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AMANDA BAHIANO PASSOS SOUSA

RESISTÊNCIA GENÉTICA DE *Musa* spp. A *Radopholus similis*: FUNDAMENTOS METODOLÓGICOS, CARACTERIZAÇÃO FENOTÍPICA E MOLECULAR

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Tese apresentada ao Programa de Pós-graduação em Biotecnologia, da Universidade Estadual de Feira de Santana como requisito parcial para obtenção do título de Doutora em Biotecnologia.

Orientador: Prof. Dr. Edson Perito Amorim
Coorientador: Dr. Leandro de Souza Rocha
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Dedico
A Nossa Senhora Aparecida.

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"Importa que Ele cresça e que eu diminua."
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RESUMO

A bananeira é uma das frutíferas mais importantes do mundo e desempenha papel fundamental na segurança alimentar global. Entre os principais fatores limitantes à produção da cultura, destacam-se os nematoides fitoparasitas, em especial *Radopholus similis*, uma das espécies mais destrutivas associadas a *Musa* spp. Nesse contexto, a resistência genética configura-se como uma estratégia sustentável e durável para o manejo desse patógeno. Este estudo realizou inicialmente uma revisão sistemática da literatura publicada entre 2011 e 2024 com o objetivo de identificar fontes de resistência genética a nematoides em *Musa* spp. Seguindo as diretrizes PRISMA, foram aplicados uma estratégia de busca padronizada e critérios pré-definidos de inclusão e exclusão em diferentes bases acadêmicas, resultando na seleção de 69 estudos. A revisão confirmou *R. similis* como o nematoide mais amplamente investigado em escala global e evidenciou a existência de fontes promissoras de resistência no germoplasma de *Musa*. Também foi avaliada a resposta à resistência de vinte genótipos de bananeira provenientes de diferentes regiões do Brasil, com base na severidade dos sintomas e no fator de reprodução do nematoide. Observou-se ampla variação de resposta entre genótipos do subgrupo Prata (AAB), enquanto os genótipos do subgrupo Cavendish (AAA) mostraram-se predominantemente suscetíveis. Adicionalmente, o processo infeccioso de *R. similis* em raízes de diploides melhorados de bananeira, previamente identificados como contrastantes quanto à resposta ao nematoide, foi investigado em nível histoquímico e molecular. As análises de expressão gênica evidenciaram eventos hormonais e estruturais associados à resistência radicular. Por fim, foi desenvolvida e validada uma escala diagramática para aprimorar a avaliação da resposta do hospedeiro à infecção por *R. similis*, com base na extensão da necrose no rizoma de mudas de bananeira. A área dos rizomas e a severidade dos sintomas foram quantificadas por meio do software ImageJ e a validação da escala envolveu dez avaliadores inexperientes que estimaram a severidade de cem rizomas com e sem o uso da escala. A aplicação da escala aumentou significativamente a concordância entre avaliadores, elevando os valores de R^2, do coeficiente de correlação de concordância de Lin (CCC) e reduzindo o viés sistemático das estimativas. De forma integrada, este trabalho reúne evidências sistemáticas, avanços metodológicos e validação experimental, oferecendo contribuições inéditas e ferramentas práticas para o avanço do melhoramento genético de *Musa* spp. com foco na resistência a *R. similis*.

Palavras-chave: Banana; nematoide cavernícola; melhoramento genético; RT-qPCR.

ABSTRACT

Banana is one of the most important fruit crops worldwide and plays a fundamental role in global food security. Among the main constraints to banana production are plant-parasitic nematodes, particularly *Radopholus similis*, one of the most destructive species associated with *Musa* spp. In this context, genetic resistance represents a sustainable and durable strategy for managing this pathogen. This study first conducted a systematic review of the literature published between 2011 and 2024 to identify sources of genetic resistance to nematodes in *Musa* spp. Following PRISMA guidelines, a standardized search strategy and predefined inclusion and exclusion criteria were applied across multiple academic databases, resulting in the selection of 69 studies. The review confirmed *R. similis* as the most extensively investigated nematode species worldwide and highlighted the existence of promising resistance sources within *Musa* germplasm. The resistance response of twenty banana genotypes from different regions of Brazil was also evaluated based on symptom severity and nematode reproduction factor. A wide variation in response was observed among genotypes of the Prata subgroup (AAB), whereas Cavendish subgroup (AAA) genotypes were predominantly susceptible. Additionally, the infection process of *R. similis* in roots of improved diploid banana genotypes, previously identified as contrasting in their response to the nematode, was investigated at histochemical and molecular levels. Gene expression analyses revealed hormonal and structural events associated with root resistance. Finally, a diagrammatic scale was developed and validated to improve the assessment of host response to *R. similis* infection, based on the extent of rhizome necrosis in banana seedlings. Rhizome area and symptom severity were quantified using ImageJ software, and scale validation involved ten inexperienced evaluators who estimated the severity of one hundred rhizomes with and without the use of the scale. The application of the scale significantly increased inter-rater agreement, improving R^2 and Lin's concordance correlation coefficient (CCC) values and reducing systematic bias in severity estimates. Taken together, this study integrates systematic evidence, methodological advances, and experimental validation, providing novel contributions and practical tools to support *Musa* breeding programs focused on resistance to *R. similis*.

Keywords: Banana; burrowing nematode; genetic breeding; RT-qPCR.

SUMÁRIO

INTRODUÇÃO GERAL

As bananas estão entre as espécies frutíferas mais cultivadas, comercializadas e consumidas no mundo, desempenhando papel central na segurança alimentar, na geração de renda e na sustentabilidade de sistemas agrícolas tropicais e subtropicais (FAO, 2024). Em diversas regiões da África Oriental, a banana constitui alimento básico essencial para milhões de pessoas e uma das principais fontes de subsistência de pequenos agricultores (Gold et al., 2002; Retkute & Gilligan, 2025). No comércio internacional, as exportações globais de banana alcançaram aproximadamente 19,7 milhões de toneladas anuais, evidenciando sua expressiva relevância econômica (FAO, 2024).

No Brasil, a bananicultura apresenta elevada importância econômica e social, estando amplamente distribuída em diferentes regiões e sistemas de produção. O país figura entre os maiores produtores mundiais, com produção superior a 7 milhões de toneladas anuais, conduzida majoritariamente por pequenos e médios agricultores (IBGE, 2025). A diversidade genética da bananeira é expressiva, com predominância de cultivares dos subgrupos Prata (AAB), Cavendish (AAA), Maçã (AAB) e Terra (AAB), além de híbridos melhorados oriundos de programas nacionais de melhoramento (Borges et al., 2011; Embrapa, 2020).

Em escala global, estima-se a existência de mais de 2.000 cultivares de bananeira, majoritariamente diploides ou triploides, resultantes de hibridizações intra e interespecíficas entre *Musa acuminata* e *Musa balbisiana*. Mesmo dentro de um mesmo grupo genômico, observa-se ampla variabilidade genética, associada, entre outros fatores, à presença de diferentes genes de resistência distribuídos nas estruturas genômicas. Evidências indicam que a resistência a doenças em *Musa* spp. é predominantemente quantitativa, controlada por múltiplos genes de efeito aditivo, o que resulta em gradientes fenotípicos de resposta aos patógenos e possibilita o desenvolvimento de variedades mais resistentes ou tolerantes por meio da seleção de mutações em cultivares existentes (Smith et al., 2006; Zuo et al., 2018).

Apesar de sua relevância socioeconômica, a bananicultura é severamente impactada por um complexo de pragas e doenças que comprometem o crescimento, o vigor e a produtividade das plantas (Nansamba et al., 2020; Anuradha et al., 2022), refletindo-se em perdas expressivas de rendimento e rentabilidade (Esguera et al., 2024). Entre esses fatores limitantes, os nematoides fitoparasitas destacam-se como um dos principais grupos de patógenos associados à cultura (Sousa et al., 2024).

As espécies de maior relevância econômica incluem o endoparasita migratório *Radopholus similis*, os nematoides das lesões *Pratylenchus coffeae* e *P. goodeyi*, o endoparasita *Helicotylenchus multicinctus* e os parasitas sedentários do gênero *Meloidogyne* (Quénéhervé, 2009; Famina et al., 2017; Luquini et al., 2019). A severidade dos danos depende de fatores edafoclimáticos, da densidade populacional do patógeno e, sobretudo, da suscetibilidade do hospedeiro, podendo assumir importância local significativa quando populações atingem níveis críticos (Quénéhervé, 2009).

Entre esses nematoides, *R. similis* destaca-se como o mais destrutivo e economicamente relevante na bananicultura (Gowen et al., 2005; Santos et al., 2023). Esse nematoide coloniza raízes e rizomas, promovendo extensas lesões necróticas que comprometem o sistema radicular funcional, reduzem a absorção de água e nutrientes e afetam a ancoragem da planta, favorecendo o tombamento do pseudocaule e a perda do cacho (Gowen & Quénéhervé, 1990; Oliveira et al., 2015).

O ciclo de vida de *R. similis* compreende seis estádios de desenvolvimento, indo de ovo, passando por quatro estádios juvenis (J1–J4), até chegar aos indivíduos adultos, com duração média de 18 a 25 dias, dependendo da temperatura, sendo otimizado entre 25 e 30 °C (Gowen & Quénéhervé, 1990; Moens et al., 2018). Embora a reprodução sexuada possa ocorrer, na ausência de machos, as fêmeas tornam-se hermafroditas, assegurando a continuidade populacional da espécie (Kaplan & Opperman, 2000; Vieira et al., 2021). Com exceção dos machos, todos os estágios móveis são capazes de se alimentar dos tecidos radiculares, promovendo danos progressivos ao hospedeiro.

A penetração do nematoide nas raízes ocorre rapidamente após a inoculação, geralmente entre 24 e 48 horas, quando juvenis móveis e fêmeas adultas iniciam a migração intracortical (Taylor & Sasser, 1978; Sarah et al., 1996). A eclosão do nematoide ocorre em aproximadamente 8 a 10 dias e os estádios juvenis subsequentes continuam a se deslocar e a se alimentar no córtex radicular, formando galerias necróticas características (Gowen & Quénéhervé, 1990).

Em nível molecular, *R. similis* secreta uma variedade de efetores que facilitam a penetração e a colonização dos tecidos da planta, incluindo enzimas de degradação da parede celular (Jacob et al., 2008), proteases como cathepsina S (Rs-CPS) essenciais para a invasão (Li et al., 2015), além de efetores imunomodulatórios e proteínas do tipo venom-allergen-like (VAP), ambos capazes de suprimir respostas de defesa da planta (Wang et al., 2016; Li et al., 2021).

Nesse contexto, o melhoramento genético visando à resistência destaca-se como a estratégia mais eficaz e sustentável para o manejo de nematoides na bananicultura (Rocha et al., 2022). Diversos programas de melhoramento de *Musa* spp. têm direcionado esforços para a incorporação de resistência a fitonematoides em diferentes regiões do mundo (Sousa et al., 2024), sendo os diploides selvagens e melhorados consistentemente descritos como fontes valiosas de resistência a *R. similis*, devido aos baixos níveis de penetração, migração e reprodução do patógeno, bem como à ativação mais eficiente de respostas de defesa (Ortiz & Vuylsteke, 1996).

Diante desse cenário, o presente trabalho teve como objetivos: 1) realizar uma revisão sistemática da literatura publicada entre os anos de 2011 e 2024 sobre fontes de resistência genética da bananeira com foco em nematoides; 2) avaliar a variabilidade genética de genótipos de bananeira quanto à resistência a *R. similis*; 3) avaliar o processo infeccioso de *R. similis* nas raízes de diploides melhorados de bananeira em nível histoquímico; 4) avaliar o processo infeccioso de *R. similis* nas raízes de diploides melhorados de bananeira em nível molecular pela quantificação da expressão de genes de resistência por qRT-PCR ao longo do tempo e 5) desenvolver e validar uma escala diagramática para classificar a resposta à infecção por *R. similis*, com base na presença e na porcentagem de necrose no rizoma de mudas de bananeira. Esses resultados trarão novas contribuições para o desenvolvimento de cultivares de bananeira resistentes a *R. similis* e ampliarão o conhecimento sobre os mecanismos envolvidos na interação planta-patógeno.

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CAPÍTULO 1

Phytoparasitic Nematodes of *Musa* spp. with Emphasis on Sources of Genetic Resistance: A Systematic Review

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plants

MDPI

Systematic Review

Phytoparasitic Nematodes of *Musa* spp. with Emphasis on Sources of Genetic Resistance: A Systematic Review

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Abstract: Bananas are a staple food that considerably contributes to both food security and income generation, especially in countries of Africa, Asia, and Central and South America. The banana plant (*Musa* spp.) is affected by various pathogens, of main concern being the plant-parasitic nematodes associated with the rhizosphere, the most important of which are *Radopholus similis* (burrowing nematode), *Helicotylenchus* sp. (spiral nematode), *Pratylenchus* sp. (root lesion nematode), and *Meloidogyne* sp. (gall nematode). Infected plants reduce their ability to absorb water and nutrients, which can lead to delayed flowering, fewer bunches, and lower fruit mass. Obtaining nematode-resistant banana cultivars through genetic improvement is an effective and sustainable option compared with chemical control with nematicides. Here, we provide the first systematic review of existing banana sources of resistance to nematodes to aid the management and control of nematodes in banana and plantain crops. Articles selected from different databases were evaluated, and searches were conducted using pre-established inclusion and exclusion criteria. We found 69 studies dealing with genetic improvement for nematode resistance in banana cultivation. Our findings revealed that sources of resistance are currently under investigation to combat the diseases caused by different nematode species in banana plants.

Keywords: *Musa* spp.; genetic resistance; parasitic nematodes; phytonematodes

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1. Introduction

Bananas and plantains (*Musa* spp.) are staple foods for more than 400 million people in the developing countries of South America, Southeast Asia, and Africa and are a key commodity in both international and local trade, particularly in Latin American and Caribbean countries [1]. These crops are ranked among the most valuable agricultural commodities in the world and are considered important for food security, as they can produce fruits throughout the year and can be cultivated in different types of environments [2]. In Africa, plantains play a crucial role in ensuring food security and income generation for more than 70 million people [3–5]. Although different banana cultivars are produced and consumed worldwide, the cultivars of the Cavendish subgroup are highly prominent, given their importance in the fruit export market [6]. Banana ranks second in fruit production, with global production estimated at approximately 167 million tons. They are mainly grown in Asia, Latin America, and Africa, and India and China are their largest producers for domestic consumption. In 2021, the estimated export volume was 20.5 million tons [7]. However, banana and plantain production is affected by various biotic and abiotic factors. Some notable pathogens include *Ralstonia solanacearum* (banana wilt) [2,8–10], *Fusarium oxysporum* f. sp. *cubense* (Fusarium wilt) [5,11–13], *Mycosphaerella fijiensis* (black Sigatoka) [14–16] *Cosmopolites sordidus* or banana fruit borer, and nematodes [17,18].

The main parasitic nematode species infecting *Musa* spp. are *Radopholus similis*, *Helicotylenchus* sp., *Meloidogyne* sp., *Pratylenchus coffeae*, and *Pratylenchus goodeyi* [19,20]. They lead to an average annual loss of 20% of banana yield worldwide [21,22]. Losses become more severe when the root system, already damaged by these parasites, experiences storms, causing the plants to topple over [23]. Additionally, the damaged roots act as a food base for fungal pathogens of the banana plant, facilitating their invasion and resulting in multiple infections, including Fusarium wilt [24–26].

Nematode management in banana plantations relies mainly on the use of chemical nematicides. However, in addition to being expensive, nematicides can cause environmental degradation, have the potential to leave residues on fruits, contaminate groundwater, and affect non-target organisms, and pose a risk of toxicity to applicators [27–29]. Cultural control measures, such as rhizome cutting followed by hot water treatment, and the use of healthy shoots or clean planting material, such as those from tissue culture, offer only temporary nematode management, as fields are usually reinfested [30,31]. Several issues, such as the frequency and method of application for biocontrol agents, particularly in field conditions, need to be addressed. In addition, the developed methods must be cost-effective, so that producers at different agricultural scales can adopt them [32].

Developing host plant resistance is an effective and economical approach to avoid yield loss due to plant-parasitic nematodes [17]. Although naturally occurring nematode resistance and tolerance have been extensively explored in many agricultural crops, nematode resistance and tolerance in banana plant have been largely overlooked, despite being present in the *Musa* gene pool [17,33,34].

Currently, banana breeding programs are focused on the introgression of various traits, including resistance and tolerance, and productivity and fruit quality [35,36], as well as the development of strategies that can surpass agricultural limitations, setting new records for yield and tolerance to biotic and abiotic stresses [37]. Banana improvement programs have made significant progress in obtaining cultivars resistant to black Sigatoka and Fusarium wilt. Based on systematic reviews on *Musa* spp. resistance to black Sigatoka and Fusarium wilt, Soares et al. [16] and Rocha et al. [5] reported that the main banana breeding programs are located in Africa, Asia, and the Americas, with around 10 genetic improvement programs for *Musa* spp. resistance worldwide [5].

Thus, various sources of resistance have already been identified and reported in *Musa* germplasm, as well as commercial cultivars resistant to the main diseases that impact banana production; however, a review of banana plant resistance to nematodes is lacking. Therefore, this systematic review was performed to determine the potential application of the current state of knowledge on *Musa* spp. resistance in genetic improvement for resistance to banana parasitic nematodes. Studies conducted between 2011 and 2024 were included in this review.

2. Results

2.1. Study Screening

Figure 1 shows the flow diagram for screening the articles analyzed in this review. Using the search *string* defined in our study, 3430 articles were found on the Google Scholar website. The first 100 pages sorted by relevance were collected, which included 1172 (31%) articles evaluated in the selection phase of this SR. The CAPES Periodicals Portal was the primary contributor for these articles, accounting for 988 articles (26%) in total. This was followed by Springer contributing to 797 articles (21%), PubMed Central with 387 articles (10%), Scopus with 312 articles (8%), Web of Science with 104 articles (3%), and MusaLit with 28 articles (1%) (Figure 1).

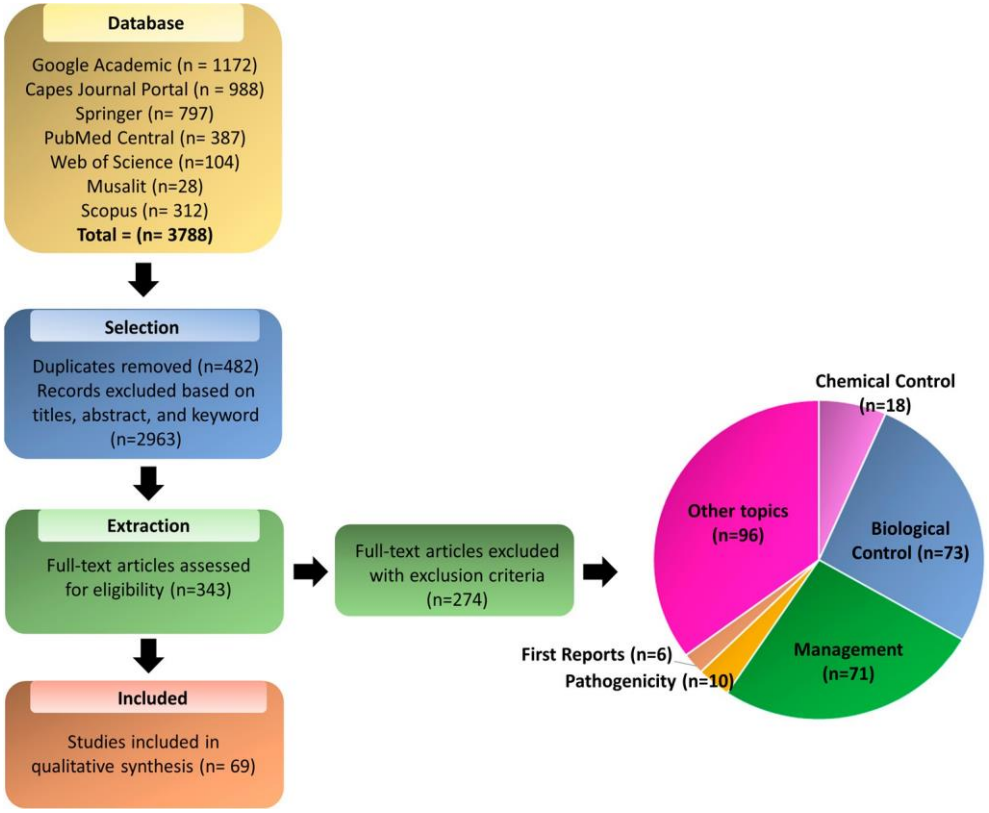

Figure 1. PRISMA flowchart for the screening process of the articles selected in this review.

2.2. Bibliometric Analysis

A total of 3788 articles were identified in the databases, of which 482 were duplicates and 2963 were rejected in the initial selection process. In the extraction phase, 343 articles were selected, of which 69 were finally included for the SR. The selected articles were stored in an open-access digital library available at: (https://doi.org/10.5281/zenodo.10854789 (accessed on 27 March 2024)).

Most of the articles excluded at the extraction stage were related to studies dealing with other topics (96) and studies on biological control of nematodes (73), followed by studies on nematode management in banana cultivation (71) (Figure 1).

To generate a keyword co-occurrence network, we defined the minimum word frequency threshold, which enabled the visualization of all keywords occurring at and above this frequency. The algorithm of the VOSviewer software version 1.6.19 generated a network based on the association method with many significant clusters. Each node in Figure 2 represents a keyword, and the radius of a node is related to the frequency of occurrence of the keyword in each article. According to the number of nodes, the central brown cluster comprises the most frequently used concepts and those that have received attention from most researchers, with the word "banana" in the center as the cluster's main theme. As shown in the map in Figure 2A, "nematode", "*Musa*", "*Radopholus similis*", and "*Meloidogyne incognita*", are among the ten most common keywords in each of the other clusters.

The graph presented in Figure 2B demonstrates the collaboration network among countries based on co-authorship. The network shows that for the topic of our study, cooperative relationships existed among nearby countries and that the clusters formed appeared to be related to the geographical proximity of each country. The largest cluster, in purple, had the largest collaboration network with other countries and was primarily made up of India with closer cooperation with the Philippines and France. The next largest clusters are shown in brown, represented by Belgium, and green, represented by Uganda. Belgium had a greater cooperation with the Philippines and African countries, such as Uganda, Nigeria, South Africa, and Kenya. The fourth largest cluster is shown in

orange, represented by Brazil, whose main collaboration networks were with the United States, Costa Rica, and Colombia, which are the closest countries geographically. Other clusters with extensive collaboration networks included the United States, China, and France (Figure 2B).

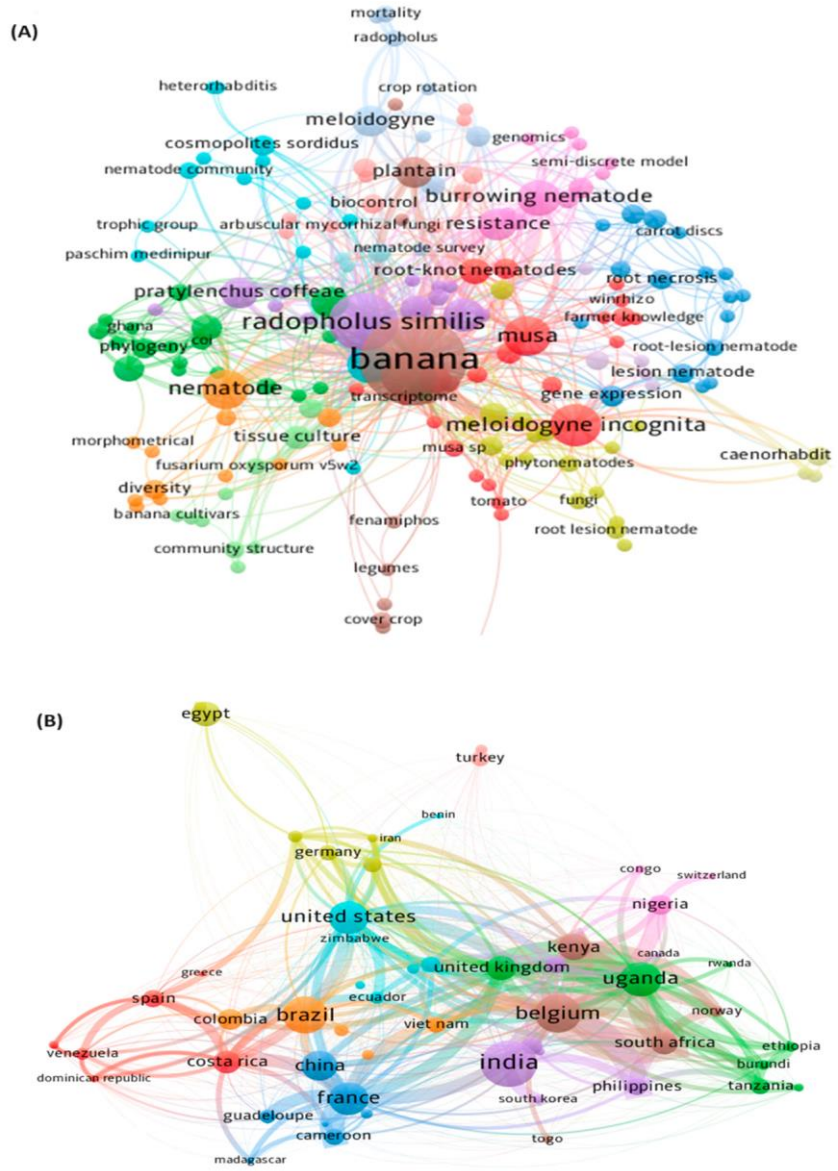

Figure 2. (**A**) Keyword co-occurrence networks and (**B**) collaboration networks between countries according to co-authorship. The size of the circle represents the frequency of occurrences of keywords or countries, with larger circles indicating higher occurrences, and the thickness of the lines reflects the strength of the collaboration, with thicker lines indicating closer collaboration networks.

We generated a network diagram for the main journals as shown in Figure 3A. The top three journals with the highest number of articles published on the subject of our study were Nematology, Nematropica, and Indian Journal of Nematology, which were distributed in five different clusters determined by the central colors. There was a greater cooperation between the journal Nematology and the journals Nematropica, European Journal of Plant Pathology, and Indian Journal of Nematology. Figure 3B also shows the relationship graph of the cooperation and co-occurrence network of authors involved in studies on banana resistance to parasitic nematodes. Seenivasan N, Das Sukhen, and Roderick Hugh were the authors who appeared in the highlighted groups, indicating that they have published

most of the articles included in our study or collaborated most with other researchers. In addition, our data showed that not many scholars were engaged in this area of banana research and no cooperative relationship existed among them.

