PLANT SCIENCE TODAY
Vol 12(3): 1-6
https://doi.org/10.14719/pst.7084

eISSN 2348-1900

RESEARCH ARTICLE

Efficacy of IBA on the performance of hardwood and softwood cuttings of Malay apple

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Received: 05 January 2025; Accepted: 26 April 2025; Available online: Version 1.0: 13 June 2025; Version 2.0: 01 July 2025

Cite this article: Bhowmick N, Singh K, Ghosh A, Chamling N, Parameshwar P, Santhoshkumar GM, Senjam BD. Efficacy of IBA on the performance of hardwood and softwood cuttings of Malay apple. Plant Science Today. 2025; 12(3): 1-6. https://doi.org/10.14719/pst.7084

Abstract

The Malay apple (*Syzygium malaccense* (L.) Merr. L.M. Perry), a minor fruit crop in the Myrtaceae family, is gaining popularity due to its attractive colour, shape, appearance, thirst-quenching appeal and nutritional value. However, large-scale cultivation is constrained by the scarcity of quality planting materials, as the species predominantly relies on seed propagation. This study aimed to identify the optimal Indole-3-Butyric Acid (IBA) concentration for the vegetative propagation of Malay apple using hardwood and softwood cuttings. The experiment was conducted at Uttar Banga Krishi Viswavidyalaya, Cooch Behar, West Bengal, using a Completely Randomized Design (CRD) with 6 treatments: T_1 (control), T_2(IBA 1000 ppm), T_3(IBA 2000 ppm), T_4(IBA 3000 ppm), T_5 (IBA 4000 ppm) and T_6 (IBA 5000 ppm). Each treatment included 60 cuttings (30 hardwood and 30 softwood) collected from 8-year-old trees. Cuttings (15 cm long with 3-4 nodes) were treated with the respective IBA concentration and planted in poly bags containing a 1:1:1 mixture of farmyard manure, soil and sand. The cuttings were maintained under polyhouse conditions for 120 days. Recorded parameters included sprouting percentage, days to sprouting, sprout length, number of roots, days to rooting, leaf number, root length and biomass. Data were analyzed using ANOVA and the LSD test in SAS (Version 9.3), with normality assessed by the Kolmogorov-Smirnov test. Results demonstrated that IBA at 3000 ppm (T_4) significantly enhanced all growth parameters, increasing rooting percentage by 127 - 130 % and survival rates by 104 - 164 % compared to the control. These findings establish 3000 ppm IBA as the optimal concentration for the successful propagation of Malay apple.

Keywords: cuttings; IBA concentration; malay apple; propagation; survival rate

Introduction

Malay apple [*Syzygium malaccense* (L.) Merr. L.M. Perry] is commonly known as Jambu and regarded as one of the essential minor fruits in West Bengal (1). The Malay apple fruit is gaining popularity for its delicious taste and thirst-quenching properties and it is commonly consumed in raw form. Southern India and Malaysia are believed to be the origin of this fruit, which has now found its way to South America, the Indian Subcontinent and sub-Saharan Africa (2). Over time, its cultivation has gained popularity worldwide, with improved techniques enhancing fruit quality and yield (3). Malay apple fruits are an excellent source of essential minerals such as calcium, iron and vitamins A and C (4). The fruit is characterized by an oblong or bell shape, ranging from 5- 10 cm in length and 2.5 - 7.5 cm in width at the apex. It has smooth, waxy skin and its flesh is red, crisp or spongy and juicy with a mild, sweet flavour (4). With a moisture content of about 91.7 %, Malay apple is classified as a low-calorie fruit, making it a refreshing choice for health-conscious individuals (5). Furthermore, it is rich in antioxidants, with significant antioxidant activity ranging from 138.4 - 144.50 g/g (6). The fruit also contains 253 IU of Vitamin A per 100 g, 13.06 - 130.08 mg of ascorbic acid per 100 g, total phenolic content between 28.0 and 30.70 mg/ 100 g and total flavonoid levels between 62.03 and 62.07 g/g.

