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Evaluation of different rooting stimulators and substrates to produce guava clone plants through softwood cuttings

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Abstract: The clonal propagation of guava (*Psidium guajava* L.) is essential to avoid segregation, and its multiplication through cuttings is the easiest and cheapest way to raise a nursery, as other propagation techniques are laborious and need expertise. The current study was planned to evaluate the best rooting stimulators and rooting substrates for guava nursery propagation. In this experiment, the guava-prepared cuttings were treated with rooting stimulators, indole butyric acid (IBA) and paclobutrazol (PB) with concentrations (0, 200, 400, and 600 ppm) and were planted in independent rooting substrates (sand, silt and topsoil) under polytunnels. The results revealed that the highest number of roots, root length and root weight were achieved in cuttings planted in the sand with IBA 400 ppm concentration. In contrast, the highest survival percentage (50%) was obtained in silt substrate with a similar concentration of IBA. However, sand's lowest and highest concentrations of both rooting hormones produced zero plants. In shooting capacity, maximum leaves (13.6) and sprouts (3.76) were obtained using IBA 400 ppm in silt substrate. The highest sprouting length (6.80 cm) and cutting height (26.2 cm) were attained with sand using PB 200 ppm. In the overall comparison, rooting stimulator IBA 400 ppm and rooting substrate silt performed better than other rooting stimulators and rooting substrates.

Keywords: clonal propagation; indole butyric acid; paclobutrazol; polytunnels; rooting

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Guava (*Psidium guajava* L.) is known as "the apple of the tropics" due to its dietary importance. It is enriched with vitamin C, dietary fibres, pectin, antioxidants, and polyphenols (Wilson et al. 2001). It is a momentous fruit among the 50 edible fruits known to grow in tropical and subtropical climates of the world. Pakistan ranks second in guava production after India and produces two crops per year under various eco-geographical conditions across the country. It is commercially and traditionally propagated through seeds, but it cannot maintain its genetic purity due to cross-pollination, and there is great segregation and recombination of characteristics observed in guava orchards, even on the same variety. That is the reason that its yield has been stagnant at 7–8 t/ha for the last decades (Shahzad et al. 2019). Therefore, the seed propagation method is not recommended in the commercial orchard as no uniformity among fruits affects their yield and quality (Singh et al. 2019).

The other major issues in guava plantation are the unavailability of quality planting material and the multiplication of plants through unreliable sources, resulting in poor-quality guava fruits that adversely affect their production. True-to-type planting material is the basic requirement of guava to confirm both quantity and quality (Akram et al. 2017). Several asexual propagation techniques have been investigated by researchers in guava, such as budding, layering, grafting, stooling, and cuttings (Abdullah et al. 2006; Abbas et al. 2013). Clonal propagation in guava avoids genetic heterogeneity of the variety, maintains the quality of fruits, and has the potential to improve yield within a short period (Qadri et al. 2018).

Nowadays, guava clonal propagation through cuttings is getting popular as it is the easiest, most cost-effective, and fastest way of asexual propagation. However, the successful multiplication of guava through stem cutting depends on climatic conditions, mother plant condition, tree age, planting time and rooting medium (Hu et al. 2020). Globally, there has been a change in the pattern of nursery management through the fusion of science and technology, and the use of rooting substrates and stimulators is becoming more common (Sardoei 2014). Worldwide, different rooting mediums, sand, silt loamy soils, perlite, sawdust, coconut, peat, and their combinations are used and preferred for the nursery (Hillel 2008), as they provide a proper environment for root initiation and permit gaseous exchange, proper water, and nutrients for plant growth (Singh et al. 2005).

Application of growth regulators observed an increased rooting efficacy, and naphthalene acetic acid (NAA), indole acetic acid (IAA), and indole butyric acid (IBA), which belong to the auxins family, are considered essential for rooting induction (Kareem et al. 2013; Akram et al. 2017). Paclobutrazol (PB) is a growth retardant and is used opposite to gibberellins as a biosynthesis inhibitor that minimises internodal growth and promotes root growth (Ayaz et al. 2004; Qadri et al. 2018). In hardy plant species, different rooting substrates and rooting chambers are being used for root success (Atak, Yalçın 2015). Further, the health of plants, plant species, and rooting hormones promotes plant success (Atak, Çorak 2024). Besides all these compounds, cutting type, size, date of cutting, and environmental factors take part in root induction (Hartmann et al. 2002). Therefore, this study was planned to develop an easy approach for getting guava true-to-type plants through softwood cuttings within a short period. Further, the objective of the study was to standardise the local best rooting substrate and rooting stimulator doses for guava nursery propagation.

MATERIAL AND METHODS

Selection and preparation of cuttings. This study was conducted at a citrus nursery sanitation project, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, to evaluate the effect of different rooting substrates and rooting stimulators for clonal propagation of guava softwood cuttings to check roots induction and the survival percentage. The cultivar 'Gola' was selected as plant material. The cuttings of this cultivar were collected from the Postgraduate Agricultural Research Station (PARS), University of Agriculture, Faisalabad, which has eight-year-old plants that are healthy and disease-free. The cuttings were taken from the juvenile apical shoot portions during September and were prepared from the terminal shoots. The prepared cutting was 12 cm in length, with 2 to 4 nodes and carrying at least 2–4 pairs of leaves. To facilitate the callusing process, the lower portion of the cuttings was injured with a budding knife. There was a total of 24 treatments in this experiment, and 10 cuttings per treatment were considered as single replicates. There were three replications per treatment, and the total number of cuttings planted in this experiment was 720. The whole procedure of the guava plantation is shown in Figure 1.

