



Propagation of grapevine through air layering using rooting hormones

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Abstract

Grapevine (*Vitis vinifera* L.) is an economically important fruit crop and the production of healthy, true-to-type planting material is a major constraint under conventional propagation methods due to poor rooting and low survival rates. Air layering, supplemented with suitable rooting hormones like IBA and NAA, can offer a promising alternative approach for rapid and efficient vegetative propagation. Keeping this in view, the present study was conducted at ICAR-National Research Centre for Grapes, Pune, Maharashtra, during 2022–23, to evaluate the influence of rooting hormones on air layering of Thompson Seedless grapevine grafted on Dogridge rootstock. The treatments consisted of IBA (2500 ppm), IBA (2500 ppm) + NAA (250 ppm), IBA (3000 ppm), IBA (3000 ppm) + NAA (250 ppm), along with an untreated control. Observations indicated that rooting hormones significantly improved air layering performance. The layers treated with 3000 ppm IBA exhibited a higher characteristic effect, viz., minimum number of days (56.00) taken for root initiation, maximum rooting percentage (78.33 %), length of the root (8.73 cm), diameter of the thickest root (3.28 mm), percentage of layers showing secondary roots (97.50 %), root dry matter (23.93 %), root volume (8.78 cm³), least number of days (15.25) required for the establishment of detached grafted layers, maximum percentage of final establishment of detached layers (94.67 %), number of new shoots per planted layer (6.83), length of shoot per planted layer (14.48 cm), length of the secondary shoot of layer (5.23 cm), layer girth (1.07 cm), leaf area (65.06 cm²), total carbohydrates (144.82 mg/g), total soluble protein (5.37 mg/g), peroxidase activity (0.133 mg/protein/min), polyphenol oxidase activity (0.790 EU/ml/min) and catalase activity (0.085 mg/protein/min). Thus, air layering supplemented with 3000 ppm IBA can provide a promising alternative for rapid vegetative propagation of grapevine.

Keywords: Thompson Seedless; Dogridge rootstock; root induction; IBA; NAA; *Vitis vinifera*

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1. Introduction

Grape (*Vitis vinifera* L.) is a highly valuable horticultural crop and one of the most important fruit crops in India, occupying an area of about 162000 hectares, which accounts for nearly 2.3 % of the total fruit crop area. India is a major exporter of fresh grapes, with a total export of 267950.39 tonnes valued at ₹2543.42 crores to various countries, including the European Union, Saudi Arabia and African countries (1).

Thompson Seedless is the most extensively cultivated commercial white table grape variety in India owing to its high yield and superior fruit quality. These vines are vigorous and adapt well to a variety of climates, making them a favoured choice for grape growers. Dogridge (*Vitis champinii* Planch.) is a natural hybrid of *Vitis rupestris* var. *candicans* which imparts great vigour to the scion. It is tolerant to salinity and well

suited for less fertile soil. It is moderately resistant to phylloxera and lime, making it one of the most widely adopted rootstocks in Indian viticulture. The Thompson Seedless-Dogridge combination is commonly used in commercial vineyards and therefore considered appropriate for the experiment.

Grapes are mainly propagated by vegetative methods, particularly hardwood cuttings, which ensure genetic uniformity in growth, fruit quality and yield. However, these methods often face constraints due to poor rooting and low survival rates, resulting in variable success rates.

Therefore, to meet the demand for genetically pure cultivars with better survivability, an alternative propagation method that is simple, rapid and efficient is required. Air layering is one of the simplest and most economical methods for producing true-to-type grape plants with improved precocity, yield and fruit quality. It

has been successfully used in several fruit crops such as litchi and guava and is one of the oldest vegetative propagation techniques (2).

The success of air layering can be further enhanced by the application of growth regulators, particularly auxins. Optimum concentrations of auxins such as IBA, NAA and IAA promote rooting in air layers, although their effectiveness varies with species and environmental conditions (3).

Although the application of auxins has been widely investigated for vegetative propagation in several fruit crops, information on the use of air layering for the propagation of grafted grapevines remains limited. Commercial grapevine multiplication is predominantly carried out through hardwood cuttings, which often exhibit variable rooting success and require considerable time to produce transplantable plants. Air layering offers a potential alternative by enabling the production of well-rooted grafted plants directly from established mother vines, thereby shortening the propagation cycle and improving planting material quality. Furthermore, limited information is available on the biochemical changes associated with auxin-induced rooting in grapevine air layers. Therefore, the present investigation was undertaken to evaluate the influence of different rooting hormone treatments on rooting behaviour, establishment, growth and biochemical attributes of grapevine air layers.