Additionally, Malay apple provides 0.31 % protein, 0.29 % fat and 0.64 mg/100 g, calcium (7). The total soluble solids range between 5.46 and 6.02 °Brix. Additionally, the fruit contains protein levels between 0.6 - 1.04 g, fibre from 0.7 - 1.93 g and carbohydrates from 5.05 - 6.48 g (8). The energy content varies from 26.07 - 29.88 kcal and phosphorus between 11.6 and 17.9 mg per 100 g of fruit. Malay apple is highly nutritious and rich in phytochemicals with significant pharmacological properties. Chalcone is one of the most important bioactive compounds in the fruit and it has a wide range of medicinal benefits. Aurentiacin is one of the predominant chalcones and proanthocyanidins like samarangenins A and B have been detected in leaf extracts. The fruit also contains essential oil, mainly composed of mono- and sesquiterpenes (9). The main anthocyanin in Malay apple is cyanidin 3-glucoside, with variants like cyanidin 3,5-diglucoside and peonidin 3-glucoside (10). Given the fruit's nutritional benefits and commercial potential, there is an increasing trend in Malay apple cultivation across different parts of West Bengal. The absence of high-quality planting material significantly hinders promoting its cultivation.

Traditionally, propagation has been done through seeds, but due to their recalcitrant nature, seeds lose viability quickly, resulting in considerable variation in fruit shape, size and colour. Auxins like IBA enhance root initiation by promoting cell division and differentiation, making them highly effective for asexual propagation of woody plants (11). While seed propagation remains standard, asexual methods using IBA-treated cuttings offer distinct advantages, including genetic uniformity and preservation of desirable traits. Techniques such as budding, cutting, air layering and grafting are effective for propagation. Among these, cuttings treated with Indole-3-Butyric Acid (IBA) have emerged as one of the quickest and most effective techniques for plant propagation (11, 12). Prior studies on *Syzygium* species suggest optimal IBA ranges between 1000 - 5000 ppm, though the ideal concentration for Malay apple remains undetermined (13). This study aims to evaluate the effects of different concentrations of Indole-3-Butyric Acid (IBA) on the rooting performance of softwood and hardwood cuttings of Malay apple. We hypothesized that applying IBA would significantly enhance root initiation, improve rooting percentage and accelerate overall growth performance, thereby ensuring a reliable technique for mass propagation of Malay apple. Despite the promising potential of IBA in propagation, the optimal concentrations for rooting success in Malay apple cuttings remain unexplored. This study aims to fill this gap by evaluating different IBA concentrations on softwood and hardwood cuttings, providing insights into developing a reliable propagation method for commercial and conservation purposes.

Materials and Methods

The present study was conducted in the Instructional Farm of Polyhouse, Department of Pomology and Post-Harvest Technology, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India during 2021 - 22. The environmental conditions during the study were characterized by daytime temperatures ranging from 22-31 ^{o}C, relative humidity levels between 67.5 % and 70 % and a photoperiod from 5 to 8 hr. The district is situated in the foothills of the eastern Himalayas at a geographic location of 28^{o}58'86" N latitude, 81^{o}66''73'' E longitude with an elevation of 42 m above mean sea level. The experiment followed a Completely Randomized Design featuring 6 treatments: T_1 (control), T_2 (IBA at 1000 ppm), T_3 (IBA at 2000 ppm), T_4 (IBA at 3000 ppm), T_5 (IBA at 4000 ppm), T_6 (IBA at 5000 ppm). Each treatment consisted of 60 stem cuttings, with 4 replications per treatment. Softwood and hardwood cuttings, each measuring 15 cm long and containing 3 - 4 nodes, were collected from 8-year-old Malay apple (*S. malaccense*) trees in June. Small sections were trimmed from both ends of the cuttings to encourage new shoot growth, just above and below the nodes. A slanted cut was made at the base of each cutting to enhance the area for absorption, promoting successful rooting. The cuttings were then treated with various concentrations of IBA solution as specified above. The layout of treatments and planting design is illustrated in Fig. 1. After treatment, the cuttings were planted in poly bags filled with a mixture of farmyard manure, soil and sand (1:1:1) as the rooting medium and kept in polyhouse conditions for 120 days. The selected IBA concentrations (1000 - 5000 ppm) were based on previous studies on *Syzygium* species and other woody perennials, demonstrating improved rooting responses within this range (13). Lower concentrations (1000 - 3000 ppm) were included to assess their effectiveness in promoting root initiation,

