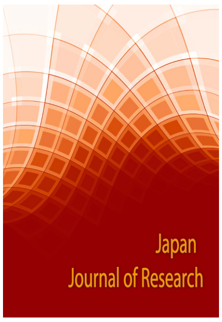
Japan Journal of Research

Original Article



Evaluation of Substrates And Indolbutiric Acid Concentration in The Propagation of Guava (*Psidium guajava L.*) By Air Layering

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- Received Date: 21 Jul 2026
- Accepted Date: 31 Jul 2026
- Publication Date: 03 Aug 2026

Keywords

vegetative propagation, auxin, rooting media, root number, root length.

Abstract

Guava (Psidium guajava) crop is economically important in many countries with tropical and subtropical climates. Internationally, Mexico ranks fourth in importance, following India, Pakistan, and Sudan. This crop is highly valued for its nutritional properties, as it contains various functional compounds with antioxidant activity. Guava propagation can be carried out by seeds (sexual reproduction) or through asexual or vegetative methods. The former results in high variability in the progeny, whereas vegetative propagation preserves the characteristics of the mother plant; consequently, the latter method is the most widely used. Although various methods of asexual propagation exist, air layering is the most commonly used in the study region. However, there is no information regarding the best type of substrate and/or on the application of phytohormones that promote greater root development. The objective of the study was to evaluate the effect of different substrates (coconut husk, peat moss, and rock wool) combined with the application of three concentrations (0%, 50%, and 100%) of indole-3-butyric acid (IBA; RADIX 10000®) on the propagation of guava by air layering. The air layers were carried out on a productive guava 15-year-old plant, typical of the region, located at the INIFAP "Los Cañones" Experimental Site in Huanusco, Zacatecas, Mexico. Fifty-one days after the layering process, the layers were separated from the mother plant, and the number and length of roots were recorded. The data were analyzed using a factorial experimental design (3 x 3), with 10 replications and one layer as the experimental unit. The results showed that substrates had a differential effect on root number and length: the highest number of roots was observed with coconut husk, while the greatest root length was obtained with peat moss, in both cases, at the 100% IBA concentration. Number and length of roots increased as the IBA concentration increased, as compared to the control that showed the lowest values. Based on the overall effects, it can be suggested that coconut husk is a suitable substrate for propagating guava via air layering, besides it is less expensive than the other two substrates. According to the results, the use of coconut husk with IBA (10,000 ppm) at 100% concentration is recommended for greater efficiency in the rooting of air layers.

Introduction

The guava tree (*Psidium guajava L.*) belongs to the Myrtaceae family; it is native to South America, and its domestication began in the southwestern Amazon [1]. It is currently distributed across many countries with tropical and subtropical climates. In Mexico, it is a major fruit crop. During the 2020–2024 period, an average of 21,422 hectares were cultivated nationwide, yielding an annual production of 313,275 tons. Currently, the largest areas dedicated to this crop are located in the states of Michoacán (61.1%), Aguascalientes (21.0%), and Zacatecas (7.4%), which are the leading producing states (Figure 1). The remaining 10.5% is distributed across other five states, where guava is cultivated in areas from 100 to 850 hectares, and another 12 states, where guava is grown in small-scale orchards covering less than 100 hectares [2].

Internationally, Mexico ranks fourth in guava production; India is the leading producer, with a cultivated area of 255,000 hectares and a fruit production of 4.1 million tons, followed by Pakistan with 66,300 hectares and 496,000 tons, and Sudan in third place with 22,000 hectares and 473,000 tons. Other significant guava producing countries include Bangladesh, Egypt, and Brazil [3]. However, guava cultivation has been reported to be economically important in more than 50 countries [4].

Citation: Padilla-Ramírez JS, González-Gaona E, Reyes-Muro L, Acosta-Díaz E, Maldonado-Ramírez JL, Acosta-Zamarripa AM. Evaluation of Substrates And Indolbutiric Acid Concentration in The Propagation of Guava (*Psidium guajava L.*) By Air Layering. Japan J Res. 2026;7(5):192.

