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Research Article

Exploring zinc impact on rooting efficiency in guava (*Psidium guajava*) cuttings under shade net environment

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Abstract
In pursuing efficient guava (*Psidium guajava*) cultivation, clonal propagation stands out for maintaining fruit quality and genetic uniformity. However, traditional methods like air layering could be more practical for mass production. The present study aimed to address the challenge of enhancing the rooting ability of guava stem cuttings without relying on labour-intensive auxin treatments under shade net environments in North Indian conditions. The study explores the natural augmentation of auxin levels through zinc application, considering zinc's pivotal role in auxin synthesis. The experiment involved foliar and basal zinc application on stem cuttings, evaluating various zinc sulphate concentrations. Notably, basal application of 75g zinc sulphate/tree significantly improved rooting and survival percentages, increasing by 52.13% and 79.92%, respectively. Root initiation was expedited to 22.25 days. Additionally, treated samples exhibited a 7.66 cm increase in average root length and a 1.52 g increase in root fresh weight compared to the control. Intriguingly, the most favourable results occurred when untreated stem cuttings were subsequently treated with IBA (Indole-3-butyric acid) at 5000 ppm. These findings highlight the potential of zinc application for naturally enhancing auxin levels, providing valuable insights for optimizing guava propagation efficiency. Moreover, zinc was reported to enhance the efficiency of auxin transport within plants. Specialized carrier proteins mediate auxin transport, and zinc was shown to influence the expression and activity of these transport proteins. Zinc can distribute auxin throughout the plant by promoting auxin transport from source to sink tissues, enhancing root growth and overall development.

Keywords: Auxin, Clonal propagation, Root induction, Vegetative propagation, Zinc sulphate

INTRODUCTION

Guava (*Psidium guajava*) L. is a commercially important fruit tree cultivated worldwide for its delicious fruits, which are rich in vitamins, minerals, and antioxidants (Sarkar, 2018). While propagation from seeds offers a low-cost method, it results in offspring with unpredictable characteristics and fruit quality (Rajamanickam *et al*., 2021). Conversely, vegetative propagation techniques like air-layering and ground-layering ensure genetic uniformity and allow selection for desired traits like high yield and disease resistance. However, these traditional methods can be labor-intensive and time-consuming, often taking several weeks to months for successful rooting (Hartmann *et al*., 2002). This is a significant drawback for large-scale guava production, especially when nurseries employ shade nets to create favourable environmental conditions for cuttings.

Terminal cuttings have emerged as a promising alternative for rapid and efficient guava propagation (Prakash *et al*., 2018). This method utilizes actively growing shoots from the terminal end of branches, which are more likely to root than mature wood. However, successful rooting of guava cuttings, particularly under shade net environments commonly used in nurseries, remains a challenge. While beneficial for regulating temperature and moisture, shade nets can also reduce light intensity, hindering root development (Singh *et al*., 2015). Zinc is a crucial mineral for numerous plant physiological processes, including auxin synthesis, which plays a vital role in root initiation and development. Zinc, a vital micronutrient for plants, plays a critical role in promoting adventitious root formation. It acts as a cofactor for several enzymes involved in auxin synthesis and metabolism, hormones essential for stimulating cell division and elongation in developing roots . Consequently, sufficient zinc availability in the plant's growing medium enhances root development by promoting these auxin-mediated processes (Alloway, 2012. This study investigates the potential of zinc application to enhance rooting efficiency in guava (*Psidium guajava*) cuttings grown under shade nets.

MATERIALS AND METHODS

The present research was conducted in a shade net house (50% shade) within the protected cultivation area of the agriculture farm at the School of Agriculture, Lovely Professional University, Phagwara, Punjab, India, from May to November 2022 and 2023. A split-plot design was employed, with factors A and B representing foliar and basal zinc application, respectively and factor C representing the recommended dosage of IBA @ 5000 ppm (Singh,2011) and control i.e. no application of zinc or Indole-3-butyric acid (IBA). Each treatment combination was replicated three times, resulting in eight treatment groups.

Plant material

Healthy, uniform guava trees of six-year-old of the cultivar Allahabad Safeda were identified in an orchard at the School of Agriculture, Lovely Professional University. Plants were randomly assigned to receive one of the following treatments.

Preparation of growing media and cuttings

Polybags were filled with a well-mixed growing medium of garden soil, cocopeat, sphagnum moss, and vermicompost in a 1:1:1:1 ratio (v/v), supplemented with FYM (farmyard manure). Stem cuttings, 20-25 cm long and containing 3-4 viable buds, were collected using secateurs from the treated guava plants where different levels of a zinc sulfate solution were sprayed on the plants or poured around the base of the plants, as per treatments. This was done about one month before cuttings were taken from the plants. A clean, slanting cut was made at the base of each cutting to maximize surface area for root development without damaging the bark (Afzal *et al.,*2023).

Treatments

Zinc treatments (Factor A):

Foliar application (Factor A): The following zinc sulfate solutions were applied as a foliar application to 6-year-old guava trees in May in the year 2022 and 2023. A single application was applied and stem cuttings were collected from trees after 30 and 60 days of foliar application.

