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A CaMK/CaMKL-targeted TaqMan qPCR assay for sensitive detection of Rhizoctonia theobromae in cassava tissue

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Research Article

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A CaMK/CaMKL-targeted TaqMan qPCR assay for sensitive detection of *Rhizoctonia theobromae* in cassava tissue

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Abstract

Reliable detection of *Rhizoctonia (Ceratobasidium) theobromae* in cassava leaves is essential for managing cassava witches' broom disease (CWBD). However, low pathogen loads and heterogeneous colonization often result in false negatives in conventional PCR assays. In this study, we developed a species-specific TaqMan real-time PCR (qPCR) assay targeting the CaMK/CaMKL locus of *R. theobromae*. The assay was validated using field samples collected from indigenous communities in Oiapoque, Amapá, Brazil. It showed high amplification efficiency (90–110%; $R^2 > 0.99$) and increased detection by 40.5% compared to conventional PCR, enabling reliable detection in leaf tissues previously classified as negative. No amplification was observed in healthy or non-CWBD samples, confirming assay specificity. This method provides a sensitive and reliable tool for pathogen detection, supporting disease surveillance and germplasm indexing.

Keywords: cassava witches' broom disease; *Rhizoctonia theobromae*; qPCR; TaqMan assay; molecular diagnostics; disease surveillance

Introduction

Cassava (*Manihot esculenta* Crantz) is one of the main food crops in the tropics, playing a central role in food security and in the economy of millions of smallholder farmers, particularly in Africa, Asia, and Latin America (Amelework et al. 2022; Scaria et al. 2024; Landicho et al. 2025). Brazil is among the world's leading cassava producers, where the crop represents an important source of carbohydrates for human and animal consumption and is widely used as a raw material in various industrial sectors (Mohidin et al. 2023; Embrapa 2024; FAOSTAT 2025).

Cassava productivity is affected by several diseases, including root rot caused by fungi and oomycetes in Brazil (Hohenfeld et al. 2024). Recently, a witches' broom disease (CWBD) has emerged in South America with severe impact on cassava production (Leiva et al. 2023; Pardo et al. 2024). The disease is characterized by leaf yellowing, proliferation of lateral shoots forming a "broom" appearance, dwarfing, leaf deformation, and vascular necrosis, leading to dieback and reduced root productivity (Leiva et al. 2023). In highly affected areas, substantial yield losses and even total crop failure have been reported.

In Southeast Asia, the fungus *Rhizoctonia theobromae* has been identified as the main pathogen associated with CWBD (Leiva et al. 2023). The genome of the pathogen was sequenced by Gil-Ordóñez et al. (2024), and its presence in cassava tissues was confirmed using RNAscope®. These authors reported irregular

colonization of vascular and epidermal tissues, which may explain the inconsistent detection of the pathogen in conventional diagnostic assays. However, despite molecular evidence, attempts to induce basidiophore formation have been unsuccessful, preventing pathogenicity testing (Pardo et al. 2024).

In French Guiana, *R. theobromae* was detected in symptomatic plants using PCR targeting the *CaMK* gene. In Brazil, high-throughput sequencing and targeted PCR assays confirmed the presence of the pathogen in cassava plants with CWBD in Amapá (Oliveira et al. 2025). Large-scale testing revealed limitations in diagnostic reproducibility, mainly due to low pathogen DNA concentration and heterogeneous distribution in plant tissues, resulting in frequent false negatives. This limitation compromises reliable detection, which is essential for disease surveillance and germplasm indexing (Gil-Ordóñez et al. 2024).

Symptoms of CWBD were first observed in Brazil in 2023 and rapidly escalated into a phytosanitary crisis, particularly in Oiapoque, Amapá, where the disease threatens indigenous farming systems. Studies have documented disease severity and its impact on cassava productivity, highlighting the need for effective diagnostic tools and management strategies (Oliveira et al. 2025).

Although a conventional PCR assay has been described (Leiva et al. 2023), more sensitive detection methods are required, especially for leaf tissues and early infection stages. In this study, we developed and validated a species-specific TaqMan real-time PCR (qPCR) assay targeting the *CaMK/CaMKL* locus of *R. theobromae* to improve detection reliability. The assay enables sensitive detection in leaf tissues and supports applications in disease surveillance and germplasm indexing.

Materials and Methods

Sample collection and DNA extraction

Cassava (*Manihot esculenta*) plants showing CWBD symptoms, including witches' broom (rosette-like structures), leaf blight, and chlorosis, were collected in the municipality of Oiapoque (Amapá, Brazil). Samples were classified by tissue type (stem, petiole, and leaf) and by the presence or absence of visible symptoms (Figure 1). A total of 100 samples were analyzed.



Figure 1. 'Witches' broom disease symptoms in cassava caused by *Rhizoctonia theobromae.* (A) Abnormal shoot growth, characterized by reduced internode length and reduced leaf proliferation. (B) White mycelial growth on the stem surface of the infected plant. (C) Stem cross-section showing internal necrosis and darkening of the vascular tissue. (D) Infection progression with necrosis and mycelium colonization in the plant.

Additional samples were collected from cassava fields in Bahia, Ceará, and Paraná, including symptomatic plants (frogskin, oversprouting, common mosaic, and vein mosaic) and asymptomatic plants (Figure 2).