2. Materials and Methods

Experimental site

The present investigation was carried out during 2022–23 at the ICAR-National Research Centre for Grapes, Manjari Farm, Pune, Maharashtra. The experimental site is located in Mid-West Maharashtra at an altitude of 559 m above mean sea level (18.32° N and 73.51° E), characterised by a tropical wet and dry climate with an average temperature of 20–28 °C, annual rainfall of 700–750 mm (June–September) and a heavy black soil (pH 7.75; EC 0.46 dS m⁻¹).

Plant material and experimental details

For this experiment, a mother block of Dogridge rootstocks, vegetatively propagated through hardwood cuttings were established by planting rooted cuttings in the field at a spacing of 3 m × 1.8 m during June 2022. Further, in February 2023, well-matured, healthy, pencil-thick shoots (approximately 8–10 mm in diameter) of the cultivar (cv.) Thompson Seedless were grafted onto the established Dogridge rootstocks. After the success of the graft union and 6–7 months of growth, healthy and uniform grafted vines were selected and used for layering during the first fortnight of October 2023. Each selected vine had 7 to 8 nodes, with an average spacing of 6–7 cm between them.

Air layering procedure

A ring of bark about 2 cm wide was completely removed between two nodes from each selected vine with the help of a sharp knife without injuring it, at 10 cm from the ground. As per treatments, solutions were applied at the girdled portion with the help of a cotton swab. Then the girdled portion was wrapped with approximately 100 g of moist sphagnum moss, which was sterilised by soaking overnight in a broad-spectrum fungicide. Excess water was squeezed out before use. The moss was then securely enclosed with a transparent 200-gauge polythene sheet (approximately 15 × 20 cm) and both ends were tied tightly using a thread.

Root emergence was monitored through the transparent polythene wrapper and the air layers were detached five days after root emergence, following sufficient root development, by making a cut just below the lower portion of the air layer with the help of secateurs. The detached layers were brought to a nursery under a 50 % shade net, where the polythene wrappers were removed gently without injuring the roots. The rooted layers were planted in planting bags containing growing medium of soil, sand and vermicompost in a 2 : 1 : 1 ratio.

Observations recorded

The observations on the rooting behavior and biochemical parameters were recorded immediately after detachment, whereas the establishment, growth measurements and final survival were assessed 45 days after detachment. Each rooted air layer served as an individual observational unit and observations were recorded separately for each layer.

Biochemical analysis

Biochemical parameters of the rooted portions were analysed, including total carbohydrates (4) and total soluble proteins (5). Enzyme activities such as peroxidase, polyphenol oxidase and catalase were estimated following standard protocols (6–8). The absorbance for estimation of total carbohydrates, total soluble proteins, peroxidase, polyphenol oxidase and catalase activities was recorded using a UV-VIS spectrophotometer.

Experimental layout and treatments

The experiment was laid out in a randomised block design (RBD) and consisted of 5 treatments with IBA and NAA concentrations viz., N1 [IBA (2500 ppm)], N2 [IBA (2500 ppm) + NAA (250 ppm)], N3 [IBA (3000 ppm)], N4 [IBA (3000 ppm) + NAA (250 ppm)] and N5 [No application (Control)] with 3 replications. Each replication comprised ten genetically uniform grafted grapevines and one air layer was prepared on each vine, resulting in 30 air layers per treatment and 150 air layers in total. The data presented represent observations recorded during a single experimental season (2022–23).

Statistical analysis

Experimental data were subjected to analysis of variance (ANOVA) using a Microsoft Excel-based statistical worksheet. The results are expressed as mean values and standard deviation (SD) following the standard procedure (9). Treatment means were compared using the critical difference (CD) test at the 5 % level of significance. Percentage data were subjected to arcsine square-root transformation prior to statistical analysis to stabilise variance, whereas back-transformed means are presented in the tables for ease of interpretation.