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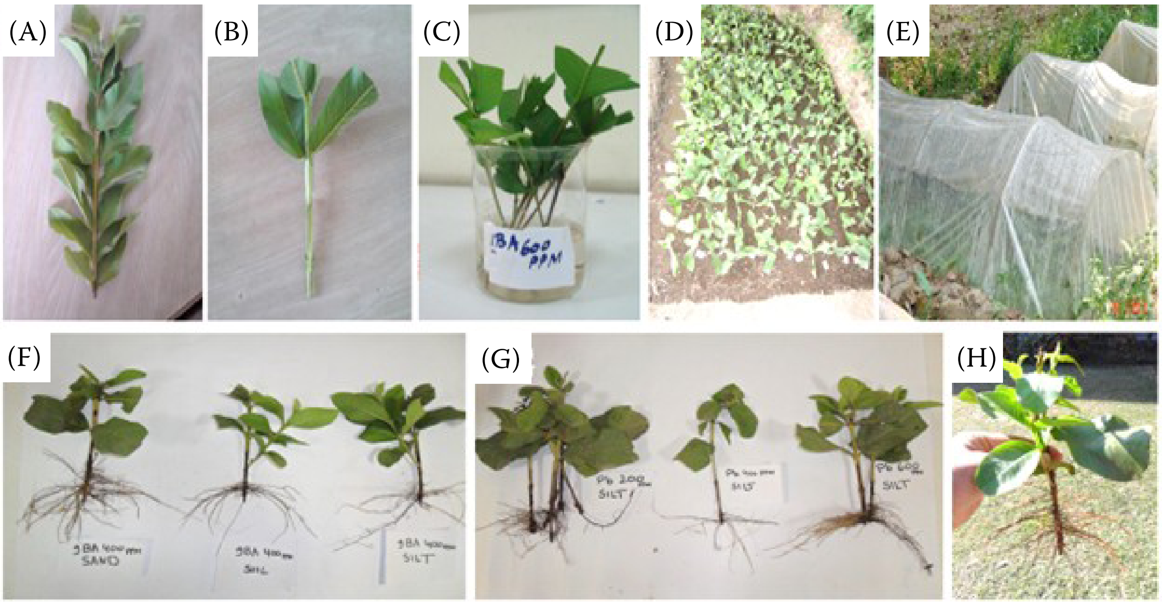

Figure 1. Steps involved in guava clonal propagation: (A) selected apical shoot, (B) prepared standard size cutting, (C) cuttings treatment with rooting stimulators, (D) planted cutting, (E) coverage of cutting with a polyethylene sheet to conserve moisture, (F) roots in cuttings obtained by IBA 400 grown in sand, soil and silt substrates, (G) roots in cutting obtained in silt substrate by PB 200, 400 and 600 ppm concentrations and (H) developed plant
IBA – indole butyric acid; PB – paclobutrazol

Preparation of growth stimulators. Cuttings were subjected to treatment with two growth rooting stimulators, indole butyric acid (IBA) and paclobutrazol (PB), at concentrations of 200, 400, and 600 ppm, serving as root-promoting stimulators. In contrast, tape water was employed as the control variant. The preparation of various IBA and PB concentrations adhered to a formula wherein 1 mg of growth stimulator was dissolved in 1 L of water, resulting in 1 ppm solution. Both the chemicals IBA and PB are available in crystal form; hence, they require ethanol to dissolve. All the equipment, like beakers and magnet stirrers, was washed with ethanol and distilled water to remove any undesired chemical contamination. All desired concentrations of IBA (200, 400 and 600 ppm) and PB (200, 400 and 600 ppm) were measured on a digital weight balance and put into different beakers to make a final volume of 1 L. First, the chemicals were dissolved in a 90% ethanol solution with the help of the magnet stirrer until the chemical precipitates had been dissolved. Distilled water was then added to the beaker slowly, after regular intervals, with continuous shaking to achieve the required concentration strength. Guava cutting was disinfected by dipping it in copper oxychloride solution (elite) for 30 s before the treatment of the cuttings (basal portion) with IBA and PB. Finally, the basal end (2.54 cm) of each guava cutting was immersed in different concentrations of IBA and PB for 5 minutes.

Preparation of rotting substrate and cutting the plantation. Three distinct rooting media, namely sand, silt, and soil, were individually applied in 6-inch layers on the ground. Before use, each rooting medium underwent a two-week sun sterilisation process under polyethene to eliminate potentially harmful pathogens. To ensure that the rooting substrate is free from stones, pebbles, and undesired material like other plants' roots and dry wood, each substrate was sieved and laid separately in parts and levelled for proper flow of water. After the pretreatment of growth stimulators IBA and PB, cuttings were planted separately in three different rooting mediums: silt, sand, and top fertile soil. These cuttings were properly tagged before irrigation was given to them. After irrigation, the cuttings were covered with a polythene sheet to maintain humidity at approximately 85%, and a temperature of 25 °C was required for proper guava rooting. Three replications were used in this experiment.

Data collection and statistical analysis. Finally, the root and shoot parameters like number of roots per cutting, root length (cm), root fresh weight (mg), survival percentage (%), number of leaves per cut-

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ting, number of sprouts per cutting, sprouting length (cm) and cutting length (cm), were recorded after 90 days of planting. The experiment was conducted under a completely randomised design with a 3-factor factorial arrangement (planting times, cutting length, and treatments). The collected data were statistically analysed by using Statistix® version 8.1 software (Tallahassee, Florida, USA) and the difference among treatment means was analysed by Tukey HSD test (Steel et al. 1997).

