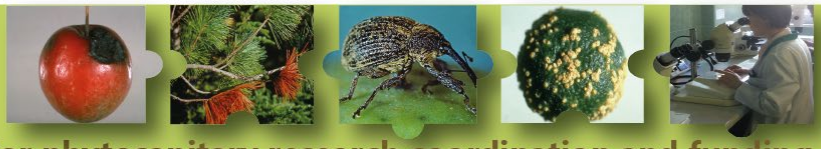
Final Report

Project title (Acronym)
Ring test for improved Potato virus Y strain detection

Project duration:

Start date:	2016-08-01
End date:	2018-06-30

Contents



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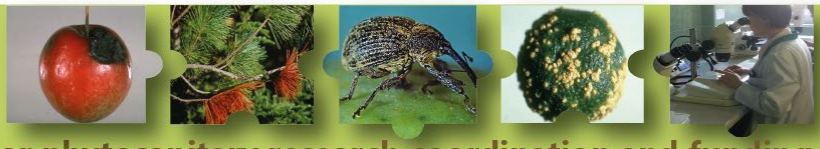

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2. Short project report

2.1 Short executive summary

Potato virus Y (PVY) is one of the most damaging virus in potato industries and it can cause crop loss up to 80%. Several strains of PVY have been identified that differ by the symptoms they cause. PVY^{O}, the common strain causes mosaic symptoms; PVY^{C} that causes stipple streak; PVY^{N}, the necrotic strain that causes mild foliage symptoms, but necrosis in tobacco plants. Mixed infections of common and necrotic strains are frequent, and genomes can mix, producing recombinant strains. Over the last years, several reports (Davie *et al.* 2017, Gray *et al*. 2010, Karasev *et al.* 2013, MacKenzie *et al.* 2018, Elwan *et al.* 2017, Chikh Ali *et al*. 2013) from all over the world are declaring that recombinant strains are becoming dominant. Those recombinant strains, mostly PVY^{N-Wi} ($PVY^{N:O}$) and PVY^{NTN} (main cause of tuber necrosis) are of increasing importance in European seed stocks. Symptoms on plants alone cannot distinguish these virus strains. Diagnosis between the different PVY strains can be difficult, as the widely used serological method (ELISA) is only able to distinguish PVY strain between O- and N-serotype but is unable to distinguish recombinant strains from non-recombinant. Recent emergence of genetically recombinant and serologically different strains of PVY has led to the development of several diagnostic protocols to determine strain identity and to detect mixed strain infections including simplex and multiplex PCR assays. Other approaches include a combination of immunological and molecular methods, though no common method for the detection of PVY and its strains has been adopted by the pathogen detection laboratories within Europe.

2.2 Project aims

The main aim of the project was to develop and validate a protocol for the detection and identification of *Potato virus Y* and its associated strains. During the project different PVY strain identification tests described in published scientific articles were considered and data on these tests were provided by project partners in order to select those that allowed PVY wide range strain identification; an interlaboratory comparison was organized and a validated protocol was agreed and is presented in Annex 1.

2.3 Description of the main activities

Activities of the project were divided into four work packages.

Work Package 1: Coordination and management of the project

During this project, a coordinator change took place. The first coordinator (AFBINI, GB) drafted the work plan, with the help of the other project partners. ARC (EE) took over the coordination at the beginning of 2017, which left little time to organize all activities, but the main tasks of the project were completed.

Work Package 2: Choosing the best methods for distinguishing PVY strains/recombinants

A mapping of several published articles on PVY strain detection and identification protocols was performed and information from the project partners that used them was collected via a survey. Based on the information collected, the most promising protocols were selected. A combined approach of serological and molecular tests appeared to be the most suitable protocol for PVY strain identification. Different laboratories used different protocols (sets of antibodies and primers), the sets are described in Annex 4. To note that partner 1 and partner 2 worked together, thus 6 (and not 7) different sets are described.

