Effects of Ultrasonic Treatment on Germination, Seedling Growth, and Root-to-Shoot Ratio in Guava (Psidium guajava L.) Seeds

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Effects of Ultrasonic Treatment on Germination, Seedling Growth, and Root-to-Shoot Ratio in Guava (*Psidium guajava* L.) Seeds

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Abstract. *Seed germination and early seedling growth are critical factors influencing plant establishment and crop productivity. This study investigates the impact of ultrasonic treatment (40 kHz) on the germination, seedling vigor, and shoot and root length of guava (Psidium guajava) seeds. Seeds were exposed to ultrasound for 5, 10, and 15 minutes, and their performance was compared against untreated controls. Guava seeds exhibited improved seedling vigor, particularly at 15 minutes of exposure. These findings suggest that ultrasonic treatment can enhance seed performance depending on species-specific responses.*

Keywords: desiccation-sensitive, ultrasonic, germination, enhance

1. INTRODUCTION

Recalcitrant seeds are metabolically active and desiccation sensitive, lacking or not expressing the defensive mechanisms associated with desiccation tolerance acquisition, which allow orthodox seeds to dry down and live at extremely low water levels [1]. Recalcitrant seeds cannot survive desiccation, and usually germinate and establish rapidly. Some recalcitrant seeds can survive for several months if kept in moist conditions, but under such conditions will tend to germinate [2].

Germination is one of the most important parameters of seed quality and it can vary based on the seed's viability and soil or environmental conditions in which they are sown. If the seed germinates rapidly and uniformly, it will be better able to withstand the field's biotic and abiotic stress factors [3]. Some different mechanisms (called dormancy) are used by plants to postpone germination and to protect the seeds until favorable conditions for seedlings are provided. Seed dormancy is defined as the ways of hindering germination for an intact viable seed. Some seeds, especially those produced in natural conditions, show different seed dormancy levels. Dormancy breaking and germination stimulation are important for the proliferation and early production of important plants. Various methods have been applied to overcome seed dormancy such as regulatory hormones, salinity, temperature, humidity, light and seed scarification, seed stratification, and fertilizers. Besides technology, the development of new techniques is suggested for breaking the seed dormancy such as magnetic water and nano-compounds [4].

Maintaining high yields will require the successful and uniform establishment of plants in the field under altered environmental conditions. To improve seed germination speed and success, much research has focused on selecting quality seeds for replanting, priming seeds before sowing, and breeding varieties with improved seed performance [5]. Recently, the use of ultrasonic waves as a safe, easy, and time-saving technique for seed dormancy breaking has attracted the researcher's attention to leverage the advancement of science, technology, and innovation for sustainable development [4] [6].

Ultrasonic treatment of seeds is a green technology that promises to have an impact on the food industry, enhancing germination and seedling development in different species through the stimulation of water and oxygen uptake and seed metabolism [7]. It has been found to improve growth, and yield by enhancing water absorption, biochemical activities, and photosynthesis, resulting in better plant vigor and productivity [8]. The treatment also improves radicle length, and tolerance to abiotic stress conditions such as salt, drought, and waterlogging [9]. Ultrasound treatment can be beneficial for many types of seeds such as chickpeas, sunflowers, barley, soybeans, caper beans, cumin, black beans, corn, and alfalfa [3] [10]. This is attributed to improved water uptake, increased seed coat porosity, and enhanced enzyme activity[11][10][12][3]. Ultrasonic seed treatment typically involves exposing seeds to ultrasonic waves with frequencies in the range of 20 kHz to 100 kHz [13] [14]. Chiu found that both 40 kHz and 80 kHz could improve seeds' hydration and sprouting rate, which might be due to the changes in seed coat structure induced by mechanical vibration caused by ultrasound [15]. This study examined the impact of 40 kHz ultrasound on the seeds of guava (*Psidium guajava* L.). Given the equipment's limitation to a single frequency and previous findings that 40 kHz can positively influence early seed growth, the research focused on how varying exposure times at this frequency affect seed germination, vigor, and the length of seedling shoots and roots.

2. MATERIALS AND METHODS

2.1 Seed Materials

The seeds were collected by extracting them from fresh fruits sourced from the local market. The gathered seeds were stored inside a refrigerator at 8°C for a day before the sonication treatment.

