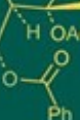
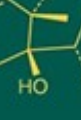
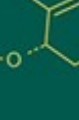
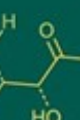
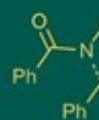


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Exogenous growth regulator mediated adventitious rooting and endogenous hormonal changes in leaf cuttings of guava (*Psidium guajava* L.)

Anushma PL¹, Rafeekher M², Satisha J³, Shivashankara KS⁴, Simi S⁵, Usharani TR⁶, Manju PR⁷, Lokesh AN⁸, Dinsha M⁹, Anupa T¹⁰

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ABSTRACT

Clonal propagation of perennial fruit species through leaf cuttings is difficult yet useful for producing true to type plants. In guava, with the advent of high density clonal orchards, development of efficient large-scale multiplication protocols through cuttings has become essential for disease-free planting material production. The present investigation was carried out in guava to test the possibility of propagating it through leaf cuttings, involving 15 treatments of plant growth regulators, replicated 30 times using completely randomized design. Exogenous application of auxins could successfully induce adventitious rooting in guava leaf bud cuttings, with highest rooting success (21.11%) and plantlet regeneration (17.78%) using IBA 1000 mg L⁻¹ + BAP 500 mg L⁻¹, while rooting and sprouting was not observed in untreated as well as BAP and coconut water treated cuttings. The plant hormone profile showed significant variations during the root initiation phase in guava. Synthetic auxins treatments of leaf cuttings induced adventitious root formation mainly through endogenous hormonal homeostasis and synergistic actions of auxins, cytokinins, gibberellins and salicylic acid. Increase in Indole-3-Acetic Acid and Gibberellic acids content accompanied by a decline in Absciscic acid and Jasmonates during the early phase of rooting was found to induce rooting in leaf cuttings.

Keywords: Rhizogenesis, Leaf bud cutting, Plant regeneration, Hormonal homeostasis

Introduction

Guava (*Psidium guajava* L.), the Myrtaceae family member, is one of the most nutritious fruit crops, widely cultivated across the globe. The crop is of great economic significance in many countries owing to its precocious and prolific bearing nature (Srivastava *et al.*, 2023)^[1] as well as nutritional and therapeutic properties of fruits (Irshad *et al.*, 2021)^[2]. The fruits as well as leaves of guava are reported to possess antioxidant, anti-diarrheal, anti-hypertensive, anti-obesity, anti-cancerous and anti-obesity properties. With the growing awareness on health benefits of consuming guavas, there has been a propending consumer preference for its fruits and processed products in the recent past (Butt *et al.*, 2025)^[3].

Though guavas were originated in the American tropics, it is currently cultivated in over 60 countries in the tropical as well as subtropical regions because of its wider adaptability to varied climatic and soil conditions. It is grown areas with average temperature ranging from 15 to 30 °C, receiving annual precipitation of 1000 to 2000 mm, located from mean sea level to higher altitudes up to 2000 m (Angulo-López *et al.*, 2021)^[4]. India, Indonesia, China, Pakistan, Brazil, Mexico, Philippines and Colombia, are the leading guava producing countries in the world, along with Peru, Ecuador, United States of America, South Africa, and Puerto Rico. As per the Ministry of Agriculture & Farmers Welfare (MoAFW), 2026^[5], guava occupies 4th position in area (3,58,140 hectare) and production (53.49 million metric tonnes), in India, following Mango, Citrus and Banana. The country has been witnessing consistent area expansion in guava over the past 10 years (2.46 lakh ha in 2014-15 to 5.34 lakh ha in 2024-25, (MoAFW, 2026)^[5], owing to its higher yield, year-round bearing and growing market demand.

Guava is propagated through sexual and asexual methods, however seed propagation is now limited to rootstocks production. Asexual methods such as layering, grafting, budding, and stem cuttings are commonly used to produce true-to-type plants that preserve the desirable traits of the variety (Ahmad *et al.*, 2025; Bhusari *et al.*, 2025)^[6, 7]. However, seedling rootstocks in addition to inducing variability in terms of root system and plant vigour, involve longer production time (18 to 24 months) and greater skills, as compared to air layers and stem cuttings (6 to 8 months). Air layering, though efficient in producing true to type plants within shorter period, requires skill and produces less number of plants per mother tree compared to shoot cuttings. With the advent of high density orchard systems and wide area expansion, the demand for quality planting material is increasing exponentially, and to meet this demand, there is a need to develop clonal

Corresponding Author:
Anushma PL
ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru, Karnataka, India

propagation protocols which could yield true to type plants in large volumes (Ahmad *et al.*, 2025; Pereira *et al.*, 2017) [6, 8]. Though cuttings are difficult to root in guava, propagation through rooting of stem cuttings by exogenous application of auxins are successful (Akram *et al.*, 2025; Vanitha *et al.*, 2023) [9, 10]. Leaf cutting would be an alternate option to stem cutting for mass multiplication of guava if it could regenerate into plantlets through appropriate interventions. Nevertheless, information on guava plant regeneration using leaf cuttings are limited. The present investigation has attempted to test the feasibility of hormonal induction of adventitious rooting in leaf cuttings and to understand the hormonal interactions in adventitious rooting of guava leaf cuttings.

Materials and methods

Experimental site: The field experiments were conducted at ICAR-Indian Institute of Horticulture Research (ICAR-IIHR), Bengaluru, Karnataka, India, which is located at 13° 58' North latitude and 78° East longitude, at an altitude of 890 m above mean sea level, and at Keeraipatti village, located at 12°0'20" North latitude, 78°31'51" East longitude and at an altitude of 650 m above mean sea level in Harur Taluk, Dharmapuri, Tamil Nadu, India during 2024-2025. The laboratory analyses were carried out at ICAR-IIHR, Bengaluru.

Plant

Material

Recently matured leaves of the current season shoots were collected from 4 years old plants of guava variety Arka Poorna. Leaves with petiole and a portion stem (leaf bud cuttings) served as the propagule. The base (2-3 cm) of the leaf cuttings were treated with auxins (Indole-3-butyric acid or Indole-3-acetic acid) at concentrations of 500 or 1000 mg L⁻¹ for 30 seconds, alone or in combination with 6-BAP (6-Benzyl amino purine) at 500 mg L⁻¹ for 30 seconds or coconut water for 30 minutes. Leaf cuttings treated with distilled water served as control. The treated leaves were planted in pro trays (50 cavity, 6.0 cm deep and 5.0 cm opening diameter) containing cocopeat and placed in a low-cost walk-in propagation chamber. The cuttings were irrigated daily once and the growth chamber was sealed tightly to maintain the temperature (34 to 37 °C during day and 25 to 28 °C during night hours) and relative humidity (>90 %) inside the chamber. The experiment was carried out in completely randomized design (CRD), involving 15 treatments, with 60 replicates per treatment. After six weeks, the rooted leaves were carefully transferred to polythene bags containing potting media of red soil, sand and well-decomposed FYM in 1:1:1 proportion and the leaf base was covered with a thin layer of potting mixture. The rooted leaf cuttings were kept under partial shading (50%) and watered daily using rose can, to prevent the cuttings from drying.