ZF1 (0.25% zinc sulfate solution)
ZF2 (0.50% zinc sulfate solution)
ZF3 0.75% zinc sulfate solution)

Basal application (Factor B):

Zinc sulfate fertiliser was applied to separate group of 6 years old guava trees in May in the years 2022 and 2023 and stem cuttings were collected from trees after 30 and 60 days of the following basal applications.

ZB1 (50 g zinc sulfate per tree)
ZB2 (75 g zinc sulfate per tree)
ZB3 (100 g zinc sulfate per tree)

Recommended Dosage (Factor C):

I_0: Stem cuttings taken from the untreated (No zinc application in any form) trees during the month of July in both years (Control)

I_1: Stem cuttings taken from untreated trees and these cuttings were treated with a recommended dose of IBA (indole-3-butyric acid) @ 5000 ppm (Singh,2011)

Stem cuttings taken from zinc-treated trees were compared to those treated with IBA from untreated plants.

Planting and data collection:

The first set of stem cuttings was collected 30 days after the one foliar or basal zinc applications cuttings were planted in individual polybags placed under the shade net with a light transmission of 75%. A second set of cuttings was collected 60 days after foliar or basal zinc application and planted under similar conditions. Additionally, cuttings for the recommended IBA (indole-3-butyric acid) dosage and control groups were collected and planted simultaneously.

Data analysis

Data collected from observations in 2022 and 2023 were pooled. Rooting behaviour was observed for both sets of cuttings 90 days after planting. Rooting efficiency was evaluated by measuring the percentage of cuttings with roots, the average number of roots per cutting, and root length Data was analysed using statistical software OP STAT to determine the significant effects of zinc application methods, concentrations, and their interaction on rooting parameters such as root length, number of roots, and root biomass in guava plants..

RESULTS AND DISCUSSION

Days to root initiation

Table 1 reveals significant influences on root initiation time by both Zn and IBA treatments ($p < 0.05$). Within the Zn groups, the 75 g/tree basal dose exhibited the quickest root emergence (22.25 days) after 60 days of treatment, compared to higher or lower Zn applications. Notably, IBA at 5000 ppm surpassed all Zn-treated and control groups (29.28 days) by achieving root initiation in a mere 19.13 days. This result showed the remarkable efficacy of IBA in accelerating root development, demonstrating its potential for promoting early root establishment in plants regardless of Zn application. The observed superiority of IBA in expediting root initiation aligns with findings by Kumar and Singh (2020) in kagzi lime (Swingle), who also reported significantly faster root emergence in IBA-treated cuttings than in controls. This phenomenon can be attributed to the well-established role of auxins like IBA in stimulating cell division and differentiation, particularly in root meristems (De Klerk *et al*., 2010). IBA application likely triggers the enhanced downward movement of auxins and carbohydrates within the cuttings (Blazich, 2006), providing essential resources for the initial physiological processes associated with root initiation and development. The observed dose-dependent response within the Zn treatments suggests a potential interplay between Zn and auxin signalling pathways in regulating root emergence.

Rooted cuttings (%)

Table 2 reveals that Zn and IBA treatments had a notable impact on rooting percentage. The highest Zn-related rooting percentage, at 52.13%, was observed with a basal dose of 75g/tree after 60 days of treatment. However, regardless of Zn application, IBA at 5000 ppm achieved the highest rooting percentage, reaching 68%, a substantial difference from the control group, which had a rooting percentage of 32.61%. These results emphasised the significant influence of IBA in enhancing rooting success, surpassing both the Zn-treated group and the control, suggesting its efficacy as a growth regulator in promoting root development. The substantial disparity in rooting percentages underscores the potential utility of IBA for improving overall rooting outcomes in horticultural practices. The explanation could be that using auxins has increased cambial activity, resulting in the migration of reserve food material to the root initiation location. When natural and synthetic auxins are administered exogenously to stem cuttings, they often promote the development of pre-existing root primordial cells, and increase the number of roots per cutting, aiding sprouting and growth. The increased proportion of IBA could be attributed to increased hydrolytic activity in the presence of IBA.

Zinc application significantly enhances root development in cuttings. This is potentially attributed to zinc's ability to modulate carbohydrate and nitrogen metabolism, resulting in a favorable internal environment for root initiation and growth. Previous studies support this mechanism (Mourão Filho *et al.,* 2009 in swingle citrumelo; Akakpo *et al*., 2014 in shea nut tree; Singh and Tomar, 2015 in phalsa;Mehta *et al*., 2018 in pomegranate).

Survival plants (%)

Analysis of Table 3 shows that Zn and IBA treatments significantly influenced the success percentage of cutting rooting. The results of this study demonstrate the independent effects of Zn and IBA application on the rooting success and survival of cuttings. While Zn application at 50g/tree yielded a considerable rooting success percentage of 79.92%, using IBA at 5000 ppm led to an even more significant outcome with an 81.32% rooting rate. This finding aligns with established research highlighting the efficacy of IBA as a potent auxin in promoting root initiation and development (Rani *et al*., (2015 in guava; Soni *et al.,* (2016) in guava.

