



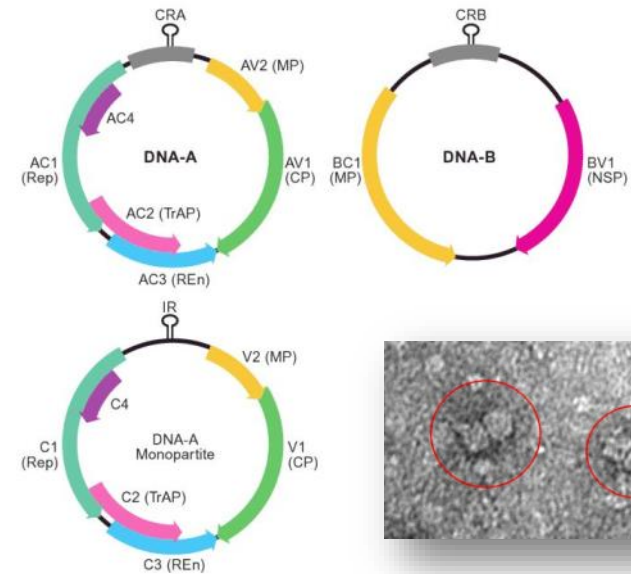
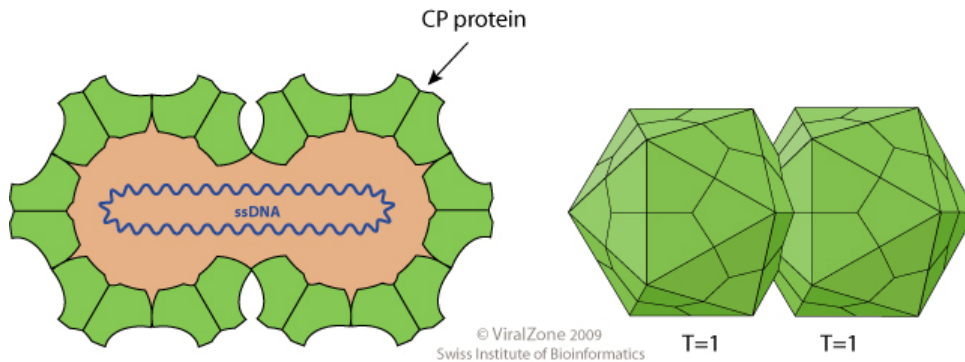
Development of a polyvalent detection method for Begomoviruses presenting a threat to the European tomato industry

A SAISON, P GENTIT – Virology team – UBVO

ANSES/ Plant health laboratory

7 rue Jean Dixmèras 49044 Angers Cedex 01 - FRANCE

Begomoviruses

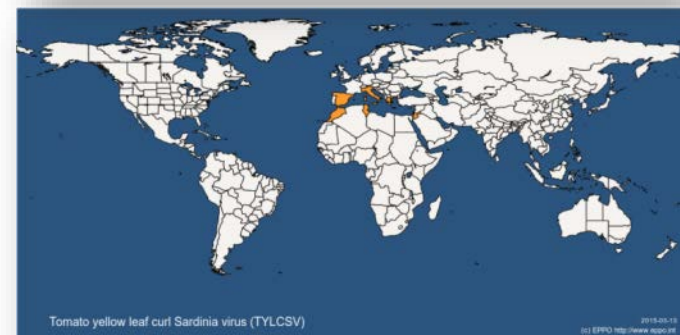
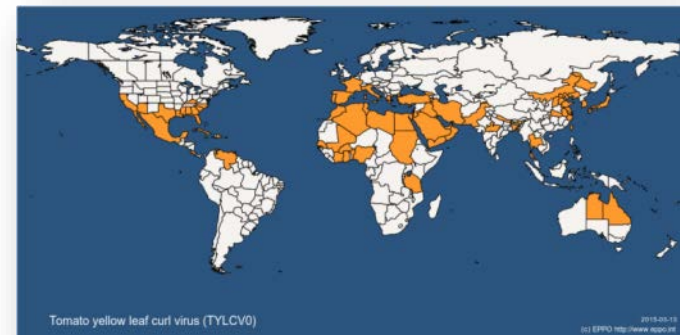
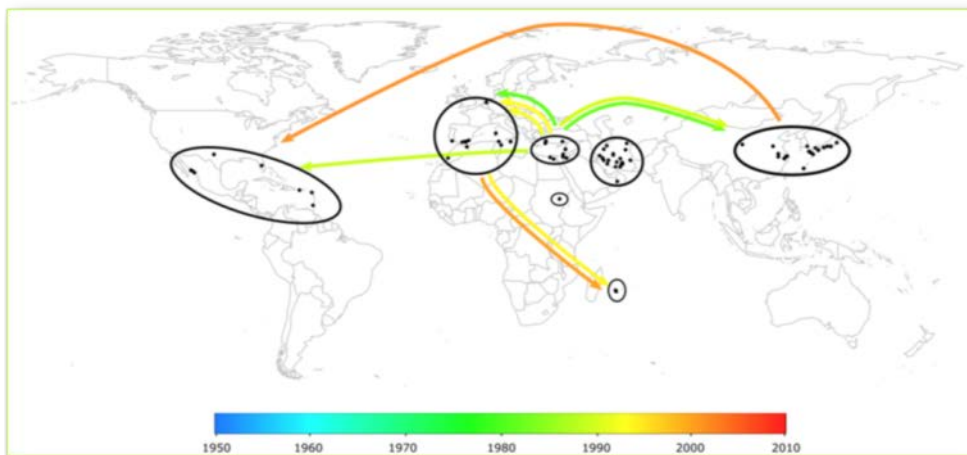