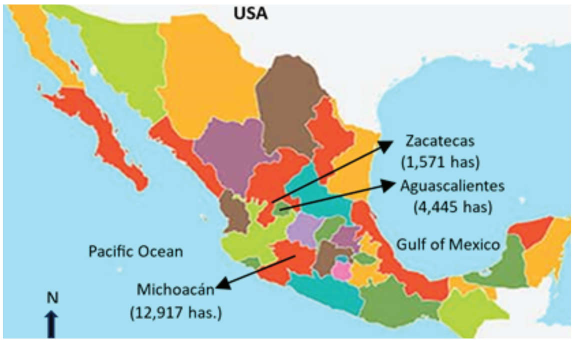

Figure 1. *Location of the main guava producing states in Mexico. Numbers in parentheses indicate the area cultivated with guava in 2024 (SIAP, 2026).*

Furthermore, the guava fruit is highly valued in the human diet for its high nutritional value, as it is rich in minerals and functional components with antioxidant properties, such as phenolic compounds and vitamins. In addition, various parts of the plant—including the leaves, fruit, stem bark, branches, and roots have been used in traditional medicine. Medicinal applications of the guava plant include antimicrobial, antispasmodic, antidiabetic, anticancer, antiallergic, anti-inflammatory, hepatoprotective, analgesic, and antidiarrheal uses, as well as treatment for stomach pain [5,6].

Regarding the propagation of the guava tree, it can be carried out sexually—using seeds—or through asexual or vegetative propagation.

Propagation by seeds

Although propagating guava trees from seed was common in the past, this method is not used, because trees grown from seed exhibit significant diversity in plant and fruit characteristics, such as tree vigor and size, growth habit, ripening time, and yield, as well as fruit shape, size, and quality [7,8]. This variation is attributed to natural cross-pollination carried out primarily by honeybees (*Apis mellifera*), which can reach rates of 35% to 41.3% and is the main cause of the variability observed in plants grown from seed [9,10].

Another disadvantage of guava trees grown from seed is that they do not bear fruit as early as those propagated vegetatively, since the latter have a shorter juvenile period before fruiting begins [3,4].

The variability in plant and fruit characteristics observed in the progeny of seed-propagated plants, along with the extended juvenile period, constitutes a significant disadvantage that limits their use. However, this method is advantageous when the goal is to obtain rootstocks for grafting productive cultivars with high fruit quality, or to generate segregating populations where variability is desired for selection in breeding programs [3,8].

Vegetative propagation

The advantage of this type of propagation is that the resulting plants retain the characteristics of the mother plant from which they originated and also have a shorter juvenile period. There are various methods of asexual propagation; among the most widely used are: rooting of semi-hardwood and non-lignified cuttings, air layering, grafting, and micropropagation [3] .

Propagation by semi-hardwood cuttings generally utilizes mature branches from the previous growth of the last year; although the guava tree is considered difficult to root, various studies cited by Sánchez et al. [11] and De Luna-Jiménez et al. [12] have reported that the use of auxins specifically indole-butyric acid (IBA) is necessary to improve cutting rooting. While this method is still practiced in some nurseries for guava propagation in Mexico, its use is declining due to low rooting percentages.

Another propagation technique that has given good results for guava is the rooting of young, non-lignified shoots (cuttings), utilizing the apical section of young stems approximately 12 cm in length—placed in rooting beds and treated with root-promoting phytohormones such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), or naphthaleneacetic acid (NAA). Additionally, it is important to reduce solar radiation and maintain high relative humidity using misting systems within the rooting chambers to keep the cuttings hydrated and their leaves turgent [13].

Propagation by grafting has certain disadvantages, as it requires the production and management of rootstocks, entailing a greater investment of human and financial resources [13,14]. In the other hand, micropropagation requires the optimization of a regeneration protocol specific to each genotype, including the culture medium and hormonal balance for each particular cultivar [15], which complicates its use in commercial propagation.

