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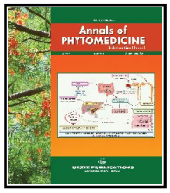

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Collisions of phytohormones on nodal and leaf cuttings in guava (*Psidium guajava* L.) cv Lucknow 49

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Abstract

In guava plants, nodal cuttings are the most efficient and effective way for propagation. Plant growth regulators that are appropriate for accelerating guava growth. To improve and stimulate the development of roots in guava nodal cuttings with the help of plant growth regulators. In this study, guava nodal cuttings were used at concentrations of 200 and 500 ppm, together with several kinds of cuttings, to examine the effects of three phytohormones, including cytokinin, indole-3-butyric acid (IBA), and indole-3-acetic acid (IAA) on single node, double node, triple node, and leaf cuts. Horticultural College and Research Institute, Periyakulam, Tamil Nadu was the site of the experiment. Nodal cuttings, double nodal cuttings, and IBA better performed the other plant growth regulators. The treatments with 500 ppm IBA in the double nodal cuttings resulted in the following: the lowest phenol content (0.030 mg/g), the lowest starch content (3.42 %), and the highest C/N ratio (8.66). The weight of the leaf was 1.73 g, the weight of the leaf after it had been dried was 0.68 g, the weight of the root (fresh) was 3.69 g, and the weight of the root (dry) was (1.86 g). With the longest root length and maximum chlorophyll content (41.94 mg/g) among all the cuttings treated with 500 ppm of IBA, the triple node cuttings stood out (27.32 cm). The cuttings collected from a single node and treated with 500 ppm IBA observed the highest amount of leaf-soluble protein at 38.92 (mg/g). It can be concluded that the combined effect of growth regulator IBA at 500 ppm double cuttings performed the best on the root and shoot parameters and also nutritional component and antioxidant activities compared to the other treatments.

1. Introduction

The guava tree, formally known as *Psidium guajava* L., is a member of the Myrtaceae family of trees. This plant kind is known as an evergreen. It goes by other names than only “apple of the tropics” and “poor man’s apple” in some areas. It is endemic to tropical and subtropical climates. Trees that produce fruit make up the majority of the 150 species in the genus *Psidium*. While most cultivars are diploid (2n = 22), others are naturally or purposefully triploid (2n = 33), producing fruits without seeds. Its original home is in the tropical Americas; namely, the region between Mexico and Peru. In terms of worldwide fruit consumption, guava ranks fifth, after apples, grapes, bananas, and citrus. Fresh guava is consumed in large quantities. Its high moisture content and high metabolic activity make it naturally at risk for accelerated degradation and spoiling (Sharma *et al*., 2023). The guava tree produces very nutrient-dense fruit, as supported by research by Naseer *et al*. (2018) and Kamath *et al*. (2008). These nutrients include vitamin C (80 mg), minerals (carotene, phenolic, beta cyanins, polyphenol, thiamin, and niacin), carbohydrates (9.1-17 mg), crude fiber (0.9-1.0 g), protein (0.1-0.5 g), flavonoids, thiamin, and cyanocobalamin. Over the past few years, guava has gained popularity and is now being utilized in international trade. This is mostly attributable to the numerous processing applications and nutritional benefits that it offers. According to Lakshmi *et al.* (2022), the list includes jam, jelly, cheese, ice cream, canned fruit, powders, nectar, sharbat, squash, and RTS. Processing foods like cheese, ice cream, and candies satisfies customers’ demands for visual appeal, flavor, and texture and enhances the functional food’s quality by supplying the appropriate concentrations of bioactive components for physiological efficacy (Rana *et al*., 2022). Guava like medicinal herbs is essential to the healing of wounds because they support blood coagulation, fight infections, and quicken the healing process. Numerous studies have shown that guava leaf ointment can heal wounds significantly faster than products available on the market (Thakur *et al*., 2020). Nutraceuticals are foods or their ingredients that provide health or therapeutic benefits, such as the prevention or treatment of health conditions (Mehrotra and Jadhav, 2021). The phenolic chemicals and flavonoids found in guava leaves are powerful antioxidants (Pandhi *et al*., 2022). Gorelick (2015) suggests that leaves are commonly regarded as the final components of plants. In contrast to shoots, which may continue to grow new shoots, leaves, flowers, and roots usually stop growing at a certain point. In simple

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terms, all vascular plants have leaves as their end-point organs. This idea also applies to all reproductive structures except flowers, which include gymnosperms, monilophytes, and lycophytes.

Asexual propagation techniques include cutting, layering, budding, and grafting; sexual propagation techniques include seeding; and there are several other methods for propagating guavas (Zamir *et al*., 2004). Chandra and Mishra (2005) research on the rooting potential of guava cuttings is lacking, rooting is undeniably the most established and widespread vegetative propagation approach (Awan *et al*., 2012). Through segregation and recombination of distinct characteristics, the direct seedling technique yields uneven progeny. Additionally, compared to plants grown from cuttings, plants developed from seeds bear fruit considerably later. Cutting-based propagation provides several benefits, including the ability to produce commercially valuable trees in a single growing season and the ability to produce plants of the true-to-type of the tree (Astha *et al.,* 2023). Different types of nodal cuts have allowed for the rapid and effective propagation of many woody perennials. Although IBA treatments produced the best performance (Akram *et al*., 2017) found that guava cuttings extracted from beheaded plants showed a much-diminished reaction to different concentrations of IAA. To maximize root initiation, root number per shoot, and average root length, the wounding section must contain an optimum concentration of IBA that promotes the mobilization and utilization of carbohydrates and nitrogen fraction in the presence of cofactors (Parmar *et al*., 2018). Auxins, which help boost root growth in cuttings, sprang to mind as they thought about how crucial it is to replicate guava from cuttings. This research utilized three auxins: indole butyric acid (IBA), cytokinin, and indole acetic acid. This study set out to investigate how different concentrations of these auxins affected the rooting process of Lucknow 49 guava cuttings (IAA).

2. Materials and Methods

2.1 Location of the experimental site

The research was conducted in a nursery at the Department of Fruit Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Tamil Nadu, India, in the academic years 2021 and 2022. At an elevation of 300 m above mean sea level, the experimental location is nestled in the range of the lower Pulney region. The mean precipitation at this location is around 105 cm. The exact coordinates of the campus were -10°72 41.882 2 North and longitude -77°352 59.282 2 East. Mild winters and hot, muggy summers were typical weather conditions in the region.

2.2 Design of the experiment details

The study's goal was to determine the combined effect of growth regulators with different nodal cuttings. The experiment was laid out with a two-component factorial completely randomized design (FCRD) to design and replicate the experiment thrice. A range of nodal cuttings, consisting of single, double, triple, and leaf cuts, were used in Factor 1 from the five-year-old Lucknow-49 guava variety. Strong mother plants were used as the source of cuttings, and only homogenous branches were chosen for use in propagation. The quick dip approach was utilized in the second component, which involved the treatment of nodal cuttings with plant development chemicals for 45 seconds. Cytokinin, indole-3-butyric acid (IBA), and indole-3-acetic acid (IAA) were found in the experiment in 200 and 500 ppm concentrations, respectively. Following the completion of the cleaning process, the assortment of nodal cuttings was placed in polybags that included sawdust, coco peat, and vermiculite as rooting media. After 35 days of misting, the terminal cuttings were shaded for 10 days. After that, they were planted in a plastic bag (8 x 10 inches) with a 1:1:1:1 ratio of red soil, sand, cocopeat, and vermicompost, and let to dry out in the open. A total of 600 cuttings were used in the research, with 20 cuttings assigned to each treatment. According to Yeboan *et al*. (2009), data was collected three months after planting by delicately removing the nodal cuttings.

