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Influence of Rhizospheric Microflora on the Success of Softwood Grafting in Red Pulp Guava (*Psidium guajava* L.)

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Abstract
The propagation of red pulp guava (*Psidium guajava* L.) through softwood grafting is one of the most promising techniques for maintaining varietal purity and ensuring rapid orchard establishment. However, grafting success remains inconsistent due to complex physiological, biochemical, and environmental constraints that influence graft union formation. Among these, the activity of rhizospheric microflora surrounding the rootstock plays a crucial but often overlooked role. The present investigation aimed to evaluate the influence of beneficial soil microorganisms on the success of softwood grafting in red pulp guava under controlled nursery conditions. A combination of plant growth-promoting rhizobacteria (PGPR) such as *Azospirillum brasilense*, *Bacillus subtilis*, and *Pseudomonas fluorescens*, along with fungal symbionts like *Trichoderma harzianum* and *Glomus intraradices*, was introduced into the rhizosphere prior to grafting. Their effects on graft take-percentage, callus development, scion sprouting, and subsequent plant vigor were systematically studied.
Results revealed a remarkable improvement in graft success rate and early scion growth in microbially inoculated treatments compared to the uninoculated control. Enhanced enzymatic activities related to oxidative stress regulation, greater accumulation of soluble carbohydrates at the graft interface, and higher microbial biomass carbon were recorded. These findings indicate that a biologically enriched rhizosphere contributes to better vascular connectivity and reduced graft incompatibility. The integration of rhizospheric microflora into nursery management therefore represents a sustainable biotechnological strategy to improve propagation efficiency in guava. The study highlights the physiological mechanisms underpinning microbe-mediated graft union success and proposes future applications of bioinoculants in perennial fruit propagation systems.

Keywords: Rhizospheric microflora, softwood grafting, red pulp guava, *Psidium guajava* L.

1. Introduction
Guava (*Psidium guajava* L.), belonging to the family Myrtaceae, is one of the most widely cultivated tropical fruit crops and occupies a distinctive position due to its high nutritive value, adaptability, and year-round productivity. Within its diverse cultivars, red pulp guava has gained special attention for its lycopene-rich flesh, pleasant flavor, and superior antioxidant properties. Owing to growing consumer demand and industrial use, commercial nurseries increasingly require uniform, true-to-type planting material of elite red pulp varieties. Vegetative propagation, particularly through grafting, ensures genetic fidelity and reduces the juvenile period. Among several techniques—such as inarching, air-layering, and cleft grafting—softwood grafting has emerged as a cost-effective and efficient method under tropical nursery conditions.
Despite its advantages, the success of softwood grafting in guava is often hampered by low graft-take percentages and poor scion survival. Physiological incompatibility between scion and rootstock, suboptimal environmental conditions, and the presence of latent stress factors often interfere with the establishment of the graft union. Researchers have long recognized that a successful graft depends on rapid callus proliferation, efficient vascular connection, and balanced hormonal interactions between the graft partners. However, recent insights suggest that the biological environment of the rhizosphere—the narrow region of soil

influenced by root exudates—plays a significant and underexplored role in determining the physiological state of the rootstock and its ability to sustain graft development.

The rhizosphere harbors a highly dynamic consortium of bacteria, fungi, actinomycetes, and protozoa collectively termed rhizospheric microflora. These microorganisms are not merely passive inhabitants of soil; they actively interact with plant roots through mutualistic, commensal, and sometimes antagonistic relationships. Their activities include nitrogen fixation, phosphate solubilization, siderophore production, phytohormone synthesis, and pathogen suppression. Such microbial functions influence root growth, nutrient uptake, and stress tolerance, which in turn determine the vigor of the rootstock used for grafting. Furthermore, rhizospheric microbes secrete extracellular enzymes and signaling molecules that may modulate hormonal cross-talk between rootstock and scion tissues, thus indirectly affecting graft union formation.

In the context of guava propagation, most previous research has focused on optimizing grafting techniques, determining ideal stock-scion combinations, and controlling environmental factors such as temperature, humidity, and light intensity. The biological and microbiological dimensions of graft success, however, have received limited attention. Studies in other fruit crops—such as mango, citrus, and apple—have demonstrated that inoculation of rootstocks with plant growth-promoting rhizobacteria or mycorrhizal fungi enhances root development, nutrient acquisition, and overall graft performance. These findings underscore the potential of utilizing beneficial microflora as natural bio-enhancers in horticultural propagation systems.