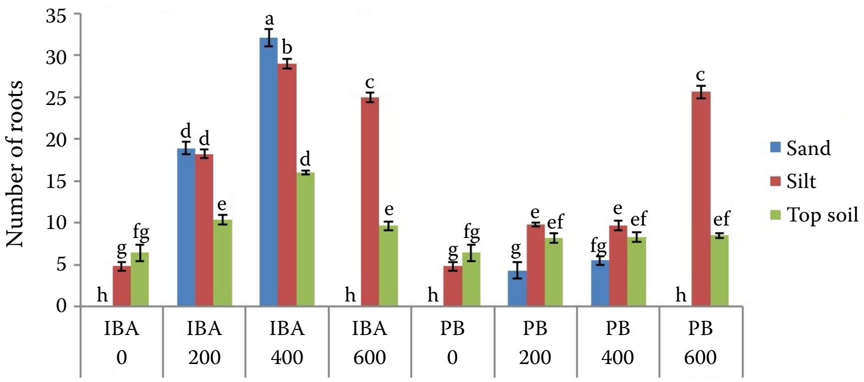
RESULTS

Rooting competency of guava softwood cutting

Number of roots. The study indicated that the number of roots is significantly affected by rooting substrate, rooting stimulators and their concentrations (Figure 2A). The results revealed that the highest number of roots (32.1) was achieved in the sand by IBA 400 ppm, followed by silt substrate (29.0) with a similar stimulator. However, sand as a rooting substrate, 0 and 600 ppm, produces zero roots with both rotting stimulators, IBA and PB. The combined interaction of rooting substrates and rooting stimulator concentrations is shown in Figure 2B. The results showed that among the rooting substrates, overall, more roots were obtained in silt (19.2), followed by sand (12.7) when treated with IBA. The lowest number of roots (2.4) was observed in sand with PB.

Average root length. This study exhibits a positive role of rooting substrate and rooting stimulators on root length, as shown in Figure 3A. The results showed that promising root lengths (13.6 cm) were obtained in sand substrate by IBA 400 ppm, followed by 200 ppm (10.35 cm) in similar rooting substrates, respectively. Our findings showed that porous rooting material promotes root length due to porosity, as maximum root lengths were observed in sand. The guava softwood cuttings planted in sand substrate treated with IBA and PB, 0 and 600 ppm concentrations, produced no roots. The combined interaction of rooting substrate and rooting stimulator concentrations is shown in Figure 3B. The results showed that the rooting stimulator IBA performed better in the induction of roots than PB.

Root fresh weight. Root fresh weight is an indicator of plant vigour, productivity and root adherence to the soil, which affects mineral absorption



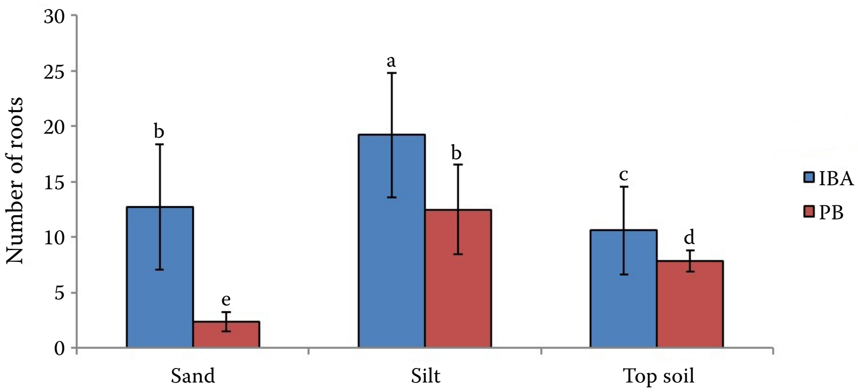


Figure 2. (A) The comparative effect of rooting substrates, rooting stimulators, and their concentrations on the number of roots of guava softwood cuttings under field conditions and (B) the combined effect of rooting substrates and rooting stimulators on guava roots

IBA – indole butyric acid (ppm); PB – paclobutrazol (ppm)

[a–h]means sharing similar letters in a column are statistically non-significant at $P > 0.05$ (Tukey HSD test)

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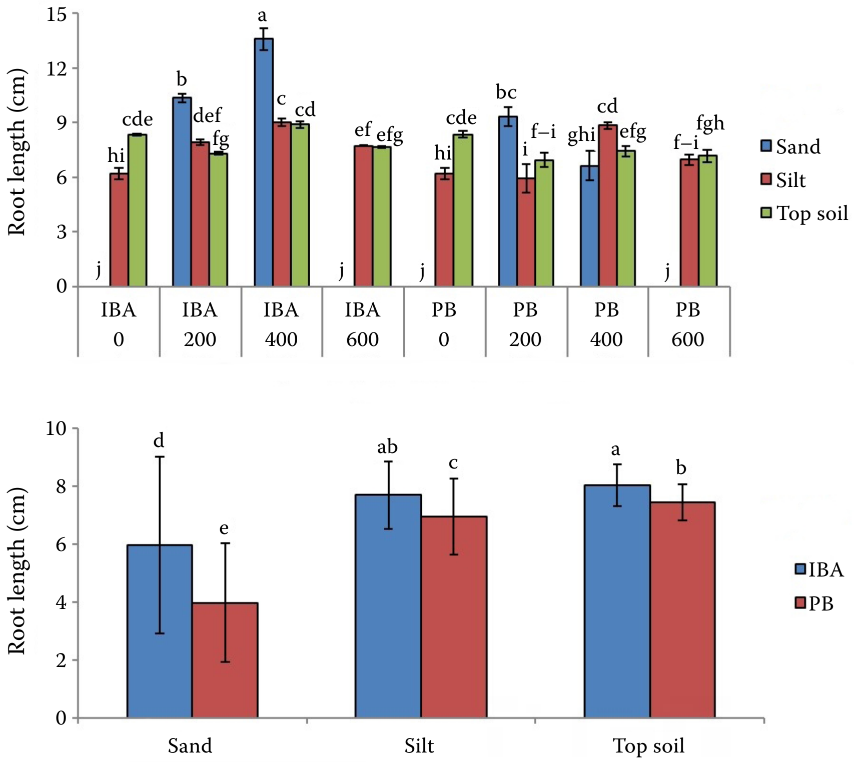