Air layering is considered an easy, rapid, and cost-effective method for cloning guava plants; consequently, it is a popular and suitable propagation technique for guava [8,16,14] and is commonly used in countries such as Taiwan, Bangladesh, Venezuela, and India [3]. Although the propagation of guava by air layering has not been employed commercially in Venezuela, despite being a fairly simple technique, it is widely used in Mexico [17]. This is particularly evident in the aforementioned major guava producing regions [18].

Air layering is an adequate method for the vegetative propagation of plants to produce uniform individuals; besides it is important to use branches of similar shape and age to ensure clonal propagation, a particularly significant factor for fruit species such as the guava [19,20]. However, a major disadvantage of air layering is its low yield regarding the number of plants obtained per mother tree compared to cuttings or grafting [3].

Although air layering is considered a suitable method for guava propagation, various factors, such as, climatic conditions (humidity and temperature), the type and concentration of auxin, the substrate used, and the type of branch, among others are important aspects to consider regarding the efficiency of propagating guava plants by air layering [8,14,19].

Singh et al. [8] indicate that this method presents no rooting issues and that the use of rooting hormones is not essential, although the authors note that root formation takes up to 2 to 3 months, depending on climatic conditions. However, several researchers mention that the exogenous application of auxins to layers stimulates root production and can shorten the time required for rooting [14,16,17,20,21].

Auxins reported for use in air layering propagation include: indoleacetic acid (IAA), naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA); these are used either individually or in combination, applied at concentrations ranging from 2,000 ppm to 9,000 ppm [3,14,17,19,20,21]. Saroj et al. [16] mentioned that high rooting percentages and optimal root characteristics (length and number) have been achieved in guava air layers through the exogenous application of IBA.

Regarding the substrates used for layering, a wide variety is reported, a fact attributed to regional availability. The most common ones are peat moss, coconut husk, river silt, phenolic foam, and poultry manure [16,17,19]. According to Punasya et al. [20], the rooting substrate must possess, among other characteristics, sufficient porosity and water-holding capacity to maintain moisture and facilitate the easy penetration and healthy growth of the roots newly formed during air layering.

Therefore, the objective of this study was to evaluate the propagation of guava by air layering using different substrates and auxin (IBA) concentrations to promote root development.

Materials And Methods

Location and climate of the experimental site

The study was conducted at the INIFAP "Los Cañones" Experimental Site (21° 44.7′ N, 102° 58.0′ W; 1,508 masl), located in the municipality of Huanusco, in the state of Zacatecas, Mexico. The experimental site lies within the guava-growing region known as "Los Cañones," situated in the southern part of the state. The prevailing climate in the study region is classified as BS$_1$hw(w), a semi-dry, semi-warm climate, according to García's [22] classification. Mean annual maximum, minimum, and average temperatures in the study area are: 31.0, 9.8, and 20.5 °C, respectively. Average annual precipitation ranges from 550 to 600 mm, occurring primarily between June and September [23].

For this study on propagation by layering, a guava mother 15-year-old plant, typical of the region and established at the "Los Cañones" experimental site was used; it produces sweet, aromatic, ovoid fruits with yellow skin, cream-colored pulp, and an average weight of 60 to 90 g per fruit (Figure 2).

Figure 2. *Guava fruit typical produced at the region around the Experimental Site "Los Cañones".*

Factors and levels evaluated

- **Substrates:** Three different substrates were evaluated: 1) Coconut husk, composed of coconut fiber (*Cocos nucifera* L.); 2) Peat moss, derived from genus *Sphagnum*; and 3) Rockwool (Agrolan®), obtained from natural volcanic rocks such as basalt. Table 1 shows the physicochemical characteristics of the substrate types used.
- **Rooting agent:** The application of auxins (Indole-3-butyric acid, "IBA") (RADIX 10000®) as a promoter of root development was evaluated, considering three concentrations: 1) 100%, only IBA; 2) 50% (50%-50% wt./wt. mixture of IBA and talcum powder); and 3) 0% (without IBA, talcum powder only).