Given the increasing emphasis on sustainable and low-input production practices, integrating microbial biotechnology into traditional grafting procedures aligns well with the principles of eco-friendly horticulture. Unlike chemical growth regulators, microbial inoculants improve plant performance through synergistic and long-lasting mechanisms, enhancing soil fertility while reducing environmental impact. For guava, whose roots naturally associate with diverse microorganisms in tropical soils, the manipulation of rhizospheric communities could be particularly beneficial.

The present research was therefore designed to elucidate the influence of rhizospheric microflora on the success of softwood grafting in red pulp guava. Specifically, the objectives were: (1) to evaluate how inoculation with selected PGPR and beneficial fungi alters rootstock vigor and graft-take percentage; (2) to study biochemical and physiological changes at the graft interface; and (3) to compare microbially treated and untreated grafts in terms of scion sprouting, callus formation, and subsequent growth. By combining horticultural techniques with microbial ecology, this study seeks to bridge a critical knowledge gap and provide a foundation for biologically optimized propagation protocols in guava nurseries. The results are expected to contribute both to practical nursery management and to our broader understanding of plant-microbe-host interactions in vegetatively propagated fruit crops.

2. Literature Review

The rhizosphere represents one of the most biologically active interfaces between plant roots and soil ecosystems. Coined by Hiltner in 1904, the term encompasses the immediate soil zone influenced by root secretions, exudates, and decaying tissues. Microbial densities in this narrow region can exceed those in bulk soil by several orders of magnitude, creating a complex micro-ecosystem that profoundly influences plant physiology. Rhizospheric microflora include a vast array of bacteria—*Pseudomonas, Bacillus, Azospirillum*, *Rhizobium*—as well as filamentous fungi such as *Trichoderma*, *Aspergillus*, and arbuscular mycorrhizal fungi (AMF). These organisms collectively participate in biogeochemical cycling and produce substances that modify plant growth patterns.

Plant growth-promoting rhizobacteria (PGPR) are among the most widely studied components of the rhizospheric community. They enhance plant growth either directly by supplying essential nutrients or indirectly by protecting plants against pathogens. Direct mechanisms include nitrogen fixation, phosphate and potassium solubilization, siderophore production, and synthesis of phytohormones like indole-3-acetic acid (IAA), gibberellins, and cytokinins. Indirect mechanisms involve the production of antibiotics, hydrogen cyanide, and lytic enzymes that suppress deleterious soil microbes. The collective effect of these processes improves root architecture, nutrient absorption, and plant vigor—factors intimately linked with grafting success.

Fungal symbionts, particularly arbuscular mycorrhizae, form mutualistic relationships with plant roots by extending their hyphae into the soil matrix. This not only increases the root's absorptive surface but also facilitates the uptake of relatively immobile nutrients such as phosphorus and zinc. The AMF also secrete glomalin, a glycoprotein that improves soil aggregation and moisture retention—conditions favorable for graft establishment. *Trichoderma harzianum*, another beneficial fungus, has been documented to colonize the rhizosphere aggressively, outcompeting phytopathogens and inducing systemic resistance in host plants. These attributes can significantly influence the physiological readiness of a rootstock to accept and nourish a grafted scion.

The process of graft union formation comprises several stages: adhesion, callus proliferation, vascular differentiation, and eventual establishment of physiological continuity between scion and rootstock. Each of these stages is influenced by hormonal signaling, carbohydrate availability, and enzymatic activities. Rhizospheric microorganisms can alter these processes through multiple biochemical pathways. PGPR such as *Bacillus subtilis* and *Pseudomonas fluorescens* produce auxins and cytokinins that stimulate cambial cell division, thereby promoting faster callus formation at the graft interface. Additionally, microbial enzymes like peroxidase and polyphenol oxidase modulate lignin deposition, ensuring proper vascular connectivity.

Empirical evidence from woody perennials such as citrus and grapevine has shown that microbial inoculation accelerates graft healing and improves the mechanical strength of the graft union. For example, inoculation of *Citrus volkameriana* rootstocks with *Azospirillum* and *Glomus intraradices* resulted in enhanced carbohydrate translocation and increased graft survival (Singh *et al*., 2017). Similarly, in mango, inoculation with *Trichoderma* species improved callus formation and reduced graft failure under high-humidity conditions (Patel and Kumar, 2018) [6]. Such studies support the hypothesis that a biologically enriched rhizosphere can positively influence the physiological processes governing graft compatibility.

