Revista de Ciências Agroveterinárias
Universidade do Estado de Santa Catarina (UDESC)

Research Article

Root architecture of guava seedlings (*Psidium guajava* L.) obtained through vegetative propagation by herbaceous cuttings

Arquitetura de raízes de mudas de goiabeira (Psidium guajava L.) obtido através de propagação vegetativa por estacas herbáceas

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Submission: November 20, 2024 | Acceptance: July 14, 2025

ABSTRACT

In guava trees, asexual propagation by cuttings is a common technique in the quest to obtain high-quality seedlings, as it ensures greater uniformity and a shorter juvenile period. For the establishment of cuttings, the dedifferentiation process is fundamental for root formation, and lesions at the base of the cutting can influence the final result. Therefore, the objective of this study was to evaluate the root system architecture of guava trees of the 'Thai' variety as a function of different types of basal cuts on the cuttings. For the trial, guava cuttings of the 'Thai' variety were subjected to four types of basal cuts (inverted wedge, normal wedge, bevel cut, and straight cut), treated with indolebutyric acid (IBA 6000 ppm), and randomly arranged in blocks. The cuttings underwent the following assessments 49 and 70 days after implantation: fresh and dry mass of the aerial and root parts, number, length, volume, diameter, and angle of insertion of the roots. The guava cuttings had a 100% survival rate under the test conditions, and the inverted wedge cut generally performed better due to its larger cut area, allowing greater exposure of meristematic tissues to the dedifferentiation process. Therefore, it can be concluded that the inverted wedge cut is superior to the others because it presents a greater number of roots, root length, fresh root mass in the first season, and greater dry root mass in the second season.

KEYWORDS: Guava. Root system. Basal cuts. Injuries.

RESUMO

Em goiabeira, a propagação assexuada por estacas é uma técnica comum na busca pela obtenção de mudas de alta qualidade pois garante maior uniformidade e menor período juvenil. Para o estabelecimento das estacas, o processo de desdiferenciação é fundamental para formação das raízes sendo que as lesões na base da estaca podem influenciar o resultado final. Portando, objetivou-se com o presente trabalho avaliar a arquitetura do sistema radicular de goiabeira da variedade 'Tailandesa' em função de diferentes tipos de cortes basais da estaca. Para o ensaio, estacas de goiabeira da variedade 'Tailandesa' foram submetidas a quatro tipos de cortes basais (cunha invertida, cunha normal, corte em bisel e corte reto), tratadas com ácido indolbutírico (AIB 6000ppm) e delineadas em blocos ao acaso. As estacas foram submetidas as seguintes avaliações aos 49 e 70 dias após a implantação: massa fresca e seca da parte aérea e radicular, número, comprimento, volume, diâmetro e ângulo de inserção das raízes. As estacas de goiabeira tiveram 100% de sobrevivência nas condições do ensaio e o corte em cunha invertido, no geral, teve desempenho superior devido à sua maior área de corte, permitindo maior exposição dos tecidos meristemáticos ao processo de desdiferenciação. Portanto, conclui-se que o corte em cunha invertida é superior aos demais por apresentar maior número de raízes, comprimento de raízes, massa fresca de raízes na primeira época e maior massa de matéria seca de raízes na segunda época.

PALAVRAS-CHAVE: Goiaba. Sistema radicular. Cortes basais. Lesões.

INTRODUCTION

Fruit growing is an activity of great economic importance for Brazil, as the country has a vast territory with distinct characteristics in each region, which allows for the cultivation of various fruit species (FONSECA 2022). Among the fruit trees grown here, guava cultivation, for example, stands out for its productive potential and popularity among consumers. In addition, it is an easily adaptable plant that is widely cultivated in several Brazilian states (GONZAGA NETO 2007).

The versatility of guava allows it to be consumed fresh, in juices, jams, and other by-products obtained from the plant for the pharmaceutical and cosmetics industries. The fruit is rich in lycopene (ROZANE & NATALE 2021) and, according to the Brazilian food composition table (TACO 2011), every 100g of raw red guava with skin contains 13g of carbohydrates, 1.10g of protein, and 0.40g of lipids.

