PROPAGAÇÃO CLONAL DE GENÓTIPOS DE *Psidium*: EFEITOS DE BIORREGULADORES DE CRESCIMENTO, SAZONALIDADE E RESISTÊNCIA A *Meloidogyne enterolobii*

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UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE DARCY RIBEIRO
CAMPOS DOS GOYTACAZES – RJ
MARÇO - 2026

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Orientadora: Cláudia Sales Marinho

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Orientadora

Para a criança que fui. Para o homem que me tornei. Foi difícil, mas aprendemos a ancorar um navio no espaço. Obrigado por todas as vezes que você não se permitiu desistir.

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RESUMO

GALVÃO, Sydney Pereira; D.Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro. Março de 2026. Propagação clonal de genótipos de *Psidium:* efeitos de biorreguladores de crescimento, sazonalidade e resistência a *Meloidogyne enterolobii***.** Orientadora: D. Sc.; Cláudia Sales Marinho.

A goiabeira (*Psidium guajava* L.) é uma das principais frutíferas tropicais cultivadas no Brasil, porém sua expansão tem sido limitada pelo declínio da goiabeira, doença complexa associada à interação entre *Meloidogyne enterolobii* e patógenos fúngicos do solo. Nesse contexto, a propagação clonal de genótipos resistentes e o uso de biorreguladores de crescimento surgem como estratégias promissoras para a produção de mudas de alta qualidade e para o desenvolvimento de porta-enxertos adaptados. Este trabalho teve como objetivo verificar os efeitos da sazonalidade e do uso de biorreguladores de crescimento sobre a eficiência da propagação clonal e o desenvolvimento de genótipos de *Psidium*, bem como avaliar a expressão da resistência a *M. enterolobii* após a multiplicação vegetativa. Os experimentos foram conduzidos em um minijardim clonal e em condições de viveiro, avaliando-se os efeitos da aplicação de ácido giberélico (GA_3) sobre o vigor das minicepas em relação ao crescimento vegetativo, emissão de brotações, produção de miniestacas em diferentes estações do ano. Adicionalmente, foi avaliado o uso do análogo de brassinosteroide, Biobrás-1,6 sobre o desenvolvimento morfofisiológico das mudas durante a aclimatação, por meio de parâmetros de crescimento, índices de pigmentos foliares e balanço de nitrogênio. A resistência a *M. enterolobii* foi avaliada em genótipos clonados, considerando o parasitismo, o crescimento das plantas e alterações fisiológicas. A sazonalidade influenciou significativamente a produção de brotações, e o crescimento das miniestacas, com respostas diferenciadas entre genótipos. A aplicação de GA_3 aumentou o vigor das minicepas e a produtividade de miniestacas, enquanto o Biobrás-16 promoveu maior desenvolvimento da parte aérea e do sistema radicular, além de melhorar parâmetros fisiológicos associados à eficiência metabólica das mudas. Houve variação genotípica em relação à resposta à inoculação com *M. enterolobii*, após a propagação clonal, com alguns genótipos possuindo menores níveis de parasitismo e melhor desempenho fisiológico. Conclui-se que a eficiência da propagação clonal de *Psidium* é influenciada pela interação

entre fatores genéticos, sazonais e hormonais, e que o uso de biorreguladores de crescimento associado à seleção de genótipos resistentes constitui uma estratégia viável para a produção de porta-enxertos de alta qualidade, contribuindo para a sustentabilidade frente ao declínio.

Palavras-chave: goiabeira, miniestaquia, brassinosteroides; ácido giberélico, nematoide das galhas.

ABSTRACT

GALVÃO, Sydney Pereira; D.Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro**.** March**,** 2026**.** Clonal propagation of *Psidium* genotypes: effects of, plant growth regulators, seasonality and resistance to *Meloidogyne enterolobii***.** Advisor: D.Sc.; Cláudia Sales Marinho.

Guava (*Psidium guajava* L.) is one of the main tropical fruit crops cultivated in Brazil; however, its expansion has been limited by guava decline, a complex disease associated with the interaction between *Meloidogyne enterolobii* and soilborne fungal pathogens. In this context, the clonal propagation of resistant genotypes and the use of plant growth regulators emerge as promising strategies for the production of high-quality seedlings and the development of adapted rootstocks. This study aimed to evaluate the effects of seasonality and the application of plant growth regulators on the efficiency of clonal propagation and the development of *Psidium* genotypes, as well as to assess the expression of resistance to *M. enterolobii* after vegetative propagation. The experiments were conducted in a clonal minigarden and under nursery conditions, evaluating the effects of gibberellic acid (GA_3) application on the vigor of ministumps in terms of vegetative growth, shoot emission, and minicutting production across different seasons of the year. Additionally, the use of the brassinosteroid analogue Biobrás-16 was evaluated on the morphophysiological development of seedlings during acclimatization, through growth parameters, leaf pigment indices, and nitrogen balance. Resistance to *M. enterolobii* was assessed in clonally propagated genotypes by analyzing parasitism, plant growth, and physiological alterations. Seasonality significantly influenced shoot production and minicutting growth, with differential responses among genotypes. GA_3 application increased ministump vigor and minicutting productivity, whereas Biobrás-16 promoted greater shoot and root development and improved physiological parameters associated with the metabolic efficiency of seedlings. Genotypic variation was observed in response to *M. enterolobii* inoculation after clonal propagation, with some genotypes exhibiting lower levels of parasitism and better physiological performance. It is concluded that the efficiency of *Psidium* clonal propagation is influenced by the interaction among genetic, seasonal, and hormonal factors, and that the use of plant growth regulators combined with the

selection of resistant genotypes represents a viable strategy for producing high-quality rootstocks, contributing to sustainability in the face of guava decline.

Keywords: guava, minicutting, brassinosteroids, gibberellic acid, root-knot nematode.

1. INTRODUÇÃO

A goiabeira (*Psidium guajava* L.) destaca-se entre as frutíferas tropicais pelo elevado valor nutricional de seus frutos e ampla adaptação a diferentes condições climáticas, sendo cultivada em diversas regiões tropicais e subtropicais do mundo (Singh et al., 2018; Das, 2020). No Brasil, a cultura tem expressiva relevância econômica e social, com participação na geração de renda e no abastecimento de mercados internos e agroindustriais. Apesar de seu potencial produtivo, a expansão sustentável da goiabeira tem sido limitada por problemas fitossanitários, entre os quais se destaca o nematoide das galhas *Meloidogyne enterolobii*.

M. enterolobii é considerado um dos fitonematoides mais agressivos associados à goiabeira, devido à sua elevada capacidade reprodutiva, ampla gama de hospedeiros e habilidade de romper mecanismos de resistência presentes em diferentes espécies vegetais (Gomes et al., 2017; Collett et al., 2021). O parasitismo radicular compromete a absorção de água e nutrientes, altera os exsudatos radiculares e favorece a colonização por microrganismos oportunistas, culminando na doença conhecida como declínio da goiabeira (Souza et al., 2023). A ausência de cultivares comerciais resistentes torna o manejo desse patógeno um grande desafio, reforçando a necessidade de estratégias baseadas no melhoramento genético e no uso de porta-enxertos tolerantes.

Nesse contexto, espécies e híbridos do gênero *Psidium* têm sido explorados como fontes de resistência a *M. enterolobii*, tendo variabilidade genética promissora para programas de seleção de porta-enxertos (Souza et al., 2018; Gomes et al., 2017). Para que esses genótipos selecionados possam ser utilizados em escala comercial, torna-se fundamental a adoção de métodos eficientes de propagação clonal, capazes de garantir uniformidade das mudas e manutenção das características genéticas de interesse. Entre as técnicas disponíveis, a miniestaquia tem se consolidado como um sistema eficiente para a multiplicação clonal de *Psidium*, permitindo produção contínua de brotações em ambiente protegido e redução do tempo de formação de mudas (Arantes et al., 2021).

O desempenho das minicepas e das mudas produzidas por miniestaquia é influenciado por fatores ambientais, especialmente pela sazonalidade, que regula o

crescimento vegetativo, a emissão de brotações e o enraizamento (Grossiord et al., 2020). Além disso, o uso de reguladores de crescimento tem sido investigado como ferramenta complementar para potencializar a eficiência do sistema de propagação clonal. O ácido giberélico (GA_3) tem atuação na elongação celular e na estruturação dos ramos, podendo aumentar o vigor vegetativo e a produção de estacas (Rademacher, 2015; Arantes et al., 2021). De forma complementar, os brassinosteroides têm amplo papel fisiológico no crescimento vegetal, na formação do sistema radicular e na modulação de respostas ao estresse, com efeitos positivos durante a fase de aclimatação de mudas (Nolan et al., 2020; Kumar et al., 2023).

Entretanto, a resposta aos reguladores de crescimento não é uniforme, sendo modulada pelo genótipo, pelas condições ambientais e pelo estágio de desenvolvimento das plantas (Tanveer et al., 2019; Chaudhuri et al., 2022). A interação entre fatores genéticos e sazonais pode alterar significativamente a magnitude dos efeitos desses compostos, o que reforça a necessidade de abordagens integradas que considerem simultaneamente genótipo, ambiente e manejo hormonal na produção de mudas clonais de *Psidium*.

Diante desses aspectos, torna-se essencial aprofundar o entendimento sobre os fatores que governam o crescimento vegetativo, a eficiência da propagação clonal e a estabilidade de características como resistência ao nematoide em genótipos de *Psidium*, considerando a influência da sazonalidade, do uso de reguladores de crescimento e das respostas fisiológicas associadas ao sistema radicular. A integração desses conhecimentos é fundamental para o aprimoramento de sistemas mais eficientes e sustentáveis de produção de porta-enxertos resistentes ao nematoide das galhas.

2. REVISÃO DE LITERATURA

2.1. O gênero *Psidium*

O gênero *Psidium* pertence à família Myrtaceae e compreende um grupo diverso de espécies neotropicais com ampla distribuição nas Américas. Revisões taxonômicas recentes indicam que o número de espécies aceitas no gênero varia conforme a base utilizada, refletindo revisões contínuas na delimitação taxonômica. De acordo com a Plants of the World Online (POWO), atualmente são reconhecidas 79 espécies aceitas para *Psidium* (Royal Botanic Gardens, Kew, 2024). Enquanto estudos filogenéticos e revisões sistemáticas apontam que novas espécies continuam sendo descritas e incorporadas ao gênero (Proença et al., 2022).

Do ponto de vista citogenético, as espécies da família Myrtaceae possuem, em geral, número básico de cromossomos x = 11, com predominância de citótipos diploides (2n = 2x = 22). Entretanto, estudos recentes evidenciam ampla variação cromossômica no gênero *Psidium*, com ocorrência frequente de poliploidia e múltiplos níveis de ploidia dentro de determinadas espécies, especialmente em *P. cattleyanum* e congêneres, com registros que variam desde 2n = 22 até valores superiores a 2n = 44 (Rojas-Gómez et al., 2020; Spadeto et al., 2022).

Esse gênero engloba a goiabeira (*Psidium guajava* L.) e diversos araçazeiros, muitos nativos do Brasil, os quais produzem frutos comestíveis e apresentam relevância econômica, agronômica, ecológica e ornamental. Entre os principais representantes destacam-se *Psidium guajava*, *Psidium guineense* e *Psidium cattleyanum*, amplamente conhecidos tanto no âmbito científico quanto popular por seu potencial produtivo (Pereira e Nachtigal, 2002; Souza et al., 2014; Souza et al., 2015; Proença et al., 2022). Além dessas espécies amplamente difundidas, outras vêm ganhando importância em sistemas produtivos e em programas de prospecção genética, como *Psidium friedrichsthalianum*, *Psidium myrtoides*, *Psidium sartorianum* e *Psidium araucanum*, reconhecidas por sua adaptação a diferentes ambientes e por características agronômicas promissoras (Bezerra et al., 2006; Franzon et al., 2009; Proença et al., 2022). Destaca-se ainda o potencial de *Psidium myrtoides* e *Psidium friedrichsthalianum* como fontes de resistência a *Meloidogyne enterolobii*, ampliando suas possibilidades de uso em programas de melhoramento genético e manejo

fitossanitário da cultura da goiabeira (Marques e Pimentel, 2012; Chiamolera et al., 2018).

2.2. Goiabeira (*Psidium guajava*)

A goiabeira (*Psidium guajava* L.), principal espécie do gênero *Psidium* do ponto de vista agrícola, é nativa da região tropical das Américas, com centro de origem situado entre o sul do México e o norte da América do Sul. Atualmente, possui ampla distribuição em regiões tropicais e subtropicais, sendo encontrada em praticamente todos os estados brasileiros (Flora Brasil 2025).

Trata-se de uma espécie arbórea de porte pequeno a médio, com copa variando entre 3 e 5 metros de altura. Possui caule tortuoso, casca lisa e delgada, geralmente quadrangular quando jovem. As folhas são opostas, oblongas, subcoriáceas, aromáticas e de coloração verde-amarelada. As flores são brancas, hermafroditas, pentâmeras, solitárias ou agrupadas em duas ou três, localizadas nas axilas das folhas e nas brotações de ramos maduros. O fruto é uma baga com variação de tamanho, forma e coloração da polpa, textura firme e numerosas sementes (Manica et al., 2001; Pereira e Nachtigal, 2002; Pommer e Murakami, 2009).

Os frutos da goiabeira possuem elevado valor nutricional, sendo ricos em vitaminas A, B1, B2, B6 e C, além de minerais como cálcio e fósforo, apresentando baixo teor calórico, elevado teor de fibras alimentares e alto potencial antioxidante (Gomes Filho et al., 2016; Rathnayake et al., 2024). Esses atributos favorecem seu consumo tanto in natura quanto na forma de produtos industrializados, como sucos, doces e polpas processadas (Franzon et al., 2009; Pereira et al., 2016).

Entre as cultivares de goiabeira produzidas no Brasil, destaca-se a cultivar ‘Paluma’, amplamente difundida. Trata-se de um clone derivado da goiaba Rubi-Supreme, obtido a partir de sementes de polinização aberta, caracterizando-se por elevada produtividade, podendo atingir valores superiores a 50 t ha^{-1}. Seus frutos possuem massa entre 140 e 250 g, forma ovoide com pescoço curto, diâmetro longitudinal de 8 a 10 cm, transversal de 7 a 9 cm, polpa de coloração vermelha intensa, baixa porcentagem de sementes (4,96%) e elevado rendimento de polpa (93,76%) (Malavolta, 2000; Pommer et al., 2006).

2.3. Araçazeiros

As espécies silvestres do gênero *Psidium* produtoras de frutos comestíveis são, de modo geral, conhecidas popularmente como araçás, com variações regionais na nomenclatura comum. Os araçazeiros possuem elevado valor nutricional e funcional, sendo fontes de compostos fenólicos, antioxidantes e óleos essenciais, o que tem despertado crescente interesse para uso alimentar, farmacêutico e agroindustrial (Franzon et al., 2009).

Entre as espécies de maior destaque encontra-se *Psidium cattleyanum* Sabine, popularmente conhecida como araçá, goiaba-morango (strawberry guava), goiaba-roxa, goiaba-amarela ou waiawi. Trata-se de uma espécie nativa da costa atlântica do Brasil, atualmente amplamente distribuída em regiões tropicais e subtropicais do mundo, incluindo ilhas do Caribe, Havaí e partes da Oceania, onde inclusive possui comportamento invasor em alguns ecossistemas (Pommer e Murakami, 2009; Denslow et al., 2024). A planta possui porte arbustivo a arbóreo, variando entre 1,8 e 4,0 m de altura, com ramos resistentes e folhas perenes, simples e opostas. As flores são solitárias, brancas e aromáticas, enquanto os frutos são pequenos, abundantes, de formato geralmente obovado, com polpa translúcida de coloração púrpura ou amarela e sabor doce-ácido característico (Mani et al., 2011; Bremenkamp, 2015).

Do ponto de vista reprodutivo, os grãos de pólen de *P. cattleyanum* apresentam elevada variabilidade morfológica, e as sementes possuem tegumento duro e impermeável, o que resulta em germinação lenta e frequentemente desuniforme (Corrêa et al., 2018).

Outro araçazeiro de grande importância é *Psidium guineense* Sw., espécie que ocorre naturalmente em diversas regiões da América do Sul. Trata-se de um arbusto ou pequena árvore que pode atingir até seis metros de altura, possui as brotações jovens frequentemente recobertas por tricomas de coloração marrom-avermelhada a cinza-amarelada. As folhas são coriáceas, de formato elíptico a obovado, com nervuras laterais bem evidentes, enquanto os frutos são globosos a piriformes, de coloração amarela quando maduros, geralmente coroados pelos remanescentes das sépalas (Landrum et al., 1995; Pommer e Murakami, 2009; Mani et al., 2011).

Além do valor alimentar, espécies de araçazeiros têm sido investigadas quanto ao seu potencial fitossanitário, especialmente como fontes de resistência a patógenos, como *Meloidogyne enterolobii*. Pesquisas apontam que algumas espécies silvestres do gênero *Psidium* apresentam respostas superiores à goiabeira comercial frente ao parasitismo por nematoides, o que reforça seu papel estratégico em programas de prospecção de porta-enxertos resistentes (Marques e Pimentel, 2012; Chiamolera et al., 2018).

2.4. Importância econômica de *Psidium*

A goiabeira, espécie de maior destaque comercial do gênero *Psidium*, possui significativa contribuição para a fruticultura brasileira e mercados regionais. A produção nacional de goiaba concentra-se principalmente nos estados do Nordeste e do Sudeste, sendo Pernambuco e São Paulo os maiores produtores do país, com aproximadamente 205,9 mil t e 182,1 mil t, respectivamente, em 2023/24. (IBGE, 2025). Outros estados que figuram entre os principais produtores: Paraná (47,1 mil t), Bahia (45,1 mil t) e Ceará (21,2 mil t), enquanto o Rio de Janeiro contribuiu com cerca de 18,1 mil t no mesmo período, reforçando a diversidade regional da produção goiabeira no Brasil (IBGE, 2025).

A área dedicada ao cultivo de goiaba no país soma cerca de 22,5 mil hectares, com uma produtividade média em torno de 25,9 t ha^{-1}, refletindo o caráter produtivo da cultura nas principais regiões brasileiras, especialmente em sistemas irrigados no Nordeste e em áreas tradicionais no Sudeste (IBGE, 2025). No contexto municipal, municípios como Santa Maria da Boa Vista (PE), Petrolina (PE) e Carlópolis (PR) destacaram-se como os maiores produtores em 2024, evidenciando a importância da fruticultura tropical para economias locais e cadeias de valor regionais (IBGE, 2025).

A goiaba (*Psidium guajava* L.) é amplamente apreciada pelo seu sabor característico, aroma e textura, fatores que contribuem para sua elevada aceitação entre consumidores, tanto in natura quanto em produtos derivados (Moon et al., 2018). Estudos indicam que aspectos físico-químicos como cor, firmeza, acidez e compostos voláteis influenciam significativamente a preferência do consumidor e o potencial de uso industrial da fruta (Costa et al., 2020).

Embora outras espécies do gênero *Psidium* também tenham potencial de exploração econômica, seja pelo consumo direto, processamento em polpas ou uso de extratos funcionais, a goiabeira permanece como a espécie de maior expressão no contexto da fruticultura nacional. Outras espécies possuem uso restrito ou extrativista, sem expressão econômica organizada (Bezerra et al., 2006; Franzon et al., 2009).

2.5. Declínio da goiabeira

O declínio da goiabeira constitui uma doença de etiologia complexa que tem provocado expressiva redução da produtividade e restringido a expansão de áreas cultivadas, resultando, em muitos casos, na erradicação de pomares adultos em plena fase produtiva (Gomes et al., 2011). A doença decorre da interação entre o fitonematoide *Meloidogyne enterolobii* e o fungo do solo *Neocosmospora falciformis*, cuja ação conjunta intensifica os danos ao sistema radicular das plantas (Castro et al., 2017).

Estudos confirmaram, por meio de análises moleculares, que o agente fúngico anteriormente associado ao complexo do declínio como *Fusarium solani* foi reclassificado como *Neocosmospora falciformis*, evidenciando avanços no entendimento da etiologia da doença (Veloso et al., 2020). Além disso, foi demonstrado que a infecção fúngica depende da presença prévia do nematoide, que atua como facilitador da penetração e colonização dos tecidos radiculares pelo patógeno, tornando o controle ainda mais desafiador (Castro, 2019).

Meloidogyne enterolobii é amplamente distribuído nos solos brasileiros, tendo sido registrado pela primeira vez no país no município de Petrolina, Pernambuco (Carneiro et al., 2001). Desde então, sua presença foi relatada em diversas regiões produtoras de goiaba, associando-se a perdas econômicas significativas (Silva e Oliveira, 2010; Gomes et al., 2017). Na região Norte do estado do Rio de Janeiro, áreas infestadas pelo nematoide tiveram queda acentuada de produtividade e viabilidade econômica dos pomares (Pereira et al., 2009).

As plantas infectadas por *M. enterolobii* manifestam como principais sintomas a formação de galhas e extensas necroses no sistema radicular. As galhas correspondem a espessamentos localizados nas raízes, resultantes da indução de células hipertrofiadas e multinucleadas pelo nematoide, formando sítios de

alimentação especializados que sustentam seu desenvolvimento sedentário (Silva et al., 2014. Essas alterações comprometem a absorção e transporte de água e nutrientes, predispondo as plantas a estresses fisiológicos.