Table 1. *Physical and chemical characteristics of the coconut husk and peat moss substrates.*

Caracteritics	Coconut husk[1]	Peat moss[1]	Rock wool[2]
Porosity (%)	86.4	80.1	97.0
Bulk density (g/cm^3)	0.05	0.15	0.04
Water holding capacity (%)	910	750	ND
Electric conductivity (mS/cm^1)	1.0	0.14	ND
pH	5.6	5.7	8.4
NH4-N (mg/L)	1.45	1.50	ND
P (mg/L)	0.08	0.02	ND
K (mg/L)	1.87	0.05	ND
Ca (mg/L)	0.50	0.30	ND

Source. [1]Arancon et al., 2015; [2]https://agrolan.com.mx/Productos; ND = Not determined.

Layering was done in early spring on actively growing branches from the previous cycle, on unpruned mother plant, at the beginning of the productive cycle, which had been induced by irrigation applied the previous month. The layers were made at 20 to 30 cm from the base of branches that were approximately 1.0 to 1.5 cm in diameter.

For each layer, a ring of bark approximately 2.0 cm wide was removed from the branch, and the corresponding rooting treatment was subsequently applied with a small brush on the ringed area. The substrates were pre-moistened, and a portion of the substrate assigned to each treatment was then applied to cover the ringed section. Finally, the substrate was wrapped in a clear plastic bag, which was tied securely at both ends to prevent moisture loss (Figure 3). The layering treatments were applied randomly to branches distributed throughout the tree canopy.

After fifty-one days, the layers were separated from the mother plant, and the substrate was removed by washing with water to expose the rootlets, taking care not to damage them (Figure 4). The variables recorded were the number and length of the largest root on each layer. Root length was measured using a Mitutoyo Series 500 digital caliper and expressed in millimeters.

Figure 3. *Details of the air-layering process for guava: From left to right: girdling the branches and applying the IBA treatment, placing the substrate to cover the girdled area, and wrapping and tying the clear plastic bag.*

The data were analyzed using the SAS statistical package version 8 [24] with a factorial experimental design (3 x 3) giving a total 9 treatments, with 10 replications per treatment. The experimental unit consisted of one layer per branch of the mother plant. When significant differences between treatments were detected, Tukey's mean comparison test was applied at a significance level of $p \leq 0.05$.

Results

The number of roots per layer showed no statistical significance for the main effects of both factors or its interaction (substrates × IBA concentration). However, root length showed a statistically significant effect regarding the substrates and the interaction with the IBA concentrations.

Figure 4. *Details of the evaluation of different substrates and IBA concentrations in guava air layers: From left to right: separation of the air layer from the mother plant, harvesting of air layers, and substrate removed for data recording.*