According to data from the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística - IBGE), in 2023 the harvested area was 22,487 hectares, with the state of Pernambuco standing out as the largest producer of the fruit in the country (205,960 tons). In Paraná, fruit production has also been highlighted, with a harvested area of 1,395 hectares and a production of 47,058 tons, with the municipality of Carlópolis being the largest producer in the state (IBGE 2023).

For the multiplication of areas, guava can be propagated sexually (via seeds) or asexually, via the production of cuttings or via grafting. Vegetative propagation has advantages because it results in uniform seedlings and a short juvenile period compared to sexually propagated plants. The propagation of herbaceous branches with intermittent misting is the main method used to produce guava seedlings and has led to significant changes in fruit production in Brazil. However, this technique requires investments in infrastructure by nursery operators, which increases the final price of the seedlings. For the production of guava seedlings, cuttings should be prepared with two nodes, keeping the pair of leaves (intact or cut in half) on the upper node and removing the basal leaves. The best substrate is vermiculite (PEREIRA et al. 2017).

In the case of seedling production by cuttings, according to TAIZ et al. (2017), during the rooting process, it is common for a mass of poorly differentiated and disorganized parenchymatous cells to form at different stages of lignification, known as callus. This scar tissue can arise from the vascular cambium, the cortex, or the pith, representing the beginning of the rooting process. According to these authors, cells that become meristematic divide and give rise to root primordia, while cells adjacent to the cambium and phloem begin to form adventitious roots, which is divided into two phases: initiation, characterized by cell division, and differentiation of cells into root primordia, resulting in adventitious root growth.

The type of basal cut that will be made on the cutting during the plant propagation process is a factor that can determine the amount of vascular cambium that will be exposed to the differentiation process and significantly influence the number of roots as well as their architecture (VERDIN-FILHO et al. 2014).

According to RODRIGUES et al. (2019), bevel cutting of guava cuttings ‘BRS Guaraçá’ resulted in 78% rooting when performed in vermiculite, while in the same substrate, but with straight cutting, only 47% of the cuttings showed roots. The results of SANTORO et al. (2010) with the guava variety '8501-9' corroborate these results,

as the authors found that exposing guava cuttings to light increases rooting by 10%. However, the authors observed no differences in the number of roots, root length, fresh mass, and dry mass of roots.

In other species, the use of lesions at the base of the cutting has proven successful in rooting. For grapevines, BETTONI et al. (2014) tested three types of lesions (no lesion, wedge-shaped lesion, and scraping) at the base of 'VR043-43' rootstock cuttings associated with two types of synthetic auxin in three concentrations and found that lesions at the base of the cuttings, associated with the application of regulators, provided greater amounts of fresh root mass, a greater number and length of roots, as well as a higher percentage of rooting and greater sprouting biomass. On the other hand, in *Coffea canephora*, AQUINO et al. (2017) studied the effects of different substrates and basal cuts on the seedling production process and found that the use of commercial substrate and straight basal cuts yielded better results for the production of clonal seedlings of this type of coffee.

Therefore, since the type of basal cut affects the rooting potential of cuttings of some economically important species and in some guava varieties, the results have presented significant conclusions in the formation of the root system of seedlings. The objective of this study was to evaluate the root system architecture of guava trees of the 'Thai' variety as a function of different basal cuts of the cutting.

MATERIALS AND METHODS

Characterization of the test site

The experiment was conducted at the Federal Institute of Education, Science, and Technology of Paraná (Instituto Federal de Educação, Ciência e Tecnologia do Paraná – IFPR), Ivaiporã campus (24°15'5.21"S, 51°42'49.21"W) from November 3, 2022, to January 13, 2023, in an arch-type agricultural greenhouse measuring 8 meters long by 12 meters wide, totaling 96 m², with a ceiling height of 3 meters, plastic film covering (transparent polyethylene), two openings at the top for temperature control, side protection with white anti-aphid screen, concrete benches measuring (0.90 x 9.0 m) and intermittent mist irrigation system controlled by an Arduino microprocessor (activated every five minutes for a period of eight seconds using Agro Jet misters, model MA-30 with a threaded base flow rate of 30.7 L h^{-1}, at a pressure of 25 m.c.a. positioned 55 cm above the stakes).