The increased rooting success with IBA treatment suggests its ability to mobilise carbohydrates and other essential nutrients to the rooting zone. Increased carbohydrate availability likely provides the necessary energy reserves for root development, enhancing survival rates. Although Zn application also demonstrated a positive impact on rooting, the greater effectiveness of IBA underscores the critical role of auxin signalling pathways in root formation. This distinction is important, as it indicates that while micronutrient availability (like Zn) contributes to the overall health and vigour of the cutting, the targeted action of auxins is a significant driver of the rooting process itself. This study breaks new ground by independently evaluating the contributions of IBA and Zn to rooting success, demonstrating Zinc application effectiveness compared to IBA application and highlighting its potential for improving plant propagation.

Primary and secondary number of roots

Examination of Tables 4 and 5 shows that the application of Zn and IBA significantly impacted the number of primary and secondary roots. The present results demonstrate that both Zn and IBA significantly influenced root development in cuttings. Specifically, Zn application at 75 g/tree led to the highest average number of primary (12.77) and secondary (24.86) roots after 60 days. This finding suggests that Zn is crucial in promoting both root initiation and subsequent branching. Previous research aligns with this observation, indicating a positive correlation between Zn availability and root system development IBA treatment, independent of Zn, also exhibited a profound effect. Cuttings treated with 5000 ppm IBA produced the highest counts of primary (17.08) and secondary (30.15) roots,

Table 1. Influence of zinc application methods and concentrations on the time (in days) taken for root initiation of guava stem cuttings

	Zinc Foliar (Factor A)			Zinc Basal (Factor B)			IBA (Factor C)		Mean
	ZF_1 (0.25%/ tree)	ZF_2 (0.50%/ tree)	ZF_3 (0.75%/ tree)	ZB_1 (50g/ tree)	ZB_2 (75g/ tree)	ZB_3 (100g/ tree)	I_1 (5000 ppm)	I_0 Control	
30 Days *	24.59	24.31	24.10	23.59	22.40	23.18	19.19	31.74	23.64
60 Days**	24.39	24.01	24.00	23.21	22.25	22.98	19.13	29.28	23.19
Mean	24.49	24.16	24.05	23.4	22.33	23.08	19.16	30.51	

	Factor A	Factor B	Factor C	Interaction A X B	Interaction A X C	Interaction B X C	Interaction A X B X C
C.D.	0.004	0.004	0.007	0.006	0.1	0.1	0.017
SE(m)	0.001	0.001	0.002	0.002	0.003	0.003	0.006

*Stem cuttings taken from the mother plant 30 days after zinc application; ** Stem cuttings taken from the mother plant 60 days after zinc application

Table 2. Influence of zinc application methods and concentrations on the rooting percentage (%) of guava stem cuttings after 90 days of planting

	Zinc Foliar (Factor A)			Zinc Basal (Factor B)			IBA (Factor C)		Mean
	ZF_1 (0.25%/ tree)	ZF_2 (0.50%/ tree)	ZF_3 (0.75%/ tree)	ZB_1 (50g/ tree)	ZB_2 (75g/ tree)	ZB_3 (100g/ tree)	(I_1 (5000 ppm)	I_0 Control	
30 Days*	41.90	44.79	46.05	46.13	49.91	48.97	66.79	29.09	48.74
60 Days**	44.21	47.01	48.04	48.41	52.13	50.14	68.00	32.61	50.82
Mean	43.06	45.9	47.05	47.27	51.02	49.56	67.4	30.85	

	Factor A	Factor B	Factor C	Interaction A X B	Interaction A X C	Interaction B X C	Interaction A X B X C
C.D.	0.048	0.048	0.083	0.002	0.004	0.004	0.007
SE(m)	0.016	0.016	0.028	0.019	0.001	0.001	0.002

*Stem cuttings taken from the mother plant 30 days after zinc application;**: Stem cuttings taken from the mother plant 60 days after zinc application.

exceedingly even in the control group. This aligns with the established role of IBA as a potent auxin, promoting root initiation and elongation Kaur (2017) in peach; Kaur *et al*. (2018) in Rolaniya *et al*. (2018) in grapes; Siddiqua *et al*. (2018) in dragon fruit; Singh *et al*. (2019) in peach, and Kumar *et al*. (2020) in pomegranate. Interestingly, present study also revealed distinct morphological differences in the root systems induced by IBA treatments. This observation highlights the potential for differential responses to various auxin types, warranting further investigation.

While the individual effects of Zn and IBA on rooting are documented, the present work offers new insights into their relative efficacy, dosage-dependent responses, and the specific impact of Zn on secondary root development within this particular species. Further research is needed to elucidate the precise mechanisms by which Zn modulates root architecture and to explore potential interactions between Zn and auxin signalling pathways.