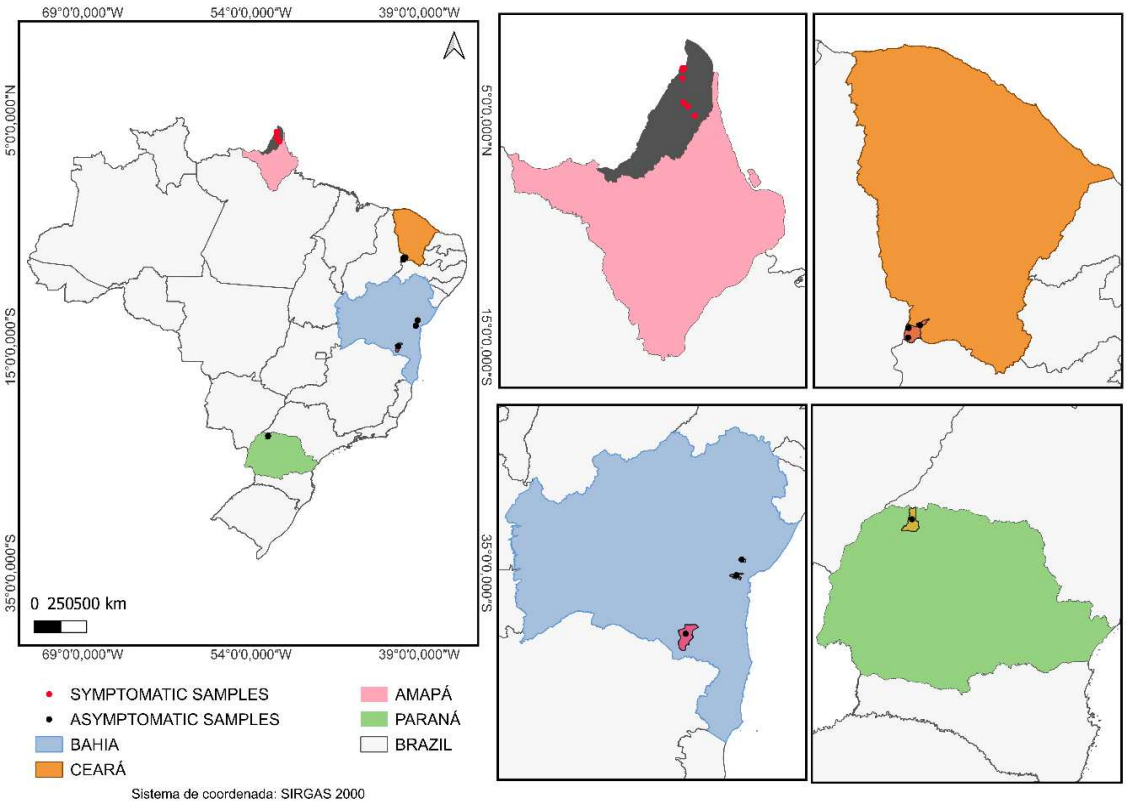


Figure 2. Collection sites in villages in Oiapoque, Amapá and cassava fields in Bahia, Ceará, and Paraná.

For DNA extraction, approximately 150 mg of tissue (stem, petiole, or leaf) was processed using the NucleoSpin® Plant II kit (Macherey-Nagel GmbH & Co. KG, Germany) following the manufacturer's protocol. DNA was eluted in 100 µL of elution buffer, verified by 1% agarose gel electrophoresis, quantified by fluorometry, and stored at −20 °C until use.

Primer design and selection

For qPCR assay development, Ca^{2+}/calmodulin-dependent protein kinase (CaMK/CaMKL) gene sequences described by Oliveira et al. (2025) were used as templates. *Rhizoctonia theobromae* sequences are available at NCBI under accession PRJEB89551 (Table S1). Consensus sequences from Brazilian isolates obtained by Illumina and Nanopore sequencing were aligned with reference genomes (GenBank accessions GCA_037974915.1, GCA_009078325.1, GCA_012932095.1). Primers and TaqMan probes were designed using Primer3 implemented in Geneious Prime (Build 2025, Dotmatics).

Two primer–probe sets were selected based on low sequence variability and amplicon length (<200 bp). Sequences and positions are shown in Table 1, and their location in the CaMK/CaMKL locus is illustrated in Figure 3.

Table 1. Primers and probes designed for the detection of *Rhizoctonia theobromae* (CaMK / CaMKL locus).

Set	Name	Sequence (5′–3′)	Approximate position	Type
1	CAMK/CAMKL-170F	GGAGAACGTTGAGCCCATCA	170–189	Advance
1	CAMK/CAMKL-296P	FAM- TGCCGTCGAGGTCTTGAAA -BHQ	296–315	Probe
1	CAMK/CAMKL-365R	TTGCTTGAGGACGGGTGTAC	346–365	Reverse
2	CAMK/CAMKL-711F	TATTTGGTGGCTCGCCTGA	711–730	Advance
2	CAMK/CAMKL-779P	FAM- CGAAAATGCGCTCCAAAGG -BHQ	760–779	Probe
2	CAMK/CAMKL-847R	ACCGGAGAAAGAGGACGAGA	828–847	Reverse

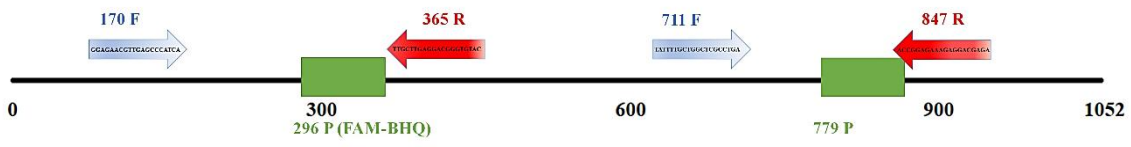


Figure 3. Schematic representation of the CaMK/CaMKL locus showing the position of the two sets of primers and Taqman probe designed for the detection of *R. theobromae*, being the first set beginning in the position of 170 (forward), 269 (probe) and 365 (reverse) and the second set beginning in the position 711 (forward), 779 (probe) and 847 (reverse).