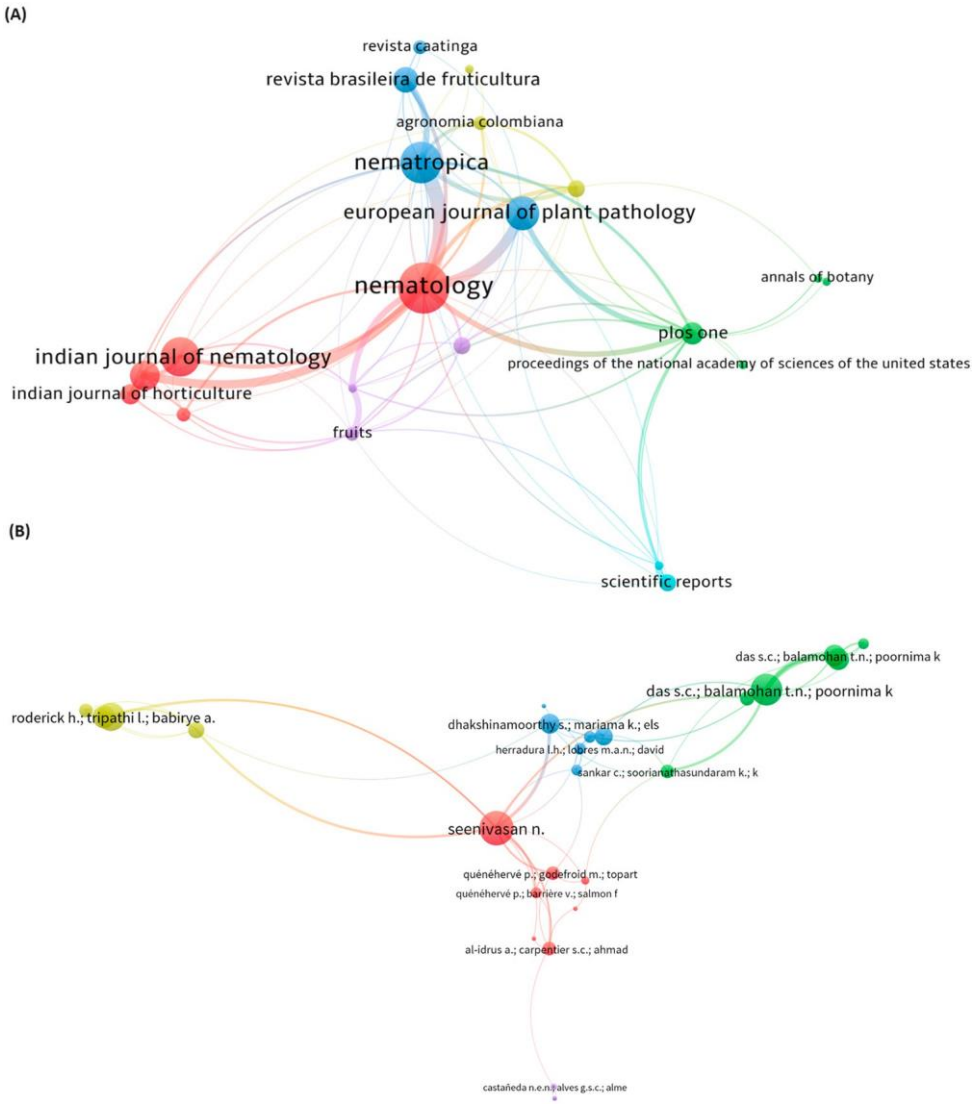

Figure 3. Bibliographic coupling of the main research sources (**A**). The size of the circle is directly proportional to the number of publications, and the thickness of the line connecting the circles is proportional to the collaboration among the journals. Networks of author collaborations on banana resistance to parasitic nematodes (**B**). The size of the circle is directly proportional to the number of articles published by the author, and the thickness of the lines is directly proportional to the closeness of the collaboration among the authors.

2.3. Places of Origin

Figure 4 shows a map with the frequency of articles by country, represented by a scale with a color grid that goes from yellow, which represents the minimum frequency (1%), to red, which represents the maximum frequency (54%). Thus, among 69 studies, 45.6% were conducted in India, 13.2% in Brazil, 8.8% in Uganda, 4.4% in Malaysia and Egypt, 2.9% in the Philippines and Costa Rica, and the remaining in countries such as Germany, Costa Rica, Ivory Coast, Belgium, Cameroon, and France.

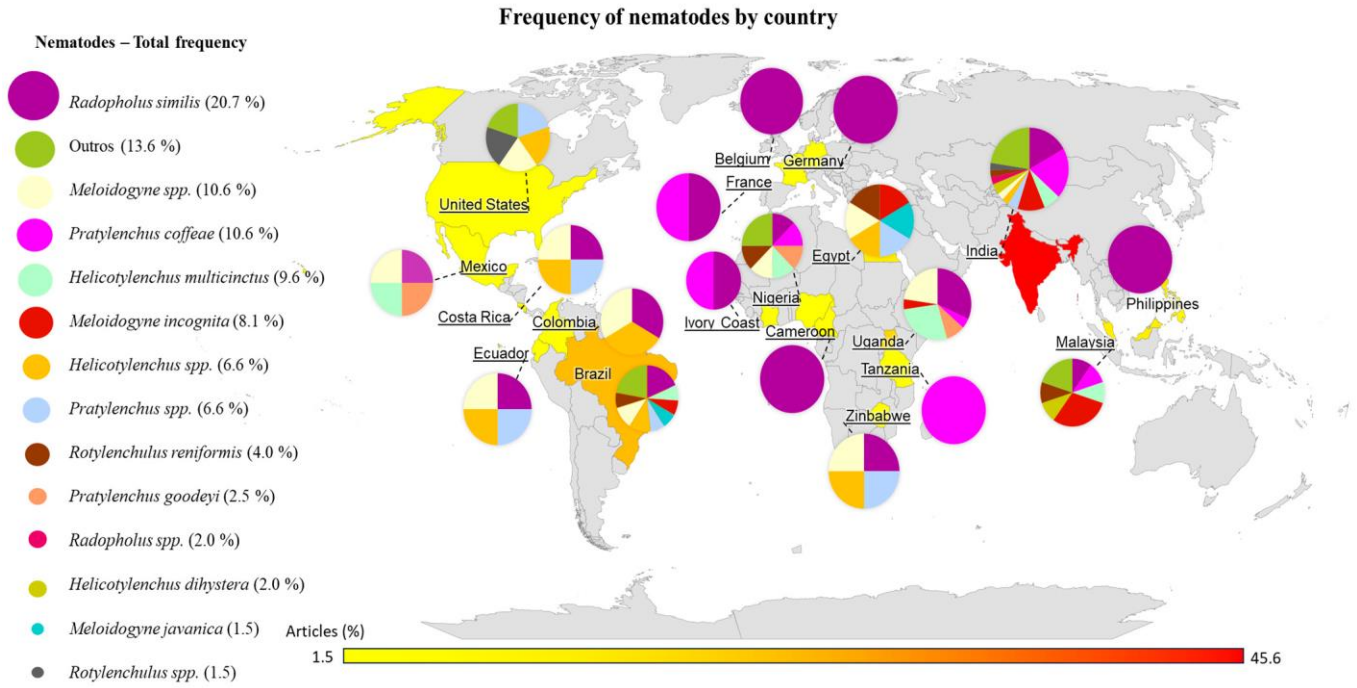

Figure 4. Frequency of articles on genetic improvement of *Musa* spp. to parasitic nematodes published in the last 13 years in different countries and species of nematodes studied per country, considering more than one nematode per publication. The map was plotted in R, using the maps, ggmap, geosphere, Eurostat, GADMTools, country code, and ggplot2 packages. lat: latitude; long: longitude.

Pie charts integrated into Figure 4 show the general frequency of nematodes studied and their distribution in the countries. Overall, *R. similis* was the most widely studied nematode, specifically in the countries of Africa, Europe, and Asia. In American countries, *Meloidogyne* spp. and *Helicotylenchus* spp. were the most frequently occurring nematodes (Figure 4). The countries with the greatest diversity of species studied were India with 11 species reported, Brazil with 8, and Malaysia with 7, in addition to other species reported at a lower frequency (Figure 4).

R. similis had the greatest frequency and distribution among the countries that have conducted studies on nematode resistance in banana (20.7%), followed by *P. coffeae* and *Meloidogyne* spp. (both 10.6%), *H. multicinctus* (9.6%), and *M. incognita* (8.1%). *Rotylenchulus reniformis*, *P. goodeyi*, *Helicotylenchus dihystera*, and *Meloidogyne javanica* appeared in 4%, 2.5%, 2%, and 1.5% of the articles. Additionally, some studies have reported other genera without identifying the species, such as *Helicotylenchus* and *Pratylenchus* (both 6.6%), as well as *Radopholus* (2%) and *Rotylenchulus* (1.5%). Other nematodes of lesser importance were also identified in 13.6% of the articles, mainly in those that dealt with population surveys in the field. The frequency considered the evaluation of more than one nematode per article (Figure 4).

The main nematodes studied in India were *P. coffeae* (n = 14), *R. similis* (n = 12), and *M. incognita* (n = 8). In Uganda, they were *R. similis* (n = 7), *H. multicinctus* (n = 6), and *Meloidogyne* spp. (n = 5), considering more than one nematode per article. In Brazil, the most studied nematode was *R. similis* (n = 5). In Costa Rica, *R. similis* (n = 2), *Helicotylenchus* spp. (n = 2), *Pratylenchus* spp. (n = 1), and *Meloidogyne* spp. (*n* = 1) were evaluated together in two different studies. Côte d'Ivoire and France studies have reported *R. similis* (n = 1) and *P. coffeae* (n = 1). Countries such as Egypt and Malaysia have evaluated nematodes such as *M. incognita* (n = 3) and *R. reniformis* (n = 2). *R. similis* and *M. incognita* have been less frequently studied by other countries.

In the selected articles, eight breeding programs from different countries were found, containing information on genetic improvement for banana resistance to parasitic nema-

todes: Brazilian Agricultural Research Corporation (Embrapa) in Brazil; Improvement Program Department of Fruticulture, National Research Centre for Banana (ICAR), and Horticultural College and Research Institute (HCRI) in India; International Institute of Tropical Agriculture (IITA) in Uganda; Institut de Recherche pour le Développement (IRD) and Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) in France; Center National for Research Agronomique (CNRA) in Ivory Coast; and Agriculture Research Center (ARC) in Egypt.

Of the 69 articles selected, 24 included population surveys of field nematodes in different regions. For this evaluation, studies that specified the nematode species, not just the genus, were separated out, thus a final restriction to 11 countries. An analysis was inserted based on a heat map, in which the population densities of different nematode species were expressed in colors ranging from red (high density), orange (medium density), and yellow (low density) (Figure 5). This analysis was associated with the world location map with the total population densities of each nematode by country (Figure 5).

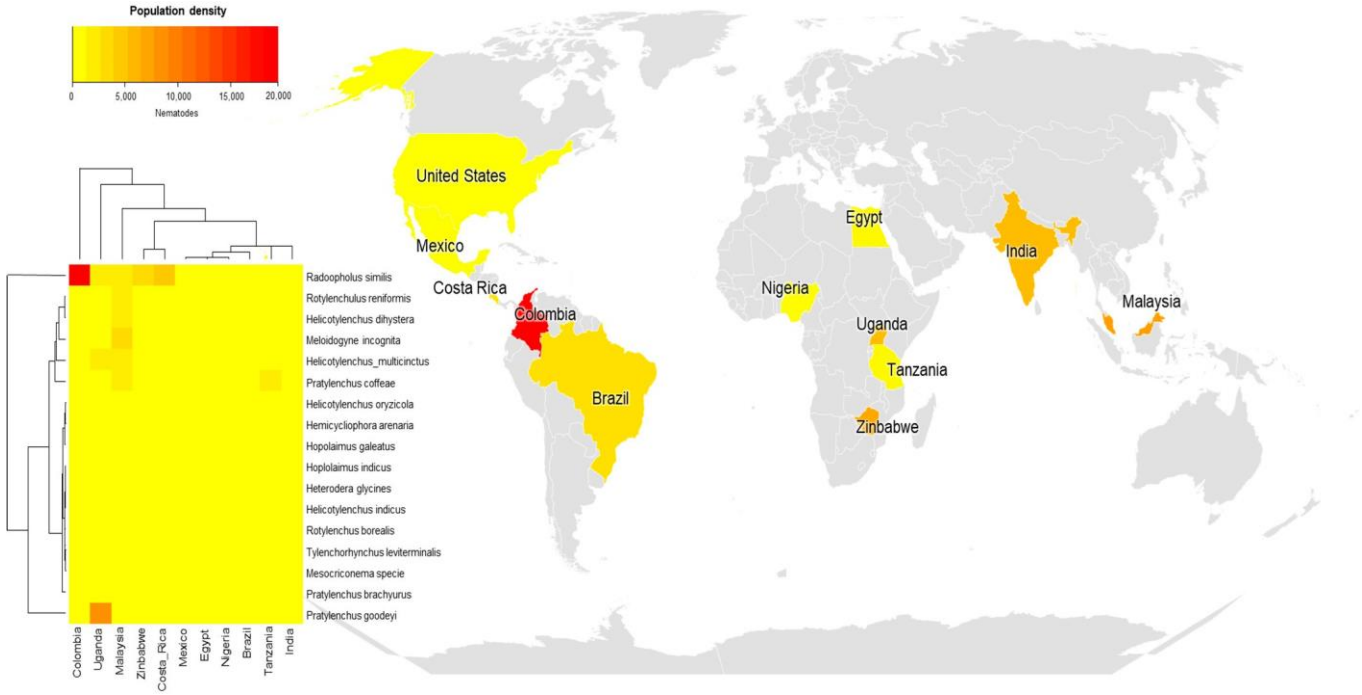

Figure 5. Population densities of banana parasitic nematode species reported in different countries in articles published in the last 13 years.

Although most of the studies have been carried out in India and larger numbers of nematode species have been evaluated in India (Figure 4), the population density of these species in banana plantations did not appear to be higher in India than in other countries. Colombia was the country with the highest population density of a single nematode, *R. similis* (19,489); in Malaysia, studies have reported higher population densities of *M. incognita* (3850), *R. reniformis* (2450), *H. multicinctus* (2450), and *H. dihystera* (2400). Uganda had a higher population density of *P. goodeyi* (8215) and intermediate densities of *H. multicinctus* (2008) and *R. similis* (1739) (Figure 5). In Zimbabwe and Costa Rica, the highest densities were for the nematode *R. similis* (3429 and 4766, respectively). In this analysis, the frequency considered the total population density across all areas evaluated per article.

2.4. Evaluation Tools and Methods

Regarding the environment in which the studies were conducted, most studies were performed in the field (44%), including population surveys in different areas, followed

by greenhouses, both of which accounted for 17% of the articles. Additionally, 6% of the studies included screenhouse experiments and 2% included in vitro and laboratory experiments. However, 9% of the articles did not specify where the experiments were conducted (Figure 6A).

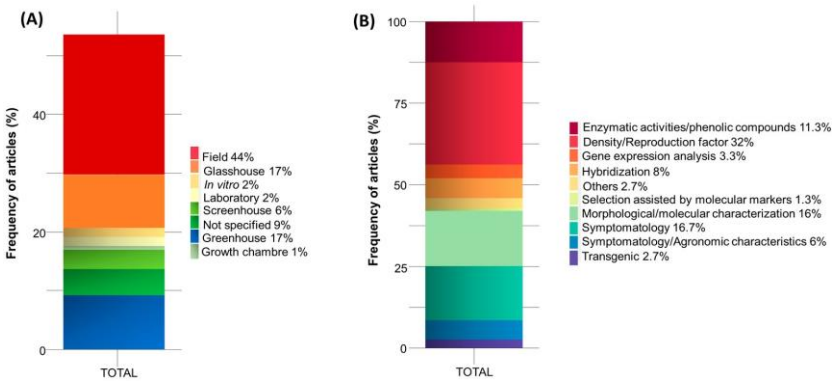

Figure 6. Stacked bar chart of the frequency of articles on nematode resistance to banana plants carried out in the last 13 years (**A**). Stacked bar graph of the frequency of articles with methods used to obtain or characterize nematode-resistant *Musa* spp. plants carried out in the last 13 years (**B**).

Regarding the main methods used to characterize nematode-resistant plants, nematode density/reproduction factor analysis was addressed in 32% of the selected articles, with the highest frequency for evaluating banana resistance to nematodes, followed by symptomatology (16.7%), morphological/molecular characterization (16%), enzymatic activities and phenolic compounds (11.3%), hybridization (8%), symptomatology/agronomic characteristics (6%), gene expression analysis (3.3%), transgenics (2.7%), selection assisted by molecular markers (1.3%), and others (2.7%). The frequency was measured considering that more than one tool was used per article (Figure 6B).

Figure 7 shows the main evaluations of the symptoms caused by different nematode species on banana trees, with some authors using rating scales. The scale, previously used by Pinochet [38], identifies susceptibility, tolerance, or resistance, and was used in six studies (27%) for *P. coffeae*, *H. multicinctus*, and *R. similis* (Table 1). There were papers that used different indices to assess root lesions and/or necrosis (19%) and the extent of root damage (15%). The articles that assessed damage caused by *M. incognita* used a root gall index scale (15%). Additionally, 8% of the articles assessed the index of necrosis in the rhizome and 4% assessed the number of functional roots. Other methods were also used to assess symptoms (12%). The frequency considered that more than one method was used per article.

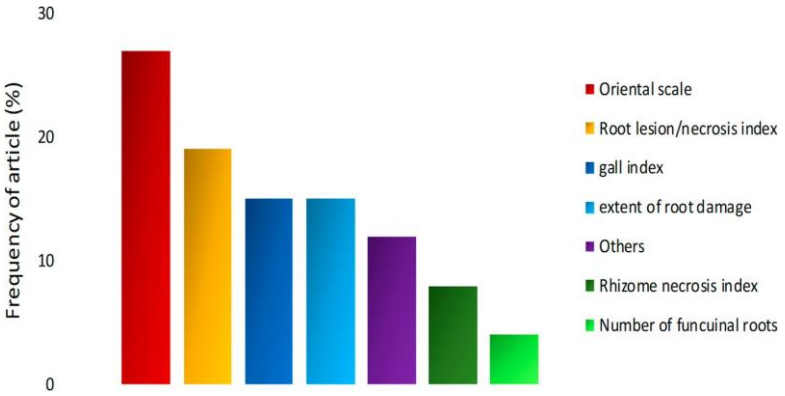

Figure 7. Methods for assessing symptoms caused by nematodes in banana plants in articles selected over the last 13 years.

Table 1. Scale to assess banana reaction to lesion nematodes according to Pinochet [38].

Plant Response	Root Lesion Index (%)	Corm Grade
Immune	0	0
Resistance	<10	<1
Tolerant	10–20	1–2
Susceptible	20–40	2–4
Highly susceptible	>40	>4

The methods for extracting nematodes in roots were also demonstrated in most of the selected articles (Table 2). Some articles only provided the reference of the protocol followed, without giving details, and other authors did not specify how they carried out the counting. To assess the population of *R. similis*, *P. coffeae*, and *Helicotylenchus* spp., the most commonly used technique was as follows: roots were first macerated in a blender for 10 s thrice with an interval of 5 s and then sieved [17,21,27,39,40]. Araya and De Waele [41] used a method where the roots were macerated in a blender for 10 s each at low and high speed, followed by sieving. Tripathi et al. [23] and Sankar et al. [42] macerated the samples in a blender for 10 s. Another method used for nematode extraction consisted of placing root samples in polypropylene bags and submerging them in 100 mL of 1% H_2O_2 solution and incubating them at room temperature for 7 days in the dark; thereafter, the nematodes were collected and counted using a microscope [43,44]. To assess the population of *Meloidogyne* spp., the most commonly used techniques were staining the roots with lactophenol acid fuchsin [29,45–47] and acid fuchsin in acetic acid [48] without macerating the root tissues. The selected articles used microscopy counting.

Table 2. Methods for extracting nematodes from *Musa* spp. roots described in articles published in the last 13 years.

Root Extration Method	Article	Nematode
Macerated roots in a blender followed by sieving	[17,40]	*Radopholus similis*
	[41]	*Radopholus similis*, *Helicotylenchus* spp., *Meloidogyne* spp., *Pratilenchus* spp.
	[49]	*Pratylenchus* spp., *Helicotylenchus* spp., *Meloidogyne* spp., *Radopholus* spp.
	[21,39]	*Helicotylenchus multicinctus*
Counting of females and juveniles after staining the roots with acid lactophenol fuchsin	[27]	*Pratilenchus coffeae*
	[29,45–47]	*Meloidogyne incognita*
Manual dissection of the lesioned roots	[50]	*Radopholus similis*
Roots in polypropylene bags submerged in 1% H_2O_2	[44]	*Radopholus similis* and mixed population (*R. similis*, *H. multicinctus*, *M. incognita*)
	[43]	*Radopholus similis*
Macerated roots in a blender and extraction using the Baermann technique	[23,42]	*Radopholus similis* and mixed population (*H. multicinctus*; *Meloidogyne* spp.)
	[51]	*Radopholus similis*, *Helicotylenchus multicinctus*, *Meloidogyne* spp.
Sifting and centrifugation with sucrose solution	[52]	*Radopholus* spp., *Helicotylenchus* spp., *Meloidogyne* spp.
Modified Baermann method and staining with acid fuchsin	[53]	*Meloidogyne javanica*, *Rotylenchulus reniformis*, *Helicotylenchus*
Maceration, flotation and centrifugation technique. And Maceration in sodium hypochlorite (NaOCL), flotation and centrifugation technique	[54]	spp., *Pratylenchus* spp. *Helicotylenchus multicinctus*, *Meloidogyne* spp., *Pratylenchus goodeyi*, *Radopholus similis*
Modified Baermann technique	[55]	*Radopholus similis*, *Pratylenchus coffeae*, *Meloidogyne incognita*, *Rotylenchulus reniformis*, *Helicotylenchus dihystera* and others
	[56]	*Radopholus similis*, *Pratylenchus* spp., *Helicotylenchus multicinctus*
	[57]	*Pratylenchus coffeae*
	[58]	*Helicotylenchus multicintus*, *Pratylenchus goodeyi*, *Radopholus similis*, *Meloidogyne* spp.
	[59]	*Radopholus similis*

Among the tools used to analyze and characterize nematode-resistant plants, the frequency of articles using reverse transcription PCR (RT-qPCR) and PCR analysis was 46.5%; histology/histochemistry, tissue culture, and chromatography accounted for 9.3% of the works evaluated; banana transcriptome, bioinformatics, and phylogenetics, accounting for 7% of the articles; and proteomics accounting for 4.7% (Figure 8A). Germplasm selection was mainly carried out through symptom evaluation, which takes into account the study environment. Consequently, greenhouse symptomology appeared in 28.6% of the publications, followed by field symptomatology (14.3%), greenhouse population evaluation (11.4%), and field agronomic characteristics (8.6%). In addition to these, there were also other germplasm selection techniques with less frequency (Figure 8B).

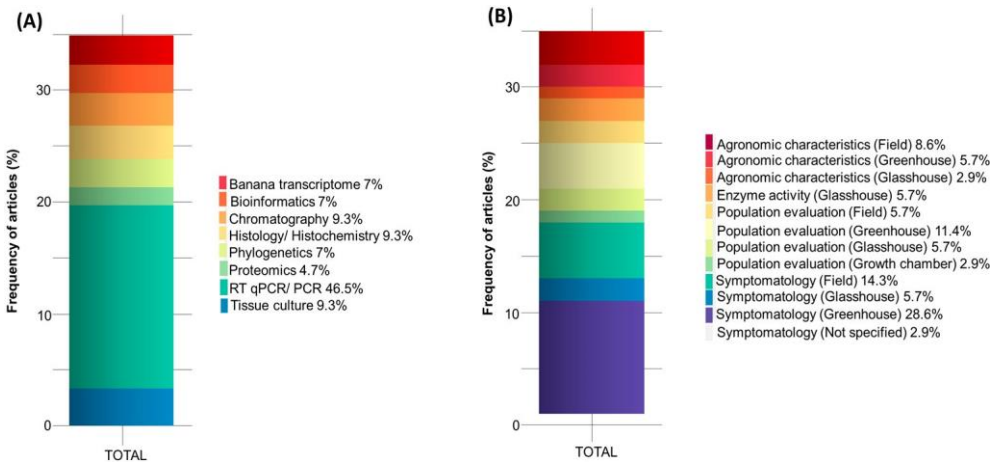

Figure 8. Stacked bar chart of article frequency with tools used in banana breeding studies for nematodes carried out over the last 13 years (**A**). Stacked bar chart of article frequency representing banana germplasm selection over the last 13 years (**B**).

2.5. Sources of Resistance

The most frequently reported known sources of resistance to nematodes were the genotypes Pisang Lilin (AA), Yangambi km5 (AAA), Karthobiumtham (ABB), and Anaikomban (AA). In addition to these, other cultivars were also used as sources of resistance to nematodes, such as the hybrids FHIA-21, FHIA-23, and FB920 (Figure 9A). Of the reported sources of resistance, the AA diploid genome predominately appeared, followed by the AAA and AAB triploids (Figure 9B).

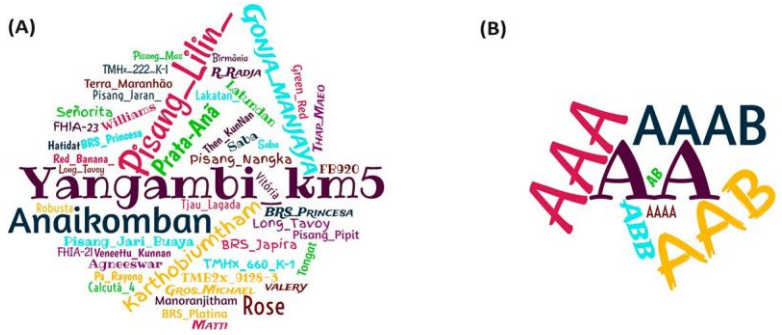

Figure 9. Word cloud generated from genotypes used as sources of resistance in articles selected to compose a systematic review on the resistance of *Musa* spp. to nematodes (**A**). Frequency of genomes associated with sources of nematode resistance in banana breeding studies carried out over the last 13 years (**B**).

Many cultivars were used to confirm or test resistance to different nematode species. Table 3 shows the genotypes that were resistant, moderately resistant, or tolerant to each

nematode. Of the genotypes reported, 41% were AA diploids, 23% were AAA triploids, 15% were AABB tetraploids, 14% were AAB triploids, 5% were AAAB tetraploids, and 3% were AB diploids (Figure 10).

Table 3. Resistance of banana genotypes to parasitic nematodes characterized in articles on genetic improvement carried out in the last 13 years.