2.2 Seed Ultrasonic Treatments

Prior to sonication treatment, the seeds were surface sterilized for 10 minutes in a 30-ppm hypochlorite solution. The seeds were then carefully cleaned with running water and then rinsed one last time with distilled water. Four (4) groups of seeds were then created: (a) controlled samples, which were soaked in distilled water for ten minutes without receiving any ultrasound treatment [T1]; (b) samples exposed to 40 kHz for five minutes [T2]; (c) samples exposed to 40 kHz for ten minutes [T3]; and (d) samples exposed to 40 kHz for fifteen minutes [T4]. For the designated periods, the classified samples were subjected to a bath-type ultrasound machine operating at a fixed frequency of 40 kHz and a temperature of 25°C. To aid in the elimination of unbound water, the samples were left at room temperature for half an hour after the ultrasonic treatment.

2.3 Experiments on Germination

Following the ultrasonic treatment, the seeds were sown in three (3) replications, each containing three subsamples of ten seeds, to test for germination. The seeds were planted on a tissue paper-lined petri dish (optimal condition). Under the observed ambient temperature conditions (27°C), the petri dishes were placed on the rack at the SU-George K. Young Feed Laboratory. Every day during the first week of the sowing period, and every two (2) days the week after, germination counts were conducted. Radicles were seen to protrude when seeds were thought to have germinated, reaching approximately 2 mm in length. Equation 1 was utilized to determine the percentage germination (PG) [16].

Equation 1. $PG = \frac{germinated\ seeds}{total\ seeds\ sown} x\ 100\%$

2.4. Seedling root and shoot length measurement and root-to-shoot ratio.

The seedlings were measured every day on the first week of the germination period, and every two (2) days the week after. A digital caliper was used to measure the lengths of the roots and shoots of the seedlings. The root-shoot ratio was also calculated using Equation 2 [16].

Equation 2. $Root - shoot\ ratio\ \frac{root\ length}{shoot\ length}$

2.5. Seedling Vigor Index

The seedling vigor index (SVI) was also calculated by the Equation 3 [16].

Equation 3. SVI = (root length + shoot length) x (PG)

2.6. Statistical Analysis

The data collected in this study, laid out in a completely randomized design (CRD), was analyzed using one-way analysis of variance and least significant difference (LSD) via the Statistical Tool for Agricultural Research (STAR) 2.0 software developed by the International Rice Research Institute to ascertain the differences between treatment means at a 5% significance level using LSD.

3. RESULTS & DISCUSSIONS

3.1 Germination Rate

The germination of guava seeds was observed on Day 6 after sowing, with seeds exposed to 40 kHz ultrasonic treatment for 15 minutes exhibiting a higher germination percentage compared to other treatments (Figure 1). This aligns with studies on *Arabidopsis thaliana*, where short ultrasonic stimulation (less than 1 minute) at 45 kHz enhanced germination by increasing seed coat porosity and water uptake [17]. Similarly, maize seeds exposed to variable-frequency ultrasonic waves (20–40 kHz) for 40 seconds showed a 10.4% increase in germination and a 230.5% increase in radicle length, along with enhanced enzyme activity crucial for seedling development [9].

Ultrasonic treatment has also shown positive effects on soybean, where exposure for up to 30 minutes improved germination, seedling vigor, and upregulated antioxidant genes [10]. Additionally, maize seeds exhibited increased resistance to abiotic stresses such as drought and salinity, highlighting ultrasonication as a promising pre-sowing treatment. These findings suggest that while ultrasonic treatment effectively enhances germination across different species, optimizing frequency, duration, and intensity is crucial for maximizing benefits.

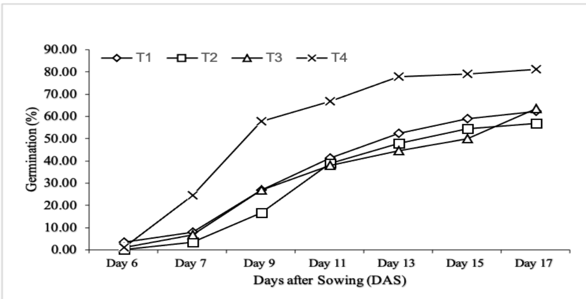

Figure 1. Germination (%) of guava after ultrasonic treatment.