Morphometric observations: Morphometric observations on rooting such as rooting success (%), number of primary and secondary roots, mean root length (cm), root fresh weight (g) and shooting viz., shooting success (%), number of sprouts, sprout length, number of leaves, leaf area (cm²) and plantlet formation rate (%) were recorded at 90 days after planting (DAP).

Estimation of plant hormones (PH) and growth regulators (GR): To investigate the changes in endogenous PH levels during the early root formation period, leaf bud cuttings from selected treatments which yielded contrasting results for rooting and shooting were analysed for major plant hormones viz., auxins (IAA & IBA), cytokinins (Zeatin, Z and Trans-Zeatin riboside, tZR) gibberellic acid (GA₃ & GA₄), abscisic acid (ABA), jasmonates and salicylic acid (SA). Fresh leaf bud cutting and representative samples of treated leaf bud cuttings were collected in dry ice at weekly intervals upto 3 weeks after planting (WAP) and stored at -80 °C until further processing and

analysis. Plant hormones were extracted following the method described by Pan *et al.* (2008) [11] as detailed hereunder.

Hormone extraction and purification: For extraction of PH and growth regulators, 3.0 g of sample (1.0 cm long segments from base of the leaf bud cuttings) was ground with liquid nitrogen and homogenized in 1-propanol:H₂O:concentrated HCl (2:1:0.002, v/v/v) solvent. The extract, after sonication at 4 °C for 30 minutes, was stored for overnight at -20 °C. Next day, dichloromethane was added to the extract and sonicated again at 4 °C for 30 minutes, followed by centrifugation at 12000 rpm for 10 minutes. The bottom layer containing hormones was transferred to clean test tube containing sodium sulphate, for removing the water traces in the extract. Further, the sample was completely dried using flash evaporator and dissolved in 1000 µl methanol: 0.05% formic acid (1:1 v/v) solvent. The extract is filtered using a nylon syringe filter (0.2 µm) and injected into LC-MS/MS (Liquid chromatography-tandem mass spectrometry) for further analysis.

Hormone profiling through LC-MS/MS: The chromatographic separation was performed on a 2.1 x 50 mm Ultra Performance Liquid Chromatography (UPLC) Bridged Ethyl Hybrid (BEH) C18 column (Waters, USA) with 1.7 µm particles, protected by a 2.1 x 5 mm BEH-C18 guard (Vanguard) column with a 1.7 µm particle size (Waters, USA). The mobile phase consisted of Solvent A (Water: Acetonitrile: Acetic acid, 95:5:0.05 v/v/v) and Solvent B (Acetonitrile: Water: Acetic acid, 95:5:0.05 v/v/v). The gradient program was set as follows: initial gradient composed of 85% solvent A and 15% solvent B, held for 1 minute; at 12 minutes, the gradient was changed to 15% solvent A and 85% solvent B, held for 1 minute and a linear gradient was followed by 85% solvent and 15% solvent B in 14 minutes, hold for 30 seconds. The system was then returned to initial conditions at 15 minutes and equilibrated for 1 minute before the next injection. The flow rate was maintained at 0.2 mL min⁻¹.

Statistical analysis: The morphological and biochemical data were subjected to analysis of variance (ANOVA) using RAISINS (R & AI Solutions for Inferential Statistics) software (Hisham *et al.*, 2025) [12, 13]. The data transformations were performed for count data (square root transformation) and for treatments which did not yield any results (log transformation).

Results

Morphological parameters

Rooting parameters

Exogenous application of GR significantly impacted the adventitious rooting of leaf bud cuttings in guava (Table 1). Highest rooting success among the treatments was observed in leaf cuttings treated with IBA 1000 mg L⁻¹ + BAP 500 mg L⁻¹ (21.11%) and IBA 500 mg L⁻¹ + BAP 500 mg L⁻¹ (20.00%). Rooting was also induced by IAA 1000 mg L⁻¹ (12.22%), IBA 500 or 1000 mg L⁻¹ alone or in combination BAP (500 mg L⁻¹). Treating leaf cuttings with IAA 500 mg L⁻¹, BAP, or coconut water and direct planting without GR exposure was not effective in inducing adventitious rooting in guava. After 90 days of planting, significantly higher number of primary roots (4.67) were produced by leaf cuttings that received IBA 500 mg L⁻¹ in combination with 500 mg L⁻¹ BAP (4.67), which were at par with IBA 1000 mg L⁻¹ + BAP 500 mg L⁻¹ and IBA 500 mg L⁻¹ + Coconut water (3.67). Treating leaf cuttings with IBA 500 or 1000 mg L⁻¹ in combination with BAP 500 mg L⁻¹ or IBA 1000 mg L⁻¹ along with coconut water produced maximum number of secondary roots (56.67, 55.67 and 61.00). Significantly longer roots (12.84 cm) were also noted in cuttings when treated with IBA 500 mg L⁻¹ in combination with 500 mg L⁻¹ BAP, followed by IBA 500 mg L⁻¹ + Coconut water (10.62 cm) and IAA 1000 mg L⁻¹ + BAP 500 mg L⁻¹ (10.50 cm).