Musa Germoplasm	Musa Genome	Level of Tolerance or Resistence	Nematode	Article
Yangambi Km5	AAA	PR	*Radopholus similis*	[28]
Yangambi Km5	AAA	R	*Radopholus similis*	[17,22,29,31,40–42,50,60–63]
Yangambi Km5	AAA	T	*Pratylenchus coffeae*	[64]
Yangambi Km5	AAA	T	*Meloidogyne incognita*	[29]
Pisang Lilin	AA	R	*Radopholus similis*	[17,22,31,40,65]
Pisang Lilin	AA	R	*Pratylenchus coffeae*	[27,55]
Pisang Lilin	AA	R	*Helicotylenchus multicinctus*	[21,39]
Pisang Lilin	AA	R	*Meloidogyne incognita*	[45–47]
Ro Im V4 6-1-1	AAA	R	*Radopholus similis*, *Pratylenchus coffeae*	[55]
Si Im V4 10-5-3	AAA	R	*Radopholus similis*, *Pratylenchus coffeae*	[55]
Anaikomban	AA	T	*Radopholus similis*, *Pratylenchus coffeae*	[55]
Anaikomban	AA	R	*Pratylenchus coffeae*	[64]
Anaikomban	AA	R	*Radopholus similis*	[17,22,40]
FB920	AAA	T	*Radopholus similis*, *Pratylenchus coffeae*	[66]
MA13	AAA	T	*Radopholus similis*, *Pratylenchus coffeae*	[66]
Pisang Jari Buaya	AA	R	*Radopholus similis*	[17,40,41]
Pisang Jari Buaya	AA	R	*Helicotylenchus* spp.	[41]
FHIA-23	AAA	R	*Radopholus similis*	[41]
Valery	AAA	R	*Helicotylenchus* spp.	[41]
Manoranjitham	AAA	R	*Radopholus similis*	[17,40]
Rose	AA	R	*Radopholus similis*	[17,22,40,67]
Matti	AA	R	*Radopholus similis*	[17,40]
Hatitat				
Pisang Mas	AB	T	*Radopholus similis*	[17,40]
Veneettu Kunnan	AB	R	*Radopholus similis*	[17,40]
Then Kunnan	AB	T	*Radopholus similis*	[17,40]
Gros Michel	AAA	T	*Radopholus similis*	[17,40]
Williams				
Red Banana (Mutant)				
Green Red				
Agneeswar				
4279-06	AA	R	*Radopholus similis*	[28]
0323-03				
0337-02				
4249-05	AA	HR	*Radopholus similis*	[28]

Table 3. *Cont.*

Musa Germoplasm	Musa Genome	Level of Tolerance or Resistence	Nematode	Article
Pisang Pipit	AA	PR	*Radopholus similis*	[28]
5854-03				
1318-01				
4285-02				
N118				
Tjau Lagada				
Calcutá 4				
1319-01				
Pa Rayong				
Birmanie				
Vitória				
Thap Maeo				
4223-06				
Pisang Jaran				
Pisang Nangka	AAAB	PR	*Radopholus similis*	[28]
FHIA-21	AAAB	R	*Radopholus similis*	[68]
Kluai Pa 26	AA	R	*Radopholus similis*	[61,69]
K. Nang Nuan	AAB	R	*Radopholus similis*	[61,69]
Pisang Papan	AAA	R	*Radopholus similis*	[61,69]
Tongat	AA	R	*Radopholus similis*	[22]
Tongat	AA	T	*Radopholus similis*	[17,40]
TMB2x 9128-3	AA	R	*Radopholus similis*	[62]
Karthobiumtham	AAB	R	*Pratylenchus coffeae*	[25,36,67,70–73]
Long Tavoy	AA	R	*Radopholus similis*	[50]
Saba	AAB	R	*Radopholus similis*	[50]
Prata-Anã	AAB	MR	*Meloidogyne javanica*	[74]
BRS Princesa	AAAB	MR	*Meloidogyne javanica*	[74]
BRS Princesa	AAAB	R	*Radopholus similis*	[26]
BRS Japira	AAAB	R	*Radopholus similis*	[26]
BRS Platina				
Latundan	AAB	PR	*Radopholus similis*	[69]
4349-05	_	R	*Radopholus similis*	[63]
H-11-08	–	R	*Radopholus similis*	[22]
H-11-21				
H-11-23				
H-11-25				
H-11-36				
H-11-69				
H-11- 70				
H-11-71				
H-11-76				

Table 3. *Cont.*

Musa Germoplasm	Musa Genome	Level of Tolerance or Resistence	Nematode	Article
H-11-02	–	T	*Radopholus similis*	[22]
H-11-03				
H-11-06				
H-11-12				
H-11-18				
H-11-24				
H-11-37				
H-11-49				
H-11-65				
H-11-78				
H201				
H912	–	R	*Radophous similis*	[42]
H914				
H916				
H926				
H943				
H 903	–	T	*Radophous similis*	[42]
H 906				
H 913				
H 915				
H923				
H934				
H939				
H 904	–	T	*Radophous similis*	[42]
			Meloidogyne incognita	[29]
H 911		T	*Radophous similis*	[42]
			Meloidogyne incognita	[29]
H 952		T	*Radophous similis*	[42]
			Meloidogyne incognita	[29]
H 921	–	T	*Meloidogyne incognita*	[29]
H 924				
H 926				
H 943				
H516	AAA	R	*Meloidogyne incognita*	[45]
			Pratylenchus coffeae	[27]
			Helicotylenchus multicinctus	[21]
			Radophous similis	[75]
H531	AAB	R	*Meloidogyne incognita*	[45]
			Pratylenchus coffeae	[27]
			Helicotylenchus multicinctus	[21]
			Radophous similis	[75]
H511	AABB	T	*Meloidogyne incognita*	[45]
			Pratylenchus coffeae	[27]
			Helicotylenchus multicinctus	[21]
			Radophous similis	[75]

Table 3. *Cont.*

Musa Germoplasm	Musa Genome	Level of Tolerance or Resistence	Nematode	Article
H534	AAB	T	*Meloidogyne incognita*	[45]
			Pratylenchus coffeae	[27]
			Helicotylenchus multicinctus	[21]
			Radophous similis	[75]
H537	AABB	T	*Meloidogyne incognita*	[45]
			Pratylenchus coffeae	[27]
			Helicotylenchus multicinctus	[21]
			Radophous similis	[75]
H571	AABB	T	*Meloidogyne incognita*	[45]
			Pratylenchus coffeae	[27]
			Helicotylenchus multicinctus	[21]
			Radophous similis	[75]
H572	AAB	T	*Meloidogyne incognita*	[45]
			Pratylenchus coffeae	[27]
			Helicotylenchus multicinctus	[21]
			Radophous similis	[75]
H589	AABB	T	*Meloidogyne incognita*	[45]
			Pratylenchus coffeae	[27]
			Helicotylenchus multicinctus	[21]
			Radophous similis	[75]
H-02-34	AABB	T	*Meloidogyne incognita*	[46]
				[47]
H-02-34	AABB	T	*Helicotylenchus multicinctus*	[39]
H-02-34	AABB	T	*Radophous similis*	[75]
H-03-05	AABB	T	*Meloidogyne incognita*	[46]
				[47]
H-03-05	AABB	T	*Helicotylenchus multicinctus*	[39]
H-03-05	AABB	T	*Radophous similis*	[75]
H-03-13	AABB	T	*Meloidogyne incognita*	[46]
				[47]
H-03-13	AABB	T	*Helicotylenchus multicinctus*	[39]
H-03-13	AABB	T	*Radophous similis*	[75]
H-03-17	AABB	T	*Meloidogyne incognita*	[46]
				[47]
H-03-17	AABB	T	*Helicotylenchus multicinctus*	[39]
H-03-17	AABB	T	*Radophous similis*	[75]
H 04-12	AABB	T	*Meloidogyne incognita*	[46]
				[47]
H 04-12	AABB	T	*Helicotylenchus multicinctus*	[39]
H 04-12	AABB	T	*Radophous similis*	[75]
H- 04-24	AABB	T	*Meloidogyne incognita*	[46]
				[47]
H- 04-24	AABB	T	*Helicotylenchus multicinctus*	[39]
H- 04-24	AABB	T	*Radopholus similis*	[75]

Table 3. *Cont.*

Musa Germoplasm	Musa Genome	Level of Tolerance or Resistence	Nematode	Article
NPH-02-01	AAB	T	*Meloidogyne incognita*	[46]
				[47]
NPH-02-01	AAB	T	*Helicotylenchus multicinctus*	[39]
NPH-02-01	AAB	T	*Radophous similis*	[75]
H 510	AABB	T	*Helicotylenchus multicinctus*	[39]
			Meloidogyne incognita	[47]

HR, R, PR, MR e T: highly resistant, resistant, partially resistant, moderately resistant and tolerant.

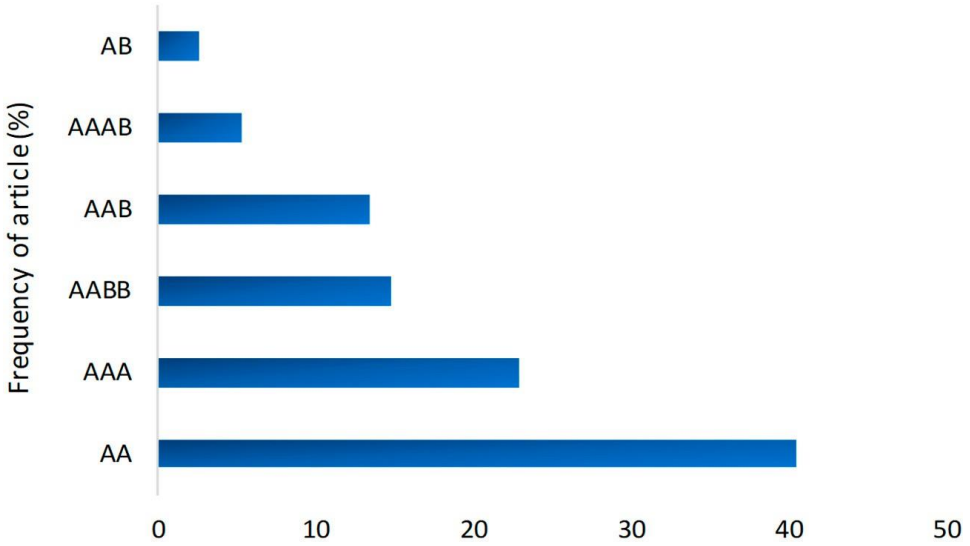

Figure 10. Bar graph of the frequency of genomes according to the resistance/tolerance of the banana genotypes studied.

2.6. Gene Expression Analysis

Most of the articles that evaluated gene expression studied the nematode *P. coffeae* [25,67,70,72]. One study reported banana resistance to *M. incognita* [76]. Table 4 shows the candidate genes that are differentially expressed and potentially involved in defense responses to different nematodes identified in the selected articles.

Table 4. Gene expression studies of banana plants infected with nematodes in articles, carried out over the last 13 years.

Tested Genotype	Genes	Sequences of Specific Primer		Nematode	Article
		Forward Primer (5′-3′)	Reverse Primer (5′-3′)		
Karthombiumtham and Nendran	AY427192.1 AM931368.1 AM931420.1 AM931401.1 AF227002.1 AM931390.1	TGATGTGTGGAATGAGAACGA CGTGGAGAGGCTTACCAAAG CCTGGAGAGCCTTACGAAAG CCTGGACAGGCTTACCATAC CAAGAGCCAGCAATGTTCAA CGTCGGGAGGCTAACCAAAG	CAAGAGCCAGCAATGTTCAA GCCAACCATTTCTGCAATCT GTACTGCGGACCTCAATGGT AACCATGTCGGCAATCTTTC GCAGTGATTTGCAAGCCTTA CCTGGTTCTCCGTACCTCAA	*Pratylenchus coffeae*	[67]
Karthobiumtham; Nendran; FHIA-1; Anaikkomban; Kunnan; Pisang Jaribuaya; Pisang lilin; Calcutta-4; Yankambi KM-5; Rasthali	poly phenol oxidase (PPO)	GACCGCATGTGGTACTTGTG	GGATCTCGACGTCTTGGTA	*Pratylenchus coffeae*	[70]

Table 4. *Cont.*

Tested Genotype	Genes	Sequences of Specific Primer		Nematode	Article
		Forward Primer (5′-3′)	Reverse Primer (5′-3′)		
Karthobiumtham and Nendran	Metallothionein	GGTCAACTCTGAGACCTGA	CCGAGGTACAGGTA GAACAT	*Pratylenchus coffeae*	[25]
	1,3 Glucanase	GGATGAGACTCTACGATCC	GCCTGATCAAGTTCTGGTTG		
	Chitinase	AGTCAAGGTGATGCTCTCCATC	TCCGGCGATGTTGAAGTCTATG		
	Lipoxygenase	TCCACCAGCTCATCAACCAC	TCAGCAGCTTGAAGATGGGG		
	Cytochrome p450	AGAGCGACTCACAGACTCGAC	CCGGGCAGGTACTTGTAGG		
	Peroxidase	TATGCTCACCATTGCTGCTC	TGATTACCATTGCGAGGACA		
	25S rRNA	ACATTGTCAGGTGGGGAGTT	CCTTTTGTTCCACACGA GATT		
Karthobiumtham and Nendran	WRKY52	TAAGGCGAAGAGGAAGGTGA	TCTCCTGTGTGCATCGGTAG	*Pratylenchus coffeae*	[72]
	WRKY92	AAAGCATCAACCCAGCAAAC	ACGGTGCATCGATAATAGGC		
	WRKY69	GAACCGGATCTGGATCTCAA	CGTTCTTCCCTTCCTCATCA		
	WRKY19	CCAGCTGAATGATCTGACGA	TTGCAATCCTGTCTGACTCG		
	WRKY41	ACGCGAATGTTAGCGTCAAT	CGTGAAGGAAGGAACGATGT		
	WRKY81	AGACAATCCATGCCCAAGAG	TGACTTGAGGTCAGGTGCTG		
Musa acuminata 4297-06 and Grad Naine	CALS7	CACCCAGAACATGGTATACTTGAAA	GGTCTCAGGCCTCGTCTTTATG	*Meloidogyne incognita*	[76]
	EXPB11	TAGCAGCAGGAAGTCCTTCGA	GTCGTTCGTCGTGCACAGAA		
	ARR18	CGGATGACGACTCTAGATGCAA	TCGGAGAGGAACACGGAAAA		
	FBXL13	TGGAGTACCTCGGCAAGTTTG	GATGAGATCGTCCTCGCTGATAC		
	EXPA26	CACCTGGGTGCCGATGAC	AAGGCTCTGCCCCACCAT		
	TIFY6B	CAACCGATAGAGTCATCCCTGC	AGTGATCGCTTCATCGAGAGCT		
	ERF4	CCCAAATGTTGGTCCGTTTC	TCGCTGTCTTCCACGATTCA		
	BETV1J	CAGCACTACCATTCGGCTACG	CGAAGAGGGTCTGCTTGCAC		
	APS1	AAGGTCAAGAAGATTGATAGGATATGTG	GTCTTCTGGGAGGTGACAACAAG		
	PER68	CCAAGAAACCACGTAGCAATCA	CAAAATGTGTATGACGTTGGATTCA		

3. Discussion

3.1. Study Screening and Places of Origin

The selection of articles in this review were restricted to genetic improvement, following the protocol developed. In total, 69 articles were included in the data synthesis and analysis. As shown in Figure 1, various articles involving nematodes and *Musa* spp. were identified, but they did not focus on genetic improvement or answer the SR questions (n = 96).

Most of the articles evaluated in this SR regarding the genetic improvement of banana plants for nematode resistance were conducted in India (45.6%), indicating that this country occupies the largest area of banana cultivation and production in the world [77]. In addition, among banana pathogens, plant-parasitic nematodes play a crucial role in crop loss by decreasing productivity [78], which can be proven by the diversity of nematode species found in India compared with other countries (Figure 4). Brazil and Uganda also contributed to the genetic improvement of *Musa* spp., accounting for 13.2% and 8.8% of the selected articles, respectively. These countries are home to important research institutions working on banana improvement, such as Embrapa in Brazil, which ranks fourth among banana producers [79], and IITA in Uganda.

Among these results, some studies conducted under controlled conditions, involved more than one nematode (n = 7). These studies compared the responses of cultivars to the genus or species of nematode used. For instance, Araya and De Waele [41] found that the horizontal and vertical distributions of *R. similis* in the root system of Valery, Gros Michel, and FHIA-18 were considerably similar. However, the distributions of *Helicotylenchus* spp. and *Meloidogyne* spp. and the total number of nematodes varied slightly among the genotypes. Vawa et al. [68] found that the hybrid FHIA 21 was resistant to *R. similis* and susceptible to *P. coffeae*. *P. coffeae* was the second most widely studied nematode and exhibits symptoms similar to those caused by *R. similis* [64]. The greater damage capacity of *P. coffeae* may be related to the parasite's ability to colonize all cellular compartments of the root system [68,80]. In addition, the biological cycle of *P. coffeae* is shorter than that of *R. similis*, and *P. coffeae* more rapidly spreads than *R. similis* [68].

We found 24 studies that performed population surveys in areas with banana plantations infested with nematodes. The majority of these researches was conducted in India (n = 7) and Brazil (n = 4). Except for the USA and Tanzania, studies from all other countries have reported the nematode *R. similis* in the rhizosphere, which is considered as one of the main causes of banana production losses in the world.

Famina et al. [81] investigated the nematodes present in the rhizosphere of banana trees in the district of Malappuram, India, and found a total of 10 species, namely *R. similis*, *H. dihystera*, *P. coffeae*, *Hopolaimus galeatus*, *R. reniformis*, *M. incognita*, *Heterodera glycines*, *Hemicycliophora arenaria*, and *Criconemella* sp. Among these, *Helicotylenchus* sp. occurred most frequently, followed by *R. similis*.

In contrast, Odala et al. [78] found that the presence of *R. similis* in the region of Attappady, India, was less common compared with other nematode species, such as *Meloidogyne* spp., *Pratylenchus* spp., and *Rotylenchulus* spp. In addition, the cultivars evaluated in the selected areas exhibited variations in their response to nematode attacks, with the Nendran cultivar being the most susceptible to phytonematodes.

The pattern of nematode infestation in banana plants found in natural habitats indicates that the occurrence and predominance of a particular species in one country may not be similar in neighboring countries [82]. However, diversity and population survey studies indicate that the genetic basis for nematode resistance in banana accessions requires further investigation [83], for which it is important to focus on the management of phytonematodes that parasitize the crop [84].

3.2. Evaluation Tools and Methods

Since nematode population directly damages the root system, causing lesions, assessing damage to the root and rhizome is of great importance [17].

The primary approach for assessing the symptoms in these studies was through rating scales, which can classify the lesions. The scale, previously used by Pinochet [38], identifies susceptibility, tolerance, or resistance, and was used to evaluate the reaction of *Musa* to *P. coffeae* [27], *H. multicinctus* [21,39], and *R. similis* [17,22,40,42]; this technique evaluates the root lesion index and the degree of the rhizomes.

Rocha et al. [26] and Kosma et al. [85] evaluated the rhizomes of plants infected with *R. similis* based on the percentage of necrosis using the scale established by Bridge [86]: necrosis in <25% of the rhizome was considered mild, from 25% to 50% moderate, from 51% to 75% severe, and >75% very severe. In addition, Rocha et al. [26] assessed the number of functional roots. Ramesh Kumar et al. [65] evaluated the root lesions caused by *R. similis* and *P. coffeae* using the index described by Sundararaju [87] and the root necrosis index described by Carlier et al. [88]. The highest index (5) was recorded in susceptible cultivars, while the lowest index (1) was recorded in the resistant cultivar Pisang Lilin. The mutants from both groups, Ro Im V4 6-1-1 and Si Im V4 10-5-3, had a relatively lower index (2). Based on the percentage of root necrosis, the Im V4 6-1-1 and Si Im V4 10-5-3 mutants were considered resistant, while the Ro Im V4-6-2-1 mutant was moderately resistant [65].

Herradura et al. [61] evaluated the root necrosis index for *R. similis* following the method of Speijer and De Waele [29]. The extent of root damage was described by the following scores: 1 if the roots were all healthy, 2 if most roots were healthy, 3 if most roots were dead, and 4 if all the roots were dead. This scoring has been used for evaluating damage caused by *R. similis* [42,69] as well as *H. multicinctus* [21,39].

In the study by Speijer and De Waele [29], the evaluation of the data obtained during nematode resistance/tolerance screening was based on a combination of nematode reproduction data and host plant response data. This included at least the number of nematodes in the roots, percentage of dead roots, and root necrosis index by preference, which were taken at different stages of plant growth. The combination of these data can give a reliable indication of whether the genotype is resistant or susceptible, tolerant, or sensitive.

In Table 2, the nematode extraction methods in roots were demonstrated. However, according to Abd-Elgawad [89], the conventional nematode extraction methods need to be optimized because they present some issues related to evaluating their populations, distribution patterns, and interactions with many other factors in the context of integrated pest management. For instance, sieving and centrifugation using a sucrose gradient can extract and quantify both dead and live nematodes, unlike the Baermann funnel method,

which can only extract live nematodes [89]. This statement highlights that the choice of method can lead to errors in nematode population assessments regarding plant damage.

Transgenic approaches (2.7%) have also been used, although only in few studies related to genetic improvement. Most of the transformation protocols employed were based on using cystatin and peptide to provide single or double defense against nematodes. One of the approaches used for transgenic resistance to nematodes involves interrupting their feeding. Cysteine proteinases are the main digestive enzymes of many nematodes, and small protein inhibitors (cystatins) from plants have mediated nematode resistance when expressed in various crops [23]. In contrast, defense based on plant roots secretions, such as synthetic peptides, disrupts nematode chemoreception and interferes with perception of host plant location [43,44]. *R. similis* and *H. multicinctus*, nematodes that were introduced in the study by Tripathi et al. [23], were unable to maintain their density in the root system of growing transgenic bananas, precisely because cystatin and the peptide provide a high level of resistance in bananas.

A total of 8% of the articles used hybrids that had been screened to detect possible genotypes resistant or tolerant to various nematode species. *R. similis, P. coffeae, M. incognita*, and *H. multicinctus* were the most commonly tested nematodes in these studies. Two hybrids, H516 (AAA) and H531 (AAB), proved to be resistant to the four nematode species mentioned above [21,27,45,75]. These hybrids were developed from genotypes considered sources of nematode resistance. H516 (AA) is a hybrid of Anaikomban x Pisang Lilin and H531 (AAB) is a hybrid of Poovan x Pisang Lilin. In addition to these, some other hybrids have been shown to be tolerant to more than one species of nematode. Traditional genetic improvements come from potential and improved diploids [21]. Tools such as marker-assisted selection, double haploids, and genomic selection can further accelerate population improvement at the diploid level [90]. Wild diploid bananas of *M. acuminata,* such as Calcutta 4, and other diploid cultivars, have AA genomes and may harbor important sources of resistance genes for the genetic improvement of triploid cultivars [14,16].

Although only few studies in this SR were related to molecular marker-assisted selection (1.3%), this tool plays an important role in the genetic improvement of any crop, allowing the analysis of genetic diversity, construction of genetic linkage maps, and development of QTLs for alleles linked to specific characteristics, such as resistance to biotic and abiotic stresses, fruit quality, and parthenocarpy [36]. For example, Afifi and Zawam [48] aimed to select nematode-resistant banana plants through induced mutation. They used ISSR molecular markers to observe the genetic similarity among the banana cultivars tested and found that this similarity was substantially low, which can be attributed to its mutagenesis effect. Backiyarani et al. [36] developed a database (MusatransSSRDB) that provides information on in silico polymorphic SSRs between contrasting banana cultivars for each stress and within the stress, thus facilitating the retrieval of results on cultivars, stresses, and polymorphism. According to the authors, the information contained in MusatransSSRDB makes it easier for banana breeders to select SSR primers based on specific objectives, including stresses caused by the nematode *P. coffeae*.

In the articles that evaluated enzyme activities and phenolic compounds (11.3%), all the genotypes considered resistant or tolerant had high levels of phenols, lignin, peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase. Enzyme activity is one of the important tools for confirming resistance to nematodes. When a pathogen infects the host tissue, a small number of specific genes is induced to produce mRNAs that enable the synthesis of a similar number of specific proteins [27,91]. Lignin and phenol are synthesized via the phenylpropanoid pathways, which confer resistance against nematode attacks [22].

3.3. Sources of Resistance

Considering that banana genotypes susceptible to nematodes are predominantly consumed globally, crop improvement to develop new cultivars that offer high quality, high yield, and resistance to biotic stresses is of great importance for the global banana industry [76].

In the present study, diploid AA genomes occurred frequently, both in the already known sources of resistance with 35.3% incidence (Figure 9B) and in those tested with 41% incidence (Figure 10). This shows that the sources of resistance related to nematodes are mostly made up of diploids. This suggests that wild diploid *M. acuminata* bananas may harbor important sources of resistance genes for the genetic improvement of triploid cultivars [14,16]. However, among the genotypes mostly used in the selected publications, in addition to the diploid Pisang Lilin, which has served as a parent for the generation of hybrids with potential resistance or tolerance, the resistant triploid Yangambi km5 also stood out.

In addition, many studies have tested nematode resistance in various hybrids. A triploid synthetic hybrid (FB920), with tolerance to yellow and black Sigatoka and partial resistance to nematodes, was tested by Quénéhervé et al. [66], who observed that tolerance to the nematodes *R. similis* and *P. coffeae*, in a field trial, was greater than for the Cavendish cultivars. However, FB920 produces small bunches and tall plants, because of which it may not be suitable for export. Nonetheless, according to the authors, these characteristics should not hinder its cultivation by small-scale producers.

3.4. Gene Expression Analysis

The vast majority of banana and plantain cultivars are interspecific hybrids of *M. acuminata* and *M. balbisiana* [92], and the sequence similarity is partially attributed to their genomic compositions [73]. In this review, 11.6% of the articles used the banana genome as an analysis tool. Of these, five were based on gene expression analysis, which used the RT-qPCR/PCR tool. RT-qPCR/PCR has been used by 46.5% of the selected articles in this review, involving molecular marker-assisted selection (1.3%) and transgenics (2.7%). Bioinformatics (7%) and histology/histochemistry (9.3%) also corroborated the results in some of these studies.

These findings imply that nematode invasion triggers multiple signaling pathways, both through tissue damage caused by the invasion of these pathogens and through the recognition of nematode elicitors by R genes [25].

Backiyarani et al. [25] found that the hydrolytic enzyme 1,3-glucanase was upregulated in resistant and susceptible cultivars during infection by *P. coffeae* but, on the 7th day after inoculation (DAI), the level of glucanase was abundant and twice as high in the resistant cultivar compared with the susceptible cultivar. A similar type of expression profile was observed for the peroxidase gene, in which the expression level of resistant cultivars was twice as high as that of susceptible ones and reached the maximum expression level on the 6th DAI [25].

The polyphenol oxidase (PPO) transcript was found in resistant and susceptible cultivars from uninoculated root samples, indicating constitutive expression of the PPO gene, while PPO mRNA levels were higher in roots of resistant plants compared with susceptible plants [70]. The differences in PPO transcript level between resistant and susceptible banana roots led to the consideration of potential PPO effects in *P. coffeae* [70]. PPOs are induced in response to biotic and abiotic stresses in plants and have been applied to various functional processes, including plant defense and regulation of plastidic oxygen levels and the phenylpropanoid pathway [93].

Many genes described in these studies represent interesting candidates for further analysis of host defense or susceptibility function. Most studies related to gene expression have tested the host response to *P. coffeae* [25,67,70,72]. The invasion caused by this parasite triggers multiple signaling pathways through wounds caused by the penetrating action of the stylet and the recognition of the presence of nematodes [25,94]. In addition, banana plants can recognize it by detecting compounds in the cuticle or secretions made by the nematode or both mediated by the triggered defense response genes [25].

In addition to evaluating gene expressions by RT qPCR/PCR, two studies used proteomics as an analysis tool for *M. incognita*. When inoculation with this nematode was tested, the PR10 protein acted against the invasion of this pathogen in Grande Naine,

showing ribonuclease activity or β-1,3-glucanase activity. This protein showed a significant decrease in abundance in *M. incognita in* the root tissues of Grande Naine at 60 DAI. PR10 activity has been associated with the plant's defense response, either through a direct antagonistic interaction with pathogens that invade cells or by increasing the plant's immunity through the induction of programmed cell death around an infection site [95]. Alldrus et al. [96] observed that 114 banana root proteins showed significant changes when inoculated with *M. incognita* at 30 and 60 DAI. The study revealed that these changes affected proteins mainly involved in fundamental biological processes, organization of cellular components, and stress responses.

3.5. Final Considerations, Limitations, and Perspectives

There is a need to develop banana cultivars that are resistant to parasitic nematodes. Therefore, future scientific objectives should prioritize increasing the benefits offered by improved banana plants with durable resistance characteristics to this stress, preferably in cultivable varieties.

This SR was highly specific to genetic improvement for the resistance of *Musa* spp. to plant-parasitic nematodes. Hence, the number of selected articles was limited to 69. We found that, over the last 13 years, some researchers have endeavored to demonstrate methods of genetic improvement to overcome attacks on *Musa* spp. by phytopathogenic nematodes. Much of this work still needs to be improved, and further studies need to be conducted to identify a safe and efficient source of resistance against nematodes. However, important tools and resources related to genetic improvement have been developed in recent years to better understand the interaction between nematodes and banana plants, including hybridization, transgenics, enzymatic data, proteomics, gene identification, and host defense response. This has enhanced our understanding of how genotypes respond to parasitic attacks and their mechanisms of defense, indicating potential for the development of resistant commercial cultivars.

Our findings revealed that the genotypes considered as resistance sources have different degrees of resistance or susceptibility to different nematode species, or even to the same species from different geographical locations. This variability is the biggest challenge for breeding programs. The genotypes Yangambi km5 and Pisang Lilin are the most widely investigated resistance sources, mainly through studies in India, and these genotypes could be targeted by other breeding programs in future studies. Work related to hybridization has also shown great potential in developing resistance against different nematode species. Selected hybrids, such as H516 and H531, showed resistance against the four most important nematode species in banana cultivation: *R. similis*, *P. coffeae*, *M. incognita*, and *H. multicinctus*. Future breeding studies need to be improved to confirm this resistance. We did not identify a method that has been studied more extensively, and there is a growing push for new, precise, and efficient technologies. However, all the methods men- tioned in the selected studies contribute to identifying cultivars with potential resistance. The functional characterization of genes, for example, can facilitate the development of new breeding strategies.

4. Materials and Methods

This systematic review (SR) was performed in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, using the open-access software StArt (State of the Art through Systematic Review) v. 3.3 Beta 03. Three stages were defined for the construction of the SR: planning, execution, and summarization.

In the planning stage, we developed a protocol for the review process, in which the title, objective, keywords, research questions, research sources, and inclusion/exclusion criteria of the articles were defined for the selection and extraction of relevant papers. This review protocol was registered in the database (https://doi.org/10.5281/zenodo.11047703 (accessed on 27 March 2024)). The main research question was structured according to

the five-component strategy PICOS (Population, Intervention, Comparison, Outcome, and Study design) [97] (Table 5).