- **Geminiviridae** family,
- **Circular genome of ssDNA**
- **Twinned particles**
- **Monopartite or bipartite** genomes
- **Whitefly** transmitted (*Bemisia tabaci*) : circulative and persistent
- **Dicotyledonous** plants
- Associated with one or two type of **satellites** (Alpha, Beta)



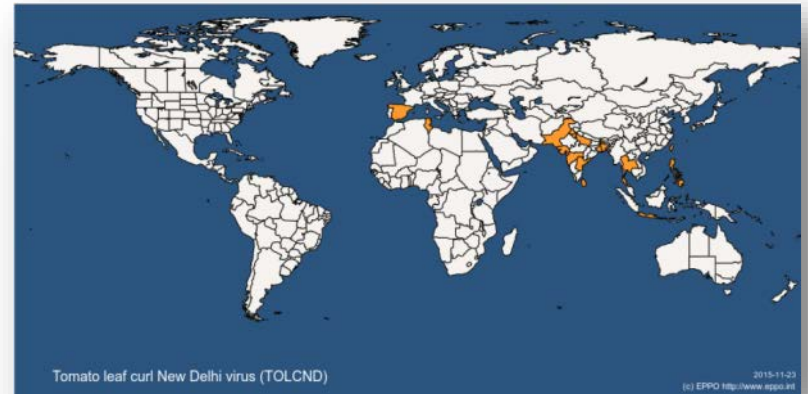
Tomato yellow leaf curl disease

- One of the **most harmful disease** of tomato widespread over the world (Hanssen *et al.*, 2010)
- Disseminated by its vector : *Bemisia tabaci*
- Associated with **several strains** of TYLCV (5) (Fauquet *et al.*, 2010) and species
- **Two main** species in Europe : **TYLCV** (strains Mld and IL) and **TYLCSV** (Just *et al.*, 2014) - Monopartite virus, 6 ORFs



Other Begomoviruses

- Three others Begomoviruses, not present in France, should be considered as a potential threat for the tomato production:
 1. **Tomato leaf curl New Delhi virus (ToLCNDV)**
 - Bipartite virus,
 - infecting solanaceous and cucurbitaceous plants (severe symptoms on Zucchini)
 - Emerging threat, already present in Europe (Spain)



Other Begomoviruses

- Three others Begomoviruses, not present in France, should be considered as a potential threat for the tomato production:
 1. Tomato leaf curl New Delhi virus (ToLCNDV)
 2. **Tomato mottle virus (ToMoV)**
 - Bipartite virus,
 - infecting solanaceous (mainly tomato)
 - Emerging threat in North and South America



Other Begomoviruses

- Three others Begomoviruses, not present in France, should be considered as a potential threat for the tomato production:
 1. Tomato leaf curl New Delhi virus (ToLCNDV)
 2. Tomato mottle virus (ToMoV)
 3. Tomato leaf curl disease (ToLCD)
 - Two different type: ToLCKMV (Comores) and ToLCMLV (Mali)
 - Monopartite viruses,
 - infecting solanaceous (mainly tomato)



Regulation

- Regulated under the directive 2000/29EC
- Included in different EPPO list according to their prevalence and risk analysis

Name		EPPO	EC	Prevalence	
				Europe	France
Tomato mottle virus	ToMoV	A1 list	Annex IA1	No	No
Tomato leaf curl virus*	ToLCV	-	Annex IA1	No	No
Tomato leaf curl New Delhi virus	ToLCNDV	Alert list	Annex IA1	Yes	No
Tomato yellow leaf curl sardinia virus	TYLCSV	A2 list	Annex IIA2	Yes	No
Tomato yellow leaf curl virus	TYLCV	A2 list	Annex IIA2	Yes	Yes