3. Results

Rooting parameters

The observations indicated that auxin treatments significantly affected the rooting behaviour of grapevine air layers (Table 1 and 2; Fig. 1 and 2). The number of days required for root initiation differed significantly among treatments. The minimum number of days for root initiation (56.0) was recorded in layers treated with IBA 3000 ppm (N₃), followed by IBA 3000 ppm + NAA 250 ppm (N₄) with 58.75 days,

whereas the maximum number of days (72.25) was observed in the control (N₅).

Rooting percentage was also significantly influenced by growth regulator treatments. The highest rooting percentage (78.33 %) was recorded in N₃, followed by N₄ (68.33 %), whereas the lowest rooting percentage (45.83 %) was observed in untreated layers (N₅). The maximum number of roots per layer (55.08) was recorded in N₄, followed by N₃ (52.33), while the minimum number of roots (10.50) was observed in N₅.

Root length varied significantly among treatments, with the maximum length (8.73 cm) recorded in N₄, followed by N₃ (8.58 cm) and the minimum (6.15 cm) in N₅. The thickest root diameter was highest in N₃ (3.28 mm), followed by N₄ (3.03 mm), while N₅ recorded the minimum diameter (1.54 mm).

The highest percentage of layers showing secondary roots was recorded in N₃ (97.50 %), which was at par with N₄ (96.67 %), while the lowest percentage (84.17 %) was recorded in N₅. Root dry matter accumulation differed significantly, with the highest value in N₃ (23.93 %), followed by N₄ (21.41 %) and the lowest in N₅ (10.51 %). Similarly, maximum root volume was recorded in N₃ (8.78 cm³), followed by N₄ (7.81 cm³), while minimum root volume (1.34 cm³) was observed in N₅.

Table 1. Effect of rooting hormones on rooting behaviour of grapevine air layers

Treatment	Days taken for root initiation	Rooting percentage (%)	Number of roots per layer	Root length (cm)	Diameter of thickest root (mm)
N ₁ (IBA 2500 ppm)	63.75	59.17 (50.52)	26.08	7.58	1.98
N ₂ (IBA 2500 ppm + NAA 250 ppm)	64.00	67.50 (55.75)	41.67	8.20	2.75
N ₃ (IBA 3000 ppm)	56.00	78.33 (65.34)	52.33	8.58	3.28
N ₄ (IBA 3000 ppm + NAA 250 ppm)	58.75	68.33 (56.50)	55.08	8.73	3.03
N ₅ (Control)	72.25	45.83 (42.39)	10.50	6.15	1.20
SE m (±)	0.54	1.49	0.61	0.05	0.03
CD (5%)	1.53	4.25	1.75	0.14	0.10

*Values represent the mean of 3 replications, each comprising ten vines. Percentage data were subjected to arcsine square-root transformation before statistical analysis; original (back-transformed) means are presented, with transformed values in parentheses.

Table 2. Effect of rooting hormones on rooting and establishment parameters of grapevine air layers

Treatment	Secondary roots (%)	Root dry matter (%)	Root volume (cm ³)	Days for establishment	Final establishment (%)
N ₁ (IBA 2500 ppm)	91.67	14.08 (22.01)	3.99	18.50	85.00 (70.03)
N ₂ (IBA 2500 ppm + NAA 250 ppm)	93.33	18.39 (25.37)	6.26	17.75	88.25 (72.49)
N ₃ (IBA 3000 ppm)	97.50	23.93 (29.25)	8.78	15.25	94.67 (78.35)
N ₄ (IBA 3000 ppm + NAA 250 ppm)	96.67	21.41 (27.54)	7.81	15.75	91.50 (75.17)
N ₅ (Control)	84.17	10.51 (18.81)	1.34	22.25	66.25 (54.17)
SE m (±)	1.27	0.10	0.11	0.16	0.56
CD (5%)	3.64	0.28	0.31	0.47	1.62

*Values represent the mean of 3 replications, each comprising ten vines. Percentage data were subjected to arcsine square-root transformation before statistical analysis; original (back-transformed) means are presented, with transformed values in parentheses.





Fig. 1. Effect of rooting hormone treatments on rooting of grapevine air layers.