Table 5. PICOS terms for the research "question" used in the systematic review of genetic improve- ment methods for nematode resistance in banana cultivation over the last 13 years.

Description	Abbrevion	Question Components
Population	P	Phytoparasitic nematodes of banana plants (*Musa* spp.)
Interest/Intervention	I	Genetic improvement strategies for nematode resistance
Comparison	C	Cultural control methods and chemical or other management strategies
Outcome	O	Tolerance or resistance of banana plants to phytoparasitic Nematodes
Study design	S	Scientific articles

Thus, the question established in the SR was as follows: "What knowledge has been generated in the last 13 years regarding the genetic improvement of *Musa* spp., which has potential applications in resistance to parasitic nematodes in banana?" The secondary research questions are listed in Table 6.

Table 6. List of questions on genetic improvement of *Musa* spp. for resistance to plant-parasitic nematodes, which will be addressed through a systematic review of studies conducted in the last 13 years.

Research Questions
Q1:What are the main nematode species that affect banana and plantain crops?
Q2:Which cultivars are recognized as resistant to nematodes?
Q3:Which banana breeding programs are focused on nematode resistance or cross-breeding for the purpose of developing resistant cultivars?
Q4:Are there any known sources of nematode resistance?
Q5:Which genes have been reported to be related to nematode resistance?
Q6:How is germplasm selected?
Q7:What are the most used methodologies for extracting nematodes from roots?
Q8:What are the methods for assessing symptoms?
Q9:What existing tools are used to characterize nematode-resistant plants? Are there any molecular markers?
Q10:Are there any studies on the topics of gene editing, cisgenics, and transgenics?
Q11:How often is the banana genome used?

For question 3, if the article did not mention the study location, the search criteria within the article were standardized to the first author's mailing address to determine the country of origin for the studies.

The execution stage involved three phases: search, selection, and extraction. The electronic searches were conducted using a search *string*, defined with the following terms: ("*Musa* spp." OR bananas OR plantains) AND (nematodes OR "plant parasitic nematodes" OR phytonematodes). The following databases were used: Web of Science, PubMed Central, Springer, CAPES Periodicals Portal, and Scopus. We also used Google Scholar, as well as MusaLit, a bibliographic database with over 18,000 references on bananas. Because MusaLit cannot identify long *strings*, the search *string* for this database was altered to (banana AND nematode), and the filtering method available in this database was used; these limitations imposed some restrictions on the results. The results were imported from each database into the BibTeX, MEDILINE, or RIS formats and then imported into the StArt software v. 3.3 Beta 03.

In the selection phase, the articles were only evaluated by reading the title, abstract, or keywords. We excluded duplicate articles, as well as articles that were not in line with the objectives of our work, such as review articles, non-English language articles, theses, dissertations, manuals, articles published before 2011, book chapters, and articles published in conference proceedings.

In the extraction phase, the articles chosen in the selection phase were read in full, and of these, only (I) the articles that answered the questions in the study protocol (Table 6) were included. The exclusion criteria for this phase were as follows: (E) first reports, (E) chemical control, (E) biological control, (E) management, (E) pathogenicity, and (E) other topics.

The summarization stage involved synthesizing all the answers to the questions proposed in Table 6. The number of articles per answer to each question was quantified, and the values were expressed as a percentage in each graph or summarized in tables. Microsoft Excel and the *ggplot2* and *dplyr* packages in the R statistical software were used to organize the data and construct the graphs. A bibliometric analysis using the VOSviewer software version 1.6.19 was inserted to check the networks of interactions among keywords, among countries, among most-publishing journals, and among authors and co-citations [98].

To avoid any risk of bias, the prism checklist was drawn up in accordance with the PRISMA standards [99]. This document is available for download at the following link: (https://doi.org/10.5281/zenodo.11047688 (accessed on 27 March 2024)).

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CAPÍTULO 2

Resistance Responses of Commercial Prata and Cavendish Banana Genotypes to the Burrowing Nematode *Radopholus similis*

Artigo: Resistance responses of commercial Prata and Cavendish banana genotypes to the burrowing nematode *Radopholus similis*, submetido à revista científica Crop Protection.

Resistance responses of commercial Prata and Cavendish banana genotypes to the burrowing nematode *Radopholus similis*

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Abstract

The burrowing nematode *Radopholus similis* is one of the most destructive soilborne pests of banana, and its management still relies heavily on chemical nematicides, raising environmental and health concerns. Host resistance represents a key component of sustainable nematode management; however, comparative information on resistance levels among commercially relevant banana genotypes remains limited. In this study, twenty banana genotypes from the Prata (AAB/AAAB) and Cavendish (AAA) subgroups, collected from growers' fields and selected based on agronomic relevance,

were evaluated for their response to *R. similis* under greenhouse conditions. Nematode reproduction factor (RF), population density in roots and soil, and the severity of root and rhizome necrosis were assessed at 90 days after inoculation. Genotypes from the Prata subgroup exhibited a wide range of responses to infection, whereas Cavendish genotypes were predominantly susceptible, showing high nematode reproduction and severe tissue damage. The tetraploid hybrids 'BRS Gerais' and 'Embrapa 03' displayed significantly lower reproduction factors and reduced necrosis severity compared with the susceptible control and were classified as moderately resistant. In contrast, all Cavendish genotypes showed high host suitability, confirming the limited availability of resistance within this subgroup. The integration of nematode population parameters with symptom severity assessments proved effective for discriminating resistance levels among banana genotypes. These results highlight exploitable variability within Prata-type materials and provide practical information to support banana breeding programs and integrated management strategies aimed at reducing the impact of *R. similis* and the reliance on chemical nematicides in banana production systems.

Keywords: Phytonematode; *Musa* spp.; screening; genetic control.

Introduction

Banana is among the most widely cultivated and consumed fruits worldwide, playing a strategic role in the agricultural economy of numerous regions, particularly in tropical and subtropical areas (Kashyap et al., 2025). More than 1.000 banana varieties have been described globally, with the Cavendish subgroup being the most commercialized, accounting for approximately 50% of global production and being almost exclusively destined for export markets (FAO, 2024). Other dessert banana

varieties, such as Gros Michel, Sukali Ndiizi, Mysore, Silk, and Pome, are also cultivated, although on a smaller scale (Tripathi et al., 2020).

In Brazil, banana cultivation has high socioeconomic relevance, contributing substantially to employment generation, income, and food security across different producing regions (Mata et al., 2024). The country has more than 468.000 hectares cultivated with banana, with production exceeding 7 million tons and an average yield of approximately 15 t ha^{-1}, highlighting the continued importance of this crop in the national agricultural landscape (IBGE, 2025).

However, banana production occurs under diverse agroecological and socioeconomic conditions, in which disease pressure varies considerably (Kema et al., 2021). Among the major constraints to the sustainability of banana cultivation is the occurrence of plant-parasitic nematodes, with particular emphasis on *Radopholus similis*, a migratory endoparasite originally described in banana roots in the Fiji Islands in 1891 (Zhang et al., 2015). This nematode penetrates feeder roots and migrates through the cortical parenchyma, causing progressive destruction of the root system (Gebremichael & Gebrehiwot, 2015), which results in plant wilting, weakening, and, in severe cases, death (Tran & Quoc, 2024).

Beyond direct damage, infection of the root system by *R. similis* plays a central role in predisposing banana plants to attacks by other soilborne pathogens. Nematode migration and feeding promote extensive necrotic lesions that act as entry points for phytopathogenic fungi and bacteria, in addition to favoring secondary colonization by opportunistic microorganisms present in the soil (Sarah et al., 1989; Gowen et al., 2005). *R. similis* is widely distributed in the main banana-producing regions of the world (Hölscher et al., 2014) and is considered the most damaging among nematodes associated with the crop. Under favorable conditions, damage caused by this species can result in

yield losses of up to 100% in plants of the Cavendish subgroup (Agrios, 2005), reflecting the high genetic susceptibility of these materials.

Management of *R. similis* in commercial plantations relies predominantly on the application of soil nematicides, usually at planting (Gowen & Quénéhervé, 1990; Atkinson et al., 2004). However, the intensive use of these products has raised concerns for decades due to their environmental impacts and risks to human health, particularly for farmers and rural workers (Atkinson et al., 2004). In this context, advances in plant biotechnology and genetic breeding have been identified as promising alternatives to mitigate productivity losses associated with this pathogen, reducing dependence on chemical inputs (Mwaka et al., 2023).

Sources of genetic resistance to nematodes can be identified both within the broad *Musa* gene pool and among hybrids developed by breeding programs conducted in different regions of the world. However, for these sources to have practical impact, resistance evaluations must consider materials representative of cultivars actually used by growers', selected based on agronomically relevant traits such as bunch weight, yield, production cycle, and performance against the main diseases affecting the crop. Early screening of these materials is therefore a desirable strategy, as it enables the identification of promising genotypes at early developmental stages, optimizing resources and accelerating breeding programs (Elsen et al., 2002).

Despite progress in this field, studies performing standardized comparative screenings for resistance to *R. similis* that include, within the same experiment, genotypes from the Prata (AAB/AAAB) and Cavendish (AAA) subgroups collected directly from growers' fields and representative of the diversity of commercially adopted cultivars remain limited. This gap is particularly relevant in the Brazilian context, where both

subgroups are of major economic importance but differ substantially in their genetic background and response to parasitism.

In this context, the present study aimed to screen banana genotypes from the Prata and Cavendish subgroups, collected from growers' fields and selected based on agronomic criteria of commercial relevance, for their response to *R. similis* infection. By integrating nematode population parameters with the expression of symptoms in the root system and rhizome, this work provides a standardized comparative assessment of nematode resistance in materials widely used by Brazilian producers, with direct implications for banana breeding programs and for the development of sustainable management strategies for *R. similis* in banana cultivation. Such information is essential for guiding genotype selection within integrated nematode management strategies in banana production.

Material and Methods

1.1. Plant materials

For this study, twenty banana genotypes belonging to the Prata (AAB/AAAB) and Cavendish (AAA) subgroups were evaluated. These genotypes were collected directly from growers' fields, constituting a representative sample of the variability observed under field conditions (Table 1).

After in vitro rooting, the genotypes were transferred to trays containing a coconut fiber-based substrate and acclimatized for 40 days in a greenhouse. The cultivar Grand Naine was used as a susceptible control (Dhakshinamoorthy et al., 2014; Sankar et al., 2017; Santos et al., 2023). All evaluations of both Prata-type and Cavendish-type genotypes were conducted within a single experiment; therefore, the

results obtained for the cultivar Grand Naine enabled direct comparison between the two subgroups.

Table 1 Banana genotypes from the Prata and Cavendish subgroups evaluated for resistance to *Radopholus similis* under greenhouse conditions.

Name	Ploidy	Subgroup	Name	Ploidy	Subgroup
BGB 159	AAB	Prata	BGB 150	AAA	Cavendish
BGB 166	AAB	Prata	BGB 268	AAA	Cavendish
BGB 170	AAB	Prata	BGB 271	AAA	Cavendish
BGB 179	AAB	Prata	BGB 151	AAA	Cavendish
BGB 208	AAB	Prata	BGB 304	AAA	Cavendish
BGB 167	AAB	Prata	BGB 154	AAA	Cavendish
BGB 168	AAB	Prata	Zelig	AAA	Cavendish
BGB 188	AAB	Prata	Grand Naine	AAA	Cavendish
Prata Anã	AAB	Prata			
Embrapa 01	AAAB	Prata			
Embrapa 03	AAAB	Prata			
BRS Gerais	AAAB	Prata			

1.2. Inoculum preparation

Purified inocula of *R. similis* were provided by the Phytopathology Laboratory of Embrapa Mandioca e Fruticultura. Nematode populations were maintained on 'Grand Naine' banana plants and extracted from the roots using a combination of maceration, sieving, and centrifugation, following the method of Coolen and D'Herde (1972), with modifications.

After extraction, nematodes were recovered on 400-mesh sieves and washed with distilled water. The resulting suspensions were collected in beakers and adjusted to a final concentration of 1,000 nematodes mL^{-1}. Only active nematodes, including juveniles, females, and males, were considered for inoculation.

1.3. Inoculation and evaluations

After the acclimatization period of approximately 40 days, plantlets were transplanted into 3-L pots containing a sterilized soil–sand mixture (3:1, v/v), previously autoclaved at 120 °C for 40 min. Approximately 40 days after transplanting, plants were inoculated with *R. similis*. For inoculation, two holes were made around each plantlet to expose the roots, into which 1 mL of the nematode suspension was applied.

At 90 days after inoculation (dai), root and soil samples were collected, placed in plastic bags, and sent to the Phytopathology Laboratory. Genotypic responses were initially determined based on infection symptoms at 90 dai. For this evaluation, rhizomes were transversely sectioned, cleaned, photographed, and analyzed for the percentage of necrosis induced by *R. similis*. Images were processed using ImageJ software, allowing delimitation of necrotic areas and total rhizome area. Infection severity was expressed as the ratio between necrotic area and total rhizome area.

Roots were washed and weighed to determine fresh mass. Subsequently, five functional primary roots, approximately 10 cm in length, were randomly selected and longitudinally sectioned. One half of each root was evaluated for the percentage of necrosis. Considering that each half could present up to 20% necrosis, the maximum cumulative value per sample was 100% for the five roots. The sum of necrosis percentages was considered the total root necrosis of the sample, as described by Speijer et al. (1997).

Nematodes present in roots and soil were extracted using modified versions of the methods of Coolen and D'Herde (1972) and Jenkins (1964), respectively. For each sample, 10 g of roots and 100 cm^3 of soil were used. Quantification of *R. similis* populations was performed using a Peters counting chamber under a light microscope. Based on these data, the reproduction factor (RF) was calculated as the ratio between the final population (Pf), corresponding to the total number of nematodes recovered from soil and roots, and the initial population (Pi).

Additionally, the reduction in reproduction factor (RRF) relative to the susceptible genotype was determined as proposed by Sasser et al. (1987), allowing discrimination of quantitative resistance levels among genotypes (Table 2). The genotype exhibiting the highest RF value was adopted as the susceptibility standard to *R. similis*, according to Moura and Régis (1987). To minimize potential bias associated with differences in root vigor among genotypes, final population values were normalized according to root fresh mass.

Table 2 Host reaction based on the percentage reduction of the nematode reproduction factor relative to the most susceptible genotype (Sasser et al., 1987).

RF Reduction (%)	Reaction
100	Highly Resistant (HR)
96 -99	Resistant (R)
76-95	Moderately Resistant (MR)
51-75	Low Resistant (LR)
26-50	Susceptible (S)
<25	Highly Susceptible (HS)

The experiment was conducted in a completely randomized design with ten replicates. Statistical analyses were performed using R software (R Core Development Team, 2016), employing the packages agricolae, ExpDes.pt, and gplot.

Results

Genotypes from the Prata (AAB) subgroup showed wide variation in their response to *R. similis* infection when compared with the susceptible control 'Grand Naine'. Based on the reduction in reproduction factor (RRF), genotypes were classified as moderately resistant (MR), low resistance (LR), or susceptible (S) (Fig. 1A). The genotypes 'BRS Gerais' and Embrapa 03 exhibited significantly lower reproduction factor (RF) values than the susceptible control and were therefore classified as moderately resistant. These genotypes showed the lowest final population (Pf) values and nematode densities, both in the soil and per gram of root, compared with the other evaluated genotypes.

The genotypes BGB 188, BGB 170, BGB 159, BGB 179, and BGB 208 were classified as low resistance, displaying intermediate RF values. The commercial cultivar Prata Anã exhibited a susceptible response, with RF values, total population, and nematode densities similar to those observed in the 'Grand Naine' control. These results demonstrate consistent differences among Prata subgroup genotypes in their ability to restrict *R. similis* multiplication.

Population density values of *R. similis* in soil and per gram of root followed the same pattern observed for the reproduction factor (Fig. 1B). Genotypes classified as MR showed the lowest densities, whereas susceptible genotypes exhibited higher values, reflecting greater root colonization and a higher total nematode population per plant (Fig. 1C).

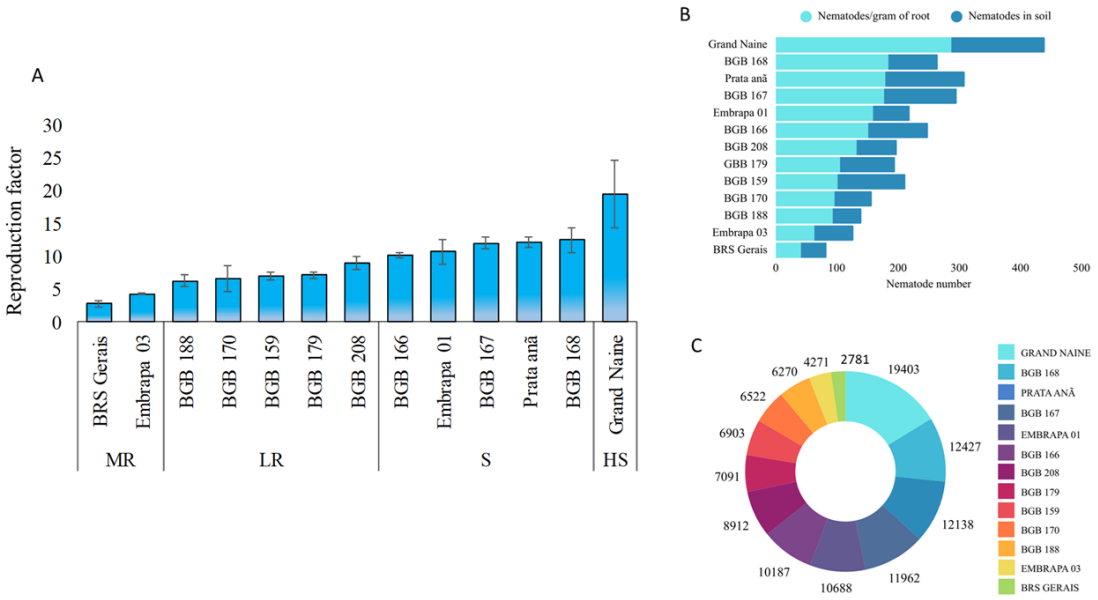

Fig. 1. Effect of *Radopholus similis* on genotypes of the Prata subgroup and on the susceptible control 'Grand Naine', evaluated under greenhouse conditions at 90 days after inoculation (dai). The figure shows the reproduction factor (RF = Pf/Pi) and the classification of genotypic reactions based on the reduction in reproduction factor (RRF), calculated as 100 − (RF of the treatment × 100 / RF of the standard), according to Sasser et al. (1987) (A); the number of nematodes in the soil and per gram of root (B); and the total nematode population per genotype (C). HS indicates highly susceptible, S susceptible, LR low resistance, MR moderately resistant, and R resistant.

The severity of necrosis in roots and rhizomes varied among genotypes of the Prata subgroup (Fig. 2). In general, genotypes classified as MR and LR exhibited low percentages of necrosis in both roots and rhizomes, whereas the susceptible control 'Grand Naine' showed the highest values, particularly in the rhizome (Fig. 2A). The genotypes 'Prata Anã' and BGB 208 displayed higher levels of rhizome necrosis compared with the other Prata genotypes, although still lower than those observed in the

susceptible control. Cluster analysis allowed the discrimination of genotypes based on the combined patterns of root and rhizome necrosis (Fig. 2B).

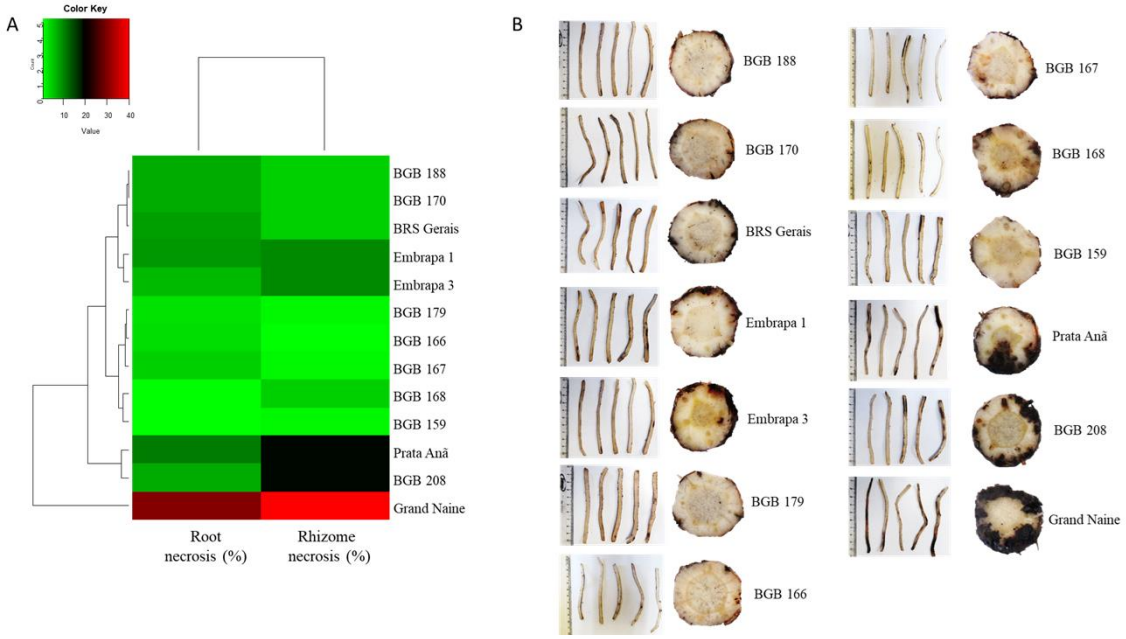

Fig. 2. (A) Cluster analysis and (B) visual representation of Prata subgroup genotypes regarding the severity of root and rhizome necrosis in the presence of *Radopholus similis* under greenhouse conditions

The eight genotypes of the Cavendish (AAA) subgroup, including the control 'Grand Naine', exhibited high susceptibility to *R. similis* infection (Fig. 3). Based on the reproduction factor, five genotypes (BGB 268, BGB 271, BGB 150, 'Grand Naine', and 'Zelig') were classified as highly susceptible, whereas BGB 154, BGB 151, and BGB 304 were classified as susceptible (Fig. 3A). The genotype 'Zelig' showed RF values higher than those of the susceptible control, indicating a high level of nematode multiplication in this material.

Cavendish subgroup genotypes exhibited high population densities of *R. similis* in the soil and per gram of root, as well as higher total population values, when compared

with genotypes from the Prata subgroup (Fig. 3B and 3C). These results indicate a greater suitability of these genotypes as hosts for the nematode.

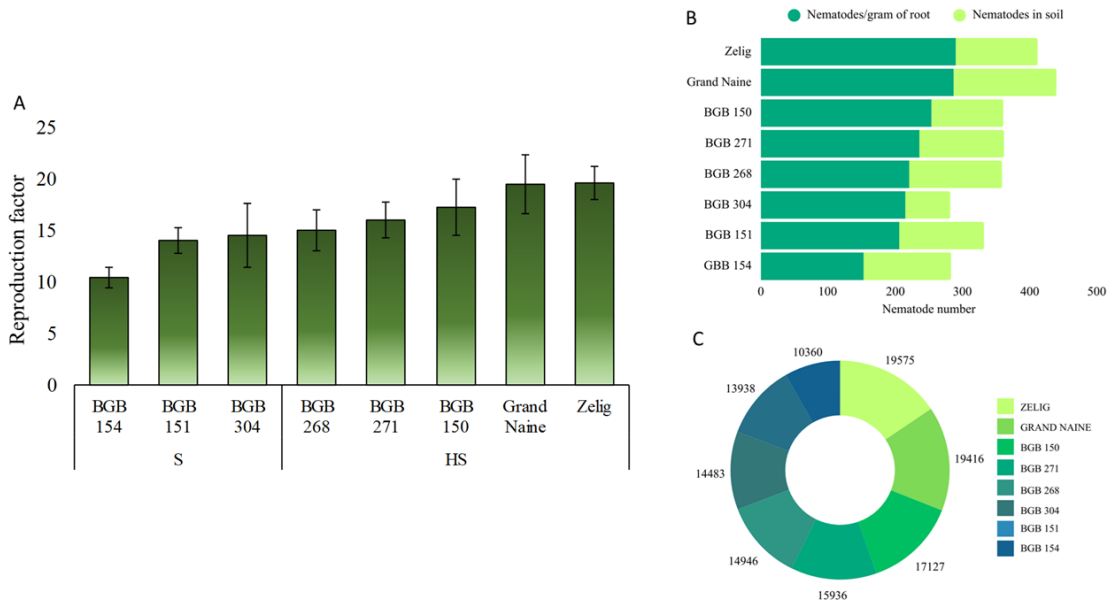

Fig. 3. Effect of *Radopholus similis* on Cavendish subgroup genotypes, evaluated under greenhouse conditions at 90 days after inoculation (dai). The figure shows the number of nematodes in the soil and per gram of root (B), the total nematode population per genotype (C), and the reproduction factor (RF = Pf/Pi), as well as the classification of genotypic reactions based on the reduction in reproduction factor (RRF), calculated as 100 − (RF of the treatment × 100 / RF of the standard), according to Sasser et al. (1987) (A). HS indicates highly susceptible and S susceptible.

The severity of necrosis in roots and rhizomes was high in most Cavendish subgroup genotypes, with a predominance of high percentages of rhizome necrosis (Fig. 4A). Some genotypes showed punctual variations in the intensity of root necrosis, whereas rhizome necrosis patterns were similar among susceptible and highly susceptible genotypes. Cluster analysis indicated closely related severity patterns among Cavendish

genotypes, reflecting a homogeneous response of this subgroup to *R. similis* infection (Fig. 4B).

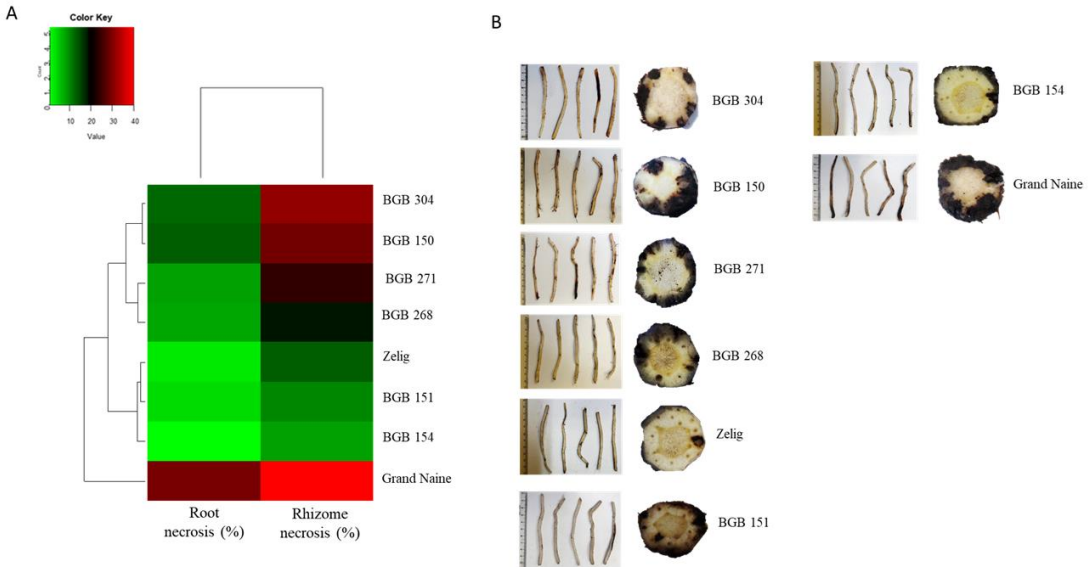

Fig. 4. (A) Cluster analysis and (B) visual representation of Cavendish subgroup genotypes regarding the severity of root and rhizome necrosis in the presence of *Radopholus similis* under greenhouse conditions.