Conventional PCR assays

Fungal DNA was detected by conventional PCR using the primers CWBD-CIAT-F2 (GGATGAGTTTAATCGCTCTAAC) and CWBD-CIAT-R2 (GCGCTCTGGTGTTTCAAGTTTG) (Leiva et al. 2023). Reactions were performed in a final volume of 25 µL containing 2.5 µL of 10× PCR buffer, 0.5 µL of each primer (10 µM), 1.87 µL of $MgCl_2$ (50 mM), 0.5 µL of GoTaq DNA polymerase (1 U; Promega), 0.5 µL of dNTPs (10 mM), 2.0 µL of template DNA (~30 ng μL^{-1}), and nuclease-free water to volume. Amplifications were carried out in a Veriti™ thermocycler (Applied Biosystems). The cycling conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 10 min.

PCR products were analyzed by electrophoresis in 1.5% agarose gels. Selected amplicons were sequenced in both directions using the same primers (ACTGene, Brazil). Sequences were quality-checked, assembled into consensus sequences, and compared with reference sequences from GenBank using BLASTn.

Real-time PCR assays

Real-time PCR (qPCR) was performed using the primer–probe sets described in Table 1 on a QuantStudio™ 5 Real-Time PCR system (Applied Biosystems). Reactions (12 µL) contained 6.25 µL of TaqMan™ Universal PCR Master Mix (1×), 0.5 µL of each primer (10 µM), 0.5 µL of probe (10 µM), 2.0 µL of template DNA (~50 ng), and nuclease-free water to volume.

The amplification program consisted of an initial denaturation at 95 °C for 2 min, followed by 50 cycles of 95 °C for 15 s and 60 °C for 30 s. Fluorescence was recorded at the annealing/extension step. Non-template controls (NTCs) and DNA from healthy plants were included in each run.

Primer validation

To validate the designed primers (Table 1), genomic DNA was extracted from cassava samples collected in Oiapoque (Amapá, Brazil) (Figure 2) showing CWBD symptoms in stems, petioles, and leaves. DNA extraction

followed Doyle and Doyle (1990), with modifications described by Ferreira et al. (2019). DNA was eluted in 100 µL of TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA) and assessed by 1% agarose gel electrophoresis.

Samples analyzed by conventional PCR were also evaluated by qPCR to enable comparison between methods. qPCR assays were performed in triplicate for each sample. Detection efficiency was expressed as the percentage of positive samples relative to the total analyzed.

Mapping the incidence and spatial representation of data.

The spatial distribution of *R. theobromae* in sampled villages in the Oiapoque region was mapped using QGIS Desktop (version 3.44.3). Georeferenced sampling points and municipal boundaries were used as the base layers. For each location, pathogen incidence was calculated as the proportion of positive samples relative to the total analyzed, considering leaf and stem tissues separately and results obtained by conventional PCR and qPCR.

Sensitivity and analytical efficiency

Serial dilutions of total DNA (800 to 0.00008 ng per reaction) were tested in triplicate. Mean *Cq* values were plotted against $\log_{10}$ DNA concentration to generate standard curves. The amplification efficiency (*E*) was determined from the slope of the linear regression line using the following equation:

$$E = \left(10^{(-1/slope)} - 1\right) \times 100\backslash\%$$

The limit of detection (LoD_{95}) was defined as the lowest concentration detected in ≥95% of replicates. The Cq cutoff was based on negative controls and the lowest consistently amplified standard curve point. Samples below this threshold were considered positive.

Estimation of the pathogen's DNA fraction

The relative proportion of fungal DNA was estimated by qPCR using the CaMK/CaMKL primer set for *R. theobromae* and a single-copy cassava nuclear gene as host reference. Reactions were performed in duplicate. The ratio between fungal and host genome copies was calculated as:

$$Copies\ ratio = \frac{E_f^{-Cq_{fungus}}}{E_h^{-Cq_{host}}}$$

where E_f and E_h are the amplification efficiencies for fungal and host targets, respectively.

Assuming similar efficiencies (E ≈ 2) the fungal DNA fraction was calculated as:

$$Mass\ fraction\ (fungus) = \frac{Copies\ ratio \times G_f}{Copies\ ratio \times G_f + G_h}$$

where $G_f$and $G_h$are the haploid genome sizes of *R. theobromae* (~45 Mb) and *M. esculenta* (~750 Mb), respectively.

Fungal DNA mass per reaction was calculated as:

$$m_{fungus} = m_{total} \times Mass\ fraction\ (fungus)$$

Where m_{total} is the total DNA per reaction.

Genome copy number was estimated from the haploid genome size of *R. theobromae*:

$$M_f\ (pg) \approx \frac{G_f\ (Mbp)}{978}$$

$$Copies = \frac{978\,000 \times m_{fungus}\ (ng)}{G_f\ (Mbp)}$$

Normalized efficiency by pathogens and LoD

Standard curves were recalculated by plotting Cq values against fungal DNA mass (m_{fungus}) or genome copy number (copies). Amplification efficiency and R^2were re-estimated from the new regression slopes.

Detection rates were recalculated based on fungal DNA input rather than total DNA. LoD_{95} was defined as the lowest fungal DNA level detected in ≥95% of replicates.

Results

CaMK/CaMKL primer performance in PCR and qPCR

The primer pair described by Leiva et al. (2023) was validated using samples previously confirmed as positive for *R. theobromae*. Amplification was consistently obtained from symptomatic stem and petiole samples, whereas conventional PCR failed to detect the pathogen in leaf tissues. Among 100 symptomatic samples tested by qPCR, 36 were positive, with no amplification observed in asymptomatic plants or plants infected by other agents (n = 100). Sanger sequencing of amplicons showed >95% identity with *R. theobromae* (CaMK/CaMKL locus), confirming specificity. No false positives were observed with the newly designed primer sets (Figure 4). Based on these results, the CaMK/CAMKL-170F/365R set and the CaMK/CAMKL-296P probe were selected for qPCR development (Figure 3).