Figure 3. (A) The comparative effect of rooting substrates, rooting stimulators and their concentrations on average root length of guava softwood cuttings under field conditions and (B) the combined effect of rooting substrates and growth stimulators on guava root length

IBA – indole butyric acid (ppm); PB – paclobutrazol (ppm)

a–j means sharing similar letters in a column are statistically non-significant at $P > 0.05$ (Tukey HSD test)

via soil solution. The statistical results of this study manifested that rooting stimulators have a significant effect on the gain of root weight (Figure 4A). In this study, maximum root fresh weight (2 670 mg) was attained in sand rooting substrate with IBA 400 ppm, followed by soil substrate (2 150 mg) with 200 ppm IBA concentration. However, no roots were obtained in the sand substrate at 0 and 600 ppm concentrations of both rooting stimulators, IBA and PB. A comparison of rooting stimulators and rooting substrates is exhibited in Figure 4B. The results indicated that the maximum root fresh weight

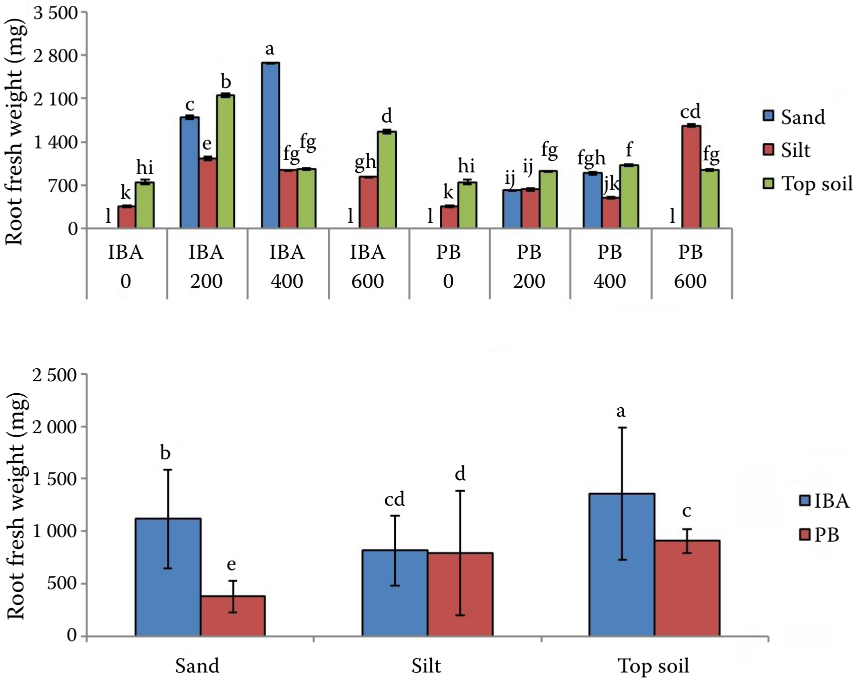

Figure 4. (A) The comparative effect of rooting substrates, rooting stimulators and their concentrations on root fresh weight of guava softwood cuttings under field conditions and (B) the combined effect of rooting substrates and growth stimulators on guava root fresh weight

IBA – indole butyric acid (ppm); PB – paclobutrazol (ppm)

a–l means sharing similar letters in a column are statistically non-significant at $P > 0.05$ (Tukey HSD test)

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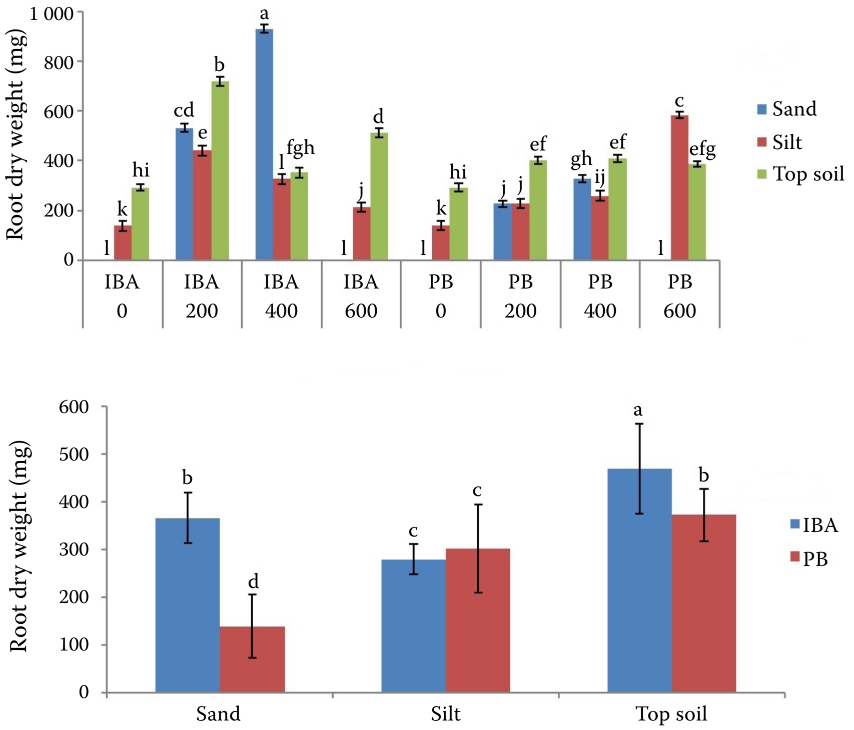

Figure 5. (A) The comparative effect of rooting substrates, rooting stimulators and their concentrations on root dry weight of guava softwood cuttings under field conditions and (B) the combined effect of rooting substrates and growth stimulators on root dry weight of cuttings

IBA – indole butyric acid (ppm); PB – paclobutrazol (ppm)

[a–l]means sharing similar letters in a column are statistically non-significant at $P > 0.05$ (Tukey HSD test)

(1 358 mg) was noted in topsoil while using IBA rooting stimulators, while the minimum root fresh weight (376.5 mg) was observed in sand with PB.