3.2 Root Length

The root length of guava seeds measured at 7 days after sowing (DAS) showed no significant difference, although 15 minutes ultrasonic (40 kHz) exposure exhibited a relatively higher root length compared to the other treatments (Figure 2). Ultrasonic treatments have been investigated for their potential to enhance early growth of various seeds, while research specifically focusing on recalcitrant seeds is limited, studies on other seed types provide insights into the effects of ultrasonication on root length. For example, a study on maize seeds exposed to variable-frequency ultrasonic waves (20–40 kHz) for 40 seconds reported a 230.5% increase in radicle length compared to untreated seeds. This treatment also led to significant increases in the activity of enzymes such as acid protease, α-amylase, and β-amylase, which are crucial for seed germination and early growth. Additionally, the ultrasonic treatment enhanced the seeds' resistance to abiotic stresses like salt, drought, and waterlogging [9]. This is advantageous for plants because longer roots allow seedlings to access deeper soil layers, improving water and nutrient absorption, especially in drought-prone or nutrient-deficient soils. This enhances seedling survival and growth under challenging conditions [18]. Also, long roots contribute to better anchorage and stability in the soil, which is crucial for survival in harsh environments. They also enable plants to compete more effectively with invasive species and adapt to variable moisture conditions [18].

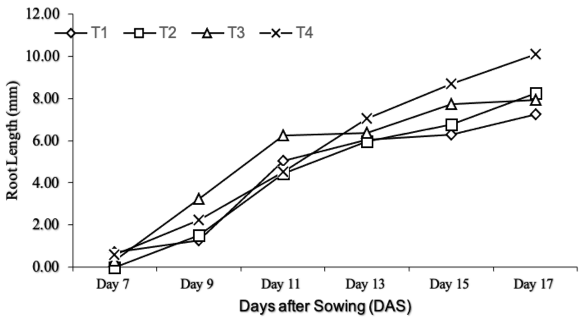

Figure 2. Root length (mm) of guava after ultrasonic treatment.

3.3 Shoot Length

The shoot length showed interesting results as application of sonication noted a relatively higher shoot length but extending the treatment to 15 minutes decreased the shoot length compared to other treatments, however, not statistically significant (Figure 3). The observed increase in shoot length with sonication, followed by a slight decrease at 15 minutes, aligns with studies showing that ultrasonic treatment enhances water absorption and enzymatic activity, promoting early growth. López-Ribera and Vicient (2017) found that short sonication improved seed hydration and metabolism in *Arabidopsis thaliana*, while Gong et al. (2024) reported increased shoot elongation in maize due to enhanced enzymatic activity [17][9]. However, prolonged exposure may induce cellular stress or oxidative damage, as seen in soybean (*Glycine max*), where extended treatment reduced growth due to reactive oxygen species accumulation [10]. Although the decline at 15 minutes was not statistically significant, these findings suggest that optimizing sonication duration is crucial to maximizing shoot development. Long shoot length improves the ability of seedlings to emerge from the soil, especially in conditions where seeds are planted deeply or when the soil surface is compacted. This is critical for establishing strong seedling vigor [19].

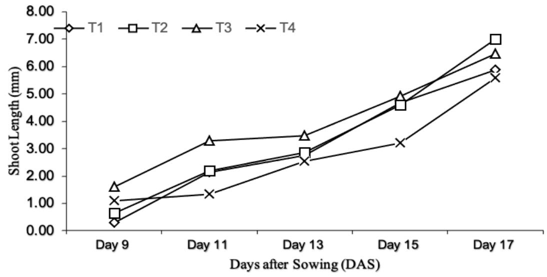

Figure 3. Shoot length (mm) of guava after ultrasonic treatment.

3.4 Root to Shoot (R/S) Ratio

The root to shoot (R/S) ratio revealed that exposing the seeds to 15 minutes sonication can increase root development over those treatments with lesser sonication exposure. The increase in the root-to-shoot (R/S) ratio with 15-minute sonication suggests that prolonged ultrasonic exposure promotes root growth more than shoot elongation. López-Ribera and Vicient (2017) discovered that sonication increased the porosity of the seed coat in *Arabidopsis thaliana*, facilitating better water uptake crucial for root growth. In a similar vein, Gong et al. (2024) observed that maize exhibited longer radicles as a result of heightened enzymatic activity—especially acid protease and α-amylase—which play key roles in nutrient mobilization for root development [17][9]. Additionally, Alfalahi et al. (2024) observed that extended ultrasonication in soybean upregulated antioxidant defenses, protecting root tissues and supporting greater root elongation [10]. These findings indicate that prolonged sonication enhances root growth by improving hydration, enzyme activation, and stress resilience, ultimately increasing the R/S ratio.