O parasitismo causado pelo nematoide torna a goiabeira suscetível ao ataque de *N. falciformis*, mesmo em plantas previamente consideradas tolerantes ao patógeno fúngico. Em poucos meses, ocorre severa degradação do sistema radicular, resultando em clorose foliar, queima das folhas, queda prematura, declínio drástico do rendimento e, frequentemente, morte das plantas, caracterizando um processo irreversível (Gomes et al., 2014; Gomes et al., 2017; Souza et al., 2023).

Dentre as estratégias de manejo para o controle do declínio da goiabeira, o uso de cultivares resistentes ou tolerantes é a alternativa mais sustentável e promissora. Métodos químicos, culturais e biológicos possuem eficácia limitada ou inviabilidade econômica em longo prazo (Freitas et al., 2017; Gomes et al., 2017). No entanto, o desenvolvimento de cultivares comerciais resistentes é um processo lento, devido ao ciclo longo das frutíferas perenes. Nesse contexto, a seleção de porta-enxertos resistentes e compatíveis com cultivares comerciais de goiabeira surge como estratégia mais viável (Castro, 2019; Ribeiro et al., 2019).

Diversos estudos têm demonstrado a existência de fontes de resistência a *M. enterolobii* em espécies silvestres do gênero *Psidium,* entretanto, observa-se elevada variabilidade de resposta entre indivíduos de um mesmo acesso quando propagados via seminífera, com alguns possuindo fator de reprodução inferior a 1, enquanto a maioria se mostra suscetível (Costa et al., 2012; Oliveira et al., 2019; Galvão et al., 2024).

Fatores como nível de inoculação, manejo nutricional, irrigação, tipo de substrato e o tempo de avaliação são determinantes para a correta identificação de genótipos resistentes(Burla et al., 2010). Estudos indicam que aproximadamente 135 dias após a inoculação (DAI), constitui o período mais adequado para avaliação do desenvolvimento do nematoide e dos efeitos sobre as plantas, permitindo amostragem representativa do progresso da infecção (Burla et al., 2010; Galvão et al., 2024).

Diante da ampla disseminação do nematoide, da limitação das estratégias químicas e culturais e da elevada variabilidade de resposta observada em materiais

propagados via sementes, a utilização de porta-enxertos geneticamente resistentes surge como a alternativa mais promissora e sustentável para o manejo da doença, reforçando a necessidade de programas de prospecção, seleção e validação de genótipos resistentes no gênero *Psidium* (Gomes et al., 2017; Castro, 2019; Galvão et al., 2024).

2.6. Propagação da goiabeira

A goiabeira pode ser propagada por sementes ou por métodos vegetativos, incluindo estaquia, enxertia e micropropagação (Singh et al., 2019). A propagação por sementes é de baixo custo e simples de implementar; contudo, devido à polinização cruzada, resulta em elevada heterogeneidade fenotípica entre plantas, dificultando o manejo de pomares comerciais e reduzindo a uniformidade desejável para produção de frutos. A propagação por sementes é atualmente utilizada principalmente na produção de porta-enxertos ou em programas de melhoramento genético. (Manica et al., 2001; Oliveira et al., 2020).

Dessa forma, a propagação vegetativa tem sido adotada como abordagem preferencial em viveiros comerciais, pois assegura uniformidade genética e desempenho previsível das mudas (Hartmann et al., 2011).

2.6.1 Propagação por estaquia

A estaquia explora a capacidade regenerativa de células vegetativas competentes para formar raízes adventícias sob condições adequadas de ambiente e manejo (Hartmann et al., 2011). Na goiabeira, a utilização de estacas herbáceas sob nebulização intermitente é uma prática comum entre viveiristas, devido à sua eficiência em favorecer enraizamento, reduzir o ciclo de produção de mudas e melhorar a uniformidade das plantas formadas (Milhem et al., 2014; Costa et al., 2019).

Estacas com baixo grau de lignificação, tendem a enraizar com maior facilidade em comparação com segmentos mais lignificados, em função da maior proporção de células indiferenciadas e menor presença de barreiras físicas ao enraizamento (Lima et al., 2006; Roussos et al., 2023). Para otimizar o enraizamento, estacas são preparadas com folhas apicais mantendo-se um par foliar parcialmente

reduzido para equilibrar transpiração e fotossíntese em condições de viveiro (Hartmann et al., 2011).

2.6.2 Propagação por miniestaquia

A miniestaquia representa uma evolução da estaquia convencional, baseada no uso de matrizes mantidas em sistema de *minijardim clonal*, que fornecem brotações juvenis altamente competentes fisiologicamente para enraizamento rápido (Xavier et al., 2009). Essa técnica tem sido adotada em culturas arbóreas e frutíferas por proporcionar vantagens importantes, como a eliminação de matrizes em campo, maior eficiência na produção de minicepas, melhor manejo de irrigação e nutrição, e redução de custos operacionais (Xavier et al., 2009; Ribeiro et al., 2021).

Em goiabeira, a miniestaquia tem demonstrado elevada eficiência, com percentuais de enraizamento superiores aos obtidos por essaquia convencional. Marinho et al. (2009) relataram taxas de enraizamento de 100% em minicepas de goiabeira cultivadas em condições controladas. Além disso, estudos em híbridos de *P. guajava* × *P. guineense* indicam que a aplicação de ácido giberélico (AG) na parte aérea pode aumentar a produtividade de minijardins e melhorar características morfométricas, sem prejuízo ao enraizamento das minicepas, com maiores respostas observadas durante o verão (Arantes et al., 2021a).

Estudos reforçam a existência de variabilidade genética entre acessos de *Psidium* quanto ao potencial de enraizamento e vigor de mudas, o que sugere a importância de selecionar genótipos com melhor performance clonal para uso em viveiros (Biazatti et al., 2018; Arantes et al., 2021a). Em *Psidium cattleyanum*, estacas herbáceas obtidas diretamente de campo frequentemente possuem baixa taxa de enraizamento, sendo necessário recorrer a estacas semilenhosas ou estratégias de manejo específicas para estabelecimento de minijardins clonais eficazes (Biazatti et al., 2018).

2.7. Uso de biorreguladores na produção de mudas da goiabeira

A utilização de biorreguladores tem se mostrado uma estratégia eficiente para aumentar a produtividade de minijardins clonais e melhorar a qualidade de mudas de goiabeira propagadas vegetativamente. Esses compostos podem atuar tanto no estímulo à emissão de brotações em minitouceiras quanto na aclimatação e no

desenvolvimento inicial das mudas após o enraizamento (Arantes et al., 2021b; Rodríguez-Solís et al., 2023).

O ácido giberélico (GA_3) é um dos reguladores de crescimento mais utilizados, desempenhando papel fundamental nos processos de divisão e alongamento celular, além de influenciar a síntese de macromoléculas e a turgescência celular, resultando em maior crescimento dos tecidos vegetais (Gao e Chu, 2020; Tang et al., 2025). Em sistemas de produção clonal, a aplicação de GA_3 tem favorecido o aumento da altura de brotos, área foliar e produtividade de miniestacas, sem prejuízo ao enraizamento, especialmente quando ajustada à época do ano (Porto et al., 2018; Arantes et al., 2021a).

A resposta ao uso de GA_3, contudo, é dependente de fatores genéticos e ambientais, incluindo o genótipo, o manejo das minicepas e a sazonalidade, que influenciam diretamente a emissão de brotações e o desempenho fisiológico das plantas, dessa forma, a produção contínua de miniestacas requer ajustes no manejo hormonal para garantir estabilidade e uniformidade ao longo do ano (Silva et al., 2019; Kumar et al., 2022).

Outro grupo de reguladores de grande relevância são os brassinosteroides (BRs), hormônios esteroides naturais que atuam na regulação do crescimento vegetativo, desenvolvimento radicular, fotossíntese e tolerância a estresses bióticos e abióticos (Mao et al., 2017; Trevisan et al., 2020). Diversos estudos recentes demonstram que os BRs promovem maior vigor das mudas, melhor absorção de nutrientes e maior capacidade de adaptação a condições ambientais adversas (Baghel et al., 2019; Tanveer et al., 2019).

Na goiabeira, aplicações foliares de análogos de brassinosteroides, como o Biobras-16, durante a fase de aclimatação têm proporcionado incrementos significativos no crescimento da parte aérea e do sistema radicular. Concentrações entre 0,3 e 0,6 mg L^{-1} resultaram em mudas de melhor qualidade em menor tempo de viveiro, otimizando o processo de produção (Arantes et al., 2021b). Estudos mais recentes reforçam que a eficiência dos BRs depende da dose, da fase de desenvolvimento da planta e das condições ambientais, sendo fundamental o ajuste desses fatores para maximizar os benefícios fisiológicos (Kumar et al., 2022).

De forma geral, o uso integrado de biorreguladores, especialmente giberelinas e brassinosteroides, constitui uma ferramenta promissora para a intensificação da produção clonal de porta-enxertos de goiabeira, permitindo maior produtividade de minijardins, uniformidade das mudas e redução do tempo de formação no viveiro (Singh et al., 2017; Arantes et al., 2021a).

3. ARTIGOS

3.1. A steroidal plant growth regulator promotes season-dependent growth responses in Psidium genotypes[1]

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ABSTRACT

Guava (*Psidium guajava L.*) is a tropical fruit species whose propagation has benefited from the use of plant growth regulators. Seedling production involves an acclimatization phase that can extend the nursery period. Bioregulators can help standardize seedling quality and accelerate their production. This study evaluated the effect of Biobrás-16 (BB-16), a steroidal plant growth regulator, during the formation of seedlings from five *Psidium* genotypes propagated by minicuttings under protected cultivation. Experiments were conducted across four seasons, assessing morphometric traits of the shoot and root system, as well as the synthesis of foliar pigments (chlorophyll, flavonoids, anthocyanins, and the nitrogen balance index). BB-16 promoted increased elongation and biomass accumulation in leaves, stems, and roots, with effects observed in all seasons, though more pronounced in spring. In the root system, increased length, area, and volume were confirmed without changes in root diameter. Integrative heatmap analysis highlighted seasonal and genotypic contrasts, with the highest responses observed in stem dry mass of genotype P01 in summer and in root length and volume in autumn. Physiologically, BB-16 increased chlorophyll and NBI values while reducing flavonoids and anthocyanins, indicating lower stress and higher photosynthetic capacity. In summary, BB-16 acted as a growth accelerator and a potential tool for reducing seedling production time.

Keywords: *Psidium guajava.* Biobrás-16. Rootstocks. Minicuttings. Acclimatization.

[1]Artigo aceito na revista South African Journal of Botany

1. Introduction

Guava (*Psidium guajava* L.) is a tropical fruit species of broad economic and social importance, cultivated in tropical and subtropical regions. Guava cultivation has expanded, driven by the nutritional value of the fruit, which is rich in vitamins, minerals and bioactive compounds; this fruit has gained market share for fresh consumption and for processing into agro-industrial products (Zaid et al., 2024).

Despite its relevance, guava faces serious phytosanitary problems. Among them, the root knot nematode (*Meloidogyne enterolobii*) stands out as a limiting pathogen that alters root exudates, promoting microbial activity that compromises root physiology and weakens plant immunity against fungal infections, thus accelerating plant deterioration (Souza et al., 2023). In this context, the use of resistant rootstocks propagated by minicuttings emerges as a competitive strategy to ensure production sustainability, enabling the use of tolerant genotypes in standardized seedling systems (Galvão et al., 2024; Vishwakarma et al., 2023).

The acclimatization phase of seedlings represents one of the critical points in production. The transition from the protected misting environment to the nursery imposes physiological stress conditions, with negative impacts on photosynthesis, membrane integrity and hormonal balance (Baghel et al., 2019). In this context, the application of growth regulators such as Biobrás-16 (a steroidal plant growth regulator) may attenuate the effects of these environmental changes, stimulating growth and improving nitrogen assimilation and related metabolic responses (Yadav et al., 2023). Brassinosteroids (BRs) are recognized as the sixth class of phytohormones, with a central role in plant growth and adaptation to different stresses, acting in signaling pathways that regulate cell division, elongation and antioxidant responses (Nolan et al., 2020).

However, the effects of BRs are modulated by several factors, such as the plant growth stage (Freitas et al., 2015), the presence or absence of environmental stress (Tanveer et al.,

2019) and the genotype (Mohammadi et al., 2020). Under lower temperatures, stem elongation is less evident due to reduced gibberellin activity and biosynthesis (Stavang et al., 2007). Exogenous BR application may mitigate damage associated with temperature changes and improve seedling performance, even though growth remains lower than that observed under optimal conditions (Sun et al., 2020). In summer, in turn, the high atmospheric demand tends to reduce stomatal conductance and limit leaf expansion, contributing to greater variability in physiological responses (Grossiord et al., 2020).

Thus, BR efficacy may depend on the developmental stage and climatic conditions, varying among species and genotypes (Chaudhuri et al., 2022). Therefore, the application of Biobrás-16 may interfere with the growth and development of *Psidium* seedlings in a differentiated way among genotypes and seasons, resulting in measurable variations in morphometric attributes during seedling formation. The present study aimed to evaluate the season-dependent effects of Biobrás-16 (BB-16), a steroidal plant growth regulator, on the development of *Psidium* seedlings during the acclimatization phase under protected cultivation, based on morphometric characteristics and foliar pigment indices.

2. Material and Methods

2.1 Study Area

The study was conducted from July 2023 to June 2024 in the northern region of the state of Rio de Janeiro (Brazil), at coordinates 21°45′44″ S and 41°17′19″ W, at an altitude of 14 m. According to the Köppen classification, the climate is Aw (tropical), with a rainy summer, dry winter, and a minimum monthly temperature above 18 °C. The experiment was carried out in a greenhouse measuring 7.0 m in width, 20 m in length, and 4.0 m in height, covered with 50% Sombrite®, with side screens and a raffia-covered floor. Air temperature and relative humidity

were monitored using an Elitech® R661 thermo-hygrometer installed inside the greenhouse at canopy height.

2.2 Experimental Design

The experiment was conducted in a randomized block design (RBD) with split-split plots, 5 × 2 × 5, with four replications and four plants per plot. The five genotypes constituted the main plots, the presence or absence of BB-16 formed the subplots, and time (five evaluation weeks) the sub-subplots. This experiment was repeated across four seasons (winter/2023, spring/2023, summer/2024, and autumn/2024) as independent trials, and data from each season were analyzed separately following the same arrangement.

2.3 Plant Material and Growing Conditions

Four hybrid *Psidium* genotypes (*P. guajava* × *P. cattleianum*): P01, P22, P90, and P140, plus the cultivar Paluma were individually cloned. The protocol used for seedling production via minicuttings was described by Arantes et al. (2020). Minicuttings were prepared with two pairs of leaves: the basal pair was removed, and the remaining pair was cut in half. The cuttings were placed in 280 cm^3 tubes filled with Basaplant® commercial substrate and then transferred to a mist chamber (spray timer of 15 seconds every 10 minutes, 7 L h^{-1} flow rate, 4.0 kgf cm^{-2} pressure) with one microsprinkler per m^2, where they remained for 60 days.

During the rooting period in the mist chamber, a high-humidity microclimate was maintained, with average air temperatures of approximately 25.7 °C in winter, 28.2 °C in spring, 30.7 °C in summer and 29.9 °C in autumn, and an average relative humidity of around 92% (ranging from approximately 85% to 99%).

After the rooting period, the minicuttings were acclimatized by transferring them from the mist chamber to a greenhouse covered with 70% Sombrite®. All seedlings were irrigated

twice daily (morning and afternoon), according to air temperature, humidity, and seedling size, with periodic adjustments. After 15 days, the tubes were arranged on benches measuring 1.15 m × 5 m, positioned 1 m above ground level, and irrigation was performed using overhead microsprinklers at a density of 0.5 units per m², with water depth adjusted according to plant water requirements. To avoid wash-off of the applied solution, BB-16 treatments were carried out at the end of the day on previously irrigated plants; irrigation was suspended at the time of application and resumed two days later. During this interval, substrate moisture was monitored, and manual irrigation was applied only when necessary to prevent water deficit.

In the greenhouse, seasonal average air temperatures were approximately 23.4 °C in winter, 26.4 °C in spring, 28.6 °C in summer and 27.4 °C in autumn, while average relative humidity values were about 75.2%, 72.5%, 72.3% and 73.4%, respectively (figure 1).

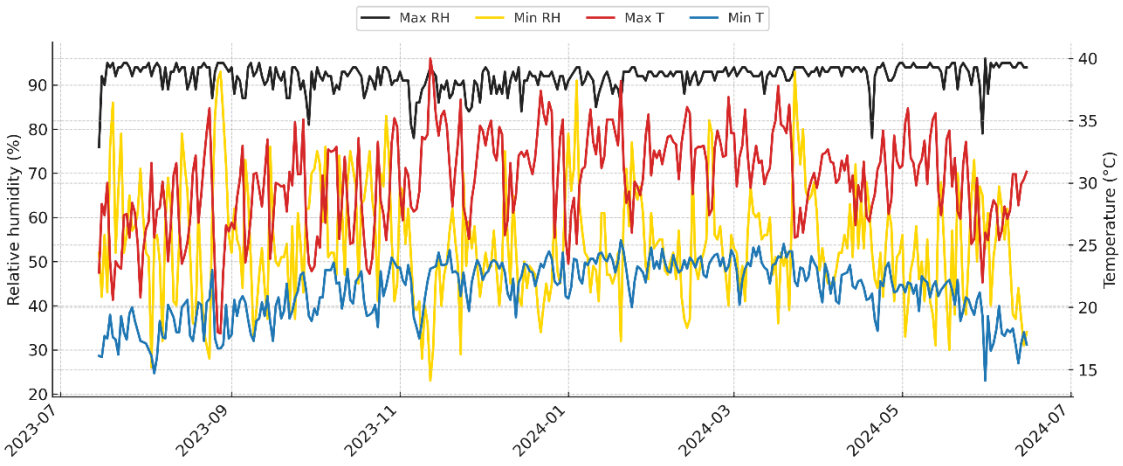

Fig. 1. Mean maximum and minimum temperatures (Tmax and Tmin) and mean maximum and minimum relative humidity (RH) inside the greenhouse between July 2023 and June 2024.

Seedlings were grown exclusively under natural light conditions throughout all seasons, with incoming radiation modulated by commercial shading nets (50% in the greenhouse and 70% during acclimatization). Light irradiance was not directly measured.

2.4 Chemicals and reagents

The compound used in this study was Biobrás-16 (BB-16), a polyhydroxylated spirostane steroid derived from diosgenin, with molecular structure (3β,5α,25R)-3,5-dihydroxyspirostan-6-one. BB-16 was supplied by the Agronomic Institute of Campinas (IAC, Brazil) and originally synthesized by the National Institute of Agricultural Sciences (San José de las Lajas, Havana, Cuba). The compound was provided as a commercial formulation (i.e., a preparation containing the active ingredient and formulation components that facilitate dispersion and application) and diluted in distilled water to obtain the working concentrations. Tween 20 (0.1%, v/v) was added as a surfactant to improve solution spreading and leaf surface coverage. Information regarding analytical purity (e.g., chromatographic analysis) and spectroscopic characterization (e.g., NMR, IR) was not provided by the supplier

2.5 Biobrás-16 Application Protocol

During acclimatization, Biobrás-16 (BB-16), a steroidal plant growth regulator, was applied. A 250 mL solution at a concentration of 0.3 mg L^{-1} of BB-16 was prepared, stored under refrigeration, and protected from light by wrapping the containers with aluminum foil to prevent degradation or precipitation (Arantes et al., 2020). Plants from the same treatment were transferred to pre-labeled trays and separated to avoid contact during spraying, and the first BB-16 application was performed on the same day as acclimatization. Sprays were carried out every 7 days for one month, totaling five applications, all performed in the late afternoon. BB-16 was applied via foliar spraying at an approximate volume of 2 mL per plant, ensuring complete coverage of the aerial part, including stems and both adaxial and abaxial leaf surfaces, using the commercial formulation of the compound with the addition of Tween 20 at 0.1% (v/v) as a surfactant to improve dispersion and contact with the leaf surface. Control plants received a

solution containing deionized water and Tween 20 at 0.1% (v/v) under the same application conditions.

After each BB-16 application, 20 mL of nutrient solution (Bolles Jones, 1954) was applied per tube weekly during the acclimatization phase. Fertilization with $Ca(NO_3)_2$ (calcium nitrate) was also performed at a concentration of 1.0 g of $Ca(NO_3)_2$ per liter of water every nine days. The first $Ca(NO_3)_2$ application was made two days after the nutrient solution was applied.

Morphometric evaluations were initiated 30 days after the beginning of the treatment applications, corresponding to the period immediately after the fifth and final application of BB-16**.**

2.6 Biometric Parameters

Thirty days after treatment application, morphometric evaluations were performed weekly, shoot length (SHL) with a measuring tape, while shoot diameter (SHD) were measured with a digital caliper. Leaf development was quantified by counting pairs of fully expanded leaves (FLP) over a total period of five weeks. The protocol was repeated across four seasons: winter/2023, spring/2023, summer/2024, and autumn/2024, maintaining the same steps and procedures described.

2.7 Foliar Pigments

To characterize plant physiological status, foliar pigment indices were measured weekly after the second BB-16 application. Chlorophyll (CHL), flavonoid (FLAV), and anthocyanin (ANTH) indices, as well as the nitrogen balance index (NBI), were recorded on the middle third of the first fully expanded leaf using a Dualex® portable meter (FORCE-A, Orsay, France). These variables were evaluated exclusively during the autumn season (2024), a transitional period with more stable environmental conditions, avoiding extreme temperatures.