Table 1. Effect of plant growth regulators on rooting of leaf cuttings

Treatment	Rooting success (%)	Number of primary roots	Number of secondary roots	Root length (cm)	Root fresh weight (g)
T ₁ - IBA 500 mg L ⁻¹ + BAP 500 mg L ⁻¹	2.00 ^a (26.565)	4.67 ^a (2.27)	56.67 ^a (7.55)	12.84 ^a (3.36)	1.88 ^a (1.77)
T ₂ - IBA 1000 mg L ⁻¹ + BAP 500 mg L ⁻¹	21.11 ^a (27.337)	3.67 ^{ab} (2.02)	55.67 ^a (7.49)	8.96 ^b (3.22)	0.55 ^c (1.52)
T ₃ - IAA 500 mg L ⁻¹ + BAP 500 mg L ⁻¹	2.22 (7.184) ^e	0.33 ^{de} (0.88)	0.00 ^c (0.71)	0.00 ^c (2.78)	0.00 ^d (1.39)
T ₄ - IAA 1000 mg L ⁻¹ + BAP 500 mg L ⁻¹	7.78 (16.123) ^c	3.33 ^b (1.94)	59.00 ^a (7.62)	10.50 ^b (3.28)	0.59 ^c (1.52)
T ₅ - IBA 500 mg L ⁻¹ + Coconut water	7.78 (16.123) ^c	3.67 ^{ab} (2.04)	61.33 ^a (7.86)	10.62 ^b (3.28)	0.71 ^c (1.55)
T ₆ - IBA 1000 mg L ⁻¹ + Coconut water	0.00 ^f (0.523)	0.00 ^c (0.71)	0.00 ^c (0.71)	0.00 ^c (2.78)	0.00 ^d (1.39)
T ₇ - IAA 500 mg L ⁻¹ + Coconut water	0.00 ^f (0.523)	0.00 ^c (0.71)	0.00 ^c (0.71)	0.00 ^c (2.78)	0.00 ^d (1.39)
T ₈ - IAA 1000 mg L ⁻¹ + Coconut water	0.00 ^f (0.523)	0.00 ^c (0.71)	0.00 ^c (0.71)	0.00 ^c (2.78)	0.00 ^d (1.39)
T ₉ - IBA 500 mg L ⁻¹	4.44 ^d (11.99)	0.00 ^c (0.71)	0.00 ^c (0.71)	0.00 ^c (2.78)	0.00 ^d (1.39)
T ₁₀ - IBA 1000 mg L ⁻¹	3.33 ^{de} (10.51)	0.67 ^{cd} (1.05)	0.00 ^c (0.71)	0.00 ^c (2.78)	0.00 ^d (1.39)
T ₁₁ - IAA 500 mg L ⁻¹	0.00 ^f (0.523)	0.00 ^c (0.71)	0.00 ^c (0.71)	0.00 ^c (2.78)	0.00 ^d (1.39)
T ₁₂ - IAA 1000 mg L ⁻¹	12.22 ^b (20.32)	1.33 ^c (1.34)	33.67 ^b (5.77)	10.10 ^b (3.27)	1.08 ^b (1.62)
T ₁₃ - BAP 500 mg L ⁻¹	0.00 ^f (0.523)	0.00 ^c (0.71)	0.00 ^c (0.71)	0.00 ^c (2.78)	0.00 ^d (1.39)
T ₁₄ - Coconut water	0.00 ^f (0.523)	0.00 ^c (0.71)	0.00 ^c (0.71)	0.00 ^c (2.78)	0.00 ^d (1.39)
T ₁₅ - Control (water)	0.00 ^f (0.523)	0.00 ^c (0.71)	0.00 ^c (0.71)	0.00 ^c (2.78)	0.00 ^d (1.39)
CD(0.05)	3.345	0.31	0.82	0.07	0.06
SE(m)	1.16	0.11	0.28	0.02	0.02
CV(%)	21.523	16.00	17.01	1.35	2.57

Sprouting parameters

The sprouting behaviour of rooted guava leaf cuttings varied significantly with the GR treatments (Table 2 and Fig. 1). Supplementation of BAP @ 500 mg L⁻¹ along with auxins (IAA or IBA) could facilitate sprouting in rooted cuttings. IAA 1000 mg L⁻¹ alone or in combination with BAP 500 mg L⁻¹ could successfully induce sprouting (100.00%) in all the rooted leaf cuttings. Treating the leaf bases with lower concentrations of auxins (IAA or IBA @ 500 mg L⁻¹) alone or using BAP or coconut water was not effective for sprout production in leaf cuttings. Leaf cuttings treated with IAA 1000 mg L⁻¹ along with BAP 500 mg L⁻¹ could sprout early (61.33 days) and put forth higher number of sprouts (1.00), sprout length (10.37 cm), maximum number of leaves (8.00) and leaf area (23.59 cm²). Among the treatments which yielded sprouting, lower sprout length (1.93 cm), number of leaves (3.67) and leaf area (1.23 cm²) were recorded in IBA 500 mg L⁻¹ + BAP 500 mg L⁻¹.

Plantlet regeneration

Exogenous GR application significantly influenced the plantlet regeneration responses from guava leaf cuttings. Maximum plantlets were produced by treating the leaf bud cuttings with IBA 1000 mg L⁻¹ + BAP 500 mg L⁻¹ (17.78%) followed by IAA 1000 mg L⁻¹ (12.22%). Treating guava leaf cuttings with lower concentration of auxins, or BAP or coconut water alone was inadequate for successful plantlet regeneration (Table 2 and Fig. 1).

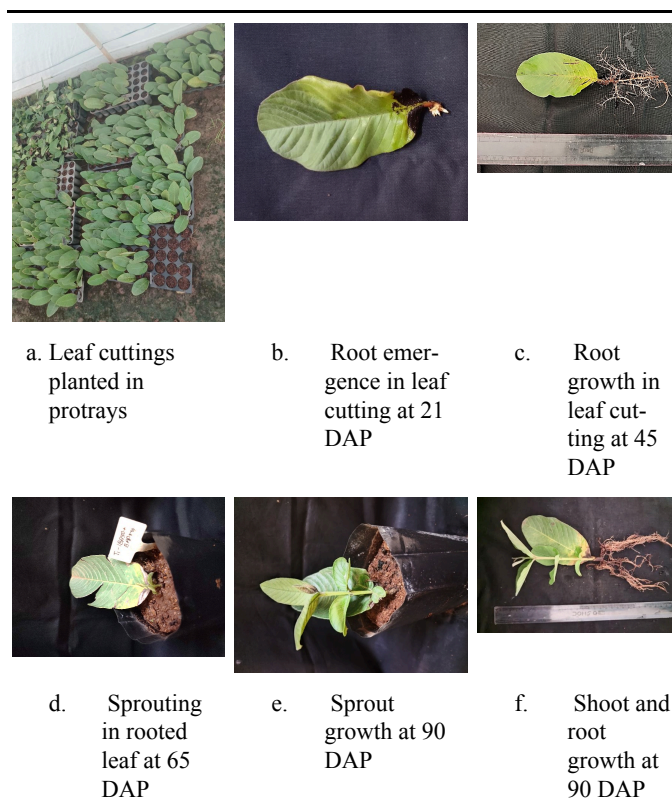
**Fig 1:** Stages of propagation in guava leaf cuttings