A high R/S ratio indicates a larger root system relative to the shoot, which can improve the plant's ability to absorb nutrients and water from the soil. This is particularly advantageous under drought or nutrient-deficient conditions [20]. Seedlings with a higher R/S ratio are better equipped to withstand environmental stresses like drought and low soil fertility due to their robust root systems [21].

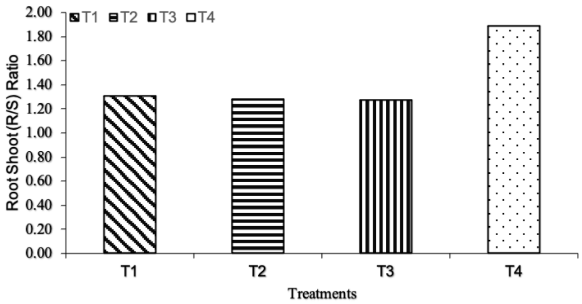

Figure 4. Root-Shoot (R/S) Ratio of guava after ultrasonic treatment.

3.5 Seedling vigor index

The seedling vigor index (SVI) of guava was significantly increased with the increased ultrasonic exposure. Seeds exposed to 15 minutes sonication had the highest seedling vigor indices. A higher seedling vigor index (SVI) signifies elevated germination rates, stronger seedling development, enhanced stress resilience, and increased storage potential. These traits contribute to reliable crop establishment, higher yields, and reduced risks of crop failure [22]. High SVI provides critical advantages in stressful environments by enhancing plants' ability to establish successfully under adverse conditions. For example, stress avoidance through rapid establishment since faster germination allows seedlings to emerge before soil moisture declines, outpace pathogen attacks and weed competition and develop roots quickly to access subsoil water reserves [19]. Seedlings with longer shoots can access sunlight more effectively, giving them a competitive advantage over weeds and neighboring plants. This is particularly beneficial in dense planting systems or weedy environments [19]. Shoot length is positively correlated with biomass accumulation, as it facilitates nutrient transport and distribution throughout the plant. This relationship is critical for overall seedling vigor and productivity [23].

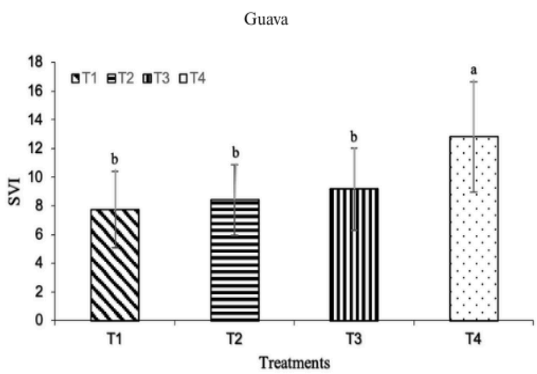

Figure 5. Seedling vigor index (SVI) of guava.

Figure 6 shows the qualitative comparison among different seeds and seedlings as influenced by ultrasonic exposure.

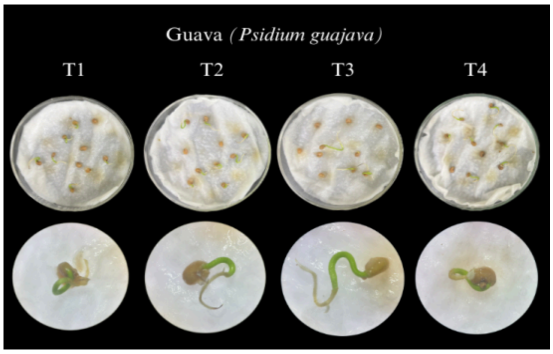

Figure 6. Germination and early emergence of seeds.