2.8 Statistical Analyses

All data were subjected to analysis of variance (ANOVA). When significant differences were detected, means were compared using Tukey's test (5%). Variables measured over multiple time points were analyzed in a split-split-plot design over time. When the time effect was significant, regression models were fitted across weeks, selected according to the significance of coefficients. Regression analyses and graphs were performed according to the significant interactions identified in the ANOVA, with models adjusted either for factor A (genotypes) or for factor B (BB-16 application), depending on which interaction with time or between factors was significant. Analyses were performed using R software.

3. Results and Discussion

The bioregulator BB-16 promoted consistent increases in growth, shoot diameter, and number of leaf pairs, with the greatest effect observed in spring. Figure 2 shows the development of shoot growth throughout the four seasons.

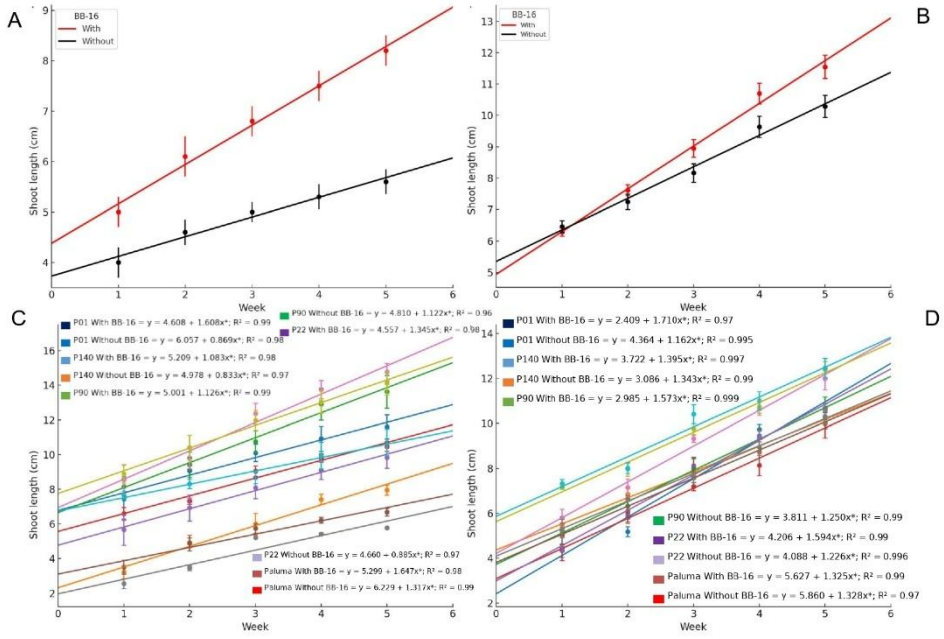

Fig. 2. Regression analysis for *Psidium* genotypes with and without Biobrás-16 (BB-16) application in winter (A), spring (B), summer (C), and autumn (D) seasons, considering the variable shoot length over five weeks. Symbols represent means. *I* = standard error, and the lines indicate regression trends over time.

During winter, elongation rates were approximately 90% higher under BB-16 application than in untreated plants. In spring, the increase was about 36%. During summer, genotypic variability was greater, but gains of around 30% were still observed under BB-16. In autumn, BB-16 maintained linear elongation rates about 52% higher in genotype P22, showing that the bioregulator supported shoot elongation capacity.

Shoot diameter was also affected by BB-16 (Figure 3). In winter, stem thickening increased by approximately 119% with the bioregulator. In spring, a significant interaction was observed between genotypes and BB-16, with greater thickening in all genotypes treated with the regulator. In summer, the effect of BB-16 was not sustained throughout the evaluation weeks. In autumn, there was an interaction between genotypes, with notable responses in P01 and ‘Paluma’ treated with BB-16.

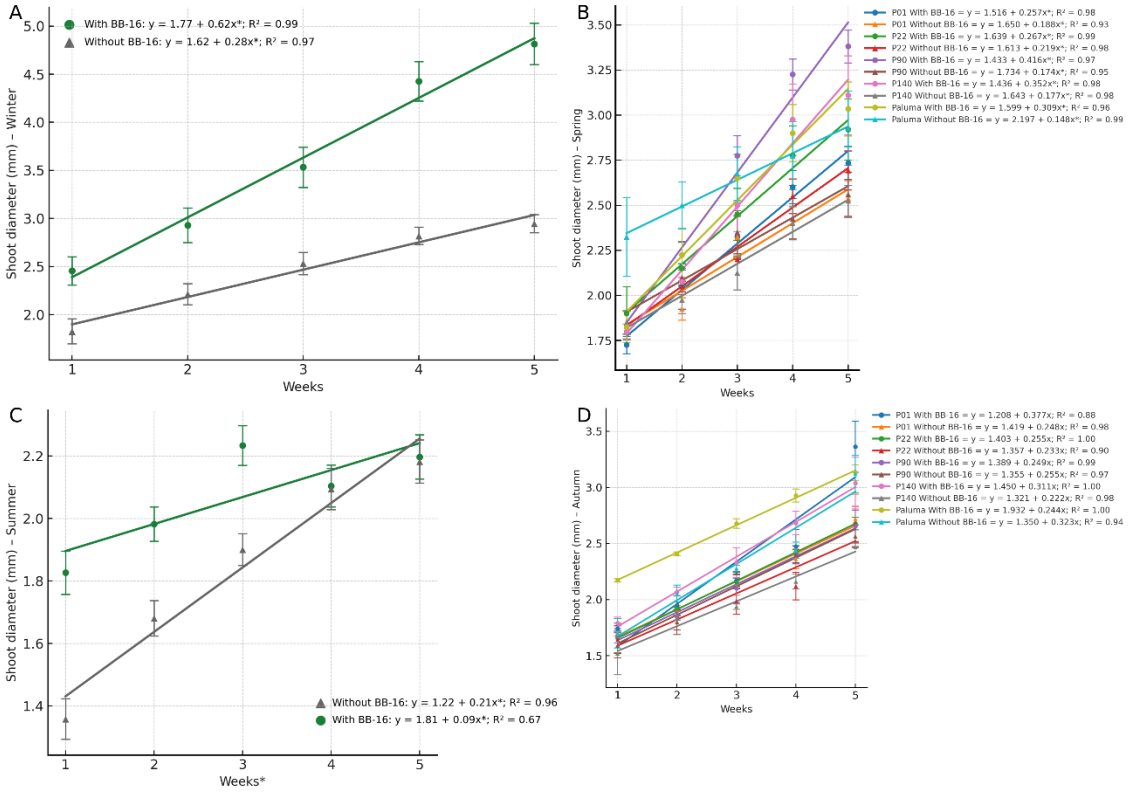

Fig. 3. Regression analysis for five *Psidium* genotypes with and without Biobrás-16 (BB-16) application, considering the variable shoot diameter over five weeks in four seasons: A = winter; B = spring; C = summer; D = autumn. Symbols represent means, *I* = standard error, and the lines indicate regression trends over time.

For the number of fully expanded leaf pairs, an accumulation was observed for this variable, positively influenced by BB-16 in most seasons (figure 4).

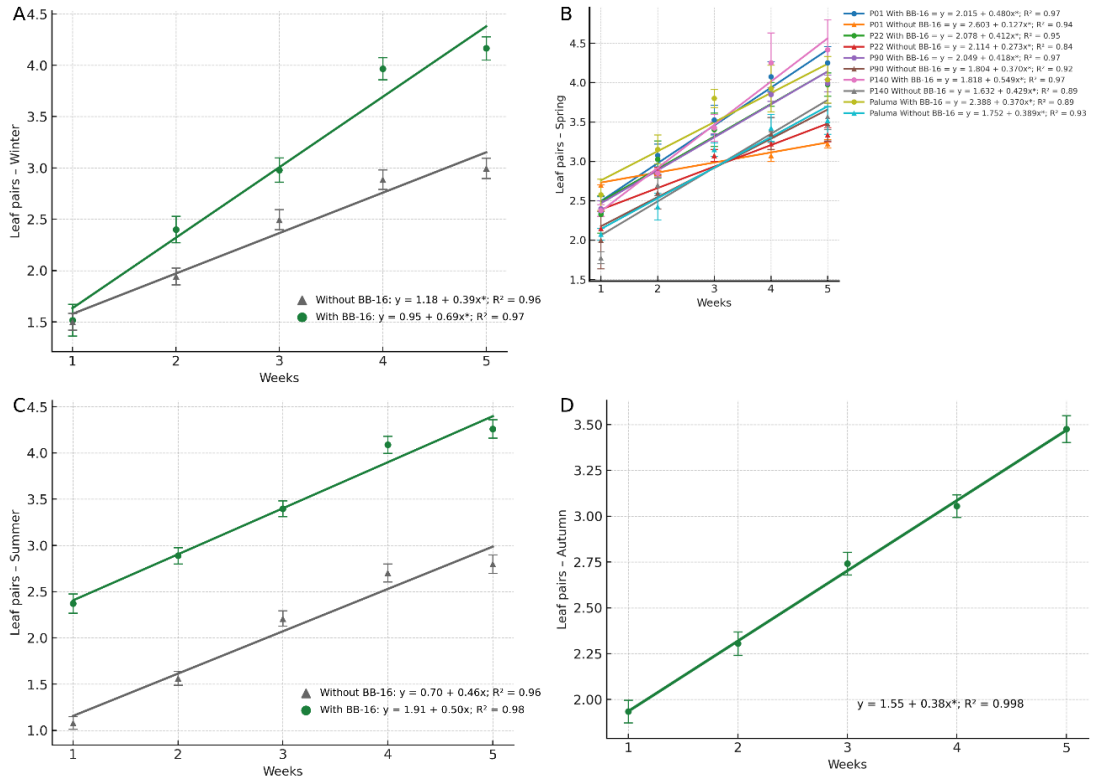

Fig. 4. Regression analysis for five *Psidium* genotypes with and without Biobrás-16 (BB-16) application, considering the variable number of leaf pairs over five weeks in four seasons: A = winter; B = spring; C = summer; D = autumn. Symbols represent means, I = standard error, and the lines indicate regression trends over time.

During winter, the bioregulator promoted a 67% increase in leaf emission. In spring, BB-16 increased the rate of leaf pair formation across all genotypes. In summer, both treated and untreated plants exhibited linear growth, although BB-16 provided an average advantage of 65%, demonstrating its ability to sustain leaf emergence under more variable environmental conditions. In autumn, no variation in the number of leaf pairs was observed in response to BB-16.

During winter, even under mild conditions that naturally reduce elongation vigor, BB-16 maintained growth stimulation, favoring elongation, thickening, and leaf emission. This

response suggests that brassinosteroids sustain cell expansion, anticipating structural development of seedlings even under lower temperatures (Sun et al., 2020).

In spring, the intensified leaf production across all genotypes reinforces that this period represents a physiological window favorable to BB-16 action, when there is greater integration between temperature, photoperiod, and hormonal response (Sahu et al., 2025).

In summer, the more variable responses reflect the modulatory effect of high vapor pressure deficit and water stress on growth (Grossiord et al., 2020). Still, the BB-16 effect indicates a contribution to greater vigor and resilience of seedlings under stress conditions.

In autumn, BB-16 sustained shoot growth, with less emphasis on rapid expansion and greater focus on canopy stabilization. This pattern is consistent with the role of brassinosteroids in supporting growth during the acclimatization of *Psidium* seedlings (Arantes et al., 2020) and in promoting cell elongation across different phenological stages in fruit species (Baghel et al., 2019; Garrido-Auñón et al., 2024).

The application of BB-16 also improved leaf area (LA) leaf dry mass (LDM), stem dry mass (SDM), and root mass (RM) throughout the evaluated period (Table 1).

Table 1.

Means values of the variables leaf area (LA), leaf dry mass (LDM), stem dry mass (SDM), and root dry mass (RM) in acclimatized *Psidium* seedlings subjected or not to the application of the plant growth regulator Biobrás-16, during the winter, spring, summer, and autumn seasons.

GEN	LA (cm²)		LDM (g)		SDM (g)		RM (g)	
WIN	+BB	-BB	+BB	-BB	+BB	-BB	+BB	-BB
P01	85.85 a	72.47 ab	0.53 cd	0.57 ab	0.15 b	0.15 ab	0.15	0.10
P140	68.50 b	78.37 a	0.66 b	0.55 b	0.13 b	0.12 b	0.17	0.17
P90	66.18 b	66.25 b	0.60 bc	0.57 ab	0.12 b	0.14 b	0.18	0.13
P22	72.66 b	70.31 ab	0.48 d	0.60 ab	0.15 b	0.13 b	0.15	0.09
Paluma	86.79 a	65.65 b	0.86 a	0.66 a	0.22 a	0.18 a	0.18	0.15
Mean	76.00 A	70.61 B	0.63 A	0.59 B	0.16 A	0.14 B	0.17 A	0.13 B
CV1%	5.59		8.00		13.20		22.08	
CV2%	5.44		7.94		10.29		22.94	
GEN	LA (cm²)		LDM (g)		SDM (g)		RM (g)	
SPR	+BB	-BB	+BB	-BB	+BB	-BB	+BB	-BB
P01	95.39 a	80.51ab	0.59 cd	0.64 a	0.17 b	0.16 ab	0.17	0.11
P140	76.11 b	87.08 a	0.73 b	0.62 a	0.15 b	0.13 b	0.19	0.17
P90	73.54 b	73.61 b	0.67 bc	0.64 a	0.14 b	0.15 b	0.20	0.15
P22	80.73 b	78.12 ab	0.53 d	0.66 a	0.16 b	0.14 b	0.16	0.10
Paluma	96.43 a	72.94 b	0.96 a	0.73 a	0.25 a	0.20 a	0.20	0.17
Mean	84.40 A	78.40 B	0.70 A	0.66 B	0.17 A	0.16 B	0.17 A	0.14 B
CV1%	5.59		8.00		13.32		22.07	
CV2%	5.44		7.93		10.28		22.96	

GEN	LA (cm²)		LDM (g)		SDM (g)		RM (g)	
SUM	+BB	-BB	+BB	-BB	+BB	-BB	+BB	-BB
P01	142.66 b	95.50 b	0.74 b	0.66 b	0.60 a	0.18 a	0.39 bc	0.26 ab
P140	116.05 c	115.25 a	0.84 ab	0.82 a	0.20 bc	0.14 a	0.42 b	0.27 a
P90	177.88 a	84.78 b	0.76 b	0.61 b	0.16 c	0.18 a	0.34 cd	0.23 ab
P22	123.14 c	93.15 b	0.76 b	0.72 ab	0.16 c	0.18 a	0.31 d	0.19 b
Paluma	155.43 b	89.89 b	0.89 a	0.65 b	0.26 b	0.13 a	0.50 a	0.25 ab
Mean	143.03 A	95.71 B	0.80 A	0.69 B	0.28 A	0.16 B	0.39 A	0.24 B
CV1%	9.13		7.96		17.38		12.35	
CV2%	5.16		6.56		13.99		8.83	

GEN	LA (cm²)		LDM (g)		SDM (g)		RM (g)	
AUT	+BB	-BB	+BB	-BB	+BB	-BB	+BB	-BB
P01	154.80 ab	85.40 b	0.73 b	0.42 c	0.24 a	0.11	0.37 bc	0.34 a
P140	146.40 bc	108.50 a	0.89 a	0.51 bc	0.21 ab	0.11	0.30 c	0.33 a
P90	149.00 ab	102.70 a	0.82 ab	0.68 a	0.20 b	0.14	0.43 b	0.24 b
P22	158.80 a	83.7 b	0.49 c	0.67 a	0.21 ab	0.12	0.30 c	0.24 b
Paluma	138.20 c	83.000 b	0.92 a	0.59 ab	0.19 b	0.11	0.51 a	0.23 b
Mean	149.40 A	92.60 B	0.77 A	0.57 B	0.21 A	0.12 B	0.38 A	0.27 B

CV1%	4.19	10.01	11.09	12.01
CV2%	3.76	9.36	11.43	9.24

Means followed by different letters, uppercase in the rows and lowercase in the columns, differ from each other by Tukey's test at 5% probability. CV1 - coefficient of variation of the plot. CV2 - coefficient of variation of the subplots. GEN - genotypes. +BB: with Biobrás-16 and -BB: without Biobrás-16. Win =winter; SPR = springer; SUM = summer; AUT = autumn.

In winter, increases reached 32.2% for leaf area, 7% for leaf dry mass, 14% for stem dry mass, and 30% for root dry mass, with the cultivar Paluma showing the highest means. In spring, the increments were 7.7%, 6%, 6%, and 21%, respectively, again with Paluma standing out. During summer, the regulator increased the variables by 49.4%, 20%, 70%, and 60%, demonstrating its effect even under stress conditions. In autumn, increases reached 61.3%, 30%, 70%, and 40%, respectively, showing broad and positive genotypic responses to BB-16 application.

Table 2 presents the values of the variables related to the root system of the seedlings.

Table 2.

Mean values of root system variables: root length (RL), root surface area (RSA), root diameter (RAD), and root volume (RV) in acclimatized *Psidium* seedlings subjected or not to the application of the plant growth regulator Biobrás-16, during the winter, spring, summer, and autumn seasons.

GEN	RL (cm)		RSA (cm²)		RAD (mm)		RV (cm³)	
WIN	+BB	-BB	+BB	-BB	+BB	-BB	+BB	-BB
P01	1061.49	969.61	96.59 b	66.40 b	0.47	0.49	1.06	0.46
P140	711.97	748.82	118.20 a	57.16 b	0.54	0.52	1.35	0.77
P90	981.01	775.11	77.44 c	51.96 b	0.45	0.48	0.89	0.71
P22	968.98	976.18	36.67 e	50.38 b	0.82	0.46	1.10	0.49
PA	981.96	932.88	53.57 d	87.43 a	0.60	0.56	0.97	0.43
Mean	941.08 A	880.52 B	76.49 A	62.67 B	0.57 A	0.50 A	1.08 A	0.57 B
CV1%	10.29%		10.70%		33.92%		27.25%	
CV2%	9.81%		12.33%		35.89%		27.20%	

GEN	RL (cm)		RSA (cm²)		RAD (mm)		RV (cm³)	
SPR	+BB	-BB	+BB	-BB	+BB	-BB	+BB	-BB
P01	917.28	687.81	80.60 b	76.57 a	0.35	0.37	0.90	0.78
P140	847.43	685.77	92.55 a	44.64 d	0.38	0.38	0.93	0.85
P90	794.79	511.37	84.71 b	54.80 c	0.34	0.36	0.73	0.64

P22	755.28	617.43	59.63 c	55.28 c	0.38	0.36	0.81	0.77
Paluma	753.17	640.65	58.62 c	62.66 b	0.42	0.40	0.84	0.72
Mean	813.59 A	628.60 B	75.22 A	58.79 B	0.37 A	0.37 A	0.84 A	0.75 B
CV1%	7.70%		5.39%		10.58%		8.11%	
CV2%	8.11%		3.89%		9.72%		9.50%	

GEN SUM	RL (cm)		RSA (cm²)		RAD (mm)		RV (cm³)	
	+BB	-BB	+BB	-BB	+BB	-BB	+BB	-BB
P01	798.06 b	431.01 b	203.40 a	115.31 bc	0.85	0.78	4.37 a	2.43 a
P140	982.88 a	622.71 a	165.80 ab	203.20 a	0.88	0.89	4.01 a	2.64 a
P90	608.56 c	247.63 c	175.74 ab	141.29 b	0.84	0.80	2.42 b	2.21 a
P22	539.30 c	258.67 c	140.19 b	91.56 c	0.80	0.65	2.60 b	0.79 b
Paluma	574.38 c	348.42 c	143.61 b	190.94 a	0.85	0.84	3.45 ab	1.85 ab
Mean	700.64 A	381.69 B	165.75 A	148.46 B	0.84 A	0.79 B	3.38 A	1.98 B
CV1%	8.30%		11.20%		8.54%		21.94%	
CV2%	11.59%		15.31%		8.25%		15.28%	

GEN OUT	RL (cm)		RSA (cm²)		RAD (mm)		RV (cm³)	
	+BB	-BB	+BB	-BB	+BB	-BB	+BB	-BB
P01	178.67 cd	143.39 ab	121.76 ab	107.44 a	0.41	0.39	2.39 b	2.15 a
P140	161.26 d	149.57 ab	90.72 c	93.90 ab	0.40	0.41	1.83 c	1.87 a
P90	203.44 ab	157.03 a	141.13 a	102.29 a	0.42	0.40	2.89 a	1.31 b

P22	221.59 a	126.52 b	135.45 a	80.76 b	0.37	0.35	2.43 b	1.20 b
Paluma	192.77 bc	144.16 ab	108.69 bc	89.64 ab	0.38	0.39	1.96 c	1.80 a
Mean	191.55 A	144.13 B	119.55 A	94.80 B	0.39 A	0.39 A	2.30 A	1.67 B
CV1%	7.52%		9.09%		5.15%		11.09%	
CV2%	6.43%		9.98%		7.85%		8.15%	

Means followed by different letters, uppercase in the rows and lowercase in the columns, differ from each other by Tukey's test at 5% probability. CV1 – coefficient of variation of the plot. CV2 – coefficient of variation of the subplots. GEN – genotypes. +BB: with Biobrás-16 and -BB: without Biobrás-16.

BB-16 promoted an increase in root length, surface area and volume, with little change in diameter. In winter, increases were 33% for length, 26% for surface area and 37% for volume, indicating expansion without thickening. In spring, increments reached 30%, 30% and 12%, respectively, confirming the role of the regulator in promoting root system expansion. During summer, BB-16 maintained increases in all variables, with the greatest contrast in length. In autumn, increases remained at 33%, 26% and 37%, while mean diameter did not change, indicating balanced growth and a positive influence of the bioregulator in genotypes such as P22 and P90.