Discussion

In this study, twelve genotypes from the Prata (AAB) subgroup and eight from the Cavendish (AAA) subgroup were evaluated for their response to *R. similis* infection under greenhouse conditions, using the cultivar Grand Naine as a susceptible control. Genotype classification was based on the reduction in reproduction factor (RRF), according to the scale proposed by Sasser et al. (1987), allowing discrimination among highly susceptible, susceptible, low-resistance, and moderately resistant materials. The combined use of the reproduction factor and symptom expression proved appropriate for a more comprehensive characterization of the plant-nematode interaction.

Although the reproduction factor is widely used to infer resistance levels by reflecting the nematode's ability to multiply in the host, it does not, by itself, describe the

intensity of damage caused to root tissues and the rhizome, which are directly associated with physiological and agronomic losses in the crop (Oostenbrink, 1966; Gowen et al., 2005). Thus, the simultaneous evaluation of population parameters and necrosis severity adopted in this study enabled a more robust characterization of genotypic responses to *R. similis* infection.

Within the Prata subgroup, the genotypes 'BRS Gerais' and Embrapa 03 stood out by exhibiting lower reproduction factor values and lower nematode population densities, and were therefore classified as moderately resistant. Both are tetraploid hybrids derived from crosses involving the diploid M53 as the male parent, whose resistance to *Fusarium oxysporum* f. sp. *cubense* race 1 (Foc 1), *F. oxysporum* f. sp. *subtropical* race 4 (Foc ST4), and *F. oxysporum* f. sp. *tropical* race 4 (Foc TR4) has already been demonstrated (Ribeiro et al., 2018; Gonçalves et al., 2019; Rocha et al., 2022). Breeding programs conducted by institutions such as Embrapa Mandioca e Fruticultura in Brazil and the Fundación Hondureña de Investigación Agrícola (FHIA) in Honduras have explored this type of germplasm in crosses between triploids and diploids to obtain tetraploid hybrids with superior agronomic performance (Silva et al., 2003; Lichtemberg & Lichtemberg, 2011; Maluf et al., 2021). The results obtained in this study indicate that these materials also perform favorably against *R. similis*, expanding their strategic potential for breeding programs.

In contrast, all Cavendish subgroup genotypes evaluated showed high susceptibility to *R. similis* infection, with high reproduction factor values, confirming the strong suitability of these materials as hosts for the nematode. This behavior is consistent with previous reports highlighting the narrow genetic base of the Cavendish subgroup, resulting from clonal propagation, and its high vulnerability to soilborne pathogens (Gowen et al., 2005; Quénéhervé, 2009). Although no resistant genotypes were identified

within this subgroup, the discrimination among susceptibility levels observed in this study provides relevant information for prioritizing materials in management and breeding strategies.

It was observed that, in some genotypes, high reproduction of *R. similis* was not necessarily associated with proportional levels of necrosis in the root system and rhizome, indicating a partial dissociation between population density and symptom severity. This pattern has been reported in previous studies and may be related to physiological, structural, or chemical differences in host root tissues that influence nematode feeding efficiency and the progression of necrotic lesions (Moens et al., 2001; Jones et al., 2013; Willig et al., 2023). Constitutive or induced chemical compounds may reduce host attractiveness or interfere with nematode feeding mechanisms, limiting damage severity even in the presence of high populations (Tomar et al., 2025).

In addition, migratory endoparasitic nematodes such as *R. similis* tend to preferentially colonize younger, less lignified roots, where structural resistance is lower and metabolic activity is higher (Jones et al., 2013). This behavior may result in high reproduction associated with more localized damage, without proportional extension of necrosis to the rhizome or thicker roots, contributing to the variability observed in symptom expression among genotypes.

Previous resistance screening studies in *Musa* spp. have reported similar patterns of variability in responses to *R. similis*, even when standardized inoculation and evaluation methodologies are employed (Fallas & Marban-Mendoza, 1994; Santos et al., 2013; Kisitu et al., 2025). These findings reinforce that host response to the nematode is influenced by multiple genetic and physiological factors, going beyond simplified classifications based solely on genomic groups.

The identification of genotypes with lower nematode reproduction or reduced damage expression is particularly relevant for genetic breeding programs and for the development of integrated management strategies. Although complete resistance remains rare in commercial cultivars, recent reviews highlight the existence of promising resistance sources within *Musa* germplasm that can be exploited through targeted crosses, marker-assisted selection, or biotechnological approaches (Sousa et al., 2024). In this context, the results obtained in this study provide practical support for the selection of priority materials and for reducing dependence on chemical nematicides.

Finally, it is important to recognize that evaluations conducted under controlled greenhouse conditions do not fully capture the complexity of biotic and abiotic interactions present in the field. Therefore, although reproduction and symptomatology parameters are fundamental for genotype comparison, field validation studies and multitemporal assessments are required to confirm the stability of the observed responses. The use of accelerated phenotyping platforms and integrated methodologies has been highlighted as a promising approach to improve the characterization of *Musa* spp. resistance to nematodes (Kisitu et al., 2025).

Conclusion

The screening of banana genotypes from the Prata and Cavendish subgroups revealed consistent differences in their interaction with *R. similis*. Cavendish genotypes showed high susceptibility, with elevated reproduction factors and greater damage severity. In contrast, Prata genotypes exhibited variable responses, with the tetraploid hybrids ‘BRS Gerais’ and Embrapa 03 identified as moderately resistant, displaying reduced nematode multiplication and lower root and rhizome necrosis. The integration of population parameters and symptom severity increased the robustness of genotype

classification. These findings indicate potential application of these materials in breeding programs and integrated nematode management, contributing to reduced reliance on chemical nematicides. Although obtained under greenhouse conditions, the results provide a basis for subsequent field validation.

CRediT authorship contribution statement

Amanda Bahiano Passos Sousa: Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anelita de Jesus Rocha**: Validation, Investigation, Formal analysis, Conceptualization. **Wanderley Diaciso dos Santos Oliveira**: Formal analysis, Conceptualization. **Kamila Vitória Mazzei Crispim**: Investigation. **Bruno Santos Louzado das Neves**: Investigation. **Liliane Santana Luquine**: Supervision, Methodology, Conceptualization. **Tamyres Amorim Rebolças**: Investigation. **Leandro Souza Rocha**: Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization**. Edson Perito Amorim**: Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization.

Consent to participate

All authors are in agreement.

Consent to publish

All authors are in agreement.

Ethical approval

This is not applicable.

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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.

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Data availability

Data will be made available on request.

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CAPÍTULO 3

Temporal Coordination of Hormonal and Cell Wall-Mediated Defense Responses Underlie Banana Root Resistance to the Migratory Nematode *Radopholus similis*

Artigo: Temporal coordination of hormonal and cell wall-mediated defense responses underlie banana root resistance to the migratory nematode *Radopholus similis*, submetido à revista científica New Phytologist.

Temporal coordination of hormonal and cell wall-mediated defense responses underlie banana root resistance to the migratory nematode *Radopholus similis*

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Summary

• Root resistance to migratory phytoparasitic nematodes requires rapid and coordinated defense responses; however, the temporal integration of hormonal, transcriptional, and structural mechanisms in banana remains poorly understood. We investigated the physiological and molecular basis of resistance to the burrowing nematode *Radopholus similis* using improved diploid genotypes with contrasting phenotypes.

• Greenhouse trials identified CNPMF 0513 as completely resistant and CNPMF 0496 as moderately resistant, whereas the commercial cultivar 'Grand Naine' was highly susceptible. Temporal analyses integrated histochemical assays and quantitative real-time polymerase chain reaction.

• Complete resistance was associated with early repression of the jasmonate-pathway repressor *TIFY5A*, rapid activation of jasmonic acid and ethylene signaling, and coordinated induction of *MYB* and *PER52*, resulting in early callose deposition, phenolic accumulation, and reinforcement of root cell walls. Delayed or transient activation led to partial nematode containment or host susceptibility.

• These findings support a model in which precise temporal coordination of hormonal, transcriptional, and structural responses determines phenotypic gradients of banana root

resistance to *R. similis*, providing a mechanistic framework for understanding banana, nematode interactions and supporting targeted genetic improvement for resistance.

Keywords: Burrowing nematode, genetic improvement, *Musa* spp., plant immunity, RT-qPCR.

Introduction

Bananas are among the most widely produced, commercially traded, and consumed fruits worldwide, with global export volumes reaching approximately 19.7 million tons in 2024 (FAO, 2025). In addition to their substantial economic relevance, bananas serve as a staple food for millions of people across tropical and subtropical regions. Despite this significance, global banana production has been progressively compromised by increasing pressure from pests and diseases, thereby affecting the productivity, stability, and sustainability of cultivation systems (Mwaka et al., 2023).

Among the principal biotic factors limiting production are phytoparasitic nematodes, which constitute key components of the rhizosphere microfauna associated with banana root systems (Cruz et al., 2005; Seenivasan, 2017). The nematode groups of greatest economic importance include root-knot nematodes (*Meloidogyne* spp.), lesion nematodes (*Pratylenchus* spp.), spiral nematodes (*Helicotylenchus* spp.), and, most notably, the burrowing nematode *Radopholus similis* (Mwaka et al., 2023). This migratory endoparasitic nematode is considered as one of the most destructive pests in intensive banana production systems, particularly in export-oriented regions, where its detrimental effects on root vigor, anchorage, and overall plant productivity are severe (Sarah et al., 1996; Gowen et al., 2005; Kilama et al., 2007; Mwaka et al., 2023).

During parasitism, *R. similis* actively penetrates and migrates through root tissues, destroying host cells and causing dark lesions, extensive necrosis, growth reduction, and, in advanced cases, wilting and eventual plant collapse (Jones et al., 2013; Li et al., 2021). This process is accompanied by the secretion of a complex repertoire of effector molecules from the nematode's esophageal glands, which are delivered directly into plant tissues via the stylet. These molecules play a central role in suppressing host defense responses and facilitating successful infection (Smant et al., 1998; Davis et al., 2008; Haegeman et al., 2012; Yang et al., 2025).

In response to infection, root cells perceive these effector molecules and activate signaling cascades that regulate the expression of defense-related-genes (Kaliyappan et

al., 2016). These responses typically involve the coordinated activation of the salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) signaling pathways, together with the induction of structural and metabolic defense mechanisms, such as cell wall reinforcement and the biosynthesis of secondary metabolites with antimicrobial activity (Castañeda et al., 2017). The integration of these signaling pathways with downstream cellular responses is particularly critical in interactions with migratory nematodes, where continuous mechanical damage and high pathogen mobility pose substantial challenges to root tissue integrity.

Although significant advances have been made in the characterization of effector genes and the transcriptome of *R. similis*, studies investigating the molecular and physiological responses of banana plants to infection remain remarkably scarce. Most of the available research focuses on biochemical analyses, including isoenzymes (Luquini et al., 2019), the accumulation of phenylphenalenone-type phytoalexins in resistant cultivars (Hölscher et al., 2014), or on targeted assessments of specific defense-related genes, such as PAL, POX, and PR-1. These studies are typically conducted via quantitative real-time polymerase chain reaction (RT-qPCR) following inoculation with nonpathogenic endophytes and subsequent challenge with *R. similis* (Paparu et al., 2007). These studies, although scientifically relevant, still provide a fragmented view of the physiological and regulatory mechanisms that underpin root resistance.

In banana plants, resistance to nematodes has been predominantly associated with diploid genotypes, which harbor a higher concentration of alleles associated with effective defense response (Sousa et al., 2024). Several diploid accessions, including Pisang Lilin (AA), Anaikomban (AA), and Calcutta 4 (AA), have been identified as important sources of resistance to *R. similis* and other economically significant nematodes (Kumar et al., 2012; Seenivasan, 2017, 2018; Sankar et al., 2017; Santos et al., 2023). However, the physiological and molecular mechanisms underlying resistance, particularly in the context of root responses to migratory parasitism, remain to be fully elucidated.

Against this framework, the present study aimed to characterize the physiological and molecular mechanisms associated with banana root resistance to *R. similis* infection. This was achieved through a temporal analysis of defense-related gene expression via RT-qPCR, integrated with histochemical evaluations of structural defense responses. For this purpose, improved banana diploid genotypes with previously characterized, contrasting resistance phenotypes were employed.

Material and Methods

1.1. Plant materials

Nine improved banana diploid genotypes developed by Embrapa were evaluated in this study (Table 1). The plants were propagated and rooted in vitro and, upon reaching approximately 6 cm in height, were transferred to trays containing a commercial coconut fiber–based substrate. The seedlings were subsequently acclimatized under greenhouse conditions for approximately 40 days, until complete vegetative establishment was achieved.

The commercial cultivar Grand Naine (AAA) was included as a susceptible control because of its well-documented sensitivity to parasitism by *R. similis* (Dhakshinamoorthy et al., 2014; Sankar et al., 2017; Santos et al., 2023).

Table 1 Improved banana diploid genotypes evaluated for resistance to *Radopholus similis* under greenhouse conditions.

Diploids	**Genealogy**
CNPMF 0557	[(M61 x Pisang Lilin)] x [(Malaccensis x Tjau Lagada)]
CNPMF 0496	[(M61 x Pisang Lilin)] x [(Terrinha x Calcutta 4)]
CNPMF 0536	[(Calcutta 4 x Madang)] x [(Borneo x Guyod)]
CNPMF 0998	[(Borneo x Guyod)] x [(Borneo x Guyod) x SH3263]
CNPMF 1323	[(Malaccensis x Sinwobogi)] x [(Calcutta 4 x Heva)]
CNPMF 0513	[(M61 x Pisang Lilin)] x [(M53 x Kumburgh)
CNPMF 0519	Self-fertilization (wild diploid Tambi)
CNPMF 0534	[(Calcutta 4 x Madang)] x [(Borneo x Guyod)]
CNPMF 0993	[(Borneo x Guyod) x (Tuugia x Calcutta 4)] x [(Khai x (Calcutta 4 x Madang)]
Control	
Grand Naine	-

1.2. Inoculum preparation

Purified *R. similis* inocula was provided by the Plant Pathology Laboratory at Embrapa Cassava and Fruit Crops. The nematode populations were maintained on ‘Grand Naine’ banana plants and extracted from the roots using a combination of grinding, sieving, and centrifugation, following the method described by Coolen and D'Herde (1972), with minor modifications.

After extraction, the nematodes were recovered using 400-mesh sieves and thoroughly washed with distilled water. The resulting suspensions were collected in

beakers and adjusted to a final concentration of 1,000 nematodes mL^{-1}. For inoculation, only active specimens were used, including juveniles, females, and males.

1.3. Inoculation and evaluations

Following an acclimatization period of approximately 40 days, the seedlings were transplanted into 3-L pots containing a sterilized soil–sand mixture at a 3:1 (v/v) ratio, previously autoclaved at 120 °C for 40 min. Approximately 40 days post-transplantation, the plants were inoculated with *R. similis*. For inoculation, two holes were created around each seedling to expose the roots, followed by the application of 1 mL of the nematode suspension.

Ninety days after inoculation (dai), root and soil samples were collected, packed in plastic bags, and transported to the Plant Pathology Laboratory. The genotypes' response was initially determined based on infection symptoms observed at 90 dai. For this evaluation, the rhizomes were transversely sectioned, cleaned, photographed, and analyzed to determine the percentage of necrosis induced by *R. similis*. The images were processed using ImageJ software, allowing the delimitation of the necrotic areas and the total area of each rhizome. The severity of infection was expressed as the ratio of the necrotic area to the total rhizome area.

The roots were washed and weighed to determine fresh root mass. Subsequently, five functional primary roots, each approximately 10 cm in length, were randomly selected and sectioned longitudinally. One half of each root was evaluated to determine the percentage of necrosis. Considering that each root half could present up to 20% necrosis, the maximum cumulative value per sample was 100% across the five roots. The sum of the necrosis percentages was considered as the total necrosis of the root sample, as described by Speijer et al. (1997).

Nematodes present in roots and soil samples were extracted using modified versions of the methods described by Coolen and D'Herde (1972) and Jenkins (1964), respectively. For each sample, 10 g of root tissue and 100 cm^3 of soil were processed for nematode extraction. Quantification of *R. similis* populations was performed using a Peters counting chamber under an optical microscope. Based on these data, the reproduction factor (RF) was calculated as the ratio between the final population (Pf), corresponding to the total number of nematodes recovered from soil and roots, and the initial population (Pi).

Additionally, the reduction in the reproduction factor (RFR) relative to the susceptible genotype was determined following Sasser et al. (1987), enabling discrimination of quantitative resistance levels among genotypes (Table 2). The genotype exhibiting the highest RF value was designated as the susceptibility standard for *R. similis*, in accordance with Moura and Régis (1987). To minimize potential biases associated with differences in root vigor among genotypes, final population values were normalized to root fresh mass.

Table 2 Host reaction based on the percentage reduction in nematode reproduction factor relative to the most susceptible genotype (Sasser et al., 1987).

RF Reduction (%)	Reaction
100	Highly Resistant (HR)
96 -99	Resistant (R)
76-95	Moderately Resistant (MR)
51-75	Low Resistant (LR)
26-50	Susceptible (S)
<25	Highly Susceptible (HS)

The experiment was conducted using a completely randomized design with ten replicates. Statistical analyses were performed using R software (R Core Development Team, 2016) utilizing the agricolae, ExpDes.pt, and gplot packages. For variables that did not satisfy the assumptions of normality (Shapiro–Wilk test at a 5% significance level), the data were log-transformed (x + 1).

1.4. Contrasting diploids evaluation

Molecular analyses by RT-qPCR along with histochemical studies, were conducted using two improved banana diploid genotypes, CNPMF 0496 and CNPMF 0513, which were previously identified as exhibiting contrasting resistance to *R. similis* based on their RF.

Subsequently, a new experiment was conducted to challenge the two improved diploid genotypes and the ‘Grand Naine’ cultivar, which served as a susceptible control. This experiment followed the same methodology adopted in the initial screening, with the exception of number of replicates, which was increased to 22 plants (clones) per genotype and arranged under a completely randomized design.

1.5. RNA extraction and cDNA synthesis

Root samples intended for total RNA extraction were collected, immediately immersed in liquid nitrogen, and stored in an ultrafreezer at -80 °C for further processing. Plants collected at 0, 3, 7, and 10 days after injury (Castañeda et al., 2017), as well as non-injured control plants, were used for total RNA extraction following the protocol modified by Ferreira et al. (2019). RNA quantity and integrity were assessed by comparative analysis on a 1% agarose gel and by spectrophotometric measurement using a Nanodrop ND-2000 device (Thermo Scientific, Waltham, MA, USA). RNA samples were treated with DNase (RNase TURBO™ DNase, Ambion), and cDNA synthesis was performed using a High-Capacity RNA-to-cDNA kit (Applied Biosystems), following the manufacturer's recommendations.

1.6. Gene expression analysis via RT-qPCR

Given that several plant defense mechanisms may be shared among different phytoparasitic nematodes and that gene expression studies involving *R. similis* remain scarce, the selection of candidate genes was based on the work conducted by Castañeda et al. (2017) for *Meloidogyne incognita*. In this study, genes associated with plant defense responses were identified through Illumina HiSeq sequencing of cDNA libraries and subsequently selected for analysis (Table 3).

Gene sequences were mapped using *Musa acuminata* spp. *malaccensis* var. Pahang (AA genome) as the reference genome, accessed through the Banana Genome Hub (https://banana-genome-hub.southgreen.fr/). Following a global transcriptomic analysis, genes were selected based on their association with plant defense processes during interactions with phytoparasitic nematodes (Castañeda et al., 2017).

The gene annotated as *MYB* (Table 3) corresponds to a partial sequence showing similarity to the At1g14600 locus of *Arabidopsis thaliana* (Castañeda, 2016). Therefore, it was considered a putative MYB in the present study. Accordingly, its functional interpretation is based on sequence similarity, as its structural integrity and biological function remain to be fully characterized.

Table 3 Primers used for gene expression analysis in the interaction between *Musa* spp. and *Radopholus similis.*

Gene	Description	Primer sequence Forward/Reverse	bp	References
Callose	*Callose synthase 3*	F-CACCCAGAACATGGTATACTTGAAA R-GGTCTCAGGCCTCGTCTTTATG	80	Castañeda et al., 2017
PR1	*Pathogenesis-related protein 1*	F-CAGCACTACCATTCGGCTACG R-CGAAGAGGGTCTGCTTGCAC	80	Castañeda et al., 2017
TIFY5A	*Putative Protein TIFY 5A*	F-CAACCGATAGAGTCATCCCTGC R-AGTGATCGCTTCATCGAGAGCT	88	Castañeda et al., 2017
MYB	*MYB family transcription factor*	F-CGGATGACGACTCTAGATGCAA R-TCGGAGAGGAACACGGAAAA	74	Castañeda et al., 2017
ERF112	*Ethylene-responsive transcription factor ERF071*	F-CCCAAATGTTGGTCCGTTTC R-TCGCTGTCTTCCACGATTCA	79	Castañeda et al., 2017
PER52	*Peroxidase 52*	F-CCAAGAAACCACGTAGCAATCA R-CAAAATGTGTATGACGTTGGATTCA	79	Castañeda et al., 2017
F1	*Elongation factor 1-alpha*	F-GCTACAACCCAGAGAAGATACCCTT R-CAGGTTGGTAGACCTCTCAATCATG	80	Castañeda et al., 2017
RPS2	*Ribosomal protein S*	F-TAGGGATTCCGACGATTTGTTT R-TAGCGTCATCATTGGCTGGGA	84	Zhang et al., 2017

RT-qPCR analyses were performed using an Applied Biosystems 7300 system (ABI, Foster City, CA, USA), SYBR Green PCR Master Mix (Ludwig Biotech), and the primers listed in Table 3. Each reaction was carried out in a final volume of 10 µL, containing 1 µL of cDNA (20 ng μL^{-1}), 0.6 µL of each primer (10 µmol L^{-1}), 5 µL of SYBR Green master mix, and 2.8 µL of nuclease-free water.

The amplification program consisted of an initial incubation at 50 °C for 2 min and an initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension 58 °C for 1 min. A melting curve analysis was performed at the end of the reaction to verify the specificity of the amplified products.

The *F1* and *RPS2* genes were used as endogenous reference genes, as previously validated by Castañeda et al. (2017) and Zhang et al. (2017), respectively. Each biological sample was analyzed in technical triplicate reactions. Relative gene expression levels were calculated using the $2^{-}\Delta\Delta Ct$ method, based on the obtained Ct values (Livak and

Schmittgen, 2001; Pfaffl et al., 2002). Results were expressed as mean ± standard deviation.

1.7. Histochemical analysis

At 0, 3, 7, 10, and 90 dai, small root fragments from genotypes CNPMF 0496, CNPMF 0513, and the susceptible control cultivar Grand Naine were collected and immediately fixed in Karnovsky's solution (Karnovsky, 1965), consisting of 1% glutaraldehyde, 4% formaldehyde, 0.1 M $CaCl_2$, and 0.2 M sodium cacodylate buffer (pH 7.2) and maintained for 48 h.

After fixation, the tissues were dehydrated through a graded ethanol series (30–100%), with 2-h intervals between successive steps. Infiltration and embedding were performed using a Historesin kit (hydroxyethyl methacrylate; Leica, Heidelberg, Germany). After polymerization, histological sections (8 µm thick) were obtained using a Leitz 1516 microtome (Thermo Fisher Scientific, Waltham, MA, USA).

Root sections were mounted on histological slides and stained with 0.05% aniline blue for 10 min, as described by Hughes and McCully (1975) for callose detection. To identify phenolic compounds, the slides were stained with ferric chloride for 3 h, following the protocol described by Johansen (1940). Control slides were prepared by replacing the staining solution with distilled water to verify the specificity of the histochemical reactions. Samples were analyzed and documented using a BX51 fluorescence microscope (Olympus Latin America Inc.).

Results

1. Screening

The improved diploids evaluated exhibited contrasting responses to *R. similis* parasitism, allowing their classification into groups with low or moderate resistance, whereas the commercial cultivar Grand Naine exhibited a highly susceptible phenotype, with the RF used as the primary evaluation criterion (Fig. 1). According to the RFR scale proposed by Sasser et al. (1987), consistent and statistically significant differences in resistance levels were observed among the tested genotypes.

The diploids CNPMF 0998, CNPMF 0519, and CNPMF 0557 exhibited intermediate levels of infestation, with RF values ranging from 3.69 to 4.73, and were therefore classified as low-resistance genotypes. In contrast, the diploids classified as moderately resistant (CNPMF 1323, CNPMF 0534, CNPMF 0536, CNPMF 0993,

CNPMF 0496, and CNPMF 0513) exhibited the lowest RF values, which ranged from 1.27 to 1.92. The cultivar Grand Naine exhibited a high RF, confirming its highly susceptible phenotype as widely reported in the literature. These results reinforce the robustness and reliability of the experimental trial.

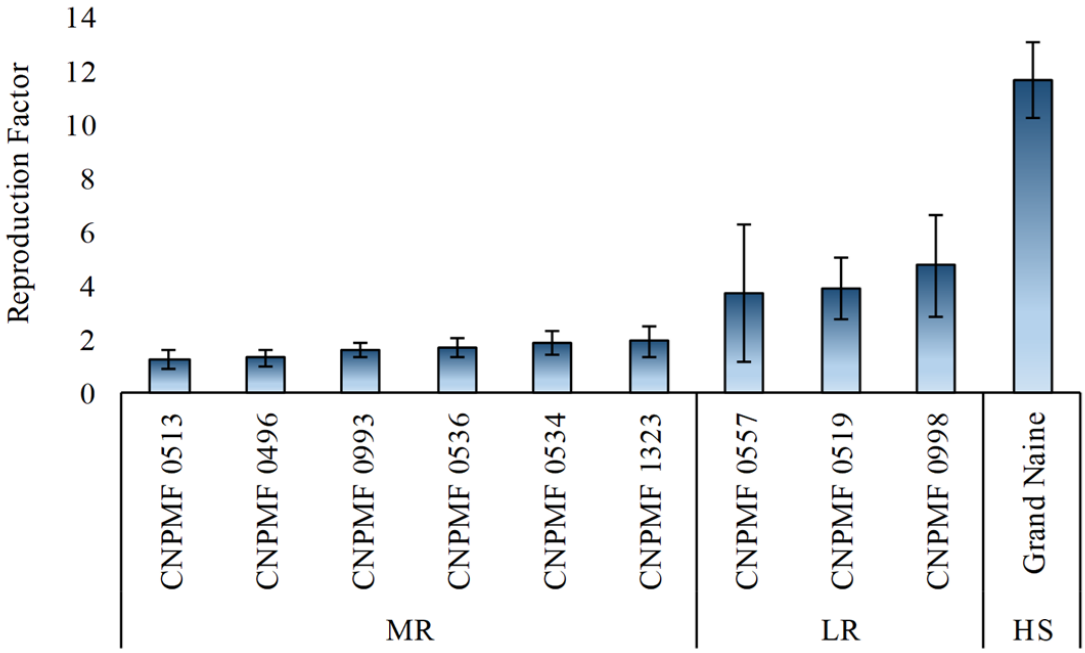

Fig. 1 Responses of improved diploids to *Radopholus similis* under greenhouse conditions (FR = pf/pi). The reaction of each treatment was defined on the reduction of the reproduction factor (RRF) = (100 – (RF treatment x 100/standard FR)) (Sasser et al., 1987). HS, highly susceptible; LR, low resistance; MR, moderately resistant.

The evaluation of visual symptoms corroborated the results based on the RF. The analysis of necrosis in roots and rhizomes revealed substantial variability in responses among the improved diploid genotypes (Fig. 2a). In general, these genotypes exhibited low levels of necrosis, evidenced by lower staining intensities, indicating greater resistance or tolerance to parasitism compared with the susceptible cultivar Grand Naine, which exhibited the highest necrosis indices, particularly in the rhizome.

Among the evaluated diploids, CNPMF 0496 and CNPMF 0534 exhibited the lowest necrosis intensities in the rhizome, whereas CNPMF 0513 exhibited the lowest necrosis intensity in roots (Fig. 2a and 2b). In contrast, genotypes such as CNPMF 0519 and CNPMF 0998 exhibited relatively higher levels of root necrosis, although these levels remained substantially lower than those observed in ‘Grand Naine’. Collectively, these

results indicate that improved diploids are capable of restricting the progression of symptoms associated with *R. similis* parasitism. This phenotypic divergence provides a robust basis for the selection of contrasting genotypes and for subsequent investigations into the physiological and molecular mechanisms underlying resistance.

