Portable advanced molecular technologies for detection of plant pathogens

EPPO Conference on Diagnostics
Vienna, 2026-04-22/24

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Instituto Valenciano de Investigaciones Agrarias (IVIA)

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Who we are

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Vienna, 2026-04-22/24

Emergent Plant Disease Prevention and Management Team

NRL Virology - IVIA

Félix Morán
María Martínez-Solsona
Ana Belén Ruiz-García
Antonio Olmos

NRL Bacteriology - IVIA

Silvia Barbé
Aminta Hernández-Marín
Ester Marco-Noales

Micology Unit - IVIA

Carlos Salas-Sierra
Ana Pérez-Sierra

Diagnostic expertise in viruses, bacteria and fungi with a common goal:

Validated tools that can move from the lab to real on-site use

Why rapid and portable devices

- Rapid detection is essential to support surveillance programs, management strategies, and safeguard plant trade at border control posts.

- Advanced molecular diagnostic technologies, are linked to laboratory facilities and specialized non portable equipment.

- Early detection and rapid response against emerging threats require validated tools able to be applied in a real diagnostic on-site context

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Portable molecular technologies

Combining laboratory robustness with on-site speed and versatility

1 Real-time RPA

Fast isothermal amplification for sensitive, specific, field-ready detection

2 Nanopore sequencing

Rapid detection and characterization with portable high-throughput sequencing

3 Magnetic induction real-time PCR

Compact PCR tool designed to bring robust molecular testing closer to the point of need

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Real-time RPA: Huanglongbing

A high-impact citrus bacterial disease

Huanglongbing (HLB) is a devastating disease that affects all commercial citrus species worldwide.

The disease is associated with bacteria of three species of the genus '*Candidatus* Liberibacter' transmitted by psyllid vectors.

HLB has no cure, so preventing its introduction into HLB-free areas is the best strategy to control its spread.

The use of accurate, sensitive, specific, and reliable detection methods is critical for good integrated management of this serious disease.

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Design of a real-time RPA assay for HLB associated '*Candidatus* Liberibacter' spp. detection