Table 2. Effect of plant growth regulators on shooting of leaf cuttings

Treatment	Sprouting success (%)	Days to sprout	Number of sprouts	Sprout length (cm)	No of leaves	Leaf area (cm ²)	Plant regeneration (%)
T ₁ - IBA 500 mg L ⁻¹ + BAP 500 mg L ⁻¹	22.22 ^b (27.82)	70.00 ^b	1.00 ^a (1.22)	1.93 ^d (2.70)	3.67 ^c (2.04)	1.23 ^d (3.45)	4.44 ^d (11.99)
T ₂ - IBA 1000 mg L ⁻¹ + BAP 500 mg L ⁻¹	83.33 ^a (74.65)	66.33 ^c	1.00 ^a (1.22)	5.47 ^c (2.92)	5.00 ^b (2.32)	5.86 ^c (3.58)	17.78 ^a (24.85)
T ₃ - IAA 500 mg L ⁻¹ + BAP 500 mg L ⁻¹	0.00 ^c (0.52)	95.00 ^a	0.00 ^b (0.71)	0.00 ^e (2.56)	0.00 ^d (0.71)	0.00 ^d (3.41)	0.00 ^e (0.523)
T ₄ - IAA 1000 mg L ⁻¹ + BAP 500 mg L ⁻¹	100.00 ^a (89.48)	61.33 ^d	1.00 ^a (1.22)	10.37 ^a (3.15)	8.00 ^a (2.92)	23.59 ^a (3.98)	6.11 ^{cd} (14.825)
T ₅ - IBA 500 mg L ⁻¹ + Coconut water	83.33 ^a (74.65)	66.00 ^c	1.00 ^a (1.22)	5.93 ^c (2.94)	5.33 ^b (2.41)	14.87 ^b (3.80)	7.18 ^c (16.123)
T ₆ - IBA 1000 mg L ⁻¹ + Coconut water	0.00 ^c (0.52)	95.00 ^a	0.00 ^b (0.71)	0.00 ^e (2.56)	0.00 ^d (0.71)	0.00 ^d (3.41)	0.00 ^e (0.523)
T ₇ - IAA 500 mg L ⁻¹ + Coconut water	0.00 ^c (0.52)	95.00 ^a	0.00 ^b (0.71)	0.00 ^e (2.56)	0.00 ^d (0.71)	0.00 ^d (3.41)	0.00 ^e (0.523)
T ₈ - IAA 1000 mg L ⁻¹ + Coconut water	0.00 ^c (0.52)	95.00 ^a	0.00 ^b (0.71)	0.00 ^e (2.56)	0.00 ^d (0.71)	0.00 ^d (3.41)	0.00 ^e (0.523)
T ₉ - IBA 500 mg L ⁻¹	0.00 ^c (0.52)	95.00 ^a	0.00 ^b (0.71)	0.00 ^e (2.56)	0.00 ^d (0.71)	0.00 ^d (3.41)	0.00 ^e (0.523)
T ₁₀ - IBA 1000 mg L ⁻¹	0.00 ^c (0.52)	95.00 ^a	0.00 ^b (0.71)	0.00 ^e (2.56)	0.00 ^d (0.71)	0.00 ^d (3.41)	0.00 ^e (0.523)
T ₁₁ - IAA 500 mg L ⁻¹	0.00 ^c (0.52)	95.00 ^a	0.00 ^b (0.71)	0.00 ^e (2.56)	0.00 ^d (0.71)	0.00 ^d (3.41)	0.00 ^e (0.523)
T ₁₂ - IAA 1000 mg L ⁻¹	100.00 ^a (89.48)	64.33 ^{cd}	1.00 ^a (1.22)	7.17 ^b (3.00)	5.33 ^b (2.41)	11.89 ^b (3.74)	12.22 ^b (20.322)
T ₁₃ - BAP 500 mg L ⁻¹	0.00 ^c (0.52)	95.00 ^a	0.00 ^b (0.71)	0.00 ^e (2.56)	0.00 ^d (0.71)	0.00 ^d (3.41)	0.00 ^e (0.523)
T ₁₄ - Coconut water	0.00 ^c (0.52)	95.00 ^a	0.00 ^b (0.71)	0.00 ^e (2.56)	0.00 ^d (0.71)	0.00 ^d (3.41)	0.00 ^e (0.523)
T ₁₅ - Control (Water)	0.00 ^c (0.52)	95.00 ^a	0.00 ^b (0.71)	0.00 ^e (2.56)	0.00 ^d (0.71)	0.00 ^d (3.41)	0.00 ^e (0.523)
CD	15.88	3.22	0	0.06	0.23	0.08	2.418
SE(m)	5.50	1.12	0.00	0.02	0.08	0.03	0.84
CV(%)	39.53	2.27	0.00	1.28	10.79	1.37	23.439

Plant hormones

Auxins

After one WAP, IAA levels were observed to be higher in leaf cuttings treated with IBA 1000 mg L⁻¹ + BAP 500 mg L⁻¹ as well as in IAA 1000 mg L⁻¹ + BAP 500 mg L⁻¹ (4.07 ng/g), as compared to the fresh leaves (2.24 ng/g). The lowest levels of IAA were noted in control (0.45 ng/g) and coconut water treated leaf cuttings (0.91 ng/g). During the second WAP, reduction in endogenous IAA content was observed in treated cuttings, with lowest contents in control (0.57 ng/g) and coconut water (0.94 ng/g) treated leaves. The IAA levels were observed to be highest in cuttings treated with IAA 1000 mg L⁻¹ + BAP 500 mg L⁻¹ (2.20 ng/g) at 3 weeks after planting, followed by IAA 1000 mg L⁻¹ + BAP 500 mg L⁻¹ (1.38 ng/g) while lowest in leaves treated with coconut water (0.44 ng/g).

IBA levels in leaf cuttings treated with exogenous IBA 1000 mg L⁻¹ alone (1.23 ng/g) or in combination with BAP 500 mg L⁻¹ (1.60 ng/g) were significantly higher at one WAP as compared to the fresh leaves (0.87 ng/g), while this IBA spike was not observed in untreated control and leaves treated with coconut water or BAP. During the sec-

ond week (root initiation phase), IBA levels were inconsistent across the treatments, showing higher contents in BAP, coconut water and distilled water treated leaf cuttings while it dropped in treatments involving exogenous IBA supply. IBA levels in leaf cuttings declined during the third WAP, which coincided with root expression phase, with lowest content in untreated control.

Table 3. Changes in endogenous auxins of leaf cuttings during root development

Cytokinins

The internal cytokinin contents showed significant variations during the early root induction to expression phase (Table 4). The Zeatin content in leaf cuttings declined after one WAP as compared to the fresh leaves, however the results did not show any definite trend during the rhizogenesis in leaf cuttings. When compared to the fresh leaves, the Trans-Zeatin Riboside (tZR) levels did not change considerably in leaf cuttings treated with IBA, IAA or BAP during the root induction phase, however, the contents were significantly higher in control (0.24 ng/g) and coconut water (0.10 ng/g) treatments.

Table 4. Changes in endogenous cytokinins of leaf cuttings during root development

Table 2. Detailed tabular representation with Multiple comparisons

Treatment	IAA (ng/g FW)			IBA (ng/g FW)		
	1 WAP	2 WAP	3 WAP	1 WAP	2 WAP	3 WAP
T ₀ -Fresh leaves	2.24 ^b	2.24 ^a	2.24 ^a	0.87 ^c	0.87 ^c	0.87 ^a
T ₂ - IBA 1000 mg L ⁻¹ + BAP 500 mg L ⁻¹	4.07 ^a	1.38 ^b	1.38 ^b	1.60 ^a	1.26 ^b	0.56 ^{bc}
T ₄ - IAA 1000 mg L ⁻¹ + BAP 500 mg L ⁻¹	4.07 ^a	1.63 ^b	2.20 ^a	0.63 ^d	0.59 ^d	0.71 ^{ab}
T ₁₀ - IBA 1000 mg L ⁻¹	1.63 ^c	1.63 ^b	0.85 ^c	1.23 ^b	0.63 ^d	0.82 ^a
T ₁₂ - IAA 1000 mg L ⁻¹	1.10 ^d	1.41 ^b	0.37 ^d	0.78 ^{cd}	0.82 ^c	0.45 ^{cd}
T ₁₃ - BAP 500 mg L ⁻¹	2.01 ^{bc}	1.59 ^b	0.53 ^{cd}	0.33 ^e	1.15 ^b	0.52 ^c
T ₁₄ - Coconut water	0.91 ^d	0.94 ^c	0.53 ^{cd}	0.30 ^e	1.64 ^a	0.48 ^{cd}
T ₁₅ - Control (Water)	0.45 ^e	0.57 ^d	0.44 ^d	0.00 ^f	0.74 ^{cd}	0.33 ^d
CD	0.38	0.36	0.34	0.21	0.18	0.17
SE(m)	0.12	0.11	0.10	0.07	0.06	0.05
CV(%)	8.11	10.99	13.92	12.96	8.19	12.57