In all seasons, the root system pattern converged toward an expansive effect of BB-16: greater length and area (and often greater volume), and with the exception of summer, mean diameter did not respond to the bioregulator in the evaluated seasons. This indicates that the bioregulator favored substrate exploration, a response consistent with brassinosteroid mechanisms that stimulate vascular differentiation, expanding the absorptive apparatus of

seedlings. According to Kandhal et al. (2025), the action of BR in roots is not only to promote division and elongation, but also to determine how carbon is allocated to greater growth in length/branching and less in thickening, in line with the pattern observed in *Psidium* treatments with BB-16.

The integrated analysis by means of a z-score heatmap (Figure 5) revealed that BB-16 application resulted in consistent gains in almost all morphological variables evaluated, with more expressive effects in summer and autumn. The most notable response was stem dry mass (SDM) of genotype P01 in summer, which increased more than threefold compared to the control. Other marked contrasts included the increase in leaf area (LA) of P90 in summer, root length (RL) of P22 in autumn, and root volume (RV) of P90 in the same period. In contrast, mean root diameter (RAD) remained practically stable, indicating that the regulator mainly stimulates foliar and root expansion without promoting significant thickening. In summary, the observed pattern reinforces that BB-16 enhances vegetative growth, especially under favorable environmental conditions.

Similar results were reported by Hazarika et al. (2025) in potato, where the use of heatmaps of standardized values allowed the identification of contrasting genotypes under different phosphorus levels. Similarly, in the present study, the heatmap highlighted differences among genotypes, seasons and treatments, proving to be an effective tool to synthesize patterns

and pinpoint the scenarios of greatest impact of BB-16 on seedling development.

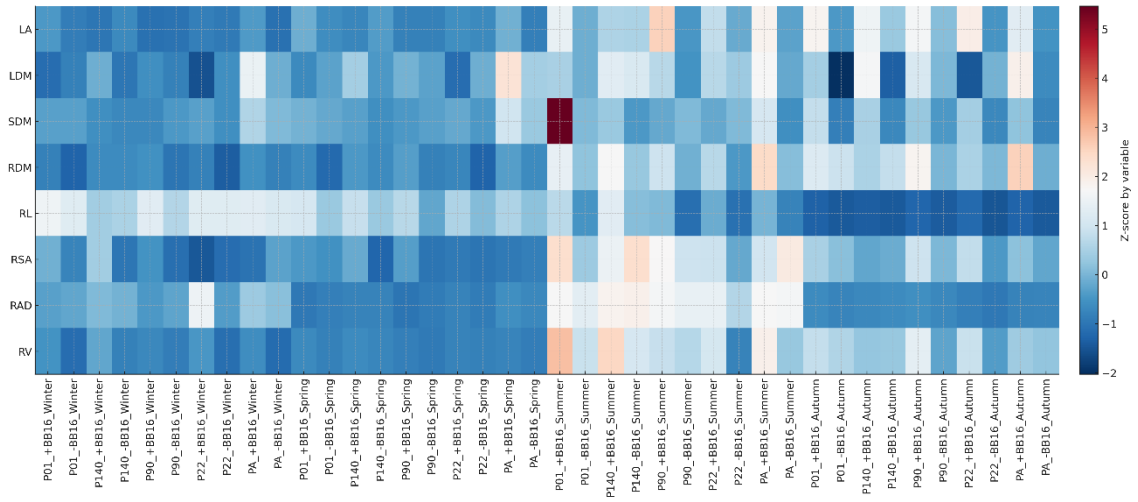

Fig. 5. Heatmap of standardized values (z-score) of morphological variables evaluated in *Psidium* seedlings grown with and without BB-16 application across four seasons. Each row represents a variable: Leaf area (LA); Leaf dry mass (LDM); Stem dry mass (SDM); Root dry mass (RDM); Root length (RL); Root surface area (RSA); Mean root diameter (RAD); Root volume (RV).

Throughout the analyzed period, clearly defined seasonal conditions were observed (figure 1). In winter, milder temperatures prevailed (Tmin 19.24 °C; Tmax 27.62 °C) and the air was more humid (RHmin 57.50%; RHmax 92.85%). In spring, the atmosphere warmed slightly (Tmin 22.16 °C; Tmax 30.73 °C), accompanied by a slight reduction in humidity (RHmin 54.47%; RHmax 90.44%). In summer, the highest temperature values were recorded (Tmin 24.13 °C; Tmax 33.10 °C), with humidity remaining high (RHmin 52.95%; RHmax 91.56%). Finally, autumn maintained intermediate temperatures (Tmin 22.96 °C; Tmax 31.87 °C) and high humidity (RHmin 53.99%; RHmax 92.86%), characterizing a smooth transition between summer and the following winter.

The effect of BB-16 varied according to seasonal conditions, with slightly more discrete differences in spring and intensified responses in summer and autumn. In these two latter seasons, greater accumulation of shoot and root biomass was observed, associated with increased shoot growth. These responses corroborate the role of brassinosteroids in cell expansion, vascular differentiation, and their function as biostimulants (Oh et al., 2020; Furuya et al., 2024).

The differences among genotypes indicate a genotypic dependence of responsiveness to the regulator, which is useful for selecting materials and adjusting the timing of application in nurseries. Similar results were reported by Núñez Vázquez et al. (2013), who demonstrated that two rice genotypes responded differently to the application of brassinosteroid analogues under saline conditions, confirming that the magnitude of BB-16 response depends on genetic background and adverse environmental conditions. Wolf et al. (2014) showed that brassinosteroids regulate the expression of cell wall-related genes such as expansins and xyloglucan endotransglycosylases (XTHs), promoting greater extensibility and cell elongation. Thus, genotypic differences in hemicellulose composition or in the basal expression of these genes may modulate the magnitude of the response to BB-16.

In practice, there was a reduction in the production cycle and an improvement in seedling morphological quality, with particularly evident gains in leaf area, number of leaves, and biomass accumulation. Beyond the direct vegetative gain, there is evidence that BRs prepare seedlings for environmental fluctuations. This effect is not limited to the endogenous BR signaling cascade: when applied exogenously, compounds such as BB-16 or 24-epibrassinolide activate defense mechanisms, modulate antioxidant activity, maintain membrane integrity, and reduce the accumulation of reactive oxygen species (ROS) (Chaudhuri et al., 2022; Avalbaev et al., 2024).

Studies have shown that one type of BR, 24-epibrassinolide, alleviates stress and maintains root growth under adverse conditions, which tends to stabilize water and nutrient uptake and sustain shoot growth in apple seedlings (Yu et al., 2023).

2.5 Foliar Pigments

There was a Significant effects were observed for factor B (BB-16 application) and factor C (time of evaluation). Thus, the differences between Biobrás-16 levels are consistent across all weeks, and the trend over time is common to all treatments (Figure 6).

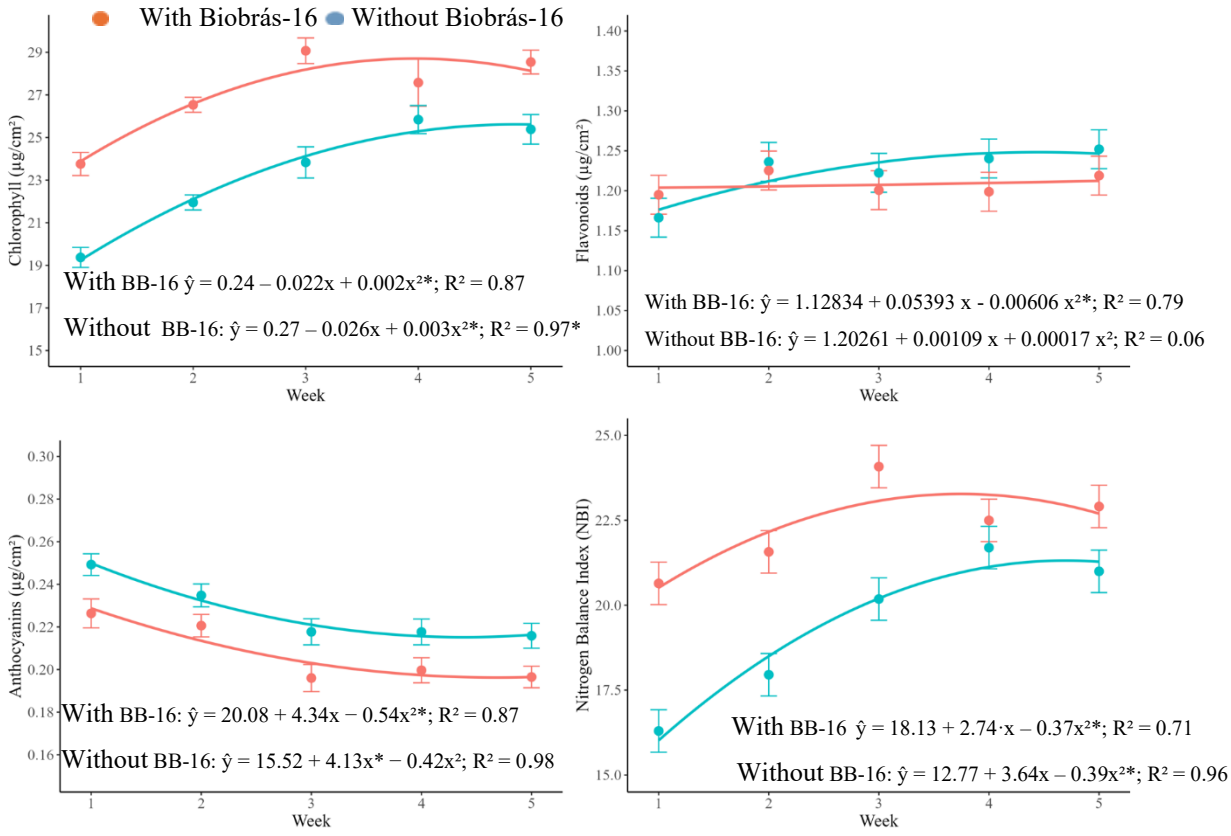

Fig. 6. Temporal dynamics of foliar pigments in *Psidium* genotypes evaluated with and without Biobrás-16 application. (A) Chlorophyll (µg cm⁻²); (B) Flavonoids (µg cm⁻²); (C) Anthocyanins (µg cm⁻²); (D) Nitrogen Balance Index – NBI, over five weeks. Symbols represent mean ± standard error, and the lines indicate regression trends over time.

Over time, an increase in the chlorophyll index was observed, reaching its maximum around the third to fourth week, followed by a slight decline. The treatment with Biobrás-16 consistently showed higher values compared to the control, especially during the peak period. The fitted curves were best described by quadratic models (Figure 7a).

For flavonoids, both time and the use of BB-16 were significant factors, and their interaction revealed a contrasting response between the BB-16 treatment and the control. The coefficient of determination (R^2) indicated that the model did not explain the variation observed in the control treatment (Figure 7b).

In the case of anthocyanins, a decline was observed over the weeks in both treatments. The fitting within each treatment followed the significance of the time factor, resulting in a predominantly decreasing trend represented by quadratic models (Figure 7c).

For the Nitrogen Balance Index (NBI), there was an increase up to approximately the fourth week, followed by a slight reduction. The Biobrás-16 treatment maintained a consistently higher NBI throughout the experimental period. The fitted models were quadratic whenever the time factor was significant (Figure 7d).

Since there was no genotype × time interaction, the differences between treatments with and without BB-16 application remained constant over the weeks: with BB-16, chlorophyll and NBI levels were higher, while flavonoids and anthocyanins were lower in all evaluations. Furthermore, the temporal trend (peaks and variations) remained similar between treatments. This set of responses is physiologically consistent with the role of brassinosteroids: higher chlorophyll and NBI indicate better photosynthetic apparatus performance and higher nitrogen content (Cerovic et al., 2012), while lower flavonoids and anthocyanins reflect reduced investment in photoprotective phenolic compounds and lower stress levels (Agati et al., 2013).

From an agronomic perspective, the use of BB-16 proved effective in enhancing photosynthetic vigor and reducing phenolic compound accumulation. The period of best

performance (third to fourth week) was the same for both treatments; BB-16 increased the values but did not shift the timing of the peak. In the study by Gözel and Gökbayrak (2022) with tomato seedlings infected by *Meloidogyne incognita*, application of 24-epibrassinolide (EBR) by different methods (immersion, irrigation, spraying) increased or better preserved chlorophyll contents, while reducing or mitigating the rise in atmospheric flavonols, correlating with improved nitrogen status. The authors attributed this effect to the ability of brassinosteroids to protect the integrity of the photosynthetic apparatus, maintain Calvin cycle activity, and modulate the antioxidant system, thereby reducing membrane peroxidation and the accumulation of secondary phenolic compounds under stress.

Studies indicate that this set of actions results from signaling through the BRI1–BZR1 pathway, which regulates the transcription of genes involved in cell wall structure, photosynthesis, and carbon metabolism, in addition to crosstalk with auxins and gibberellins (Garrido-Auñón et al., 2024).

Overall, these results corroborate that BRs preserve the photosynthetic machinery and reduce the production of phenolic compounds as stress indicators, in agreement with the findings of this study (higher CHL and NBI, and lower FLAV and ANTH). Moreover, they confirm that the concentration of 0.3 mg L^{-1} of Biobrás-16 used in this experiment can be effective across different genotypes.

Therefore, beyond the quantitative data, the difference in the size and vigor of *Psidium* seedlings is clearly visible 65 days after acclimatization (Figure 7), highlighting the practical potential of BB-16 in enhancing nursery growth performance.

Fig. 7. *Psidium* seedling treated and untreated with BB-16 at 65 days after acclimatization.

4. CONCLUSIONS

The response of *Psidium* genotypes to BB-16 is season-dependent under greenhouse conditions. Although the compound promotes shoot elongation and biomass accumulation in leaves, stems, and roots, the magnitude of these responses varies across seasons, indicating environmental modulation of its effects. The concentration of 0.3 mg L^{-1} is effective across genotypes, but its performance is influenced by seasonal conditions. Root responses are predominantly expansive, with increases in length, area, and volume without changes in diameter, while BB-16 application is associated with increased chlorophyll and nitrogen balance indices and reduced flavonoid and anthocyanin levels, indicating changes in plant physiological status. Overall, these findings indicate that seasonal variation is a key factor regulating plant responses to BB-16, provides insights for optimizing its application in nursery systems, and highlights the need for further studies addressing the underlying biochemical and molecular mechanisms.

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3.2 Changes in root environment affect *Meloidogyne enterolobii* resistance and pigment synthesis in *Psidium*[2]

HIGHLIGHTS:

Propagation method and physiological conditions affect Psidium resistance to nematodes.

Leaf pigment indices are early indicators of nematode stress.

Chlorophyll and nitrogen balance index decline with increased nematode multiplication in the root environment.

ABSTRACT: Guava (*Psidium guajava*) can be parasitized by the plant-parasitic nematode *Meloidogyne enterolobii*, which causes a complex disease. Research on resistance involves studying infection, nematode multiplication in roots, and early symptom expression. This study aimed to confirm *Meloidogyne enterolobii* resistance in *Psidium* genotypes selected as potential guava rootstocks after their clonal propagation and determine the symptomatology associated with plant infection by evaluating leaf pigmentation indices and seedling growth rates. The experiment was conducted in a protected environment at the Research Support Unit of the State University of Northern Rio de Janeiro, in Campos dos Goytacazes, Rio de Janeiro state (RJ), between August 2023 and March 2024. A randomized block design was used, with seven treatments, 12 replicates, and one plant per plot. Seedlings, obtained through mini-cutting, were inoculated with 2,000 eggs and second-stage juveniles (J_2) of *M. enterolobii*, and evaluated over 135 days. The resistance levels previously identified in seed-propagated seedlings were not consistently confirmed after vegetative propagation. Early parasitism symptoms coincided with changes in leaf pigment indices. The susceptibility of most genotypes, previously classified as resistant, suggests that epigenetic factors may influence the expression of resistance to *M. enterolobii*.

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Keywords: root-knot nematode, rootstock, *Psidium cattleianum*, *Psidium guajava,* vegetative propagation

Mudanças no ambiente radicular afetam resistência a *Meloidogyne enterolobii* e síntese de pigmentos em *Psidium*

RESUMO: A goiabeira (*Psidium guajava*) pode ser parasitada pelo fitonematoide *Meloidogyne enterolobii*, causando uma doença complexa. Estudos da resistência envolvem a infecção, multiplicação do nematoide nas raízes e expressão precoce de sintomas. Portanto, este estudo teve como objetivo reavaliar a resistência a *M. enterolobii* em genótipos de *Psidium* selecionados como potenciais porta-enxertos para goiabeira após propagação clonal e determinar a sintomatologia associada à infecção das plantas por meio de índices de pigmentação foliar e das taxas de crescimento de mudas. O experimento foi conduzido em ambiente protegido na Unidade de Apoio à Pesquisa da Universidade Estadual do Norte Fluminense, município de Campos dos Goytacazes-RJ, entre agosto de 2023 e março de 2024. Utilizou-se o delineamento em blocos casualizados, com sete tratamentos, com 12 repetições e uma planta por parcela. As mudas foram obtidas por miniestaquia, inoculadas com 2.000 ovos e J_2 de *M. enterolobii* e avaliadas durante um período de 135 dias. Os níveis de resistência previamente encontrados em mudas de origem seminífera não foram todos confirmados após a propagação vegetativa. Sintomas precoces do parasitismo ocorreram juntamente com alterações nos índices de pigmentos foliares. A suscetibilidade da maioria dos genótipos, anteriormente classificados como resistentes, sugere que fatores epigenéticos atuem na expressão da resistência a *M. enterolobii*.

Palavras-chave: nematoide das galhas, porta-enxerto, *Psidium cattleianum*, *Psidium guajava,* propagação vegetativa

INTRODUCTION

Guava (*Psidium guajava* L.) is a highly nutritious fruit commercially cultivated in tropical and subtropical regions worldwide (Das, 2020). It is consumed fresh and widely used in the food industry due to its rich nutritional profile (Singh et al., 2018).

Currently, the primary challenge in guava production in Brazil is guava decline, a plant health disorder caused by a complex interaction between the root-knot nematode *Meloidogyne enterolobii* and the fungus *Neocosmospora falciformis*. Parasitism by *M. enterolobii* alters root exudates, promoting microbial activity that compromises root physiology and weakens plant immunity against fungal infection, thereby accelerating plant deterioration (Souza et al., 2023).

Given the environmental toxicity, high cost, and limited effectiveness of currently available synthetic nematicides, developing integrated and sustainable management strategies is essential for effective control of plant-parasitic nematodes. One promising strategy is the development of *M. enterolobii*-resistant genotypes. Since no resistance has been reported in guava cultivars, research has focused on identifying resistance in other *Psidium* species, such as *P. guineense* (Souza et al., 2018) and *P. cattleianum* (Gomes et al., 2017). A hybrid between *P. guajava* and *P. guineense* was the first rootstock officially registered in Brazil for sustainable coexistence with nematodes (Souza et al., 2018), combining resistance with desirable fruit quality (Simões et al., 2023).

Genetic diversity in rootstocks is essential for boosting fruit yield and sustainability. However, nematode populations within the same genotype can be influenced by environmental factors, and resistance may vary due to its polygenic nature (Gomes et al., 2017). Consequently, resistance observed in a single juvenile plant might be affected after clonal propagation, due to epistatic interactions.

Monitoring physiological traits under nematode stress provides crucial insights into plant stress responses. Biotic stress conditions trigger physiological alterations in plants, often reflected in altered pigment levels, such as chlorophyll and anthocyanins (Baskar et al., 2018).

Studies show that *Psidium* species susceptible to *M. enterolobii* exhibit secondary symptoms, including leaf bronzing and post-inoculation chlorosis, attributed to root system damage and reduced water and nutrient uptake (Chiamolera et al., 2018). Similarly, Gomes et al. (2008) associated the onset of chlorosis and leaf wilting with nutritional deficiencies caused by nematode-induced physiological stress. A decline in chlorophyll indices indicates damage, given that these leaves would otherwise be at their peak assimilate production (Silva et al., 2016). In addition to evaluating plant survival and nematode reproduction, monitoring physiological traits under nematode stress provides valuable data on plant responses.

In this context, physiological indicators such as chlorophyll and anthocyanin content, along with pigment dynamics, can serve as early stress markers in plants and help elucidate the underlying mechanisms of nematode tolerance among different *Psidium* genotypes. Reduced chlorophyll content in guava plants under water deficit conditions (Roque et al., 2025) underscores the importance of pigment dynamics as early indicators of plant stress. Assessing these parameters alongside nematode reproduction provides a broader understanding of plant-nematode interactions and supports the selection of resilient genotypes for integrated management strategies.

Therefore, this study aimed to confirm *M. enterolobii* resistance in *Psidium* genotypes selected as potential rootstocks for guava after clonal propagation and determine the symptomatology associated with plant infection by evaluating leaf pigmentation indices and seedling growth rates.

MATERIAL AND METHODS

The experiment was conducted from August 2023 to March 2024 in the northern region of Rio de Janeiro state (RJ), Brazil (21° 45′ 44″ S, 41° 17′ 19″ W, altitude of 14 m). According to Köppen's classification, the climate of the region is classified as Aw, tropical with a rainy summer, dry winter, and minimum monthly temperature above 18 °C.