Fig. 1. (a) Collection of cuttings from the Malay Apple mother plant grown at the Instructional Farm, UBKV (b & d) Preparation of softwood cuttings with leaves (c) Preparation of hardwood cuttings (e) Cuttings treated with fungicide (f) Preparation of IBA solution (g) Record keeping with cuttings (h) Placement of cuttings under the polyhouse at the Instructional Farm, UBKV.

while higher concentrations (4000 - 5000 ppm) were tested to evaluate whether increased auxin levels further enhance rooting or lead to toxicity. The control (0 ppm) was included for comparison. Previous research has shown that auxin application significantly improves root development in *Syzygium* species and other woody species, justifying the selected concentration range (14, 15). Each replication randomly selected 5 cuttings for recording morphological characters, including rooting percentage, number of roots per cutting, root length, shoot length, number of leaves per cutting and survival percentage. Statistical analysis was performed using analysis of variance (ANOVA) for each parameter with the Proc Glm procedure in SAS software (Version 9.3). Mean separation among accessions across different parameters was conducted using the Least Significant Difference (LSD) test at a significance level of P ≤ 0.05. Normality of residuals under the assumptions of ANOVA was tested using the Kolmogorov-Smirnov test with the Proc-Univariate procedure of SAS (Version 9.3).

Results

The data presented in Table 1 reveal significant variation among the treatments regarding sprouting per cent, sprouting days and sprout length in hardwood cuttings and softwood cuttings of Malay apple. Among the treatments, the maximum sprouting percentage and sprout length were observed in hardwood cuttings treated with IBA at 3000 ppm, showing a 161.19 % increase in sprouting percentage over the control, followed by softwood cuttings with a 151.56 % increase. The shortest sprouting time in Malay apple was recorded in hardwood cuttings treated with IBA 3000 ppm, with 30.16 % reduction in days taken for sprouting compared to the control, followed by a 19.91 % decrease in softwood cuttings. Similarly, sprout length in both softwood and hardwood cuttings was significantly influenced by IBA at 3000 ppm. The significant increase in sprout number might be attributed to the effect of IBA at different concentrations.

Table 2 shows that Malay apple cuttings treated with concentrations of IBA at 3000 ppm exhibited the maximum number of leaves, with hardwood cuttings and softwood cuttings showing a 140.70 % and 117.01 % increase over the control respectively. The shortest duration taken for rooting was observed in hardwood and softwood cuttings treated with IBA at 3000 ppm, with a reduction of 15.48 % and 12.61 % over the control. Additionally, root numbers were maximum in hardwood and softwood cuttings with concentrations of IBA 3000 ppm showing 104.31 % and 60.47 % respectively, over the control. The increment of leaves might be attributed to robust development induced by IBA, which improved nutrient uptake, stimulated shoot growth and enhanced node production.

Data presented in Table 3 show that Malay apple cuttings treated with IBA at 3000 ppm exhibited the highest rooting percentage with 127.43 % and 129.92 % in hardwood and softwood cuttings. The maximum root fresh weight and dry weight were also observed with IBA concentration at 3000 ppm, showing an increment of 104.86 % and 137.54 % in hardwood cuttings and 109.18 % and 165.98 % in softwood cuttings, respectively over control.