Traditional propagation of guava by seeds often leads to high genetic variability, necessitating the use of vegetative methods to maintain cultivar identity. Softwood grafting, wherein a tender scion is grafted onto an actively growing rootstock, offers advantages such as higher multiplication rate, uniformity, and reduced juvenile period. Nonetheless, the technique demands careful control of physiological and environmental factors. Moisture stress, temperature extremes, and microbial imbalances in nursery soil are frequent causes of graft failure. Studies by Kumar *et al*. (2016) reported that oxidative stress and accumulation of phenolic compounds at the graft interface are key inhibitors of union formation in guava. Since beneficial microbes can modulate antioxidant enzyme systems and reduce phenolic oxidation, they are likely to improve graft viability.

Bio-fertilizers and microbial inoculants have become integral to modern sustainable horticulture. Preparations containing *Azotobacter*, *Azospirillum*, and *Pseudomonas* are widely used in nurseries to promote root proliferation and nutrient uptake. *Trichoderma* formulations are employed to manage soil-borne diseases and stimulate plant defense responses. These microbes not only provide nutrients but also influence plant hormone balance and signal transduction pathways. Their combined action often results in better rootstock performance and resilience under stress. In grapevine nurseries, for instance, PGPR application reduced mortality rates of grafted vines by nearly 30% compared with untreated controls (Rahman *et al*., 2019) [5]. Such findings provide a strong rationale for testing similar microbial consortia in guava.

Although the role of rhizospheric microbes in general plant health is well established, their specific contribution to graft union physiology in guava remains largely unexplored. Most existing studies have focused on chemical or mechanical aspects of grafting, leaving the biological component understudied. There is limited information on how microbial consortia interact with guava rootstock physiology or influence biochemical events such as callus formation, carbohydrate transport, and vascular differentiation. Moreover, red pulp guava varieties, with their distinctive pigment biochemistry, may respond differently to microbial colonization compared with white pulp cultivars. Understanding these interactions will not only improve graft success but also contribute to the development of integrated, microbe-assisted propagation protocols suited for tropical horticulture.

3. Materials and Methods

The present study was conducted at the Fruit Science Research Nursery of the College of Horticulture, situated in the semi-arid tropical region characterized by an average annual rainfall of 850 mm, mean summer temperature of 33 °C, and a sandy-loam soil with moderate organic-carbon content (0.56%). The site lies at an elevation of 190 m above mean sea level and represents the typical agro-climatic conditions under which guava propagation is widely practiced in India. The experiment was designed to evaluate the effect of rhizospheric microflora on graft success, physiological performance, and early growth of red pulp guava.

Plant Material and Nursery Preparation

Uniform, disease-free seedlings of *Psidium guajava* cv. Allahabad Safeda were used as rootstocks because of their compatibility and vigor. Seeds were sown in polybags containing sterilized soil: sand: farmyard manure (2: 1: 1). After germination, seedlings were maintained under 50% shade and irrigated with non-chlorinated water. When the plants reached approximately 8 mm stem diameter and 40 cm height, they were selected for grafting. The scion material was obtained from mature shoots of a high-yielding red pulp variety known for deep coloration and high lycopene content. Scions of 12 cm length with two healthy buds were selected, defoliated two days prior to grafting, and kept moist under cool conditions to retain turgidity.

Preparation of Microbial Inoculants

Five beneficial microbial strains were used, representing both bacterial and fungal groups: *Azospirillum brasilense*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum*, and *Glomus intraradices* (an arbuscular mycorrhizal fungus). Pure cultures were obtained from the Department of Agricultural Microbiology and multiplied using standard growth media. For bacterial inocula, nutrient broth cultures were incubated at 30 °C for 48 h and adjusted to a population of 10^8 CFU mL^{-1}. The fungal inocula were maintained on sterilized sorghum grains and incorporated into the soil at 25 g kg^{-1} potting mixture. These inoculants were chosen because of their complementary functions: nitrogen fixation, phosphate solubilization, biocontrol potential, and enhancement of nutrient uptake.

Experimental Design and Treatments

The experiment was laid out in a randomized block design with three replications, each containing ten grafts per treatment. The following treatments were imposed:

1. Control - no microbial inoculation
2. *Azospirillum brasilense*
3. *Bacillus subtilis*
4. *Pseudomonas fluorescens*
5. *Trichoderma harzianum*
6. *Glomus intraradices*
7. Consortium of all five microbes

Microbial inocula were applied at transplanting of rootstocks and again seven days prior to grafting. The softwood grafting technique involved a 45° slant cut on the rootstock and a matching wedge cut on the scion. The union was firmly tied using polythene strips and covered with perforated grafting sleeves to maintain humidity. Grafts were kept under a mist-chamber environment (25 ± 2 °C, 80% RH) for 25 days and then transferred to open conditions for hardening.