Root dry weight. The results indicated that root dry weight was significantly affected by rooting substrate and rooting stimulator treatments (Figure 5A). In this finding, maximum root dry weight (930 mg) was attained in a sand rooting substrate with IBA of 400 ppm, followed by soil substrate (719.6 mg) with 200 ppm IBA and PB concentrations. However, no roots were obtained in the sand substrate at 0 and 600 ppm concentrations of both rooting stimulators, IBA and PB. A comparison of rooting stimulators and rooting substrates is exhibited in Figure 5B. The results indicated that the maximum root dry weight (468.8 mg) was noted in topsoil using IBA rooting stimulators, while the minimum root fresh weight (139 mg) was observed in sand with PB.

Survival percentage. The attribute of guava softwood cutting regarding survival percentage significantly varied with rooting substrate, rooting stimulators and their concentrations (Figure 6A). The results indicated that maximum survival (52%) of guava softwood cutting was obtained in silt substrate by using IBA rooting stimulator, followed by PB 400 ppm in a similar rooting substrate (45.4%). However, no cuttings survived in the sand substrate with 0 and 600 ppm of rooting stimulators IBA and PB. A comparison of rooting substrate and rooting stimulators is presented in Figure 6B. Regarding the rooting substrate, the highest survival percentage was obtained from the silt substrate, followed by topsoil and sand. In the rooting stimulators comparison, the survival percentage was higher in IBA-treated cuttings than in PB-treated cuttings.

Shooting capability of guava softwood cuttings

Number of leaves per cutting. The results of this study revealed that growth stimulators and rooting substrate had a significant effect on the number of leaves. The comparison of three growing substrates and two growth stimulators indicated that the number of leaves in cuttings was significantly affected individually by the rooting substrate and stimulators and within their combined interaction. The results showed that growth stimulators IBA and PB had a pronounced effect on an increase in the number of leaves (8.53 and 6.36, respectively) as compared to untreated or control variants (4.33). Meanwhile, in the rooting substrate, the highest number of leaves was obtained in the silt substrate (9.80), and overall, 400 ppm concentration of both growth stimulators gave maximum leaves (9.77). Likewise, the overall interaction between rooting substrate, rooting stimulators, and their concentrations was highly significant and showed that the maximum number of leaves (13.6) was recorded with silt by using IBA 400 ppm, followed by sand substrate (13.1) at a simi-

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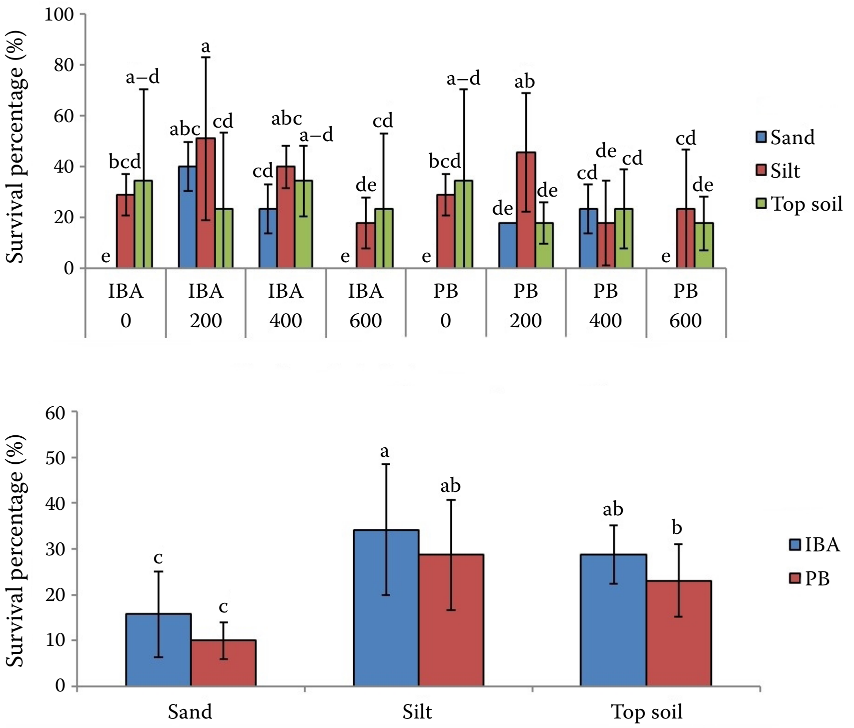

Figure 6. (A) The comparative effect of rooting substrates, rooting stimulators and their concentrations on the survival percentage of guava softwood cuttings under field conditions and (B) the combined effect of rooting substrates and growth stimulators on the survival percentage of cuttings

IBA – indole butyric acid (ppm); PB – paclobutrazol (ppm)

$^{a-e}$means sharing similar letters in a column are statistically non-significant at $P > 0.05$ (Tukey HSD test)

lar concentration. However, the sand substrate with the control variant and other highest concentrations (600 ppm) of both IBA and PB produced zero leaves in guava softwood cutting, as shown in Table 1.

Number of sprouts per cutting. The results related to the number of sprouts were significantly affected by rooting substrate, growth stimulators, and growth stimulator concentrations (Table 2). In the rooting stimulators comparison, maximum sprouts were recorded in IBA (2.53) compared to PB (2.16). Among growing substrates, maximum sprouts (2.99) were obtained in topsoil, at par with silt (2.97), with cuttings treated with IBA. However, the comparative interaction of rooting substrate, stimulators, and their concentrations showed that maximum sprouts (3.76) were observed in silt substrate at par with topsoil substrate (3.50) when treated with IBA 400 ppm concentration. However, no new sprouts were observed in cuttings planted in sand substrate with the control variant and the highest concentration (600 ppm) of both rooting stimulators, IBA and PB.