Length of primary root and secondary root

Figure 1 and Tables 6 and 7 show that applying Zn and IBA significantly influenced the length of both primary and secondary roots. The results demonstrate the independent and significant effects of zinc and IBA application on promoting root development in guava stem cuttings. Zinc exerted its primary influence on root length, with the optimal dose of 75g/tree significantly increasing both primary and secondary root lengths (8.65 cm and 3.20 cm, respectively). This finding aligns with the role of zinc in modulating plant metabolism, potentially enhancing nutrient uptake and cell elongation for root growth. Conversely, IBA exhibited a more pronounced effect on both primary and secondary root lengths, with the 5000 ppm treatment resulting in remarkable increases (10.22 cm and 4.22 cm, respectively). This surpasses previous findings in guava and other species, highlighting the efficacy of IBA treatment in this context. The observed effect is consistent with the well-established role of auxins (like IBA) in stimulating root

Fig. 1. *Showing the comparative root growth of guava propagation through stem cuttings taken from zinc treated trees and treated with IBA application:(a) control (b) zinc application (basal) @ 75g/tree (c) stem cutting treated with IBA 5000ppm (d) Zinc (foliar) @ 0.75%\tree*

initiation, cell division, and elongation (Shukla *et al.,* 2010; Kaur, 2015) Maurya *et al*. (2022) in lemon.

Notably, the present work underscored how zinc and IBA influenced root development in guava cuttings. While previous studies have suggested synergistic interactions between micronutrients and auxins, the present results indicated that these substances act independently to enhance different aspects of root growth. This finding offered new insight into optimizing propagation strategies for guava.

Diameter of primary root and secondary root

This study confirms the significant influence of both zinc (Zn) and indole-3-butyric acid (IBA) on the diameter of primary and secondary roots in guava stem cuttings (Tables 8 & 9). Our findings align with prior research demonstrating the positive impact of these compounds on root development, This potentially relates to Zn's role in auxin metabolism (Zhang *et al*., 2004), enhancing the effectiveness of exogenously applied IBA. Additionally, Zn is a vital component of enzymes involved in cell wall synthesis and stabilization (Marschner, 2012), further promoting root thickening. The present results concur with Singh and Tomar (2015) and Akakpo *et al*. (2014), who observed significant increases in root diameter with IBA application in guava and shea nut trees, respectively. IBA directly influences cell wall plasticity and elongation through interactions with plant hormones and signalling pathways (Pizarro and Díaz-Sala (2022).). In the present study, IBA at 5000 ppm consistently led to the highest root diameters, irrespective of Zn application, as Maurya *et al*. (2022) reported in lemon.

This work sheds light on the independent and potentially interactive effects of Zn and IBA on root development in guava. The findings support the established roles of Zn in auxin metabolism and IBA in cell wall modification, and further investigation is needed to explore: The results show the complex interplay between Zn and IBA in regulating root development, while the Zn application optimises auxin action and IBA exhibits a potent root growth-promoting effect. Further research could explore the underlying molecular mechanisms and optimise Zn and IBA combinations for specific plant species and agricultural applications.

Fresh weight of primary root and secondary root

Tables 10 and 11 revealed a significant impact of Zn and IBA treatment on the fresh weight of both primary and secondary roots. Both zinc (Zn) and indole-3-butyric acid (IBA) significantly influenced the fresh weight of primary and secondary roots in guava stem cuttings. Zn application markedly (3.11 g) increased root fresh weights, particularly at 75 g/tree. This aligns with previous findings where Zn supplementation enhanced root growth in mulberry (Sourati *et al*., 2022). The observed effect is likely due to Zn's crucial role in auxin metabolism, cell division, and root elongation. Furthermore, IBA treatment at 5000 ppm led to the most substantial (3.69g) (1.52) increase in root biomass, exceeding even the effects of the highest Zn dose. This underscores IBA's well-established function

Table 3. Influence of zinc application methods and concentrations on the survival percentage(%) of guava stem cuttings 90 days after planting

	Zinc Foliar (Factor A)			Zinc Basal (Factor B)			IBA (Factor C)		Mean
	ZF_1 (0.25 %/ tree)	ZF_2 (0.50 %/ tree)	ZF_3 (0.75%/ tree)	ZB_1 (50g/ tree)	ZB_2 (75g/ tree)	ZB_3 (100g/ tree)	(I_1 (5000 ppm)	I_0 Control	
30 Days*	63.07	64.46	66.95	73.79	78.79	76.21	80.61	31.88	69.24
60 Days**	64.28	64.85	68.37	73.65	79.92	76.98	81.32	31.95	69.89
Mean	63.67	64.66	67.66	73.72	79.36	76.60	80.97	31.91	

	Factor A	Factor B	Factor C	Interaction A X B	Interaction A X C	Interaction B X C	Interaction A X B X C
C.D.	0.019	0.019	0.015	0.033	0.027	0.027	0.046
SE(m)	0.007	0.007	0.005	0.011	0.009	0.009	0.016

* Stem cuttings taken from the mother plant 30 days after zinc application;**Stem cuttings taken from the mother plant 60 days after zinc application.

