

Effect of Different Growth Regulators on the Rooting of Hardwood Cuttings of *Bougainvillea glabra* var. "Mohan"

Abstract

An experiment about the effect of different growth regulators on the rooting of hardwood cuttings of *Bougainvillea glabra* var. "Mohan" titled "Effect of Different Growth Regulators on the Rooting of Hardwood Cuttings of *Bougainvillea* var. "Mohan" was conducted during the year 2020-21 at the Faculty of Horticulture, Bidhan Chandra Krishi Vidyalaya, Mohanpur, Nadia, West Bengal, India. The study employed a completely randomized design with ten treatments, each replicated three times. The findings of this investigation revealed that among the various concentrations of IAA, IBA, and NAA, hardwood cuttings treated with IAA at 1500 ppm exhibited the earliest bud initiation (7 days). The best result for the first root initiation (7 days) was observed with IAA at 1000 ppm. IBA at 1500 ppm resulted in the highest number of leaves per cutting (25), while IBA at 1000 ppm produced the highest number of buds per cutting (4). Furthermore, the highest number of roots (9.2) and the greatest average root length (8.7 cm) were achieved with IBA at 1500 ppm.

Introduction

Bougainvillea belongs to the dicot family Nyctaginaceae and is native to tropical and subtropical South America, making it well-adapted to similar climatic conditions. There are about 10 species of *Bougainvillea*, but only three are horticulturally important: *B. spectabilis*, *B. peruviana*, and *B. glabra*. This plant can tolerate both semi-shaded areas and full sun, though it thrives best in full sunlight. A minimum of 5 hours of direct sunlight per day is required for good blooming, and more hours of sunlight are even better. *Bougainvillea* does not require a lot of water and is prized by gardeners for its wide range of habitats, prolonged flowering seasons, and variety of flower colours. It also has numerous uses in landscaping- (Bhardwaj *et al.*, 2020).

Bougainvillea blooms in the winter, starting in October when the weather becomes cool and pleasant, with a second flush of flowers in the summer. However, some varieties, like Snow Queen and Snow-White, only bloom once during the winter. The plant can be used as a shrub or climber and can be trained to grow in pergolas. It can grow over 30 feet high, with large, simple, mostly ovate leaves that may have rippling edges and hairs underneath. The flowers are usually surrounded by three brightly coloured bracts, while the actual flowers are small, tubular, and often inconspicuous, located in the centre of the bracts. Bract colours include white, light mauve, magenta, pink, deep mauve, orange, yellow, and red. *Bougainvillea* has a fragile root system that does not form a good root ball and has many adaptations to its climate and environment- (Bhardwaj *et al.*, 2020)

Betacyanin's and flavanols are extracted from the bracts of *Bougainvillea glabra* (Heuer *et al.*, 1994), and pinitol, an anti-diabetic compound, has been isolated from the leaves of *Bougainvillea spectabilis* (Narayanan *et al.*, 1987). *Bougainvillea* is a valuable ornamental plant with culinary uses and is also utilized in traditional medicine for treating common ailments. It is traditionally used against several diseases, including diarrhoea, hypotension, intestinal disorders, stomach-ache, nausea, inflammation-related ailments, and pain

Comment [p1]: If the authors want to mention the name of the Variety in the title, the scientific name should be written in full and correctly.

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Comment [p2]: An opening sentence should be written about the importance of the plant and the importance of improving its propagation.

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Comment [p3]: These treatments must be described. What growth regulators are used and what is their concentration?

Comment [p4]: The containers, the planting media, and the conditions for incubating the cuttings should be mentioned briefly.

Comment [p5]: This is the first mention of these abbreviations here, so the full name should be mentioned.

Comment [p6]: It is preferable to use the unit mg/L, which is equivalent to the unit parts per million (ppm). Expression in ppm is considered a somewhat old unit.