The region's climate is classified as humid subtropical, with hot summers and mild winters, characterized by mild temperatures and high humidity throughout the year, with frequent rainfall (APARECIDO et al. 2016). During the trial period, temperatures were in line with historical averages for the municipality of Ivaiporã, ranging from 22.1–23 °C, 23.1–24 °C, and 23.1–24 °C for the months of November, December, and January, respectively (NITSCHE et al. 2019).

Characterization of plant matrices and cuttings

The cuttings were obtained from a commercial guava production area of the Thai cultivar, with four-year-old plants spaced 7 x 4 meters apart on November 3, 2022. The cultivar was selected for the study because it has good commercial characteristics, such as oblong fruits, with colors ranging from light green to yellow, reddish pulp, a significant amount of seeds, and slightly rough skin (SANTOS et al. 2019).

The cuttings were selected from the best plants, visibly free from diseases and pests, in order to obtain quality seedlings. The branches were cut from the production area with the aid of pruning shears and transported from the field to the greenhouse in Styrofoam boxes that provided a cool, humid environment.

For the preparation of the cuttings, herbaceous parts of the branches with two nodes and a pair of leaves on the upper node were used, with an average length of 10 cm and a diameter of 14 mm.

Experimental design and treatments

The test adopted a randomized block design with four replicates. Initially, the herbaceous cuttings were prepared using pruning shears and a grafting knife, keeping two nodes per cutting with the pair of leaves on the upper node cut in half and removing the basal leaves.

The treatments consisted of different types of cuts at the base of the cuttings near the first node with a 1 cm long lesion, namely: inverted wedge (Figure 1A), which consists of a V-shaped lesion prepared with a pocketknife; normal wedge (Figure 1B), made with the aid of a pocketknife, obtaining a wedge at the base of the cutting near the first pair of buds; bevel cut (Figure 1C) prepared with pruning shears at a 45-degree angle, and straight cut (Figure 1D) produced with pruning shears, consisting of a flat cut near the first pair of basal buds. The plot (sample unit) consisted of the average of twelve cuttings from each treatment in four randomized blocks, totaling 96 cuttings, which were divided into two evaluation periods.

After obtaining the treatments, the cuttings were treated with indolebutyric acid (IBA - powder) to stimulate rooting, since according to SINGH et al. (2019), the application of auxin improves histological characteristics, such as callus and tissue formation and vascular tissue differentiation, and there may be cases of absence of rooting. The dose was adjusted to 6,000 ppm because it ensures the highest survival rate of cuttings, according to WAHAB et al. (2001). After applying IBA, the cuttings were planted 3 cm deep in 200 mL transparent plastic cups containing vermiculite as substrate and kept in the location described in the characterization of the test site.