In a subset of 38 samples, qPCR detected all samples previously positive by conventional PCR and identified 13 additional positives in leaf tissues. No amplification was observed in healthy or non-CWBD samples, indicating higher sensitivity of qPCR for pathogen detection in leaves (Figure 4).

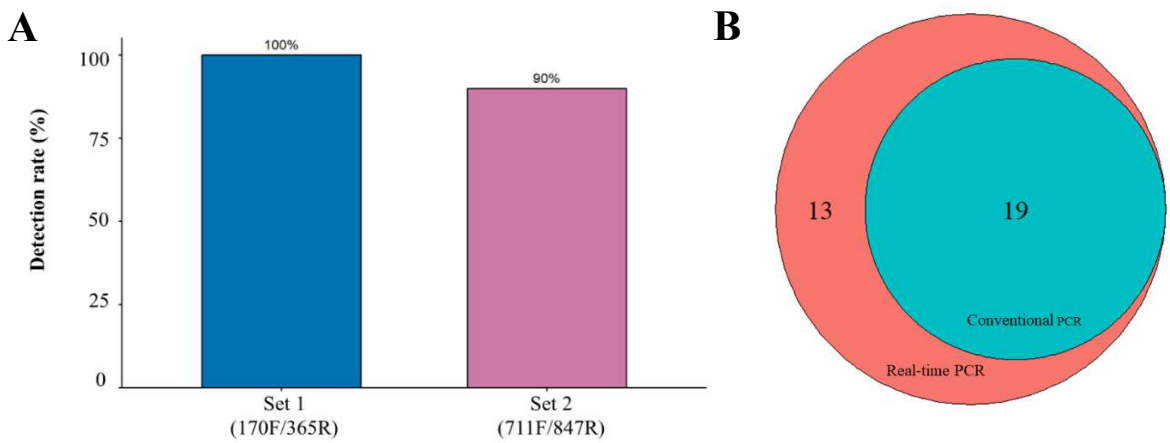


Figure 4. Performance of primer set 1 (CaMK/CaMKL-170F/365R) and primer set 2 (CaMK/CaMKL-711F/847R) in conventional PCR. **(A)** Bar graph comparing detection rates; error bars represent 95% binomial confidence intervals. **(B)** Venn diagram comparing the detection of *Rhizoctonia theobromae* in infected cassava tissues using conventional versus real-time PCR.

Primer validation

Primer performance was further evaluated using serial dilution assays. Specific amplification was confirmed by agarose gel electrophoresis (Supplementary Figure S1). qPCR detected low amounts of fungal DNA, with reliable amplification at 0.008 ng in leaf samples and 0.003 ng in stem samples (Figure 5A). Standard curves showed high linearity ($R^2 > 0.99$) and amplification efficiency between 90–110% for both tissues (Figure 5B–C), indicating robust assay performance.

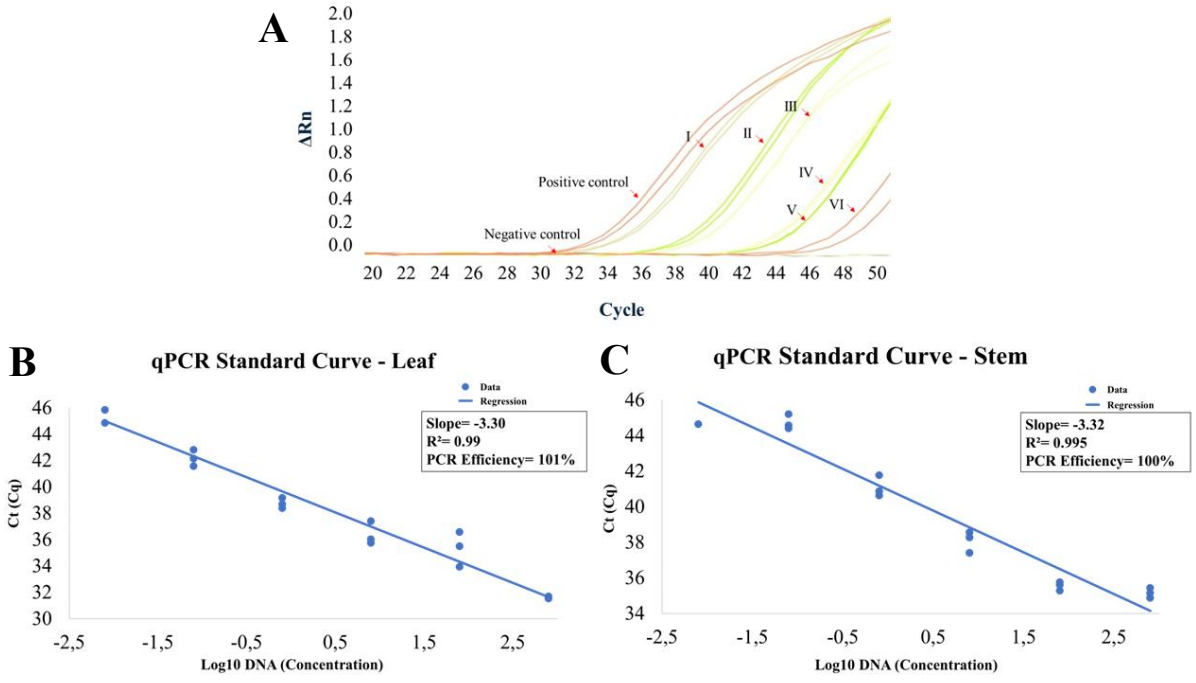


Figure 5. Validation of *CaMK/CaMKL* primers for the detection of *R. theobromae* in cassava samples using serial dilution of total DNA. **(A)** qPCR detection curves (amplification plots) for leaf and stem samples, roman numerals indicate serial dilutions: I = 800 ng, II = 80 ng, III = 0.8 ng, IV = 0.08 ng, V = 0.008 ng, and VI = 0.0008 ng (10-fold serial dilution); **(B)** linear regression and standard curve for leaf samples; **(C)** linear regression and standard curve for stem samples.

