A new diagnostic tool for the identification of four beet yellows viruses by multiplex RT-qPCR

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Yellows Resistbeet: PNRI Axis 2 project
→ **P1.3-009**

Context

Virus yellows diseases in sugar beet (VY)

- Viral diseases that can cause yield losses of up to 50 %
- In Europe, complex of different viruses belonging to three viral *genera* present as mono- or co-infections
- Viruses transmitted by aphids whose populations can be controlled by the use of neonicotinoids (NNIs)

National Research and Innovation Plan (PNRI)

- NNIs banned since 2018 and 30 % yield losses in 2020
- Launch of a PNRI by the French government to fund projects with the aim of finding operational alternatives to NNIs against VY
- PNRI Yellows Resistbeet project is developing a sugar beet varietal evaluation protocol against VY (more details on **P1.3-009**)

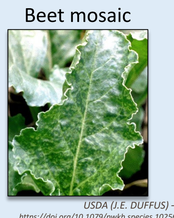
Virus detection and identification method

- Essential to have detection and identification test of the different viruses for varietal evaluation assays
- ELISA available, but not all viruses can be accurately identified.
- RT-PCR method available, but simultaneous identification of all viruses requires the development of multiplex RT-qPCR

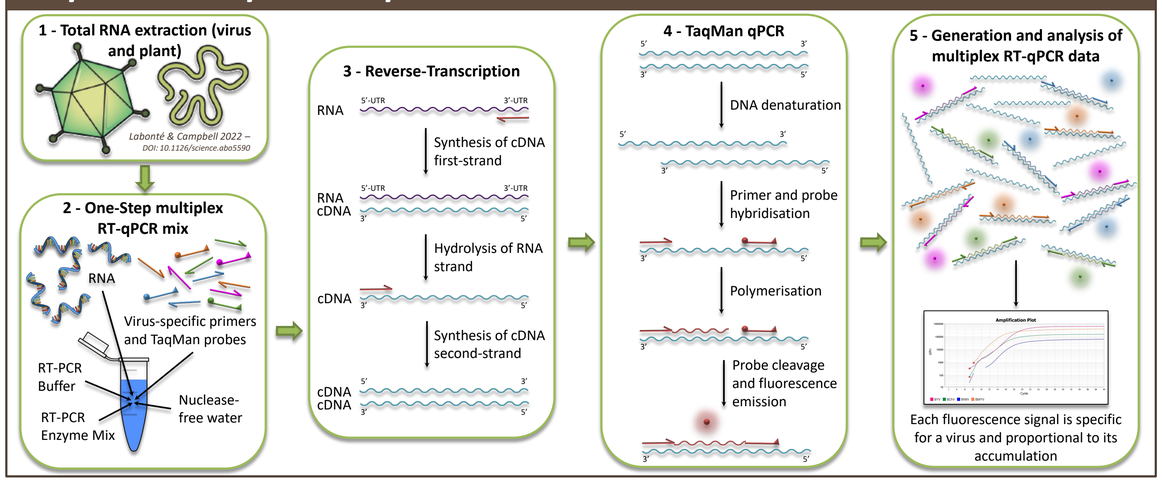
Virus yellows diseases in sugar beet

VY in sugar beet is caused in Europe by a complex of four different virus species present as mono- or co-infections: BChV, BMYV, BYV and BtMV. All viruses are mainly transmitted by the aphid *Myzus persicae.*