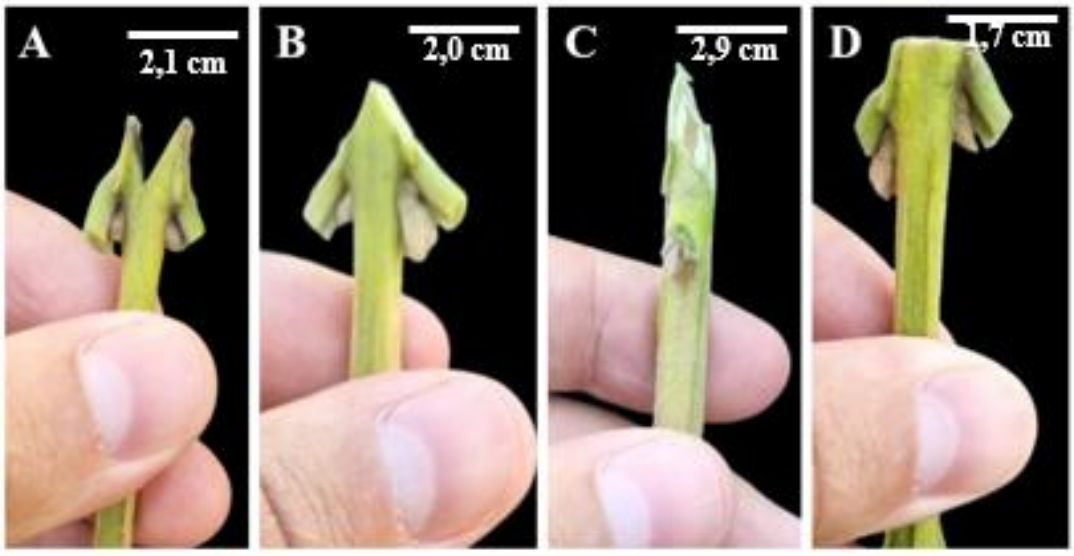

Figure 1. Types of basal cuts used in obtaining cuttings of the 'Thai' guava cultivar: A - inverted wedge cut, B - wedge cut, C - bevel cut, and D - straight cut.

Guava tree stake reviews

The evaluations were carried out at two stages, referred to as Stage 1 (49 days after the start of treatment) and Stage 2 (70 days after the start of treatment).

Seasons 1 and 2 were evaluated at the Agroecology and Plant Science Laboratory of the Federal Institute of Paraná, Ivaiporã Campus, with 48 cuttings randomly selected for each evaluation (Figures 2 and 3).

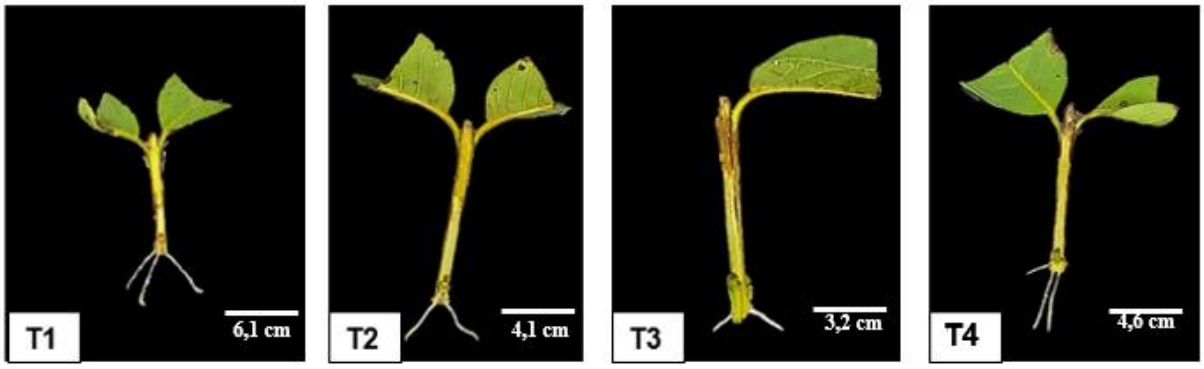

Figure 2. Development of adventitious roots in guava cuttings at 49 days into the experiment (Season 01). T1 - inverted wedge cut, T2 - wedge cut, T3 - bevel cut, and T4 - straight basal cut.