Test assessment: Performance criteria

- Definition of performance criteria evaluated (EPPO PM7/76 or ISO16140)
 - **Diagnostic sensitivity** Proportion of infected / infested samples testing positive compared to results from an alternative test (or combination of tests).
$$\text{Sensitivity} = \text{true positives (PA)} / (\text{true positives (PA)} + \text{false negatives (ND)})$$
 - **Diagnostic specificity** Proportion of uninfected samples (true negatives) testing negative compared to results from an alternative test (or combination of tests).
$$\text{Specificity} = \text{true negatives (NA)} / (\text{true negatives (NA)} + \text{false positives (PD)})$$
 - **Repeatability** Level of agreement between replicates of a sample tested under the same conditions
 - **Analytical sensitivity** or Limit of detection is the smallest amount of target that can be detected reliably

Antisera assessment

- Evaluation of the sensitivity and specificity of 4 different antisera commonly used by accredited laboratories for official controls of the TYLCV
 - **Antisera :**
 - Adgen (EPPO 7/50) (Sulfite)
 - Bioreba with two extraction buffers (Bioreba and sulfite)
 - Loewe (Sulfite)
 - Sediag (Sulfite)
 - **Targets**
 - TYLCV (11 Israel, 3 Mild), TYLCSV (2)
 - **Non targets**
 - Pepper (1), Tomato (3), ToSRV (1), ToMoV (1)

Antisera assessment

- evaluation of the sensitivity and specificity of 4 different antisera commonly used by accredited laboratories for official controls of the TYLCV

	Antisera ADGEN	Antisera BIOREBA			Antisera LOEWE	Antisera SEDIAG
	Sulfite buffer	Sulfite Buffer	Bioreba buffer		Sulfite buffer	Sulfite buffer
			Ind = PA	Ind = ND		
Samples	25	25	25	25	23	23
NA	7		7	7	6	
PA	6		10	6	5	
ND	11		8	12	12	
PD	1	2	0	0	0	0
Sensitivity	35%	83%	56%	33%	29%	18%
specificity	88%	71%	100%	100%	100%	100%

Antisera assessment

- Evaluation of the sensitivity, specificity and repeatability of the selected antisera
 - **Antisera :**
 - Bioreba with sulfite extraction buffers
 - **11 x 4 : Targets**
 - TYLCV (3 Israel, 1 Mild), TYLCSV (2), **ToLCNDV, ToMoV, ToLCV, ACMV**
 - **8 x 4 : Non targets**
 - TICV, ToCV, PepMV, EMDV, TSWV, Healthy (3)

Antisera assessment

	Sample	OD	Result	Sample	OD	Result
T a r g e t	EI-3 / TYLCV	0,107	pos	EI-3 / TYLCV	0,159	pos
		0,094	pos		0,161	pos
	EI-4 / TYLCSV	0,347	pos	EI-4 / TYLCSV	0,396	pos
		0,355	pos		0,434	pos
	EI-9 / TYLCV	0,002	neg	EI-9 / TYLCV	0,006	neg
		0,007	neg		0,006	neg
	EI-14 / TYLCV	0,201	pos	EI-14 / TYLCV	0,223	pos
		0,209	pos		0,272	pos
	EI-18 / TYLCSV	0,031	pos	EI-18 / TYLCSV	0,042	pos
		0,034	pos		0,046	pos
	EI-20 / ToLCV	0,002	neg	EI-20 / ToLCV	0,009	neg
		0,010	neg		0,008	neg
	13/398.8 - TYLCV	0,001	neg	13/398.8 - TYLCV	0,003	neg
		0,002	neg		0,001	neg
	EI-25 / ToLCV	0,869	pos	EI-25 / ToLCV	0,666	pos
		0,928	pos		0,719	pos
EI-28 / ToMoV	3,034	pos	EI-28 / ToMoV	3,328	pos	
	2,991	pos		3,386	pos	
EI-29 / ACMV	0,021	pos	EI-29 / ACMV	0,030	pos	
	0,025	pos		0,033	pos	
EI-35 / ToLCNDV	0,056	pos	EI-35 / ToLCNDV	0,056	pos	
	0,055	pos		0,062	pos	

Antisera assessment

• Conclusions

- Only one antisera can be recommended for routine analysis of TYLCD viruses
- Lack of sensitivity for some TYLCV isolates (false negatives) : 73% of sensitivity
- Cross reaction with different others Begomoviruses without possibility of correct identification