To account for host DNA interference, a pathogen-normalized analysis was performed using fungal DNA estimates derived from paired host and pathogen assays. The β-tubulin gene was used as a plant endogenous control.

Standard curves generated for the β-tubulin gene showed consistent amplification in leaf and stem samples (Supplementary Figure S2A–B), indicating DNA quality and absence of inhibition. ΔCt-based quantification showed similar distributions between tissues (Supplementary Figure S2C–D). A single peak in dissociation curves confirmed amplification specificity (Supplementary Figure S3), and replicate consistency was supported by heat map analysis (Supplementary Figure S2E).

Conventional PCR detected 71.2% of samples, whereas qPCR detected 100% (Figure 6A). Of 104 positive samples, 55 were detected by both methods, increasing to 74 when including previously overlapping samples. qPCR identified 30 additional positive samples not detected by conventional PCR, demonstrating higher sensitivity, particularly for low fungal loads (Figure 6B). No samples were exclusively detected by conventional PCR. Sequencing confirmed all qPCR amplicons as *R. theobromae*.

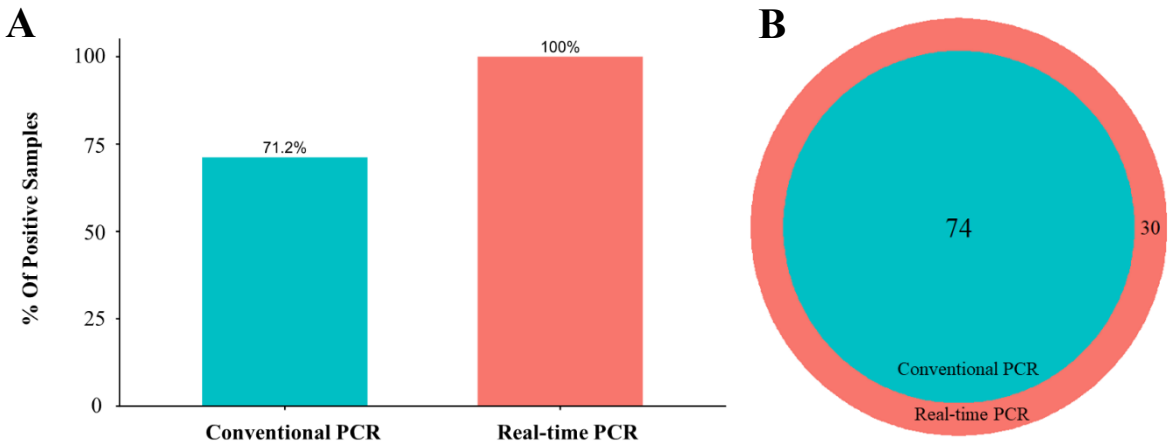


Figure 6. Conventional PCR and qPCR detection results of *R. theobromae* comparisons. For the **(A)** bar graph representing the percentage of detection for each method. **(B)** Venn diagram showing the positive samples exclusive to each technique and those detected by both.

The CaMK/CaMKL locus assay enabled detection of *R. theobromae* in cassava samples from multiple villages in the Oiapoque region (Figure S4). All locations showed pathogen presence, with detection rates varying by method and tissue. qPCR consistently showed higher detection rates than conventional PCR, including in Yanawaka, where no positives were detected by conventional PCR. These results indicate widespread pathogen occurrence and confirm the higher sensitivity of qPCR for field detection.

Discussion

This study addresses limitations in the detection of *R. theobromae* in cassava tissues using conventional PCR, which is affected by low pathogen DNA concentration and heterogeneous distribution. These constraints are well recognized in plant pathology and often lead to false-negative results, particularly in early infection stages or unevenly colonized tissues (Okubara et al. 2005; Hadidi et al. 2017). The TaqMan real-time PCR (qPCR) assay targeting the CaMK/CaMKL locus developed here enabled reliable detection in leaf tissues, overcoming these limitations.

The use of a probe-based qPCR assay improves both sensitivity and specificity compared to conventional PCR, allowing detection of low pathogen titers and reducing non-specific amplification (López et al. 2008; Aglietti et al. 2024). In this study, qPCR identified additional positive samples not detected by conventional PCR, confirming its suitability for detecting low fungal loads and early infections. The few qPCR-negative samples require further investigation to assess possible sequence variation affecting probe binding.

Early detection using sensitive molecular tools is critical for disease surveillance and phytosanitary management, as it enables identification of infections before symptom development and supports rapid response measures (Luchi et al. 2020; Aglietti et al. 2024). This is particularly relevant for CWBD, where latent infections and heterogeneous pathogen distribution complicate diagnosis.

Field surveillance confirmed the presence of *R. theobromae* in all sampled villages in Amapá, indicating widespread distribution. Higher detection rates in stem tissues compared to leaves suggest that stems are a more consistent source of fungal DNA for diagnosis. However, the ability of qPCR to detect the pathogen in leaf tissues expands its applicability for non-destructive sampling strategies.

Overall, the qPCR assay provides a sensitive and reliable tool for *R. theobromae* detection, supporting disease surveillance and early diagnosis. Its application may improve phytosanitary monitoring and contribute to the production of pathogen-free planting material.

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Funding statement

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Conflict of interest

The authors declare that they have no conflict of interest.