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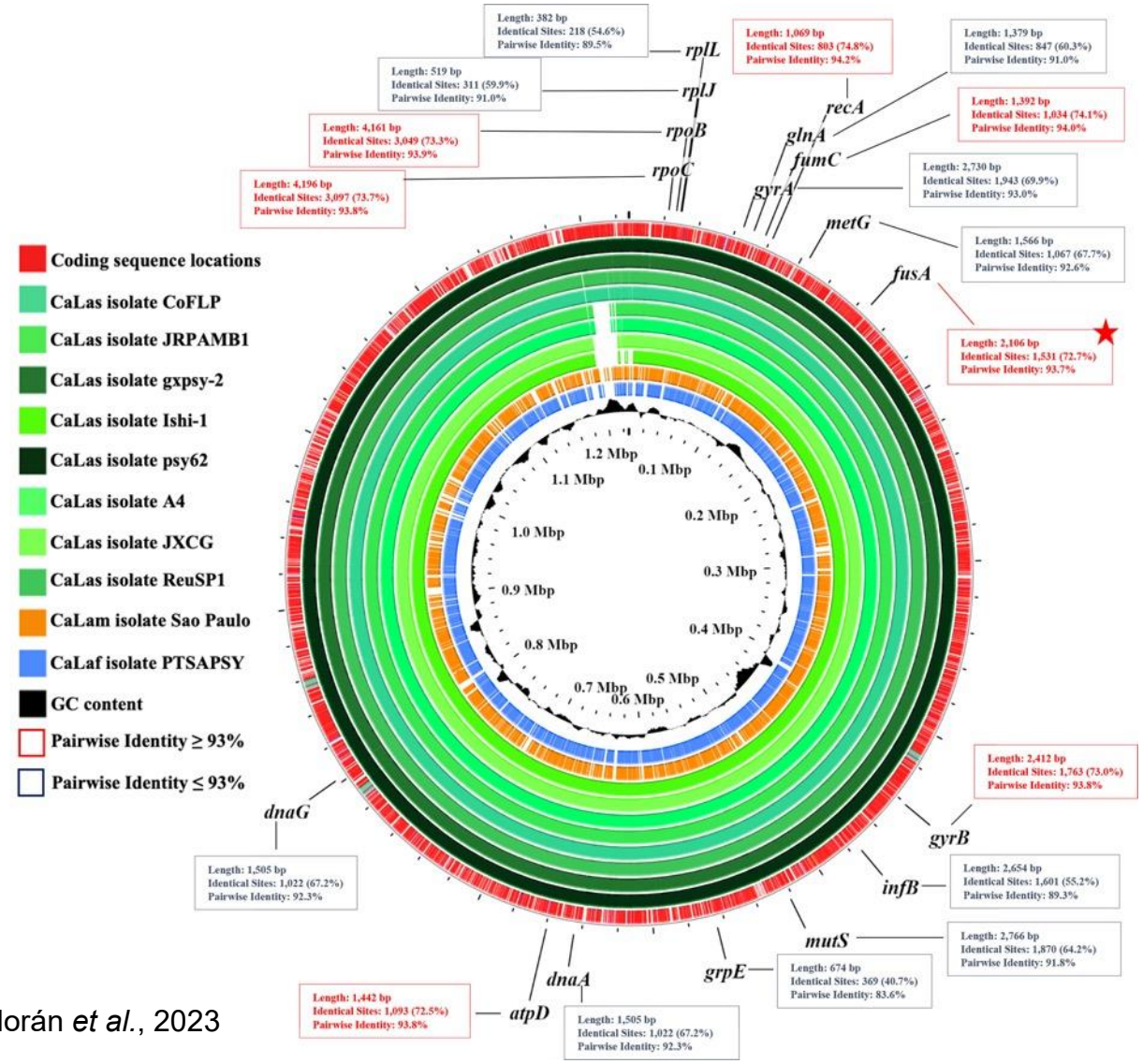
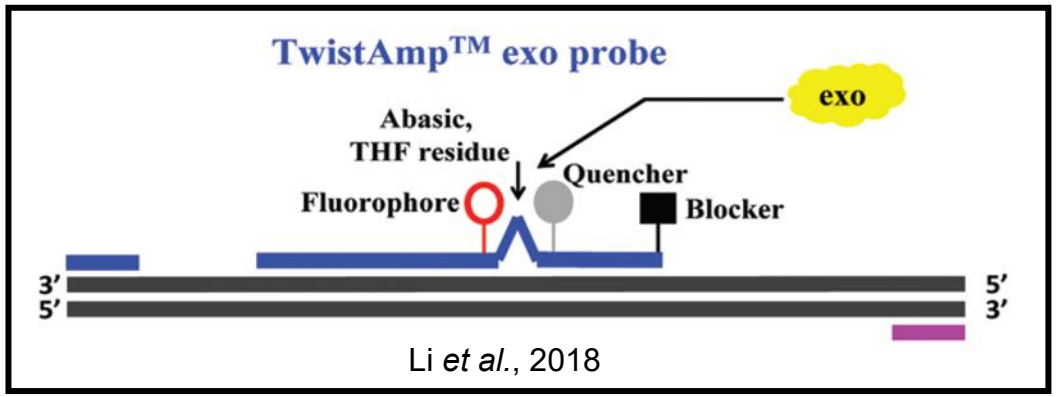


Morán *et al.*, 2023

Alignment of 11 complete genomes and evaluation of 17 housekeeping genes



Li *et al.*, 2018

Detection of the three HLB-associated species: CaLas, CaLaf, and CaLam.

The assay targets a 201 bp region of the *fusA* gene chosen based on its high conservation (average identity 93.7%).

Validation according EPPO standards (PM7/98)

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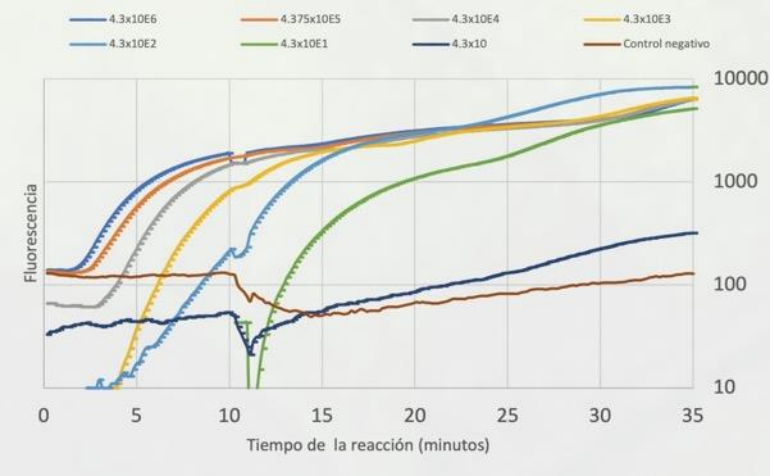
Analytical sensitivity:

- 4-8 copies/µl synthetic DNA
- 40 copies/µl in infected plant material



Selectivity:

- *Citrus sinensis, Citrus hystrix, Citrus reshni*
- Rusk citrange
- *Diaphorina citri*

Morán *et al.*, 2023

Analytical specificity:

Inclusivity

- Able to detect CaLas, CaLaf, and CaLam
- Different geographic origins (Cuba, Brazil, Costa Rica, USA, South Africa)

Exclusivity

- No cross-reactions with non-target bacteria or negative samples

Repeatability and Reproducibility:

Relatively low titer plant samples analyzed in triplicate by different operators, different testing days using two portable fluorimeters (Genie II, AmplifyRP XRT)


I.V.I.A.


Run Description:
1. Well One
2. Well Two
3. Well Three
4. Well Four
5. Well Five
6. Well Six
7. Well Seven
8. Well Eight
Protocol: RPA Basic 40 001
Reaction: 35 min @ 40°C
Cancel

Performance in field conditions

226 samples

- Brazil
- Costa Rica
- Spain

Life for Citrus

		Real-time PCR		
		Positive	Negative	Total
Real-time RPA	Positive	99	0	99
	Negative	19	108	127
	Total	118	108	226
Diagnostic sensitivity		83.89 %		
Diagnostic specificity		100 %		

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Nanopore sequencing: Citrus black spot

A major citrus fungal disease

Citrus black spot (CBS) is caused by the fungus *Phyllosticta citricarpa*

CBS is a major citrus disease with strong impact on fruit quality, external appearance, and marketability

Early and reliable detection is essential to support rapid containment and particularly relevant at border inspection

A strong target for portable, fast, and onsite diagnostic tools

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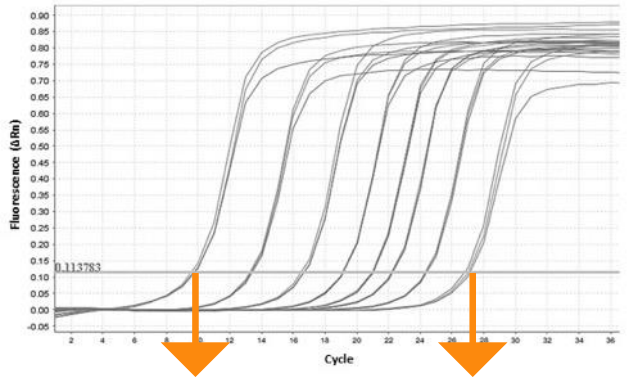
Nanopore sequencing for *P. citricarpa* detection

Artificial inoculations and selection of samples





Selection of high and low fungal titer samples to determine detection limits of the Nanopore sequencing method



Real time qPCR Van Gent-Pelzer et al., 2007.

EmergeNOW

BENAKI PHYTOPATHOLOGICAL INSTITUTE

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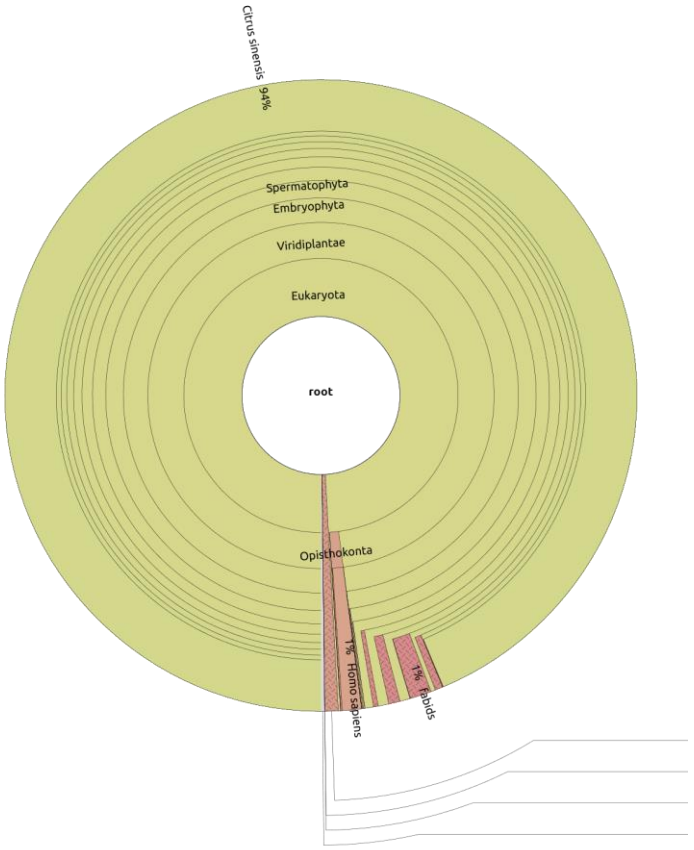
Application of nanopore sequencing for CBS detection