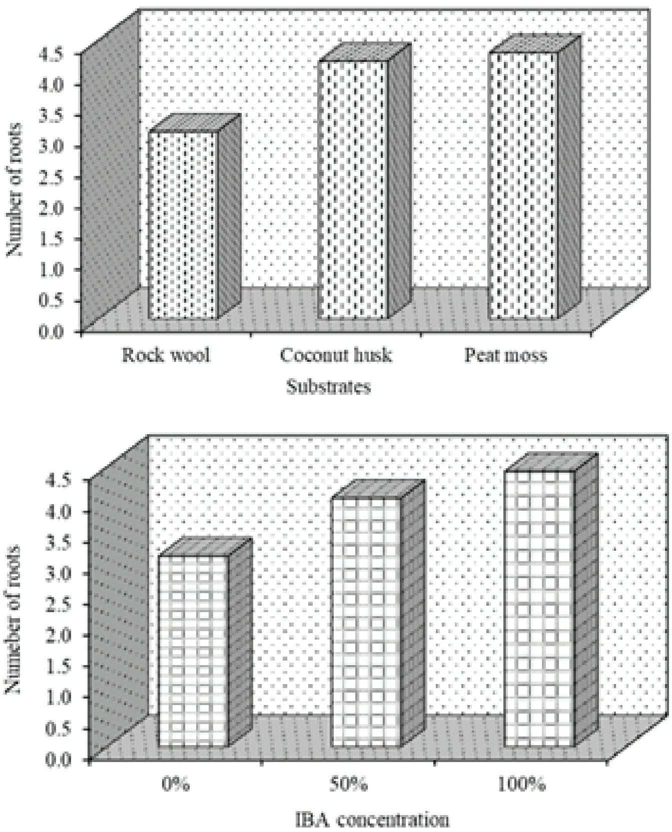

Figure 5. *Main effects of substrate type (left) and IBA concentration (right) on the number of roots in guava layers.*

Clear trends regarding the influence of both factors on guava air layers were observed in the number of roots. Regarding the effect of the substrates, coconut husk and peat moss performed similarly, with an average of 4.2 and 4.3 roots respectively, while rock wool was the substrate with the lowest average number of roots (3.0). Meanwhile, IBA concentrations also showed variation in the average number of roots per air layer; the 100% IBA treatment outperformed the control without IBA (4.4 vs. 3.1), whereas the 50% IBA treatment showed intermediate values (4.0) (Figure 5).

Regarding the effects of the interaction between substrates and IBA concentrations on the number of roots, the best combination was observed to be coconut husk with the IBA at 100% concentration, which showed the highest number of roots (6.5 roots/layer); conversely, the lowest number of roots was recorded for the coconut husk treatment without IBA and the rock wool treatment with 100% IBA, both producing only 2.3 roots/layer (Figure 6).

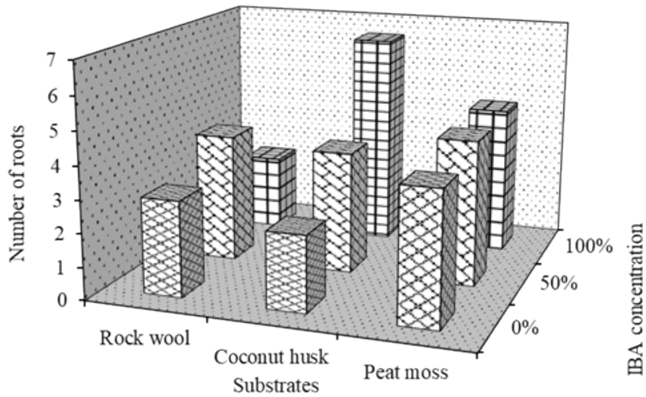

Figure 6. *Effect of the interaction between substrate type and IBA concentration on the number of roots in guava layers.*

The length of the longest root on the layers was statistically different for the substrate type ($p \leq 0.05$), and the substrate × IBA concentration interaction ($p \leq 0.01$). Mean root length was similar in the rockwool and coconut husk substrates (41.6 mm), whereas in peat moss, root length was greater, averaging 56.2 mm. Regarding IBA concentrations, although no statistical differences were detected, the trend showed that the 100% IBA treatment achieved the greatest mean root length (50.3 mm), followed by the 50% concentration (46.7 mm), while the control without auxin exhibited the shortest roots, averaging only 42.3 mm (Figure 7).

The interaction between substrates and IBA concentration showed differential effects on the root length of the layers; in the rockwool substrate, a decrease was observed as the auxin concentration increased, dropping from 54.0 mm without auxin to just 25.0 mm when the rooting agent was applied at 100% concentration. In contrast, root length increased with higher IBA concentrations in both the coconut husk and peat moss substrates. In the coconut husk substrate, root length went from 28.1 mm without auxin to 55.4 mm with 100% IBA, while in peat moss, root length was 44.8 mm without IBA versus 70.5 mm at the highest concentration, the latter being the best treatment, showing the greatest root length (Figure 8).