Laboratory 1 used only RT-PCR methods with three different primer sets: set 1 as *Potyvirus* general primers (Marie-Jeanne *et al.* 2000), set 2 as PVY general primers (Bostan *et al.* 2009) and set 3 to distinguish PVY strains (Lorenzen *et al.* 2006).
Laboratory 2 used antibodies for general PVY detection (Bioreba) and RT-real-time PCR primer set 1 for general PVY detection (Kogovšek *et a*l. 2008).
Laboratory 3 used also Bioreba company antibodies (general and N specific) and primer set 4 (Weidemann *et al.* 1996) for general PVY detection and primer set 3 (Lorenzen *et al.* 2006) for distinguishing between strains.
Laboratory 4 used four different antibodies produced in their own institute (general PVY, 2xN-specific and O/C-specific) and two commercial antibodies (Bioreba N-specific and Neogen O/C-specific). In laboratory 4, concerning the molecular methods, they used four different RT-real-time PCR primer sets, two of them: set 1 (Kogovšek *et al.* 2008) and set 2 (Boonham *et al.* 2009) were general PVY detection and two were N or O/C specific (set 3 and set 4 Jacquot *et al.* 2005).
Laboratory 5 used own produced antibodies for PVY general detection as well as O- and N-specific. From molecular methods, laboratory 5 used RT-PCR with primer set 3 (strain differentiation (Lorenzen *et al*. 2006) and RT-real-time-PCR they used primer sets 2 (Boonham *et al*. 2009) and set 5 (Massart *et al*. 2014) for general PVY detection.
Laboratory 6 used two different sets of commercial antibodies: SASA and Agdia (N- and O/C-specific). From molecular methods laboratory 6 used RT-PCR with primer set 3 (Lorenzen *et al*. 2006) and RT-real-time-PCR primer set 2 (Boonham *et al*. 2009).
Overall, for serological identification, fourteen antibody sets were used, three different wide spectrum PVY general antibodies, six N-specific antibodies and five O-specific antibodies were used. For molecular detection with RT-PCR four different primer sets were used (Annex 4), sets 1,2 and 4 were suitable for overall PVY detection and primer set 3 are able to differentiate between most common PVY strains, this set was used in four different laboratories. In case of RT-real-time PCR five different primer sets were used, sets 1, 2 and 5 are for general PVY detection, set 3 is N-specific and set 4 is O-specific. General PVY primer set 1 were used in two laboratories and set 2 were used in three laboratories simultaneously.

Work Package 3: Organizing the interlaboratory comparison for PVY strain identification
To organize the interlaboratory comparison, different PVY strain isolates were collected from project partners. All partners received 23 samples, 20 of which represented isolates of all main PVY strains and 3 negative controls. The isolates represented 11 non-recombinant PVY isolates (seven PVY^{O}, three PVY^{N} and one PVY^{C}), 7 recombinant isolates (three PVY^{N-Wi}, three PVY^{EU-NTN} and one PVY^{NA-NTN}) and 2 isolates representative of mixed infection of PVY^{N-Wi} and PVY^{NTN}. Isolates origin and previous identification are showed in Annex 2. Isolates were in different forms, some as freeze-dried plant tissues, and others as fresh plant material. To provide equal material for ring test, all received isolates were used to infect tobacco plants for multiplying the virus. Two weeks after virus inoculation, plants were tested with Bioreba wide spectrum PVY antibodies, to confirm successful inoculation. Since four isolates from Italy arrived late, they were not tested prior to the ring test and material was sent out for interlaboratory comparison. As the results showed later, isolates from Italy (three PVY^{O} and one PVY^{N}) proved to be un-infectious and multiplication on tobacco failed.

Work Package 4: interlaboratory comparison
All project partners participated in the interlaboratory comparison with the methods they were using in their laboratories.