Table 2. Detailed tabular representation with Multiple comparisons

Treatment	Zeatin IAA (ng/g FW)			tZR IAA (ng/g FW)		
	1 WAP	2 WAP	3 WAP	1 WAP	2 WAP	3 WAP
T ₀ -Fresh leaves	0.37 ^a	0.37 ^b	0.37 ^b	0.04 ^d	0.04 ^f	0.04 ^f
T ₂ - IBA 1000 mg L ⁻¹ + BAP 500 mg L ⁻¹	0.29 ^b	0.19 ^{cd}	0.07 ^e	0.04 ^d	0.05 ^{ef}	0.10 ^e
T ₄ - IAA 1000 mg L ⁻¹ + BAP 500 mg L ⁻¹	0.03 ^e	0.00 ^e	0.55 ^a	0.04 ^d	0.13 ^b	0.13 ^{cd}
T ₁₀ - IBA 1000 mg L ⁻¹	0.29 ^b	0.43 ^b	0.12 ^{de}	0.09 ^c	0.11 ^c	0.13 ^c
T ₁₂ - IAA 1000 mg L ⁻¹	0.26 ^b	0.20 ^{cd}	0.21 ^c	0.12 ^b	0.08 ^d	0.11 ^{cde}
T ₁₃ - BAP 500 mg L ⁻¹	0.17 ^c	0.13 ^d	0.12 ^{de}	0.05 ^d	0.16 ^a	0.25 ^a
T ₁₄ - Coconut water	0.11 ^d	0.25 ^c	0.18 ^{cd}	0.10 ^{bc}	0.08 ^d	0.18 ^b
T ₁₅ - Control (Water)	0.25 ^b	0.52 ^a	0.40 ^b	0.24 ^a	0.06 ^e	0.11 ^{de}
CD	0.05	0.08	0.09	0.03	0.02	0.02
SE(m)	0.02	0.02	0.03	0.01	0.01	0.01
CV(%)	9.8	13.3	14.9	14.1	7.8	7.5

Gibberellins

The endogenous gibberellic acid (GA) contents varied significantly post-plant GR treatments (Table 5). At one WAP, the GA₃ content was found to be highest in cuttings that received IAA 1000 mg L⁻¹ + BAP 500 mg L⁻¹ (0.76 ng/g), followed by IAA 1000 mg L⁻¹, while it was lowest in the untreated control cuttings. Cuttings treated with coconut water or BAP 500 mg L⁻¹ also possessed low GA₃ levels (0.16 ng/g

and 0.19 ng/g respectively) during the root induction phase. During the root initiation phase, GA₃ content was higher in cuttings exposed to IBA 1000 mg L⁻¹ + BAP 500 mg L⁻¹ (0.60 ng/g), which was at par with IAA 1000 mg L⁻¹ + BAP 500 mg L⁻¹ (0.52 ng/g). In leaf cuttings treated with BAP 500 mg L⁻¹, coconut water or distilled water, a spike in GA₃ level was noted during the second WAP. During the root expression phase i.e., 3 weeks post GR treatment, endogenous GA₃

was maximum in control cuttings (0.60 ng/g) while it was minimum in treatment involving IBA 1000 mg L⁻¹+ BAP 500 mg L⁻¹ (0.18 ng/g). In general, treatments involving auxins showed lower levels of GA₃ at expression phase as compared to the initiation phase.

Leaf cuttings underwent significant changes in GA₄ contents during early phase of adventitious rooting (Table 5). A surge in GA₄ levels was noted in leaf cuttings during the first WAP, as compared to the fresh leaf cuttings (0.18 ng/g). The highest content was noted in BAP 500 mg L⁻¹ (4.93 ng/g) treated cuttings, followed by coconut water or

IBA 1000 mg L⁻¹ treated ones (4.63 ng/g). At 2 weeks after planting, leaf cuttings treated with IAA 1000 mg L⁻¹+ BAP 500 mg L⁻¹ presented higher GA₄ content (4.74 ng/g) as compared to the control (0.95 ng/g) as well as coconut water treated cuttings (0.00 ng/g). During the third week, GA₄ levels were higher in BAP 500 mg L⁻¹ (3.98 ng/g), while it was not detected in coconut water treated or control cuttings (Table 5).

Table 5. Changes in endogenous gibberellins of leaf cuttings during root development

Table 2. Detailed tabular representation with Multiple comparisons

Treatment	GA ₃ (ng/g FW)			GA ₄ (ng/g FW)		
	1 WAP	2 WAP	3 WAP	1 WAP	2 WAP	3 WAP
T ₀ -Fresh leaves	0.20 ^e	0.20 ^d	0.20 ^{de}	0.54 ^e	0.54 ^f	0.54 ^e
T ₂ - IBA 1000 mg L ⁻¹ + BAP 500 mg L ⁻¹	0.26 ^d	0.60 ^a	0.18 ^e	4.17 ^{bc}	3.22 ^c	2.46 ^{bc}
T ₄ - IAA 1000 mg L ⁻¹ + BAP 500 mg L ⁻¹	0.76 ^a	0.52 ^{ab}	0.30 ^{bcd}	3.60 ^c	4.74 ^a	2.65 ^b
T ₁₀ - IBA 1000 mg L ⁻¹	0.34 ^c	0.46 ^b	0.31 ^{bc}	4.36 ^{ab}	2.27 ^d	1.90 ^{cd}
T ₁₂ - IAA 1000 mg L ⁻¹	0.41 ^b	0.21 ^d	0.34 ^b	2.46 ^d	1.11 ^e	1.71 ^d
T ₁₃ - BAP 500 mg L ⁻¹	0.19 ^e	0.26 ^{cd}	0.23 ^{cde}	4.93 ^a	3.98 ^b	3.98 ^a
T ₁₄ - Coconut water	0.16 ^e	0.34 ^c	0.30 ^{bcd}	2.65 ^d	0.00 ^g	0.00 ^e
T ₁₅ - Control (Water)	0.00 ^f	0.32 ^{cd}	0.60 ^a	4.36 ^{ab}	0.95 ^{ef}	0.00 ^e
CD	0.06	0.12	0.11	0.76	0.49	0.73
SE(m)	0.02	0.04	0.03	0.23	0.15	0.22
CV(%)	8.71	14.26	15.01	9.71	10.06	19.02

Abscissic acid (ABA) content in guava leaf cuttings showed significant changes post-exposure to GR treatments (Fig. 2). Fresh leaf cuttings possessed high ABA (8.63 ng/g) which declined during the adventitious rooting phase. At one WAP, ABA was highest in leaf cuttings treated with coconut water (2.75 ng/g), which was on par with IBA 1000 mg L⁻¹ treated ones (2.58 ng/g) while it was lowest in IAA or BAP treated cuttings. At second week, high ABA levels were

recorded in IAA 1000 mg L⁻¹+ BAP 500 mg L⁻¹ (2.99 ng/g), while it was low in coconut water treated cuttings. The root expression phase witnessed a spike in ABA levels, with maximum content in IBA 1000 mg L⁻¹ treatment (5.73 ng/g). The ABA content was comparatively lesser in BAP (1.30 ng/g) or coconut water (2.98 ng/g) treated cuttings and in control ones (3.19 ng/g) as compared to the auxin treated cuttings that successfully induced rooting.