Sequencing → Taxonomic classification



P. citricarpa detection

Dilution	Ct	Mapped reads
10^{-1}	17.231	243
10^{-2}	21.208	378
10^{-3}	25.609	254
10^{-4}	28.261	150
10^{-5}	32.117	74
10^{-6}	36.468	11
10^{-7}	39.864	1

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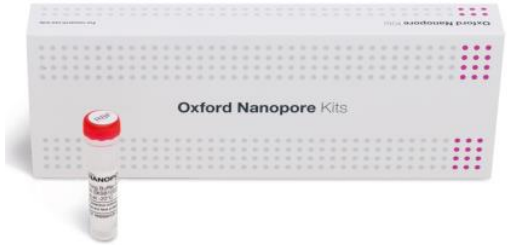
Rapid DNA Extraction adapted to Nanopore sequencing

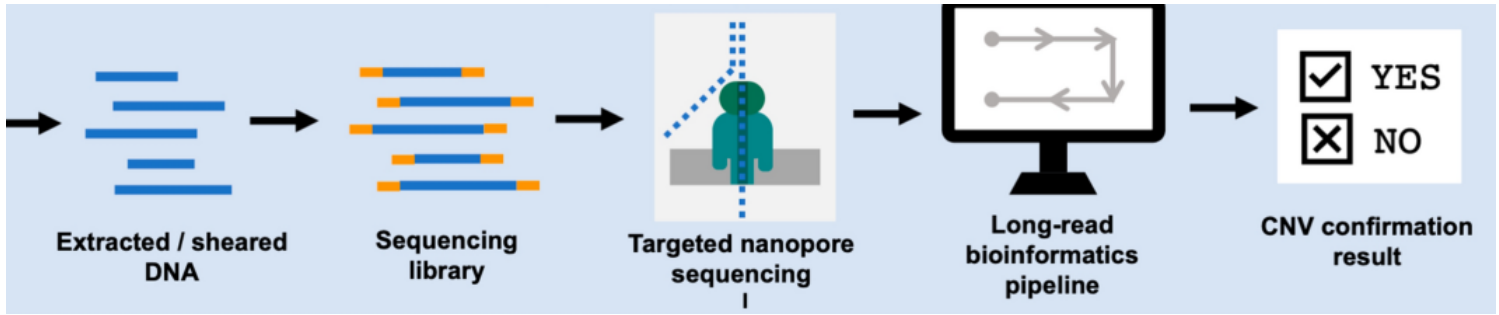
DNA



Transposon based rapid sequencing kit
20 minutes

Rapid DNA extraction methods to be adapted to the HTS workflow are being evaluated

Automatic Pipeline designed in our Lab



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Magnetic induction real-time RT-PCR: yellow vein clearing

An emerging citrus viral disease

Citrus yellow vein clearing virus (CYVCV) is an emerging virus included in EPPO alert list (2022)

CYVCV is associated with yellow vein clearing, leaf curling, and shoot symptoms, especially in susceptible hosts

First detections in EU (Italy, 2024; Spain 2025)

A case for rapid, sensitive, and field-applicable diagnostic tools

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Obtaining complete CYVCV Spanish genomes

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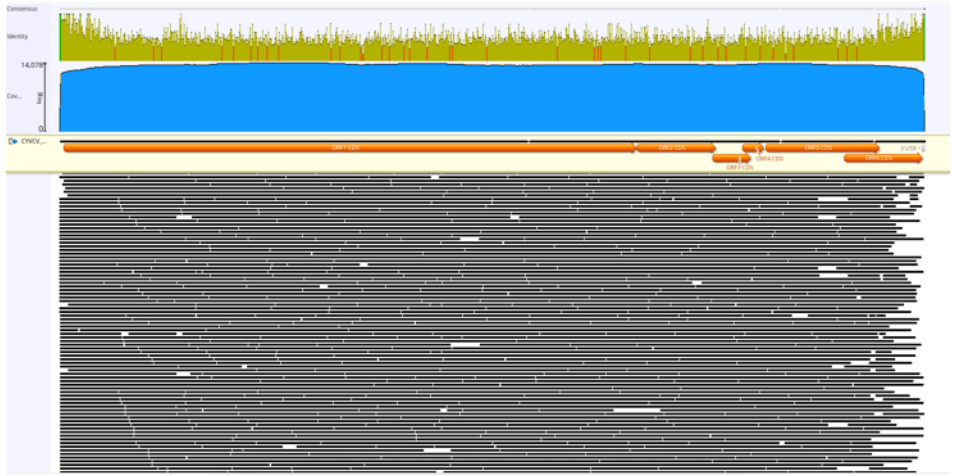
CYVCV infected plants