The genotypes evaluated in this study were derived from Galvão et al. (2024), and the selection process is summarized in Figure 1. Six genotypes with a reproduction factor lower than one were identified and selected for cloning using the mini-cutting technique (Arantes et al., 2021), along with the 'Paluma' guava cultivar, which served as the susceptible control. Each resistant plant was identified in an established clonal mini-garden and subsequently transplanted into 30-L pots maintained in a greenhouse (7.0 m wide, 20 m long, and 4.0 m high) without climate control, covered with 50% Sombrite® shade cloth and equipped with lateral mesh and a raffia-covered floor. The soil used in the pots was classified as Ultisol (Soil Survey Staff, 2022). Each pot was fertilized with 8.5 g L^{-1} of limestone and 2.74 g L^{-1} of single superphosphate. Additionally, a slow-release Osmocote® fertilizer (17-07-12 formulation) was added at a rate of 6.6 g L^{-1}. Irrigation was automated via a drip irrigation system, using two drippers per pot, each with a flow rate of 2.0 L h^{-1}. The irrigation schedule was adjusted based on the plants' water requirements, which varied according to temperature. The genotypes remained under these conditions for approximately one year, after which their branches were pruned to stimulate the production of herbaceous shoots used for cuttings in the seedling production process, following the protocol described by Arantes et al. (2021).

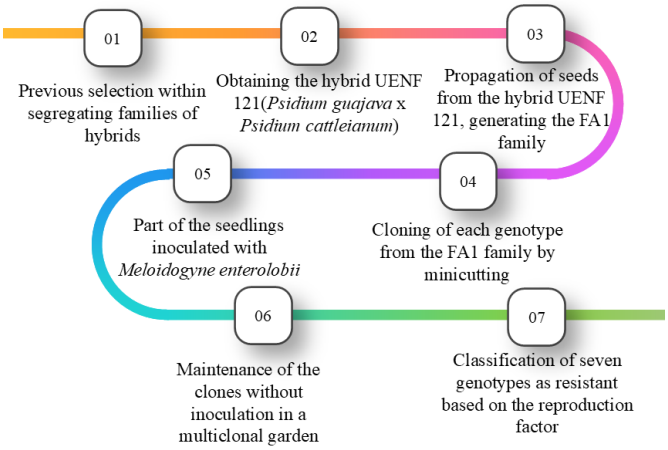


Figure 1. Flowchart for classifying Psidium genotypes as resistant based on the reproduction factor

Mini-cuttings were prepared with two pairs of leaves. The basal pair was removed, and the upper pair trimmed to half its original size. The cuttings were placed in 280 mL tubes filled with commercial Basaplant® substrate. They were subsequently maintained in a mist chamber for 60 days (15-second spraying intervals every 10 min, flow rate of 7 L h^{-1}, under a pressure of 4.0 kgf cm^{-2}, with one micro-sprinkler per m^2).

After acclimatization, the plants were transplanted into 5 L pots filled with a 2:1:1 mixture of washed river sand, local soil (from the established clonal garden), and cattle manure. This mixture underwent a 72-hour solarization process to eliminate any pre-existing nematodes. After transplanting, the plants were placed on benches (1.15 m wide × 5 m long) 1 m above the ground in the greenhouse. The plants were irrigated via overhead micro-sprinklers at a density of 0.5 micro-sprinkler per m^2, with irrigation adjusted according to plant water requirements.

For this experiment, a randomized block design (RBD) was used. The experimental treatments consisted of six previously identified *Psidium* genotypes, along with the 'Paluma' cultivar, totaling seven treatments. Each treatment was replicated 12 times, with one plant per plot.

Throughout the experimental period, climate data were collected using a datalogger inside the greenhouse. The average conditions recorded were a maximum temperature of 36.3 °C, minimum temperature of 21.8 °C, maximum relative humidity of 92.0%, and minimum relative humidity of 53.8%. These values indicate an environment with consistently high temperatures and elevated humidity levels.

Inoculation with *M. enterolobii* was performed when the plants possessed three to five pairs of leaves (30 days after transplanting). A pure isolate maintained in tomato plants (*Solanum lycopersicum* L.) was used as the inoculum source. To extract the inoculum, infected roots were processed using a modified Coolen e D'Herde (1972) extraction method, omitting the use of kaolin. The resulting nematode suspension was passed through stacked 65- and 500-mesh sieves and collected in a beaker. Under a stereoscopic microscope, using a Peters slide, the suspension was calibrated to a concentration of 2,000 *M. enterolobii* eggs + J_2 per 10 mL of water. Each seedling was inoculated with 10 mL of the suspension, which was distributed into four 1 cm-deep holes made in the soil around the plant collar.

Resistance was evaluated at 135 DAI (days after inoculation). The plants were removed from their pots and the shoot was separated from the root system. To extract eggs and second-stage juveniles (J_2), the roots were processed using the same method previously described for obtaining the inoculum. The only modification was agitating the roots in a 6% aqueous solution of sodium hypochlorite (2% active chlorine), instead of using pure water. The egg and J_2 suspension obtained from each plant was homogenized, and three 1 mL aliquots were used for counting with a Peters slide, expressed as the final nematode population (FP).

The reproduction factor (RF) was calculated according to Oostenbrink (1966) by dividing the final population (number of eggs + J_2) by the initial inoculated population (eggs + J_2). RF = FP / 2000), where RF = 0 indicates immunity, RF < 1 resistance, and RF > 1 susceptibility (Oostenbrink, 1966).

The percentage reduction in RF (PRRF%) was also calculated. This was determined similarly to the RF FP/IP ratio (final population/initial population). The population with the highest reproduction index was considered the susceptibility reference. Next, the reference reproduction index was compared with that of the other populations, calculating the reduction percentage for each, following the methodology described by Moura e Régis (1987). Based on these values, the resistance levels of each *Psidium* genotype to *M. enterolobii* were defined as: HS - highly susceptible: PRRF% from 0 to 25%; S - susceptible: PRRF% from 26 to 50%; LR - low resistance: PRRF% from 51 to 75%; MR - moderately resistant: PRRF% from 76 to 95%; R - resistant: PRRF% from 96 to 99%; and HR - highly resistant/immune: PRRF% of 100%.

The ratio of the number of eggs + J_2 per gram of root (NOJ/G) was also calculated.

On the day of inoculation, SPAD index (leaf greenness index) readings were initiated using a portable chlorophyll meter (Minolta SPAD - 502 “Soil Plant Analyzer Development”, Japan) to indirectly and non-destructively measure total chlorophyll. Three consecutive readings were taken from the most recently matured leaf pair (the third pair from the apex) and the oldest leaf pair on each plant.

The nitrogen balance index (NBI) and relative chlorophyll (CHL), anthocyanin (ANTH), and flavonoid (FLV) contents were measured using a portable Dualex® meter (Scientific, FORCE-A model). Evaluations were conducted on the middle third of the last fully developed leaf at two time points: at inoculation (day 0), to evaluate plant status before pathogen development in the rhizosphere, and 135 days after *M. Enterolobii* inoculation, selected as the optimal time point for assessing infestation severity. A different fully developed third leaf was sampled for each evaluation.

Biometric data, including plant height, stem diameter, and number of leaf pairs, were collected immediately after *M. Enterolobii* inoculation. Subsequent evaluations occurred at 0,

28, 55, 82, 109 and 135 days after inoculation (DAI), totaling six assessments, including the initial measurement (Sikandar et al., 2024).

Additional biometric data were obtained at 135 DAI, including: leaf area (cm^2), measured with a LI-3100 LICOR bench-top meter (Lincoln, NE, USA); stem and leaf dry mass (g), determined after drying plant material in a forced-air oven at 70 °C for 72 hours, and shoot dry mass, measured using a precision balance. Fresh root system mass was also assessed with a precision balance, and root volume was determined by water displacement in a graduated cylinder after root system immersion.

All data underwent the Shapiro-Wilk normality test (5% probability). Non-normal data were transformed using Log (x + 1) before analysis of variance (ANOVA). Means were compared using Tukey's test (5% probability), in R software with the ExpDes.pt package. Principal component analysis (PCA) was conducted using Paleontological Statistics Software (PAST 4.03).

SPAD index and growth indices over time were analyzed using a split-plot design (genotypes as the main plot and evaluation times as subplots). Biometric evaluations conducted at multiple time points were also analyzed using a split-plot design in time. Data were subjected to regression analysis (5% probability), and means were compared using Tukey's test (5% probability).

RESULTS AND DISCUSSION

The *Psidium* genotypes tested for resistance to *M. enterolobii* exhibited significant variations in the number of eggs per gram of root, final nematode population, reproduction factor (RF), and RF reduction percentage (Table 1).

Table 1. Number of eggs per gram of root, reproduction factor (RF), and RF reduction percentage (%) of *Psidium* genotypes 135 days after inoculation with *M. enterolobii*

Genotypes	Number of eggs per gram of root	Final population	Reproduction fator (RF)	RF reduction percentage (PRRF%)
P01	1528.83 ab	63618.18 abc	31.80 abc	40.72
P22	820.29 c	24745.38 c	12.37 c	76.94
P55	1356.24 ab	52630.91 abc	26.31 abc	50.96
P77	3438.70 a	104750.00 a	52.37 a	2.39
P90	1192.50 b	33397.92 bc	16.70 bc	68.88
P140	2057.17 ab	107312.50 ab	53.65 ab	x
Paluma	1848.06 ab	50681.82 abc	25.34 abc	52.77
CV (%)	11.6	8.21	26.24	17.29

Means followed by the same lowercase letters in the column do not differ according to Tukey's test (5% probability). CV - Coefficient of variation. x = The genotype with the highest reproduction factor (RF) index was established as the susceptibility reference, and the RF reduction percentage (%) of the other genotypes was calculated relative to it.

Genotype P22 stood out for exhibiting the lowest number of eggs per gram of root. However, its final population and RF did not differ significantly from those of other genotypes or from the 'Paluma' guava, the conventional susceptibility standard, except for P77 and P140, which had higher susceptibility indicators.

According to the nematode resistance classification established by Oostenbrink (1966), none of the analyzed genotypes achieved an RF of 0 or < 1, thus all were classified as susceptible. Under the classification based on the percentage reduction in the reproduction factor (PRRF%), established by Moura e Regis (1987), with genotype P140 serving as the susceptibility reference due to its highest reproduction factor, genotype P22 was classified as moderately resistant. The other genotypes were classified as susceptible, except for P77, which was classified as highly susceptible, and P90 and 'Paluma' as slightly resistant.

Ribeiro et al. (2019) classified thirty-four *Psidium* genotypes as resistant according to Oostenbrink (1966), while Moura e Regis (1987) identified forty-two resistant genotypes Similarly, Cavalcanti Júnior et al. (2021) found the same number of resistant genotypes using both classifications.

While resistance classification criteria are essential for grouping genotypes by their resistance levels, they should be used with caution. Depending on the infestation severity, genotypes with very similar nematode populations might be classified differently (e.g., one as resistant and another as susceptible with RF = 0.9 and 1.1, respectively). Likewise, genotypes with high nematode population growth may be deemed highly resistant when compared to those with very high populations. Therefore, when assessing resistance, the final population should always be considered first, and classification methods carefully assessed to determine the most suitable approach.

Studies such as that by Oliveira et al. (2019) highlight the importance of selecting robustly resistant genotypes, especially in regions where nematode infestation is common, given the significant damage caused by this disease. The high susceptibility observed in this study suggests that the evaluated genotypes would not be suitable as rootstocks in nematode-infested areas.

Additionally, it is important to note that genotypes previously classified as resistant at a certain developmental stage may exhibit changes in resistance depending on environmental conditions and management practices. Oliveira et al. (2019) emphasized that *Psidium* genotypes may not maintain consistent resistance under natural field infestation. In the present study, despite uniform inoculation pressure, the physiological age of the genotypes influenced their resistance patterns, underscoring the need to consider this factor in breeding programs for nematode resistance.

Silva et al. (2024) reported that, although vegetative propagation is generally expected to produce identical offspring, epigenetic changes can lead to a shift in susceptibility. These authors observed that epigenetic variations in the expression of the analyzed variables, including the reproduction factor, can alter nematode resistance. Jirtle e Skinner (2007) also emphasized that environmental factors, such as stress and nutrient availability, can induce epigenetic changes, which may alter a plant's vulnerability to diseases.

The absence of resistance observed in this study may result from a complex interaction between genetic and epigenetic factors, influenced by the cloning process. Genotypes originally identified as resistant may lose resistance due to epigenetic reprogramming during vegetative development, as observed in guava accessions by Silva et al. (2024). According to Collett et al. (2023), *M. enterolobii* can complete its life cycle approximately 15 days after inoculation; thus, the 135-day period allowed for multiple reproductive cycles and a thorough assessment of plant response to nematode pressure.

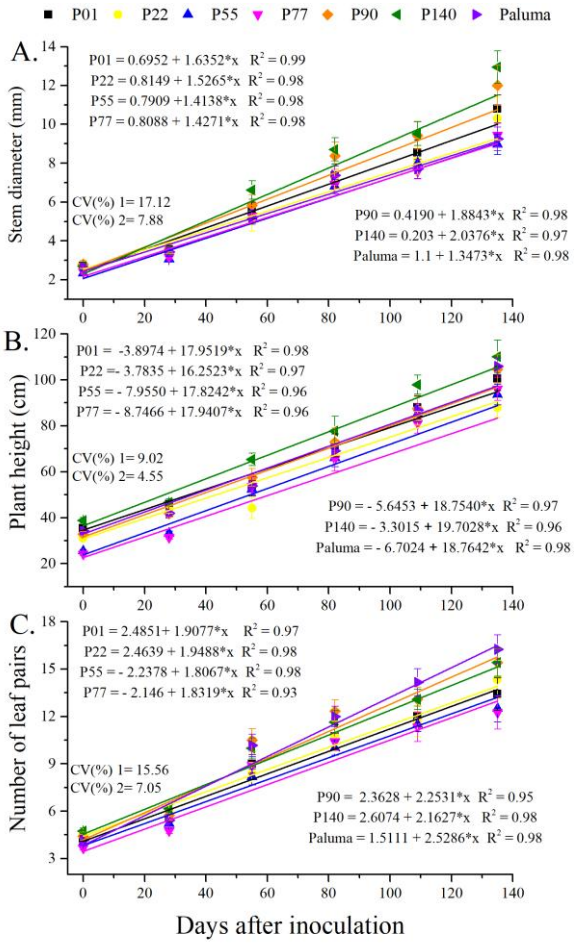
An important factor to consider is that the plants evaluated in this study developed an adventitious root system originating from stem tissues or the base of mini-cuttings. By contrast, the genotypes previously classified as resistant were propagated via seeds and developed a taproot (axial) system, characterized by a dominant, deep-growing primary root. In the study by Oliveira et al. (2024), seed-propagated seedlings displayed underdeveloped root systems with low fresh mass at the time of evaluation, which may have limited nematode parasitism and reproduction due to root suberization. By contrast, all the clones obtained through vegetative propagation produced a larger number of adventitious roots, potentially facilitating parasitism and increasing nematode reproduction.

A previous study by Galvão et al. (2024) classified the same *Psidium* genotypes as resistant to *M enterolobii*. when evaluated as young plants with a taproot system. However, after a cycle of vegetative propagation via cuttings and development of an adventitious root system, their

resistance classification changed. In the present study, these previously identified genotypes showed increased RF values, exceeding the threshold of 1.0. This change may be related to the loss of vigor and alterations in the typical root system architecture of seed-propagated plants, since adventitious roots often exhibit lower efficiency in nutrient and water uptake and may create a different chemical and structural environment for nematode development (Li, 2021). Additionally, physiological and epigenetic changes induced during clonal propagation processes may alter the plant's defense mechanisms against biotic stress (Silva et al., 2024).

In summary, these results underscore the importance of carefully selecting *Psidium* genotypes for *M. Enterolobii* management. The findings of this study highlight the need for thorough evaluation before planting in high-risk areas. Moreover, epigenetic variation during clonal propagation should be considered in future studies, since it may affect the stability of resistance traits.

Analysis of variance revealed significant effects of evaluation periods and significant differences among genotypes. However, no significant genotype x evaluation period interaction was detected, indicating that although genotypes differed in growth rates, their response patterns over time remained consistent. A linear model appropriately described stem diameter growth of the different genotypes within the observed range (Figures 2A, B and C), with the slopes reflecting the varying growth rates.



The vertical bars represent the standard error; * $p \leq 0.05$ (F test); CV1 - Coefficient of variation among genotypes (main factor). CV2 - Coefficient of variation within time (subplots)

Figure 2. Stem diameter (A), plant height (B) and number of leaf pairs (C) of *Psidium* genotypes over 135 days after inoculation (DAI) with *Meloidogyne enterolobii*

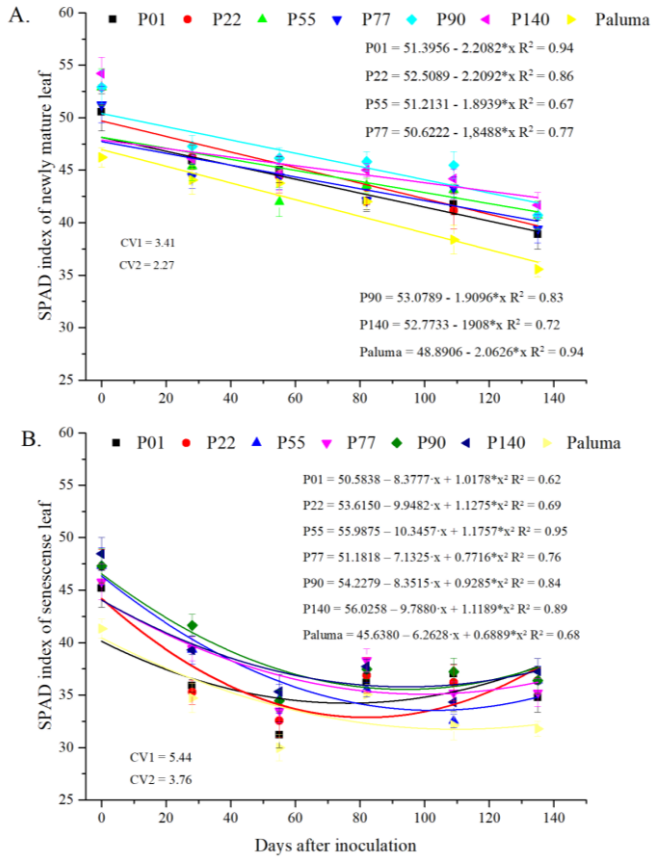
Genotypes P140 and P90 achieved the largest final stem diameter (Figure 2A). P140 also stood out in height, while P22 exhibited slower growth (Figure 2B). By contrast, the P55 guava tree had the highest number of leaf pairs (Figure 2C).

With respect to height growth, the growth curve of genotype P140 diverged from the other curves, reaching 110 cm at 135 DAI (Figure 2B). On the other hand, genotype P22 showed slower growth, but still reached an average height exceeding 87 cm at 135 DAI.

In relation to the number of leaf pairs, the ‘Paluma’ guava outperformed the other genotypes, particularly P55.

Overall, despite nematode parasitism, several evaluated genotypes displayed greater vigor than the ‘Paluma’ guava, particularly in stem diameter. This trait is crucial for rootstock selection, since increased vigor could shorten seedling production time (Mir et al., 2023). Combining vigor in the nursery with nematode resistance is a key goal for ensuring viable seedling production.

In terms of plant physiology, SPAD indices varied among genotypes, but a decreasing trend was observed over time in the newly mature leaves (Figure 3A).



The vertical bars represent the standard error; * $p \leq 0.05$ (F test); CV1 - Coefficient of variation among genotypes (main factor). CV2 - Coefficient of variation within time (subplots).