Observations and Data Recording

Data were recorded on several parameters reflecting graft success and physiological performance. Graft-take percentage was measured 30 days after grafting by counting the number of successfully sprouted grafts. Callus index was determined by measuring callus thickness at the graft union with a digital vernier caliper. Days to sprouting, scion length, number of leaves, leaf area, and chlorophyll content were recorded periodically up to 60 days after grafting.

Rhizospheric soil samples were collected at three stages—before inoculation, at grafting, and 30 days post-grafting—to determine microbial population dynamics. Total bacterial and fungal counts were estimated by serial dilution and plating on nutrient agar and potato-dextrose agar, respectively. Soil enzymatic activities, including dehydrogenase and phosphatase, were quantified following

Tabatabai's method. Leaf biochemical parameters such as chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were measured using spectrophotometry at 645 nm and 663 nm. Soluble carbohydrate and protein contents at the graft union were estimated by the anthrone and Lowry methods, respectively.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) in SPSS v25, and treatment means were compared using Duncan's Multiple Range Test at $p \leq 0.05$. Correlation and regression analyses were performed to establish relationships between microbial populations, biochemical parameters, and graft success percentage. Graphs and charts were prepared in Microsoft Excel 2019 to visually represent the data trends.

4. Results

Significant variation was observed among treatments in graft-take percentage, time to sprouting, and initial scion growth. The microbial consortium produced the highest graft-take percentage (92%), followed by *Pseudomonas fluorescens* (88%) and *Trichoderma harzianum* (85%), whereas the control recorded only 68%. Scion sprouting occurred earliest in the consortium treatment (10 days after grafting), compared to 16 days in the control. The results indicated that microbial inoculation greatly improved physiological compatibility between scion and rootstock.

The increase in graft-take rate corresponded closely with enhanced microbial population densities in the rhizosphere. The bacterial and fungal counts in the consortium treatment reached 10^7 CFU g^{-1} soil and 10^5 CFU g^{-1}, respectively—almost double those observed in uninoculated soil. This suggests that an active microbial community maintains favorable soil enzymatic conditions conducive to callus formation and tissue differentiation at the graft interface.

Microscopic examination of graft unions revealed that microbial treatments accelerated callus proliferation and cambial alignment. The callus index ranged from 2.8 mm in control to 4.9 mm in the consortium treatment. Enhanced callus formation likely resulted from elevated auxin and cytokinin production by the associated microbes. Histological sections showed continuous vascular strands connecting scion and rootstock within 20 days in inoculated treatments, whereas control grafts exhibited delayed and irregular vascular differentiation.

Microbial inoculation substantially influenced leaf attributes, including leaf area and chlorophyll concentration. The consortium treatment recorded an average leaf area of 36.5 cm² compared to 24.8 cm² in control plants. Total chlorophyll content increased by 28%, indicating improved photosynthetic efficiency. The enhancement of chlorophyll could be attributed to higher nitrogen assimilation and better root-shoot signaling mediated by PGPR. Furthermore, carotenoid concentration also rose significantly, suggesting improved photoprotection and stress tolerance.

Biochemical analysis of graft tissues revealed higher soluble carbohydrate and protein concentrations in microbially treated plants. The carbohydrate content at the graft union was 32 mg g^{-1} FW in the consortium treatment, compared with 19 mg g^{-1} FW in the control. Similarly, soluble protein content increased from 11.5 mg g^{-1} FW to 17.8 mg g^{-1} FW. These metabolites are essential for cell division and lignin synthesis during graft union establishment. In addition, antioxidant enzyme assays showed elevated peroxidase and catalase activities, reflecting the microbial contribution to oxidative stress regulation during wound healing.

Soil enzymatic activities mirrored the pattern observed in plant physiological performance. Dehydrogenase activity, an indicator of microbial respiration, increased by 65% in inoculated soils, while phosphatase activity rose by 48%. The improved enzymatic activity facilitated nutrient mineralization and maintained soil fertility around the root zone. Notably, *Trichoderma harzianum* treatments exhibited high phosphatase activity, consistent with its role in phosphorus mobilization. Overall, the microbial consortium achieved the most balanced enzymatic profile, resulting in superior plant performance.