Total RNA Purification

cDNA synthesis and library preparation

NovaSeq X (Illumina)



Obtaining complete genomes of CYVCV isolates

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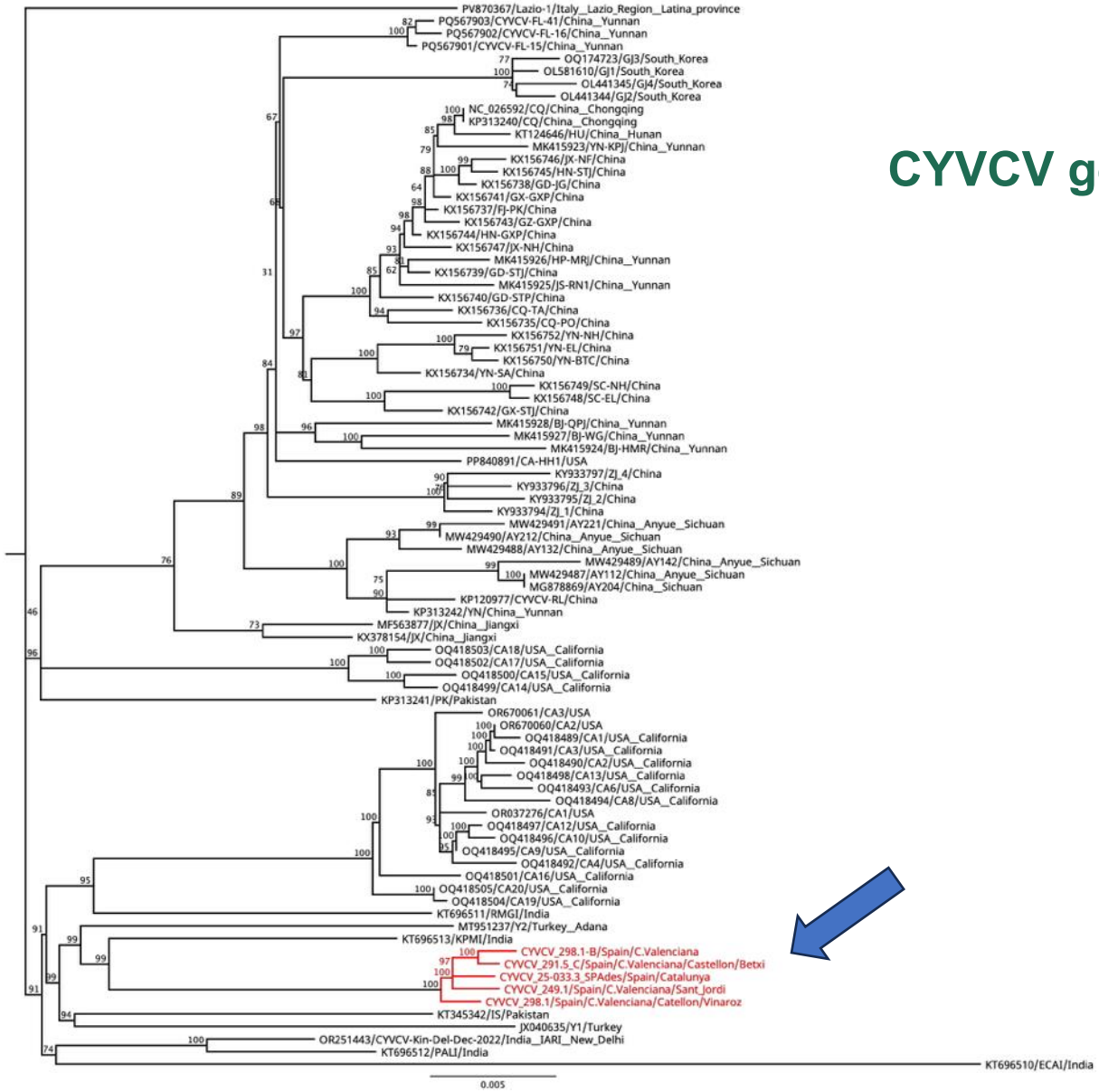