2.3 Biochemical analysis

2.3.1 Estimation of phenol content (mg/g)

The concentration of phenol was measured in mg/g using the methodology outlined by Bates *et al*. (1973). The plant sample was prepared by dissolving 5 mg in 10 ml methanol. To 300 µl of this solution taken in a test tube, 1 ml methanol, 3.16 ml distilled water, and 200 µl Folin-Ciocalteu reagent were added and the mixture was incubated at room temperature for 8 min. After incubation, 600 µl sodium carbonate solution (10%) was added and the test tube was incubated in a water bath maintained at 40°C for 30 min. A blank was set up by following the same procedure wherein the plant extract was replaced with an equal volume of methanol. Similarly, a Gallic acid standard curve was furnished using the same procedure. Finally, the absorbance of the samples and standards were measured against the blank using a UV-visible spectrophotometer at 765 nm. Total phenolic content was calculated using the regression equation,

$Y = 0.03262x + 0.02949$ ($R^2 = 0.9962$)

where,

y is the absorbance at 765 nm and x is the amount of gallic acid (µg/ml).

Results were expressed as micrograms of gallic acid equivalents (GAE) per milligram extract.

2.3.2 Estimation of chlorophyll content (mg/g)

Arnon and Daniel (1949) described a technique for calculating total leaf chlorophyll content, which was then represented as mg/g.

$$\text{Total chlorophyll} = \frac{[20.2(A645) + 8.02(A663)] \times V}{1000 \times W \times a}$$

where,

A = Absorbance at specific wavelengths (645 cm and 663 cm)

V = Volume of the extract (10 ml)

W = Fresh weight of the sample (100 mg)

a = Path length of light in the cuvette (1 cm)

2.3.3 Estimation of starch (%)

The anthrone technique, which was developed by Sadasivam and Manickam (1992), was used to determine the amount of starch. Percentages relative to dry weight were used to represent the outcomes. Sugar extraction was performed on 50 mg of the ground sample using 80% warm ethanol for 1 hour. The ethanol in the extract was eliminated using a centrifugal evaporator (CVE-200D, Tokyo Rika Kikai Co. Ltd., Tokyo, Japan). The aqueous portion was diluted to a volume of 100 ml with distilled water and utilized

for assessing sugar content. Subsequently, the remaining residue was dried for 24 h in a draft oven maintained at 60°C, followed by starch extraction using perchloric acid. The quantification of sugars and starch was conducted using the anthrone method. Specifically, 6 ml of anthrone reagent was mixed with 1 ml of the sample, heated at 100°C for 10 min, and subsequently cooled to room temperature using running tap water. Following this, the absorbance at 620 nm was determined using a spectrophotometer (V-630; JASCO, Tokyo, Japan). The sugar and starch levels were then calculated based on sucrose and glucose equivalents, respectively.

2.3.4 Total protein content (mg/g)

Protein was estimated by Lowry *et al.* (1951) method.

Reagent A: 1% Na_2CO_3 in 0.5 N NaOH; Reagent B: 1% $CuSO_4$. $5H_2O$; Reagent C: 2% sodium tartrate ($Na_2C_4H_4O_6$); Reagent D: Mix 0.5 ml reagent C with 0.5 ml reagent B and 10 ml reagent A and Reagent E: Folin 0.2 N.

Soluble proteins were extracted from 2 g dry weight of each sample into 5 ml Tris-HCl buffer (pH=8.0) containing 26.8 ml 0.2 N HCl 17.2% sucrose, 1% ascorbic acid, and centrifuged. 1 ml of reagent D was added into 0.05 ml of resulted solution and kept in a temperature room. Then, 3 ml of reagent E was added and the sample was kept in Bain-marie at 50°C. The absorbance was measured spectrophotometrically at 625 nm.

2.3.5 Carbohydrate to nitrogen ratio (C/N ratio)

The complete amount of carbohydrates in the shoot was divided by the total amount of nitrogen to get the C: N ratio.

2.4 Physiological parameters

2.4.1 Root fresh weight (g)

In each treatment and replication, five rooted cuttings were chosen at random. After extracting and washing the roots from each cutting, we used an electronic scale to record their fresh weight. The average was determined and given in (g).

2.4.2 Root dry weight (g)

Following the determination of the cutting's fresh weight, the roots were wrapped in brown paper and subjected to a hot air oven drying process set at 55°C to keep track of the dry weights, an electronic balance was used. The average root weight was calculated.

2.4.3 Leaf fresh weight (g)

Fresh leaf weight was measured in five randomly selected cuttings on each treatment and replications after 60 days of planting and expressed in grams (g).

2.4.4 Leaf dry weight (g)

We dried the leaves from each replicate in a hot air oven set at 55°C for 24 h after picking them. We measured the leaf's dry weight in (g).

2.4.5 Root length (cm)

After 90 days of development, the root was chosen as the main root, and the maximum length of the rooted cuttings was measured using the meter scale. The average of maximum root length was calculated randomly by selecting five cuttings, then measured and noted in cm.

2.5 Statistical analysis

The experimental findings were statistically examined by using Panse *et al.* (1954) factorial completely randomized design (FCRD). The framework of Snedecor and Cochran was used to calculate the correlation between success rate and environmental factors. All the data were recorded at the nursery level and biochemical parameters were recorded in the laboratory. Applications "AGDATA," "AGRES.exe," and OPSTAT were used to investigate treatment differences, and the data was subjected to statistical analysis using the analysis of variance approach (accessible online at www.hau.ernet.in). Some research findings have used similar software (Sharma *et al*., 2023)

3. Results

Tables 1, 2, 3, 4, and 5 show the physical parameters (Weights of roots and leaves in both fresh and dry states, along with root length.), as well as the biochemical features (total chlorophyll content, total phenol content, starch, leaf protein content, C/N ratio) treated with different growth regulators under mist chamber conditions. Figures 1, 2, 3, 4, and 5 show the same kinds of data.

3.1 Biochemical analysis

3.1.1 Chlorophyll content (mg/g)

At 90 days of post-planting, there were noticeable differences in the total chlorophyll content of nodal cuttings with the application of different growth regulators and the combined effects of these regulators (Figure 1). With a maximum of (37.74 mg/g), the triple node cuttings (C_3) had the highest chlorophyll content, while the leaf cuttings had the lowest, measuring 26.52 mg/g (C_4). In the presence of various growth regulators, the highest chlorophyll content (36.23 mg/g) was found in the IBA 500 ppm (G_2) group, whereas the control group showed the lowest chlorophyll content (27.94 mg/g) in their leaves (G_7). Cuttings treated with different concentrations of IBA resulted in significantly different chlorophyll contents. Cuttings of triple nodes treated with 500 ppm of IBA showed the maximum chlorophyll content (41.94 mg/g), while cuttings treated with 200 ppm of IBA showed the lowest chlorophyll content (23.46 mg/g) with combination (C_4G_7).