Table 1. Influence of IBA on sprouting characters of Malay apple cuttings

Treatment	Sprouting percentage (%)		Days taken for sprouting		Sprout length (cm)	
	Softwood cuttings	Hardwood cuttings	Softwood cuttings	Hardwood cuttings	Softwood cuttings	Hardwood cuttings
T_1 (control)	56.25 ^{d} (100. 00)	54.75 ^{d} (100. 00)	18.43^{a}	12.40^{a}	15.83^{f}	17.09^{f}
T_2 (1000 ppm)	65.25 ^{bc} (116.00)	82.25 ^{b} (150.23)	16.21^{d}	9.79^{d}	24.46^{d}	26.60^{c}
T_3 (2000 ppm)	70.25 ^{b} (124.89)	84.75 ^{ab} (154.79)	15.80^{e}	9.21^{e}	28.94^{b}	27.96^{b}
T_4 (3000 ppm)	85.25 ^{a} (151.56)	88.25 ^{a} (161.19)	14.76^{f}	8.66^{f}	32.52^{a}	31.74^{a}
T_5 (4000 ppm)	67.75 ^{bc} (120.44)	73.50 ^{c} (134.25)	17.28^{c}	11.80^{c}	26.88^{c}	21.97^{d}
T_6 (5000 ppm)	59.75 ^{cd} (106.22)	72.25 ^{c} (131.96)	18.21^{b}	12.19^{b}	21.11^{e}	21.05^{e}
S.Em. (±)	1.83	1.33	0.02	0.02	0.66	0.31
CD at ≤ 5 %	5.47	3.97	0.05	0.05	1.97	0.92

* Means with same letter within a column are not significantly different at P ≤ 0.05. Values in parenthesis indicate angular transformed values. The normality of residuals was tested using the Kolmogorov-Smirnov test with the Proc-Univariate procedure in SAS (version 9.3).

Table 2. Influence of IBA on the leaf count, rooting time and root number in Malay apple cuttings

Treatments	Number of leaves		Days taken for rooting		Number of roots	
	Softwood cuttings	Hardwood cuttings	Softwood cuttings	Hardwood cuttings	Softwood cuttings	Hardwood cuttings
T_1 (control)	20.75^{e}	22.53^{d}	27.82^{a}	21.76^{a}	12.93^{d}	13.50^{d}
T_2 (1000 ppm)	32.03^{c}	44.75^{b}	25.41^{c}	19.10^{d}	15.70^{bc}	18.60^{b}
T_3 (2000 ppm)	38.65^{b}	45.23^{b}	24.82^{d}	18.50^{e}	17.85^{b}	19.18^{b}
T_4 (3000 ppm)	45.03^{a}	54.23^{a}	24.31^{e}	18.39^{f}	20.75^{a}	22.58^{a}
T_5 (4000 ppm)	30.58^{cd}	33.13^{c}	25.48^{c}	20.14^{c}	15.25^{cd}	16.90^{bc}
T_6 (5000 ppm)	28.88^{d}	32.93^{c}	27.06^{b}	21.17^{b}	14.73^{cd}	15.55^{cd}
S.Em. (±)	0.90	0.92	0.07	0.02	0.82	0.84
CD at ≤ 5 %	2.69	0.96	0.20	0.05	2.46	2.51

*Means with the same letter within a column are not significantly different at P ≤ 0.05. The normality of residuals was tested using the Kolmogorov-Smirnov test with the Proc-Univariate procedure in SAS (version 9.3).

Table 3. Influence of IBA on rooting percentage, fresh and dry weight of roots in Malay apple cuttings