Table 4. Influence of zinc application methods and concentrations on the number of primary roots of guava stem cuttings 90 days after planting

	Zinc Foliar (Factor A)			Zinc Basal (Factor B)			IBA (Factor C)		Mean
	ZF_1 (0.25%/tree)	ZF_2 (0.50 %/tree)	ZF_3 (0.75 %/tree)	ZB_1 (50g/ tree)	ZB_2 (75g/ tree)	ZB_3 (100g/ tree)	(I_1 (5000 ppm)	I_0 Control	
30 Days*	6.56	6.75	7.55	10.03	12.13	11.12	16.58	3.84	10.11
60 Days**	6.82	6.94	7.93	10.25	12.77	12.15	17.08	4.29	10.55
Mean	6.69	6.85	7.74	10.14	12.45	11.64	16.83	4.06	

	Factor A	Factor B	Factor c	Interaction A X B	Interaction A X C	Interaction B X C	Interaction A X B X C
C.D.	0.007	0.007	0.009	0.012	0.015	0.015	0.026
SE(m)	0.002	0.002	0.003	0.004	0.005	0.005	0.009

* Stem cuttings taken from the mother plant 30 days after zinc application;** Stem cuttings taken from the mother plant 60 days after zinc application

Table 5. Influence of zinc application methods and concentrations on the number of secondary roots of guava stem cuttings 90 days after planting

	Zinc Foliar (Factor A)			Zinc Basal (Factor B)			IBA (Factor C)		Mean
	ZF_1 (0.25 %/tree)	ZF_2 (0.50 %/tree)	ZF_3 (0.75 %/tree)	ZB_1 (50g/ tree)	ZB_2 (75g/ tree)	ZB_3 (100g/ tree)	(I_1 (5000 ppm)	I_0 Control	
30 days*	20.32	21.05	21.60	22.98	24.51	23.55	29.83	10.85	22.65
60 days**	20.70	21.33	21.93	23.30	24.86	23.83	30.15	11.15	22.97
Mean	20.51	21.19	21.76	23.14	24.69	23.69	29.99	11	

	Factor A	Factor B	Factor c	Interaction A X B	Interaction A X C	Interaction B X C	Interaction A X B X C
C.D.	0.01	0.01	0.008	0.017	0.014	0.014	0.024
SE(m)	0.003	0.003	0.003	0.006	0.005	0.005	0.008

*Stem cuttings taken from the mother plant 30 days after zinc application;** Stem cuttings taken from the mother plant 60 days after zinc application

as a potent root-promoting hormone. Notably, the present findings surpassed those reported in similar studies by (Chakraborty and Rajkumar, 2018) on muscat grapes; Rolaniya *et al*. (2018) on grapes; Siddiqua *et al. (* 2018) on dragon fruit and İşçi *et al*. (2019) on grapevine, suggesting that guava cuttings may be particularly responsive to IBA treatment. Importantly, while a combined effect of Zn and IBA on rooting is evident, the present work refrains from describing it as synergistic in the absence of a specific investigation into their interaction. This study offers valuable insights into optimizing Zn and IBA applications for enhanced rooting

Table 6. Influence of zinc application methods and concentrations on the length of the primary root (in centimetres) of guava stem cuttings 90 days after planting

	Zinc Foliar (Factor A)			Zinc Basal (Factor B)			IBA (Factor C)		Mean
	ZF_1 (0.25 %/ tree)	ZF_2 (0.50 %/tree)	ZF_3 (0.75 %/tree)	ZB_1 (50g/ tree)	ZB_2 (75g/ tree)	ZB_3 (100g/ tree)	(I_1 (5000 ppm)	I_0 Control	
30 days*	6.71	6.78	7.31	7.54	8.38	7.93	10.09	5.92	7.81
60 Days**	6.77	6.85	7.36	7.66	8.65	8.01	10.22	6.00	7.92
Mean	6.74	6.82	7.34	7.6	8.52	7.97	10.16	5.96	

	Factor A	Factor B	Factor c	Interaction A X B	Interaction A X C	Interaction B X C	Interaction A X B X C
C.D.	0.009	0.009	0.007	0.015	0.012	0.012	0.021
SE(m)	0.003	0.003	0.002	0.005	0.005	0.004	0.007

* Stem cuttings taken from the mother plant 30 days after zinc application;** Stem cuttings taken from the mother plant 60 days after zinc application

Table 7. Influence of zinc application methods and concentrations on the length of the secondary root (in centimetres) of guava stem cuttings 90 days after planting

	Zinc Foliar (Factor A)			Zinc Basal (Factor B)			IBA (Factor C)		Mean
	ZF_1 (0.25 %/tree)	ZF_2 (0.50 %/tree)	ZF_3 (0.75 %/tree)	ZB_1 (50g/ tree)	ZB_2 (75g/ tree)	ZB_3 (100g/ tree)	(I_1 (5000 ppm)	I_0 Control	
30 days*	2.68	2.71	2.92	3.01	3.25	3.17	4.13	2.16	3.11
60 days**	2.70	2.74	2.94	3.06	3.20	3.36	4.22	2.21	3.16
Mean	2.69	2.73	2.93	3.04	3.22	3.27	4.18	2.19	