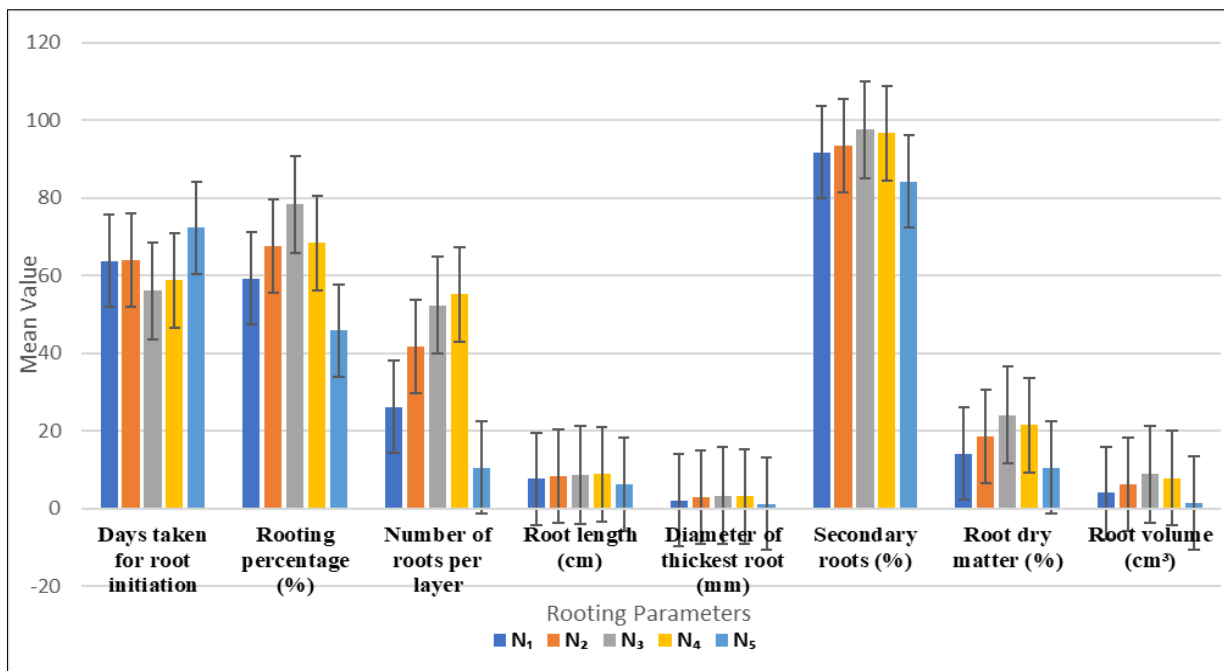


Fig. 2. Effect of rooting hormones on rooting behaviour.

Establishment parameters

The findings demonstrated that auxin treatments significantly affected the establishment parameters of grapevine air layers (Table 2; Fig. 3). The number of days required for the establishment of detached layers differed significantly among treatments. The minimum number of days (15.25) was recorded in

layers treated with IBA 3000 ppm (N₃), which was at par with N₄ (15.75 days), whereas the maximum number of days (22.25) was observed in N₅ (Control). The maximum final establishment percentage (94.67 %) was recorded in N₃, followed by N₄ (91.50 %), while the minimum establishment (66.25 %) was observed in N₅.