Comment [p7]: There are clear differences between the recorded characteristics for each treatment and each concentration. What recommendation do the authors consider useful for the farmer who wants to produce this plant (*Bougainvillea glabra* var. "Mohan") early? This recommendation can be written here in the A ... [1]

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Comment [p13]: Do the authors mean Bhardwaj and Kumar (2020)???

Comment [p14]: abbreviate the genus name (*B. glabra*)

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45 | management (Saleem *et al.*, 20202021). The leaves have anti-inflammatory potential (Giri *et*
46 | *al.*, 1988), and traditional practitioners in Mandsaur, India, use the leaves to treat various
47 | gastrointestinal disorders like diarrhoea and acidity. Additionally, the anti-diarrheal activity
48 | of *Bougainvillea glabra* is related to its antimicrobial properties.
49 |

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Comment [p18]: abbreviate the genus name (*B. glabra*)

Comment [p19]: Where are the references?
See :
https://link.springer.com/content/pdf/10.1007/978-981-15-1554-5_2-1.pdf

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Comment [p20]: The authors have exaggerated the importance of the plant and have not mentioned any information about the problem of the present study. Rooting of cuttings in *Bougainvillea* is quite difficult and there are many studies that have done anatomical and hormonal studies to explain this. This should be mentioned in detail in a separate paragraph here.

Please see this recent work:
Horticulturae 2022, 8(12),
1156; <https://doi.org/10.3390/horticulturae8121156>

Comment [p21]: Add references

Comment [p22]: IAA and IBA are also used commercially in the field and in tissue culture, and this should also be noted.

Comment [p23]: The introduction cannot end like this. Please explain the importance of the current study and the purpose of the experiment.

Comment [p24]: Please add location coordinates (longitude and latitude)

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Comment [p25]: Thickness is written in centimeters like length.

Comment [p26]: Please note that: 1 mg/L = 1 part per million (ppm) for dilute aqueous solutions. The authors probably mean 1 g/L. Please revise and correct.
Hormone concentrations are better stated later, please delete this from here.

Comment [p27]: The name and country of the company of the growth regulator must be mentioned.

50 | Adventitious root formation is a crucial step in the vegetative propagation of woody or
51 | horticultural species, and problems associated with rooting cuttings can result in significant
52 | economic losses. The application of exogenous auxin/rooting hormones can help overcome
53 | these challenges. Commercially available exogenous auxins that aid in the formation of
54 | adventitious roots include Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), and
55 | Naphthalene Acetic acid (NAA). Naturally occurring auxins like IAA and IBA favor
56 | apical dominance, help control xylem differentiation, and aid in cell division. NAA is a
57 | synthetic plant hormone in the auxin family and is an ingredient in many commercial plant
58 | rooting products. It is used as a rooting agent for the vegetative propagation of plants from
59 | stem and leaf cuttings and in plant tissue culture (Memon *et al.*, 2013).

60 | Materials and methods

61 | The experiment was conducted at a site in Jalpaiguri, West Bengal, India, using hardwood
62 | cuttings of *B. glabra* var. "Mohan" collected from the premises of the Faculty of Horticulture,
63 | Bidhan Chandra Krishi Vidyalaya, Mohanpur, Nadia, West Bengal, India. The hardwood
64 | cuttings, each 15 cm in length and with the thickness of a pencil, were prepared in January. A
65 | quick dip treatment (10 seconds) at the basal portion with different concentrations of IAA,
66 | IBA (Indole-3-butyric acid), and NAA (Naphthalene acetic acid) was applied. The stock
67 | solutions were prepared by dissolving 1 mg/liter of NAA or IBA in distilled water, using
68 | ethyl alcohol to aid dissolution.