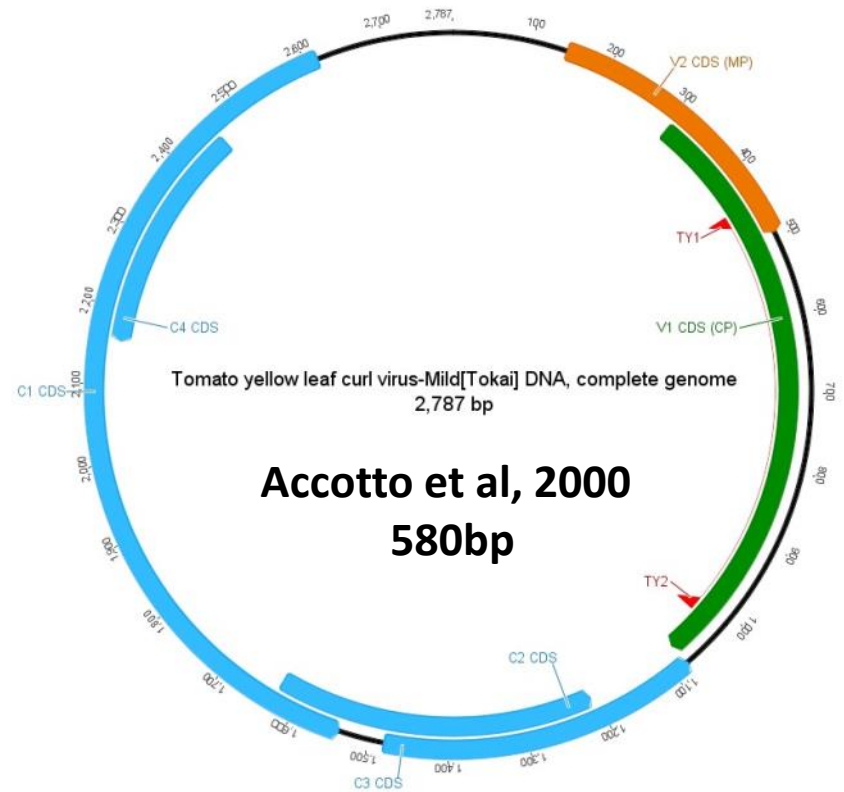
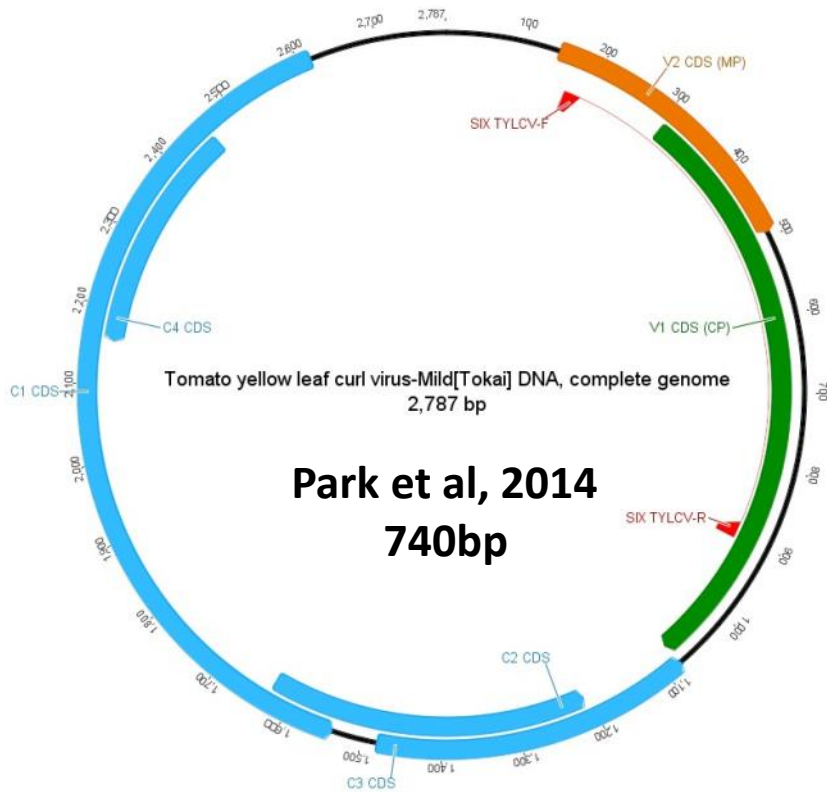
	ELISA Bioreba Sulfite Buffer
Samples	80
NA	36
PA	32
ND	12
PD	0
Sensitivity	73%
Specificity	100%
Repeatability	100%