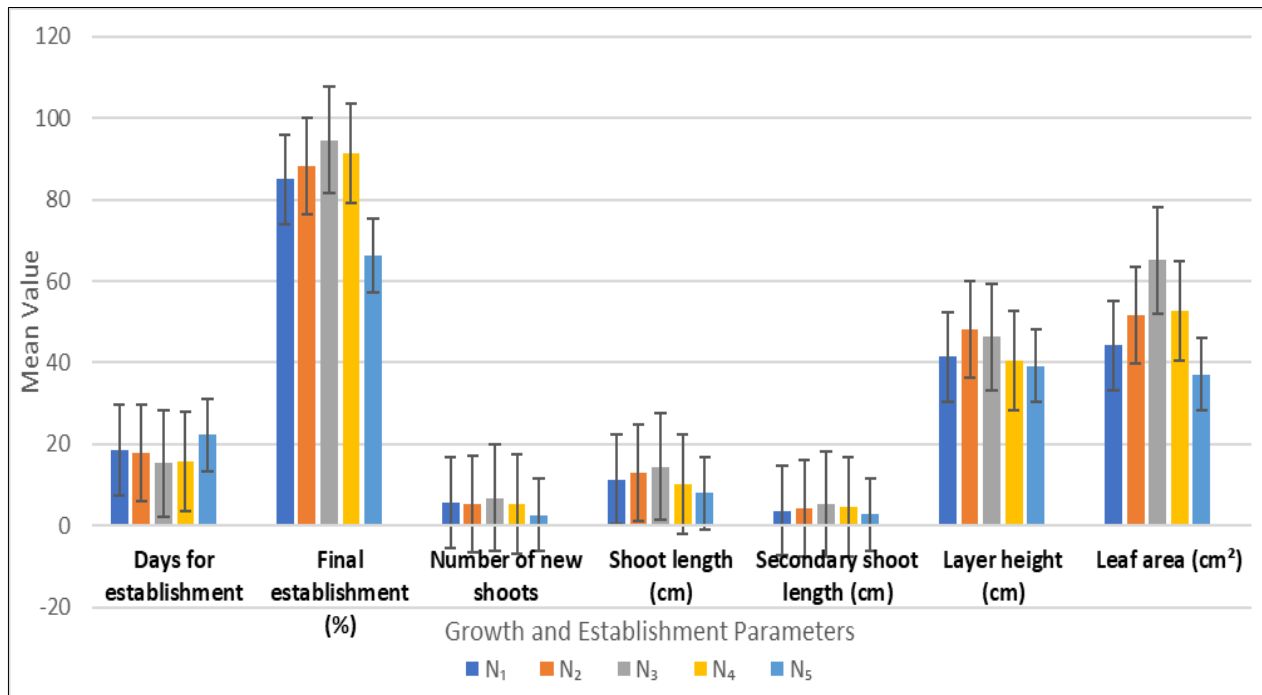


Fig. 3. Effect of rooting hormones on establishment and growth parameters of grapevine.

Growth parameters

The data indicated that auxin treatments significantly affected the growth parameters of grapevine air layers (Table 3; Fig. 3). The maximum number of new shoots per planted layer (6.83) was recorded in N₃, followed by N₁ (5.58), while the minimum (2.67) was noted in N₅. Shoot length was maximum in N₃ (14.48 cm), followed by N₂ (12.88 cm), while the minimum (8.0 cm) was recorded in N₅. Similarly, maximum secondary shoot length (5.23 cm) was recorded in N₃, followed by N₄ (4.58 cm), with the minimum (2.68 cm) in N₅.

The maximum layer height (48.02 cm) was recorded in N₂, followed by N₃ (46.30 cm), whereas the minimum (39.17 cm) was observed in N₅. Layer girth was maximum in N₃ (1.07 cm), followed by N₄ (0.87 cm), while the minimum (0.62 cm) was recorded

in N₅. The maximum leaf area (65.06 cm²) was recorded in N₃, followed by N₄ (52.77 cm²), while the minimum (37.09 cm²) was observed in N₅.

Biochemical parameters

The biochemical parameters of grapevines were significantly influenced by the different concentrations of auxin (Table 4; Fig. 4). Total carbohydrate content differed significantly among treatments. The maximum total carbohydrate content (144.82 mg g⁻¹) was recorded in N₃, followed by N₄ (135.48 mg g⁻¹), while the lowest carbohydrate content (121.23 mg g⁻¹) was observed in N₅. Similarly, total soluble protein content was significantly higher in hormone-treated layers. The maximum protein content (5.37 mg g⁻¹) was recorded in N₃, followed by N₄ (5.25 mg g⁻¹), whereas the minimum (3.92 mg g⁻¹) was observed in N₅.

Table 3. Effect of rooting hormones on growth parameters of established grapevine air layers (45 days after detachment)

Treatment	Number of new shoots	Shoot length (cm)	Secondary shoot length (cm)	Layer height (cm)	Layer girth (cm)	Leaf area (cm ²)
N ₁ (IBA 2500 ppm)	5.58	11.38	3.71	41.38	0.75	44.22
N ₂ (IBA 2500 ppm + NAA 250 ppm)	5.17	12.88	4.21	48.02	0.80	51.67
N ₃ (IBA 3000 ppm)	6.83	14.48	5.23	46.30	1.07	65.06
N ₄ (IBA 3000 ppm + NAA 250 ppm)	5.33	10.18	4.58	40.37	0.87	52.77
N ₅ (Control)	2.67	8.00	2.68	39.17	0.62	37.09
SE m (±)	0.20	0.12	0.05	0.327	0.01	0.45
CD (5%)	0.58	0.33	0.14	0.936	0.02	1.29

*Values represent the mean of 3 replications, each comprising ten vines.