3.1.2 Phenol content (mg/g)

Figure 2 displays data on phenol content at 90 DAP. This data demonstrates that several growth regulators were applied to many nodal cuttings, and the cumulative effect of these treatments was significant. Double node cuts (C_2) had the lowest total phenol concentration (0.035 mg/g), whereas leaf cutting had the highest (0.057 mg/g) (C_4). Among the growth regulators, the control group had the highest levels (0.054 mg/g) and the lowest amounts of phenol when compared to the other study groups (0.035 mg/g). It was discovered that IAA 500 ppm (G_4) (0.064 mg/g) contained the least level of phenol (0.035) observed in (G_2). Double node cuttings that were treated with IBA 500 ppm (C_2G_4) had the lowest phenol content (0.030 mg/g), followed by those that were treated with IAA 500 ppm (G_4). Out of all the cuttings tested, those treated with 500 ppm of IBA had the lowest phenol level (0.030 mg/g).

3.1.3 Starch content (%)

Nodal cuttings treated with various growth regulators and the combined impact of these exhibited significantly variable starch contents 90 days after dissemination (Figure 3). Among different

types of cuttings revealed that the leaf cuttings (C_4) showed the maximum starch content (5.62 %), and the minimum starch content (3.42 %) was recorded in double node cutting (C_2). Among growth regulators, the maximum starch content (5.28 %) was observed in control (G_7), and the minimum starch content (3.42 %) was recorded in IBA 200 ppm (G_2). Single-node cuttings that were treated with control (C_1G_7) had an interaction effect that resulted in a maximum starch content of (6.17 %). In contrast, double-node cuttings that were treated with IBA at a concentration of 500 ppm had the lowest starch level, which was (2.09 %) with interaction (C_2G_2).

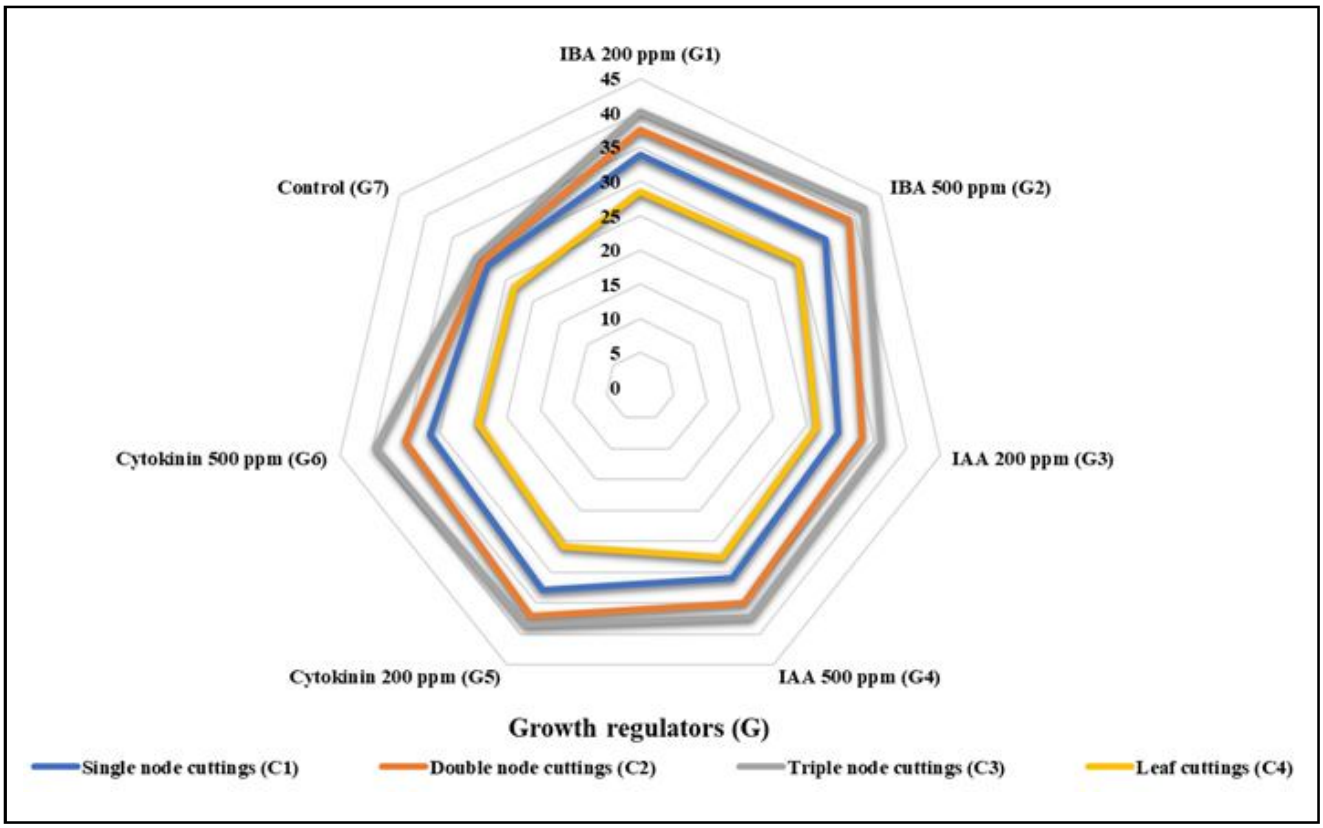

Figure 1: Influence of growth regulators with different nodal cuttings on chlorophyll (SPAD) in guava at 90 DAP.

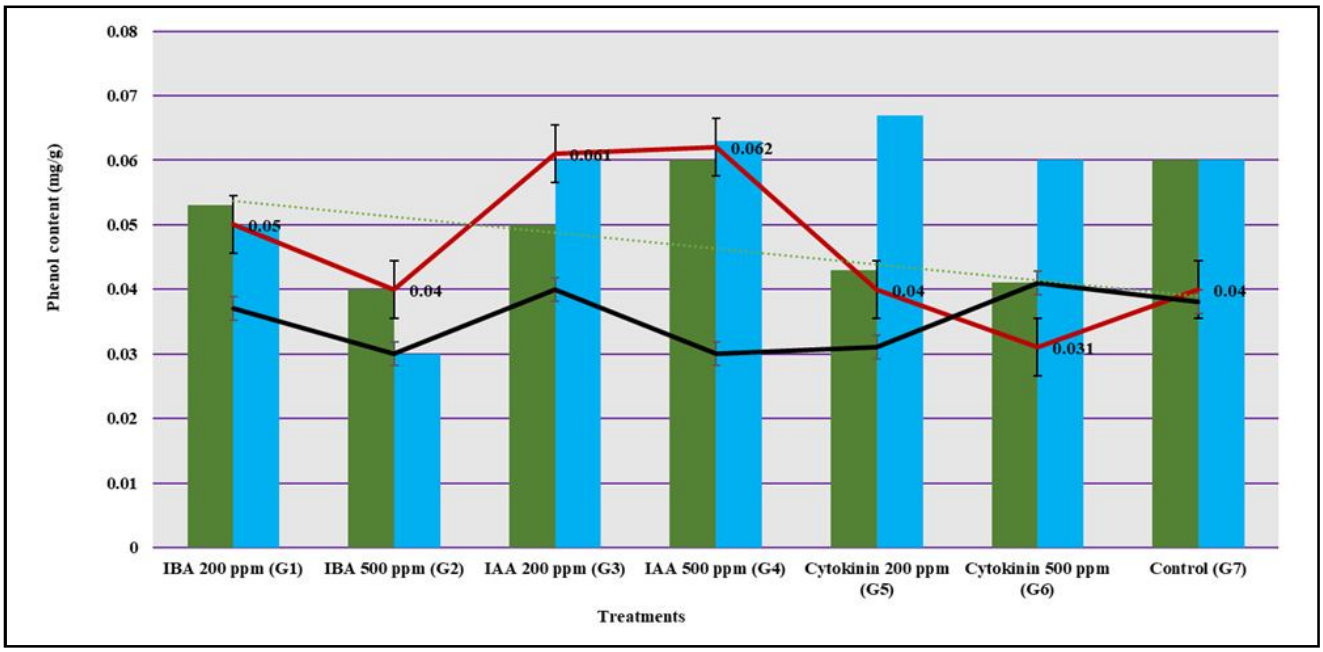

Figure 2: Influence of growth regulators with different nodal cuttings on phenol content in guava at 90 DAP.