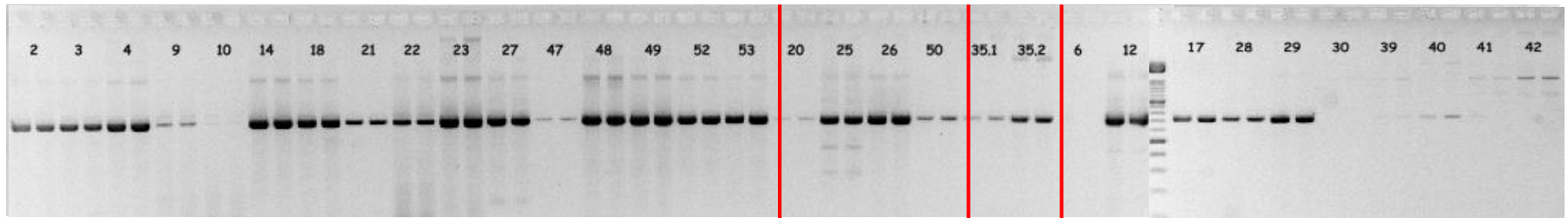
Development of a polyvalent PCR test

PCR assessment

- Evaluation of two selected PCR
 - Accotto et al. (2000) : Recommended by EPPO (PM 7/50)
 - Park et al. (2014)



PCR assessment: Park et al, 2014



TYLCV / TYLCSV

ToLCV

ToLCNDV

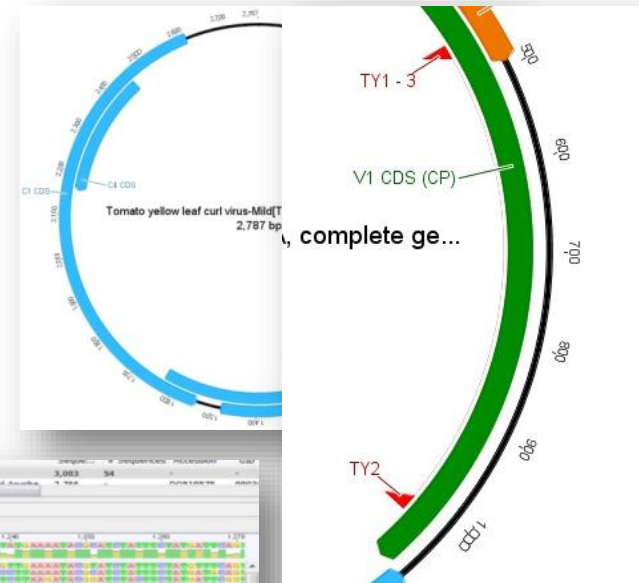
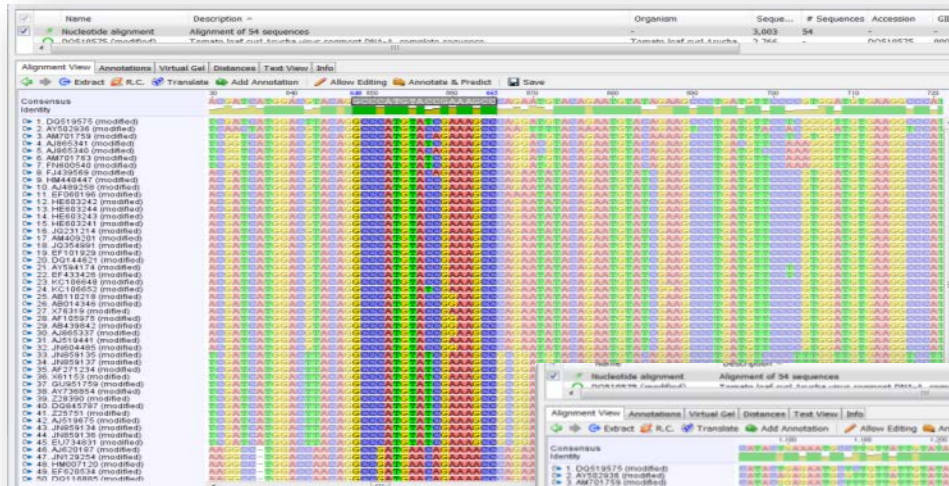
Sample	Begomovirus
EL-6	ToSRV
EL-12	ACMV
EL-17	ToCMoV
EL28	ToMoV
EL 29	ACMV
EL 30	SLCV
EL 39	WmCSV
EL 40	ICMV
EL 41	SLCMV
EL 42	BGMV