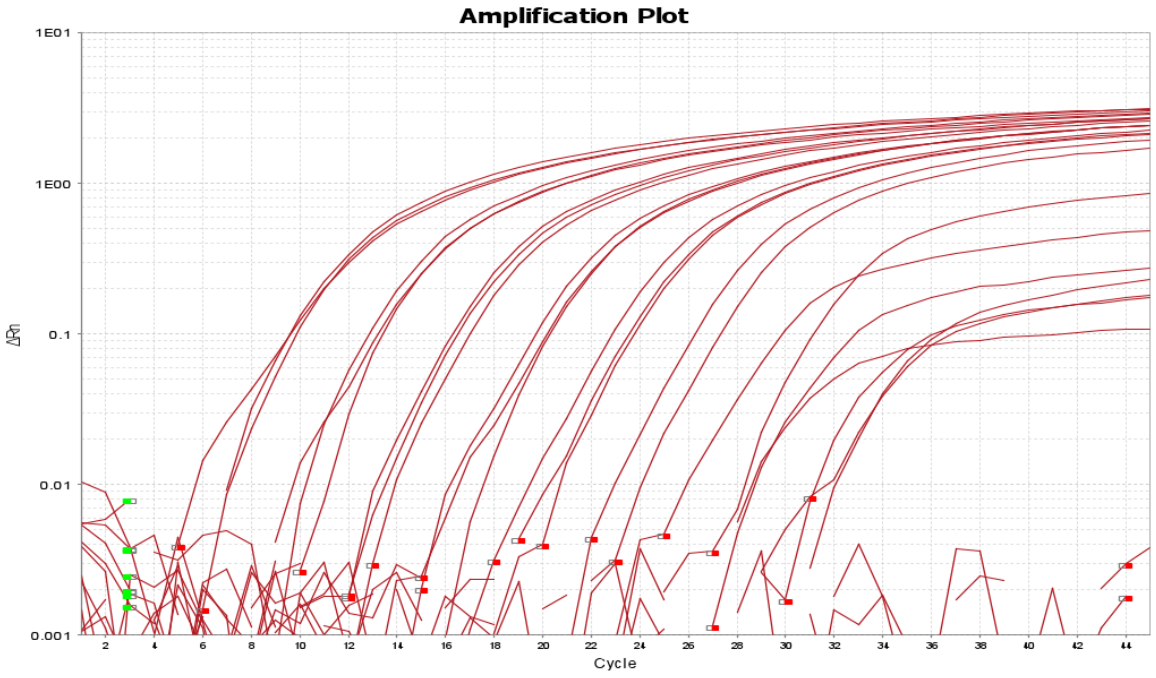
CYVCV genomic diversity including Spanish isolates

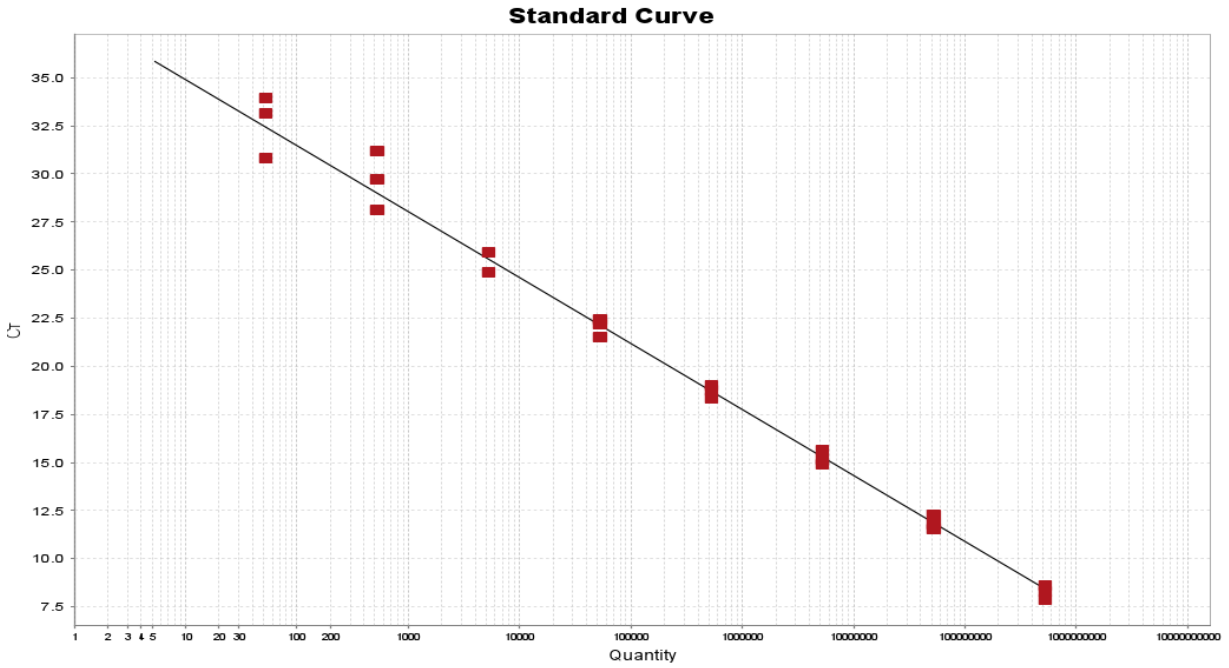
Design of an inclusive real time RT-qPCR
EPPO Validation
Reference method