Sprouting length (cm). The sprouting length of guava softwood cutting was significantly increased with the application of rooting stimulators and rooting substrate, as shown in Table 3. The results depicted that the new sprouting length was highly improved by the application of PB (3.20 cm) as compared to IBA (2.51 cm). However, the statistical results

Table 1. The comparative effect of rooting substrates, rooting stimulators (IBA, PB) and their concentrations on the number of leaves of guava softwood cuttings under field conditions

Concentration used (ppm)	IBA			PB			Mean
	sand	silt	topsoil	sand	silt	topsoil	
0	0.00^{h}	5.00^{g}	8.00^{ef}	0.00^{h}	5.00^{g}	8.00^{ef}	4.33^{D}
200	12.80^{ab}	9.21^{de}	8.33^{e}	8.00^{ef}	9.27^{de}	5.66^{g}	8.87^{B}
400	13.10^{ab}	13.60^{a}	10.66^{cd}	5.50^{g}	9.66^{cde}	6.33^{fg}	9.80^{A}
600	0.00^{h}	11.30^{bc}	10.50^{cd}	0.00^{h}	12.80^{ab}	6.16^{fg}	6.79^{C}
Mean	6.47^{B}	9.77^{A}	9.37^{A}	3.37^{C}	9.18^{A}	6.53^{B}	
		8.53^{A}			6.36^{B}		

IBA – indole butyric acid; PB – paclobutrazol

means sharing similar letters in a column are statistically non-significant at $P > 0.05$ (Tukey HSD test)

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Table 2. The comparative effect of rooting substrates, rooting stimulators (IBA, PB) and their concentrations on the number of sprouts of guava softwood cuttings under field conditions

Concentration used (ppm)	IBA			PB			Mean
	sand	silt	topsoil	sand	silt	topsoil	
0	0.00^{e}	1.83^{de}	3.16^{ab}	0.00^{e}	1.83^{de}	3.16^{ab}	1.66^{C}
200	3.27^{ab}	2.99^{abc}	3.00^{abc}	2.00^{cde}	2.88^{a-d}	2.66^{a-d}	2.80^{B}
400	3.33^{ab}	3.76^{a}	3.50^{a}	3.16^{ab}	2.66^{a-d}	3.33^{ab}	3.29^{A}
600	0.00^{e}	3.33^{ab}	2.33^{bcd}	0.00^{e}	2.33^{bcd}	2.00^{cde}	1.66^{C}
Mean	1.65^{CD}	2.97^{A}	2.99^{A}	1.29^{D}	2.42^{BC}	2.78^{AB}	
		2.53^{A}			2.16^{B}		

IBA – indole butyric acid; PB – paclobutrazol
means sharing similar letters in a column are statistically non-significant at $P > 0.05$ (Tukey HSD test)

Table 3. The comparative effect of rooting substrates, rooting stimulators (IBA, PB) and their concentrations on the sprouting length of guava softwood cuttings under field conditions

Concentration used (ppm)	IBA			PB			Mean
	sand	silt	topsoil	sand	silt	topsoil	
0	0.00^{h}	2.91^{c-f}	2.49^{fg}	0.00^{h}	2.91^{c-f}	2.49^{fg}	1.80^{D}
200	4.97^{b}	2.49^{fg}	3.49^{c}	6.80^{a}	2.64^{efg}	3.20^{cde}	3.93^{A}
400	3.21^{cde}	3.30^{cd}	2.62^{efg}	4.79^{b}	4.95^{b}	3.43^{c}	3.71^{B}
600	0.00^{h}	2.05^{g}	2.67^{d-g}	0.00^{h}	4.45^{b}	2.85^{c-f}	2.00^{C}
Mean	2.04^{E}	2.68^{DE}	2.81^{CD}	2.89^{B}	3.73^{A}	2.99^{C}	
		2.5^{B}			3.20^{A}		

IBA – indole butyric acid; PB – paclobutrazol
means sharing similar letters in a column are statistically non-significant at $P > 0.05$ (Tukey HSD test)

of the interaction between rooting substrate, growth stimulators, and growth stimulator concentrations revealed that the highest sprouting length (6.80 cm) was achieved within the sand by using PB 200 ppm concentration, followed by IBA 200 ppm at a similar rooting substrate (4.97 cm). Moreover, no new sprouts were examined in guava softwood cuttings grown in the sand substrate by treating with 0 and 600 ppm concentrations of IBA and PB.

Cutting length. Treatment of guava softwood cuttings with rooting stimulators showed an encouraging effect on their length (Table 4). The statistical analysis showed that among rooting stimulators, the highest lengths were obtained in PB (17.30 cm), followed by IBA (15.89 cm), and among rooting substrates, silt performed better and showed more gain in height when treated with PB (20.9 cm) and IBA (19.8 cm). Likewise, the interaction between rooting substrate, rooting stimulators and their concentrations exhibited that the highest cutting height (26.2 cm) was attained with sand by using PB 400 and 200 ppm concentrations. In contrast, the sand substrate control variant (0 ppm) and the highest concentration of 600 ppm of both rooting stimulators (IBA and PB) produced zero cutting lengths.