2.4 Main results

Serological tests results (Annex 3, Table 1) showed that the three wide-spectrum PVY antibodies were all able to detect the 16 isolates used in the interlaboratory comparison. With serotype specific antibodies the results were variable, since PVY was represented with many strains and several different serotypes were described. Five isolates (no. 2, 5, 12, 13 and 14) out of six belonging to PVY N-serotype (PVY^{N} and PVY^{NTN}) were determined as PVY N-serotype with all used antibodies, while one isolate (no. 7) was detected with 5 N-specific antibodies but was negative with Agdia N antibody. Concerning the O-serotype (PVY^{O}, PVY^{C} and $PVY^{N\text{-}Wi}$) isolates (no. 1, 3, 6, 10, 11 and 17), most antibodies performed well and detected the isolates correctly. Only in one case, with PVY^{C} (no. 11) isolate laboratory 5 own O-specific antibody missed the infection. In addition, one isolate (no. 1) was also detected as positive with Agdia N-specific antibody. This phenomenon has been described before and serotype like that has been named PVY^{O}-O5. With double (PVY^{NTN} and $PVY^{N\text{-}Wi}$) infection samples antibodies performed good, only again Laboratory 5 own O-specific antibody missed the infection.

Regarding the conventional RT-PCR tests, four different primer sets were used as described in WP2. The tests with general PVY primer sets (by Marie-Jeanne *et al*. 2000 and Bostan *et al.* 2009) were able to detect all PVY isolates, primers from Weidemann *et al.* 1996 missed one PVY^{O} isolate (no. 6). The strain specific test from Lorenzen *et al*. 2006 identified all isolates correctly in all four laboratories with one exception (mixed infection isolate no. 16) resulted negative in laboratory 5. In case of PVY^{C} (no. 11) isolate, laboratories marked the results as unidentified. This is due to the fact that published primers (Lorenzen *et al*. 2006) were not designed to detect PVY^{C} but according to our results it can be said that in PVY^{C} infection one PCR product is produced with size of approximately 267 bp. Results obtained from that study, confirm that widely used primers from Lorenzen *et al.* 2006 performed well and are fit for purpose to identify common PVY strains.

On the RT-real-time PCR tests panel, general primer sets by Kogovšek *et al*. 2008, Boonham *et al*. 2009 and Massart *et al*. 2014 were all proved to be suitable for PVY detection, only laboratory 6 missed one isolate PVY^{NTN} (no. 7). With specific PVY primers by Jacquot *et al.* 2005, only PVY belonging to N or O strain group is achieved. The RT-real-time PCR tests evaluated in this study were not able to identify strains, especially in PVY^{N} group (PVY^{N}, PVY^{NTN}, $PVY^{N\text{-}Wi}$).

2.5 Conclusions and recommendations to policy makers

From the interlaboratory comparison results it can be concluded that general PVY detection antibodies and primers used in this study are performing well. Since the aim of the study was to validate a fast and accurate test for PVY strain identification, the primers described by Lorenzen *et al.* 2006 with modified and simplified one-step RT-PCR protocol (Annex 1) can be recommended to use as easy and cost-effective primary tests for PVY population studies. This method can provide good discrimination of the most common PVY strains. Nevertheless, due to PVY high variability one method alone cannot provide conclusive identification. There are PVY sub strains (PVY^{O}-O5 and PVY^{NTN}-AST, for example), that differ from each other at serological level (isolate no. 1 in this study) without having major changes at molecular level to be able to identify with PCR primers. For a comprehensive and accurate PVY identification it is recommended to use a combination of several antibodies (especially different N-specific antibodies should be used) and RT-PCR based methods. Another solution might be the sequencing of the full genome.

2.6 Benefits from trans-national cooperation

The project was of high importance to all participating organizations, to confirm if the methods used in their laboratories are suitable for detection and correct identification of PVY strains. The collaboration allowed experts to know each other and to share knowledge and experience useful for the project; strong links were developed that provide the foundation for future collaborations. The collaboration allowed to share isolates from different origins that were essential for the evaluation of the tests.

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3. Publications

1.1. Article(s) for publication in the EPPO Bulletin

None.

1.2. Article for publication in the EPPO Reporting Service

None.

1.3. Article(s) for publication in other journals

None.

4. Open Euphresco data

Relevant data will be published in the EPPO Database for diagnostic expertise.