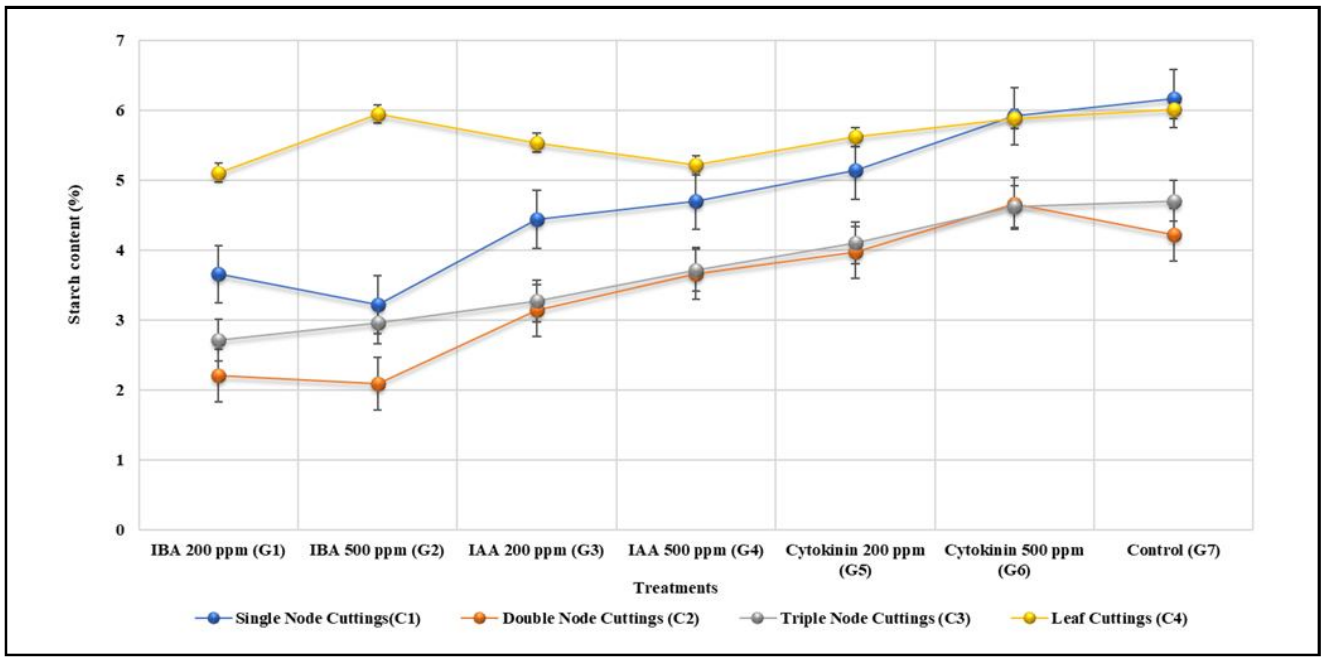

Figure 3: Influence of growth regulators with different nodal cuttings on starch content in guava at 90 DAP.

3.1.4 Total protein (mg/g)

Based on data on leaf soluble protein content at 90 DAP, several growth regulators were applied to distinct nodal cuts, and the combined effect was considerable. The total leaf soluble protein content was maximum (10.80 mg/g) showed in single node cuttings (C_1), and the minimum protein content (4.62 mg/g) was observed in double node cutting (C_2). Among growth regulators, the maximum total leaf soluble protein content (8.22 mg/g) was recorded in IAA 500 ppm (G_4), and the minimum protein content (7.85 mg/g) was observed in the IBA 200 ppm (G_1). A maximum of (12.50 mg/g) of protein was found in single node cuttings treated with 500 ppm of IBA (C_1G_2), followed by 200 ppm of IAA (G_1), according to the interaction effect. In comparison, the lowest protein content of (3.34 mg/g) was found in double node cuttings treated with 500 ppm of IBA (C_2G_2).

3.1.5 Carbohydrate to nitrogen ratio (C/N ratio)

The impact of various growth regulators, nodal cutting kinds, and the C/N ratio is substantial. The findings revealed that the C/N ratio was 6.34 in (C_2) cuts, the highest, and 3.22 in leaf cuttings, the lowest (C_4). In terms of growth regulators, the C/N ratio was lowest in the control (G_7) group (2.53) and highest in the IBA 500 ppm (G_2) group (6.81). Nodal cuttings of various kinds interact with growth regulators in interesting ways. The carbon-to-nitrogen ratio was observed to be greatest (8.66) in double-node cuttings treated with 500 ppm of IBA (C_2G_2), subsequently 200 ppm of IBA (C_2G_1), and lowest (1.95) in leaf cuttings treated with control (C_4G_7).

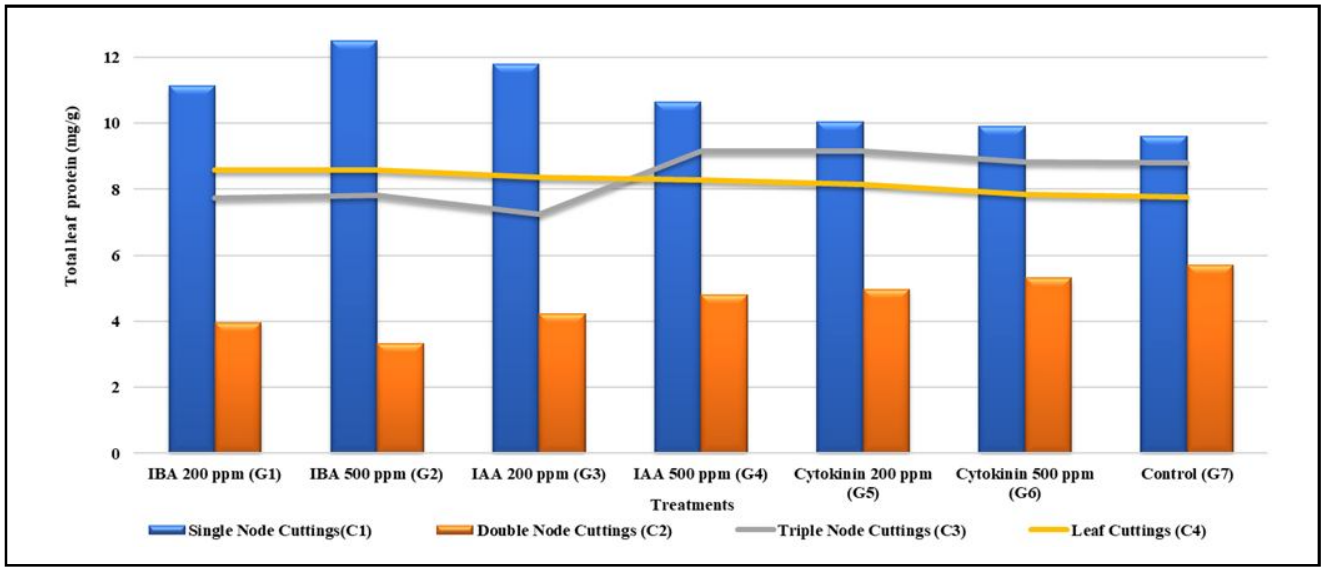

Figure 4: Influence of growth regulators with different nodal cuttings on total leaf soluble protein in guava at 90 DAP.