Treatments	Rooting percentage (%)		Fresh weight of root (g)		Dry weight of root (g)	
	Softwood cuttings	Hardwood cuttings	Softwood cuttings	Hardwood cuttings	Softwood cuttings	Hardwood cuttings
T_1 (control)	66.00 ^{c} (100.00)	72.00 ^{e} (100.00)	10.76^{e}	12.94^{d}	4.88^{d}	6.64^{d}
T_2 (1000 ppm)	71.75 ^{c} (108.71)	81.25 ^{c} (112.85)	18.48^{c}	19.88^{b}	8.98^{bc}	11.96^{b}
T_3 (2000 ppm)	81.50 ^{ab} (123.48)	85.75 ^{b} (119.10)	21.58^{b}	25.58^{a}	10.21^{b}	12.77^{a}
T_4 (3000 ppm)	85.75 ^{a} (129.92)	91.75 ^{a} (127.43)	25.56^{a}	26.51^{a}	12.98^{a}	13.89^{a}
T_5 (4000 ppm)	77.75 ^{b} (117.80)	79.75 ^{cd} (110.76)	16.97^{c}	16.44^{c}	8.90^{bc}	9.11^{c}
T_6 (5000 ppm)	77.50 ^{b} (117.42)	76.50 ^{d} (106.25)	14.11^{d}	14.59^{dc}	7.95^{c}	8.09^{c}
S.Em. (±)	1.21	0.77	0.55	1.03	0.49	0.45
CD at ≤ 5 %	3.63	2.32	1.65	3.08	1.46	1.36

*Means with the same letter within a column are not significantly different at P ≤ 0.05. Values in parentheses indicate angular transformed values. The normality of residuals was tested using the Kolmogorov-Smirnov test with the Proc-Univariate procedure in SAS (version 9.3).

Data presented in Table 4 highlights the significant effects of IBA on the success and survival rates of Malay apple cuttings. Maximum success rates in hardwood and softwood cuttings were recorded with IBA at 3000 ppm, showing an increase of 163.68 % and 152.51 % respectively, compared to the control. Similarly, survival rates were also enhanced by 128.06 % and 125.86 % in Malay apple's hardwood and softwood cuttings respectively.

Discussions

Auxin, particularly indole-3-butyric acid (IBA), plays a crucial role in stimulating root development in hardwood cuttings and softwood cuttings of various fruit crops by promoting cell elongation, division and differentiation in the rooting zone (16–18). The present study observed that leaves on cuttings often fell during the root induction phase as a strategy to allocate more energy to root initiation. This highlights how various factors synergistically promote root and shoot initiation in cuttings. Similarly, the significant impact of different IBA concentrations on sprouting time in Malay apple cuttings may be attributed to a high level of stored carbohydrates and low to moderate nitrogen concentrations, which facilitate shoot development by hydrolysing, mobilizing and using nutritional reserves. This finding aligns with the research emphasising nutrient availability's significant influence on shoot initiation and growth. An optimal concentration of IBA was found to enhance nutrient uptake, leading to enhanced photosynthesis, which provided the necessary energy for cell division and elongation. This ultimately improved the sprouting in the cuttings, which aligns with the earlier finding (15). Similarly, applying IBA at 3000 ppm improved root and shoot development in *Pinus caribaea* var. *hondurensis* Morelet cuttings (19). The increased rooting percentage in Malay apple cuttings observed in our study may be attributed to IBA's role in stimulating root cell development through its conversion into synergy with endogenous IAA (20). The increase in root numbers in Malay apple cuttings treated with an IBA concentration of 3000 ppm is likely due to IBA's ability to stimulate cambial activity, promote lateral root formation and accelerate carbohydrate transport to the basal portion of the cuttings.

Additionally, it increases cell wall plasticity, facilitating rapid cell division, callus formation and root growth, consistent with the reports in guava (21). The application of IBA promoted rooting percentage by accelerating the root primordia development and improving sugar transport to the nodes of the cuttings, leading to better root formation (22). Similar results were also observed with IBA application at 3000 ppm (23). Root and shoot formation in cuttings are

Table 4. Influence of IBA on success rate and survival percentage of Malay apple cuttings