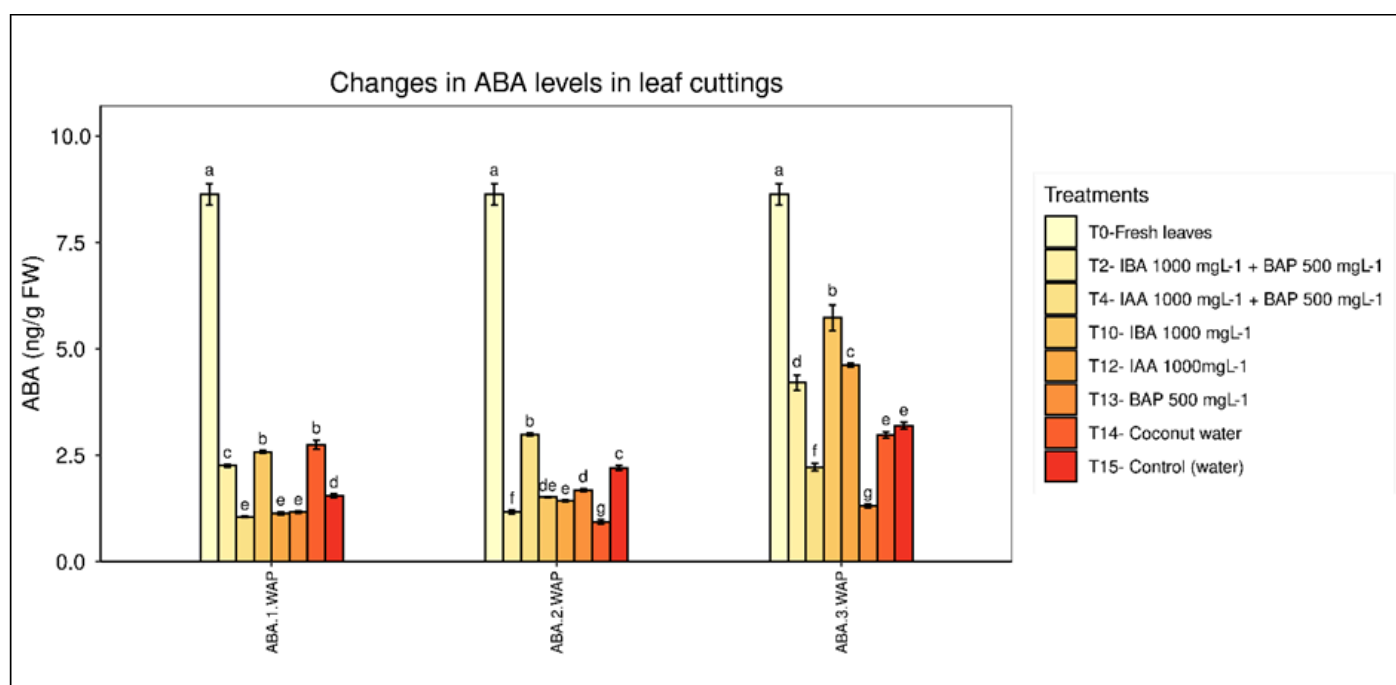


Figure 2. Changes in ABA content in leaf cuttings during rooting phase

Jasmonates, the lipid derived GRs showed significant variations during the early rooting phase in guava leaf cuttings (Table 6). JA in fresh leaf cutting (5.69 ng/g) declined during the root induction and initiation phase i.e., up to two weeks of planting. The lowest JA content was observed in BAP 500 mg L⁻¹ treated leaf cuttings (0.22 ng/g), followed by IAA 1000 mg L⁻¹ treated ones (0.66 ng/g), while it

was highest in coconut water treatment (2.00 ng/g). At two weeks of planting, cuttings treated with IBA 1000 mg L⁻¹+ BAP 500 mg L⁻¹ showed high JA content (1.43 ng/g), while it was minimum in the control (0.07 ng/g) and coconut water treated cuttings (0.15 ng/g). During the root expression phase (3 WAP), JA in leaf cuttings increased, with maximum content in coconut water treated leaves (7.02

ng/g) followed by control leaves (6.45 ng/g) while the minimum in BAP (1.64 ng/g). JA contents were low in IBA or IAA treated leaf cuttings, alone or in combination with BAP.

Changes in endogenous Cis-Jasmone contents during early phase of adventitious rooting were apparent in guava leaf cuttings (Table 6). Cis-Jasmone content in fresh leaf cuttings (5.66 ng/g) declined considerably after one WAP, with lowest content in control (0.03 ng/g) and coconut water treated cuttings (0.06 ng/g). Maximum Cis-

Jasmone was observed in IBA 1000 mg L⁻¹+ BAP 500 mg L⁻¹(0.43 ng/g), followed by IBA 1000 mg L⁻¹(0.39 ng/g) and IAA 1000 mg L⁻¹+ BAP 500 mg L⁻¹(0.34 ng/g). A gradual increase in Cis-Jasmone levels was shown during second and third WAP, with highest levels seen in IBA 1000 mg L⁻¹+ BAP 500 mg L⁻¹(0.66 and 0.83 ng/g respectively at 2 and 3 weeks). However, the Cis-Jasmone contents were noted to be lowest in control leaf cuttings (0.15 ng/g at 2 WAP) and BAP 500 mg L⁻¹(0.25 ng/g at 3 WAP).