Author Contributions

Vanessa Pierote Silva: Investigation, Data curation, Writing original draft; Maria Selma Alves Silva Diamantino: Methodology, Investigation, Validation, ; Andresa Priscila de Souza Ramos: Methodology, Data curation; Claudia Fortes Ferreira: Supervision, Methodology, Writing review and editing; Cintia Paula Feitosa Souza: Investigation; Samar Sheat: Methodology, Validation, Writing review and editing; Natalia Florencio Martins: Supervision, Writing review and editing; Stephan Winter: Supervision, Funding acquisition, Writing review and editing; Saulo Alves Santos de Oliveira: Conceptualization, Supervision, Funding acquisition, Writing review and editing.

Data Availability Statement

Data are available within the article and its Supplementary Material. Additional data are available from the corresponding author upon request.

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SUPPLEMENTARY MATERIAL

Summary:

Table S1. Description of *Rhizoctonia* -like isolates used for phylogenetic analysis based on the ITS rDNA region, including collection details and GenBank accession numbers.

Fig. S1. Agarose gel electrophoresis of the *CaMK/CaMKL* locus amplification

Fig S2. Evaluation of the efficiency and specificity of the qPCR system using the *β-tubulin* endogenous control.

Fig. S3. Dissociation curve (melt curve) of the qPCR assay.

Fig. S4. Map of *Rhizoctonia theobromae* incidence in cassava sample's locations collected at villages in the Oiapoque region.

Supplementary Table S1. Description of *Rhizoctonia* -like isolates used for phylogenetic analysis based on the ITS rDNA region, including collection details and GenBank accession numbers.

GenBank	Taxonomic assignation	Location	Host	Isolation source
AB122145	*Ceratobasidium* sp. AG-k	Japan	*Allium cepa*	mycelium
AB196647	*Ceratobasidium* sp.	Japan	*Arachis hypogaea*	mycelium
AB196650	*Ceratobasidium* sp. AG-I	Japan	*Artemisia* sp.	mycelia
AJ427407	*Ceratobasidium* sp. AGR	USA	*Cucumis* sp.	mycelia
AF354095	*Ceratobasidium* sp. AGQ	Japan	Cynodon_Soil	mycelia
KY075643	*Ceratobasidium theobromae*	China	*Lonicera japonica*	--

KP119764	*Ceratobasidium theobromae*	China	*Lonicera japonica*	--
KP119766	*Ceratobasidium theobromae*	China	*Lonicera japonica*	--
KP119765	*Ceratobasidium theobromae*	China	*Lonicera japonica*	--
OR145521	*Ceratobasidium theobromae*	Cambodia	*Manihot esculenta*	petiole
OR145522	*Ceratobasidium theobromae*	Vietnam	*Manihot esculenta*	petiole
OR145523	*Ceratobasidium theobromae*	Lao PDR	*Manihot esculenta*	petiole
AB286940	*Ceratobasidium* sp. AG-P	Japan	--	--
AB290017	*Ceratobasidium* sp. AG-E	Japan	--	--
AB000019	*Rhizoctonia solani*	Japan	--	--
AY154313	*Thanatephorus cucumeris*	Brazil	--	--
AF354108	*Thanatephorus cucumeris*	Veracruz	*Solanum tuberosum*	mycelia
AF153804	*Rhizoctonia solani*	Australia	*Pterostylis acuminata*	--
AB286930	*Ceratobasidium* sp. AG-Ba	Japan	Rice	--
AB196659	*Ceratobasidium* sp. AG-T	Japan	*Rosa odorata*	--
AB196653	*Ceratobasidium* sp. AG-L	Japan	Soil	mycelium
AB000003	*Rhizoctonia solani*	Japan	Soil	--
AF153800	*Rhizoctonia solani*	Australia	Soil	--
AB196649	*Ceratobasidium* sp. AG-H	Japan	Soil	mycelia
AB290021	*Ceratobasidium* sp. AG-C	Japan	Sugar beet	mycelia
KU255726	*Ceratobasidium theobromae*	Indonesia	*Theobroma cacao*	leaf
KU319572	*Ceratobasidium theobromae*	Indonesia	*Theobroma cacao*	leaf
KU255724	*Ceratobasidium theobromae*	Indonesia	*Theobroma cacao*	petiole
KM523668	*Ceratobasidium theobromae*	Indonesia	*Theobroma cacao*	mycelium
KU319573	*Ceratobasidium theobromae*	Indonesia	*Theobroma cacao*	leaf
KU310940	*Ceratobasidium theobromae*	India	*Theobroma cacao*	petiole
KU310941	*Ceratobasidium theobromae*	India	*Theobroma cacao*	petiole
AB000004	*Rhizoctonia solani*	U.S.A.	Tobacco	--
AB198702	*Ceratobasidium* sp. AG-D	Japan	*Zoysia japonica*	mycelia
MZ159527	*Scotomyces subviolaceus*	Scotland	--	--

MT340869	*Heteroacanthella acanthophysa*	--	--	--
PQ192611	*Ceratobasidium theobromae*	Brazil	*Manihot esculenta*	petiole
PQ192612	*Ceratobasidium theobromae*	Brazil	*Manihot esculenta*	stem

Adapted from Oliveira et al. (2025).