Figure 3. SPAD indices measured in newly mature (A) and senescent leaves (B) of *Psidium* genotypes over 135 days after inoculation (DAI) with *Meloidogyne enterolobii*

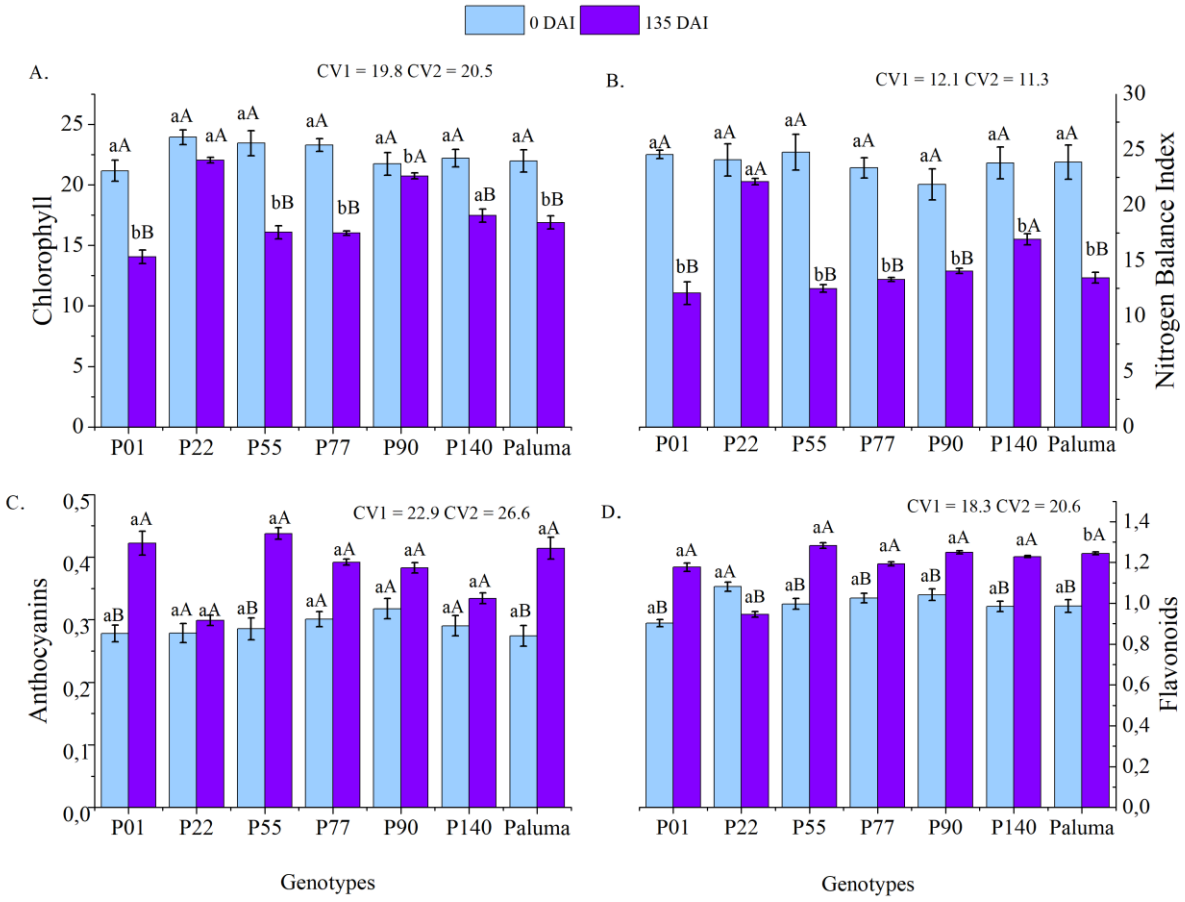
This reduction in SPAD index may be associated with nutritional deficiencies resulting from *M. enterolobii* infestation, which damages the root system through gall formation and reduces the functional root area (Collett et al., 2023). This damage impairs the translocation of key nutrients that act as cofactors in chlorophyll biosynthesis and maintenance (Hajihassani et al., 2013). The resulting nutrient deficiency limits chlorophyll production and accelerates leaf senescence, contributing to the decline in SPAD index and photosynthetic capacity. Additionally, nutrient imbalances can exacerbate oxidative stress in plant tissues, triggering the accumulation of secondary metabolites such as anthocyanins and flavonoids as protective responses (Leonetti e Molinari, 2020). The decrease in chlorophyll in newly matured leaves signals damage, given that these leaves are expected to exhibit peak assimilate production (Silva et al., 2016), while in senescent leaves, declining chlorophyll levels would be standard. Although nitrogen, a mobile nutrient in plants, is typically redistributed during senescence, this translocation may be slower under more favorable environmental conditions (Bieker e Zentgraf, 2013).

Overall, a decline in chlorophyll indices in senescent leaves (Figure 3B) was a common trend among the analyzed genotypes, but the intensity of this decline varied depending on genotype and stage of senescence. The quadratic pattern observed in most cases suggests an initial reduction in chlorophyll levels, followed by stabilization in the later evaluation periods (Figure 3B).

The decrease in chlorophyll content results from nematode-induced root system damage, hindering the plants' ability to absorb essential nutrients required for maintaining green pigmentation (Hajihassani et al., 2013). The quadratic decline of SPAD indices in senescent leaves likely stems from an initial rapid chlorophyll degradation due to nutrient remobilization under nematode stress, followed by stabilization as the plant’s capacity to remobilize nutrients

diminishes and leaf senescence progresses. Additionally, natural abscission and variations in the physiological state of sampled leaves over time contribute to fluctuations in SPAD values (Hajihassani et al., 2013; Taiz et al., 2015).

Figure 4 displays the combined indices of chlorophyll, flavonoids, anthocyanins, and nitrogen balance for all the genotypes at both 0 and 135 DAI. For most genotypes, chlorophyll indices (Figure 4A) were higher at 0 DAI, except for P22 and P90, which maintained high levels at both time points. The other genotypes, however, showed a sharp reduction in their chlorophyll index at 135 DAI.



The vertical bars represent the standard error; Different lowercase letters indicate significant differences between genotypes at each time point, while different uppercase letters indicate significant differences between time points for the same genotype, according to Tukey's test ($p \leq 0.05$); CV1 - Coefficient of variation among genotypes (main factor). CV2 - Coefficient of variation within time (subplots). DAI - days after inoculation.

Figure 4. Mean values of chlorophyll (A), nitrogen balance index – NBI (B), anthocyanins (C), and flavonoids (D) in guava genotypes (P01, P22, P55, P77, P90, P140, Paluma) at two different time points (0 DAI and 135 DAI)

With respect to the nitrogen balance index (NBI) (Figure 4B), a trend similar to that of chlorophyll was observed, with higher values at the initial time point and a significant decrease in the second. All genotypes, except for P22, exhibited a substantial reduction in NBI, suggesting a possible genotype x environment interaction and differing responses to biotic stress. The NBI, calculated as the ratio between chlorophyll and flavonoids, is an indicator of

the plant's nutritional status. Under conditions of low nitrogen (N) availability, plants allocate carbon to the synthesis of polyphenols, such as flavonoids (Cerovic et al., 2015). A decline in NBI reflects nitrogen deficiency, which compromises plant growth and development (Coelho et al., 2012).

Chlorophyll and NBI indices declined by an average of 21.9 and 37.3%, respectively, while anthocyanin (Figure 4C) and flavonoid (Figure 4D) levels increased by an average of 30.4 and 31.1% in the two evaluation periods. These changes help explain nematode proliferation in the root environment of the genotypes. However, genotype P22 exhibited a different profile, with significantly smaller reductions in chlorophyll (-7.9%) and NBI (-8.1%), along with smaller increases in antioxidant compounds (+7.4% for both anthocyanins and flavonoids). This reflects its lower parasitism levels and supports its classification as moderately resistant.

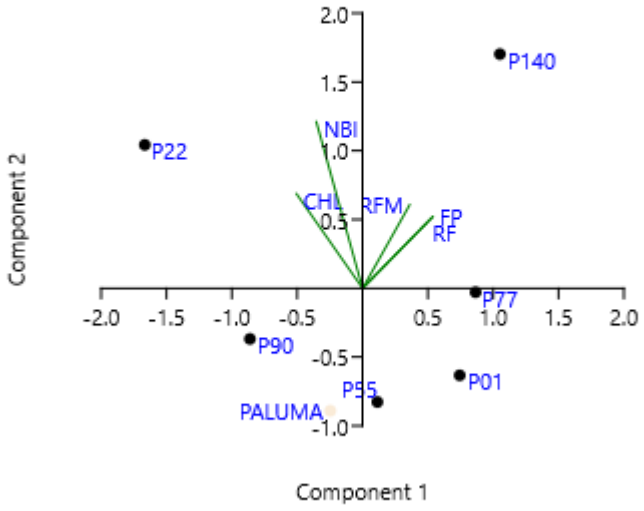
The physiological differences observed between the two time points also demonstrate the effect of parasitism on genotype performance. At the first time point (0 DAI), plants generally exhibited higher chlorophyll and NBI values, while at 135 DAI, these indices declined significantly. Flavonoids and anthocyanins increased, highlighting an adaptive stress response to nematode infection. These findings are important for identifying more stable genotypes better adapted to environmental variations, such as P22, which was less susceptible to temporal variations when compared to other genotypes.

The results become even more relevant when we consider that the genotypes were newly inoculated at the first time point, but had already undergone a period of *M. enterolobii* multiplication by the second. At the initial time point, higher levels of chlorophyll and NBI levels reflected the plants' baseline physiological status, not yet significantly affected by nematode infestation. However, a sharp reduction in both chlorophyll and NBI was observed at the second evaluation point, indicating that the nematode might be impairing nutrient absorption and the plants' photosynthetic capacity.

By 135 DAI, all genotypes exhibited the negative effect of *M. enterolobii,* which compromises the plant root system, hindering water and nutrient uptake, and consequently affecting their growth and development (Souza et al., 2023). On the other hand, the increase in flavonoid and anthocyanin levels at the second time point could be a direct response to nematode infestation stress. Plants might be activating defense mechanisms to mitigate the damage, given that these compounds are known for their protective role against environmental stresses, including pathogen parasitism (Baskar et al., 2018).

Photosynthetic pigment indices provide insights into photosynthetic efficiency and overall plant health (Agati et al., 2021). Hamdane et al. (2022) investigated the use of remote sensing, including flavonoid content and NBI, to evaluate stress in plants inoculated with *M. incognita*. Their findings indicated that plants with greater resistance to the nematode showed lower flavonoid concentrations and senescence indices, suggesting enhanced protection against pathogen-induced damage. Therefore, the reduction in chlorophyll and NBI levels, along with increasing flavonoid and anthocyanin concentrations, may reflect similar physiological defense mechanisms in response to *M. eterolobii* infection.

Principal component analysis (PCA) was performed to investigate the relationships between *M. enterolobii* resistance traits and physiological indices, thereby enabling the identification and visualization of correlations among variables, simplifying the interpretation of interactions (Figure 5)



RFM - Root fresh mass, CHL - Chlorophyll, NBI - Nitrogen balance index, FP - Final population, RF - Reproduction factor

Figure 5. Principal component analysis (PCA), showing the distribution of seven *Psidium* genotypes based on the variables associated with resistance to *M. enterolobii*

The analysis reveals that the first two principal components (PC1 and PC2) together explained 83.82% of the total data variability, with PC1 accounting for 60.68% and PC2 23.14%. PC1 was positively influenced by the variables FRM, FP, and RF, indicating a correlation among these variables. This component highlights the overall differences in nematode resistance among the genotypes. Genotypes located at the positive extremes of PC1 (such as P01, P77, and P140) exhibited higher values for these variables, reflecting lower resistance due to increased pathogen reproduction. On the other hand, CHL and NBI were oriented in the opposite direction, albeit at a low angle, suggesting a subtle negative correlation with PC1. This implies that as FRM, FP, and RF increase, CHl and NBI levels tend to decrease. Genotypes with higher nematode reproduction and root biomass showed lower levels of these indicators and more pronounced symptoms of infection.

PC2, in turn, was more strongly associated with CHL and NBI. Genotypes more displaced along the negative axis of PC2 (such as P22 and P90) appeared to be less impacted by these indices, possibly reflecting genotypes with greater resistance or a weaker physiological response to nematode-induced stress. Therefore, genotypes at the positive extremes of PC1 tend to exhibit greater susceptibility to the pathogen, while those more displaced along the negative axis of PC2 may reflect greater resistance or a weaker response to the evaluated traits, especially with respect to nematode reproduction.

PC1 clearly distinguishes between genotypes with different levels of nematode resistance. These findings corroborate those of recent studies, emphasizing the importance of variables such as FP, RF, and FRM in assessing resistance to nematode parasitism, where genotypes with a lower reproduction factor and less FRM exhibit greater resistance (Oliveira et al., 2024).

Table 2 shows that, at 135 DAI with *M. enterolobii*, the evaluated *Psidium* genotypes exhibited similar vegetative performance, suggesting that all were equally affected in their vegetative traits.

Table 2. Leaf area, stem and leaf dry mass, root fresh mass, and root volume of *Psidium* genotypes 135 days after inoculation with *M. enterolobii*

Genotypes	Leaf area (cm^2)	Stem dry mass (g)	Leaf dry mass (g)	Root fresh mass (g)	Root volume (cm^3)
P01	2317.13 a	16.85 a	22.29 a	45.50 a	45.16 a
P22	2736.90 a	19.92 a	21.57 a	36.88 a	41.92 a
P55	2136.87 a	17.46 a	20.10 a	40.52 a	38.90 a
P77	2627.20 a	17.17 a	21.13 a	36.68 a	43.75 a
P90	2684.38 a	21.55 a	25.73 a	38.69 a	35.50 a
P140	3118.31 a	23.85 a	27.77 a	48.25 a	46.04 a
Paluma	2303.27 a	21.30 a	19.47 a	35.93 a	31.18 a
CV (%)	6.11	13.56	13.65	15.41	16.32

Means followed by the same lowercase letters in the column do not differ according to Tukey's test (5% probability). CV - Coefficient of variation

The vegetative similarity observed among the *Psidium* genotypes at 135 DAI indicates that all genotypes were significantly affected by *M. enterolobii* infection. High levels of root galling, egg masses, and nematode reproduction were recorded across all treatments, suggesting more susceptibility after vegetative propagation. This outcome may be associated with the aggressive parasitism typical of *M. enterolobii*, as reported by Oliveira et al. (2024) and Silva et al. (2024).

These findings align with those reported by Biazatti et al. (2016), who observed statistical differences in shoot and root system parameters among resistant *Psidium* genotypes but not among susceptible accessions. Furthermore, wild *Psidium* species resistant to *M. enterolobii* tend to exhibit lower vegetative vigor, as demonstrated by Freitas et al. (2014).

CONCLUSIONS

The resistance level assessed in seedlings of seminal origin was not fully confirmed after vegetative propagation, given that most genotypes showed susceptibility to *Meloidogyne enterolobii*. However, genotype P22 maintained moderate resistance, with a 76% reduction in the reproduction factor. Early symptoms of parasitism were also expressed as changes in leaf pigment indices.

The physiological responses of the genotypes to *M. enterolobii* infestation confirmed the association between nematode parasitism and alterations in chlorophyll content, nitrogen balance, and antioxidant compound production. Among the evaluated genotypes, P22 exhibited greater physiological stability under infestation, thereby supporting its classification as moderately resistant.

The susceptibility of genotypes previously classified as resistant suggests a possible influence of epigenetic factors on the expression of resistance to *M. enterolobii.* Although the extent of this influence was not determined in this study, it may partially explain the variability observed after vegetative propagation. Further research is needed to elucidate the extent of this effect on nematode resistance in *Psidium* genotypes.

Author's Contributions: Sydney P. Galvão was responsible for the conceptualization, formal analysis, investigation, methodology, visualization, and drafting the manuscript. Cláudia S. Marinho was responsible for data curation, funding acquisition, project administration, resource management, supervision, and writing — review and editing. She also advised, supervised the work, handled administration, and worked on data analysis and revisions. Raudielle F. dos Santos and Rachel M. R. Silva were responsible for the visualization, original drafting, and writing — review and editing. Antonio E. Silva and Mariana Z. Mangeiro contributed to research, data acquisition, data analysis, and manuscript writing. Ricardo M. Souza worked on data acquisition, manuscript improvements, and revisions.

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3.3 Sasonal variation in the efficiency of minicutting production in *Psidium* under gibberellic acid application[3]

ABSTRACT

Minicutting is an important technique for *Psidium* propagation, particularly for the multiplication of genotypes in breeding programs. This study evaluated the effects of environment (season and GA_3 application) on vegetative growth, minicutting production, and rooting in different *Psidium* genotypes. Six hybrids resistant to *Meloidogyne enterolobii* and the commercial cultivar 'Paluma' were evaluated, with or without GA_3 application, across four seasons. The experiment was conducted under protected conditions in a randomized block design. Individual analyses were performed for each season, and a combined analysis considered environments defined by the interaction between season and GA_3 application. Shoot length and diameter, internode spacing, number of leaf pairs, number of shoots, number of minicuttings, and rooting were measured. GA_3 significantly affected most variables, particularly those related to shoot growth. The genotype × environment interaction was significant for traits associated with shoot emission and minicutting production, indicating that genotype performance depends on environmental conditions. 'Paluma' showed the highest mean values for vegetative traits across seasons, while genotypes P140 and P90 stood out for shoot emission and minicutting production, respectively. Environment was the main determinant of stock plant growth, and GA_3 effects were more pronounced under favorable growth conditions, without consistent effects on minicutting rooting.

Keywords: *Psidium guajava; Psidium cattleianum;* Rootstock; Vegetative propagation; genotypes.

[3]Artigo em revisão na revista Scientia Horticulturae

Introduction

The production of grafted seedlings onto rootstocks resistant to *Meloidogyne enterolobii* has become strategic for maintaining the guava production chain, requiring the clonal propagation of both scion and rootstock (Gomes et al., 2011; Collett et al., 2021). In this context, the availability of clonal rootstocks to ensure genetic fidelity is essential to maintain traits such as resistance, tolerance, and vigor throughout the propagation process, particularly under increasing biotic pressure from this pathogen (Rawls et al., 2015).

Among clonal propagation methods, minicutting has been established as an efficient technique for both the operational expansion of clonal mini-gardens and the final multiplication stage in breeding programs. Stock plants maintained under protected conditions allow continuous cycles of shoot production, enabling frequent harvesting of minicuttings and reducing the time required to multiply superior genotypes (Arantes et al., 2021). Thus, minicutting has proven to be an effective tool for the fixation and multiplication of selected clones.

In this context, plant growth regulators may enhance the efficiency of genotype multiplication by improving stock plant architecture, vigor, and the regularity of propagule production. Among these regulators, gibberellic acid (GA_3) promotes cell elongation and expansion, influencing plant height, leaf area, and shoot development (Ritonga et al., 2023). In addition, GA_3 responses may interact with environmental signals, indicating that its effects are genotype-dependent and influenced by growing conditions (Gupta e Chakrabarty, 2013).

In *Psidium*, Arantes et al. (2021) demonstrated that foliar application of GA_3 can increase minicutting production; however, this study was conducted using a single genotype, leaving uncertainty regarding the influence of genetic variability on the response to this regulator. Therefore, in multiclonal mini-gardens, genotypic differences and their interaction

with environmental conditions may alter genotype performance rankings, directly affecting minicutting production and the progress of rootstock breeding programs in guava.

Thus, this study aimed to evaluate the interaction between genotypes and environments, defined as the combination of seasons and GA_3 application, on vegetative traits and the efficiency of minicutting production in *Psidium* genotypes.

Material and Methods

The experiment was conducted in a greenhouse covered with plastic film and equipped with 50% shading mesh, lateral screening, and raffia-covered floor at the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) In Rio de Janeiro, Brazil. The plant material consisted of six genotypes resistant to the nematode *Meloidogyne enterolobii* (P90, P01, P22, P77, P140, and P55), selected from a segregating population derived from the hybrid UENF 121 (*P. guajava* × *P. cattleianum*), in addition to the susceptible cultivar 'Paluma', used as a control.

Seedlings obtained by vegetative propagation through minicutting were used to establish a clonal mini-garden in 5-L pots. The pots were filled with a commercial substrate (Basaplant®), amended with lime (30 g L^{-1}), and fertilized with single superphosphate (6 g L^{-1}) and Osmocote® (6.6 g L^{-1}, 14-14-14 formulation). During the mini-garden management phase, topdressing fertilization was performed with 7.6 g of urea, 24.7 g of single superphosphate, and 2.8 g of potassium chloride per pot, split into three applications at 14-day intervals (Biazatti et al., 2018).

The mini-garden was arranged on a bench (1.15 m × 5.0 m) at 1 m above ground level, with spacing of 0.70 m between rows and 0.27 m between plants. Plants were trained to a single stem, and after reaching the pruning stage (lignified tissues), the first cut was performed,

leaving four pairs of mature leaves. Subsequent shoots formed the stock plants, which were managed with two main branches.

Plants were grown under natural light conditions. Light irradiance was not controlled, depending on external environmental conditions and attenuated by the greenhouse structure.

Microclimatic conditions inside the greenhouse were monitored daily throughout the experimental period using a digital thermo-hygrometer installed in the central area. Seasonal mean maximum and minimum air temperatures were 30.1 and 22.3 °C in autumn, 30.2 and 21.8 °C in winter, 32.2 and 23.8 °C in spring, and 33.1 and 24.7 °C in summer, respectively. Relative humidity showed mean maximum values ranging from 86.3% to 89.0% and mean minimum values between 34.3% and 36.0%, depending on the season. The periods corresponding to each season, considered as experimental evaluation windows, are shown in Figure 1.

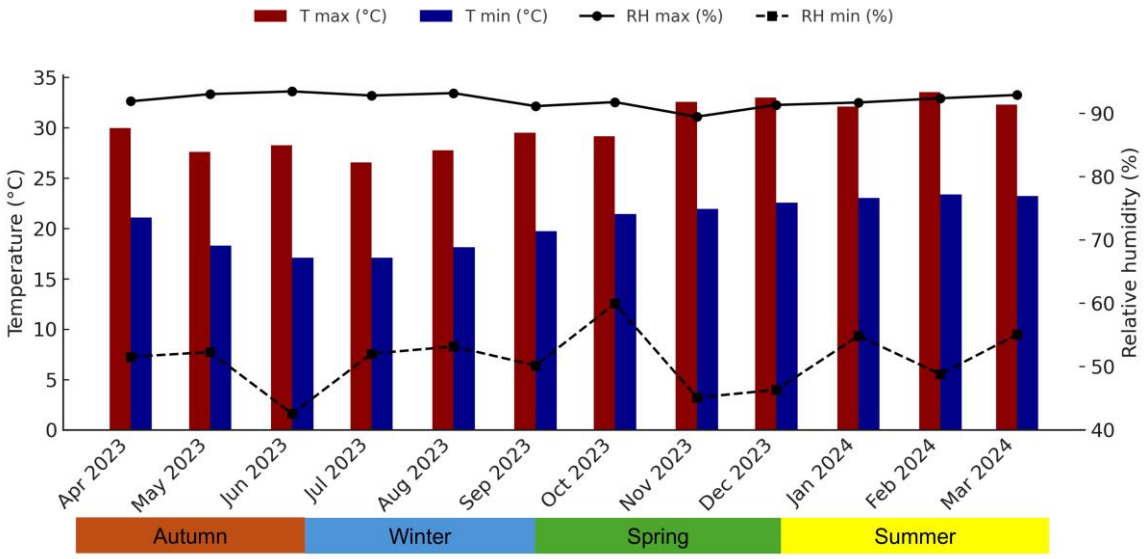


Fig. 1. Maximum (Tmax) and minimum (Tmin) air temperature (°C) and maximum (RHmax) and minimum (RHmin) relative humidity (%) under protected conditions during the experimental period, from April 2023 to March 2024.