Real Time RT-qPCR for CYVCV detection

Analytical sensitivity of up to 3 copies with transcripts and 30 viral copies in plant material

Efficiency: 99%





Martínez-Solsona *et al.*, 2026

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Sampling is a critical step

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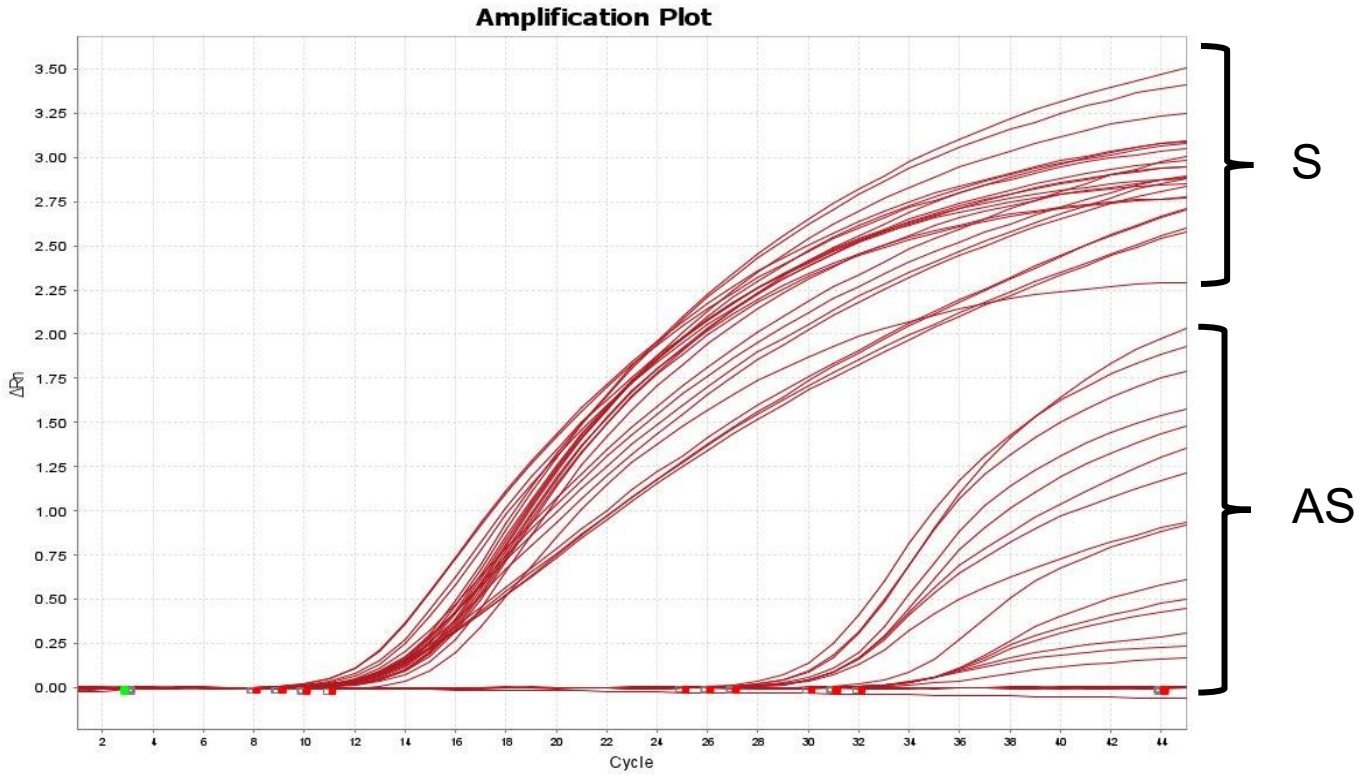
Asymptomatic Branch (AS)