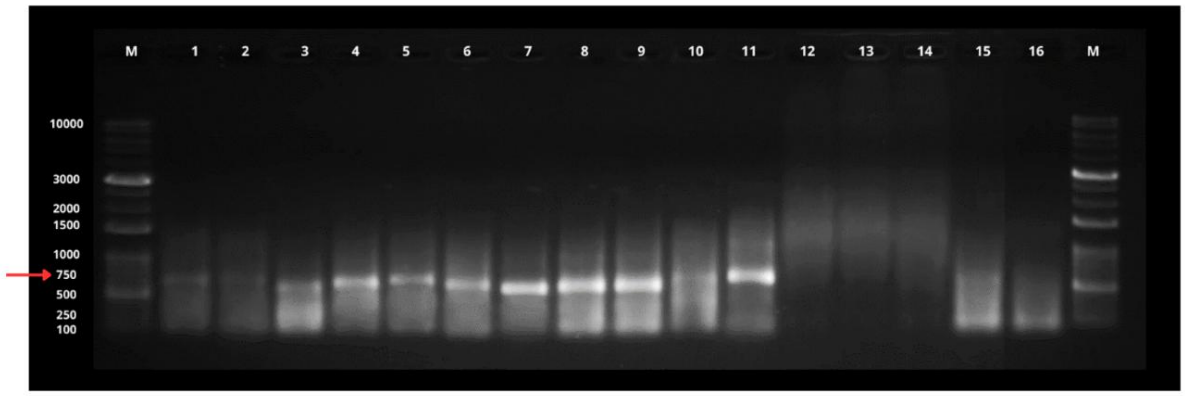
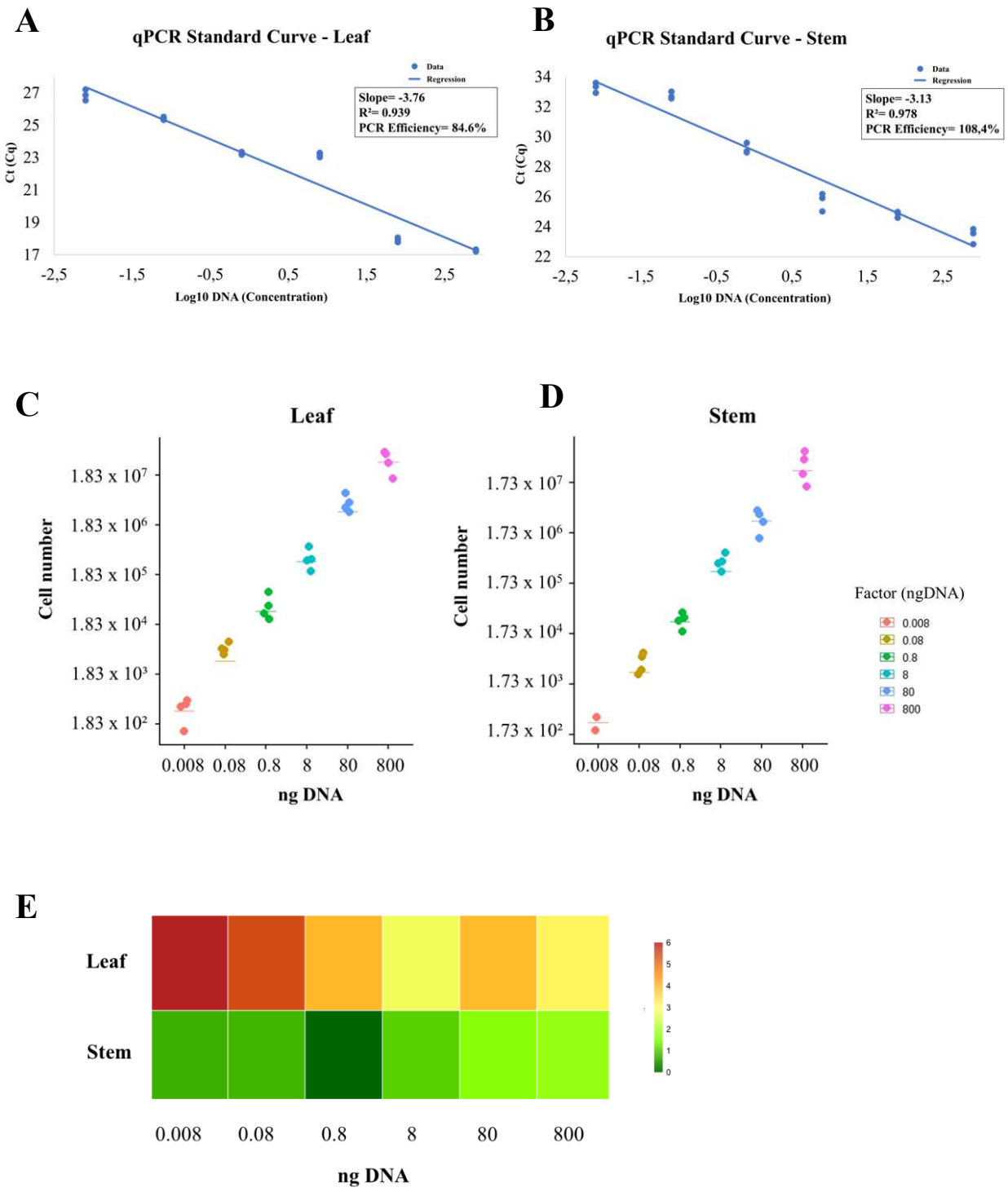
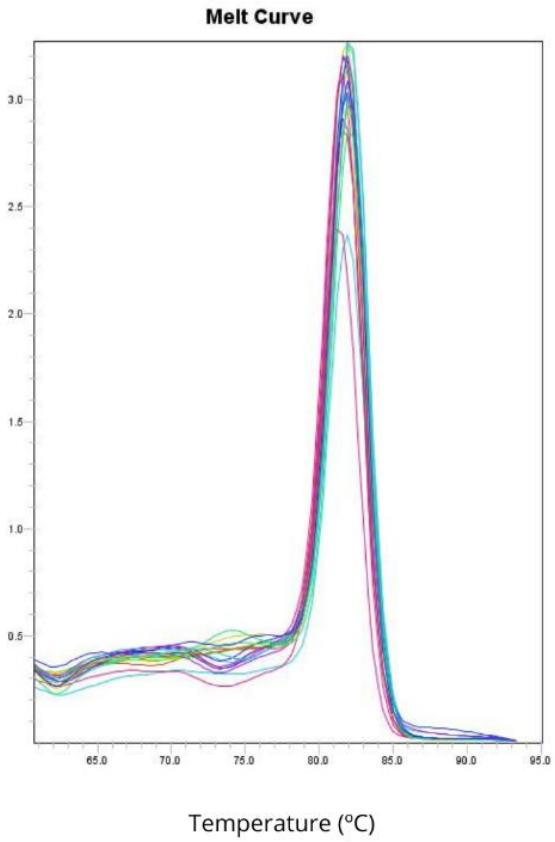