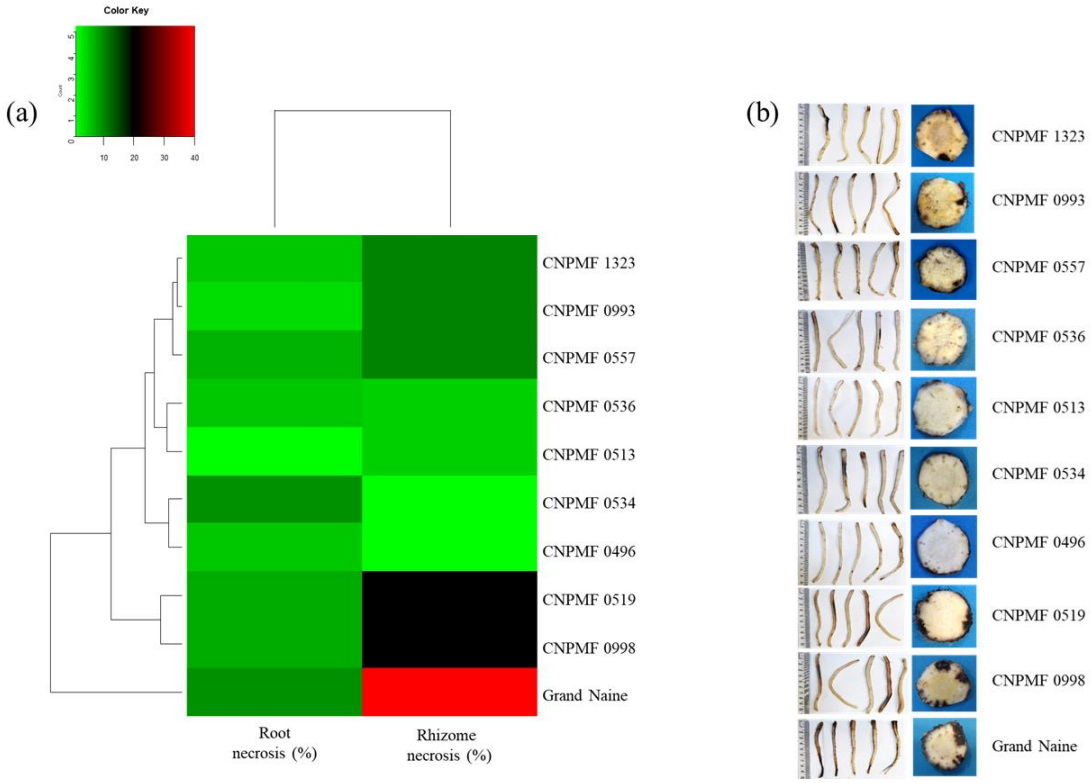

Fig. 2 (a) Cluster analysis and (b) visual representation of improved banana diploid genotypes based on necrosis severity in roots and rhizomes following *Radopholus similis* infection under greenhouse conditions.

2. Contrasting diploids evaluation

2.1 Population density and symptoms

Following the initial screening, the improved diploids CNPMF 0496 and CNPMF 0513 were selected for further analysis in a subsequent experiment. This selection was based on an integrated assessment of the RF results and the intensity of symptoms recorded, which indicated contrasting performance and justified their selection for more detailed evaluation.

At 90 dai, the population density of *R. similis* and the genotypes' responses were evaluated. Figure 3a illustrates the mean fresh root weight for each genotype. The nematode population in the soil differed significantly among genotypes, with the highest

density observed in the susceptible control 'Grand Naine' (n = 99) (Fig. 3b). Analysis of the number of nematodes per gram of root revealed that the diploid CNPMF 0513 had the lowest infestation level, with a mean of 4 nematodes g^{-1}, whereas CNPMF 0496 exhibited 46 nematodes g^{-1}. The 'Grand Naine' control exhibited the highest nematode density, with 227 nematodes g^{-1}, differing significantly from all other genotypes (Fig. 3b).

The improved diploid CNPMF 0513 had a Pf of 436 nematodes (Fig. 3c), an RF < 1 (Fig. 3d), and was classified as resistant (98%) according to the RFR scale (Figure 3e). In contrast, CNPMF 0496 had a Pf of 6,594 nematodes, whereas the 'Grand Naine' control reached 29,939 nematodes (Figure 3c), and were classified as moderately resistant (78%) and highly susceptible (0%), respectively (Fig. 3e).

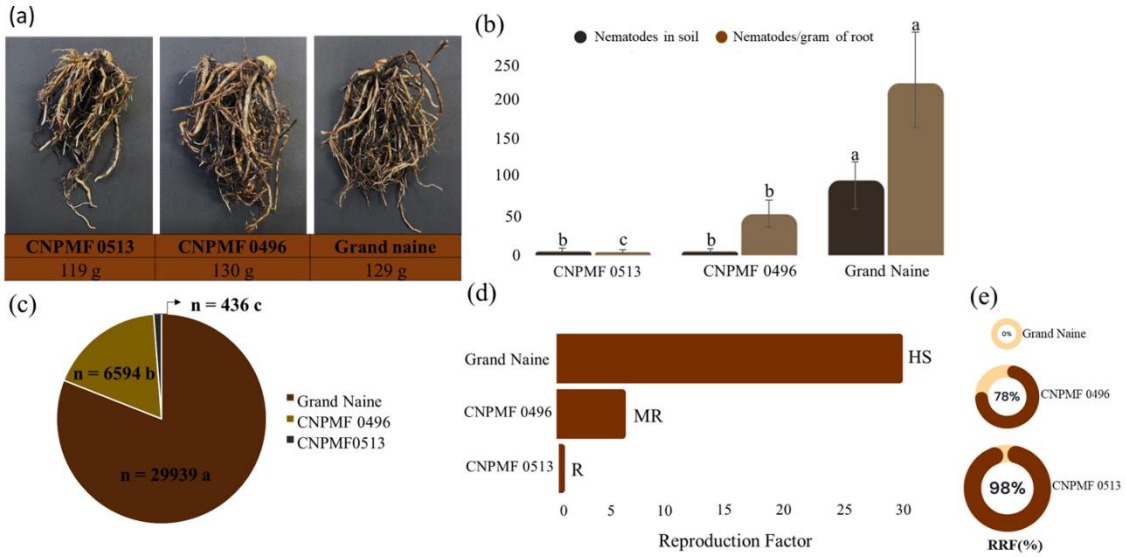

Fig. 3 Effect of *Radopholus similis* on improved banana diploid genotypes and the susceptible control 'Grand Naine,' evaluated under greenhouse conditions 90 days after inoculation. The figure illustrates mean fresh root weight, number of nematodes in soil per gram of root, total nematode population per genotype, reproduction factor (RF = Pf/Pi), and genotype reaction classes based on the reduction in the reproduction factor (RFR), calculated as 100 − (RF_treatment × 100 / RF_standard), according to Sasser et al. (1987). HS indicates highly susceptible; MR moderately resistant; and R resistant. Means followed by the same letter do not differ significantly according to the Scott-Knott test ($p \leq 0.05$), with data log-transformed [(x + 1)] for statistical analysis.

Figure 4 illustrates greater necrosis severity in the susceptible control, particularly in the rhizome, as evidenced by more intense red staining. In contrast, both improved diploids exhibited substantially lower levels of necrosis, characterized by a predominance of green tones that indicate reduced tissue damage. Representative images confirmed these patterns: while 'Grand Naine' exhibited darkened roots with continuous lesions and rhizomes containing extensive areas of necrotic tissue, the improved diploids displayed better-preserved roots with discrete or absent necrotic spots. Furthermore, their rhizomes exhibited more uniform coloration, without evident signs of tissue degradation. These findings demonstrate that CNPMF 0496 and CNPMF 0513 exhibit greater resistance to the pathogen, as reflected by the limitation of necrosis progression in both roots and rhizomes following inoculation.

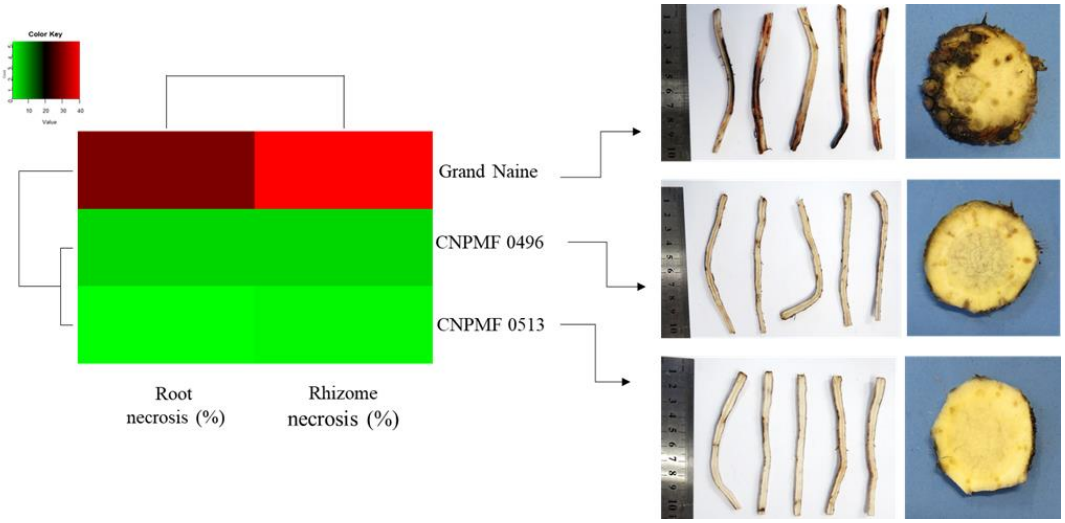

Fig. 4 Cluster analysis of improved banana diploid genotypes and the 'Grand Naine' control based on the severity of necrosis in roots and rhizomes following *Radopholus similis* infection, 90 days after inoculation under greenhouse conditions.

2.2 Histochemical analysis

Histochemical analyses revealed pronounced temporal differences in root defense responses among the evaluated genotypes. At 3 dai, the fully resistant genotype CNPMF 0513 exhibited pronounced callose deposition and phenolic compound accumulation at infection sites, whereas these responses were weaker or delayed in the moderately resistant genotype CNPMF 0496 and were largely absent in the susceptible cultivar Grand Naine.

Histological analysis of root sections stained with aniline blue revealed callose deposition in the roots of the evaluated genotypes, as evidenced by the characteristic blue

green fluorescence. A progressive increase in callose deposition was observed starting at 3 dai in both the control and the diploid genotypes, indicating early activation of defense-related mechanisms (Fig. 5).

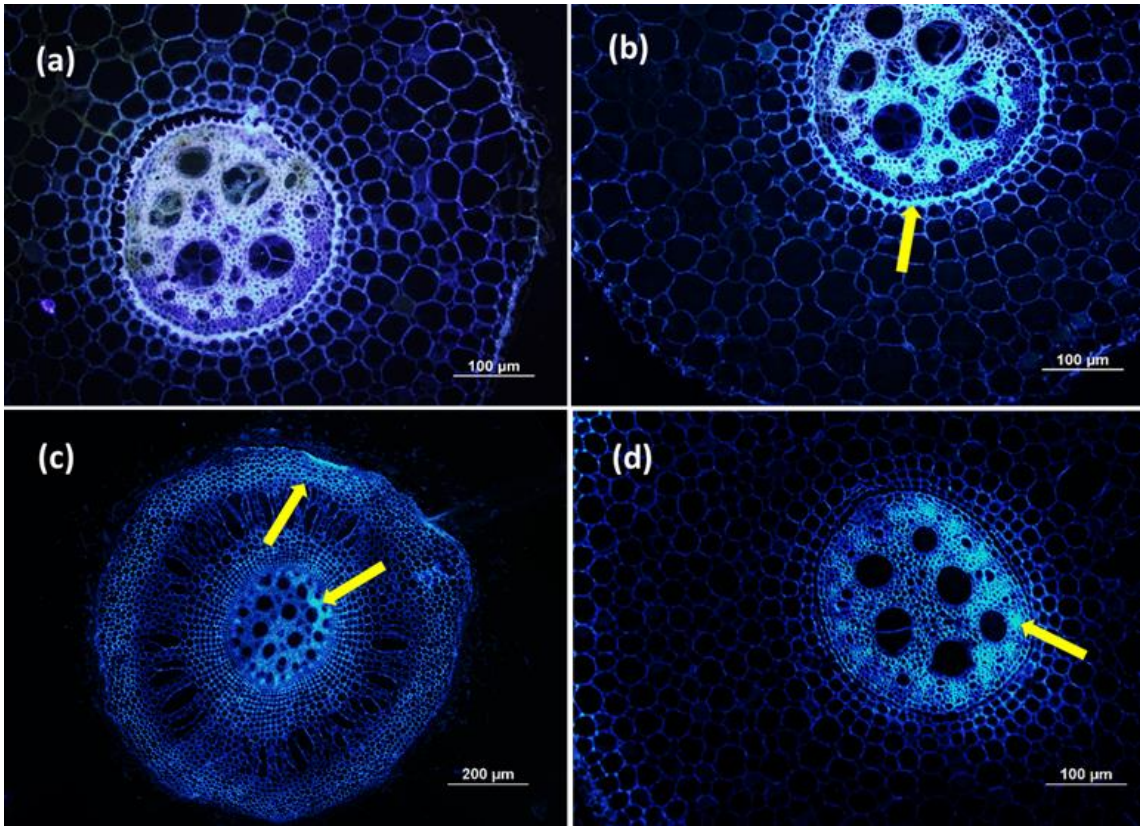

Fig. 5 Histochemical detection of callose deposition in banana roots using aniline blue staining, 3 days after inoculation with *Radopholus similis*. (a) Slide without dye; (b) control (cv. Grand Naine); (c) CNPMF 0513; and (d) CNPMF 0496. Yellow arrows indicate second-degree blue–green fluorescence associated with callose accumulation in root tissues.

The histochemical analysis for the detection of phenolic compounds in roots using ferric chloride revealed the presence of these metabolites in all evaluated plants, evidenced by small dark-brown spots in the root tissue (Fig. 6). The diploid CNPMF 0513 showed accumulation of phenolic compounds from 3 dai, indicating an earlier defense response. In contrast, the diploid CNPMF 0496 and the control ‘Grand Naine’ exhibited accumulation of these compounds from 7 dai onward.

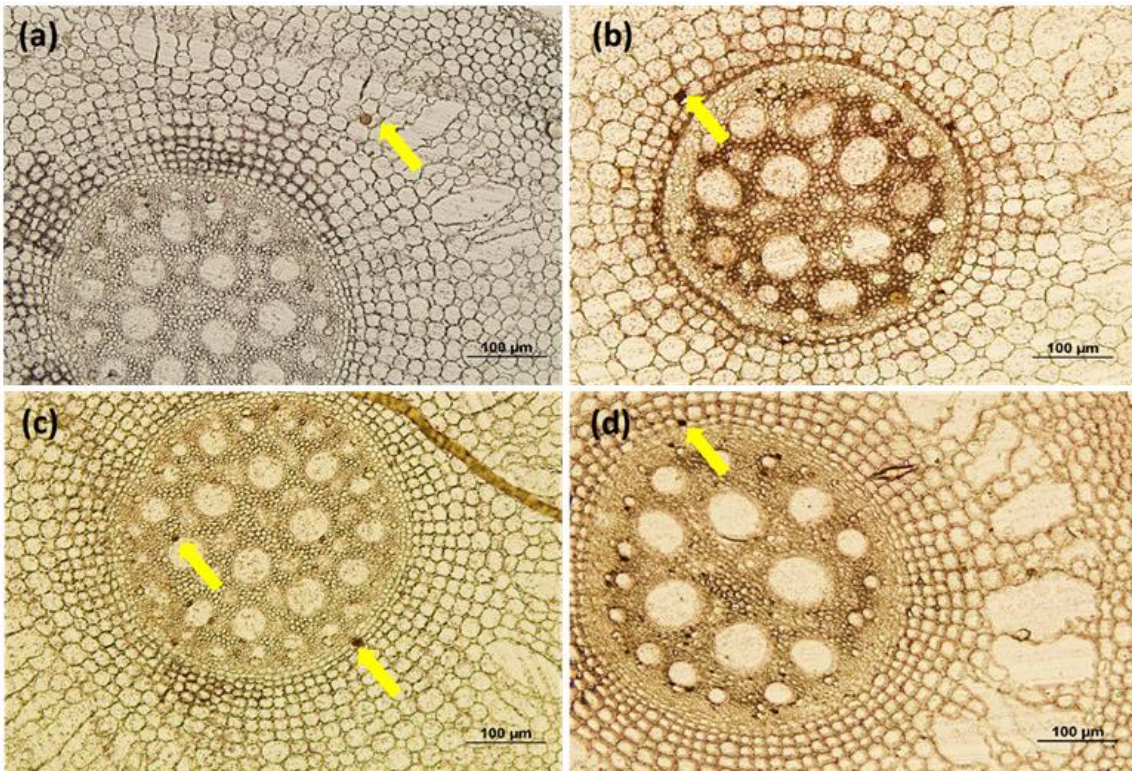

Fig. 6 Histochemical detection of phenolic compounds in banana roots using ferric chloride staining following inoculation with *Radopholus similis*. (a) Slide without dye; (b) Control (cv. Grand Naine), 7 days after inoculation (dai). (c) CNPMF 0513, 3 dai; (d) CNPMF 0496, 7 dai. Yellow arrows indicate regions with phenolic compound accumulation.

2.3 Gene expression analysis

Temporal analysis of defense-related gene expression revealed clear differences in both the timing and magnitude of responses among banana genotypes exhibiting complete resistance (CNPMF 0513), moderate resistance (CNPMF 0496), and susceptibility ('Grand Naine'). While the fully resistant genotype exhibited early and sustained transcriptional activation of defense-associated pathways, the moderately resistant genotype displayed a delayed and transient response, whereas the susceptible control failed to mount or sustain effective defense signaling.

The expression of defense-related genes exhibited contrasting patterns between the 'Grand Naine' control and the diploids CNPMF 0496 and CNPMF 0513 across the three evaluated time points (Supplementary Table 1).

The gene encoding the *TIFY5A* protein was downregulated in all genotypes, reflecting a possible suppression of the jasmonate signaling pathway throughout the

infection process (Fig. 7). In contrast, expression of the *MYB* transcription factor revealed a clear distinction between susceptible and resistant genotypes. The Grand Naine cultivar exhibited initial induction followed by strong repression at 10 dai, whereas both improved diploids exhibited a gradual increase in expression particularly CNPMF 0513, suggesting the involvement of this regulator in the resistance response, possibly through modulation of phenolic compound biosynthesis (Fig.7).

The *CALS3* gene was positively regulated in the 'Grand Naine' control at 7 dai, whereas both diploids exhibited continuous repression. This repression was more pronounced in CNPMF 0513 at 10 dai, indicating that callose deposition is not a central defense response in these resistant genotypes (Fig. 7). For *PR1*, early positive regulation was observed in 'Grand Naine' at 3 dai, followed by repression at later time points, suggesting an initially activated but ultimately ineffective salicylic acid mediated defense pathway. In the diploid genotypes, *PR1* expression remained consistently low and stable, without pronounced temporal fluctuations (Fig. 7).

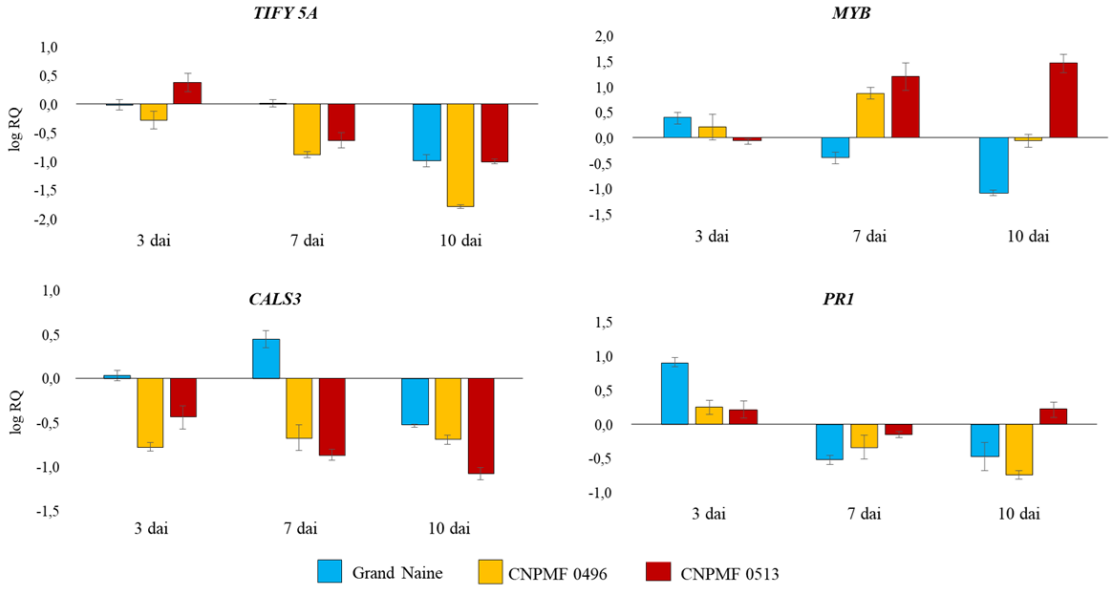

Fig. 7 Relative expression levels of four defense-related genes in banana genotypes at 3, 7, and 10 days after inoculation with *Radopholus similis*. Relative gene expression was analyzed by RT-qPCR for pathogenesis-related protein 1 (*PR1*), putative *TIFY5A* protein (*TIFY5A*), callose synthase 3 (*CALS3*), and an MYB family transcription factor (*MYB*). Data represents standard deviations from three biological replicates.

The expression of *ERF112*, an ET-responsive transcription factor, showed early induction in the diploid genotypes at 3 dai, whereas the 'Grand Naine' control remained close to baseline expression levels. However, all genotypes exhibited transcriptional repression from 7 dai onward, with more pronounced repression in 'Grand Naine' (Fig. 8). This expression pattern suggests the possible involvement of the ET signaling pathway during the initial phase of pathogen recognition, followed by a transition to alternative defense mechanisms. Finally, the peroxidase 52 gene (*PER52*) exhibited consistent upregulation in CNPMF 0513 at 3 dai and in CNPMF 0496 at 3 and 7 dai, whereas 'Grand Naine' exhibited strong repression at the last two evaluated time points (Fig. 8).

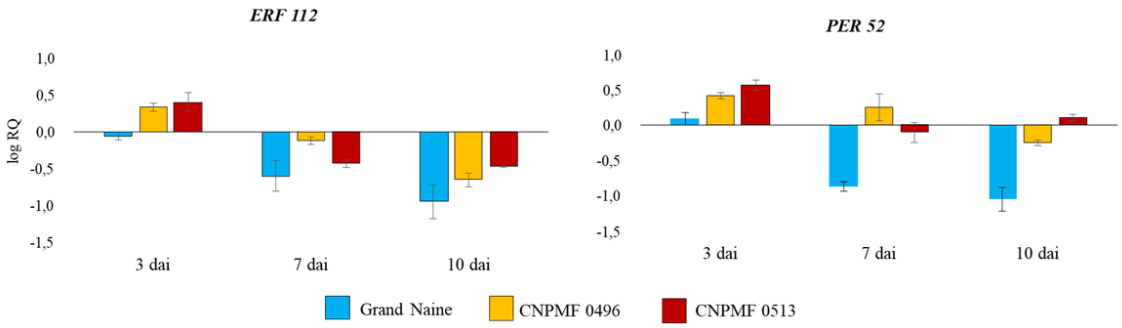

Fig. 8 Relative expression levels of two defense-related genes in banana genotypes at 3, 7, and 10 days after inoculation (dai) with *Radopholus similis*. Relative gene expression was analyzed by RT-qPCR for the ethylene-responsive transcription factor *ERF112* and Peroxidase 52 (*PER52*). Data represent means ± standard deviations from three biological replicates.

Discussion

The results of this study indicate that banana plants' resistance to the burrowing nematode *R. similis* is not solely associated with the isolated activation of defense-related genes; rather it critically depends on the timing, intensity, and coordination of physiological responses mobilized in roots during the early stages of infection. The genotype exhibiting complete resistance demonstrated early and sustained activation of hormonal pathways and structural defense mechanisms. Conversely, the late or transient responses, as observed in the moderately resistant genotype, resulted in only partial containment of the nematode. In contrast, the susceptible genotype exhibited an inefficient or unsustained defense response, thereby allowing extensive colonization of host tissues.

In this study, nine improved banana diploid genotypes were initially evaluated for resistance to *R. similis* under greenhouse conditions. This screening revealed genotypes with varying levels of resistance, contrasting sharply with the control 'Grand Naine', which was classified as highly susceptible and exhibited more severe symptoms than the diploids. Based on symptom intensity and RF, diploid genotypes CNPMF 0496 and CNPMF 0513 were selected for subsequent analyses of population density, symptomatology, gene expression, and histochemical responses. In the initial screening, both genotypes were classified as moderately resistant, reflecting a defensive response sufficient to reduce, but not completely eliminate, the progression of the pathogen (Fig. 1). However, in the subsequent experiment, CNPMF 0496 maintained a moderately resistant phenotype, whereas CNPMF 0513 began to exhibit complete resistance, indicating phenotypic stability in the former and greater defensive efficiency in the latter.

The differences observed between the initial screening and the subsequent experiment may be partially associated with variations in plant vigor and overall nutritional status. In the second experiment, the plants received additional mineral nutritional supplementation, including NPK fertilization and foliar application of a mixed mineral fertilizer, which likely contributed to improved vegetative growth and root development. Adequate mineral nutrition is widely recognized as a key factor in modulating plant defense responses, as it can promote the formation of structural and metabolic barriers and enhance the efficiency of resistance mechanisms (Agrios, 2005; Walters & Bingham, 2007). Although mineral nutrition alone does not determine resistance, it can substantially influence the expression and intensity of defensive traits.

Symptom evaluations consistently indicated that the susceptible control 'Grand Naine' exhibited greater necrosis intensity in both roots and rhizomes, reflecting extensive colonization of host tissues by *R. similis*. In contrast, the improved diploids exhibited little to no necrotic tissue, indicating reduced tissue degradation and a greater capacity to contain pathogen progression (Fig. 4). This differential necrosis pattern suggests that the evaluated diploids possess more efficient defense mechanisms, including structural barriers and molecular responses capable of limiting nematode progression.

These diploids are among the materials currently used as progenitors in genetic improvement programs, due to their reported resistance to *Fusarium oxysporum* f. sp. *cubense* (Santana et al., 2024) and *Pseudocercospora fijiensis* (Gonçalves et al., 2021). Molecular studies further indicate that these improved diploids maintain high genetic

variability and strong combinability with commercial triploids, thereby favoring the development of superior hybrids with integrated resistance (Sampaio et al., 2024). Although these aspects reinforce their applied relevance, the present study focuses on the physiological and molecular foundations that underpin the resistance of these genotypes to *R. similis*.

Analyses of population density and RF identified the diploid CNPMF 0513 as resistant, due to the significant reduction in nematode number (Fig. 3). Previous studies involving *F. oxysporum* f. sp. *cubense* race 1 and *F. oxysporum* f. sp. *subtropical* race 4 demonstrated that CNPMF 0496 and CNPMF 0513 are highly resistant to Foc R1 and resistant to Foc ST4 (Santana et al., 2024). These results reinforce the potential of these genotypes as valuable sources of multiple resistance traits. The resistance observed in both diploids likely derives from alleles inherited from parental genotypes such as Calcutta 4, the improved diploid M53, or Pisang Lilin, which is recognized for its high resistance to *R. similis* (Kumar et al., 2012; Seenivasan, 2017; Sankar et al., 2017).