PCR assessment

Sample	Virus	PCR Accotto et al. (2000)	PCR Park et al (2014)	Sample	Virus	PCR Accotto et al. (2000)	PCR Park et al (2014)
EL-2	TYLCV	+++	+++	EL-20	ToLCV	+++	+
EL-3	TYLCV	+++	+++	EL-25	ToLCV	+++	+++
EL-4	TYLCSV	+++	+++	EL-26	ToLCV	+++	+++
EL-9	TYLCV	+++	+	EL-50	ToLCV	+++	+
EL-10	TYLCV	+	-	EL-35.1	ToLCNDV	-	+
EL-14	TYLCV	+++	+++	EL-35.2	ToLCNDV	-	+++
EL-18	TYLCSV	+++	+++	EL-28	ToMoV	-	+++
EL-21	TYLCSV	+++	+++	EL-17	ToMoV	+	+++
EL-22	TYLCV+TOCV	+++	+++	EL-6	ToSRV	+	-
EL-23	TYLCV	+++	+++	EL-12	ACMV	+++	+++
EL-27	TYLCV	+++	+++	EL-29	ACMV	+	+++
EL-47	TYLCSV	+++	+	EL-30	SLCuV	-	-
EL-48	TYLCV	+++	+++	EL-39	WmCSV	-	-
EL-49	TYLCSV	+++	+++	EL-40	ICMV	+++	+
EL-52	TYLCV	+++	+++	EL-41	SLCMV	+++	-
EL-53	TYLCV	+++	+++	EL-42	BGMV	-	-

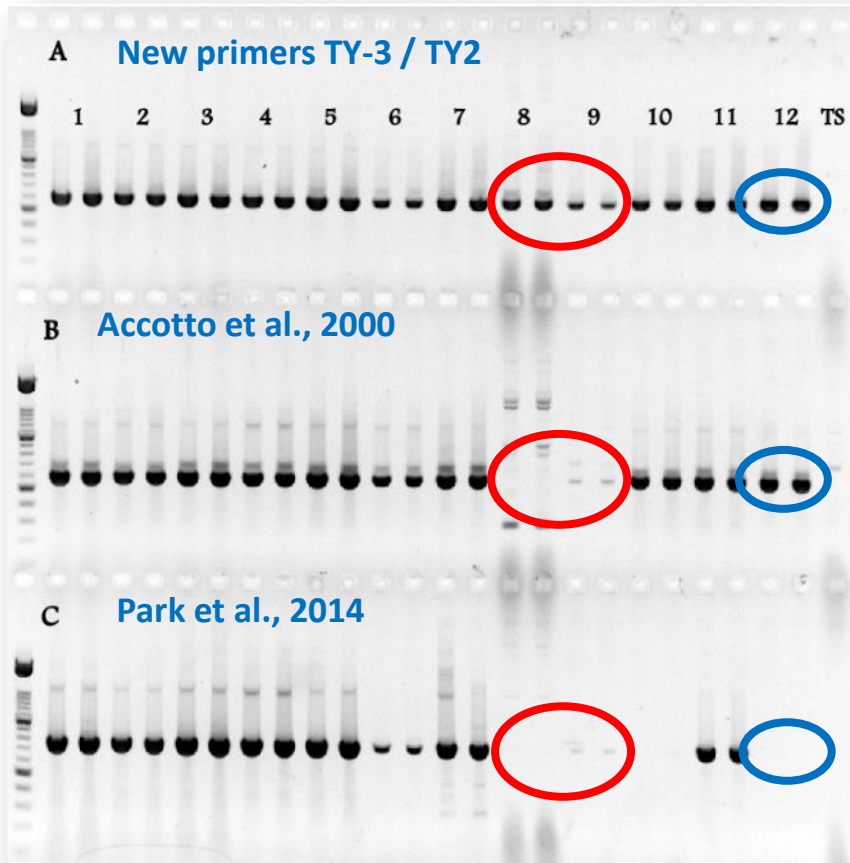
PCR assessment

A multiple sequence alignment of 29 TYLCV, 9 TYLCSV, 7 TOLCV and 9 ToLCNDV sequences available at NCBI database and from different geographical regions was made using the ClustalX program with Genious software.