Annex 1 Proposed RT-PCR protocol for detection of PVY strains

The proposed protocol is modified from the RT-PCR test described by Lorenzen *et al*. 2006. To simplify the detection of the different PVY strains, one-step RT-PCR is proposed, additional oligo-dT primers are added and RT-PCR conditions are optimized to improve the detection and identification of single and mixed PVY strain infections. Primer sequences, reaction components and RT-PCR program are presented in the tables. PCR products are separated on 1% agarose gel and visualized with GelRed® (Biotium).

This simplified protocol is an option for easy and accurate PVY strain identification, though primer sets reported in Lorenzen *et al*. 2006 have been proved to work also with other protocols.

Table 1 Primers used for PVY strain identification (Lorenzen *et al*. 2006)

Primer name	Primer sequence
n2258	GTCGATCACGAAACGCAGACAT
n2650c	TGATCCACAACTTCACCGCTAACT
o2172	CAACTATGATGGATTTGGCGACC
o2439c	CCCAAGTTCAGGGCATGCAT
n5707	GTGTCTCACCAGGGCAAGAAC
A6032m	CTTGCGGACATCACTAAAGCG
S5585m	GGATCTCAAGTTGAAGGGGAC
o6266c	CTCCTGTGCTGGTATGTCCT

Table 2 Additional Oligo-dT

Primer name	Primer sequence
Oligo-dT-Not I	AACTGGAAGAATTGGCGGCCGCAGGAATTTTTTTTTTTTTTTT

Table 3 One-Step RT-PCR Master mix

Component	25 µl reaction	Final concentration
RNase-free water	8.7 µl	-
5x QIAGEN One-Step RT-PCR Buffer	5 µl	1x
dNTP Mix (10 mM of each dNTP)	1.5 µl	400 µM of each dNTP
Primers (8+oligo-dT)	0.7 µl x 9	0.14 µM of each
QIAGEN One-Step RT-PCR Enzyme Mix	1 µl	-
Template RNA	1 µl	variable

Table 4 One-Step RT-PCR conditions

Step	Temperature	Time	
RT-step	50°C	30 min	
RT enzyme deactivation	95°C	15 min	35 cycles
Denaturation	94°C	30 sec	
Annealing	50°C	30 sec	
Extention	72°C	90 sec	
Final extention	72°C	7 min	

Annex 2 PVY isolates used for the proficiency test

Sample no	Origin	Name of the isolate	Strain identification
1	France, FN3PT	PVY^{O}-139	PVY^{O}
2		PVY^{N}-605	PVY^{N}
3		PVY^{O}-LW	PVY^{O}
4		PVY^{N}-Sp17	PVY^{N-Wi}
5*	France, ANSES	PVY #5 (4)	PVY^{N}
6*		PVY #3	PVY^{O}
7*		PVY #2	PVY^{EU-NTN}
8	United Kingdom, SASA	neg	neg
9		SASAN-W	PVY^{N-Wi}
10		SASA PVY^{O}	PVY^{O}
11		$SASAPVY^{C}$	PVY^{C}
12		SASA PVY^{NA-NTN}	PVY^{NA-NTN}
13	Estonia, ARC	Secura NTN	PVY^{EU-NTN}
14		AxF 42	PVY^{EU-NTN}
15		AxA 85	PVY^{NTN}+PVY^{N-Wi}
16		AxA 76	PVY^{NTN}+PVY^{N-Wi}
17		AxF 12	PVY^{N-Wi}
18 **	Italy, CREA	485/7E	PVY^{O}
19 **		324/925	PVY^{N}
20 **		497/477	PVY^{O}
21 **		600/13	PVY^{O}
22	Estonia, ARC	Virus free material	neg
23	Estonia, ARC	Virus free material	neg

*Strain identification for isolates 5, 6 and 7 was based on previous information provided by ANSES (FR). According to the results obtained by all partners, the information was correct, with the exception of isolate 5 which was identified as NA-N/NTN.

** Isolates 18, 19, 20 and 21 (CREA, IT) were shipped late and successful multiplication into tobacco plants were not confirmed prior to the interlaboratory comparison. The material turned out to be noninfectious and those samples were considered negative samples.