To investigate defense responses at the transcriptional level, the expression of six genes associated with plant immunity against nematodes was evaluated, encompassing markers of hormonal signaling pathways, transcription factors, PR proteins, and genes related to PAMP perception. The regulation patterns observed in response to *R. similis* contrasted with those described for *M. incognita* by Castañeda et al. (2017), particularly in the susceptible control. This divergence likely reflects fundamental differences in the biology and parasitism modes of these nematodes. *R. similis* is a migratory endoparasite that causes direct mechanical damage through tissue perforation and continuous displacement within the root cortex, resulting in extensive microcracks, loss of cellular integrity, and intense oxidative stress (Jones et al., 2013; Li et al., 2021; Espada et al., 2025). In contrast, *M. incognita* establishes a sedentary biotrophic interaction by forming giant cells and galls, which requires active cell wall remodeling, continuous suppression of hosts defenses, and prolonged hormonal signaling (Goverse & Smant, 2014; Choi & Klessig, 2016; Kaloshian & Teixeira, 2019). Thus, each nematode species activates and represses distinct defense pathways over time.

Previous studies have demonstrated that plants can recognize molecular patterns associated with different groups of pathogens, thereby activating specific defense responses for limiting colonization (Dangl & Jones, 2001; Nürnberger & Scheel, 2001; Holt et al., 2003; Berrocal-Lobo & Molina, 2004). In this context, the *MYB* transcription factor associated with lignin accumulation, phenolic compound biosynthesis, and cell

wall strengthening (Luo et al., 2025), exhibited positive regulation in the analyzed diploids, with strong induction in CNPMF 0513 at 7 and 10 dai (Fig. 7). These compounds play an essential role in containing migrating parasites by forming physical and chemical barriers (Liu et al., 2015; Chezem & Clay, 2016), in addition to participating in the biosynthesis of flavonoids such as anthocyanins, flavonols, and proanthocyanidins (Yu et al., 2023; Luo et al., 2025). These results are consistent with the histochemical analyses, which demonstrated deposition of phenolic compounds from 3 dai in CNPMF 0513 and from 7 dai in CNPMF 0496 (Fig. 6).

In studies involving *R. similis*, banana cultivars considered tolerant, such as Yangambi km5 and Gros Michel (*Musa acuminata* AAA), exhibited a higher number of cells containing phenolic compounds than the susceptible cultivar Grand Naine (Mateille, 1994; Fogain & Gowen, 1996; Wuyts et al., 2007). In the present study, 'Grand Naine' exhibited limited deposition of phenolic compounds only at 7 dai and exhibited repression *MYB* repression at 7 and 10 dai, suggesting a structural inability to activate effective cell wall reinforcement mechanisms against nematode advancement.

The positive regulation of *PER52* (Fig. 8) reinforces this interpretation, as peroxidases participate directly in cell wall strengthening and catalyze the oxidation of phenolic compounds through the reduction of hydrogen peroxide (Wuyts et al., 2006). In contrast, 'Grand Naine' exhibited negative regulation of *PER52* expression. Thakker et al. (2013) observed increased peroxidase activity in 'Grand Naine' when challenged by *F. oxysporum* f. sp. *cubense*, particularly when the pathogen was inactivated, suggesting that viable pathogens can suppress host defense responses. This pattern is consistent with the results observed in the present study, in which the lower expression of *PER52* in 'Grand Naine' may be associated with the action of *R. similis* effectors that negatively modulate plant oxidative pathways (Gillet et al., 2017).

Although the *CALS3* gene showed reduced expression in the diploid genotypes, histochemical analyses revealed clear callose deposition following inoculation. This apparent discrepancy can be attributed to the fact that callose synthesis is controlled by a multigene family of callose synthases with partially redundant and functionally specialized roles (Chen & Kim, 2009; Cui & Lee, 2016). Furthermore, callose deposition is regulated by post-transcriptional mechanisms that depend on calcium signaling, reactive oxygen species, and protein–protein interactions (Wu et al., 2018; Wang et al., 2021; Vu et al., 2023). It is also probable that other CALS isoforms were induced before 3 dai and were not captured at the time of sample collection, while callose deposition

persisted in the tissues (Voigt, 2014). Collectively, these factors account for the accumulation of callose despite the reduced expression of the evaluated gene.

The negative regulation of *TIFY5A*, one of the main repressors of the JA pathway, is consistent with the biology of *R. similis*, as nematode migration triggers typical wound responses and rapid JA activation (Erb & Reymond, 2019). This process promotes proteasomal degradation of TIFY/JAZ family proteins, thereby releasing MYC transcription factors that activate downstream defense responses (Shyu et al., 2012). Similar suppression of *TIFY5A* was observed in resistant banana genotypes during interaction with *F. oxysporum* f. sp. *cubense* (Costa, 2013). The more pronounced repression of *TIFY5A* at 10 dai in CNPMF 0496 reinforces the hypothesis of robust activation of the JA pathway in these diploids, which contributed to the restriction of parasite mobility.

In 'Grand Naine', strong initial induction of *PR1* was observed at 3 dai, followed by downregulation at later time points (Fig. 7). *PR1* is a classic marker of the salicylic acid (SA) - dependent signaling pathway, which predominates in responses against biotrophic pathogens (Vlot et al., 2009; Han et al., 2023), such as nematodes of the genus *Meloidogyne*. This transient activation suggests initial recognition of infection but an inability to maintain effective SA signaling. In contrast, resistant diploids showed low and stable expression of *PR1*, indicating that their resistance does not primarily depend on the SA pathway but rather on JA-mediated and structural defense mechanisms. Considering the well-established antagonism between the SA and JA signaling pathways (Kunkel & Brooks, 2002), this differential balance likely contributes to the contrasting patterns of susceptibility and resistance observed.

The expression profiles of *PR1* and *TIFY5A* are consistent with a differential shift between the SA and JA pathways that contributes to both the susceptibility of 'Grand Naine' and the resistance of the evaluated diploids. In 'Grand Naine,' early induction of *PR1* may antagonize JA pathway activation by inhibiting JA-Ile formation and the degradation of JAZ/TIFY repressors, thereby blocking the release of transcription factors, such as MYC2, required for defense against migrating pathogens (Vlot et al., 2009; Zhu, 2014; Howe et al., 2018). In contrast, resistant diploids exhibited more pronounced repression of *TIFY5A* at 7 and 10 dai and low expression of *PR1*, a pattern compatible with JA activation and the implementation of structural defenses such as callose deposition and lignification.

Finally, expression of *ERF112*, a regulator of the ET signaling pathway, was negatively regulated in 'Grand Naine' and transiently induced in resistant diploids at 3 dai, followed by repression at 7 and 10 dai (Fig. 8). Studies in *Arabidopsis* demonstrate broad overlap among responses regulated by ET, JA, and SA following hormonal treatments or pathogen infection (Schenk et al., 2000; Glazebrook et al., 2003; Berrocal-Lobo & Molina, 2004). Under conditions associated with necrotrophic pathogens or mechanical damage, JA/ET pathways tend to suppress SA signaling, resulting in functional antagonism (Glazebrook, 2005; Pieterse et al., 2009). Thus, the sustained repression of *ERF112* in 'Grand Naine' suggests dysregulation of JA/ET–ABA pathway integration, whereas the transient induction observed in resistant diploids is consistent with an initial prioritization of JA/ET pathways, which are more suitable for responding to the cellular damage caused by *R. similis*.

Collectively, these results support a model in which banana plants' resistance to *R. similis* depends on early recognition of parasitism, coordinated activation of JA/ET hormonal pathways, induction of transcriptional regulators associated with cell wall reinforcement, and the implementation of effective structural barriers, ultimately restricting nematode migration and multiplication.

In summary, the integrative model presented in Fig. 9 demonstrates that banana roots resistance to *R. similis* is not determined solely by the activation of defense-related genes but rather by the timing and coordination of hormonal, transcriptional, and structural responses. Complete resistance is associated with early repression of *TIFY5A*, rapid activation of JA/ET pathways, and coordinated induction of *MYB* and *PER52*. This integrated molecular response results in early callose deposition, accumulation of phenolic compounds, and effective cell wall reinforcement, thereby restricting nematode migration. In contrast, delayed or transient activation of these defense mechanisms, as observed in moderately resistant or susceptible genotypes, results in partial or inefficient containment of the pathogen. This temporal framework provides a mechanistic explanation for the observed phenotypic gradients of resistance, highlighting the importance of early and coordinated defense activation in the interaction between banana plants and migratory endoparasitic nematodes.

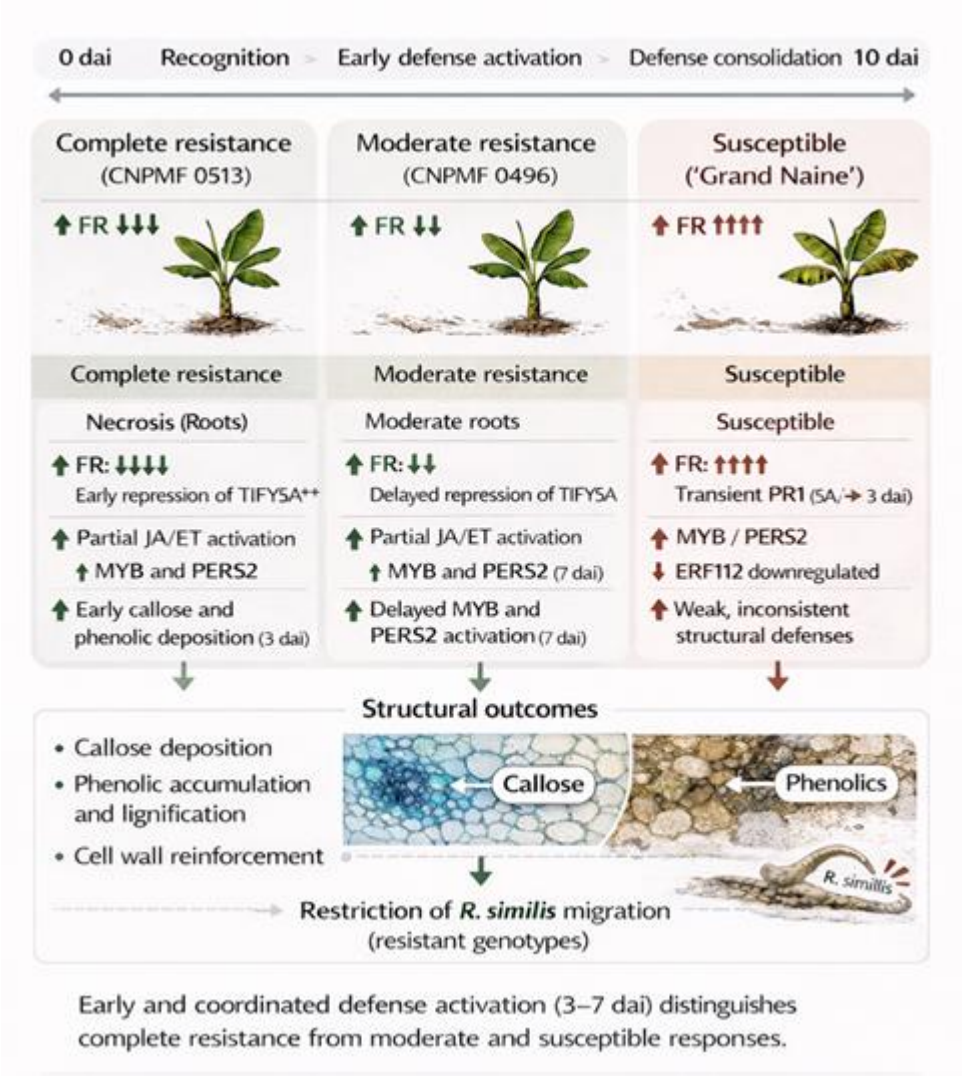

Fig. 9 Temporal coordination of hormonal, transcriptional, and structural defense responses underlying banana root resistance to *Radopholus similis*.

Conclusion

The findings of this study indicate that resistance of banana to *R. similis* is strongly associated with the precise timing and coordinated activation of defense responses in root tissues during the early stages of infection. Genotypes with complete resistance exhibited early repression of *TIFY5A*, faster activation of JA/ET pathways, and coordinated induction of *MYB* and *PER52*, accompanied by early callose deposition, accumulation of phenolic compounds, and cell wall reinforcement. Collectively, these events are associated with the limitation of nematode migration and multiplication within root tissues.

In contrast, genotypes that exhibited late or transient responses exhibited intermediate or reduced levels of pathogen containment, reflecting phenotypes of

moderate resistance or susceptibility. Although the present study does not establish direct causal relationships between specific genes and the observed resistance phenotypes, the results obtained support an integrative conceptual model in which early and coordinated activation of hormonal signaling pathways and structural defense mechanisms emerge as central components of root resistance to *R. similis*.

These findings provide a physiological and molecular basis for elucidating the phenotypic gradients of resistance observed in banana plants and highlight the importance of considering the temporal dynamics of defense responses in the identification and deployment of diploid genotypes in breeding programs.

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Competing interests

None declared.

Author Contributions

Conceptualization: ABPS. Methodology: ABPS, LSL, WDdSO, AdJR, BSLdN, KVMC, LSR, TAR, APdSR and EPA. Original draft preparation: ABPS. Data interpretation: ABPS, CFR and FH. Funding acquisition, supervision, manuscript review and editing: EPA.

Data Availability

The data supporting the findings of this study are provided in the Supporting Information as Excel file (Dataset S1).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Relative gene expression data (RT-qPCR) used for temporal analysis of defense-related genes in banana genotypes following *Radopholus similis* infection (Excel file).

CAPÍTULO 4

Development and Statistical Validation of a Diagrammatic Scale for Assessing Rhizome Necrosis Severity in Banana Caused by *Radopholus similis*

Artigo: Development and statistical validation of a diagrammatic scale for assessing rhizome necrosis severity in banana caused by *Radopholus similis*, submetido à revista científica European Journal of Plant Pathology.

Development and statistical validation of a diagrammatic scale for assessing rhizome necrosis severity in banana caused by *Radopholus similis*

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Abstract

Accurate quantification of rhizome necrosis caused by *Radopholus similis* is essential for resistance studies, phenotyping, epidemiological analyses, and nematode management in banana. However, visual assessment of rhizome necrosis remains poorly standardized and highly subjective. This study aimed to develop and statistically validate a diagrammatic scale to quantify the severity of rhizome necrosis in banana plants infected by *R. similis*. One hundred rhizomes exhibiting a wide range of necrosis levels were selected from greenhouse-grown, inoculated plants. Rhizomes were photographed, digitally processed, and the necrotic area was quantified using ImageJ software. Based on these measurements, a diagrammatic scale with eight severity levels (0, 5.4, 9.6, 20.0, 30.5, 40.1, 56.7, and 83.4%) was constructed. Scale validation was performed by ten inexperienced evaluators who estimated disease severity in two independent assessments conducted with and without the aid of the proposed scale. Accuracy, precision, and agreement were evaluated using linear regression analyses, coefficients of determination (R^2), and Lin's concordance correlation coefficient (CCC). Use of the diagrammatic scale significantly improved evaluator performance, resulting in higher R^2 and CCC values and a substantial reduction in systematic bias. The results demonstrate that the proposed diagrammatic scale is a robust, accurate, and reproducible tool for standardizing the

assessment of banana rhizome necrosis caused by *R. similis*, with direct applicability to resistance screening and banana breeding programs.

Keywords: Accuracy; Banana; Disease severity; Diagrammatic scale; Burrowing nematode

Introduction

Banana is an important staple food crop and a major source of income for smallholder farmers in approximately 150 tropical and subtropical countries (Tripathi et al. 2022). In 2023, global banana exports reached approximately 19.1 million tons (FAO 2024). Despite its socioeconomic relevance, banana production is affected by a wide range of pests and diseases (Santos et al. 2023), among which the burrowing nematode *Radopholus similis* is one of the most destructive. This nematode is widely distributed in banana-growing regions worldwide and causes substantial yield losses (Gowen and Quénéhervé 1990; Sarah 1990; Chabrier and Quénéhervé 2003).

High infestations of *R. similis* cause cracking along the roots, after which the nematodes migrate to the rhizome, leading to tissue decay and the formation of large and dark cavities (Agrios 2005). This process compromises water and nutrient uptake, resulting in reduced sucker growth, lower bunch weight, and plant toppling (Gowen and Quénéhervé 1990; Sarah 1999; Blomme et al. 2004; Lafont et al. 2007).

Given the importance of banana as a staple food and income source, together with the significant impact of nematodes on productivity, greater efforts are required to develop *Musa* genotypes resistant or tolerant to these pathogens (Viaene et al. 2003). Resistant cultivars represent one of the most sustainable management strategies and are equally accessible to subsistence farmers and commercial producers (Wuyts et al. 2007). Several approaches are used to characterize plant resistance, tolerance, or susceptibility to nematodes. In banana, the most common methods include nematode population density and reproduction factor analyses, morphological and molecular characterization, enzymatic activity, gene expression analyses, agronomic traits, and symptom evaluation (Sousa et al. 2024). Symptoms caused by *R. similis* can be assessed using lesion indices, the number of functional roots, and/or rhizome necrosis indices (Sousa et al. 2024). Disease severity is usually estimated visually as the percentage of damaged tissue relative to the total affected area (Duarte et al. 2013; Ruiz et al. 2021).

The use of scales to support disease severity assessment is more common for foliar diseases (Goulart 2018). Despite the importance of *R. similis* in banana production, no quantitatively validated diagrammatic scale is currently available for assessing rhizome necrosis severity in banana, limiting the comparability of results across studies.

According to Sousa et al. (2024), two scales have been described for evaluating rhizome necrosis caused by phytoparasitic nematodes in banana; however, both present limitations, including the absence of visual support. The scale proposed by Pinochet (1988), based on lesion size and number on the rhizome surface, can be impractical for large sample sizes. The scale proposed by Bridge (1988), adapted from root necrosis assessments, may also be limited because its intervals were not derived from actual rhizome measurements.

To improve disease quantification accuracy, several strategies have been proposed, among which diagrammatic scales stand out. These scales consist of illustrated representations of plant organs showing different disease severity levels and should be easy and fast to use under diverse conditions, producing accurate, precise, and reproducible results (Berger 1980; Bergamin Filho and Amorim 1996; Martins et al. 2004).

When developing diagrammatic scales, it is essential to consider upper and lower limits corresponding to the maximum and minimum disease severity observed (Horsfall and Barratt 1945; James 1974; Bergamin Filho and Amorim 1996; Godoy et al. 2006). Furthermore, diagrammatic scales must be validated before being proposed as standard assessment tools and should be corrected if they produce unsatisfactory results (Martins et al. 2004; Godoy et al. 2006).

Given the growing need for studies on *R. similis* control in banana and plantain, together with the lack of standardized methods for disease quantification, the objective of this study was to develop and statistically validate a diagrammatic scale to classify plant responses to *R. similis* infection based on the presence and percentage of rhizome necrosis in banana seedlings grown under greenhouse conditions.

Material and Methods

Development of the Diagrammatic Scale

The diagrammatic scale was developed using 100 rhizomes obtained from a greenhouse experiment involving 340 banana plants, including genotypes from the Prata

(AAB) and Cavendish (AAA) subgroups, as well as somaclones of the cultivar Grand Naine. Sample selection was structured to ensure complete coverage of the observed severity spectrum, encompassing the full range of rhizome necrosis recorded in the original population, thereby ensuring adequate phenotypic representativeness for scale development.

Plants were inoculated approximately 40 days after planting with *R. similis* by applying a suspension containing 1.000 nematodes per plant, following the methodology described by Rocha et al. (2020). Samples were collected 90 days after inoculation (dai). Rhizomes were immediately separated from roots and shoots, washed, and longitudinally sectioned with a knife. All rhizomes were then photographed. Selected images were processed using ImageJ software to determine total and necrotic areas.

Based on these measurements, rhizomes exhibiting the lowest and highest necrosis levels were identified, establishing the lower and upper limits of the scale. Six intermediate severity levels were then defined to represent intervals observed in the original sample set. These levels were selected to reflect lesion severity, tissue integrity, and the typical distribution and shape of lesions for each severity class, following the methodology proposed by Franceschi et al. (2020).

Once disease severity percentages were established, a standard rhizome of known area was illustrated eight times, starting from the minimum severity level and following a logistic increment of disease severity. This process reproduced symptoms observed in rhizomes at 90 dai under greenhouse conditions, resulting in the final diagrammatic scale.

Scale Validation

Scale validation was performed using images of the same 100 rhizomes representing all severity levels. Images were randomly arranged on individual slides and displayed using Microsoft® PowerPoint® 2010 (Microsoft Corporation, Redmond, WA, USA).

Ten evaluators with no prior experience in disease severity assessment were selected to test the robustness and applicability of the scale under non-specialized conditions, simulating real-use scenarios. In the first assessment, evaluators estimated rhizome necrosis severity without the aid of any scale. Seven days later, the same evaluators assessed the same images using the proposed diagrammatic scale.

The number of evaluators (n = 10) followed established methodologies for diagrammatic scale development, as previous studies have successfully employed similar

sample sizes to ensure reliability and repeatability of visual estimates (Braga et al. 2020; Borges Junior et al. 2020; Figueiredo et al. 2022; Pietrobon et al. 2022).

Statistical Analyses

The precision and accuracy of each evaluator's visual estimates were assessed using linear regression analysis, considering actual disease severity as the independent variable and estimated severity as the dependent variable. Precision was evaluated using the coefficient of determination (R^2) and the variance of absolute errors (estimated minus actual severity). Accuracy was assessed using t-tests applied to the intercept (β_0) and slope (β_1) of the regression equations.

Scale reproducibility was evaluated using coefficients of determination from linear regressions between severity estimates of evaluator pairs, as proposed by Nutter Jr. and Schultz (1995) and Nutter Jr. et al. (1993).

In addition to linear regression, Lin's concordance correlation coefficient (CCC) was used to evaluate agreement between estimated and actual severity values (Lin 1989). Five parameters were assessed: Lin's concordance coefficient, scale shift coefficient, location shift coefficient, bias correction factor, and Pearson's correlation coefficient. Confidence intervals (95%) were calculated to test differences ($P < 0.05$) between assessments performed with and without the diagrammatic scale (Figueiredo et al. 2022). All statistical analyses were performed using R software (R Core Development Team 2016) with the packages ggplot2, dplyr, broom, and ggpubr. The epi.ccc function from the epiR package was used to calculate CCC values.

Results

Among the evaluated rhizomes, 97% exhibited severity levels between 0 and 59.99%, whereas only 3% showed necrosis levels above 60% (Fig. 1). This distribution is associated with host defense mechanisms and the presence of plants with different susceptibility levels within the evaluated population. These results are consistent with subsequent analyses related to the reproduction factor (RF) of *R. similis* (Sasser et al. 1987). Furthermore, even in highly susceptible plants, nematode populations were predominantly concentrated in secondary and superficial roots. Consequently, different degrees of rhizome necrosis were observed within the same group of plants. Therefore, the absence of severe rhizome symptoms does not necessarily indicate resistance but rather reflects the dynamics of the plant-nematode interaction at the time of evaluation. For this reason, the present study did not aim to characterize the resistance status of

individual rhizomes; instead, it focused on representing distinct levels of rhizome necrosis to develop a reliable diagrammatic scale.

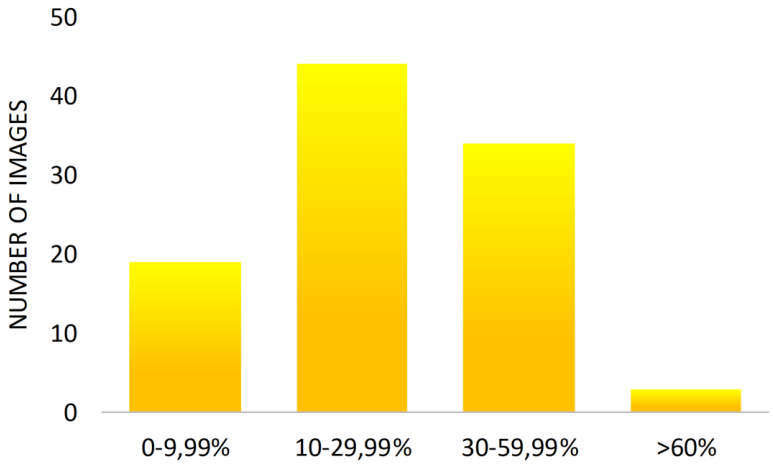

Fig. 1 Distribution of severity levels based on 100 rhizome images used to develop the standard area diagrammatic scale for assessing banana rhizome necrosis caused by *Radopholus similis*

Based on the analysis of the 100 selected rhizomes, the diagrammatic scale developed in this study comprised eight severity levels, with lower and upper limits of 0% (no symptoms) and 83.4% necrotic area, respectively (Fig. 2). Severity values above 83.4% were not included in the scale, as they were not observed under greenhouse conditions.

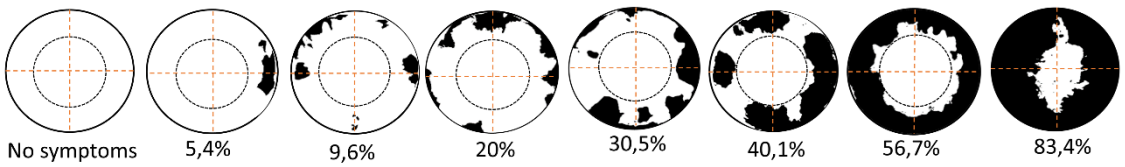

Fig. 2 Diagrammatic scale of banana rhizome necrosis caused by *Radopholus similis* at 90 days after inoculation under greenhouse conditions

To classify plant responses to *R. similis*, severity intervals and corresponding grades were assigned as follows: no symptoms (0); <10% necrosis (grade 1); 10–29.99% necrosis (grade 2); 30–59.99% necrosis (grade 3); and >60% necrosis (grade 4) (Table 1). Grade 0 corresponded to rhizomes without visible necrosis; grade 1 was characterized by small, dark, diffuse necrotic lesions emerging from the rhizome margin; grade 2 consisted of moderate, diffuse dark lesions appearing at multiple marginal points; grade 3 was defined by large, dark necrotic lesions covering most of the rhizome margin and

192 extending toward the central region; and grade 4 was characterized by extensive dark
193 necrosis affecting more than 60% of the rhizome, encompassing the entire marginal
194 region and part of the central tissue.
195
196 **Table 1** Classification scale used to evaluate banana plant responses to
197 *Radopholus similis* based on rhizome necrosis severity.

Rhizome Lesion Index (%)	Grade
No symptoms	0
<10%	1
10% - 29.99%	2
30% - 59.99%	3
>60%	4

198
199 To ensure the applicability of the proposed scale, ten inexperienced evaluators
200 assessed images of the selected rhizomes in two independent sessions, performed with
201 and without the diagrammatic scale and separated by a seven-day interval.
202 Joint analysis of the regression coefficients revealed substantially higher accuracy
203 and precision when the diagrammatic scale was used compared with assessments
204 performed without the scale (Table 2; Fig. 3). Without the scale, several evaluators
205 exhibited intercepts significantly different from zero (evaluators 1, 2, 5, 6, and 10),
206 indicating systematic bias. In addition, slopes significantly different from one were
207 observed for evaluators 1, 5, 6, and 7, indicating distortion in severity estimates as disease
208 severity increased. Although R^2 values without the scale ranged from moderate to high
209 (approximately 0.57–0.78), considerable variability and estimation errors were evident.
210 In contrast, when the diagrammatic scale was used, intercepts for nearly all
211 evaluators did not differ significantly from zero, and slopes did not differ from one,
212 indicating elimination of systematic bias. Coefficients of determination increased
213 substantially for most evaluators; for example, R^2 values increased from 0.7271 to 0.9361
214 for evaluator 1, from 0.7559 to 0.9173 for evaluator 2, and from 0.7510 to 0.8633 for
215 evaluator 10. Even evaluators who showed poor performance without the scale (e.g.,
216 evaluator 4, $R^2 = 0.57$) exhibited marked improvement when using the scale ($R^2 =$
217 0.8180). These results demonstrate that the diagrammatic scale enhanced evaluator
218 accuracy and consistency while effectively eliminating statistically significant bias.

Table 2 Intercept (β_0), slope (β_1), and coefficient of determination (R^2) of linear regression equations relating visual severity estimates performed by evaluators without and with the use of the diagrammatic scale.