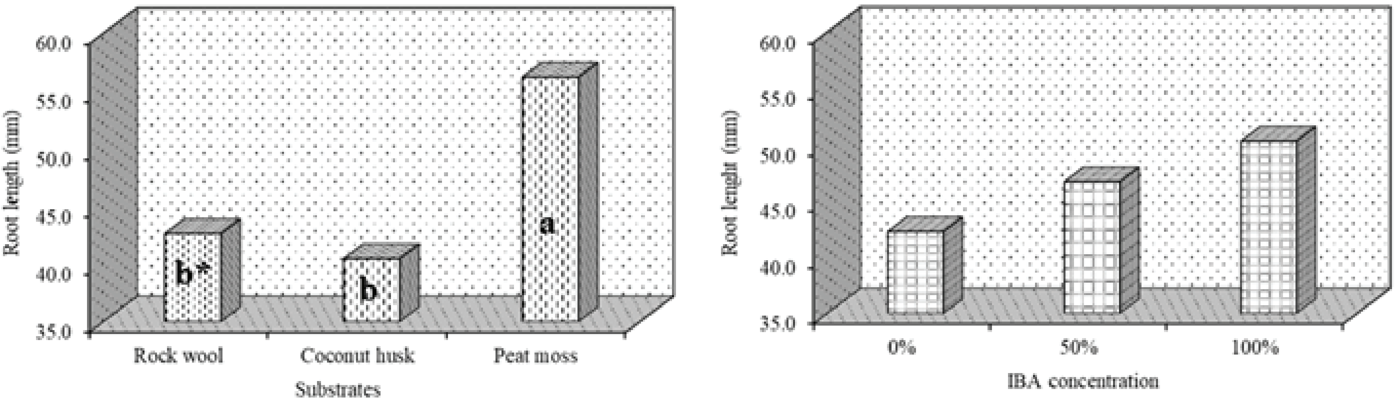

Figure 7. *Main effects of substrate type (left) and IBA concentration (right) on root length in guava air layers. *Means with different letters in the substrate column are statistically different (Tukey, p ≤ 0.05).*

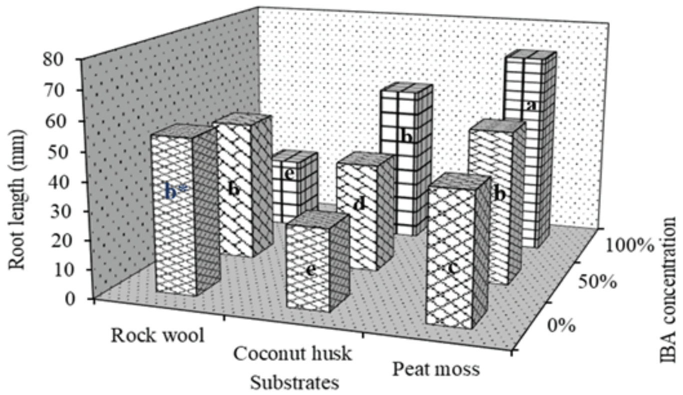

Figure 8*. Effect of the interaction between substrate type and IBA concentration on root length in guava layers. *Means with different letters are statistically different (Tukey, p≤ 0.05).*

Discussion

The values for root number and length found in this study are similar to those reported by Albany et al. [17] in a study on guava layering; they evaluated IBA and NAA at 5,000 ppm as root development promoters and concluded that NAA promoted a greater number and length of primary roots compared to IBA (7.8 vs. 4.2 and 41.3 mm vs. 28.4 mm, respectively). Regarding the substrates evaluated, a mixture of river sand and phenolic foam performed better than river sand alone. In contrast, Sánchez-Urdaneta et al. [19] describe a study evaluating four IBA concentrations ranging from 0 to 6,000 ppm in guava layers; where not significant effect was observed on the number or length of the primary root, with an average of only 0.96 roots per layer, all measuring less than 10 mm in length. The absence of the IBA effect was attributed to the fact that endogenous auxin levels were insufficient to promote root development, as well as to the substrate used (phenolic foam + wood shaving) and the method of application (IBA was applied as a paste, prepared with odorless petrolatum Vaseline®). In our study, the substrate rock wool showed negative effect on number and length of roots when IBA concentration increased, as in Sánchez-Urdaneta et al., [19] with foam + wood shaving, which root development was deficient, even at 6000 ppm IBA.