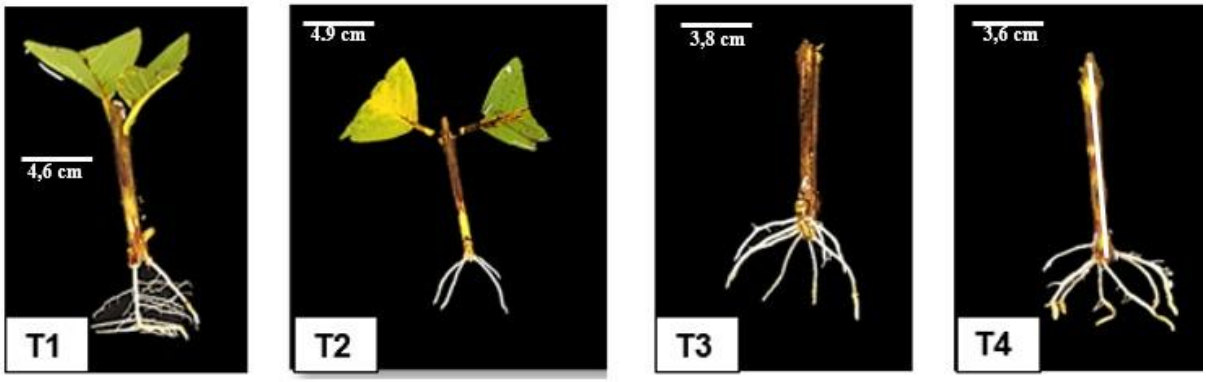

Figure 3. Development of adventitious roots in guava cuttings at 70 days into the experiment (Season 02). T1 - inverted wedge cut, T2 - wedge cut, T3 - bevel cut, and T4 - straight basal cut.

To remove the cuttings from the plastic cups with vermiculite, a 15-liter bucket of water was used to submerge the plants and then wash the root system without causing damage. The plants were photographed in a plastic container with water, using a black sheet as a background, for subsequent evaluation of the number, length, volume, diameter of the roots, and angle of insertion of the roots using the images with the SmartRoot® extension of the ImageJ software.

After obtaining the images, for the analysis of the cuttings, the aerial parts and roots were separated and then placed in Kraft paper bags for measurements of the fresh and dry mass of the aerial and root parts. The fresh mass was weighed on a precision scale immediately after separation, while the dry mass was dried in a forced-air oven at 70 °C until it reached a constant weight (BRAUN et al. 2010).

Statistical analysis of data

The data obtained from the SmartRoot® software were exported to a table, and the average values were calculated for each sample unit. Statistical analysis was performed using the Friedman test at 5% significance in R Studio software.

RESULTS AND DISCUSSION

The cuttings developed well throughout the trial, with a total survival rate of 100%. Between the first (49 days) and second (70 days) evaluation periods, an increase in root development was observed, which enabled evaluation in both proposed periods (Figures 2 and 3).

Additionally, although a statistical analysis of the leaf retention parameter was not initially proposed, it was observed that the inverted wedge and normal wedge basal pruning treatments presented persistent leaves until the end of the trial (Figure 3). According to TAIZ et al. (2017), leaves have the capacity to produce auxin, a hormone related to plant rooting, which can increase the rooting rate of cuttings.

The parameters evaluated by ImageJ software, number of roots and total root length, were statistically significant between the different basal sections at 49 DAI (season 1), however, the same effect was not observed for the evaluation at 70 DAI (season 2). The results (Table 1) showed that the inverted wedge basal cut had a greater number and length of roots in season 1 compared to the bevel cut and straight cut. On the other hand, the normal wedge treatment showed intermediate behavior for these two parameters, indicating no significant difference between any type of cut. No differences were observed between the types of basal cuts for root volume, root diameter, and root insertion angle.

Table 1. Average results of number of roots (NR), root length (CR), root volume (VR), root diameter (DR), and root insertion angle (AIR) of guava seedlings subjected to different types of basal cuts evaluated at 49 DAI (Season 1) and 70 DAI (Season 2).
Tabela 1. *Resultados médios de número de raízes (NR), comprimento de raízes (CR), volume de raízes (VR), diâmetro de raízes (DR) e ângulo de inserção de raízes (AIR) de mudas de goiaba submetidas a diferentes tipos de cortes basais avaliadas aos 49 DAI (Época 1) e 70 DAI (Época 2).*