Evaluator	Without scale			With scale		
	β_0	β_1	R^2	β_0	β_1	R^2
1	-10.0890**	1.3354**	0.7271	-1.2634^{ns}	1.0472^{ns}	0.9361
2	-6.7782**	1.1251^{ns}	0.7559	-1.7506^{ns}	0.9499^{ns}	0.9173
3	3.2118^{ns}	1.0218^{ns}	0.7525	2.4000*	1.0029^{ns}	0.8813
4	5.3566^{ns}	1.0030^{ns}	0.5706	2.3543^{ns}	0.9416^{ns}	0.8180
5	10.0204**	0.8030**	0.7419	5.7299**	0.9519^{ns}	0.7298
6	-3.4895**	0.7425**	0.7789	0.8796^{ns}	0.8600**	0.8603
7	-0.9204^{ns}	1.3563**	0.7501	-2.5421^{ns}	1.0953^{ns}	0.8261
8	2.5973^{ns}	0.9520^{ns}	0.7769	0.1563^{ns}	0.9428^{ns}	0.8719
9	-6.7133^{ns}	1.2156^{ns}	0.5236	-5.6085*	1.1260^{ns}	0.6217
10	4.4664*	1.0843^{ns}	0.7510	-0.3105^{ns}	1.0274^{ns}	0.8633

** and * significant at the 1% and 5% levels, respectively, according to the t-test. ns, not significant ($\beta_0 = 0$ and $\beta_1 = 1$).

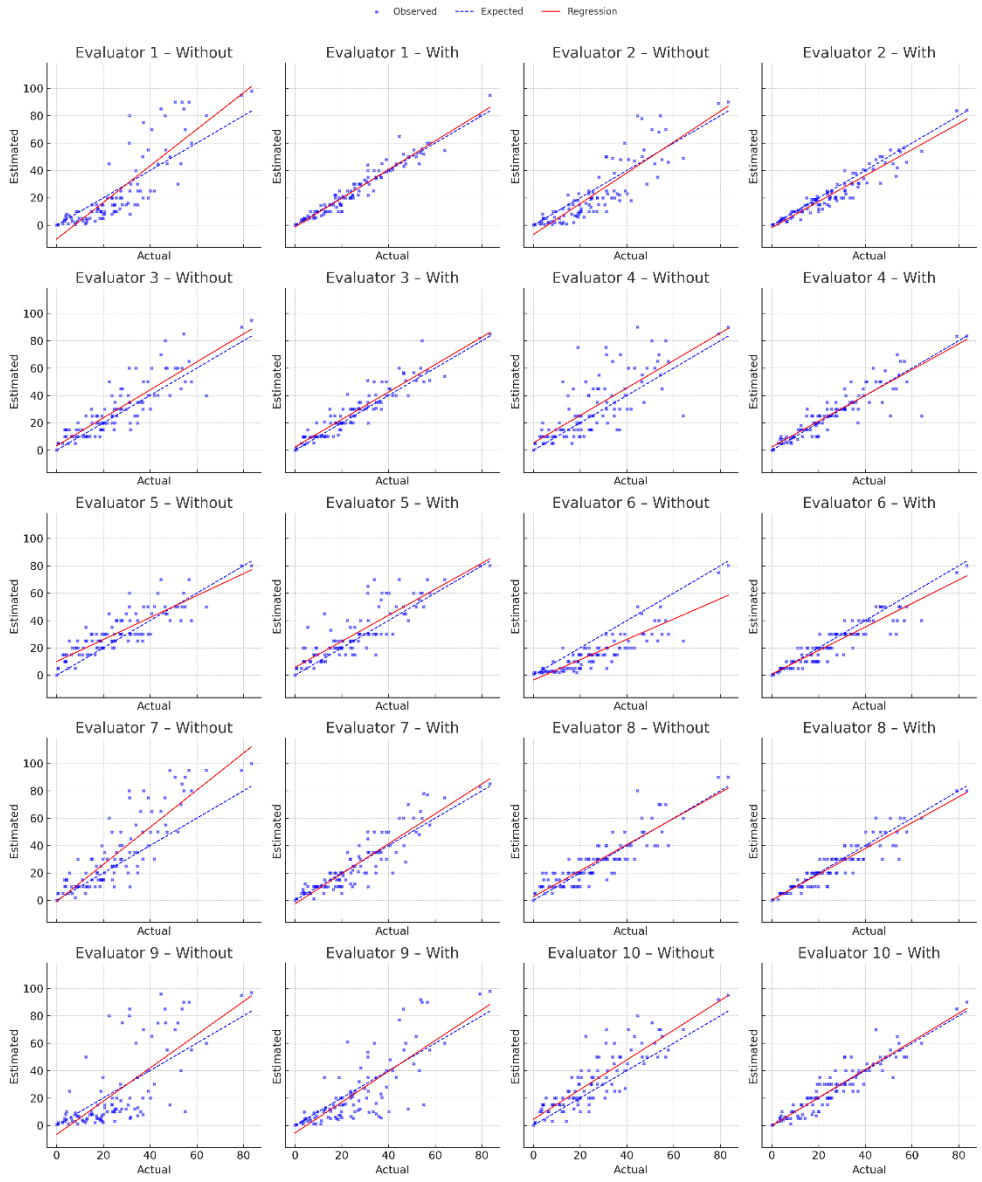

Fig. 3 Linear regression models relating actual and visually estimated values of disease severity caused by *Radopholus similis*, assessed by evaluators without and with the use of the diagrammatic scale

Residual analysis further confirmed the improvement in estimation quality provided by the scale (Fig. 4). Without the scale, residuals were widely dispersed and frequently distant from the zero line, reflecting low precision. Some evaluators showed a systematic tendency to overestimate (positive residuals) or underestimate (negative residuals) disease severity, particularly at intermediate and high severity levels. In contrast, when the scale was used, residuals were more tightly clustered around zero, with reduced dispersion, indicating greater consistency and a marked reduction in estimation error.

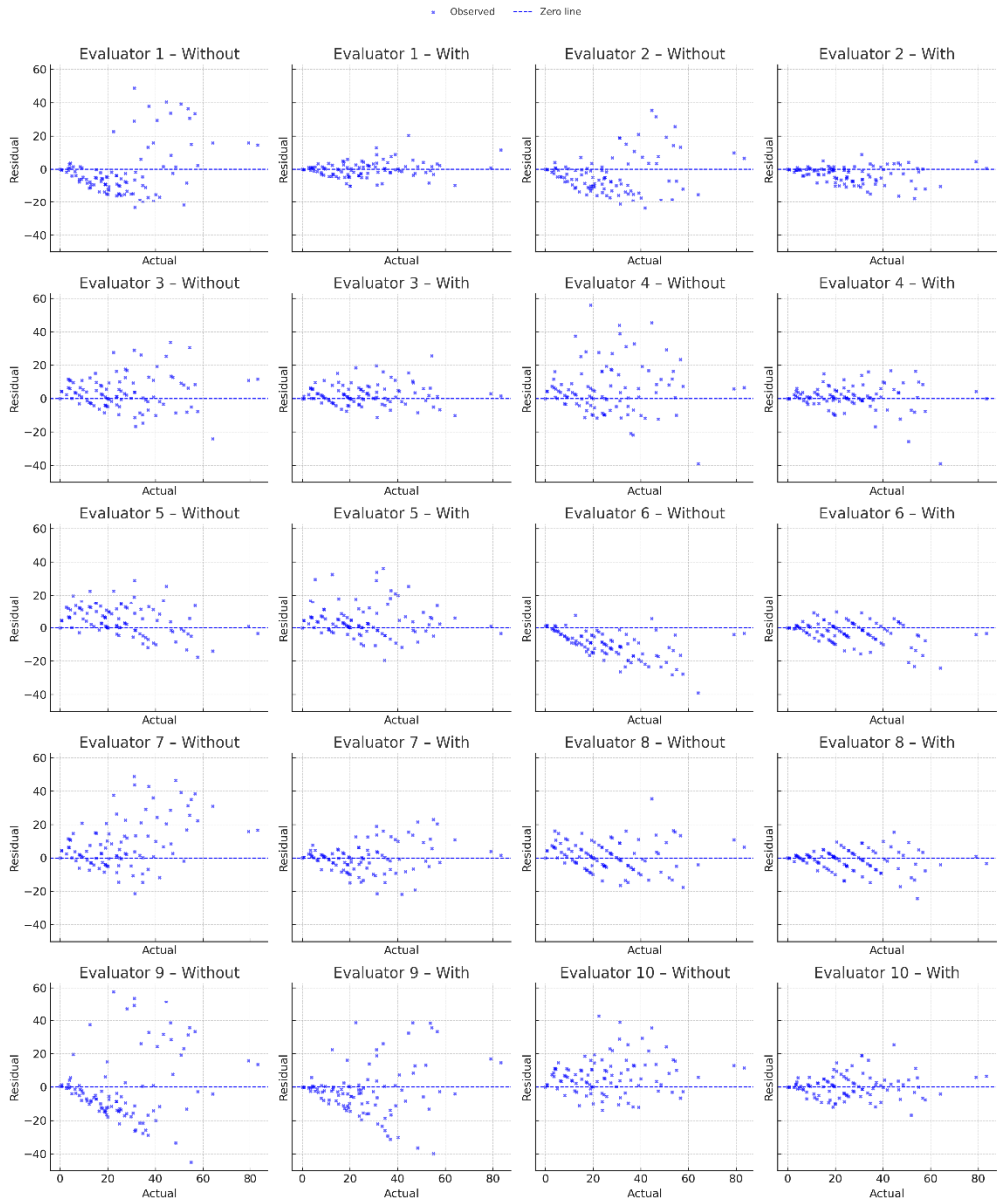

Fig. 4 Residual distribution (estimated severity − actual severity) for disease caused by *Radopholus similis* assessments performed without and with the use of the diagrammatic scale

Analysis of inter-evaluator agreement using coefficients of determination (R^2) revealed substantial heterogeneity when assessments were performed without the scale (Table 3). While some evaluator pairs exhibited relatively high agreement (e.g., evaluators 1 × 2, $R^2 = 0.88$; evaluators 6 × 7, $R^2 = 0.87$), others showed weak agreement (e.g., evaluators 4 × 10, $R^2 = 0.60$), indicating the absence of a common assessment standard. When the diagrammatic scale was applied, R^2 values generally increased and became more consistent, typically ranging from 0.70 to 0.92. Notably, evaluator pairs

with previously low agreement showed marked improvement (e.g., evaluators 4 × 10, R^2 increased from 0.60 to 0.82), demonstrating that the scale harmonized severity estimates among evaluators.

Table 3 Coefficients of determination (R^2) from linear regression analyses between evaluator pairs for assessments of necrosis caused by *Radopholus similis* performed without and with the diagrammatic scale.

Evaluators	2	3	4	5	6	7	8	9	10
					Without scale				
1	0,88	0,77	0,64	0,75	0,76	0,87	0,83	0,74	0,75
2		0,85	0,68	0,79	0,85	0,76	0,87	0,77	0,77
3			0,70	0,78	0,85	0,74	0,87	0,75	0,72
4				0,71	0,63	0,62	0,65	0,67	0,60
5					0,78	0,71	0,82	0,71	0,79
6						0,70	0,87	0,69	0,71
7							0,78	0,61	0,73
8								0,70	0,74
9									0,65
					With scale				
1	0,92	0,91	0,81	0,78	0,87	0,85	0,88	0,67	0,88
2		0,85	0,78	0,73	0,84	0,81	0,84	0,63	0,85
3			0,82	0,79	0,84	0,84	0,80	0,73	0,88
4				0,69	0,81	0,79	0,79	0,58	0,82
5					0,68	0,80	0,74	0,62	0,79
6						0,73	0,79	0,61	0,86
7							0,83	0,60	0,83
8								0,55	0,85
9									0,65

These patterns were clearly illustrated in the heatmap representation of inter-evaluator agreement (Fig. 5). Without the scale, the heatmap displayed pronounced heterogeneity, with wide variation in agreement levels across evaluator pairs. With the use of the diagrammatic scale, the heatmap became more homogeneous and darker,

reflecting consistently higher R^2 values. Several evaluator pairs achieved agreement values ≥0.80, including pairs that previously exhibited poor concordance.

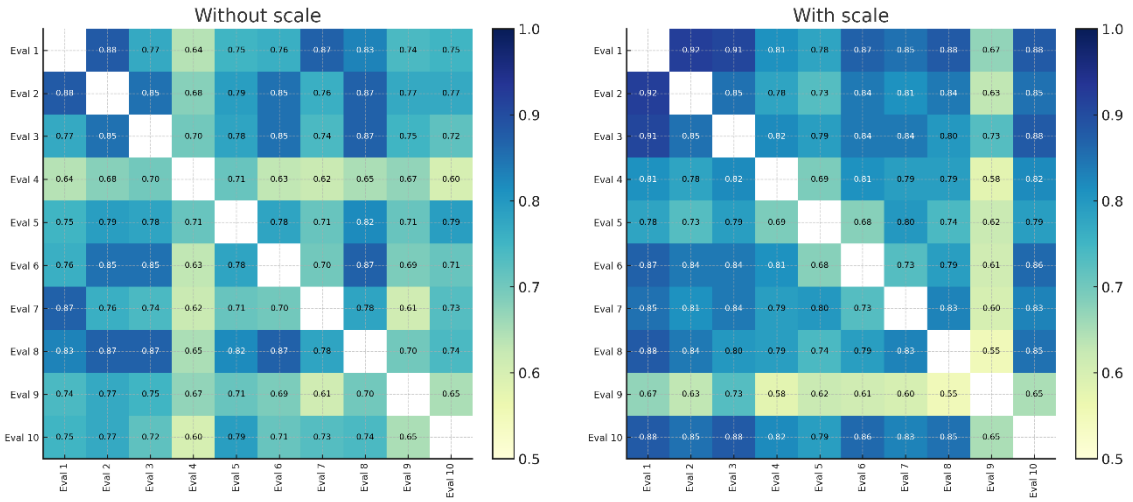

Fig. 5 Heatmap of coefficients of determination (R^2) between evaluator pairs for assessments of necrosis caused by *Radopholus similis* performed without and with the diagrammatic scale

Lin's concordance correlation coefficient (CCC) analysis further supported the effectiveness of the proposed scale (Table 4). Without the scale, the CCC indicated moderate agreement, with a scale shift coefficient of 0.7489 and a negative location shift (–0.0731), reflecting systematic underestimation. When the scale was used, the CCC increased substantially, the scale shift coefficient rose to 0.8984, and the location shift approached zero (0.0078), indicating near elimination of systematic bias. The bias correction factor (Cb) approached unity, and Pearson's correlation coefficient increased, confirming improvements in both accuracy and precision.

Table 4 Comparison of visual severity estimates performed by ten evaluators without and with the use of the diagrammatic scale based on Lin's concordance statistics.

Statistics	**Without scale**	**95% confidence interval**	**With scale**	**95% confidence interval**
Coefficient of agreement[a]	0.7626	[0.730; 0.788]	0.8883	[0.869; 0.905]
Scale deviation[b]	0.7489	[0.717; 0.782]	0.8984	[0.870; 0.927]
Location deviation[c]	-0.0731	[-0.116; -0.028]	0.0078	[-0.021; 0.036]
Bias correction factor[d]	0.9571	[0.943; 0.969]	0.9943	[0.990; 0.997]

Correlation coefficient[e]	0.7967	[0.769; 0.821]	0.8935	[0.875; 0.910]

[a] Lin's concordance correlation coefficient: combines measures of precision and accuracy to assess the degree of agreement with the actual values.

[b] Scale shift coefficient: coefficient relative to perfect agreement (1 = perfect agreement between x and y).

[c] Location shift coefficient: coefficient relative to perfect agreement (0 = perfect agreement between x and y).

[d] Bias correction factor: measures the extent to which the fitted regression line deviates from the 45° line. No bias occurs when Cb = 1. It is a measure of accuracy calculated from the scale and location shift coefficients.

[e] Pearson's correlation coefficient: measures precision (the strength of the linear relationship).

Graphical representation of Lin's method further illustrated these improvements (Fig. 6). Without the scale, estimated severity values were widely dispersed around the identity line, and Lin's regression line deviated substantially from the 45° line, indicating bias and low agreement. In contrast, when the scale was used, estimates clustered closely around the identity line, and Lin's regression line closely overlapped the line of perfect concordance, demonstrating high accuracy and precision.

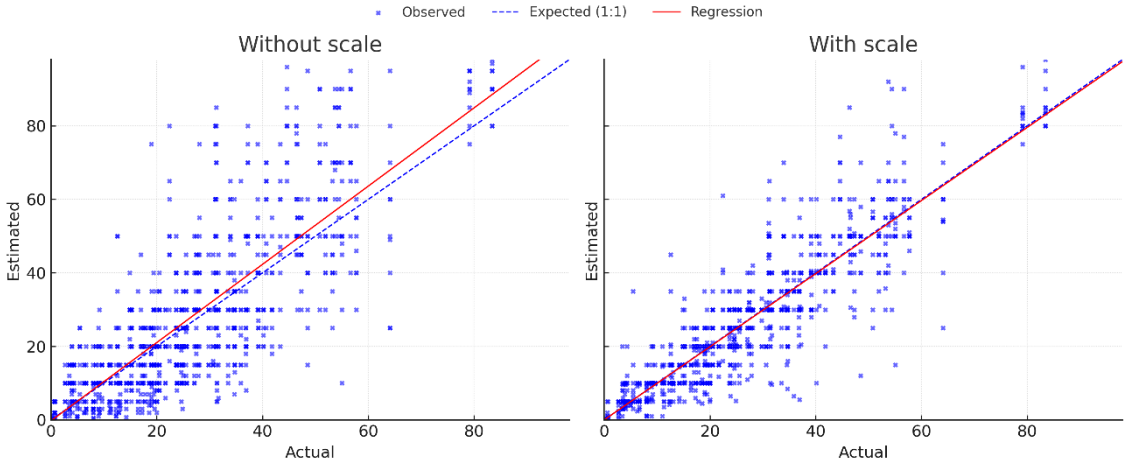

Fig. 6 Lin's concordance analysis between actual and estimated severity values caused by *Radopholus similis* for assessments conducted without and with the diagrammatic scale. Each point represents one of 100 estimates performed by each evaluator

Finally, residual plots derived from Lin's method confirmed these findings (Fig. 7). Without the scale, residuals were large and inconsistent, indicating low agreement between estimated and actual severity values. With the diagrammatic scale, residuals were more homogeneous and closer to zero, confirming that the scale effectively standardized disease severity assessments and improved their reliability.

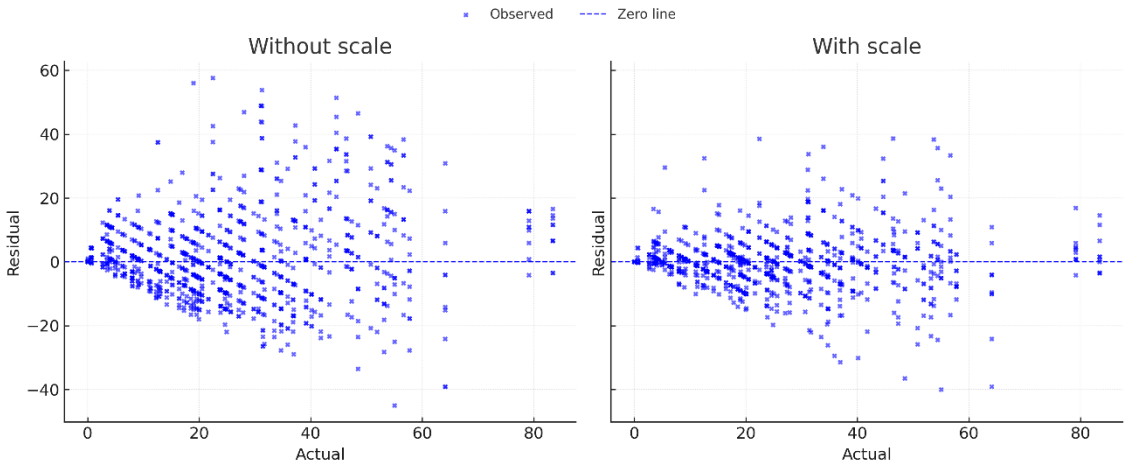

Fig. 7 Residual plots derived from Lin's concordance method for assessments performed without and with the diagrammatic scale. Points represent 100 estimates per evaluator

Discussion

The diagrammatic scale proposed in this study did not include severity values above 83.4%, as such levels were not observed under greenhouse conditions. This outcome is directly related to host behavior at the evaluation time. As nematode populations increase, secondary symptoms associated with root necrosis emerge (Agrios 2005; Gebremichael 2015), facilitating nematode migration toward the rhizome and leading to tissue decay and the formation of extensive dark cavities (Agrios 2005). A genotype is considered susceptible when it allows greater nematode reproduction and exhibits more extensive lesions in roots or rhizomes following infection (Stoffelen et al. 2000).

In this study, we developed and validated the first diagrammatic scale specifically designed to assess *R. similis* severity in banana rhizomes. Lesion areas were objectively quantified using digital image analysis in 100 rhizomes, enabling the construction of a diagrammatic scale based on real severity values. This approach is fundamental for disease assessment, as it reduces the subjectivity inherent to visual estimations (Martins

et al. 2004; Lopes et al. 2022). Consequently, evaluators produced estimates closer to actual values, achieving reliable results even under varying conditions (Nutter Jr.? et al. 1991; Nutter Jr.? and Schultz 1995; Lopes et al. 2022).

Visual estimators are naturally prone to overestimating disease severity in the absence of diagrammatic references, often relying on lesion size and number as primary cues (Capucho et al. 2011; Lopes et al. 2022). In this context, evaluator training has been shown to be an effective strategy for reducing bias and improving accuracy (Teramoto et al. 2011; Sousa et al. 2014). These findings reinforce the concept that assessment quality depends not only on the evaluation tool but also on individual factors such as training, experience, and visual perception (Amorim and Bergamin Filho 2018; Figueiredo et al. 2022). Indeed, targeted training can significantly enhance estimation quality (Michereff et al. 2000). Conversely, lesion morphology complexity, psychological responses to visual stimuli, fatigue, and emotional state may negatively affect assessment precision (Kranz 1988; Sherwood et al. 1983; Nutter Jr. et al. 2006; Sachs et al. 2011; Sousa et al. 2014).

Inaccurate visual estimates may compromise experimental conclusions (Parker et al. 1995). Therefore, the establishment of a standardized system for quantifying banana rhizome necrosis represents a valuable contribution to studies on *R. similis* severity. In the present study, disease severity assessments performed with the proposed scale showed high precision among evaluators. Estimated severity values closely matched actual measurements, confirming the scale's accuracy. In this context, accuracy is defined as the proximity between observed and estimated values and can be evaluated through regression intercept (β_0) and slope (β_1). Deviations from zero (β_0) and one (β_1) indicate constant or systematic errors in estimation (Belasque Jr. et al. 2005; Sussel et al. 2009; Lenz et al. 2010; Sousa et al. 2014). Use of the proposed scale resulted in greater homogeneity and closer agreement with actual values, confirming its effectiveness in standardizing and validating visual severity assessments.

Accordingly, the results demonstrate that the diagrammatic scale significantly reduced overestimation errors, particularly among inexperienced evaluators (Sousa et al. 2014). For this reason, similar studies proposing diagrammatic scales have been increasingly reported for different crops and pathosystems. For example, the diagrammatic scale developed by de Andrade et al. (2024) represents a significant advance in assessing soybean seed coating quality by standardizing a process traditionally subject to evaluator subjectivity. That scale was constructed using artificial intelligence

tools to objectively quantify coating coverage, enabling reliable visual classification into quality categories.

Likewise, Figueiredo et al. (2022) established a diagrammatic scale for whole leaves of *Coffea arabica* with seven severity levels, which demonstrated greater precision, accuracy, and reproducibility after validation with ten evaluators, making it suitable for both field and laboratory assessments. Rojas-Chacón et al. (2024) adapted this approach to leaf discs of mutagenized plants, developing a specific scale (0–52.15%) capable of discriminating genotypes with different resistance levels and providing practical benefits for breeding programs. Similarly, Todd et al. (2025) proposed a standardized diagrammatic scale for assessing Fusarium yellows severity in sugar beet, which outperformed traditional ordinal scales and showed high precision and accuracy (Cb = 0.99) when tested by inexperienced evaluators.

Unlike ordinal classification systems, the diagrammatic scale developed in the present study allows precise determination of the percentage of affected area using real reference values (Stonehouse 1994; Godoy et al. 1997; Leite and Amorim 2002; Belasque Jr. et al. 2005; Michereff et al. 2006; Spolti et al. 2011; Yadav et al. 2013). This feature enhances symptom quantification and makes the proposed scale particularly relevant for banana breeding programs worldwide. Moreover, the results confirm its applicability in future research conducted under greenhouse conditions.

Finally, although lesion classification scales are an important approach for symptom evaluation in phytoparasitic nematode studies, they should not be used in isolation. Complementary parameters, such as nematode population density, percentage of dead roots, and root necrosis indices, must also be considered. Integration of these indicators provides a more reliable assessment of genotype resistance or susceptibility (Sousa et al. 2024).

Conclusion

This study presents the first quantitatively defined and statistically validated diagrammatic scale for assessing rhizome necrosis severity in banana caused by *R. similis*. The developed scale demonstrated high accuracy, precision, and reproducibility, effectively reducing systematic bias and variability associated with visual assessments, even when applied by inexperienced evaluators. The use of real severity values obtained through digital image analysis enabled the construction of a robust and representative tool, fully aligned with methodological principles recommended for diagrammatic scale

development. Statistical validation confirmed its applicability as a standardized method for quantifying banana rhizome necrosis under greenhouse conditions. The proposed scale represents a practical and reliable tool for resistance studies, phenotyping, and banana breeding programs, contributing to the standardization of disease assessments and improving comparability among experiments. When used in combination with other phytopathological and nematological parameters, this scale can strengthen the characterization of *Musa* genotype responses to *R. similis* infection.

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Author Contributions Conceptualization: ABPS; methodology: ABPS, AdJR, BSLdN, LSL, KVMC, LSR, and EPA; original draft preparation: ABPS; data interpretation: ABPS and CAdSL; funding acquisition, supervision, manuscript review and editing: EPA.

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Declarations

Conflict of Interest The authors declare no conflict of interest.

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CONSIDERAÇÕES FINAIS

À luz dos resultados obtidos, fica evidente que ainda há um caminho longo a ser explorado no patossistema *Musa* spp. × *Radopholus similis*. A resistência genética se confirma como a estratégia mais sustentável e eficiente de manejo, reforçando a necessidade de continuidade em programas de melhoramento que integrem avaliação fenotípica cuidadosa, análises histoquímicas e ferramentas moleculares.

A variabilidade observada entre os subgrupos genômicos, especialmente no subgrupo Prata, demonstra que a diversidade genética de *Musa* spp. é um recurso valioso e ainda pouco explorado. Investigações com populações segregantes, avaliações em diferentes ambientes e estudos multilocacionais serão essenciais para confirmar a estabilidade da resistência e selecionar materiais com desempenho consistente em campo.

Do ponto de vista biológico, a evidência de uma resposta coordenada envolvendo sinalização hormonal e reforço da parede celular amplia a compreensão dos mecanismos de defesa contra *R. similis*. Esse avanço abre espaço para estudos proteômicos, metabolômicos e transcriptômicos, permitindo identificar genes, proteínas e metabólitos associados à limitação dos danos causados pelo nematoide. A integração dessas abordagens pode revelar redes regulatórias envolvidas na resposta de defesa e acelerar a identificação de alvos estratégicos.

A partir dessas descobertas, será possível desenvolver e validar marcadores moleculares mais robustos, tornando a seleção assistida mais precisa e eficiente. Além disso, o conhecimento gerado sobre genes e vias regulatórias poderá fundamentar, no futuro, estratégias de edição gênica por meio do sistema CRISPR/Cas, sempre acompanhadas de validação funcional e avaliação agronômica rigorosa.

Por fim, a escala diagramática validada neste estudo representa uma contribuição prática importante, ao padronizar as avaliações de severidade e aumentar a confiabilidade e comparabilidade dos dados. Em conjunto, os resultados desta tese não apenas ampliam o entendimento sobre a interação entre *Musa* spp. e *R. similis*, mas também oferecem bases sólidas para o avanço do melhoramento genético, com foco no desenvolvimento de cultivares mais resistentes, estáveis e produtivas.