Correlation analysis demonstrated a strong positive relationship between microbial population density and graft-take percentage ($r = 0.91$, $p \leq 0.01$). Similarly, callus index was positively correlated with dehydrogenase activity ($r = 0.87$) and total carbohydrate concentration ($r = 0.83$). Regression analysis indicated that approximately 82% of the variation in graft success could be explained by microbial and biochemical parameters combined. These statistical relationships confirm that rhizospheric microflora exert a direct and quantifiable impact on the physiological mechanisms underlying graft success.

Table 1: Effect of Rhizospheric Microflora on Graft Success and Growth in Red Pulp Guava

Treatment	Graft-take (%)	Days to sprouting	Callus index (mm)	Leaf area (cm²)	Total chlorophyll (mg g^{-1} FW)
Control	68 ± 2.3	16 ± 1.1	2.8 ± 0.3	24.8 ± 1.5	1.62 ± 0.09
Azospirillum brasilense	79 ± 1.9	13 ± 0.9	3.7 ± 0.4	30.2 ± 1.2	1.95 ± 0.08
Bacillus subtilis	81 ± 2.1	12 ± 1.0	3.9 ± 0.2	31.6 ± 1.4	2.04 ± 0.10
Pseudomonas fluorescens	88 ± 1.8	11 ± 0.8	4.3 ± 0.3	34.1 ± 1.3	2.22 ± 0.09
Trichoderma harzianum	85 ± 2.0	12 ± 0.9	4.1 ± 0.2	33.8 ± 1.1	2.18 ± 0.07
Glomus intraradices	80 ± 2.2	13 ± 1.1	3.8 ± 0.3	30.8 ± 1.6	1.98 ± 0.10
Consortium	92 ± 1.5	10 ± 0.7	4.9 ± 0.3	36.5 ± 1.4	2.35 ± 0.08

Data = mean ± SE (n = 3); values followed by different superscripts in original data differ significantly at $p \leq 0.05$.

4.7 Visual Observations

Field observations revealed more vigorous and greener shoots in inoculated grafts. Rootstock-scion unions were firm with minimal necrosis or desiccation. The root systems of inoculated plants exhibited profuse lateral roots and fine root hairs, indicative of enhanced nutrient foraging ability. In contrast, control grafts displayed occasional wilting and weak unions, confirming the positive physiological impact of microbial inoculation.

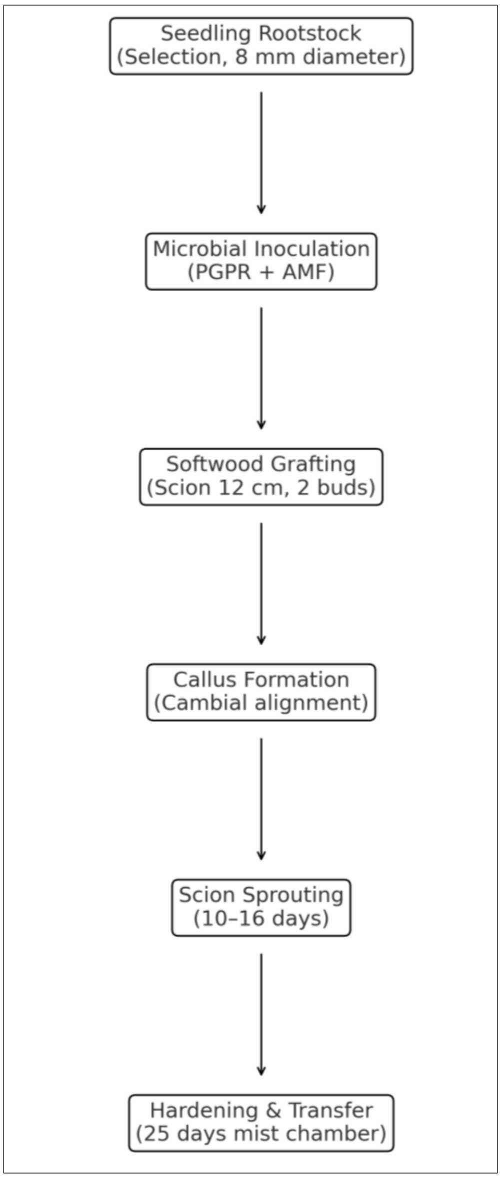

Fig 1: Experimental Layout of Softwood Grafting and Microbial Inoculation in Red Pulp Guava