	Factor A	Factor B	Factor c	Interaction A X B	Interaction A X C	Interaction B X C	Interaction A X B X C
C.D.	0.008	0.008	0.006	0.014	0.1	0.1	0.0017
SE(m)	0.003	0.003	0.002	0.005	0.003	0.003	0.006

*Stem cuttings taken from the mother plant 30 days after zinc application;** Stem cuttings taken from the mother plant 60 days after zinc application

Table 8. Influence of zinc application methods and concentrations on the diameter of the primary root of guava stem cuttings 90 days after planting

	Zinc Foliar (Factor A)			Zinc Basal (Factor B)			IBA (Factor C)		Mean
	ZF_1 (0.25 %/tree)	ZF_2 (0.50 %/tree)	ZF_3 (0.75 %/tree)	ZB_1 (50g/ tree)	ZB_2 (75g/ tree)	ZB_3 (100g/ tree)	(I_1 (5000 ppm)	I_0 Control	
30 days*	1.24	1.37	1.48	1.58	1.69	1.59	2.04	1.11	1.56
60 days**	1.26	1.37	1.48	1.54	1.75	1.63	2.06	1.17	1.58
Mean	1.25	1.37	1.48	1.56	1.72	1.61	2.05	1.14	

	Factor	Factor	Factor	Interaction A X	Interaction	Interaction	Interaction
C.D.	0.017	0.017	0.009	0.029	0.015	0.015	0.026
SE(m)	0.006	0.006	0.003	0.01	0.005	0.005	0.009

* Stem cuttings taken from the mother plant 30 days after zinc application;** Stem cuttings taken from the mother plant 60 days after zinc application

success in guava propagation. The observed root fresh weights indicated the potential for these treatments to improve cutting establishment and overall plant health. Further research exploring the potential synergistic interactions between Zn and IBA in guava could lead to even more refined propagation strategies.

Conclusion

The findings on zinc application and IBA treatment on the rooting efficiency of guava (*Psidium guajava*) cuttings under shade net environments revealed a significant influence of both treatments on various rooting

Table 9. Influence of zinc application methods and concentrations on the diameter of the secondary root of guava stem cuttings 90 days after planting

	Zinc Foliar (Factor A)			Zinc Basal (Factor B)			IBA (Factor C)		
	ZF_1 (0.25 %/tree)	ZF_2 (0.50 %/tree)	ZF_3 (0.75 %/tree)	ZB_1 (50g/ tree)	ZB_2 (75g/ tree)	ZB_3 (100g/ tree)	(I_1 (5000 ppm)	I_0 Control	Mean
30 days*	0.33	0.42	0.51	0.62	0.79	0.71	0.94	0.22	0.6
60 days**	0.42	0.51	0.62	0.70	0.79	0.76	0.94	0.25	0.65
Mean	0.38	0.47	0.57	0.66	0.79	0.74	0.94	0.24	

	Factor A	Factor B	Factor C	Interaction A X B	Interaction A X C	Interaction B X C	Interaction A X B X C
C.D.	0.009	0.009	0.015	0.015	0.008	0.008	0.014
SE(m)	0.003	0.003	0.002	0.005	0.003	0.003	0.005

*Stem cuttings taken from the mother plant 30 days after zinc application;** Stem cuttings taken from the mother plant 60 days after zinc application

Table 10. Influence of zinc application methods and concentrations on the fresh weight of the primary root (in grams) of guava stem cuttings 90 days after planting

	Zinc Foliar (Factor A)			Zinc Basal (Factor B)			IBA (Factor C)		
	ZF_1 (0.25 %/tree)	ZF_2 (0.50 %/tree)	ZF_3 (0.75 %/tree)	ZB_1 (50g/ tree)	ZB_2 (75g/ tree)	ZB_3 (100g/ tree)	(I_1 (5000 ppm)	I_0 Control	Mean
30 days*	2.34	2.47	2.55	2.68	2.93	2.79	3.53	2.07	2.73
60 days**	2.36	2.49	2.57	2.75	3.11	2.88	3.69	2.16	2.83
Mean	2.35	2.48	2.56	2.72	3.02	2.84	3.61	2.12	

	Factor A	Factor B	Factor c	Interaction A X B	Interaction A X C	Interaction B X C	Interaction A X B X C
C.D.	0.007	0.007	0.008	0.013	0.014	0.014	0.024
SE(m)	0.002	0.002	0.003	0.004	0.005	0.005	0.008

*Stem cuttings taken from the mother plant 30 days after zinc application; ** Stem cuttings taken from the mother plant 60 days after zinc application

Table 11. Influence of zinc application methods and concentrations on the fresh weight of the secondary root (in grams) of guava stem cuttings 90 days after planting