In another study, Awasthi et al. [14] evaluated the rooting response of the 'Shweta' guava cultivar to different IBA concentrations (0 to 9,000 ppm), substrates, and two air-layering timings. Sixty days after the air layers were made, they observed that the highest IBA concentration showed the greatest average number and length of roots compared to the control (20.2 vs. 8.8 and 59.7 vs. 25.3 mm, respectively) and that a peat moss and coconut husk mixture outperformed the control using soil as the substrate. The authors concluded that the combination of a 9,000 ppm IBA concentration, the mixture of peat moss + coconut fiber, and doing the air-layering in August represented the best option for efficient rooting of guava air layers. The number of roots reported by Awasthi et al. [14] was considerably higher than those found in our study (4 - 7 roots per layer), although root length was similar in both studies (59.7 vs 55.4 mm). It is possibly that leaving the air layers for a little longer (60 to 65, instead of 51 days) attached to the mother plant, the number of roots may be increased.

Punasya et al. [20] conducted a study to evaluate different IBA concentrations (2,000, 4,000, and 6,000 ppm), substrates (peat moss and coconut husk), and polyethylene colors (black and white) for covering guava air layers. The results showed a trend toward an increase in the number and length of roots in response to higher IBA concentrations; the average number of primary roots per air layer was 17.8, 22.8, and 27.6, while root length was 75.0, 90.0, and 110.6 mm for 2,000, 4,000, and 6,000 ppm of IBA, respectively. These results are in concordance with those observed in our study, as both, root number and length increased with the IBA concentrations. Punasya et al. [20] attribute this effect to the fact that the amount of auxin reaching the cambium capable of initiating root primordia increases as the concentration of IBA is increased. In addition, peat moss yielded better results, likely due to its high moisture-retention capacity and high porosity, which promotes better aeration. Coconut husk was less efficient due to its low water-retention capacity. The best response was observed with the treatment involving the application of 6,000 ppm IBA, peat moss, and black polyethylene covers. However, in our case, coconut husk showed a good response in promoting root number when IBA was applied at higher concentrations, similar to peat moss.

Regarding the results for the substrates, Punasya et al. [20] noted that peat moss was the best substrate due to its superior moisture-retention capacity compared to coconut fiber; meanwhile, Awasthi et al. [14] reported that a mixture of peat moss + coconut fiber was the best substrate for rooting guava air layers. In contrast, our study observed a differential effect: coconut fiber promoted a greater number of roots, whereas peat moss favored root length, suggesting that a mixture of both substrates could be a suitable option, as mentioned by Awasthi et al. [14].

Conclusions

Propagation of guava by air layers at one of the main producing region of Mexico, showed good root development observed at 51 days after the layering process was initiated. The highest number of roots was observed with coconut husk, whereas the greatest root length was achieved with peat moss; in both instances, at 100% IBA concentration. Furthermore, both concentrations of the IBA contributed to an increase in the number and length of roots, although the 100% concentration showed the best results. The control treatment (without IBA) showed the lowest values for both, root number and length. Based on these results, it is concluded that coconut husk is a suitable substrate for propagating guava by air layering, and it is also less expensive than the other substrates tested. Using coconut husk combined with IBA is recommended to enhance rooting efficiency in guava air layers.

Acknowledgments

We thank the technical staff of the INIFAP- "Los Cañones" Experimental Site for their support with fieldwork activities.

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