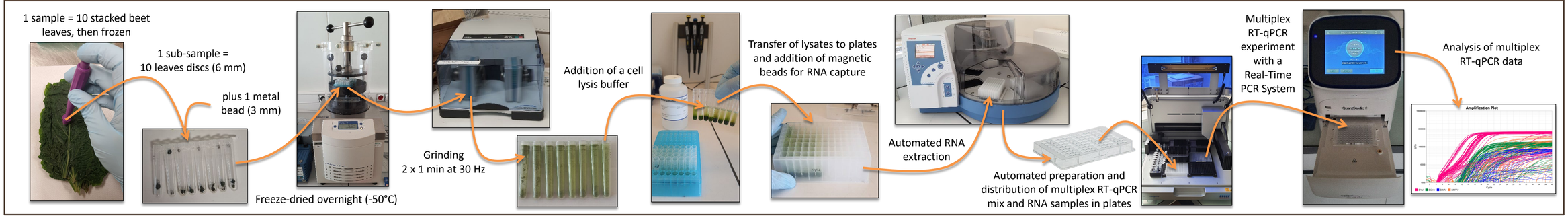
Myzus persicae - ITB - www.itbfr.org

BChV beet chlorosis virus	BMYV beet mild yellowing virus	BYV beet yellows virus	BtMV beet mosaic virus
Polerovirus		Closterovirus	Potyvirus
Persistent acquisition: 12-72h - retention: aphid all life		Semi-persistent acq.: few hours - ret.: 48h-72h	Non-persistent acq.: few min. - ret.: few min.
around 30 % yield loss		40-50 % yield loss	low yield loss
Moderate beet yellowing		Severe beet yellowing	Beet mosaic
			
GEVES – PNRI Yellows Resistbeet		*ITB – PNRI Yellows Resistbeet*	*USDA (J.E. DUFFUS) – https://doi.org/10.1079/pwkb.species.10256*

TaqMan multiplex RT-qPCR



Detection and identification method of the 4 viruses responsible for sugar beet yellows



Analytical specificity

Definition: Ability of an assay to detect the targeted pathogens (inclusivity) while excluding the non-targeted ones (exclusivity)

Method:

- Beet leaf samples for ***in vitro* testing**:
  - Infected with ≠ isolates of the 4 targeted viruses
  - Healthy
  - Infected with 2 non-targeted viruses
- For ***in silico* testing** with Primer-BLAST, all sequences belonging to :
  - Polerovirus
  - Closterovirus
  - Potyvirus

Sample tested \ Virus detected	BChV	BMYV	BYV	BtMV
BChV	+	-	-	-
BMYV	-	+	-	-
BYV	-	-	+	-
BtMV	-	-	-	+
Healthy beet	-	-	-	-
BWYV	-	-	-	-
TuYV	-	-	-	-
Inclusivity	100 %	100 %	100 %	100 %
Exclusivity	100 %	100 %	100 %	100 %
Analytical specificity	100 %	100 %	100 %	100 %

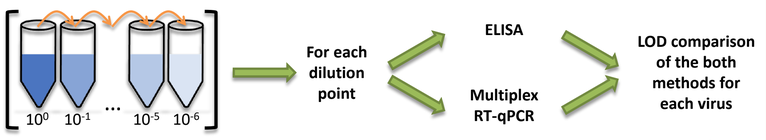
Conclusion: Inclusivity and exclusivity of all primers and TaqMan probes = 100 %

Multiplex RT-qPCR enables the specific detection of each of the four targeted beet yellows viruses

Analytical sensitivity

Definition: Smallest amount of the targeted pathogen that can be detected i.e. limit of detection (LOD)

Method:



Virus	Method	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	LOD
BChV	ELISA	2 + and 1 -	3 -	3 -	3 -	3 -	3 -	NA	$> 10^0$
	RT-qPCR	**3 +**	**3 +**	**3 +**	2 + and 1 -	1 + and 2 -	3 -	NA	10^{-2}
BMYV	ELISA	2 + and 1 -	3 -	3 -	3 -	3 -	3 -	NA	$> 10^0$
	RT-qPCR	**3 +**	**3 +**	**3 +**	**3 +**	1 + and 2 -	1 + and 2 -	3 -	10^{-3}
BYV	ELISA	**3 +**	**3 +**	1 + and 2 -	3 -	3 -	3 -	NA	10^{-1}
	RT-qPCR	**3 +**	**3 +**	**3 +**	**3 +**	**3 +**	2 + and 1 -	1 + and 2 -	10^{-4}
BtMV	ELISA	2 + and 1 -	2 + and 1 -	3 -	3 -	3 -	3 -	NA	$> 10^0$
	RT-qPCR	**3 +**	**3 +**	**3 +**	**3 +**	**3 +**	**3 +**	1 + and 2 -	10^{-5}