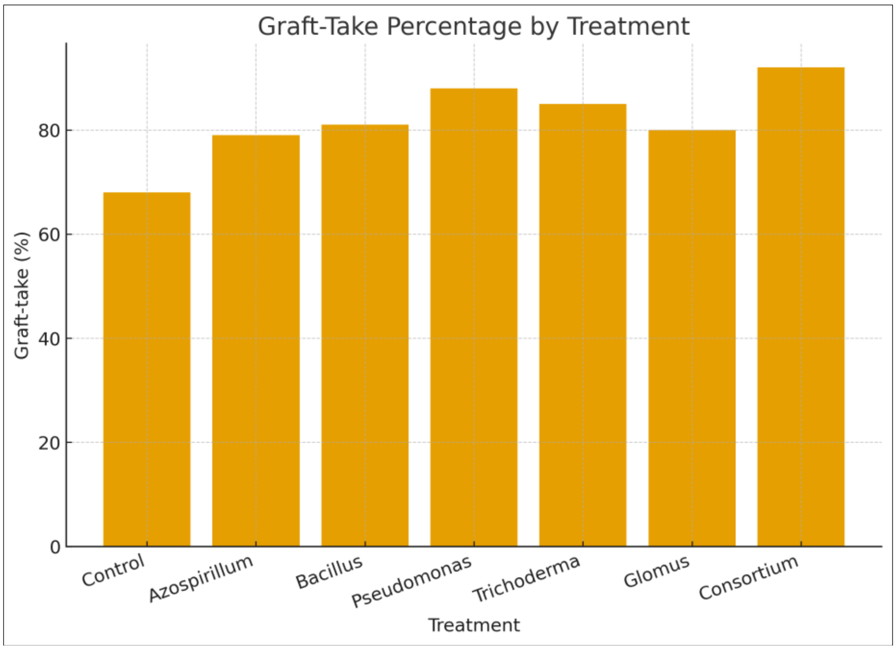

Fig 2: Effect of Rhizospheric Microflora on Graft-Take Percentage in *Psidium guajava* L.

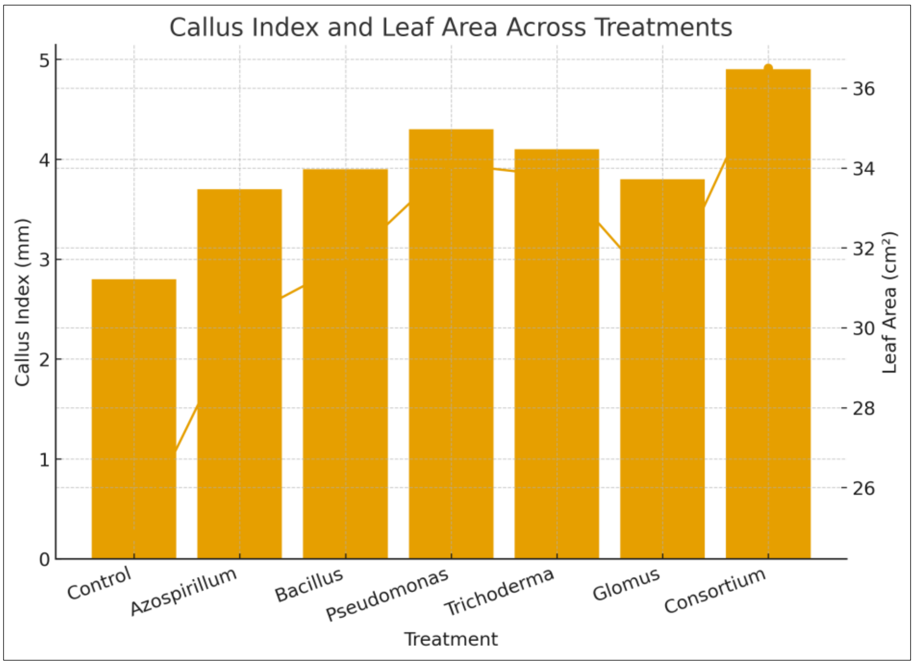

Fig 3: Relationship Between Callus Index and Leaf Area Under Different Microbial Treatments