PCR assessment

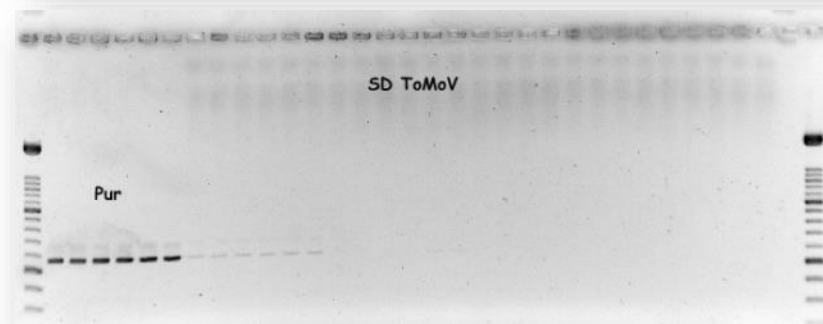
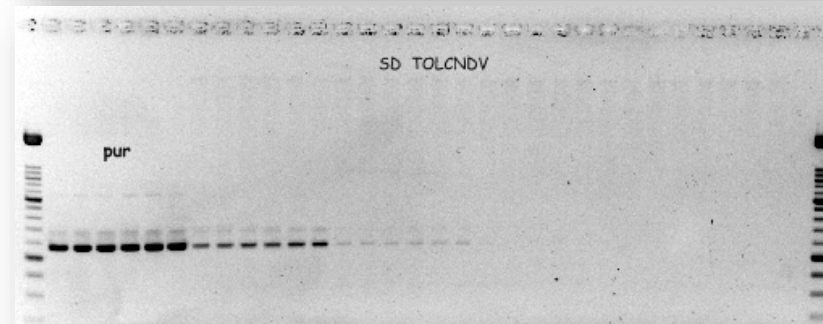
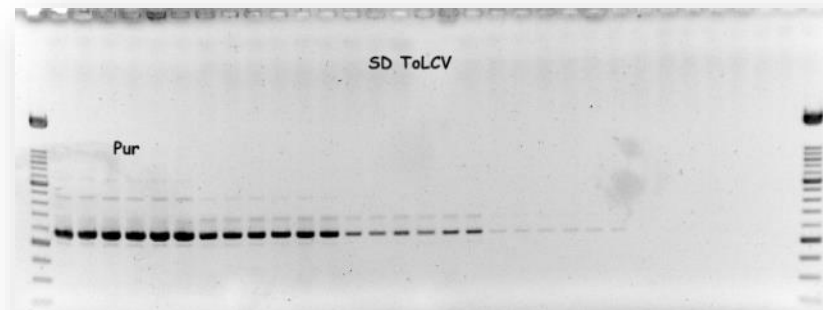
	Amorces		nt	Sequence	T° hybridisation	PCR Product
New primers	Forward	TY1-6	447	5'- GCC CAT GWA YMG RAA RCC -3'	58 °C	580 bp
New primers	Forward	TY1-3	447	5'- GCC CAT GTA YCG RAA RCC -3'		
Accotto et al, 2000	Reverse	TY2	1024	5'- GGR TTA GAR GCA TGM GTA C -3'		



Sample	N° Gel	Virus
EL-14	1	TYLCV
EL-18/19	2	TYLCSV
EL 23	3	TYLCV
EL 48	4	TYLCV
EL 49	5	TYLCSV
EL 20	6	ToLCV
EL 25	7	ToLCV
EL35	8	ToLCNDV
EL 28	9	ToMoV
EL-6	10	ToSRV
EL 29	11	ACuMV
15/103	12	BGMV

PCR assessment : analytical sensitivity

Sample	Virus	Species	Origin
EL-14	<i>TYLCV</i>	Tomate	PACA
EL-18	<i>TYLCSV</i>	Tomate	Espagne
EL 25	<i>ToLCV</i>	Tomate	Cirad
EL35	<i>ToLCNDV</i>	Courgette	Alicante, Spain
EL 28	<i>ToMoV</i>	Tomate	Floride USA



Analytical sensitivity New primers		Repeatability
TYLCSV	10^{-3}	100%
TYLCV	10^{-3}	100%
ToLCV	10^{-3}	100%
ToLCNDV	10^{-2}	100%
ToMoV	10^{-1}	100%

PCR assessment

- Evaluation of the sensitivity, specificity, repeatability of the new primers against a list of target and non target samples

N°	Virus
EL 32	ToCV
EL 31	TICV
EL 33	PepMV
EL 34	EMDV
EL 36	TS
EL 36bis	TS
EL 37	ToTV
EL 38	TSWV
EL 43	AMV
EL 44	CMV
EL 45	ZYMV
EL 56	ToMV
EL 57	PVY "C"
EL 58	PRSV
EL 59	PVX
EL 70	TS