Symptomatic Branch (S)



Detection can be challenging due to uneven distribution, seasonal effects, and asymptomatic infections

Martínez-Solsona *et al.*, 2026

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Magnetic induction real-time PCR: a portable tool

Quantabio Q real-time thermal cycler

- Magnetic induction technology
- Compact portable equipment
- 48 samples / device
- Up to 10 devices conected

Dr. Campbell Costello, Queensland

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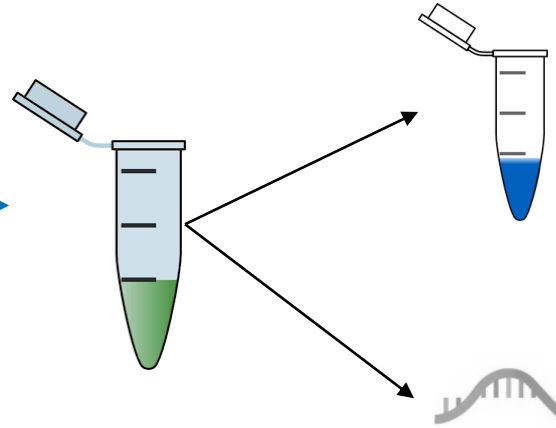
Magnetic induction real-time PCR for CYVCV detection

Field sampling

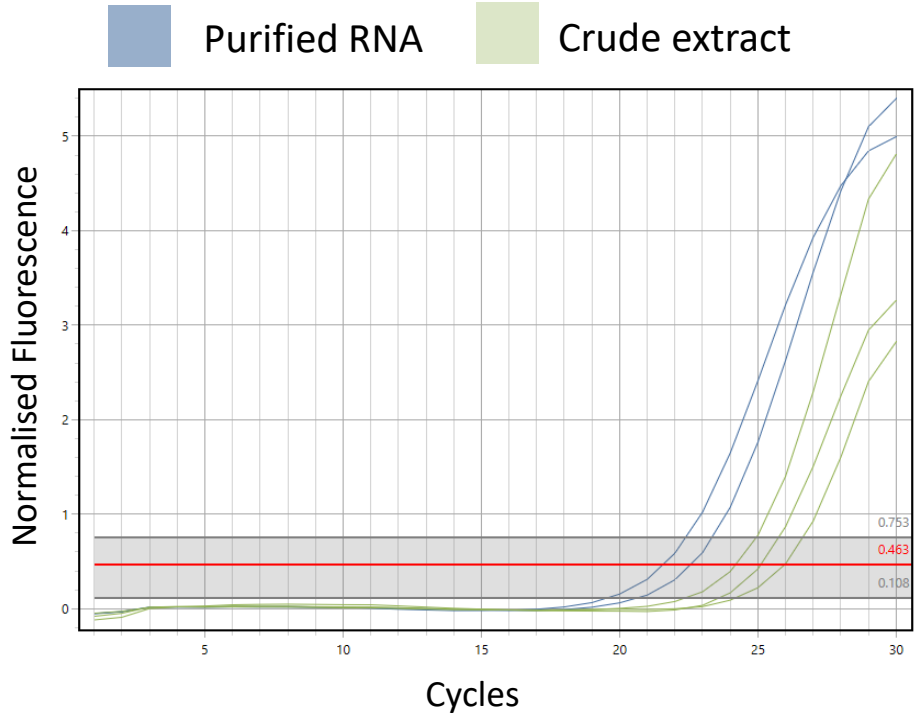
Sample processing



Diluted crude extract

RNA purification

In less than an hour



Towards more accessible plant pathogen detection

- Portable diagnostics are moving plant pathogen detection closer to where decisions need to be made
- The examples presented here show that portable approaches can be applied across different pathogen groups. This includes bacteria, fungi, and viruses of clear phytosanitary relevance
- Their main advantage is to enable rapid testing for surveillance, early detection, and phytosanitary response
- Under those conditions, portable devices can make a meaningful contribution to modern plant health systems

Emergent Plant Disease Prevention and Management Team

Thank you for your attention