Table 4. Effect of rooting hormones on biochemical parameters of rooted portions of grapevine air layers

Treatment	Total carbohydrates (mg g ⁻¹)	Total soluble protein (mg g ⁻¹)	Peroxidase (mg protein ⁻¹ min ⁻¹)	Catalase (mg protein ⁻¹ min ⁻¹)	Polyphenol oxidase (EU ml ⁻¹ min ⁻¹)
N ₁ (IBA 2500 ppm)	123.41	4.72	0.082	0.060	0.590
N ₂ (IBA 2500 ppm + NAA 250 ppm)	127.10	5.21	0.095	0.052	0.630
N ₃ (IBA 3000 ppm)	144.82	5.37	0.133	0.085	0.790
N ₄ (IBA 3000 ppm + NAA 250 ppm)	135.48	5.25	0.116	0.077	0.630
N ₅ (Control)	121.23	3.92	0.062	0.048	0.610
SE m (±)	0.69	0.03	0.003	0.002	0.033
CD (5%)	1.99	0.08	0.007	0.007	0.094

*Values represent the mean of 3 replications, each comprising ten vines.

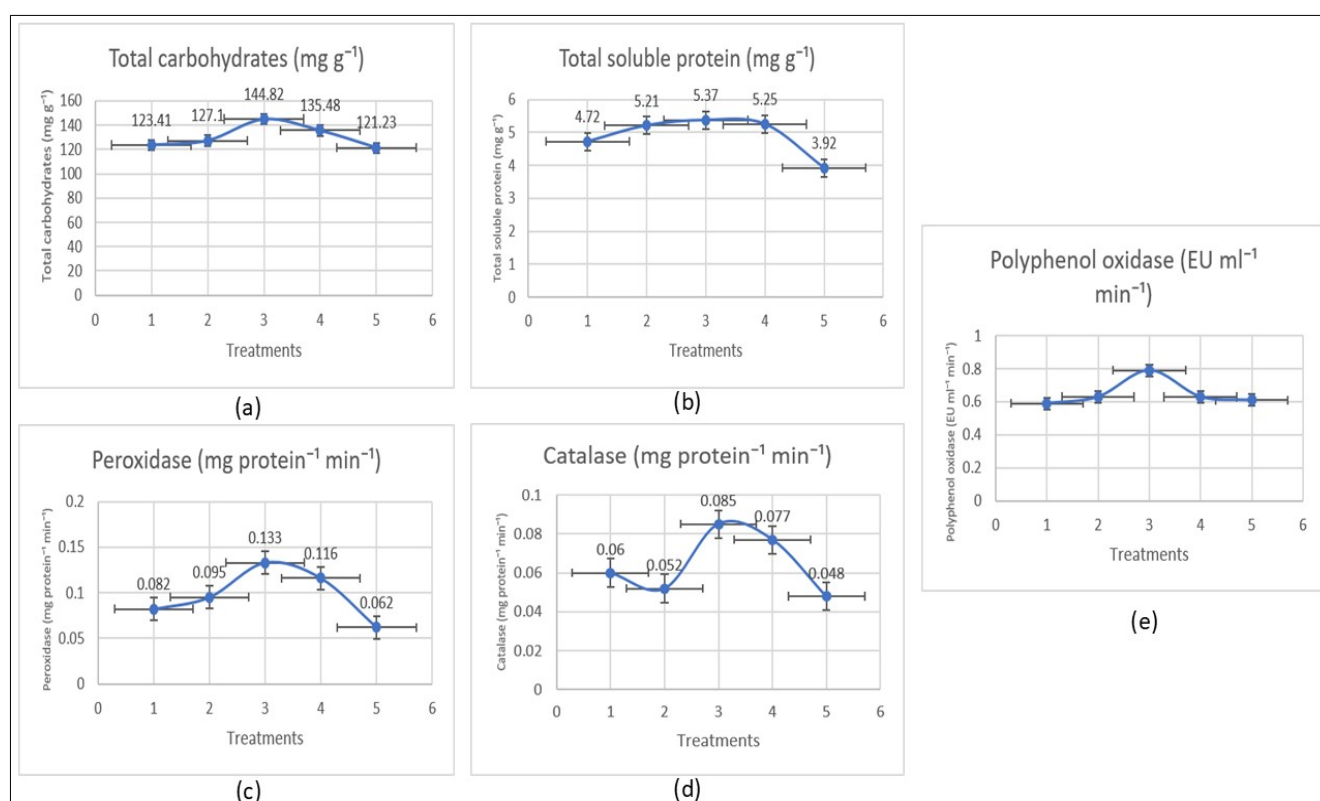


Fig. 4. Effect of auxin treatments on biochemical parameters of grapevine air layers: (a) total carbohydrates (mg g⁻¹); (b) total soluble protein (mg g⁻¹); (c) peroxidase activity (mg protein⁻¹ min⁻¹); (d) catalase activity (mg protein⁻¹ min⁻¹); and (e) polyphenol oxidase activity (EU ml⁻¹ min⁻¹).

Peroxidase activity differed significantly, with the highest value in N₃ (0.133 mg protein⁻¹ min⁻¹), followed by N₄ (0.116 mg protein⁻¹ min⁻¹), while the lowest activity (0.062 mg protein⁻¹ min⁻¹) was recorded in N₅. Polyphenol oxidase activity differed significantly among treatments, with the maximum value (0.790 EU ml⁻¹ min⁻¹) recorded in N₃, followed by N₄ and N₂ (0.630 EU ml⁻¹ min⁻¹). The lowest activity (0.590 EU ml⁻¹ min⁻¹) was observed in N₅. Catalase activity differed significantly, with the maximum value in N₃ (0.085 mg protein⁻¹ min⁻¹), followed by N₄ (0.077 mg protein⁻¹ min⁻¹), while the minimum activity (0.048 mg protein⁻¹ min⁻¹) was recorded in N₅.

4. Discussion

The present investigation demonstrated that auxin treatments significantly improved rooting behaviour, establishment and growth performance of grapevine air layers. Among the treatments, higher concentration of IBA 3000 ppm consistently enhanced early root initiation, rooting percentage, root number and establishment. This improved response may be attributed to efficient utilisation of endogenous reserves and stimulation of physiological and biochemical processes involved in root initiation and development.