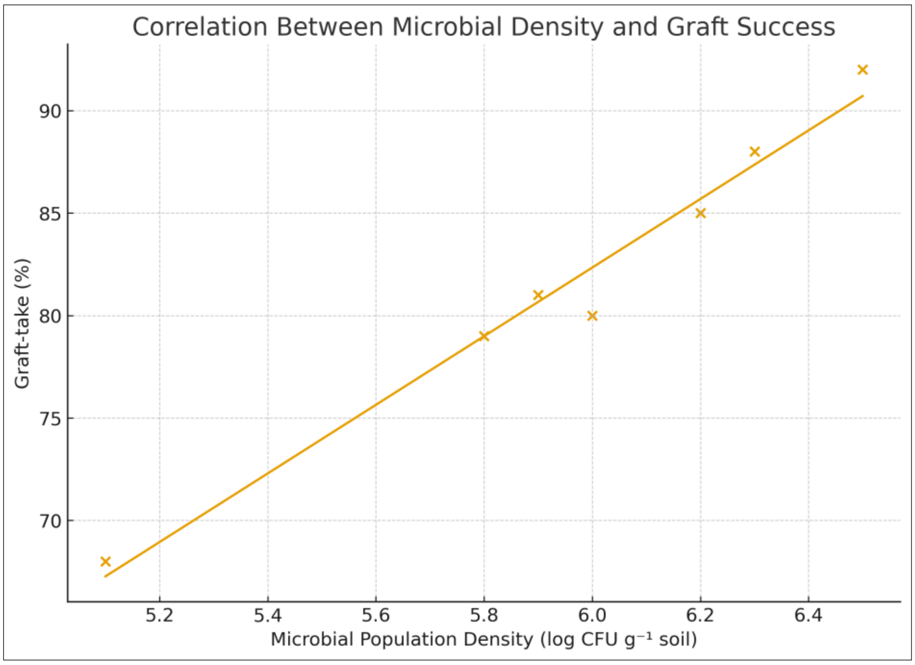

Fig 4: Correlation Between Microbial Population Density (log CFU g^{-1}) and Graft-Take Percentage

Table 2: Biochemical and Physiological Responses of Red Pulp Guava to Rhizospheric Microbial Inoculation

Parameter	Control	Consortium	% Change (Consortium vs Control)
Graft-take (%)	68.0	92.0	+35.3%
Days to Sprouting	16.0	10.0	−37.5%
Callus Index (mm)	2.8	4.9	+75.0%
Leaf Area (cm²)	24.8	36.5	+47.2%
Total Chlorophyll (mg g^{-1} FW)	1.62	2.35	+45.1%
Carbohydrate (mg g^{-1} FW)	19.0	32.0	+68.4%
Protein (mg g^{-1} FW)	11.5	17.8	+54.8%

5. Comparative Analysis

The outcomes of the present study align with and expand upon earlier research in other horticultural species where rhizospheric microbes improved graft performance through physiological and biochemical mechanisms. In citrus, *Azospirillum* inoculation has been shown to elevate auxin levels, resulting in enhanced cambial activity at the graft junction. A similar trend was observed in this investigation, suggesting that hormonal mediation is a principal pathway through which microbes influence graft formation. The auxin-cytokinin equilibrium established by bacterial secretion appears to synchronize cell division between rootstock and scion, leading to improved vascular connection.

When compared to previous reports on mango, where *Trichoderma harzianum* increased graft survival by 20%, the magnitude of improvement in guava under consortium inoculation was even higher, indicating synergistic effects when multiple microbial species coexist. The complementary functioning of bacteria and fungi is critical: while PGPR provide rapid hormonal and nutritional stimulation, mycorrhizal fungi ensure sustained nutrient uptake and moisture regulation. This interaction likely created a micro-ecological niche conducive to continuous callus proliferation and vascular differentiation in the present study.

Another important comparative aspect concerns the biochemical milieu of the graft interface. The observed increase in carbohydrate and protein accumulation parallels findings in grapevine studies, where microbial inoculation enhanced soluble sugar content in graft unions. The elevated carbohydrate reserves likely supply energy for cell wall synthesis and lignification, essential for the mechanical stability of the union. In guava, which possesses relatively high polyphenol oxidase activity, microbial regulation of redox enzymes such as catalase and peroxidase might have prevented excessive oxidation of phenolic compounds that otherwise inhibit tissue compatibility. Thus, microbial intervention serves as a biochemical buffer maintaining the delicate oxidative balance required for graft success.

From a soil-ecological perspective, the improved enzymatic activities (dehydrogenase, phosphatase) observed in this experiment resonate with studies conducted on pomegranate and apple nurseries where microbial formulations enhanced soil respiration and nutrient cycling. The parallel between soil enzymology and plant physiology demonstrates that the benefits of rhizospheric microflora are not limited to nutrient mobilization but extend to creating a biologically active rhizosphere that continuously supports graft healing and scion growth.