Treatments	NR	CR (cm)	VR (cm^3)	DR (cm)	AIR (º)
	.....Season 1 (49 DAI).....				
Inverted wedge	2.42 A*	7.10A*	0.0708 A	0.0963 A	57.24 A
Normal wedge	1.16 AB	1.54 AB	0.0345 A	0.1227 A	103.98 A
Bevel cut	0.58 B	1.49 B	0.0258 A	0.0585 A	132.31 A
Straight cut	0.33 B	1.42 B	0.0288 A	0.0622 A	104.17 A
	.....Season 2 (70 DAI)				
Inverted wedge	2.83 A	8.96 A	0.204 A	0.1280 A	87.16 A
Normal wedge	2.10 A	5.69 A	0.103 A	0.1437 A	120.31 A
Bevel cut	1.18 A	4.06 A	0.0545 A	0.0778 A	94.03 A
Straight cut	2.00 A	3.24 A	0.0800 A	0.0558 A	120.31 A

*Averages followed by different capital letters are significant according to the Friedman test at 5%.

As for the results of fresh and dry mass measurements of the aerial part and root, there was a significant difference for fresh root mass in the first evaluation period and dry root mass in the second evaluation period (Table 2). It was found that the inverted wedge basal cut had the highest fresh mass values for the root system at 49 DAI compared to the bevel cut and the straight cut, while at 70 DAI, the same result was observed for dry mass of the root system. In both cases, the normal wedge cut showed no differences between treatments. These data reaffirmed the previous results (Table 1), where the inverted wedge treatment stood out in relation to the others.

The rooting potential of cuttings used in plant propagation may be influenced by the type of basal cut made on the cuttings, as the size of the wound can affect the degree of exposure of the vascular cambium (secondary meristem) to the substrate, in addition to enabling greater or lesser uniformity of auxin in the area (AQUINO et al. 2017).

Table 2. Average results for fresh shoot mass (MFPA), fresh root mass (MFPR), dry shoot mass (MSPA), and dry root mass (MSPR) of guava seedlings subjected to different types of basal cuts evaluated at 49 DAI (Season 1) and 70 DAI (Season 2).
Tabela 2. *Resultados médios obtidos para massa fresca de parte aérea (MFPA), massa fresca da parte radicular (MFPR), massa seca de parte aérea (MSPA) e massa seca de parte radicular (MSPR) de mudas de goiaba submetidas a diferentes tipos de cortes basais avaliadas aos 49 DAI (Época 1) e 70 DAI (Época 2).*

Trat.	MFPA (g)	MFPR (g)	MSPA (g)	MSPR (g)
	.....Season 1 (49 DAI).....			
Inverted wedge	4.40 A	0.251 A*	1.16 A	0.138 A
Normal wedge	4.72 A	0.030 AB	1.28 A	0.098 A
Bevel cut	3.82 A	0.028 B	1.06 A	0.098 A
Straight cut	4.11 A	0.010 B	1.15 A	0.026 A
	.....Season 2 (70 DAI)			
Inverted wedge	4.57 A	0.281 A	1.33 A	0.038 A*
Normal wedge	4.10 A	0.121 A	1.26 A	0.015 AB
Bevel cut	3.72 A	0.061 A	1.08 A	0.007 B
Straight cut	3.00 A	0.104 A	0.81 A	0.012 B

*Averages followed by different capital letters are significant according to the Friedman test at 5%.

In the case of guava trees, the use of lesions at the base of the cutting, resulting in greater exposure of the cambium in the '8501-9' variety, increased the number of rooted cuttings by 15% compared to the use of cuttings without lesions (SANTORO et al. 2010). COLOMBO et al. (2008) also observed a significant difference between the methods of preparing guava cuttings of variety '8501-1' (with and without lesions), where cuttings with lesions showed a higher percentage of callus compared to those without lesions.

Similar results have been observed in other species. VILLA et al. (2022) observed that in the rhizogenesis process of black raspberry stem cuttings with two types of basal cuts and four substrates, the highest rooting rate occurred in the bevel cut compared to the straight cut, with the best result observed in 75% of the substrates tested. In Okinawa peach cuttings, CAMOLESI et al. (2007) found that the percentage of rooted cuttings was higher for cuttings with lesions, corroborating the results of SOUZA et al. (1995), who observed that damage to the base of plum tree (*Prunus salicina*) cuttings increased the rooting percentage from 54.5% (without damage) to 81.7% (with damage). PAIVA et al. (2015) identified that leafless pomegranate (*Punica granatum L.*) cuttings with incisions at the base were superior in the seedling production process compared to cuttings with leaves and leafless cuttings.

