially in hard-to-root plant species, as it promotes the formation of more root primordia, leading to better rooting success (Geneve and Heuser, 1982; Yan *et al*., 2014). Compared to 2,4-D, NAA promotes direct root formation with less callus production, leading to more efficient adventitious rooting (De Klerk *et al*., 1999). Additionally, NAA has a longer-lasting effect on promoting rooting than IBA, which degrades more quickly under some conditions (Dalbro, 1975). Conversely, cuttings treated with 2,4-D presented the lowest rooting percentage, corroborating the results of Sabagh *et al*. (2021), who noted the inhibitory effects of 2,4-D on root formation. The interaction between cultivar and cutting type further emphasized that woody cuttings of the red cultivar presented the highest rooting percentage, which is in line with the observations of Rademacher (2015) regarding the superior rooting potential of woody cuttings.

Practical implications for growers and horticulturists: The findings of this study have significant practical implications for growers and horticulturists. Hardwood cuttings, with their higher success rates and lower susceptibility to environmental stress, offer a cost-effective and reliable propagation method. This is particularly beneficial for large-scale production, where consistency and efficiency are critical. Additionally, the use of hardwood cuttings reduces the need for specialized equipment or controlled environments, making it accessible to small-scale growers and nurseries with limited resources (Dhillon, 2017). By adopting this method, growers can improve propagation outcomes and reduce losses, ultimately enhancing productivity and profitableility. Furthermore, the use of NAA as a PGR can significantly improve rooting success, especially for hard-to-root species. Growers should consider the specific needs of the plants being propagated and choose the most appropriate auxin to achieve optimal rooting results. The advantages of NAA, such as faster root initiation, stronger root systems, and reduced callus formation, make it a valuable tool in the propagation of a wide variety of plant species (Yan *et al*., 2014).

CONCLUSION

The results indicated that the timing (winter vs. spring) had no significant effect on the rooting percentage. However, a significant difference was observed in branching, with the shortest average branch length (2.6 cm) occurring in early winter and the longest (4.2 cm) occurring in early spring. Significant differences were also found between the tested varieties, with the red variety outperforming the white variety in all the measured factors. While no significant difference was noted between the soft and semiwoody cuttings, the woody cuttings presented significantly better results. Among the PGRs, those treated with 250 mg L^{-1} IBA or NAA presented the highest rooting percentages. Increasing concentrations of these auxins resulted in decreased rooting, with the lowest rooting percentage observed at 1000 mg L^{-1} 2,4-D. This study demonstrated that the type and concentration of auxins significantly affect the rooting and branching of bougainvillea cuttings, with optimal results achieved using 250 mg L^{-1} IBA and NAA. These findings suggest that careful selection of the auxin type and concentration, as well as consideration of the cutting type and timing, are crucial for the successful propagation of bougainvillea. The findings of this study highlight the critical role of PGRs, particularly NAA, in enhancing rooting success and overall plant growth. The results also emphasize the importance of the cutting type and cultivar in determining rooting and growth outcomes. While seasonal timing did not significantly impact the rooting percentage, number of roots, or root length, it did influence branch length, with spring cuttings showing the longest shoot length. These insights can inform horticultural practices and propagation strategies, particularly for woody plants, by identifying optimal conditions for root initiation and plant development. Future research could further explore the interactions between different PGRs, cutting types, and environmental conditions to refine propagation techniques and improve plant growth outcomes.

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