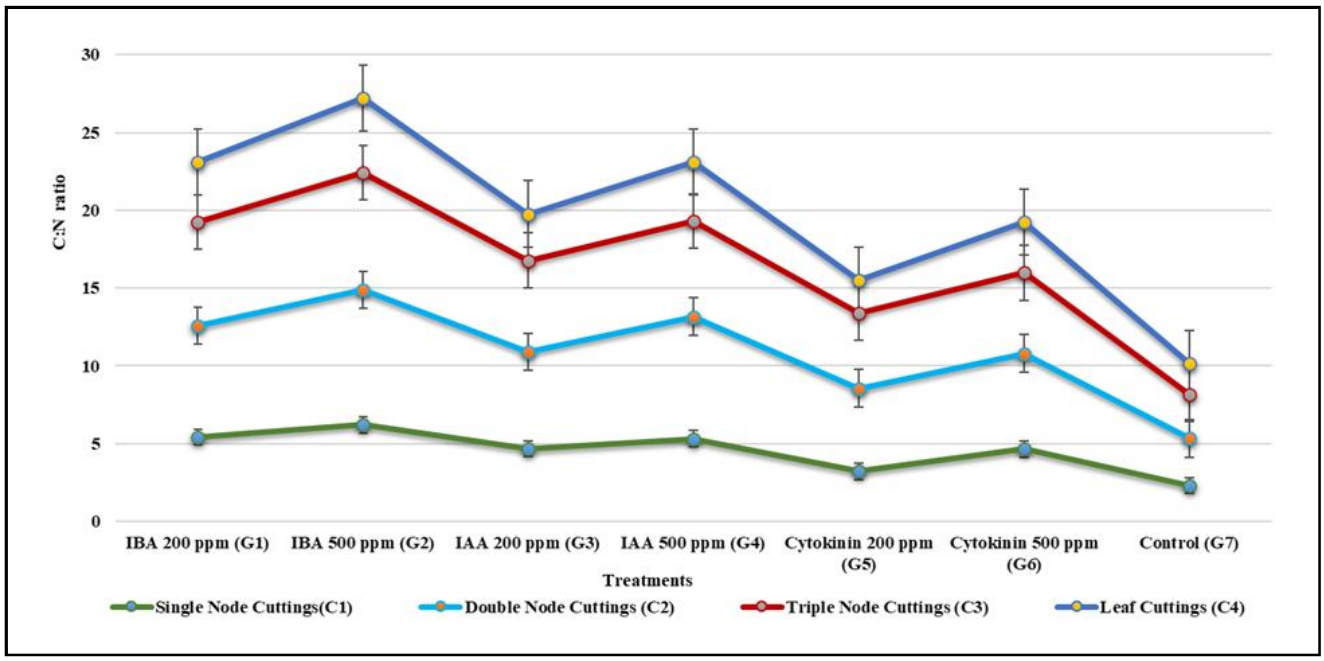

Figure 5: Influence of growth regulators with different nodal cuttings on carbohydrate to nitrogen ratio (C/N ratio) in guava at 90 DAP.

3.2 Physiological characters

3.2.1 Root length (cm)

Both growth regulators, the types of nodal cuts, and the interactions between the two had a substantial impact on root length sixty days after planting (Table 1 and Figure 6). Triple node cuttings (C_3) had the longest roots (10.44 cm), whereas leaf cuttings had the shortest (7.14 cm) (C_4). Among the growth regulators, root length was found to be (11.01 cm) in the IBA 500 ppm (G_2) group and (5.79 cm) in the control group (G_7). Cuttings treated with varying doses of IBA were assessed for root length in centimeters. Cuttings with triple nodes (12.33 cm), double nodes (11.97 cm), and leaf cuttings (4.55 cm) treated with the control had the shortest root lengths (C_4G_7). The relationship between the various growth regulators and the nodal cuttings treated with them substantially influenced the length of the plant's roots. The results showed that root length ranged from (12.30 cm) in leaf cuttings to (21.73 cm) in triple node cuttings (C_3) and (C_4). Among the growth regulators, root length was found to be (22.30 cm) in the IBA 500 ppm (G_2) group and (11.05 cm) in the control group (G_7). The cuttings with the longest roots were those with triple nodes and were treated with 500 ppm of IBA (C_3G_2), followed by 200 ppm of IBA (G_2), whereas the cuttings with the shortest roots were those with leaves and were treated with a control (C_4G_7) shown in Figure 6.

3.2.2 Root fresh weight (g)

Table 2 indicated that the root fresh weight was significantly influenced by various types of nodal cuttings, growth regulators, and their interactions. Root fresh weight was found to be (0.65 g) for leaf-cutting samples and (1.76 g) for (C_2) cuttings, the highest root fresh weight group (C_4). Root fresh weight was (1.77 g) when IBA 500 ppm (G_2) was the growth regulator of choice, whereas control had the lowest at (0.80 g) in (G_7). A great deal of interaction occurs between the different kinds of nodal cuttings that have been treated with different growth regulators. A root fresh weight of (2.68 g) was recorded for double node cuttings treated with IBA at a concentration of 500 ppm (C_2G_2), followed by 200 ppm (C_2G_1), and the lowest root fresh weight of (0.35 g) was recorded for leaf cuttings that served as a control (C_4G_7). When the plant was planted ninety days later, the new root weight was influenced by the specific type of nodal cutting, the growth regulators, and the method in which they interacted with one another. Leaf cuttings had the lowest root fresh weight (1.45 g), whereas double node cuttings (C_2) had the highest root fresh weight (2.74 g). Leaf cuttings were the ones that had the lowest root fresh weight (C_4). The control group (G_7) had the minimum fresh root weight of (1.78 g), while the group treated with IBA 500 ppm (G_2) had the heaviest at (2.77 g). The cuttings from the double nodes that were exposed to 500 ppm of IBA (C_2G_2) had the highest root fresh weight at (3.69 g), although the cuttings from the leaves that were left untreated had the lowest, at (1.02 g). The complex interplay between several growth regulators and the many varieties of nodal cuts proved this (C_4G_7).