Application of exogenous auxins supplements endogenous auxin levels at the rooting zone, thereby maintaining an optimum hormonal balance required for root induction. This interaction enhances cell division, vascular differentiation and subsequent root primordium development, particularly in woody perennial species (10, 11).

Early root initiation observed in auxin-treated layers can be attributed to enhanced mobilisation of stored starch into soluble carbohydrates, increased protein synthesis and activation of meristematic activity at the rooting zone. These findings are supported by previous studies in guava (12). Improved rooting percentage under IBA application may be due to greater accumulation and utilisation of carbohydrates at the root primordia, which is essential for successful root formation. Similar observations were reported earlier in litchi (13). Higher root number per layer may result from the synergistic interaction of auxins with endogenous hormones, carbohydrates and nitrogenous compounds, promoting rapid root differentiation. These findings are consistent with that of olive (14). Improved root length under auxin treatment may be due to enhanced respiratory activity and rapid hydrolysis of starch into physiologically active sugars, supporting meristematic cell elongation. Comparable observations were reported earlier (15). The increase in root diameter may be attributed to auxin-induced cell enlargement and tissue differentiation (16). The higher percentage of secondary roots may be due to hormonal regulation and basipetal movement of growth substances towards the rooting zone (17). Elevated root dry matter may be attributed to enhanced root initiation and biomass accumulation (18).

Faster establishment at a higher IBA concentration may be attributed to increased root number and root growth, resulting in improved absorption of nutrients and minerals, leading to vigorous plant growth and early establishment. A similar trend was documented earlier (19). Higher root volume may be associated with increased root number and biomass accumulation (3). Improved establishment percentage may be due to early root initiation, higher root number and root length, and better plant vigour. These findings are similar to those observed in guava (12). The enhanced performance under IBA may also be due to improved balance between rooting cofactors, nutritive substances and auxin (20).