Figure S1. PCR products amplified using the CWBD-CIAT-F2/R2 primer set from 16 cassava samples, analyzed by 1% agarose gel electrophoresis stained with GelRed and visualized under ultraviolet light. **M:** molecular weight marker (1 kb DNA Plus Ladder, Invitrogen); **lanes 1–11:** leaf and stem samples positive for *R. theobromae*; **lanes 12–15:** leaf and stem samples positive for *R. theobromae* only by qPCR; **lane 16:** negative control (healthy cassava leaf sample).



Supplemental Figure S2. Evaluation of the efficiency and specificity of the qPCR system using the *β-tubulin* endogenous control. **(A)** Regression curve for leaf samples; **(B)** Regression curve for stem samples; **(C)** and **(D)** Boxplots representing the number of copies detected in leaves and stems, respectively; **(E)** Heat map representing the average number of copies obtained from ΔCt values.



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365 **Figure S3.** Dissociation curve (melt curve) analysis of the qPCR system. The presence of a single, sharp peak
366 indicates high specificity of the amplification and the absence of non-specific products or primer-dimers,
367 confirming the reliability of the *CaMK/CaMKL* probe-based assay.

368

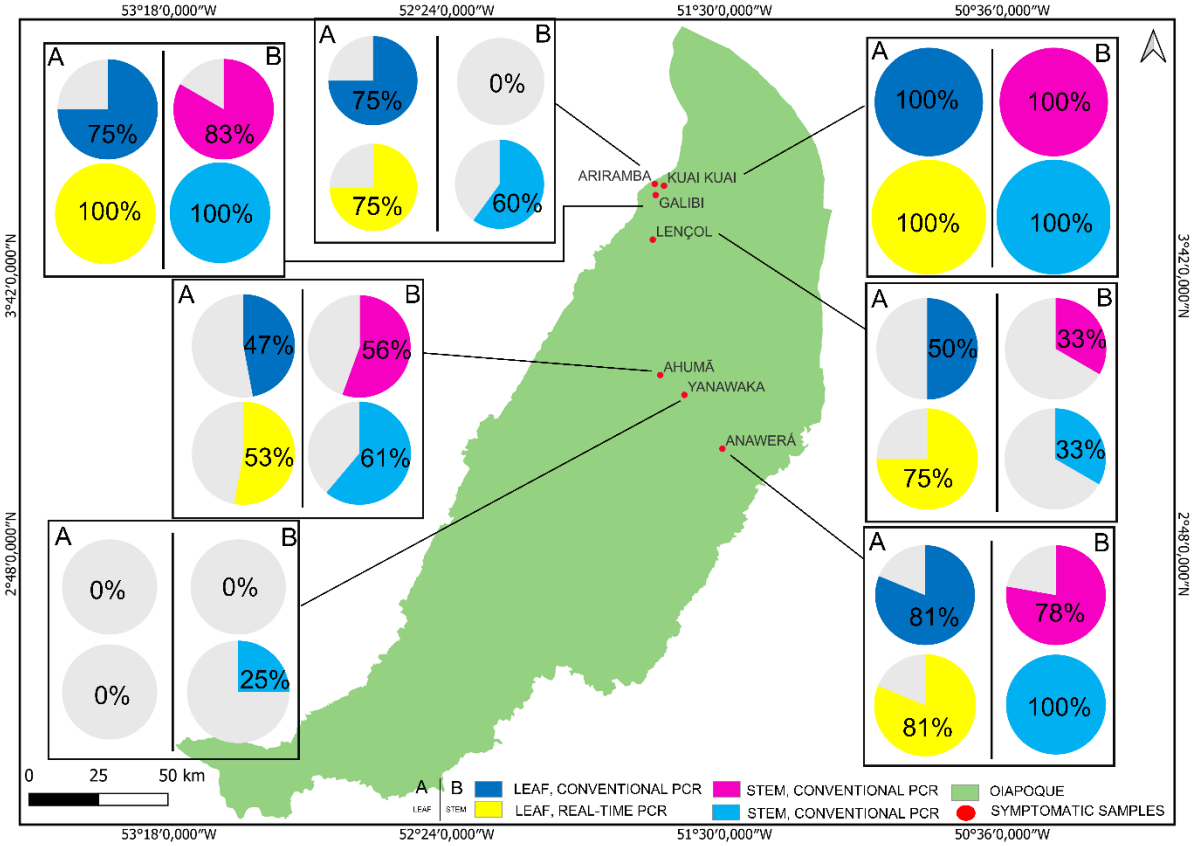


Figure S4. Map of *Rhizoctonia theobromae* incidence in cassava sample's locations collected at villages in the Oiapoque region (Kuai-Kuai, Ariramba, Galibí, Lençol, Ahumã, Anawerá, and Yanawaka). The red dots indicate the collection sites. The pie charts represent the results of molecular detection by both methods: dark blue (leaf, conventional PCR); pink (stem, conventional PCR); yellow (leaf, qPCR); and light blue (stem, qPCR). Column **A** corresponds to the leaf samples, and column **B** corresponds to the stem samples. The green area represents the Oiapoque municipality.

Supplementary Files

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