On the other hand, according to WAGNER JÚNIOR et al. (2004), the different types of lesions at the base of blueberries (*Vaccinium sp*.) did not influence the rooting and development of the roots of herbaceous cuttings from four cultivars. Similar results

were observed by TREVISAN et al. (2008) also in blueberries, where they observed that the lesion at the base of the cuttings with the application of indolebutyric acid did not influence the rooting of the herbaceous blueberry cuttings evaluated.

Therefore, based on the results obtained in this test, it was found that the type of basal cut in cuttings of the 'Thai' variety of guava directly influences the rooting and development of the cuttings. The best results obtained in the inverted wedge basal cutting were due to early rooting compared to the other cuttings. As an advantage, this cut provides greater exposure of the vascular cambium tissue for dedifferentiation, as well as a larger area of contact with auxin, since the shape of this cut makes it difficult for the plant regulator to leach out. As a disadvantage, this type of cut requires more care and time when performed with a pocket knife, which could be circumvented by using equipment that provides this practical cut.

Regarding other advantages for inverted wedge basal cutting, the greater number of roots in season 1 and the greater dry mass of roots in season 2 resulted in a standardization of root system development as well as the quality of the seedlings formed. The basal cuts currently used (straight and bevel cuts) were inferior in these two parameters, which is disadvantageous for nursery operators because in everyday situations where there is a lack of electricity or water to supply the irrigation system, the lower number of initial roots or lower dry root mass could result in increased seedling mortality.

Another benefit is the early transplanting of material treated with inverted wedge cutting, since, as observed in this study, after forty-nine days the cuttings have good root development and can be transplanted into larger containers with suitable substrate, promoting rapid growth of the new seedlings.

CONCLUSION

Among the basal cuts studied in the 'Thai' guava variety, the inverted wedge cut is superior to the others because it has a greater number of roots, root length, fresh root mass early on (first season), and greater dry matter mass in the second season.

NOTES

AUTHORS' CONTRIBUTIONS

Conceptualization, methodology, and formal analysis**, Matheus Vinicius Oliveira Brunk, Christiane dos Santos Silva,** and **Denis Santiago da Costa;** software and validation**, Matheus Vinicius Oliveira Brunk** and **Denis Santiago da Costa;** investigation**, Matheus Vinicius Oliveira Brunk;** resources and data curation**, Denis Santiago da Costa;** writing - preparation of the original draft, **Matheus Vinicius Oliveira Brunk, Christiane dos Santos Silva,** and **Denis Santiago da Costa;** writing - revision and editing**, Matheus Vinicius Oliveira Brunk, Christiane dos Santos Silva,** and **Denis Santiago da Costa;** visualization**, Matheus Vinicius Oliveira Brunk, Christiane dos Santos Silva,** and **Denis Santiago da Costa;** supervision**, Denis Santiago da Costa;** project management**, Denis Santiago da Costa;** funding acquisition**, Denis Santiago da Costa**. All authors have read and agreed to the published version of the manuscript.

FINANCING
This paper was not supported by any funding agency.

STATEMENT BY THE INSTITUTIONAL REVIEW BOARD
Not applicable to studies that do not involve humans or animals.

INFORMED CONSENT STATEMENT
Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT
Data can be made available upon request.

ACKNOWLEDGEMENTS
The authors would like to thank the IFPR Ivaiporã Teaching, Research, and Extension Support Group (Grupo de Apoio ao Ensino, Pesquisa e Extensão – GAEPE) for their support in field and laboratory activities. In particular, we would like to thank Thais Fernanda Monteiro Schultz for organizing the spaces and monitoring the activities.

CONFLICTS OF INTEREST
The authors declare that there are no conflicts of interest.

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