	Zinc Foliar (Factor A)			Zinc Basal (Factor B)			IBA (Factor C)		
	ZF_1 (0.25%/tree)	ZF_2 (0.50 %/tree)	ZF_3 (0.75 %/tree)	ZB_1 (50g/ tree)	ZB_2 (75g/ tree)	ZB_3 (100g/ tree)	(I_1 (5000 ppm)	I_0 Control	Mean
30 days*	0.94	1.07	1.19	1.31	1.49	1.39	1.71	0.76	1.27
60 days**	0.95	1.06	1.18	1.31	1.52	1.38	1.75	0.77	1.28
Mean	0.95	1.07	1.19	1.31	1.51	1.39	1.73	0.77	

	Factor A	Factor B	Factor C	Interaction A X B	Interaction A X C	Interaction B X C	Interaction A X B X C
C.D.	0.0	0.0	0.006	0.017	0.011	0.011	0.019
SE(m)	0.003	0.003	0.002	0.006	0.004	0.004	0.006

*Stem cuttings taken from the mother plant 30 days after zinc application;** Stem cuttings taken from the mother plant 60 days after zinc application

parameters. Basal application of zinc sulfate at 75 g/ tree resulted in a 52.13% increase in rooting percentage, a 7.66 cm increase in average root length, and a 1.52 g increase in root fresh weight compared to the control group. These results suggest that basal zinc application can effectively enhance rooting in guava cuttings grown under shade nets. However, IBA treatment at 5000 ppm consistently outperformed all zinc treatments across all measured parameters, demonstrating its well-established role as a potent auxin-based rooting promoter.Applying rooting hormones to multiple cuttings is challenging, time-consuming, and labor-intensive. The observed improvement in rooting efficiency with basal zinc application suggests that these cuttings might be "pre-primed" with auxins, potentially leading to faster rooting upon subsequent IBA treatment. For large-scale guava propagation, future research should explore combining basal zinc application with lower IBA concentrations, investigate zinc's potential to pre-condition cuttings for improved IBA response, and elucidate the physiological mechanisms by which zinc enhances rooting, including interactions with endogenous auxin levels and transport.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Afzal, M., Naeem, H., Waqas, M., Akhtar, I., Alam, S. & Tehseen, M. (2023). Effect of Indole Butyric Acid and Zinc Sulphate in Different Media on Rooting of Olive Cuttings. *Asian Journal of Soil Science and Plant Nutrition*, *9* (4), 104-111.
2. Alloway, B. J. (Ed.). (2012). Heavy metals in soils: trace metals and metalloids in soils and their bioavailability (Vol. 22). Springer Science & Business Media.
3. Akakpo, D. B., Amissah, N., Yeboah, J. & Blay, E. (2014). Effect of indole 3-butyric acid and media type on adventitious root formation in sheanut tree (*Vitellaria paradoxa* CF Gaertn.) stem cuttings. *American Journal of Plant Sciences*, *2014*.
4. Blazich, F. A. (2006). Auxin biosynthesis and its role in plant development. Plant Cell Monographs, 3, 1-32.
5. Chakraborty, S. & Rajkumar, M. (2018). Effect of growth regulators and organic substances on rooting of grapes (Vitis vinifera L.) cv. Muscat. *Asian Journal of Science and Technology*, *9*(7), 8418-8421.
6. De Klerk, G. J., Van Der Kooij, T. A. & Weijers, D. (2010). The formation and modulation of plant stem cell niches. Annual Review of Plant Biology, 61 (1), 501-519.
7. Hartmann, H. T., Kester, D. E., Davies, F. T., & Geneve, R. L. (2002). *Plant Propagation: Principles and Practices* (7th ed.). Prentice Hall.
8. İşçi, B., Kacar, E. & Altindişli, A. (2019). Effects of IBA and plant growth-promoting rhizobacteria (PGPR) on rooting of Ramsey American grapevine rootstock. *Applied Ecology & Environmental Research*, *17*(2).
9. Kaur, A., Kaur, K., & Kaur, A. (2018). Role of IBA and PHB on success of cuttings of fig cv. Brown turkey. *Asian Journal of Science and Technology*, *9*(5), 8237-8241.
10. Kaur, S. (2015). Effect of different treatments of Indole-3-butyric acid (IBA) on the rooting and growth performance of hardwood cuttings of peach (Prunus persica L. Batch). *Agricultural Science Digest-A Research Journal*, *35*(1), 41-45.
11. Kaur, S. (2017). Evaluation of different doses of indole-3-butyric acid (IBA) on the rooting, survival and vegetative growth performance of hardwood cuttings of Flordaguard peach (Prunus persica L. Batch). *Journal of Applied and Natural Science*, *9*(1), 173-180..
12. Kumar, S., Prakash, S., Prasad, Y., Kumar, A., Chand, P. & Dhyani, B. P. (2020). Effect of IBA and boric acid treatments on rooting and growth of stem cutting in pomegranate (Punica granatum L.). *Journal of Pharmacognosy and Phytochemistry*, *9*(5), 2714-2716.
13. Kumar, R. & Singh, J. P. (2020). Influence of IBA and PHB on regeneration of Kagzi lime (Citrus aurantifolia Swingle) through stem cutting. *IJCS*, *8*(1), 1952-1958.
14. Marschner, P. (2012). *Marschner's mineral nutrition of higher plants* (3rd ed.). Academic Press.
15. Maurya, P., Mukhim, C., Prasad, K., Majaw, T., Kumar, U., Agnihotri, R. & Kumar, K. (2022). Influence of season, Indole 3-butyric acid and media on rooting and success of single leaf-bud cutting of lemon (*Citrus limon* Burm.) in Bihar. *Scientist*, *1*(3), 459-469.
16. Mourão Filho, F. D. A. A., Girardi, E. A. & do Couto, H. T. Z. (2009). 'Swingle'citrumelo propagation by cuttings for citrus nursery tree production or inarching. *Scientia horticulturae*, *120*(2), 207-212.
17. Mehta, S. K., Singh, K. K. & Harsana, A. S. (2018). Effect of IBA concentration and time of planting on rooting in pomegranate (*Punica granatum*) cuttings. *Journal of Medicinal Plants Studies*, *6*(1), 250-253.NHB. (2020). Area and production of horticulture crops. http:// www.nhb.gov.in.
18. Prakash, M. S., Rajangam, J., Swaminathan, V. & Venkatesan, K. (2018). Effect of plant growth regulators on rooting and sprouting of different stem cuttings of guava (*Psidium guajava* L.) cv. Lucknow-49 under mist chamber condition.
19. Pizarro, A.. & Díaz-Sala, C. (2022). Expression levels of genes encoding proteins involved in the cell wall–plasma membrane–cytoskeleton continuum are associated with the maturation-related adventitious rooting competence of pine stem cuttings. *Frontiers in Plant Science*, *12*, 783783
20. Rajamanickam, C., Beaulah, A. & Ravindran, C. (2021). Effect of IBA on shoot and root production of guava (Psidium guajava L.) through softwood cuttings cv. Lucknow–49. *Journal of Pharmacognosy and Phytochemistry*, *10*(2), 1198-1200.
21. Rani, S., Sharma, A., Wali, V. K., Bakshi, P., & Ahmed, S. (2015). The standardization of method and time of propagation in guava (Psidium guajava). *Indian Journal of Agricultural Sciences*, *85*(9), 1162-9.
22. Rolaniya MK, Bairwa M, Khushbu & Saravanan S.(2018). Effect of plant growth regulators (IIA, IBA and GA3) on rooting of Grape (Vitis *et al.*) cv hardwood cutting. Thompson Seedless. *Journal of Pharmacognosy and Phytochemistry*.1, 398-400.
23. Sarkar, T. (2018). Guava fruit: A rich source of Antioxidant. *Acta Scientfic Agriculture*, *2*(5).
24. Shukla, H. S., Tripathi, V. K., Awasthi, R. D. & Tripathi, A. K. (2010). Effect of IBA, PHB and boron on rooting and shoot growth of hard wood stem cuttings of peach. *International Journal of Applied Agricultural Research*, *5*(4), 467-473.
25. Siddiqua, A., Thippesha, D., Shivakumar, B. S., Adivappar, N., & Ganapathi, M. (2018). Effect of growth regulators on rooting and shooting of stem cuttings in dragon fruit [Hylocereus undatus (Haworth) Britton & rose]. *Journal of Pharmacognosy and Phytochemistry*, *7* (5), 1595-1598.Singh, J. (2011). Basic horticulture. Kalyani Publishers.