3.2.3 Root dry weight (g)

Table 3 shows data on root dry weight at 60 days after planting. Distinct growth regulators were applied to different nodal cuttings, and the influence of this interaction was also significantly impacted. According to the findings, the root dry weight of leaf cuttings was the lowest (0.74 g), while the root dry weight of double node cuttings was the greatest (1.27 g) in (C_2). The root dry weight of the group using the growth regulator was (1.25 g) for the group using IBA 500 ppm (G_2), whereas the root dry weight of the control group was (0.77 g) in (G_7). The double node cuttings underwent treatment with 500 ppm of (IBA) as revealed by the findings (C_2G_2) had the highest root dry weight of (1.62 g). After this, the cuttings were treated with 200 ppm of IBA (C_2G_1), while the leaf cuttings treated with control (C_4G_7) had the lowest root dry weight of (0.57 g) and (0.49 g). Root

dry weight at 90 days after planting (DAP) data from Table 3 demonstrated that various nodal cuttings were treated with growth regulators and that the interaction impact was also substantially affected. The results revealed that root dry weight was greatest in double node cuttings (C_2) at (1.49 g), and lowest in leaf cuttings at (0.85 g). Among the growth regulators, control had the lightest root dry weight (0.87 g) while IBA 500 ppm (G_2) had the heaviest (1.45 g) in (G_7). The cuttings that were treated with 500 ppm of IBA (C_2G_2) had the highest root dry weight of (1.86 g), followed by the cuttings that were treated with 200 ppm of IBA (C_2G_1), and the leaf cuttings that were exposed with control (C_4G_7) had the lowest root dry weight (0.54 g).

Table 1: Influence of growth regulators with different types of cuttings on root length (cm) in guava cuttings at 60 and 90 DAP

Growth regulators (G)	Root length (cm) at 60 DAP				Mean	Root length (cm) at 90 DAP				Mean
	Types of cuttings (C)					Types of cuttings (C)				
	Single node cuttings (C_1)	Double node cuttings (C_2)	Triple node cuttings (C_3)	Leaf cuttings (C_4)		Single node cuttings (C_1)	Double node cuttings (C_2)	Triple node cuttings (C_3)	Leaf cuttings (C_4)	
IBA 200 ppm (G_1)	9.34	10.46	11.56	8.12	**9.87**	17.21	21.22	25.12	13.02	**19.14**
IBA 500 ppm (G_2)	10.68	11.97	12.33	9.05	**11.01**	20.64	24.37	27.32	16.87	**22.30**
IAA 200 ppm (G_3)	8.34	9.56	10.78	6.98	**8.91**	15.22	18.54	21.36	12.24	**16.84**
IAA 500 ppm (G_4)	9.32	10.24	11.31	8.24	**9.77**	18.31	21.12	24.64	15.01	**19.77**
Cytokinin 200 ppm (G_5)	7.01	8.73	9.72	6.12	**7.89**	12.23	14.26	17.59	9.35	**13.36**
Cytokinin 500 ppm (G_6)	8.51	9.52	10.32	6.96	**8.82**	14.56	17.33	20.86	11.12	**15.97**
Control (G_7)	5.23	6.34	7.07	4.55	**5.79**	9.57	11.05	15.21	8.52	**11.05**
Mean	**8.34**	**9.54**	**10.44**	**7.14**	**8.86**	**15.37**	**18.27**	**21.73**	**12.30**	**16.91**
Factor	C	G	C × G			C	G	C × G		
S.E (d)	0.06	0.08	0.17			0.14	0.19	0.38		
CD at 5%	0.13**	0.17**	0.35**			0.29**	0.38**	0.77**		

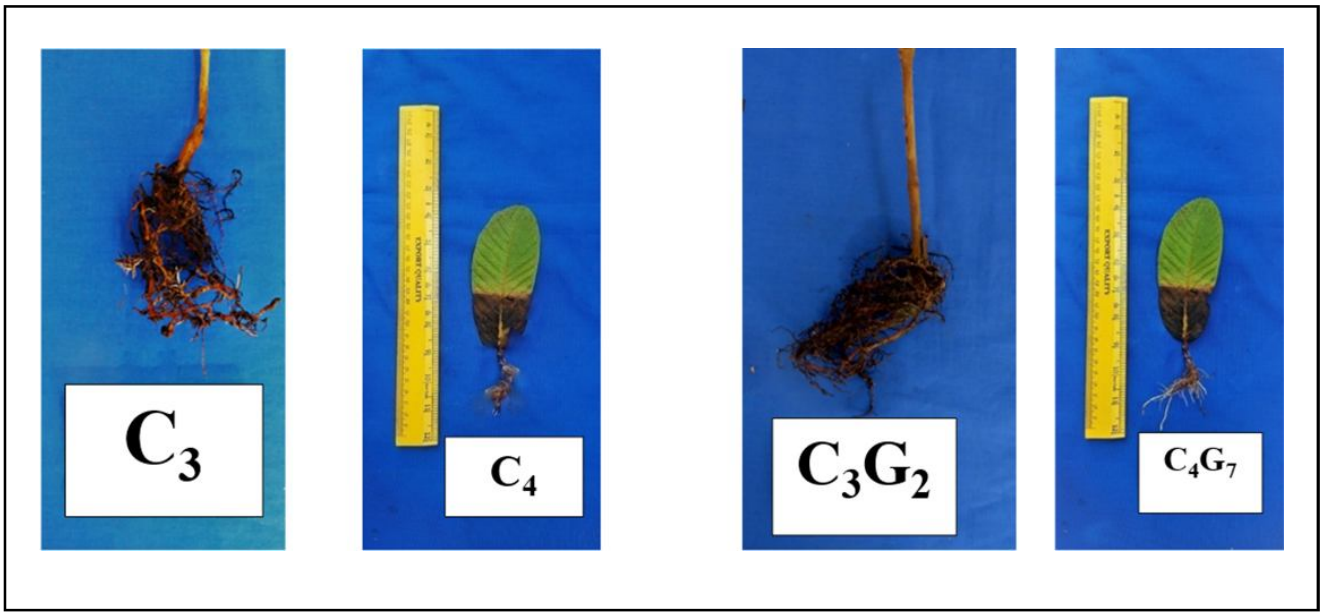

Figure 6: Influence of growth regulators with different types of cuttings on root length.

3.2.4 Leaf fresh weight (g)

Table 4 presents the impact of several growth regulators and nodal cuts on the leaf's fresh weight 60 days after planting. The leaf fresh weight (0.85 g) was greatest for the double node cuttings (C_2) and lowest for the leaf cuttings (0.46 g) in (C_4). IBA 500 ppm (G_2) had the heaviest fresh leaf weight (0.91 g) among the growth regulators, whereas control had the lowest (0.34 g) in (G_7). In comparison to the control group of cuttings, which had the lowest leaf fresh weight (0.28 g), the double node cuttings treated with 500 ppm of IBA (C_2G_2) had the greatest leaf fresh weight (1.18 g) in (C_4G_7). When, it comes to the leaf fresh weight at 90 days after planting, there was a significant amount of heterogeneity between growth regulators, different types of nodal cuttings, and the interactions and interactions

between these factors. In terms of fresh weight, double node cuttings (C_2) had the greatest value, coming in at (0.82 g), while leaf cuttings (0.61 g) had the lowest value (C_4). The leaf fresh weight measurements were taken about the growth regulators, with the control group showing the lowest weight at (0.29 g) and IBA 500 ppm showing the highest weight at (1.32 g) in (G_7). Standard surgery of leaf cuttings with 500 ppm of IBA (C_2G_2), the control group's fresh weight ranged from (0.24 g) to (1.73 g) in (C_4G_7).