Annex 3 Tables of results and decisions

Table 1. ELISA results: Some antibodies are produced by the laboratories themselves* Results are given as negative/positive or as OD values

Sample no	Previously identified PVY isolate	PVY all strains antibodies				PVY N-specific antibodies							PVY O- or O- and C-specific antibodies				
		Bioreba all		Lab own all AB*	Lab own all AB*	Bioreba N		Lab own YN19G6*	Lab own 05YN312*	Lab own N*	SASA N	Agdia N	Lab own YO15C10*	Neogen O/C	Lab own O*	SASA O/C	Agdia O/C
		Lab 2	Lab 3	Lab 4	Lab 5	Lab 3	Lab 4	Lab 4	Lab 4	Lab 5	Lab 6	Lab 6	Lab 4	Lab 4	Lab 5	Lab 6	Lab 6
1	O	4,507	0,5017	2,922	Positive	0,0708	0,075	0,092	0,082		0,0525	2,662	2,755	2,98	Positive	2,205	1,4945
2	N	4,447	0,4470	0,682	Positive	0,4207	0,804	0,572	0,324	Positive	0,6465	0,53	0,17	0,101		0,0455	0,036
3	O(Lw)	4,424	0,3300	1,568	Positive	0,0763	0,067	0,085	0,076		0,051	0,0955	2,402	3,449	Positive	1,782	0,5545
4	W	0,931	0,0954	1,222	Positive	0,0749	0,084	0,115	0,089		0,053	0,0685	2,544	3,447	Positive	1,877	1,5025
5	N	1,398	0,1095	3,02	Positive	0,5581	2,962	3,087	2,523	Positive	3,4765	1,924	0,179	0,105		0,048	0,0385
6	O	4,382	0,4251	0,928	Positive	0,0757	0,064	0,078	0,074		0,051	0,08	1,373	2,871	Positive	1,109	0,666
7	NTN	4,491	0,6233	0,978	Positive	0,4646	1,512	0,766	0,432	Positive	1,3525	0,0885	0,163	0,102		0,0465	0,0345
8	neg	0,082	0,0816	0,068	Negative	0,0821	0,079	0,089	0,081		0,047	0,038	0,164	0,109		0,0475	0,035
9	W	4,484	0,6034	1,081	Positive	0,0750	0,081	0,097	0,088		0,052	0,0295	2,075	2,833	Positive	2,8425	1,466
10	O	4,541	0,6376	2,243	Positive	0,0743	0,08	0,105	0,09		0,055	0,0405	2,953	3,447	Positive	3,463	1,61
11	C	4,363	0,3626	1,492	Positive	0,0789	0,079	0,101	0,091		0,0545	0,038	2,073	3,02	NB	3,0875	0,333
12	NTN	4,507	0,5424	1,03	Positive	0,6259	1,051	0,772	0,527	Positive	1,4225	0,6875	0,164	0,095		0,05	0,0345
13	NTN	4,428	0,5419	0,388	Positive	0,7462	0,451	0,374	0,196	Positive	0,8735	0,466	0,171	0,097		0,0515	0,0365
14	NTN	4,321	0,5229	0,446	Positive	0,5100	0,587	0,412	0,223	Positive	0,9745	0,6565	0,165	0,093		0,049	0,0365
15	NTN+W	4,372	0,5018	0,872	Positive	0,5148	0,626	0,389	0,222	Positive	1,2595	0,6325	0,895	1,885	Positive	2,232	0,4015
16	NTN+W	4,377	0,4452	0,505	Positive	0,5083	0,419	0,266	0,166	Positive	1,3455	0,5465	0,633	1,22	NB	1,939	0,367
17	W	4,434	0,3850	0,332	Positive	0,0741	0,082	0,1	0,092		0,0575	0,044	0,799	1,736	Positive	2,6305	1,1025
18	O	0,084	0,0794	0,056	Negative	0,0746	0,061	0,081	0,069		0,052	0,044	0,144	0,097		0,0535	0,04