Enhanced shoot emergence at a higher IBA concentration may be associated with improved root development and nutrient uptake. These results are consistent with previous reports (17). Enhanced shoot growth with IBA application may be due to better root growth and supplementation of nutrients (17). Improved layer height may be due to enhanced root initiation and efficient nutrient absorption in auxin application (21). Increased layer girth may be due to auxin-induced enhancement of vascular differentiation and callus formation (22). Greater leaf area at a higher IBA concentration may be attributed to improved vegetative growth under favourable physiological conditions (23).

Elevated carbohydrate accumulation in auxin treatment may be due to enhanced storage and translocation of photosynthates at the rooting zone along with increased cortex parenchymatous cell activity (13). Besides serving as an energy source, soluble carbohydrates provide carbon skeletons for actively dividing cells and support the differentiation of root primordia, making carbohydrate availability a critical determinant of successful adventitious root formation (11, 24). Increased protein accumulation due to auxin application has been widely reported (24) and is positively associated with adventitious root formation (25).

Adventitious root initiation is accompanied by increased metabolic activity and transient production of reactive oxygen species (ROS), which function as signalling molecules during root induction. Antioxidant enzymes such as peroxidase and catalase regulate ROS homeostasis, thereby protecting developing tissues from oxidative damage while supporting root primordium formation (11, 25). Enhanced peroxidase activity following auxin treatment is associated with cell wall synthesis and plays a critical role during the later stages of root formation (26). Similar auxin-induced changes in peroxidase activity during rooting have been reported earlier (27). Polyphenol oxidase plays a key role in adventitious root induction by modifying IAA-oxidase activity and acting as a rooting cofactor (28). Polar auxin transport is essential for establishing local auxin maxima at the rooting zone, thereby initiating adventitious root primordia. In addition, auxin interacts with ethylene signalling during root induction and coordinated auxin-ethylene crosstalk contributes to cell differentiation and adventitious root development (29, 30). Enhanced catalase activity following auxin application may reduce H_2O_2 levels, thereby protecting root cells from oxidative damage during active root formation (31). Maintenance of cellular redox homeostasis is essential for successful adventitious rooting, as excessive oxidative stress can impair cell division and differentiation, whereas controlled ROS accumulation acts as an important signal during early root development (10).

The superior performance of IBA over the combined IBA + NAA treatment may be attributed to its efficient conversion to the biologically active auxin, indole-3-acetic acid (IAA), thereby sustaining auxin availability at the rooting site. In contrast, combining two synthetic auxins may modify endogenous auxin homeostasis, resulting in a less favourable hormonal balance for adventitious rooting (32, 33). Simultaneous application of different auxins does not necessarily produce additive effects and species-specific differences in auxin uptake, metabolism and signalling may reduce rooting efficiency compared with a single optimal auxin treatment (34).

5. Conclusion

The present investigation demonstrated that auxin treatments significantly influenced rooting, establishment, growth and biochemical attributes of grapevine air layers. Among the different treatments, IBA at 3000 ppm consistently produced superior results with respect to early root initiation, higher rooting percentage, increased root number and volume, and improved survivability and establishment of detached layers. Enhanced biochemical activity under this treatment further supported better rooting and plant growth. The study indicates that air layering combined with IBA at 3000 ppm is an efficient, promising and reliable technique for the rapid propagation of grapevine. Future research should compare the rooting efficiency, propagation duration and field performance of air layering with conventional propagation methods, including hardwood cuttings and grafting, to establish their relative advantages under commercial nursery conditions. Further research should also focus on validating this protocol under diverse agro-climatic conditions, evaluating its performance in different grapevine cultivars and rootstocks, and investigating the physiological and molecular mechanisms underlying auxin-mediated adventitious root formation to further optimise propagation efficiency.

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Authors' contributions

RW and PN conceptualised the research. RW, SJ and PN designed the experiments. AS and PN provided experimental materials. RW, MK, VN and AJ conducted the field and laboratory experiments and collected data. RW, AAA, AS, TJD and IM performed data analysis and interpretation. RW, SJ, AJ and PN prepared and revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT 5.5 to assist with language, sentence structure and grammar. The AI tool was not used for data generation, data analysis, interpretation of results or drawing scientific conclusions. All scientific content, data, interpretations and the final manuscript were critically reviewed, verified and approved by the authors, who take full responsibility for the accuracy and integrity of the work. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the accuracy and integrity of the manuscript publication.

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