Stem diameter (mm). The findings of this study revealed that growth stimulators and rooting substrate had a significant effect on the stem diameter (Table 5). The results showed that growth stimulators IBA and PB significantly impacted the stem diameter (4.36 and 3.37 mm, respectively). The interaction between rooting substrate, growth stimulators, and growth stimulator concentrations was significant and maximum stem diameter was obtained with sand substrate by using IBA at 400 ppm concentration (6.50 mm) and with IBA 600 ppm in silt substrate (6.18 mm), respectively. Still, the interaction among them was not significant. However, sand substrate with the control variant and other highest concentrations (600 ppm) of both IBA and PB produced zero stem diameter. The cutting rooting behaviour planted

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Table 4. The comparative effect of rooting substrates, rooting stimulators (IBA, IPB) and their concentrations on the cutting length of guava softwood cuttings under field conditions

Concentration used (ppm)	IBA			PB			Mean
	sand	silt	topsoil	sand	silt	topsoil	
0	0.00^{j}	18.00^{g}	19.50^{def}	0.00^{j}	18.00^{g}	19.50^{def}	12.50^{B}
200	19.30$^{d–g}$	20.10^{def}	23.00^{b}	26.20^{a}	20.70^{cd}	16.40^{h}	20.90^{A}
400	19.00^{efg}	19.50^{def}	16.50^{h}	26.20^{a}	23.00^{b}	20.20^{de}	20.70^{A}
600	0.00^{j}	21.90^{bc}	14.50^{i}	0.00^{j}	22.00^{bc}	18.60^{fg}	12.80^{B}
Mean	9.57^{E}	19.80^{B}	18.30^{C}	13.10^{D}	20.90^{A}	17.92^{C}	
		15.89^{B}			17.30^{A}		

IBA – indole butyric acid; PB – paclobutrazol
means sharing similar letters in a column are statistically non-significant at $P > 0.05$ (Tukey HSD test)

Table 5. The comparative effect of rooting substrates, rooting stimulators (IBA, PB) and their concentrations on the stem diameter of guava softwood cuttings under field conditions

Concentration used (ppm)	IBA			PB			Mean
	sand	silt	topsoil	sand	silt	topsoil	
0	0.00^{i}	3.10^{h}	3.83^{fgh}	0.00^{i}	3.10^{h}	3.83^{fgh}	2.31^{D}
200	5.67^{abc}	5.19^{bcd}	5.58^{abc}	4.00$^{e–h}$	4.49$^{d–g}$	3.63^{gh}	4.76^{B}
400	6.50^{a}	5.80^{ab}	4.81^{c-e}	3.82^{fgh}	4.62^{def}	4.92^{bcd}	5.08^{A}
600	0.00^{i}	6.18^{a}	5.73^{ab}	0.00^{i}	4.30$^{d–g}$	5.01^{cd}	3.53^{C}
Mean	3.04^{C}	5.06^{A}	4.99 ^{A}	1.95^{D}	4.12^{B}	4.35^{B}	
		4.36^{A}			3.47^{B}		

IBA – indole butyric acid; PB – paclobutrazol
means sharing similar letters in a column are statistically non-significant at $P > 0.05$ (Tukey HSD test)

in different rooting substrates treated with different rooting stimulators is shown in Figure 7.

Correlation of traits. The correlation analysis showed amazing facts, such as that each parameter is positively correlated with others and that no negative interaction was observed among the studied parameters (Table 6). The highest positive correlation (0.996) was observed between the number of leaves

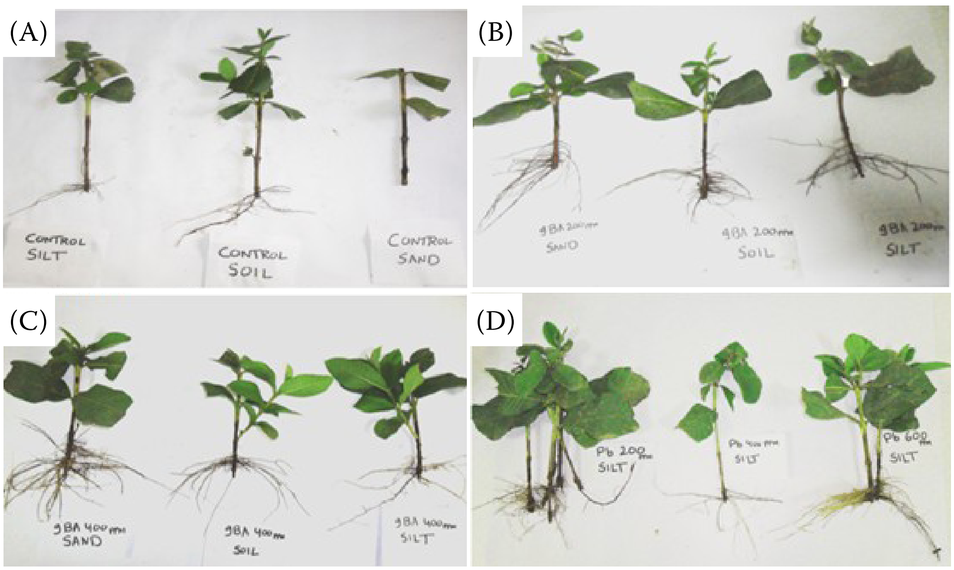

Figure 7. Cutting growth behaviour to different rooting substrates and rooting stimulators: (A) without rooting hormones treatment, (B) with IBA 200 ppm, (C) with IBA 400 ppm and (D) with PB (200, 400, 600 ppm)
IBA – indole butyric acid; PB – paclobutrazol

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Table 6. Coefficient of correlation among morphological traits of guava cuttings

	Number of leaves	Number of roots	Number of sprouts	Root dry weight	Root fresh weight	Stem diameter	Survival percentage	Shoot length	Sprouting length
Number of roots	0.8587**								
Number of sprouts	0.7724*	0.6272*							
Root dry weight	0.7154*	0.6726*	0.5984						
Root fresh weight	0.7274*	0.7023*	0.5771	0.9811**					
Stem diameter	0.8883**	0.7271*	0.8118**	0.7740*	0.7591*				
Survival percentage	0.6272*	0.439	0.6405*	0.4024	0.3853	0.6273*			
Shoot length	0.9991**	0.4491	0.7114*	0.5714	0.5212	0.8192**	0.6117*		
Sprouting length	0.5183	0.2495	0.3935	0.4444	0.3983	0.5330	0.3187	0.7798*	
Root length	0.8503**	0.6746	0.7754*	0.7667*	0.7329*	0.8873**	0.5987	0.8001**	0.6427*

**very strong correlation (0.8–1), *strong correlation (0.6–0.79), moderate correlation (0.4–0.59), weak correlation (0.2–0.39)

and shoot length, followed by root fresh weight and root dry weight (0.9811). Similarly, stem diameter showed maximum correlation with the number of leaves (0.8883) and root length (0.8873). The analysis also revealed that the number of roots has a positive correlation with the number of leaves (0.8503).