Table 6. Changes in endogenous Jasmonates during root formation

Table 2. Detailed tabular representation with Multiple comparisons

Treatment	JA (ng/g FW)			Cis Jasmone (ng/g FW)		
	1 WAP	2 WAP	3 WAP	1 WAP	2 WAP	3 WAP
T ₀ -Fresh leaves	5.69 ^a	5.69 ^a	5.69 ^c	5.66 ^a	5.66 ^a	5.66 ^a
T ₂ - IBA 1000 mg L ⁻¹ + BAP 500 mg L ⁻¹	1.57 ^c	1.43 ^b	3.86 ^e	0.43 ^b	0.66 ^b	0.83 ^b
T ₄ - IAA 1000 mg L ⁻¹ + BAP 500 mg L ⁻¹	0.84 ^{ef}	0.65 ^c	3.43 ^f	0.34 ^{cd}	0.74 ^b	0.26 ^e
T ₁₀ - IBA 1000 mg L ⁻¹	1.04 ^{de}	0.47 ^d	3.79 ^e	0.39 ^{bc}	0.22 ^{cde}	0.51 ^c
T ₁₂ - IAA 1000 mg L ⁻¹	0.66 ^f	0.39 ^d	4.38 ^d	0.16 ^e	0.18 ^{de}	0.41 ^{cd}
T ₁₃ - BAP 500 mg L ⁻¹	0.22 ^g	0.45 ^d	1.64 ^g	0.33 ^d	0.24 ^{cd}	0.25 ^e
T ₁₄ - Coconut water	2.01 ^b	0.15 ^e	7.02 ^a	0.06 ^f	0.30 ^c	0.36 ^{de}
T ₁₅ - Control (Water)	1.29 ^{cd}	0.07 ^e	6.45 ^b	0.04 ^f	0.15 ^e	0.37 ^{de}
CD	0.298	0.12	0.19	0.058	0.081	0.126
SE(m)	0.091	0.037	0.060	0.018	0.025	0.039
CV(%)	7.757	4.475	1.856	2.711	3.456	5.068

Variations in Salicylic acid (SA) content in leaf cuttings upon exogenous GR application was evident in the early rooting phase (Fig. 3). While the fresh leaves contained 269.46 ng/g SA, an upsurge of SA was observed at one week post-planting of cuttings. Highest concentration of SA at one WAP was detected in cuttings that received IBA 1000 mg L⁻¹+ BAP 500 mg L⁻¹(1246.91 ng/g). During second week, SA concentration decreased in the cuttings irrespective of the treatments, with lowest levels in coconut water treated cuttings (102.96

ng/g), control (122.02 ng/g) and in BAP 500 mg L⁻¹treated cuttings (209.72 ng/g). High levels of SA were retained by cuttings treated with auxins, alone or in combination with BAP. During the expression phase, cuttings treated with IAA 1000 mg L⁻¹+ BAP 500 mg L⁻¹retained higher SA content (400.38 ng/g), followed by IAA 1000 mg L⁻¹(240.23 ng/g) and IBA 1000 mg L⁻¹ (236.42 ng/g), while SA content depleted in control treatment (45.76 ng/g) and coconut water treated cuttings (99.14 ng/g).

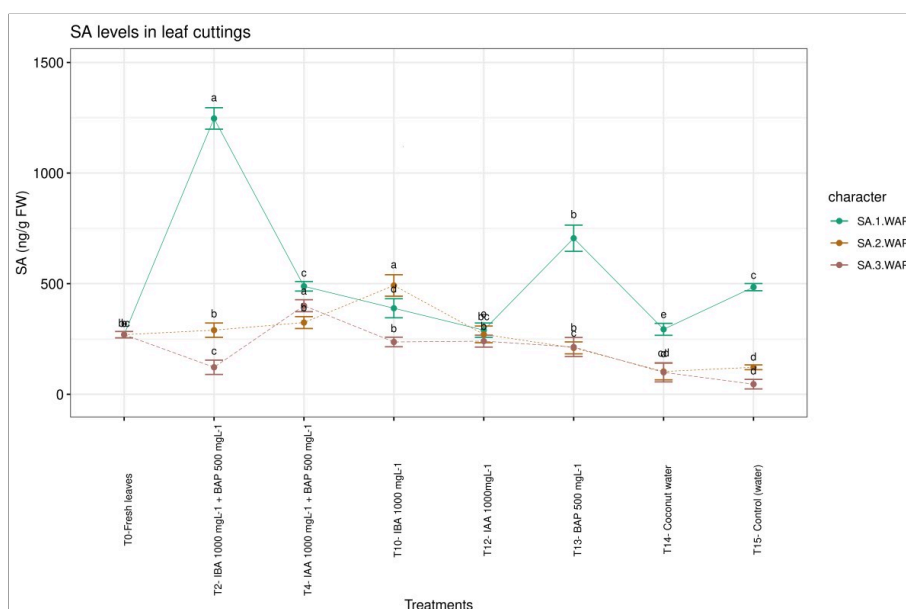


Figure 3. Changes in salicylic acid content in leaf cuttings during rooting phase

Discussion

Induction of adventitious rooting

Clonal propagation is an important aspect of guava cultivation, as it is crucial for maintaining true to type and uniform plants in the orchard. Leaf propagation is conventionally adopted in herbaceous plants and succulents and is rare in woody perennial species. Although protocols

for propagating guava using stem cutting are available, information of successful propagation through leaves or leaf bud cuttings is limited. In the present investigation, adventitious rooting of leaf bud cuttings of guava variety Arka Poorna was successfully achieved through exogenous application of auxins (IBA and IAA) alone or in combination with BAP. Treatment with auxins was essential to induce rooting in guava leaf cuttings while untreated leaves did not root. Similar find

ings were reported by Choudhary *et al.* (2022)^[14] in guava var. Lucknow-49 and Sundarajan (2024)^[15]. However, lower concentration of IAA (500 mg L⁻¹) was insufficient for root induction. In addition, treatments with coconut water and BAP failed to induce rooting of leaf cuttings. Successful rooting of cuttings using coconut water as natural source of PH was previously reported by Nwankwo *et al.* (2023)^[16], Ibironke *et al.* (2016)^[17], Agampodi and Jayawardena (2008)^[18], however the results of the present study is not agreement with the these reports. This might be due to low concentration of hormones in coconut water which could not meet the crop demand for root induction. Even though sole application of BAP and coconut water failed to induce roots, supplementing these compounds along with synthetic auxins could enhance the root number and growth. Findings of Sundarajan (2024) ^[15] was in agreement with these results, wherein better root production of guava leaf cuttings was achieved using auxins. As reported by De Klerk *et al.* (2001) ^[19], cell division initiated by endogenous cytokinins immediately after wounding is a crucial step for adventitious root formation. In our study, presence of BAP and coconut water could increase the endogenous BA content during the root induction phase thereby promoting cell division and overall root growth.

Sprouting and plantlet formation

Sprouting is another crucial step in propagation by leaf cuttings, following rooting. Rooted cuttings fail to sprout if optimal conditions are not present. In our study, rooted cuttings of IBA (500 and 1000 mg L⁻¹) treatments failed to produce sprouts. Nevertheless, supplementing BAP (500 mg L⁻¹) along with higher dose of IAA or IBA (1000 mg L⁻¹) could promote sprouting in rooted leaf cuttings. Similar results of better bud sprouting and plantlet formation in leaf cuttings through combined exogenous application of IBA and BAP is reported in Chinese Fir (Li *et al.*, 2017; Zhou *et al.*, 2018) ^[20, 21]. Auxins and cytokinins interact to regulate the various growth and development processes. While the core functions of cytokinins are to maintain the structure and size of shoot apical meristems, new leaf formation from the apical meristem commences with accumulation of auxins. However, cytokinins are essential for promoting the growth of shoot meristem for leaf initiation (Schaller *et al.*, 2015; Wu *et al.*, 2021)^[22, 23]. In the present study, treatment of leaf cuttings to BAP might have protected and maintained the axillary bud shoot meristem from degeneration during the process of adventitious root production, rather than their direct role in induction of rooting. The well-established roots in the cuttings might also attribute as source of cytokinins for accelerating sprouting from the buds, in addition to better water and nutrient absorption from the growing medium might have attributed to sprout initiation and growth of cuttings.