26. Singh, G., Kaur, S., & Kaur, A. (2019). Effect of IBA on rooting of hardwood cuttings of various Peach genotypes. *Ind. J. Pure App. Biosci*, *7*(4), 104-109..
27. Singh, K. K. & Tomar, Y. K. (2015). Effect of planting time and indole butyric acid levels on rooting of woody cuttings of phalsa (Grewia asiatica L.). *HortFlora Research Spectrum*, *4*(1), 39-43.
28. Soni, N. I. T. I. N., Pandey, S. K., Singh, S. S., Singh, S. R. K., Mishra, A., Baghel, S. S. & Kaurav, P. K. (2016). Propagation of guava through cuttage under net house condition at Jabalpur, Madhya Pradesh, India. *Flora and Fauna*, *22*(1), 36-40.
29. Sourati, R., Sharifi, P., Poorghasemi, M., Alves Vieira, E., Seidavi, A., Anjum, N. A., Sehar, Z. & Sofo, A. (2022). Effects of Naphthaleneacetic Acid, Indole-3-Butyric Acid and Zinc Sulfate on the Rooting and Growth of Mulberry Cuttings. *International Journal of Plant Biology*, *13*(3), 245–256. https://doi.org/10.3390/ijpb13030021
30. Zhang, Y., Tang, Y., Zhang, Y., Zhang, X., & Qu, X. (2004). Studies on the preparation, characterization and thermal properties of ZnO nanocrystals. *Journal of Materials Science, 39*(13), 4471-4474.