19	N	0,085	0,0693	0,061	Negative	0,0734	0,074	0,091	0,08		0,057	0,044	0,151	0,093		0,055	0,0415
20	O	0,086	0,0789	0,065	Negative	0,0746	0,076	0,096	0,081		0,0565	0,0435	0,149	0,096		0,0555	0,041
21	O	0,094	0,0825	0,073	Negative	0,0717	0,077	0,095	0,083		0,0615	0,0455	0,173	0,093		0,055	0,0435
22	neg	0,085	0,0744	0,066	Negative	0,0747	0,08	0,098	0,087		0,0565	0,0425	0,167	0,092		0,051	0,0395
23	neg	0,085	0,0791	0,069	Negative	0,0720	0,076	0,095	0,083		0,0595	0,047	0,172	0,091		0,0545	0,038

Table 2. RT-PCR results (+++ strong positive, ++ positive, + weak positive, unidentified-)

Sample no	Previously identified PVY isolate	PVY all strains			PVY specific strains (O, N, NTN, N-Wilga, NA-N/NTN)			
		Primer set 1 (potyviruses)	Primer set 2 PVY	Primer set 4 PVY	Primer set 3 (Multiplex)			
		Lab 1	Lab 1	Lab 3	Lab 1	Lab 3	Lab 5	Lab 6
1	O	(+++)	(+++)	(+++)	O	O	O	O
2	N	(+++)	(+++)	(+)	N	N	neg	N
3	O(Lw)	(+++)	(+++)	(+++)	O	O	O	O
4	W	(+++)	(+++)	(+)	N:O	W	N:W	W
5	N	(+++)	(+++)	(++)	NA-N/NTN, NA-NTN	NA-N/NTN	NA-NTN	NA-N/NTN
6	O	(+++)	(+++)	neg	O	O	O	O
7	NTN	(+++)	(+++)	(+++)	NTN	NTN	NTN	NTN
8	neg	neg	neg	neg	neg	neg	neg	neg
9	W	(+++)	(+++)	(++)	N:O	W	N:W	W
10	O	(+++)	(+++)	(+++)	O	O	O	O
11	C	(+++)	(+++)	(+++)	unidentified	unidentified	unidentified	267 bp
12	NA-NTN	(+++)	(+++)	(+++)	NA-N/NTN, NA-NTN	NA-N/NTN	NA-NTN	NA-N/NTN
13	NTN	(+++)	(+++)	(+++)	NTN	NTN	NTN	NTN
14	NTN	(+++)	(+++)	(+++)	NTN	NTN	NTN	NTN
15	NTN+W	(+++)	(+++)	(+++)	NTN+N:O	NTN+W	NTN + N:W	NTN+W
16	NTN+W	(+++)	(+++)	(+++)	NTN+N:O	NTN+W	NTN	NTN+W

17	W	(+++)	(+++)	(+++)	N:O	W	N:W	W
18	O	(+)	(+)	neg	unidentified	neg	neg	neg
19	N	neg	neg	neg	neg	neg	neg	neg
20	O	neg	neg	neg	neg	neg	neg	neg
21	O	neg	neg	neg	neg	neg	neg	neg
22	neg	neg	neg	neg	neg	neg	neg	neg
23	neg	neg	neg	neg	neg	neg	neg	neg

Table 3. RT-real-time-PCR results (results are given in Ct values)