Endogenous hormonal changes during adventitious root formation

The complex process of adventitious root formation is largely dependent on endogenous PH and GRs concentration. IAA and IBA, the common auxin hormones in plant are known for its direct involvement in adventitious root formation (Fattorini *et al.*, 2017)^[24]. Exogenous application of auxins (IBA or IAA) have resulted in increased IAA content in leaf cuttings at one WAP, coinciding with the root induction phase, whereas this IAA spike did not happen in the non-treated control, BAP and coconut water treated cuttings. The IAA levels were further declining in the later phases of root initiation (2 WAP) and expression (3 WAP). Our results were in agreement with the findings in *Magnolia* cuttings by Wang *et al.*, (2022) ^[25]. Early phase peaking of IAA levels is crucial for the formation of root primordia from vascular cambial cells (Ahkami *et al.*, 2013) ^[26]. The reduction in IAA during the later phases might have resulted from catabolism (Wang *et al.*, 2022) ^[25]. The internal IBA content were found to increase in cuttings involving exogenous IBA treatments during the first WAP, which in turn might have aided rooting. This finding is in agreement with Chen *et al.* (2023) ^[27] in *Morus* cuttings. Cytokinins play significant role in root development. They seem to promote the initial cell proliferation but inhibit the cell reprogramming during root formation (Lakehal and Bellini, 2018) ^[28]. The low

BA content in control and coconut water treatments and maintenance of BA in auxin treated cuttings during the inductive phase shows its complementary action with IAA in root formation. As reported by Druege *et al.* (2019) ^[12], the root primordial cells undergo dedifferentiation prior to adventitious root induction. Presence of moderate BA might have aided in this dedifferentiation by enhancing the auxin responsiveness. However, tZR content at induction phase was higher in untreated leaves than the fresh and auxin treated leaf cuttings, showing its inhibitory effects on adventitious root formation. Previous studies have also shown the inhibitory action of higher ZR contents in adventitious root formation in *Morus* (Chen *et al.*, 2023) ^[27] and cucumber hypocotyls (Kuroha *et al.*, 2002) ^[29].

Gibberellic acids (GAs) are the group of bioactive hormones influencing various developmental processes in plants. In the present study, endogenous GA₃ levels in auxin treated cuttings witnessed a spike during the early root induction phase (1 WAP) that promoted rooting, while decline in GA₃ levels in non-treated, BAP or coconut water treated cuttings led to rooting failure. This failure was also associated with gradual increase in GA₃ levels during root initiation. GA₄ levels were also high during the root induction phase, gradually decreasing during root initiation and expression stages. Tissues in control and coconut water treated cuttings failed to retain GA₄ levels while maintenance of GA₄ levels during the later phases in auxin treated tissues might have helped in the cell enlargement and elongation leading to root emergence. High levels endogenous GA₃ in the early rooting phase, followed by gradual decline in root expression has been reported in *Morus* (Chen *et al.*, 2023) ^[27].

Abscisic acid (ABA) is an inhibitory plant hormone, mainly associated with stress-responses. Role of ABA in adventitious rooting has contradicting reports. It was reported to inhibit adventitious root formation, mainly interfering with IAA, gibberellin and ethylene signalling pathways (Lakehal and Bellini, 2018) ^[28]. However, in our study, ABA content decreased at the root induction phase, followed by gradual increase during the initiation and expression phase. Exogenous IBA applied leaf cuttings retained a higher level of ABA at one week, while IAA contents were lower in IAA treated ones. Increase in ABA contents, associated with root expression was evident in auxins treated cuttings. Untreated, BAP and coconut water treated leaves had low ABA at 3 WAP, showing low rooting. In the present study, a conclusive regulatory role of ABA was not derived.

In the present study, JA contents showed a declining trend until root initiation phase followed by a slight increase during root expression. During root induction phase, JA levels were comparatively higher in control and coconut water treatments which ultimately failed to root, as compared to the auxin treated cuttings. In addition, high JA levels in auxin treated cuttings at root initiation phase shows a complementary action of auxins with JA in promoting rooting, while non-rooted cuttings presented low to JA levels at initiation phase. Cis Jasmones, though declined at root induction phase, was showing complementing effect in root formation in auxin treated cuttings. Control, BAP and coconut water treated cuttings failed to root, and showed low levels of Cis-Jasmones during the later phases also. Previous studies in petunias, potato and tobacco, reported that JA promoted root formation in presence of external auxin (Ravnikar *et al.*, 1992; Fattorini *et al.*, 2009) ^[31, 30]. Involvement of JA in adventitious root formation may be species specific and could stimulate callus development and cell regeneration, thereby supporting adventitious rooting as described by Chen *et al.* (2023) ^[27].

Salicylic acid (SA) levels in cuttings were found higher than the fresh leaf cuttings during root induction phase, which might be due to the wound responsive surge. High concentrations of SA in exogenous auxin treated cuttings during the initiation phase hinders its harmonious role in root initiation whereas cuttings without exogenous auxins presented considerably low SA, failing to root. These findings are in agreement with Chen *et al.* (2023) ^[27] indicating SA synthesis during root initiation period can accelerate adventitious root development.

Conclusions

This study demonstrates the potential of leaf-bud cuttings as an alternative propagation method in guava and provides new insights into the hormonal regulation of adventitious rooting. In summary, exogenous application of auxins could successfully induce adventitious rooting in guava leaf bud cuttings. Exogenous cytokinins (BAP) application along with auxins could induce sprouting and plantlet regeneration, while sole application of cytokinins was insufficient in root induction. Treating leaf bud cuttings with synthetic auxins could develop roots mainly through accelerating endogenous IAA and IBA levels during the first WAP, in addition to complementary hormonal interactions of BA, GA and SA. Further investigations on refining the propagation conditions (temperature & relative humidity) and GRs treatment are essential to increase the plantlet formation for scaling up the propagation of guava through leaf cuttings.

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Disclosure statement

The authors declare that they have no competing interests.

Data availability statement

All the data pertaining to the experiment are already included in the manuscript.

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Authors

Anushma PL

ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru, Karnataka, India

Rafeekher M

College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

Satisha J

ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru, Karnataka, India

Shivashankara KS

ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru, Karnataka, India

Simi S

College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

Usharani TR

ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru, Karnataka, India

Manju PR

College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

Lokesha AN

ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru, Karnataka, India

Dinsha M

ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru, Karnataka, India

Anupa T

ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru, Karnataka, India