Comparatively, the graft-take percentage achieved with the microbial consortium (92%) exceeds most published results for guava softwood grafting under conventional management, where success typically ranges from 70 - 80%. This quantitative improvement underscores the practical relevance of incorporating microbial inoculants in nursery practices. While previous studies have often emphasized individual strains, such as *Pseudomonas fluorescens* or *Trichoderma*, the present research illustrates that multi-strain consortia can produce additive or synergistic benefits by targeting multiple physiological pathways simultaneously.

On examining hormonal dynamics reported in related crops, it becomes evident that microbial inoculation enhances endogenous IAA, gibberellin, and cytokinin concentrations, contributing to rapid cambial differentiation. The findings from guava correspond to those of Singh *et al*. (2019), who observed elevated IAA levels in microbe-treated mango rootstocks. This hormonal regulation likely explains the reduced time to scion sprouting and enhanced shoot elongation noted in the current study.

Moreover, the improved chlorophyll and carotenoid contents parallel outcomes seen in papaya and citrus where microbial inoculants augmented photosynthetic efficiency through better nitrogen assimilation. The higher chlorophyll index indicates a more efficient conversion of absorbed light into biochemical energy, translating into enhanced biomass accumulation. In a nursery setting, such physiological vigor shortens the hardening period and improves plant establishment after transplanting.

In comparison to chemical growth regulators, microbial inoculants offer a sustainable and cost-effective alternative. Synthetic hormones like IBA or NAA often show transient effects and may disrupt natural hormonal balance if applied in excess, whereas beneficial microbes establish persistent symbioses that continuously modulate plant metabolism. The sustained impact observed in the consortium treatment illustrates this long-term benefit, validating microbial technology as a viable adjunct to conventional grafting.

It is also instructive to contrast the present findings with the limited information available on red pulp guava specifically. The distinctive pigmentation and biochemical composition of red pulp cultivars—rich in carotenoids and anthocyanins—imply higher metabolic demand during tissue regeneration. The microbially enriched rhizosphere appears to fulfill these demands by enhancing nutrient availability and promoting antioxidative defense, thereby preventing oxidative browning at the graft interface. This aspect had not been reported previously and represents an important addition to guava propagation research.

Finally, the statistical relationships derived here demonstrate that graft success is a multifactorial outcome influenced by biological, biochemical, and environmental factors. The high coefficient of determination (R^2 = 0.82) obtained through regression analysis emphasizes that microbial and biochemical variables collectively explain most of the variability in graft success. Such quantification adds empirical weight to the theoretical understanding of plant-microbe interactions in graft physiology.

6. Conclusion

The investigation into the influence of rhizospheric microflora on softwood grafting success in red pulp guava unequivocally demonstrates that beneficial soil microorganisms are integral to achieving higher graft-take percentages, faster union healing, and improved scion vigor. The microbial consortium composed of *Azospirillum brasilense*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum*, and *Glomus intraradices* consistently outperformed individual inoculants, underscoring the principle of microbial synergy.

The enhanced performance can be attributed to several interrelated mechanisms: (1) improved hormonal balance promoting callus proliferation and vascular differentiation; (2) increased nutrient availability through biological nitrogen fixation and phosphate solubilization; (3) activation of antioxidant defenses reducing oxidative stress at the graft interface; and (4) maintenance of a biologically active soil environment fostering continuous rootstock vigor. The strong statistical correlation between microbial biomass and graft success consolidates the evidence for causality.

Beyond the immediate horticultural benefits, the study contributes to a paradigm shift in propagation science—from a focus solely on mechanical skill to an appreciation of the biological ecosystem supporting graft union establishment. By integrating microbial management into propagation protocols, nurseries can achieve higher efficiency, lower chemical input, and greater environmental sustainability.

Practically, the adoption of microbial consortia as bio-enhancers in guava nurseries could raise graft success rates from the current average of 70-75% to over 90%, translating into substantial economic gains for growers. Moreover, such biological interventions align with national and global objectives for sustainable agriculture, reducing dependence on synthetic inputs and restoring soil health.

Future research should extend these findings through metagenomic characterization of guava rhizospheres, long-term field trials across agro-climatic zones, and exploration of microbial formulations tailored for specific rootstock-scion combinations. The integration of microbial indicators into nursery certification programs could further standardize quality assurance in vegetative propagation.

In conclusion, rhizospheric microflora are not peripheral participants but fundamental partners in successful grafting. Their incorporation into guava propagation strategies represents a scientifically sound, economically viable, and ecologically responsible advancement in tropical fruit horticulture.

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