Improved seedling vigor index (SVI) in guava, a perennial fruit tree, has significant long-term Key implications for orchard establishment, productivity, and stress resilience. For example, higher germination rates and synchronized emergence ensure even-aged plantations, reducing gaps and optimizing space utilization and vigorous seedlings exhibit longer radicles and plumules, enabling rapid root establishment and canopy development [24]. Then, there would be reduced replanting costs due to reliable germination and vigor-driven early canopy development enhances photosynthetic capacity, directly influencing fruit set. While, dense root systems improve nutrient and water uptake, supporting sustained yields in mature trees [24][25].

4. CONCLUSIONS

This study demonstrated that ultrasonic treatment at 40 kHz positively influenced the germination and early growth of guava seeds. Seeds exposed to 15 minutes of ultrasound exhibited the highest germination percentage and enhanced root development compared to other treatments. The improved root-to-shoot ratio following 15 minutes of sonication suggests better root system development, which could enhance seedling survival under challenging conditions. Overall, ultrasonic treatment, particularly at optimized exposure times, presents a promising, green technique for improving seed vigor and early growth in guava.

5. RECOMMENDATIONS

Based on the findings, ultrasonic treatment at 40 kHz shows potential as a seed enhancement technique, though its effects vary by species. For guava, 15-minute exposure is recommended to improve seedling vigor and germination. Further studies should explore frequency modulation and longer-term effects to maximize the benefits of ultrasound treatment for different seed types.

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7. REFERENCES

[1] P. Berjak and N. W. Pammenter, “Recalcitrance,” in *Encyclopedia of Applied Plant Sciences*, Elsevier, 2017, pp. 532–539. doi: 10.1016/B978-0-12-394807-6.00048-4.

[2] L. Colville, “Seed Storage,” in *Encyclopedia of Applied Plant Sciences*, Elsevier, 2017, pp. 335–339. doi: 10.1016/B978-0-12-394807-6.00080-0.

[3] S. Kavak, “Ultrasonic Treatments to Enhance Seed Germination and Related Parameters,” *J. Sci. Eng. Res.*, vol. 11, no. 4, pp. 234–238, 2024.

[4] M. Nazari, “Impacts of Ultrasonic Waves on Seeds: A Mini-Review,” *Agric. Res. Technol. Open Access J.*, vol. 6, no. 3, Apr. 2017, doi: 10.19080/ARTOAJ.2017.06.555688.

[5] R. C. Reed, K. J. Bradford, and I. Khanday, “Seed germination and vigor: ensuring crop sustainability in a changing climate,” *Heredity*, vol. 128, no. 6, pp. 450–459, Jun. 2022, doi: 10.1038/s41437-022-00497-2.

[6] E. Mutisya, “Leveraging the Advancement Science, Technology, and Innovation for Sustainable Development,” *Proc. Int. Exch. Innov. Conf. Eng. Sci. IEICES*, vol. 10, pp. 30–31, Oct. 2024, doi: 10.5109/7323431.

[7] A. Nogueira, H. Puga, H. Gerós, and A. Teixeira, “Seed germination and seedling development assisted by ultrasound: gaps and future research directions,” *J. Sci. Food Agric.*, vol. 104, no. 2, pp. 583–597, Jan. 2024, doi: 10.1002/jsfa.12994.

[8] S. Huang *et al.*, “Ultrasonic seed treatment improved seed germination, growth, and yield of rice by modulating associated physio-biochemical mechanisms,” *Ultrason. Sonochem.*, vol. 104, p. 106821, Mar. 2024, doi: 10.1016/j.ultsonch.2024.106821.

[9] M. Gong *et al.*, “Ultrasonic treatment can improve maize seed germination and abiotic stress resistance,” *BMC Plant Biol.*, vol. 24, no. 1, p. 758, Aug. 2024, doi: 10.1186/s12870-024-05474-x.

[10] A. O. Alfalahi *et al.*, “Ultrasonic Treatment Enhances Germination and Affects Antioxidant Gene Expression in Soybean (Glycine max L. Merr),” *Agronomy*, vol. 12, no. 10, p. 2446, Oct. 2022, doi: 10.3390/agronomy12102446.

[11] A. C. Miano, J. D. C. Pereira, N. Castanha, M. D. D. M. Júnior, and P. E. D. Augusto, “Enhancing mung bean hydration using the ultrasound technology: description of mechanisms and impact on its germination and main components,” *Sci. Rep.*, vol. 6, no. 1, p. 38996, Dec. 2016, doi: 10.1038/srep38996.