Treatments	Success rate		Survival percentage (%)	
	Softwood cutting	Hardwood cutting	Softwood cutting	Hardwood cutting
T_1 (control)	54.75^{d} (100.00)	53.00^{d} (100.00)	73.03^{d} (100.00)	72.80^{e} (100.00)
T_2 (1000 ppm)	64.75^{bc} (118.26)	80.50^{b} (151.89)	84.76^{abc} (116.04)	87.73^{bc} (120.56)
T_3 (2000 ppm)	69.50^{b} (126.94)	84.00^{ab} (158.49)	87.25^{ab} (119.48)	90.64^{ab} (12455)
T_4 (3000 ppm)	83.50^{a} (152.51)	86.75^{a} (163.68)	91.92^{a} (125.86)	93.23^{a} (128.06)
T_5 (4000 ppm)	64.00^{bc} (116.89)	69.25^{c} (130.66)	80.90^{bcd} (110.81)	82.06^{cd} (112.76)
T_6 (5000 ppm)	57.25^{cd} (104.57)	71.00^{c} (133.96)	78.21^{cd} (107.09)	79.05^{de} (108.59)
S.Em.(±)	1.80	1.58	2.27	1.67
CD at ≤ 5 %	5.38	4.73	6.81	5.01

*Means with same letter within a column are not significantly different at P ≤ 0.05. Values in parenthesis indicate angular transformed values. The normality of residuals was tested using the Kolmogorov-Smirnov test with the Proc-Univariate procedure in SAS (version 9.3).

influenced by various factors such as genotype, cutting age, hormone levels, wood type, environment and root-promoting hormones. Our results align with those who observed a significant interaction of IBA at 3000 ppm in Water Yam (*Dioscorea alata* L.) (24). Furthermore, our study findings corroborate research on *Prunus Africana*, which demonstrated that rooting medium, auxin concentration and leaf area significantly influenced rooting success. In *P. africana,* sawdust provided the highest rooting success (80 %) and leaf area played a crucial role, with cuttings possessing larger leaves exhibiting more significant root formation (25).

Additionally, auxin application in *P. africana* showed improved rooting at an optimal 100 - 200 µg IBA range, beyond which supraoptimal effects were observed. Likewise, studies on *Solanum procumbens* demonstrate the species-specific response to auxins in vegetative propagation. A study evaluating the effects of IAA, IBA and NAA on *S. procumbens* stem cuttings found that IBA at 500 ppm resulted in the highest sprouting rate (92.34 %), root number (32.25), root length (6.8 cm), root weight (0.574 g), sprout length (11.7 cm) and leaf-pair number (7.5) (26). The diminishing effects of higher auxin concentrations observed in *S. procumbens* align with the supraoptimal effects reported in *Prunus africana*. This suggests that auxin sensitivity varies across species and must be carefully optimized for successful propagation. Additionally, similar findings in seedless wax apple propagation, where hardwood cuttings treated with 2500 ppm IBA achieved maximum survival rates, likely due to the presence of compounds like phenol, gibberellins and abscisic acid promoting root generation (27). Contrary to our findings, applying IBA at 3000 ppm significantly enhanced survival rates due to better root and shoot development in cuttings (28).

Conclusion

This study provides valuable insights into the propagation of Malay apple under the sub-Himalayan Terai region of West Bengal. By evaluating the effects of different Indole-3-Butyric Acid (IBA) concentrations, this study has contributed to developing an efficient vegetative propagation method that can benefit commercial cultivation and germplasm conservation. Identifying IBA 3000 ppm as the optimal treatment supports nursery growers and orchardists in improving rooting success and plant establishment. Future research should refine the propagation technique, investigate the genetic basis of rooting responses and assess the field performance and economic feasibility of IBA-treated cuttings to support large-scale adoption.

Acknowledgements

Authors acknowledged the research assistant provided by the Uttar Banga Krishi Viswavidyalay for successfully conducting the research.

Authors' contributions

NB supervised the experiment, developed the concept, conducted the experiment and prepared the manuscript. KS performed the experiment, kept the data and prepared the manuscript. AG carried out the data analysis. NC kept the data and made the review work. PP kept the data and made the review work. SGM edited the manuscript. BDS edited the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The Authors do not have any conflict of interest to declare.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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