69 | The experiment followed a Randomized Block Design (RBD) with three replications and ten
70 | treatments as follows:

- 71 | • T1: IAA 500 ppm
- 72 | • T2: IAA 1000 ppm
- 73 | • T3: IAA 1500 ppm
- 74 | • T4: IBA 500 ppm
- 75 | • T5: IBA 1000 ppm
- 76 | • T6: IBA 1500 ppm
- 77 | • T7: NAA 500 ppm
- 78 | • T8: NAA 1000 ppm
- 79 | • T9: NAA 1500 ppm
- 80 | • T10: Control (No chemical)

81 | A total of 210 hardwood cuttings of *Bougainvillea* var. "Mohan," each 20 cm long and of
82 | pencil thickness, were prepared. The beds were drenched with fungicide before planting the
83 | cuttings, and black polythene was used below the bed for bottom heating to encourage faster
84 | rooting. IAA solutions of 500 ppm, 1000 ppm, and 1500 ppm were prepared by dissolving
85 | 0.5 g/l, 1 g/l, and 1.5 g/l, respectively, in distilled water. IBA and NAA solutions were
86 | prepared in the same manner. ~~The cuttings were treated with the growth regulators at the~~
87 | ~~basal portion and then planted in the beds following the Randomized Block Design.~~

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Comment [p29]: What are the details of the propagation media, the planting containers used, the conditions for incubating the cuttings, the temperature, humidity, lighting, etc.?

88 The observations recorded included:

- 89 • Days taken for first bud initiation in cuttings
- 90 • Days taken for first rooting of cuttings
- 91 • Number of leaves per cutting
- 92 • Number of buds per cutting
- 93 • Number of roots per cutting
- 94 • Length of roots in cuttings

95 Final data for the parameters (number of leaves, number of buds per cutting, number of roots
96 per cutting, and length of roots) were recorded after 8 weeks. Data were also collected in the
97 1st, 3rd, 4th, 6th, and 8th weeks after planting the cuttings.

Comment [p30]: Why is the survival rate not recorded after 8 weeks?

98 Statistical analysis

Comment [p31]: How was the data statistically analyzed? What is the method followed and what software was used?

99 4.RESULTS AND DISCUSSIONS

100 The experiment concluded that treatment T₃ (IAA 1500 ppm) showed the earliest bud
101 initiation, taking the fewest days (7) for the first bud to appear. Treatments T₉ (NAA 1500
102 ppm) and T₇ (NAA 500 ppm) produced similar results, with the first buds appearing in 8 and
103 9 days, respectively. In contrast, the control treatment T₁₀, which did not use any hormones,
104 resulted in late bud initiation (15 days). This suggests that the exogenous application of auxin
105 helps break down starch into simple sugars, which are essential for new cell production and
106 increased respiratory activity during the regeneration of tissue at the time of new primordial
107 initiation. These results are consistent with the findings of Kumar *et al.* (2014) in Carnation.

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108 For the days taken for first rooting of cuttings, treatment T₂ (IAA 1000 ppm) gave the best
109 results, with root initiation starting very quickly (7 days). However, this treatment did not
110 produce a large number of roots later on. Treatments T₄ (IBA 500 ppm), T₆ (IBA 1500 ppm),
111 T₃ (IAA 1500 ppm), and T₅ (IBA 1000 ppm) also performed well, with root initiation
112 occurring in 8 to 9 days. This indicates that the appropriate concentration of plant growth
113 regulators can enhance cell division, cell elongation, and early differentiation of callus tissue
114 toward root formation, resulting in early growth in cuttings. These findings align with the
115 observations of Netam *et al.* (2018) and Bhardwaj and Kumar (2020) in Bougainvillea.

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116 The highest number of leaves was observed in treatment T₆ (IBA 1500 ppm) with 25 leaves.
117 Treatments T₅ (IBA 1000 ppm) and T₄ (IBA 500 ppm) also performed well, with 22 and 19
118 leaves, respectively. However, treatments T₁ (IAA 500 ppm) and T₇ (NAA 500 ppm)
119 produced fewer leaves (13 and 12, respectively), and the control treatment T₁₀ had the fewest
120 leaves (10). The highest number of buds per cutting was found in treatment T₅ (IBA 1000
121 ppm) with 4 buds, followed closely by T₄ (IBA 500 ppm) and T₆ (IBA 1500 ppm). The
122 control treatment performed poorly in this regard. This can be attributed to IBA's ability to
123 produce healthier and longer roots, which absorb more nutrients and water, resulting in more
124 leaves. Additionally, the vigorous growth and early root initiation induced by growth
125 regulators allow for better nutrient absorption and increased leaf production. These results are
126 consistent with the findings of Sahariya *et al.* (2013) and Kale and Bhujbal (1972) in
127 Bougainvillea.