[12] E. Mihaylova, M. Marcheva, and N. Peruhov, “Ultrasound seed treatment for organic farming.,” *Bulg. J. Agric. Sci.*, vol. 27, 2021.

[13] A. Babaei‐Ghaghelestany, M. T. Alebrahim, D. R. MacGregor, S. A. Khatami, and R. Hasani Nasab Farzaneh, “Evaluation of ultrasound technology to break seed dormancy of common lambsquarters (*Chenopodium album*),” *Food Sci. Nutr.*, vol. 8, no. 6, pp. 2662–2669, Jun. 2020, doi: 10.1002/fsn3.1547.

[14] N. MemiŞ, E. S. NjiE, S. MuhiE, G. ŞahiN, and İ. DemiR, “Effect of ultrasonic treatment on seed germination and seedling emergence in seven vegetable species,” *ANADOLU J. Agric. Sci.*, Dec. 2021, doi: 10.7161/omuanajas.890033.

[15] K.-Y. Chiu, “Changes in Microstructure, Germination, Sprout Growth, Phytochemical and Microbial Quality of Ultrasonication Treated Adzuki Bean Seeds,” *Agronomy*, vol. 11, no. 6, p. 1093, May 2021, doi: 10.3390/agronomy11061093.

[16] M. C. Alvarado, A. Jr. D. Bulfa, and A. L. Aquino, “Ultrasonic Influence on Germination and Early Growth of Bell Pepper Seeds,” *Proc. Int. Exch. Innov. Conf. Eng. Sci. IEICES*, vol. 10, pp. 19–25, Oct. 2024, doi: 10.5109/7323237.

[17] I. López-Ribera and C. M. Vicient, “Use of ultrasonication to increase germination rates of Arabidopsis seeds,” *Plant Methods*, vol. 13, no. 1, p. 31, Dec. 2017, doi: 10.1186/s13007-017-0182-6.

[18] D. Z. Atwater, J. J. James, and E. A. Leger, “Seedling root traits strongly influence field survival and performance of a common bunchgrass,” *Basic Appl. Ecol.*, vol. 16, no. 2, pp. 128–140, Mar. 2015, doi: 10.1016/j.baae.2014.12.004.

[19] J. Dong *et al.*, “The Function of SD1 on Shoot Length and its Pyramiding Effect on Shoot Length and Plant Height in Rice (Oryza sativa L.),” *Rice*, vol. 17, no. 1, p. 21, Dec. 2024, doi: 10.1186/s12284-024-00699-8.

[20] L. Bláha, “Importance Of Root-Shoot Ratio For Crops Production,” *Agron. Agric. Sci.*, vol. 2, no. 2, pp. 1–7, Nov. 2019, doi: 10.24966/AAS-8292/100012.

[21] R. Harris, “Root-Shoot Ratios,” *Arboric. Urban For.*, vol. 18, no. 1, pp. 39–42, Jan. 1992, doi: 10.48044/jauf.1992.009.

[22] H. Cheng, M. Ye, T. Wu, and H. Ma, “Evaluation and Heritability Analysis of the Seed Vigor of Soybean Strains Tested in the Huanghuaihai Regional Test of China,” *Plants*, vol. 12, no. 6, p. 1347, Mar. 2023, doi: 10.3390/plants12061347.

[23] H. Liu, F. Fiorani, O. Jäck, T. Colombi, K. A. Nagel, and M. Weih, “Shoot and Root Traits Underlying Genotypic Variation in Early Vigor and Nutrient Accumulation in Spring Wheat Grown in High-Latitude Light Conditions,” *Plants*, vol. 10, no. 1, p. 174, Jan. 2021, doi: 10.3390/plants10010174.

[24] M. S. Hosseini, S. M. Zahedi, N. F. Hoveizeh, L. Li, M. Rafiee, and M. Farooq, “Improving seed germination and seedling growth of guava under heat and osmotic stresses by chemical and hormonal seed treatments,” *Bragantia*, vol. 79, no. 4, pp. 512–524, Dec. 2020, doi: 10.1590/1678-4499.20200155.

[25] A. Dinesh and S. Padmapriya, “Effect of different pre-sowing treatments on seed germination, seed coat morphology and survival of guava (Psidium guajava L.),” *J. Pharmacogn. Phytochem.*, vol. 11, no. 3, pp. 265–269, 2022.