16 Non targets

2 repetitions

N°	Virus	N°	Virus
EL-4	TYLCSV	EL-12	ACMV
EL 21	TYLCSV	EL 29	ACMV
EL 47	TYLCSV	EL 30	SLCV
EL 49	TYLCSV	EL-17	ToCMoV
EL-18	TYLCSV	EL35 Ext 1	ToLCNDV
EL-2	TYLCV	EL35 Ext 2	ToLCNDV
EL-3	TYLCV	EL 20	ToLCV
EL-9	TYLCV	EL 25	ToLCV
EL-11	TYLCV	EL 26	ToLCV
EL-13	TYLCV	EL 50	ToLCV
EL-14	TYLCV	EL 51	ToLCV
EL 23	TYLCV	EL 28	ToMoV
EL 27	TYLCV	EL-6	ToSRV
EL 48	TYLCV	EL 39	WmCSV
EL 52	TYLCV	EL 40	ICMV
EL 53	TYLCV	EL 41	SLCMV
EL 54	TYLCV	EL 42	BGMV
EL 60	TYLCV		
EL 61	TYLCV		
EL 62	TYLCV		
EL 63	TYLCV		
EL 22	TYLCV+ToCV		

39 Targets

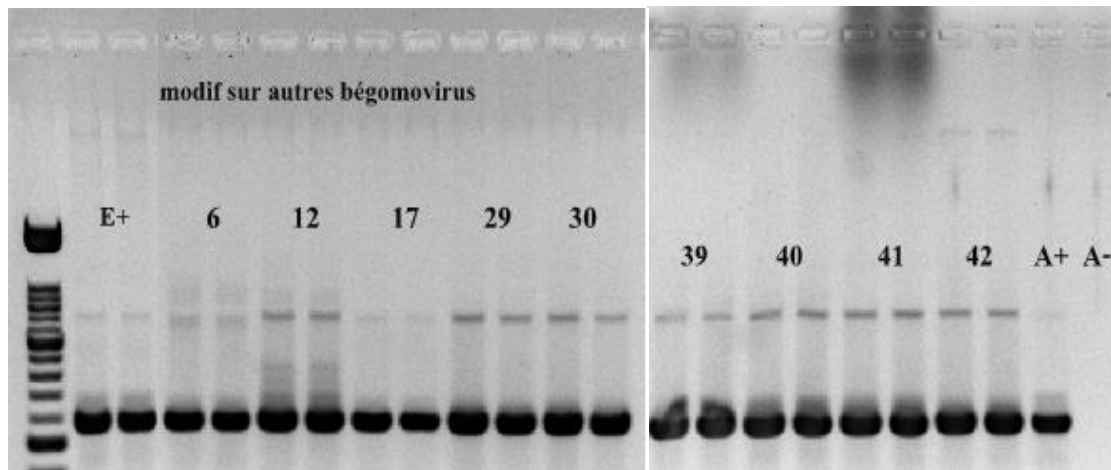
PCR assessment

	New primers	ELISA Bioreba TP Sulfite
Samples	90	76
NA	30	36
PA	60	28
ND	0	12
PD	0	0
Sensitivity	100%	70%
specificity	100%	100%
Repeatability	100%	100%

- Detection of a wide range of TYLCD associated viruses, ToLCD associated viruses, ToLCNDV, ToMoV and some others Begomoviruses

PCR assessment

Sample	Virus	PCR Accotto et al., 2000	PCR Park et al., 2014	New primers
EL-6	Tomato severe rugose virus (ToSRV)	+	-	+++
EL-12	African cassava mosaic virus (ACMV)	+++	+++	+++
EL-17	Tomato chlorotic mottle virus (ToCMoV)	+	+++	+++
EL 29	African cassava mosaic virus (ACMV)	+	+++	+++
EL 30	Squash leaf curl virus (SLCV)	-	-	+++
EL 39	Watermelon chlorotic stunt virus (WmCSV)	-	-	+++
EL 40	Indian cassava mosaic virus (ICMV)	+++	+	+++
EL 41	Sri Lankan cassava mosaic virus (SLCMV)	+++	-	+++
EL 42	Bean golden mosaic virus (BGMV)	-	-	+++



Conclusion

- Among 4 different antisera, only one reach an acceptable level of sensitivity
- None antisera leads to distinguish TYLCV from other Begomoviruses
- A set of polyvalent degenerated primers was developed to allow the detection of the main Begomoviruses presenting a threat for the EU : TYLCV, TYLCSV, ToLCNDV, ToMoV, ToLCD associated viruses
- This set allow the detection of a range of 8 Begomoviruses



**Thank you for your
attention**



Anne Saison