Table 2: Influence of growth regulators with different types of cuttings on root fresh weight (g) in guava cuttings at 60 and 90 DAP

Growth regulators (G)	Root fresh weight (g) at 60 DAP					Root fresh weight (g) at 90 DAP				
	Types of cuttings (C)				Mean	Types of cuttings (C)				Mean
	Single node cuttings (C_1)	Double node cuttings (C_2)	Triple node cuttings (C_3)	Leaf cuttings (C_4)		Single node cuttings (C_1)	Double node cuttings (C_2)	Triple node cuttings (C_3)	Leaf cuttings (C_4)	
IBA 200 ppm (G_1)	1.58	2.12	1.99	0.93	**1.65**	2.68	3.16	2.72	1.72	**2.57**
IBA 500 ppm (G_2)	1.64	2.68	1.91	0.86	**1.77**	2.74	3.69	2.85	1.83	**2.77**
IAA 200 ppm (G_3)	1.87	1.89	1.73	0.79	**1.57**	2.45	2.56	2.51	1.64	**2.29**
IAA 500 ppm (G_4)	1.72	1.98	1.67	0.62	**1.49**	2.22	2.89	2.31	1.45	**2.21**
Cytokinin 200 ppm (G_5)	1.53	1.45	1.55	0.58	**1.27**	2.18	2.56	2.23	1.32	**2.07**
Cytokinin 500 ppm (G_6)	1.41	1.21	1.41	0.43	**1.11**	2.09	2.27	2.14	1.21	**1.92**
Control (G_7)	0.98	1.03	0.85	0.35	**0.80**	1.99	2.06	2.05	1.02	**1.78**
Mean	**1.53**	**1.76**	**1.58**	**0.65**	**1.38**	**2.33**	**2.74**	**2.40**	**1.45**	**2.23**
Factor	C	G	C x G			C	G	C x G		
S.E (d)	0.01	0.01	0.02			0.01	0.02	0.04		
CD at 5%	0.02**	0.03**	0.05**			0.03**	0.04**	0.08**		

Table 3: Influence of growth regulators with different types of cuttings on root dry weight (g) in guava cuttings at 60 and 90 DAP

Growth regulators (G)	Root dry weight (g) at 60 DAP					Root dry weight (g) at 90 DAP				
	Types of cuttings (C)				Mean	Types of cuttings (C)				Mean
	Single node cuttings (C_1)	Double node cuttings (C_2)	Triple node cuttings (C_3)	Leaf cuttings (C_4)		Single node cuttings (C_1)	Double node cuttings (C_2)	Triple node cuttings (C_3)	Leaf cuttings (C_4)	
IBA 200 ppm (G_1)	1.21	1.52	1.29	0.96	**1.24**	1.24	1.72	1.68	1.02	**1.41**
IBA 500 ppm (G_2)	1.16	1.62	1.18	1.05	**1.25**	1.35	1.86	1.59	1.11	**1.47**
IAA 200 ppm (G_3)	1.11	1.24	1.05	0.87	**1.06**	1.18	1.53	1.48	0.97	**1.29**
IAA 500 ppm (G_4)	1.04	1.35	0.99	0.71	**1.02**	1.09	1.65	1.39	0.85	**1.25**
Cytokinin 200 ppm (G_5)	0.98	1.14	1.03	0.61	**0.93**	1.03	1.41	1.26	0.78	**1.12**
Cytokinin 500 ppm (G_6)	0.91	1.05	0.89	0.52	**0.84**	0.96	1.32	1.18	0.69	**1.03**
Control (G_7)	0.82	0.98	0.82	0.49	**0.77**	0.89	0.98	1.09	0.54	**0.87**
Mean	**1.03**	**1.27**	**1.03**	**0.74**	**1.02**	**1.11**	**1.49**	**1.38**	**0.85**	**1.21**
Factor	C	G	C x G			C	G	C x G		
S.E (d)	0.01	0.01	0.02			0.01	0.01	0.01		
CD at 5%	0.02**	0.02**	0.04**			0.02**	0.03**	0.02**		

Table 4: Influence of growth regulators with different types of cuttings on leaf fresh weight (g) in guava cuttings at 60 and 90 DAP

Growth regulators (G)	Leaf fresh weight (g) at 60 DAP				Mean	Leaf fresh weight (g) at 90 DAP				Mean
	Types of cuttings (C)					Types of cuttings (C)				
	Single node cuttings (C_1)	Double node cuttings (C_2)	Triple node cuttings (C_3)	Leaf cuttings (C_4)		Single node cuttings (C_1)	Double node cuttings (C_2)	Triple node cuttings (C_3)	Leaf cuttings (C_4)	
IBA 200 ppm (G_1)	0.85	1.12	0.75	0.60	**0.83**	1.12	1.32	1.21	1.02	**1.16**
IBA 500 ppm (G_2)	0.93	1.18	0.83	0.72	**0.91**	1.18	1.73	1.29	1.10	**1.32**
IAA 200 ppm (G_3)	0.56	0.87	0.49	0.35	**0.56**	0.75	0.62	0.76	0.64	**0.69**
IAA 500 ppm (G_4)	0.63	0.96	0.56	0.44	**0.64**	0.83	0.69	0.82	0.71	**0.76**
Cytokinin 200 ppm (G_5)	0.42	0.62	0.41	0.37	**0.45**	0.33	0.45	0.26	0.28	**0.33**
Cytokinin 500 ppm (G_6)	0.59	0.78	0.51	0.47	**0.59**	0.39	0.51	0.32	0.31	**0.38**
Control (G_7)	0.36	0.42	0.33	0.28	**0.34**	0.29	0.41	0.25	0.24	**0.29**
Mean	**0.62**	**0.85**	**0.55**	**0.46**	**0.62**	**0.69**	**0.82**	**0.72**	**0.61**	**0.71**
Factor	C	G	C x G			C	G	C x G		
S.E (d)	0.004	0.005	0.011			0.006	0.008	0.006		
CD at 5%	0.008**	0.011**	0.022**			0.012**	0.016**	0.012**		

Table 5: Influence of growth regulators with different types of cuttings on leaf dry weight (g) in guava cuttings at 60 and 90 DAP

Growth regulators (G)	Leaf dry weight (g) at 60 DAP				Mean	Leaf dry weight (g) at 90 DAP				Mean
	Types of cuttings (C)					Types of cuttings (C)				
	Single node cuttings (C_1)	Double node cuttings (C_2)	Triple node cuttings (C_3)	Leaf cuttings (C_4)		Single node cuttings (C_1)	Double node cuttings (C_2)	Triple node cuttings (C_3)	Leaf cuttings (C_4)	
IBA 200 ppm (G_1)	0.32	0.72	0.51	0.21	**0.44**	0.57	0.86	0.74	0.32	**0.62**
IBA 500 ppm (G_2)	0.38	0.85	0.75	0.23	**0.55**	0.63	0.91	0.82	0.38	**0.68**
IAA 200 ppm (G_3)	0.29	0.36	0.33	0.17	**0.28**	0.43	0.73	0.55	0.22	**0.48**
IAA 500 ppm (G_4)	0.27	0.42	0.31	0.19	**0.29**	0.51	0.76	0.59	0.30	**0.54**
Cytokinin 200 ppm (G_5)	0.23	0.29	0.24	0.16	**0.23**	0.44	0.64	0.63	0.20	**0.48**
Cytokinin 500 ppm (G_6)	0.26	0.31	0.28	0.18	**0.25**	0.39	0.72	0.73	0.26	**0.53**
Control (G_7)	0.22	0.20	0.20	0.14	**0.19**	0.28	0.37	0.34	0.19	**0.29**
Mean	**0.28**	**0.45**	**0.37**	**0.18**	**0.32**	**0.46**	**0.71**	**0.63**	**0.27**	**0.51**
Factor	C	G	C x G			C	G	C x G		
S.E (d)	0.002	0.002	0.004			0.005	0.006	0.012		
CD at 5%	0.003**	0.004**	0.009**			0.009**	0.012**	0.025**		