The experiment was established in a randomized block design, arranged in a split–split-plot scheme (7 × 2 × 4), with seven *Psidium* genotypes as the main factor and GA_3 application

(with or without) as the second factor, evaluated across four seasons. Four replicates were used, with two plants per experimental unit. For treatments with GA_3, a concentration of 200 mg L^{-1} was adopted based on previous recommendations (Arantes et al., 2021), while control treatments were sprayed with distilled water under the same conditions.

The clonal mini-garden was maintained for 12 months, during which five pruning cycles were carried out for propagule collection, with average intervals of approximately 75 days between cycles. Immediately after each pruning, plants were subjected to the treatments through foliar spraying with 25 mL of the respective solution per experimental unit. The commercial product Progibb® (40% GA_3) was used as the source of gibberellic acid. Applications were always performed in the morning.

Thirty days after the first GA_3 application in each pruning cycle (season), six vegetative variables were evaluated. Shoot length was measured using a millimeter ruler from the base to the apex of the shoot. Shoot diameter was determined with a digital caliper at the mid-portion of the shoot. Internode spacing was calculated as the average distance between consecutive nodes, considering the first three developed internodes. The number of leaf pairs was obtained by counting fully expanded leaves along the shoot. Propagule production capacity was estimated as the number of potential cuttings, calculated as the total shoot length divided by the mean internode length. The number of shoots was recorded as the total number of active shoots emitted after pruning, reflecting the regrowth intensity of each genotype.

After pruning of the stock plants to induce shoot emission, the vegetative material was allowed to grow until reaching the appropriate stage for collection, characterized by uniform shoots with herbaceous to semi-hardwood consistency. Minicuttings were prepared with two pairs of leaves: the basal pair was removed, and the remaining pair was cut in half. Cuttings were inserted into 280-mL tubes filled with commercial substrate (Basaplant®) and transferred

to an intermittent mist chamber (15 s every 10 min, flow rate of 7 L h^{-1}, pressure of 4.0 kgf cm^{-2}, with one microsprinkler per m^2), where they remained for 60 days.

Rooting was evaluated at the end of the rooting period (60 days). Cuttings were considered rooted when they showed visible adventitious root formation and maintenance and/or growth of the aerial part. Rooting percentage was calculated as the ratio between the number of rooted cuttings and the total number of cuttings per experimental unit. Twelve cuttings were used per experimental unit, and results were expressed as percentage. Evaluations were performed at the end of each shoot production cycle.

Morphological variables were subjected to individual analysis of variance for each season (E1–E4), considering a randomized block design with genotypes evaluated under two levels of GA_3 application (with and without). The following model was used: $Y_{ijl} = \mu + G_i + B_l + \varepsilon_{ijl}$, where μ is the overall mean, Gi is the effect of the i-th genotype, Bl is the effect of the l-th block, and εijl is the experimental error.

For the combined analysis, environments were defined as the combination of the four seasons and GA_3 application, resulting in eight experimental environments. The adopted model was: $Yijk = \mu + Gi + Aj + (GA)ij + Bk(Aj) + \varepsilon ijk$, where μ is the overall mean, Gi is the effect of the i-th genotype (considered fixed), Aj is the effect of the j-th environment (defined as season × GA_3), $(GA)ij$ represents the genotype × environment interaction, $Bk(Aj)$ is the effect of the k-th block within the environment, and ε_{ijk} is the residual error.

Individual and combined analyses provided mean squares for the sources of variation and the coefficient of experimental variation [CV (%)]. Means were compared using Tukey's test at the 5% probability level. When the genotype × environment interaction was significant, the effects were further analyzed by comparing genotypes within each environment.

Percentage data for rooting were transformed using the arcsine square root transformation [arcsin(√x/100)] to meet the assumptions of analysis of variance, and results are

presented on the original scale. When the G × E interaction was significant, the interaction was further explored by comparing genotypes within each environment.

Additionally, Pearson correlation coefficients were estimated among the evaluated traits using genotype means within each season, and correlations were calculated separately for each seasonal condition.

All analyses were performed using R software.

Results

In the individual analyses by season, all variables showed significant differences among genotypes, indicating consistent phenotypic variability across evaluation periods. In the combined analysis of variance, considering genotypes as a fixed effect and environments defined by the combination of season and GA_3 application, significant effects of genotype (G) and environment (E) were observed for all evaluated traits. The G × E interaction was significant for most variables, indicating that the relative performance of genotypes varied according to environmental conditions. Only shoot length did not show a significant interaction, suggesting a more stable response across environments. These results indicate that both genotypic variability and seasonal conditions associated with GA_3 application influence the growth and vegetative production of *Psidium* stock plants (Table 1).

Table 1. Summary of the combined analysis of variance considering genotypes as a fixed effect and environments defined by the combination of season and GA_3 application for six clonal mini-garden productivity traits evaluated in seven *Psidium* genotypes over a one-year period.

Source of variation	DF	NS	SL	LP	SD	IS	NC	RT%
Genotype (G)	6	**	**	**	**	**	**	**
Environment (E)	7	**	**	**	**	**	**	**
G × E	42	**	ns	**	*	**	**	**
CV(%)		71.2	18.3	12.6	9.8	14.2	21.7	12.3

NS: number of shoots; SL: shoot length; LP: leaf pairs; SD: shoot diameter; IS: internode spacing; NC: number of cuttings; RT: rooting percentage; CV (%): coefficient of variation; DF: degrees of freedom; ns, *, **, and ***: not significant and significant at 5%, 1%, and 0.1% probability, respectively, by the F test.

Figure 2 summarizes the mean response of vegetative traits to GA_3 application relative to the untreated control, based on overall mean values of the experiment. In general, differences in response magnitude were observed among the evaluated traits, with greater effects on structural growth descriptors, whereas variables associated with shoot emission showed lower relative variation. These results should be interpreted as a general trend, considering environments defined by the combination of season and GA_3 application.

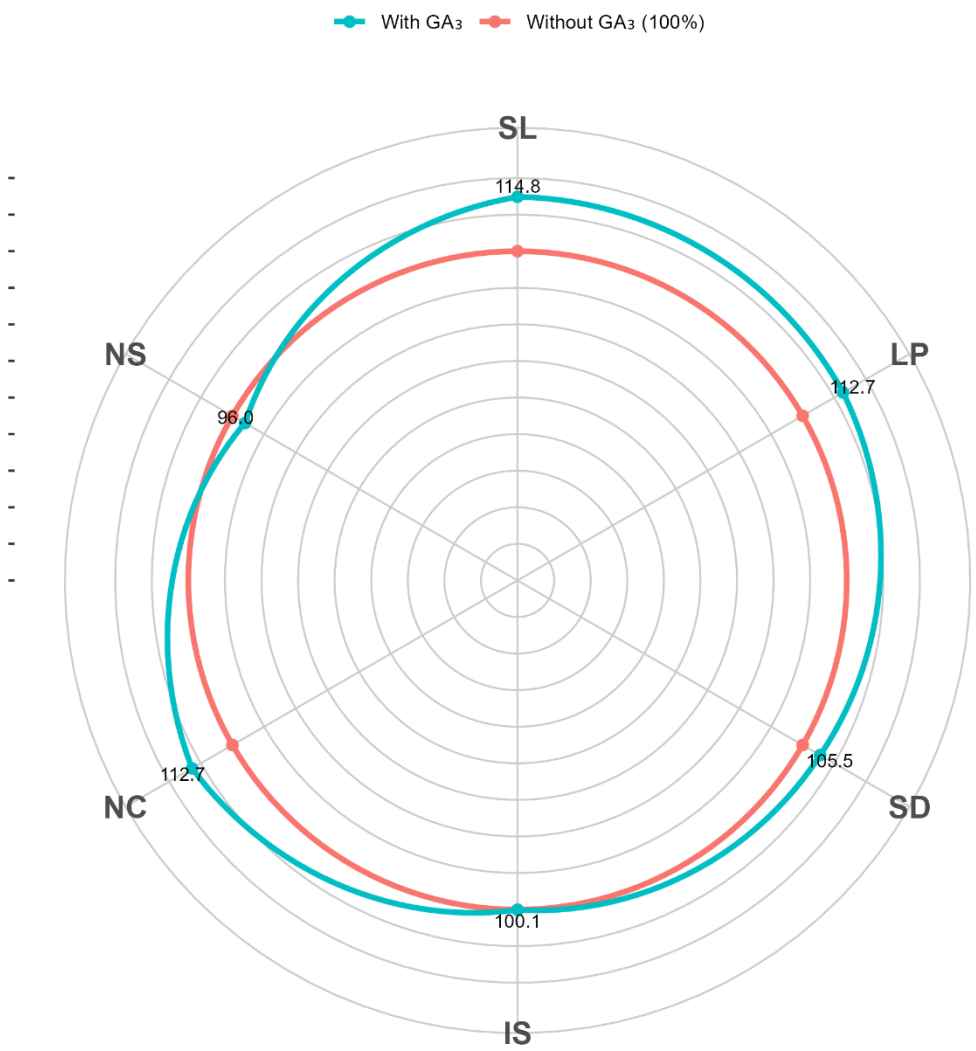
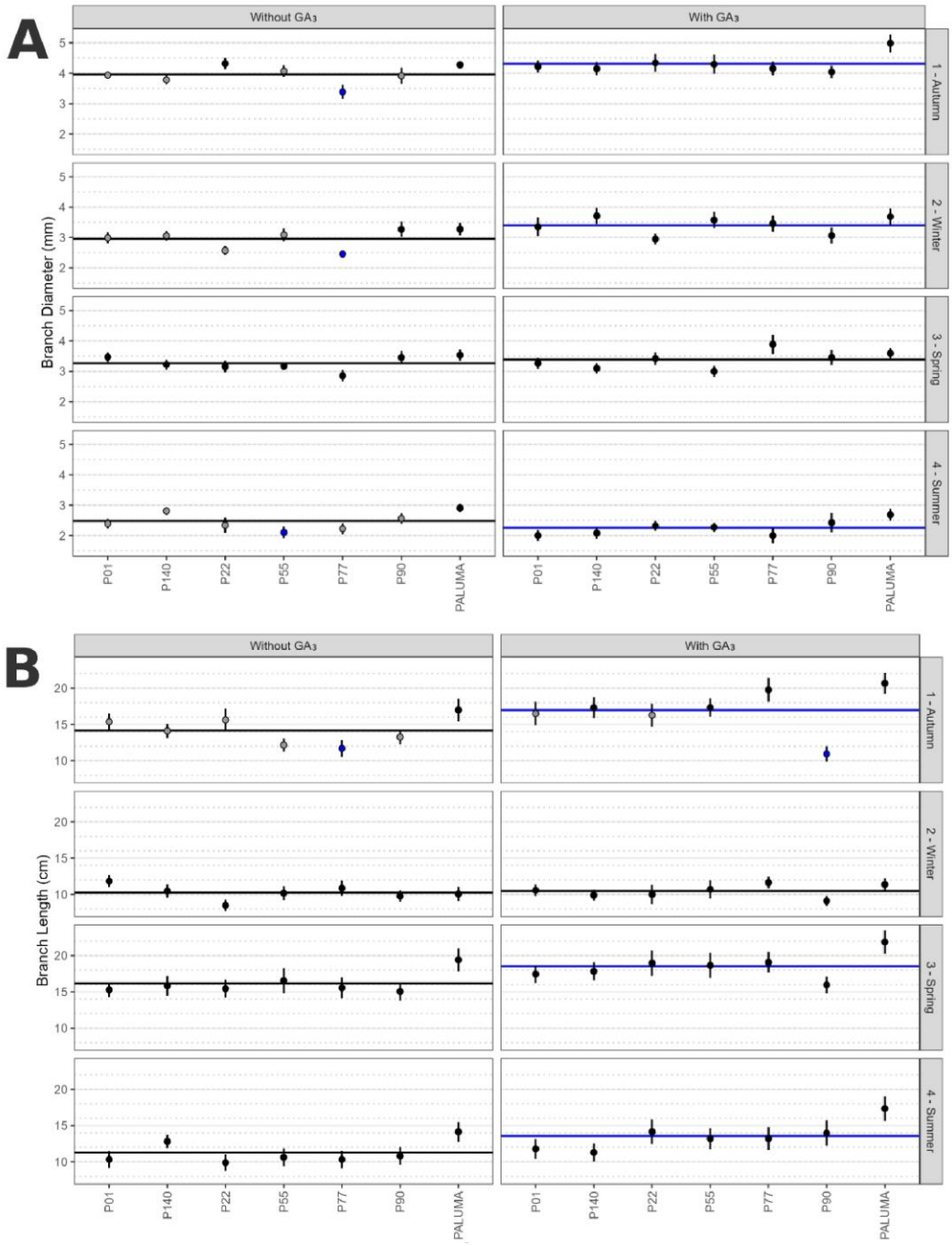
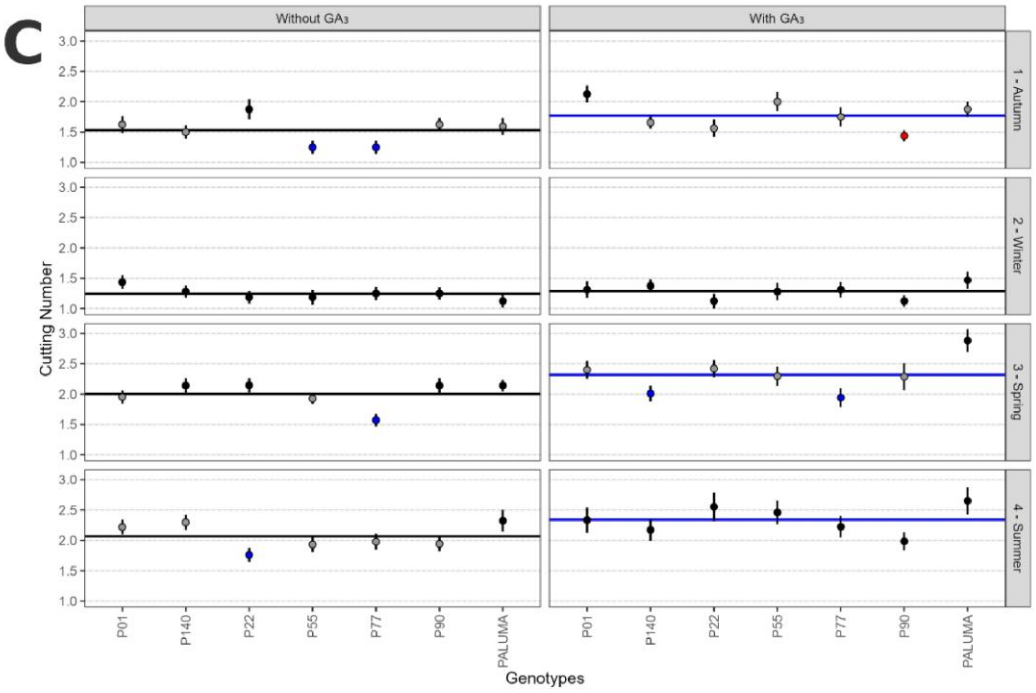


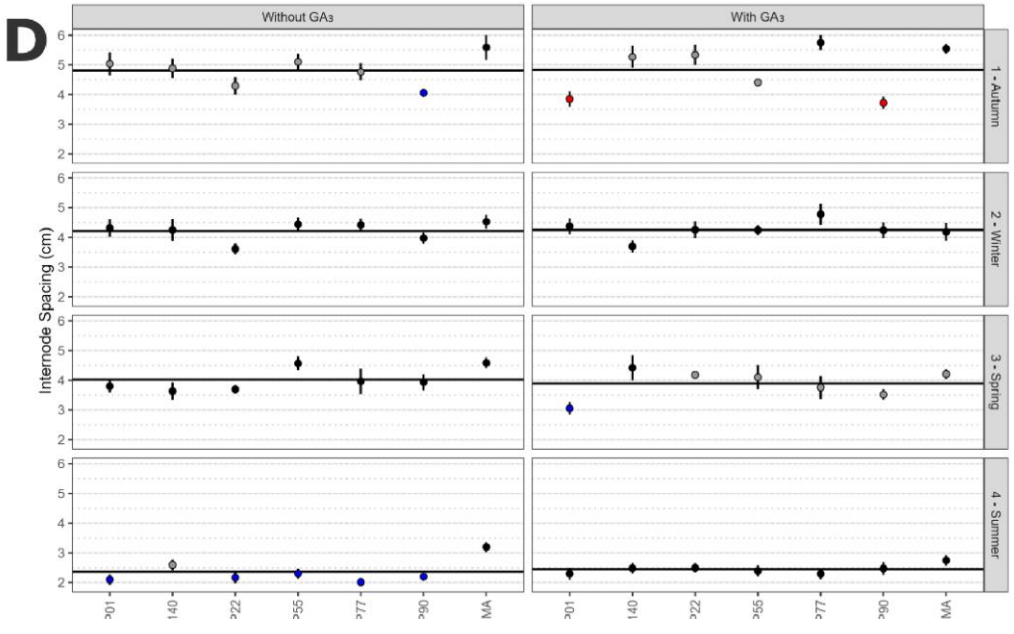
Fig. 2. Relative performance of vegetative traits under GA_3 application in relation to the untreated control (100%), based on overall means of the experiment. Values represent percentage differences between treatments and are presented for descriptive purposes only. Traits: SL – shoot length; LP – leaf pairs; SD – shoot diameter; IS – internode spacing; NC – number of cuttings; NS – shoots.

Figure 3 shows differences among *Psidium* genotypes across the evaluated environments, defined by the combination of season and GA_3 application, for traits related to vegetative growth. In general, higher mean values were observed in environments

corresponding to spring and summer. Comparisons among environments indicate variation in the magnitude of responses across traits, with more pronounced differences for structural growth descriptors. For some traits, such as leaf pairs and number of shoots, no consistent differences were observed between environments with and without GA_3 application within the same season. These results indicate a significant environmental effect and variation in the relative performance of genotypes across environments, without consistent changes in genotype ranking.


A
Without GA3
With GA3
1 - Autumn
2 - Winter
3 - Spring
4 - Summer
Branch Diameter (mm)
P01
P140
P22
P55
P77
P90
PALUMA
B
Without GA3
With GA3
1 - Autumn
2 - Winter
3 - Spring
4 - Summer
Branch Length (cm)
P01
P140
P22
P55
P77
P90
PALUMA


C
Without GA3
With GA3
1 - Autumn
2 - Winter
3 - Spring
4 - Summer
Cutting Number
3.0
2.5
2.0
1.5
1.0
P01
P140
P22
P55
P77
P90
PALUMA
Genotypes


D
Without GA3
With GA3
1 - Autumn
2 - Winter
3 - Spring
4 - Summer
Internode Spacing (cm)
6
5
4
3
2

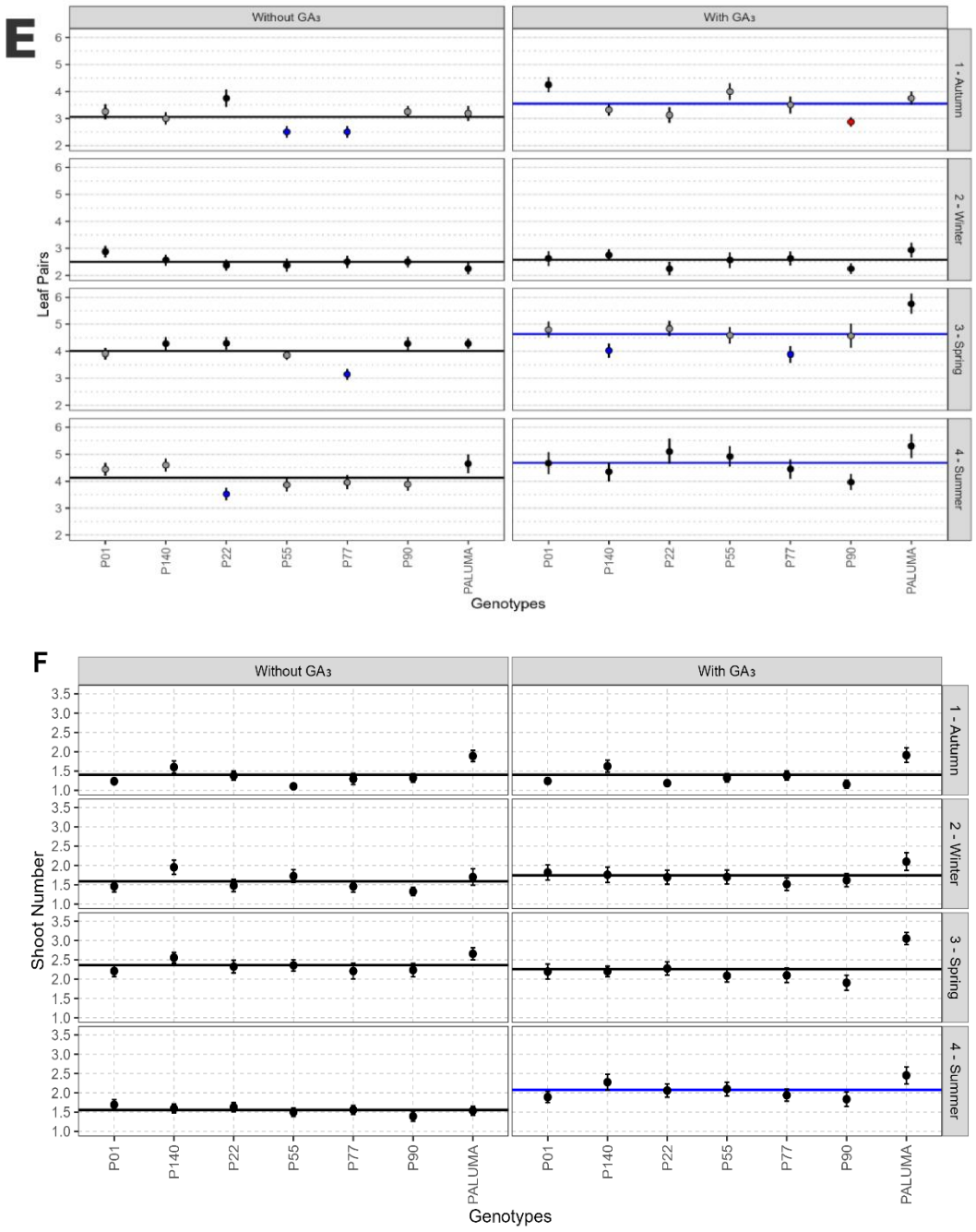


Fig. 3. Performance of *Psidium* genotypes across four seasons under treatments with and without GA_3 for vegetative traits: shoot diameter (A); shoot length (B); number of cuttings (C); internode spacing (D); leaf pairs (E); and shoot number (F). Each point represents the genotype mean with its respective standard error. Points with different colors indicate significant differences among genotypes according to Tukey's test ($p \leq 0.05$). The horizontal line represents the overall mean of the trait in each season, allowing comparison of genotype

performance within and across treatments. Lines with different colors (black and blue) indicate contrasting responses relative to the overall seasonal pattern according to Tukey's test ($p \leq 0.05$).