Sample no	Previously identified PVY isolate	PVY all strains						PVY^N or PVY^O specific	
		Primer set 1 (Kogovšek *et al.* 2008)		Primer set 2 (Boonham *et al.* 2009)			Primer set 5 (Massart *et al.*, 2014)	Primer set 3 PVY^N (Jacquot *et al.*, 2005)	Primer set 4 PVY^O (Jacquot *et al.*, 2005)
		Lab 2	Lab 4	Lab 4	Lab 5	Lab 6	Lab 5	Lab 4	Lab 4
1	O	10,71	25,94	23,53	16,07	17,02	20,27	36,55	29,97
2	N	10,71	26,95	30,46	20,91	21,31	19,13	28,14	40
3	O(Lw)	10,72	23,64	23,21	16,48	16,95	30,84	35,92	26,93
4	W	9,48	25,68	25,29	16,99	16,75	29,24	26,95	40
5	N	10,36	23,27	24,34	16,9	18,14	29,19	25,84	40
6	O	11,63	27,59	27	18,29	17,88	25,37	36	31,58
7	NTN	12,79	26,17	32,45	24,29	40	24,18	26,85	40
8	neg	29,32	35,62	37,04	N/A	40	32,53	37,75	40
9	W	9,92	21,31	20,92	19,2	16,94	30,09	21,64	40
10	O	9,06	24,11	23,85	16,5	16,85	29,63	40	28,81
11	C	10,82	27,58	28,97	18,38	21,59	24,65	37,97	40
12	NTN	10,64	25,71	26,52	19,07	17,41	27,65	29,36	40
13	NTN	10,58	25,4	26,78	20,95	17,34	25,68	25,43	40
14	NTN	12,04	22,39	24,7	22,24	18,99	28,42	23,64	40
15	NTN+W	10,35	27,29	26,73	18,81	17,03	27,2	28,27	40

16	NTN+W	11,4	26,16	27,67	22,18	18,44	28,14	27,18	40
17	W	10,37	23,15	22,64	18,51	15,93	27,5	24,43	40
18	O	27,48	40	35,41	N/A	28,62	27,19	37,46	40
19	N	27,37	36,22	34,93	N/A	29,21	27,8	37,57	40
20	O	31,01	35,91	36,94	N/A	30,19	24,54	37,13	40
21	O	27,16	36,06	35,55	37,56	29,62	20,99	>40	40
22	neg	28,85	>40	36,45	33,74	37,23	21,24	>40	40
23	neg	31,74	>40	40	N/A	32,75	20,28	36,33	40

Annex 4. Tables of methods (primers and antibodies used) used in the interlaboratory comparison

Table 1. List of antibodies

ELISA							
	Specificity	**Lab 1**	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 5**	**Lab 6**
Bioreba	Wide spectrum PVY						
Cocktail anti-PVY	Wide spectrum PVY						
Lab 5 own	Wide spectrum PVY						
SASA	O/C-specific						
Agdia Mab2	O/C-specific						
YO15C10	O/C-specific						
Neogen	O/C-specific						
Lab 5 own	O-specific						
Bioreba	N-specific						
SASA	N-specific						
Agdia 1F5	N-specific						
YN19G6	N-specific						
05YN312	N-specific						
Lab 5 own	N-specific						

Table 2. Primer sets used for RT-PCR and RT-real-time PCR

RT-PCR						
Sequences on primer sheet	**Lab 1**	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 5**	**Lab 6**
Primer set 1						
Primer set 2						
Primer set 3						
Primer set 4						
RT-real-time PCR						
Sequences on primer sheet	**Lab 1**	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 5**	**Lab 6**
Primer set 1						
Primer set 2						
Primer set 3						
Primer set 4						
Primer set 5						

Table 3. Primer sequences for RT-PCR and references

RT-PCR		
Primer set 1	potyv MJ1	ATGGTHTGGTGYATHGARAAYGG
	potyv MJ2	TGCTGCKGCYTTCATYTG
Primer set 2	PVYf	AAGCTTCCATACTCACCCGC
	PVYr	CATTTGTGCCCAATTGCC
Primer set 3	n2258	GTCGATCACGAAACGCAGACAT
	n2650c	TGATCCACAACTTCACCGCTAACT
	o2172	CAACTATGATGGATTTGGCGACC
	o2439c	CCCAAGTTCAGGGCATGCAT
	n5707	GTGTCTCACCAGGGCAAGAAC
	A6032m	CTTGCGGACATCACTAAAGCG
	S5585m	GGATCTCAAGTTGAAGGGGAC
	o6266c	CTCCTGTGCTGGTATGTCCT
Primer set 4	PVY-1	TTCCAAAGTGTCCTTTGAG
	PVY-3	CAAGACTGATGCCCAGAT