3.2.5 Leaf dry weight (g)

Among the growth regulators, IBA 500 ppm produced the leaves with the heaviest fresh weight (1.32 g). Leaf dry weight data from propagated guava plants revealed substantial variance at 60 days post-multiplication (Table 5). The largest leaf dry weight (0.45 g) was seen in double node cuttings (C_2), while the least leaf dry weight (0.18 g) was reported in leaf-cutting when comparing the various nodal cuts (C_4). The leaf dry weight was 0.55 g at the highest concentration of IBA 500 ppm (G_2) and (0.19 g) at the lowest concentration in the control group, according to the growth regulators (G_7). Cuttings with two nodes subjected to a treatment involving 500 ppm of IBA (C_2G_2) had the heaviest leaf dry weight (0.85 g), whereas control cuttings had the lightest (0.14 g) in (C_4G_7). Significant variances were seen in the data on leaf dry weight after 90 days following the propagation of guava plants, as shown in Table 5. When comparing the different nodal cuttings, double node cuts (C_2) had the greatest leaf dry weight (0.71 g), whereas leaf-cutting (C_4) had the lowest (0.27 g). Among the growth regulators, the control group had the lowest leaf dry weight (0.29 g) in (G_7), whereas the group treated with 500 ppm of IBA (G_2) had the highest (0.68 g). The double node treatment with 500 ppm IBA (C_2G_2) resulted in the

highest leaf dry weight (0.91 g), followed by the 200 ppm treatment (G_1), and the control group of leaf cuttings had the lowest (0.19 g) leaf dry weight (C_4G_7).

4. Discussion

Plant growth regulators also referred to as "Phytohormones," are synthetic compounds designed to replicate the effects of natural plant hormones produced in minuscule quantities within plants. The primary categories of plant growth regulators include auxin, gibberellins, ethylene, and abscisic acid. Auxin formulations are used for commercial distribution and commercial root-promoting products. Auxins as the main active ingredient on their labels are frequently mentioned or used widely as rooting hormones but in the scientific literature, the term hormone is usually restricted to naturally occurring auxins (IAA and IBA) and other endogenous substances that are active at low concentrations, produced at multiple plant sites, and that cause a range of morphogenetic and growth responses. The number of food reserves in cuttings could illustrate that they have the longest root per cutting. By hydrolyzing and transporting nitrogenous compounds and carbohydrates to the base of the cuttings, auxin, also known as IBA, may hasten cell elongation and cell division (Singh and Sujatha, 2003). The larger shoot width could have been caused by either a larger root system or an increase in the number of leaves. This would have resulted in the plant being better able to absorb water and minerals from the soil, which would have led to improved digestion of carbohydrates and overall improvement in vegetative development. The maximal fresh and dry weight of leaves and higher photosynthetic activity are outcomes of larger leaf areas, both in terms of length and width. The present work is supported by previous research on guava (Gotur *et al*., 2017), lemon (Bhilare *et al*., 2018), and tamarind (Deshmukh *et al*., 2018).

According to Deepak *et al*. (2015), auxins when added to a medium at lower concentrations increase root development. Previous research on the effects of auxin on the average fresh and dry root weight per cutting has been corroborated by investigations in *Piper nigrum* (Garande *et al*., 2022). The reason dried roots weigh less than fresh roots can be due to proper aeration, an abundance of nutrients, and a high water-holding capacity. Basal cuts function best due to the high glucose content and the presence of highly developed tissues. It is possible that the increase in auxin concentrations and leaf area triggered photosynthesis, increasing the amounts of chlorophyll in each cutting. The maximal fresh and dry weight of leaves and higher photosynthetic activity are outcomes of larger leaf areas, both in terms of length and width. Reasons for the root's dry weight include its excellent water-holding capacity, robust nutrient availability, and sufficient aeration. In the cases of *Bohemia nivea*, Garden rue, and *Piper nigrum* (Bendre *et al*., 2020) obtained similar results.

Research findings on guava by Gotur *et al*. (2017), lemon by Bhilare *et al*. (2018), and tamarind by Deshmukh *et al*. (2018) corroborate the current study. Auxin activates specific proteins in the cell membrane, promoting the movement of H^+ ions into the cell wall, and promoting protein synthesis, plasticity, and cell elongation (Setiawan *et al*., 2021). The cell wall is composed of cellulose chains, and to partially dehydrogenate them, certain enzymes must be activated by the presence of hydrogen ions (H^+ ions) in the cell membrane through the cell wall. Cells grow and elongate due to water entering by osmosis. In addition to encouraging cell lengthening, which in turn allows roots and stems to grow longer, water osmosis induces cells to enlarge and lengthen the combination of auxin and gibberellin will promote the development of vessel tissue and stimulate cell division in the vessel cambium and thus support the formation of stem diameter. The amount and location of nutrient uptake by plants are largely regulated by hormones (Galston and Petter Devies, 1972).

These findings might imply that terminal cuttings with comparatively larger total phenol content cannot promote better roots. Increased concentrations of phenols may not stimulate better rooting; carbohydrates, auxins, and root-promoting co-factors are also required in sufficient quantities to cause roots. Because they have the potential to create endogenous auxins, also known as carbohydrates, through the process of photosynthesis, leaves are essential for rooting cuttings (Vallejos *et al*., 2021). According to Bannoud *et al*. (2021), IBA may have improved roots by producing endogenous IAA, raising internal auxin levels, or changing IAA's function in a complementary manner. This complex influences the microstructure of the cell, showing division, and differentiation into root initials. The Folin-Ciocalteu reagent's oxidation serves as the basis for assessing phenolic compounds. This reagent is made by combining phosphotungstic acid, and phosphomolybdic acid after oxidizing phenols. Reducing it to molybdenum and blue tungsten oxides is the next step (Monika Moond *et al*., 2023). It is evident that the intensity of the blue absorption peak, which takes place at around 730 nm, is directly proportional to the amount of phenolic chemicals that are present in the extract. When there is an abundance of endogenous free auxin at the cutting's base, rooting is postponed because the phenolic co-factors function as free agents that hinder cell growth. Similar outcomes were also attained by Abidin and Metali (2015) using *Bursera penicillata* cuttings and *Jasminum auriculatum* Cv. Parimuliai, respectively. According to the findings of Deepak *et al*. (2015), cytokinins did not promote strong shoot multiplication in this experiment; rather, they only created one shoot per axil. This finding is in agreement with the previously mentioned findings.

5. Conclusion

Plant growth regulators significantly affected guava-cutting growth metrics as shown in the research. The application of plant growth regulators in guava-selected cuttings influences the nutritional components (Protein and starch), antioxidant properties (Phenol), and pigment (chlorophyll). Among other advantages the rapid commencement of root and shoot growth, a high survival rate, ease of multiplication, and early bearing. The highest root length and leaf-soluble protein content were seen with IBA 500 ppm, in comparison to other growth regulators. Double-node cuttings treated with IBA had superior outcomes in terms of C/N ratio, carbon content, starch content, fresh weight, dry weight, and fresh weight of the roots compared to the control, IAA, and cytokinin groups. The triple node cuttings with the longest roots and highest chlorophyll content were those treated with 500 ppm of IBA. According to the research, to propagate guava trees, it is advised to use double node cuttings in conjunction with 500 ppm of IBA. This indicates that there is a possibility that this method may be utilized in a commercial setting in the future.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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