Figure 4 shows the genotypic correlations among variables related to minicutting production. In autumn, the set of variables exhibited predominantly positive genotypic correlations among shoot length, leaf pairs, shoot diameter, internode spacing, number of cuttings, and number of shoots, indicating a consistent association among vegetative descriptors. No relevant negative correlations were observed in this season.

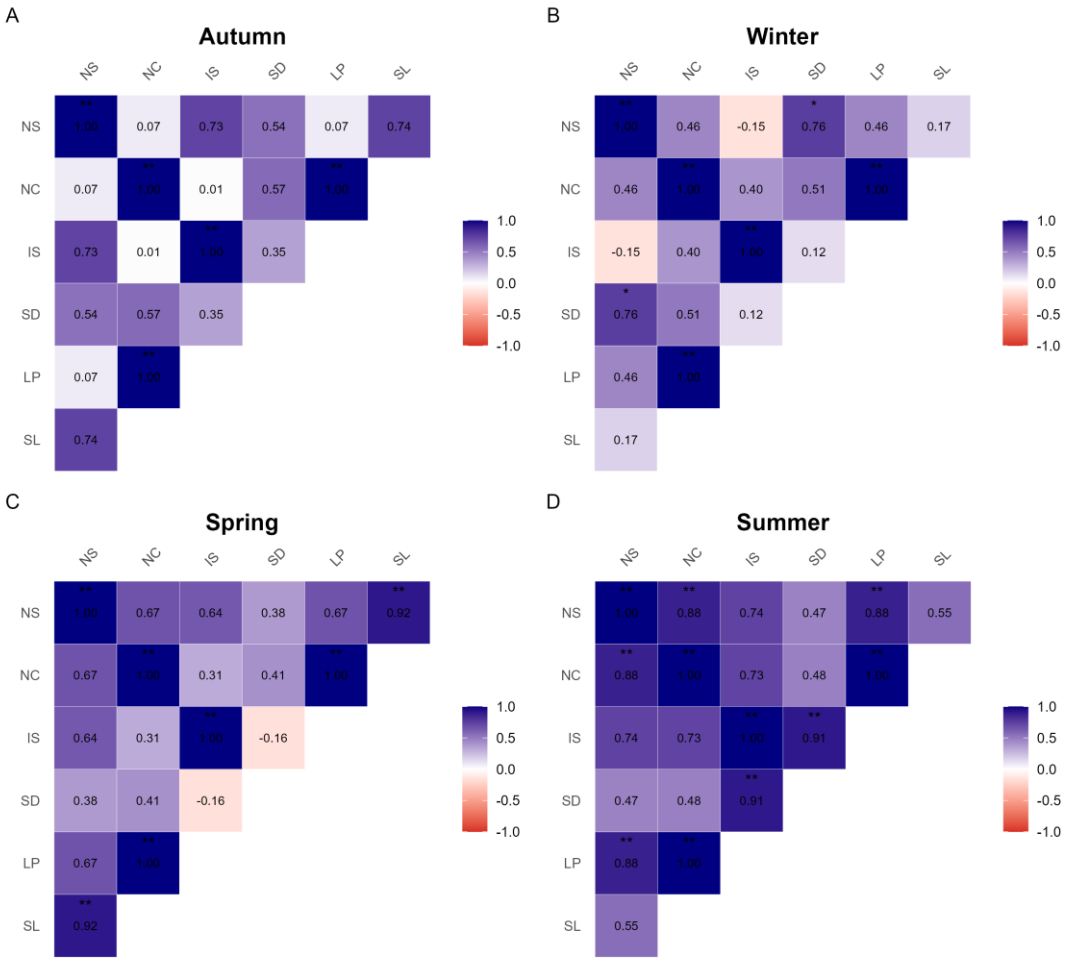


Fig. 4. Estimates of Pearson's correlation coefficient (r) represented as a heatmap among six morphological variables: number of shoots (NS), number of cuttings (NC), internode spacing (IS), shoot diameter (SD), leaf pairs (LP), and shoot length (SL), evaluated in seven *Psidium* genotypes based on mean values within each season: autumn (A), winter (B), spring (C), and summer (D).

In winter, the correlation pattern showed greater heterogeneity, with both positive and negative associations. Shoot diameter, number of cuttings, and number of shoots were positively correlated with each other, whereas internode spacing showed negative correlations with part of the remaining variables, indicating a dissociation between shoot elongation and other components of vegetative growth.

In spring, correlations were predominantly positive, with intermediate intensity among variables. Most traits varied in the same direction, while internode spacing showed negative correlations with some variables, indicating a distinct response of this trait.

In summer, genotypic correlations were predominantly positive and stronger compared to other seasons. All variables were associated in the same direction, with no negative correlations observed, indicating greater integration among vegetative growth components.

To complement the analysis of seasonal effects and GA_3 application, Figure 5 shows the mean values of vegetative traits in genotypes under both hormonal treatments across seasons.

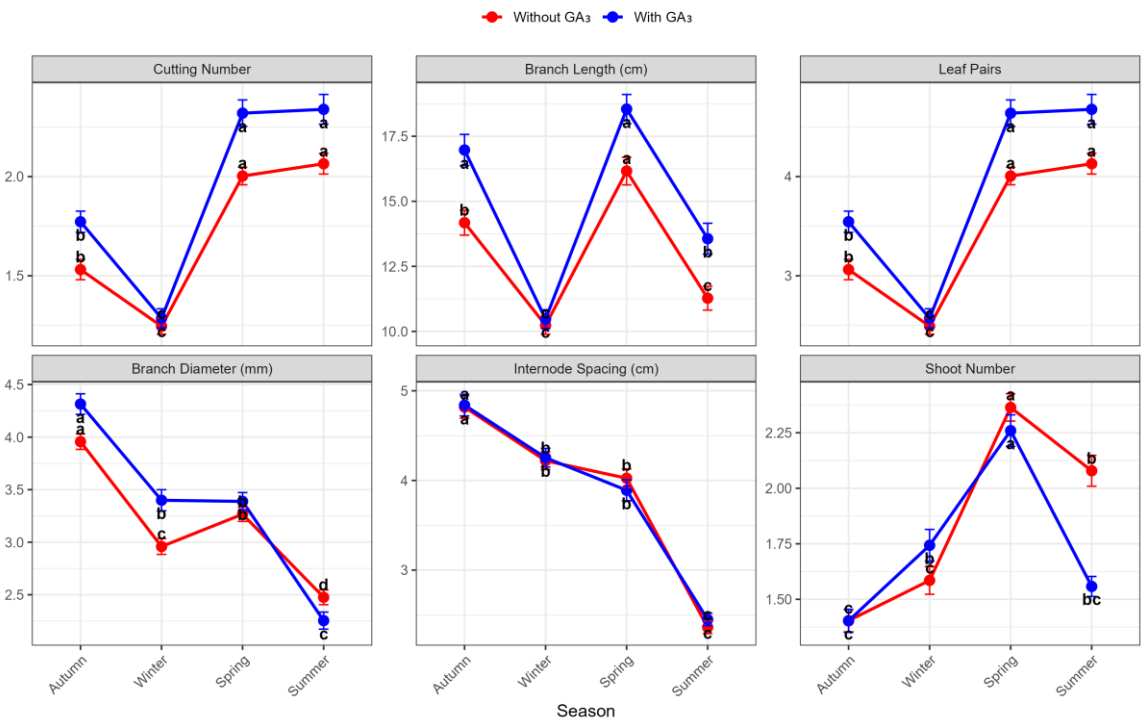


Fig. 5. Mean values of number of cuttings, shoot length, leaf pairs, shoot diameter, internode spacing, and number of shoots of genotypes treated with and without GA_3 across four seasons (autumn, winter, spring, and summer). Values correspond to means ± standard error for each treatment x season combination, with the treatment without GA_3 represented by red points and the treatment with GA_3 by blue points. Letters above the points indicate statistical differences according to Tukey's test ($p < 0.05$). Uppercase letters compare treatments within each season,

whereas lowercase letters compare seasons within each treatment. Different letters indicate statistically significant differences.

Rooting percentage showed variation as a function of the genotype × environment interaction, with environments defined by the combination of season and GA_3 application (Table 2). In general, most genotypes exhibited high rooting across the evaluated environments. Genotypes P77, P140, and ‘Paluma’ showed consistent performance across environments, whereas genotype P22 exhibited greater variation in rooting across the evaluated combinations. For genotypes P90 and P01, no consistent pattern associated with GA_3 application was observed across environments.

Table 2. Rooting (%) of minicuttings of six *Psidium* genotypes and the cultivar 'Paluma' as a function of environment, defined by season and without GA_3 application ($–GA_3$) and with GA_3 application ($+GA_3$).

Genotype	Autumn ($–GA_3$)	Autumn ($+GA_3$)	Winter ($–GA_3$)	Winter ($+GA_3$)	Spring ($–GA_3$)	Spring ($+GA_3$)	Summer ($–GA_3$)	Summer ($+GA_3$)
P90	83.33 aA	91.67 aA	91.67 aA	91.67 aA	91.67 abA	91.67 abA	91.67 aA	100.00 aA
P01	91.67 aA	83.33 aA	83.33 aA	83.33 aA	91.67 abA	91.67 abA	91.67 aA	91.67 aA
P22	66.67 bBC	75.00 bBC	83.33 aAB	83.33 aAB	83.33 bBC	83.33 bBC	91.67 aA	83.33 aA
P77	91.67 aA	91.67 aA	91.67 aA	100.00 aA	100.00 aA	100.00 aA	100.00 aA	100.00 aA
P140	100.00 aA	91.67 aA	100.00 aA	100.00 aA	100.00 aA	100.00 aA	91.67 aA	100.00 aA
P55	83.33 aA	91.67 aA	91.67 aA	91.67 aA	100.00 aA	100.00 aA	91.67 aA	100.00 aA
Paluma	100.00 aA	100.00 aA	100.00 aA	100.00 aA	91.67 abB	91.67 abB	100.00 aA	100.00 aA

Means followed by the same lowercase letter within a column do not differ among genotypes within each environment, and means followed by the same uppercase letter do not differ among environments for each genotype, according to Tukey's test at the 5% probability level.

Discussion

The variability observed among *Psidium* genotypes across seasons indicates high phenotypic plasticity in woody species, associated with responses to seasonal conditions, as reported for other economically important Myrtaceae species (Blackman et al., 2017).

The environmental influence observed on morphological traits of *Psidium* stock plants is consistent with the physiological responses of perennial woody species in tropical environments, in which periods of active vegetative growth alternate with phases of reduced growth, regulated by temperature, photoperiod, and water availability (Davies, 2010; Ritonga et al., 2023). In mature guava plants, studies on pruning management and vegetative growth show that shoot emission and bud formation vary markedly across seasons, with greater vigor

under warmer conditions and reduced growth under milder conditions, reflecting the strong dependence of the crop on environmental conditions (Serrano et al., 2007; Silva et al., 2017). This pattern explains the lower mean values of growth and shoot emission in winter and the concentration of more pronounced responses in spring and summer, as observed in the present study.

In the context of minicutting and clonal mini-gardens, the interaction between season and vegetative response has also been consistently reported. Arantes et al. (2021) showed that minicutting productivity of the hybrid 'BRS Guaraçá' is season-dependent, with greater shoot length, stem diameter, and propagule production in summer compared to winter. Studies on minicutting in guava and araçazeiro reinforce that temperature and relative humidity affect both the regrowth capacity of stock plants and the quality of the produced cuttings, conditioning the performance of propagation systems to local climatic regimes (Marinho et al., 2009; Altoé et al., 2011; Milhem et al., 2015). Thus, the observed peaks in growth variables during spring and summer, even under the same GA_3 management, are consistent with the greater expression of vegetative traits under favorable seasonal conditions.

GA_3 application primarily affected structural traits such as shoot length, stem diameter, leaf pairs, and number of cuttings, whereas buds, shoot emission, and internode spacing were less responsive. This pattern reflects the classical effect of gibberellins on cell elongation, tissue expansion, and biomass accumulation, particularly in actively growing tissues (Blázquez et al., 2020). In clonal mini-gardens of *Psidium*, Arantes et al. (2021) observed linear increases in shoot length, stem diameter, and mini-stump productivity with increasing GA_3 concentration in summer, whereas responses were more limited in winter, indicating that the regulator enhances growth mainly when environmental conditions are already favorable.

Similarly, studies have shown that GA_3 application can enhance vegetative growth and vigor-related traits, particularly under favorable environmental conditions (Shah et al., 2023).

Comparable results have been reported in other fruit crops, in which GA_3 promotes shoot elongation and increased vegetative growth in seedlings and young plants under appropriate management (Dantas et al., 2012; Li et al., 2024).

The low differentiation between treatments for number of shoots and, in some cases, internode spacing suggests that GA_3 does not substantially alter primary meristematic activity (Faizan et al., 2026). In several woody fruit species, bud activation is regulated by a complex hormonal balance involving cytokinins, endogenous gibberellins, growth inhibitors, and gradients of light and carbohydrate reserves (Chen et al., 2023). Studies on rooting environments and juvenile guava material indicate that microenvironmental factors (light, humidity, nutritional status) influence the emission of new tissues and the quality of cuttings, with variation even in genetically homogeneous materials (Altoé et al., 2011; Dias et al., 2012; Milhem et al., 2015). This helps explain why, in the present study, GA_3 was more effective in traits related to shoot elongation and thickening than in variables associated with initial shoot emission.

In contrast, the number of shoots, although showing high genotypic variability, tends to present high experimental coefficients of variation, reflecting the strong influence of microenvironmental conditions, the occurrence of many low or zero values, and the intrinsic plasticity of this trait. Similar patterns have been reported in temperate fruit species, in which bud number per node, floral bud density, and effective fruiting vary widely among cultivars, years, and locations, with significant genotype × environment interactions (Penso et al., 2018). Therefore, evaluation across multiple seasons and environments is necessary, as recommended for perennial species (Partelli et al., 2022).

The significant G × E interaction indicates that the relative performance of genotypes varies according to environmental conditions, demonstrating that phenotypic expression in *Psidium* is environment-dependent, in agreement with the classical concept of genotype ×

environment interaction. This interaction reflects differential genotype responses to environmental variation, resulting in changes in relative performance across environments and representing a major challenge for the selection of stable and adapted genotypes in breeding programs (Napier et al., 2022; Amin et al., 2025).

Genotype × environment interaction is one of the main factors influencing adaptation and phenotypic stability of cultivars, and its consideration is essential in experiments conducted across multiple environments (Cooper et al., 2014; Crossa et al., 2021). Arantes et al. (2021) observed in 'BRS Guaraçá' that GA_3 increased shoot length and minicutting productivity, while maintaining the relative performance pattern across concentrations and seasons.

The results indicate that GA_3 does not uniformly modify vegetative descriptors, with effects dependent on environmental conditions, exerting greater influence on structural traits and limited effects on primary meristematic activity. This pattern is consistent with the physiological role of gibberellins in promoting cell elongation and organ expansion, whereas bud activation and shoot emission depend mainly on hormonal balance with cytokinins, as well as local microclimatic conditions and resource availability (Davies, 2010; Dias et al., 2012; Ritonga et al., 2023). Thus, GA_3 application is more advantageous when the agronomic objective is to increase shoot size and vigor and enhance the potential for minicutting production under favorable environmental conditions, with limited contribution to stimulating initial shoot emission under restrictive conditions.

Genotypic variation in response to GA_3 is associated with differences in hormone perception and signaling pathways, involving specific receptors and regulation of DELLA protein degradation, which act as growth repressors (Blázquez et al., 2020). More vigorous genotypes tend to exhibit greater sensitivity to GA_3, possibly due to more efficient DELLA degradation and enhanced activation of cell elongation processes, whereas less responsive

materials may maintain higher DELLA stability or stronger influence of antagonistic regulators, resulting in reduced hormonal effects (Shi et al., 2022).

GA_3 application increased shoot length and number of leaf pairs without affecting internode length. This combination results in a higher number of nodes potentially available for propagation, which may favor cutting yield per stock plant. This effect was more evident during periods of higher vegetative activity, particularly in spring and summer, and less pronounced in autumn, while no consistent response was observed in winter. Therefore, GA_3 can be considered a complementary tool to enhance cutting production under favorable environmental conditions, whereas its application during winter appears unnecessary under the conditions evaluated.

Although GA_3 application may inhibit adventitious root development under certain conditions due to its effects on hormonal balance (Zhang et al., 2021), this effect was not observed in the present study. This may be attributed to the high rooting capacity of shoots derived from stock plants, the juvenile nature of the propagative material, and favorable environmental conditions during the experiment (Izhaki et al., 2018; Porfírio et al., 2016). Additionally, the interval between GA_3 application and shoot collection may have reduced transient physiological effects, since GA_3 is primarily associated with cell elongation rather than inhibition of adventitious rooting (Zhang et al., 2021). Thus, at the time of cutting preparation, shoots may have reached similar physiological conditions across treatments, explaining the lack of consistent GA_3 effect on rooting and highlighting the stronger influence of genotypic and environmental factors (Porfírio et al., 2016).

Finally, the consistently superior performance of the cultivar ‘Paluma’ for several growth and vegetative production traits reinforces its well-known vigor and adaptability under different cultivation conditions. Field studies with ‘Paluma’ under different management systems, seasons, and pruning intensities have shown high regrowth capacity and stable

productivity, demonstrating its physiological plasticity (Serrano et al., 2007; Silva et al., 2016). These results support the use of ‘Paluma’ as a reference genotype for clonal propagation and as a highly adaptable material capable of sustaining productive clonal mini-gardens across seasons, serving as a benchmark for comparison with new *Psidium* hybrids in breeding programs.

Conclusions

Differences were observed among the evaluated genotypes in terms of vegetative growth and minicutting production in a *Psidium* clonal mini-garden, influenced by the environment defined as the combination of season and GA_3 application. Variation in the relative performance of genotypes highlights the importance of multi-environment evaluation. GA_3 application increased growth-related traits, such as shoot length and number of leaf pairs, with more evident effects in autumn, spring, and summer. The high rooting of minicuttings, regardless of GA_3 application, indicates that this process was mainly determined by the environment, without negative effects of the regulator. The cultivar ‘Paluma’ showed greater stability, while two *Psidium* genotypes also stood out for shoot emission and minicutting production.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. We have read and understood your journal's policies, and we believe that neither the manuscript nor the study violates any of these.

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4. CONCLUSÕES

A estação do ano influencia o crescimento vegetativo, a emissão de brotações e a produção de miniestacas de *Psidium* conduzidos em minijardim clonal, evidenciando respostas diferenciadas entre os genótipos avaliados. A aplicação de GA_3 favorece características associadas ao vigor das minicepas e aumenta a eficiência da propagação clonal.

O uso do análogo de brassinosteroide Biobrás-16 durante a fase de aclimatação promove maior desenvolvimento da parte aérea e do sistema radicular das mudas, além de melhorar parâmetros fisiológicos relacionados aos pigmentos foliares e ao balanço de nitrogênio, indicando maior equilíbrio metabólico das plantas.

Além disso, foram identificadas respostas contrastantes à infestação por *Meloidogyne enterolobii*, com alguns genótipos de *Psidium* tendo maior equilíbrio fisiológico e potencial de resistência.

A eficiência da propagação clonal de *Psidium* é influenciada pela interação entre fatores ambientais e hormonais, sendo a propagação vegetativa uma ferramenta para a multiplicação e validação de genótipos previamente selecionados quanto à resistência a *Meloidogyne enterolobii*.

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