DISCUSSION

The propagation of plants through cutting is the easiest way to avoid segregation. However, the propagation of guava through cuttings is difficult due to enzymatic oxidation. In clonal propagation, root induction is one of the basic steps for its survival, and in vegetative propagation, root induction and initiation are essential for root development (Heloir et al. 1996). In the present study, rooting substrate and rooting stimulators significantly promoted root and shoot characteristics. In rooting stimulators, the highest number of roots, rooting length, root fresh weight, number of leaves, sprouts, sprouting length, and survival percentage were achieved in cuttings treated with IBA. Similar results were observed by other researchers who found that the application of rooting stimulator IBA has enhanced the ability of root formation in guava (Kareem et al. 2016; Akram et al. 2017). The findings were also in harmony with those of Li et al. (2017), who stated that root-stimulating stimulators trigger cambium activity and promote root initiation. Similarly, the most favourable results in terms of rooting percentage, root length, and number of roots were observed when employing IBA at a concentration of 3 000 ppm, according to findings by Vale et al. (2008).

However, in our study regarding PB, a more significant number of roots, root length, and root fresh weight were observed at low concentrations compared to high levels. Rahman et al. (2004) exhibited that the rooting rate of guava cuttings was 71.22% when treated with PB 100 ppm solution in comparison to NAA 1 000 ppm concentration. It has also been observed that the applications of PB have increased fine roots by improving plant root recovery after damage (Watson 2004). In our findings, the optimum doses of rooting stimulators have increased the number of roots, their lengths, and fresh weights, which may be due to the application of auxins, which have inhibited gibberellins production and increased the supply of carbohydrates to roots. The outcomes

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of this study are consistent with the results of Darwesh et al. (2013), who reported that when endogenous stimulator levels and climatic factors are favourable, then growth regulators help to stimulate roots and are indirectly helpful in stimulating the production of secretions that have an active role in the process of sprouting (Shahzad et al. 2019).

In our study, besides rooting stimulators, the rooting substrate also played a significant role in root promotion. Among rooting substrates, the highest roots and root length were observed in silt substrate, followed by sand and topsoil. While in the interaction of rooting stimulators and rooting substrate, the highest number of roots and root lengths were examined in the sand substrate with IBA 400 ppm concentration, followed by the silt substrate. This increase in root promotion is due to the availability of pore spaces in sand substrate that promote its production; however, the non-availability of nutrients in the sand substrate is a major issue for its survival. The rooting medium is the basic need for healthy plants, and the silt substrate produces more sprouting and rooting than other substrates due to the availability of nutrients and pore spaces. Similar results were observed by Adams et al. (2003), who got more sprouts in silt substrate, and the interaction effects of rooting substrate and rooting stimulators can significantly affect their roots and shoot parameters (Qadri et al. 2018). Similarly, AlHattali et al. (2024) depicted that the rooting medium with good porosity has maximum nutrient absorption capability and promotes shoot growth. The interaction of the rooting substrate and the rooting stimulator has a significant effect on shoot growth, and maximum heights were achieved in the coco peat-perlite substrate at IBA 2 500 ppm (Rahimi, Moghaddam 2012). Likewise, Khandaker et al. (2022) observed that IBA with the best rooting media vermicompost improves root initiation, root length and survival percentage in wax apple asexual propagation. Further, the type of cutting, planting material, nutrient availability, and environmental conditions greatly affect the guava root induction and its survival percentage (Akram et al. 2017).

Our findings showed that the number of leaves has a positive correlation with shoot length, stem diameter, number of roots, and root length. Leaves are the major source of photosynthesis that provides energy to plants for several physiological activities and root development. Similar findings were reported by Hartmann et al. (2011), who stated that optimal leaf numbers are necessary for root initiation and provide sufficient carbohydrates for root initiation and development. Likewise, Druege et al. (2000) observed that optimal leaves in cuttings improve rooting as it balances water uptake and loss. Therefore, the optimum number of leaves on cuttings results in a better root system (Howard, Harrison-Murray 2011).

In this study, softwood cutting of guava was selected for its propagation as juvenile apical shoots have more potential for rooting induction and take less time to sprout (Goel, Behl 2004). In clonal propagation, the selection of the optimum size of cuttings is essential as carbohydrates reserved in shoots supply food to shoots for their bud sprouting (Akram et al. 2017). The rooting stimulators directly affect sprouting and may increase the guava cuttings' growth and survival percentage (Rahman et al. 2004). The guava clones can be successfully propagated in nurseries by the selection of optimum rooting stimulators and rooting substrate. However, the optimum doses are required for cutting survival, as high doses of rooting stimulators harm shooting and rooting attributes.

CONCLUSION

This endeavour concluded that softwood cuttings can propagate guava successfully for commercial nursery production. In rooting substrate, silt is preferable to a better substrate than sand and topsoil. While in rooting stimulators, IBA is better in root induction parameters than PB. Therefore, to develop clone plants on a large scale, guava softwood cuttings treated with IBA 400 ppm concentration are highly preferred. However, there is a need to evaluate other local substrates such as bagasse, perlite, rice and wheat husk response for guava cutting rooting and survival.

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