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128 Treatment T₆ (IBA 1500 ppm) also produced the highest number of roots and had the best
129 average root length. Treatments T₄ (IBA 500 ppm) and T₅ (IBA 1000 ppm) also performed

130 well. In contrast, the control treatment had poor rooting results. This suggests that IBA
 131 increases cell wall plasticity, cell division, callus development, and root growth. Cuttings
 132 treated with the appropriate concentration of auxin showed early and better root initiation,
 133 leading to a higher number of roots per cutting. Increased root length in IBA-treated cuttings
 134 may be due to enhanced carbohydrate hydrolysis, new protein synthesis, cell enlargement,
 135 and cell division induced by auxins. Auxins also initiate the synthesis of structural enzyme
 136 proteins involved in adventitious root formation, increasing root length through acidification.
 137 These results align with the findings of Singh *et al.* (2017) in Bougainvillea and Bhatt and
 138 Chauhan (2012) in Marigold marigold

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Comment [p36]: The authors used 3 types of auxins and there are significant differences in the rooting response of the cuttings for these types. What is the reason for the superiority of auxin IBA over the other types in most variables (according to the authors' conclusion)? It is known that auxin IAA is naturally present and therefore there is an internal enzyme that breaks it down and reduces its activity, what about auxin NAA which is synthetic? This should be explained with relevant references.

140

141 **Conclusion**

142 The experiment aimed to study the effect of growth regulators on the sprouting and rooting of
 143 *Bougainvillea* hardwood cuttings, and the findings were significant. It was observed that IAA
 144 at 1500 ppm resulted in the earliest bud initiation, while NAA at both 1500 ppm and 500 ppm
 145 produced similar results for bud initiation. For root initiation, IAA at 1000 ppm gave the best
 146 results. The highest number of leaves were seen in cuttings treated with IBA at 1500 ppm,
 147 and the most buds per cutting were observed with IBA at 1000 ppm. In terms of root
 148 development, the number of roots and the average root length were highest in cuttings treated
 149 with IBA at 1500 ppm. These results indicate that IBA at a higher concentration (1500 ppm)
 150 yielded the best overall outcomes for rooting *Bougainvillea* hardwood cuttings var. "Mohan,"
 151 demonstrating a significant positive impact on both shoot and root growth.

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152 **Table 1. Outcome of the different treatments**

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Treatment	Days taken to first bud	Days taken to first rooting of cutting	Number of leaves per cuttings±	Number of buds per cuttings
T ₁	12	8	13	3
T ₂	13	7	18	2.7
T ₃	7	9	15	2.5
T ₄	10	8	19	3.3
T ₅	11	9	22	4
T ₆	11	8	25	3.3
T ₇	9	11	12	3
T ₈	12	13	14	3.2
T ₉	8	12	17	3
T ₁₀	15	15	10	2
SEm	0.80	0.87	1.55	0.18
CD @-at5%	2.31	2.60	4.48	0.51

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157 **Table 2. Average root length in each treatment**

Treatment	Number of roots per cuttings	Avg. root length (cm)
T ₁	7	3.5
T ₂	8.2	5.2
T ₃	8.9	3
T ₄	9	5
T ₅	10	8.5
T ₆	15	8.7
T ₇	8.6	3
T ₈	9.2	3.5
T ₉	8	4
T ₁₀	5	3.2
SEm	0.85	0.72
CD @ 5%	2.46	2.09

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