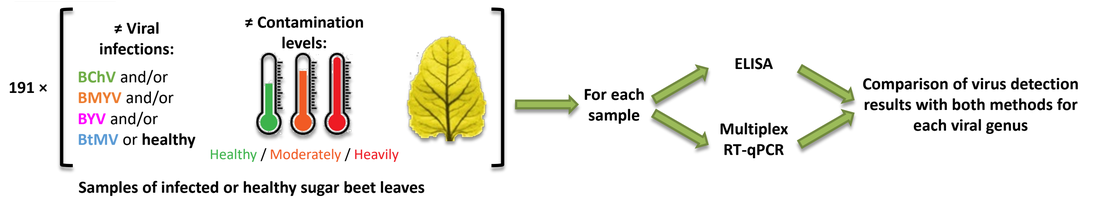
Conclusion: Multiplex RT-qPCR is always more sensitive than ELISA

Depending on the virus, LOD for multiplex RT-qPCR is 100 to 10,000 times lower than LOD for ELISA

Diagnostic sensitivity

Definition: Capacity to give a positive result when the pathogen is present (no false-negatives)

Method:



BChV + BMYV	RT-qPCR +	RT-qPCR -	Diagnostic sensitivity
ELISA +	58	0	100 %
ELISA -	61	40	

BYV	RT-qPCR +	RT-qPCR -	Diagnostic sensitivity
ELISA +	74	0	100 %
ELISA -	3	77	

BtMV	RT-qPCR +	RT-qPCR -	Diagnostic sensitivity
ELISA +	9	0	100 %
ELISA -	15	40	

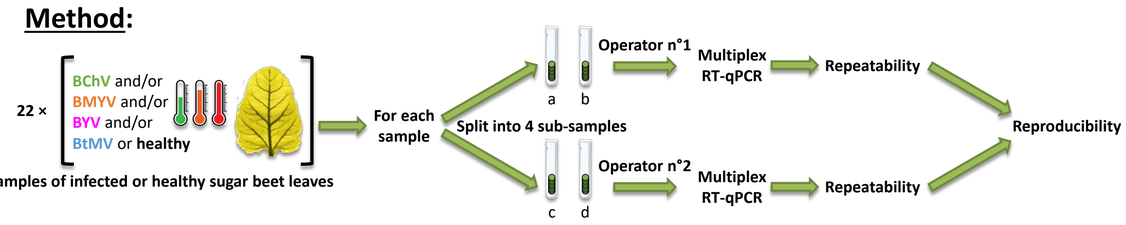
Conclusion: Diagnostic sensitivity for the four targeted viruses = 100 %

Multiplex RT-qPCR produces no false-negative results for all targeted viruses

Repeatability and Reproducibility

Definition: Capacity to produce the same result in the same lab by the same operator (repeatability) and the same result under different experimental conditions (reproducibility)

Method:



Virus detected	BChV	BMYV	BYV	BtMV
Repeatability n°1 (a+b)	100 %	100 %	100 %	100 %
Repeatability n°2 (c+d)	94.21 %	100 %	98.85 %	99.28 %
Reproducibility (1+2)	96.97 %	100 %	95.83 %	97.92 %

Conclusion: Repeatability > 94 % (for both operators) and Reproducibility > 95 %

For all targeted viruses, multiplex RT-qPCR is repeatable and reproducible

Prospects

Yellows Resistbeet project

- Control of *inocula* (upstream) and of inoculations (downstream) for varietal evaluation assays (more details on **P1.3-009**)
- Possibility valuation of using multiplex RT-qPCR semi-quantitative data and corresponding to virus accumulation to characterise the resistance or tolerance to VY of the sugar beet varieties

Within GEVES

- Providing services for customers
- Control tool for the Value for Cultivation, Use, and Sustainability testing (VCUS) for the registration of new sugar beet varieties in the French Catalogue, on the proposal of the Permanent Technical Committee for Plant Breeding (CTPS)

Further achievements

- Publication in a scientific journal in collaboration with INRAE
- Potential use of multiplex RT-qPCR as part of epidemiological surveillance to monitor the development of beet yellows in the field over time

Poster ref. P4.2-012

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SFP Société Française de Phytopathologie
ISPP INTERNATIONAL SOCIETY FOR PLANT PATHOLOGY