Primer set 1	Marie-Jeanne V, Ioos R, Peyre J, Alliot B, Signoret P, 2000. Differentiation of Poaceae potyviruses by reverse transcription-polymerase chain reaction and restriction analysis. Journal of Phytopathology 148, 141–51. [doi:10.1046/j.1439-0434.2000.00473.x]
Primer set 2	Bostan H, Peker PK, 2009, The feasibility of tetraplex RT-PCR in the determination of PVS, PLRV, PVX and PVY from dormant potato tubers. African Journal of Biotechnology Vol. 8 (17), pp. 4043-4047 of PVS, PLRV, PVX and PVY from dormant potato tubers
Primer set 3	Lorenzen, J. H., Piche, L. M., Gudmestad, N. C., Meacham, T., and Shiel, P. 2006. A multiplex PCR assay to characterize Potato virus Y isolates and identify strain mixtures. Plant Dis. 90: 935-940.
Primer set 4	Weidemann H. L. and Maiss E. (1996). Detection of the potato tuber necrotic ringspot strain of potato virus Y (PVYNTN) by reverse transcription and immunocapture polymerase chain reaction. Journal of plant diseases and protection, 103, 4: 337-345

Table 4. Primers and probes sequences for RT-real-time PCR and references

RT-real-time PCR primers and probes				
Primer set 1	Primer 1	Univ-F	CATAGGAGAAACTGAGATGCCAACT	all
	Primer 2	Univ-R	TGGCGAGGTTCCATTTTCA	
	Probe	Univ-probe	TGATGAATGGGCTTATGGTTTGGTGCA	
Primer set 2	Primer 1	PVY 411F	GGGCTTATGGTTTGGTGCA	all
	Primer 2	PVY 477R	CCGTCATAACCCAAACTCCG	
	Probe	PVY probe	TGAAAATGGAACCTCGCCAAATGTCA	
Primer set 3	Primer 1	FpN	AACCATGATGGATCTGGCTACAA	N (HC-Pro)
	Primer 2	RpN	TTCTAGGCAGTTCTGCATCATGAA	
	Probe	Probe-N400	CTCAAATGAAAATATTCTAC	
Primer set 4	Primer 1	FpO	ATGGATTTGGCGACCACTTGT	O (HC-Pro)
	Primer 2	FpR	TAAACTAGGCAGCTCTGCATC	
	Probe	Probe-O400	CTCAAATGAGAATATTCTA	
Primer set 5	Primer 1	Multipot-F	GGTTTCGTAATGTTCCTCACCAA	
	Primer 2	Multipot-R	AAAGGTATTTATCCAGCAGTAGATCCTT	
	Probe	Multipot probe	Cy5-CATGGTTGACGTTGAAT-BHQ2	XS probe

Primer set 1	Kogovšek P. et al., 2008, Single-step RT real-time PCR for sensitive detection and discrimination of Potato virus Y isolates
Primer set 2	Boonham N, Laurenson L, Weekes R et al (2009) Direct detection of plant viruses in potato tubers using real-time PCR. In: Burns R (ed) Methods in molecular biology, vol 508, Plant pathology. Humana Press, New York, pp 249–258
Primer set 3	Jacquot, E., Tribodet, M., Croizat, F., Balme-Sinibaldi, V., Kerlan, C., 2005. A single nucleotide polymorphism-based technique for specific characterization of YO and YN isolates of Potato virus Y (PVY). J. Virol. Methods 125, 83–93.
Primer set 4	Jacquot, E., Tribodet, M., Croizat, F., Balme-Sinibaldi, V., Kerlan, C., 2005. A single nucleotide polymorphism-based technique for specific characterization of YO and YN isolates of Potato virus Y (PVY). J. Virol. Methods 125, 83–93.
Primer set 5	Massart, S.; Nagy, C.; Jijakli, M.H., 2014. Development of the simultaneous detection of Ralstonia solanacearum race 3 and Clavibacter michiganensis subsp. sepedonicus in potato tubers by a multiplex real-time PCR assay. Eur. J. Plant Pathol. 138, 29–37.