# Assessment of performance criteria of '*Candidatus* Liberibacter solanacearum' detection methods

Loiseau M., Cousseau-Suhard P., Renaudin I., Lucas P.-M., Gentit P.

- ANSES-LSV, 7 rue Jean Dixméras, 49044 Angers cedex 01 - France

Ca. L. solanacearum: « Zebra chip » diseases

Haplotypes A and B: *Ca.* L. solanacearum on *Solanaceae*

- Regulations: list A1 of EPPO – In France, survey of potatoes import

- Geographical distribution: North and Central America, New Zealand

- Vector: *Bactericera cockerelli* (absent from European territory)

- Transmission by potato tubers

- Symptoms on potatoes:
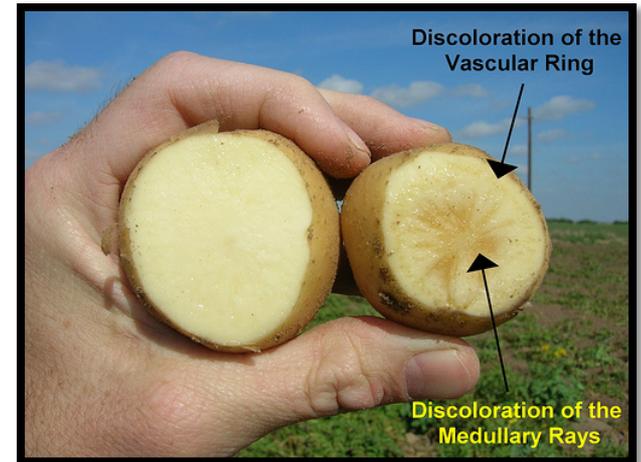
Source : Crosslin *et al.*, 2010

Discoloration of the Vascular Ring

Discoloration of the Medullary Rays

- Economic impact: up to 80% loss, marketing pb, export pb, quality of seeds and tubers degraded…

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Ca. L. solanacearum: vegetative disorders on *Apiaceae*

Haplotypes C, D and E : *Ca.* L. solanacearum on *Apiaceae*

- Regulations: unregulated

- Geographical distribution: Europe (Finland, Norway, Sweden, Spain, France and Germany) and Morocco

- Vectors: *Trioza apicalis*, *Bactericera trigonica*

- Symptoms:

Source : ANSES-LSV, 2014

- Economic impact: still difficult to assess, little or no seed production, export pb, marketing difficulties of roots...

Evaluated methods

- ## DNA extraction methods:
  - CTAB 3%
  - Dneasy plant mini kit (Qiagen®)
  - QuickPick™ SML Plant DNA (Bio-Nobile®)

- ## DNA Amplification methods:
  - End point PCR: Ravindran *et al.*, 2011 with primers targeting 16S-23S ITS;
  - Real-time PCR: Li *et al.*, 2009 and Teresani *et al.*, 2014, both targeting 16S rDNA

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Previous results

Based on literature

Method	Inclusivity (give a positive result with the method)	Specificity (give a negative result with the method)	Analytical sensitivity
Li *et al.*, 2009	16 *Ca.* L. solanacearum from potato 2 *Ca.* L. solanacearum from tomato	3 *Ca.* L. asiaticus 3 *Ca.* L. africanus 3 *Ca.* L. americanus Potato leaf roll virus Clover proliferation phytoplasma *Ca.* P. americanum *Xylella fastidiosa* PD strain *Xyllela fastidiosa* CVC strain	100 fold more sensitive than end point PCR of Liefting *et al.*, 2008
Ravindran *et al.*, 2011	9 isolats of *Ca.* L. solanacearum from potato	1 *Ca.* L. asiaticus 1 potato	40 fold more sensitive than end point PCR of Liefting *et al.*, 2008
Teresani *et al.*, 2014	3 *Ca.* L. solanacearum from celery 2 *Ca.* L. solanacearum from psyllids 4 *Ca.* L. solanacearum from carots	13 *Ca.* L. asiaticus 2 *Ca.* L. africanus 2 *Ca.* L. americanus 16 strains of bacterial species 81 unidentified bacterial	The same as Li *et al.*, 2009

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Evaluation of DNA extraction methods

- samples:
  - 2 samples of leaves from infected carrots diluted in healthy carrot leaves;
  - 2 infected carrot seeds samples diluted in PBS buffer;
  - 2 infected samples from apiaceous diluted in PBS buffer.

- 10 fold dilution series to pur sample from 10^{-3} ; 2 repetitions.

- Evaluation of analytical sensitivity

Evaluation of DNA amplification methods

- 15 target samples, 2 repetitions

Bacterial strains or healthy plant (-)	Sample type	origine
Ca. L. solanacearum	Carrot seeds	France
	Carrot leaves	France
	Celery leaves	France
	B. trigonica	France
	B. trigonica	Canary Islands
	Carrot leaves	Canary Islands
	Celery leaves	Austria
	Carrot leaves	Austria
	Carrot seeds	-
	B. cockerelli	NZ
	Tomato	NZ
	Potato leaves	NZ
	Solanaceous	-
	Apiaceous	-
	Carrot seeds	France

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Evaluation of DNA amplification methods

- 15 non target samples, 2 repetitions

Bacterial strains or healthy plant (-)	Sample type	origine
Ca. L. europaeus	Pear	Italy
L. crescens	Carica x heilbornii var. pentagona	USA, Puerto Rico DSMZ 26877
Ca. L. asiaticus	*Citrus*	LSV-RAPT
Ca. L. africanus	*Citrus*	LSV-RAPT
S. citri	*Citrus*	LSV-SQ
-	Carrot seeds	-
-	Carrot leaves	France
-	Celery leaves	France
-	Tomato leaves	France
-	Potato leaves	France
-	Apiaceous	France
-	Celery	France
Ca. Phytoplasma asteris	Oinion	France
Ca. Phytoplasma solani	Tomato	France
-	Solanaceous	France

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Performance criteria

Ref.: EPPO PM 7/98, ANSES Guide NB1/V09

» Analytical specificity: diagnostic sensitivity and diagnostic specificity

» Analytical sensitivity

» Repeatability

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Results : DNA extraction methods

Sample			CTAB		DNeasy kit		QuickPick kit	
Type of samples comtaminated by LSO	Level of dilution	Use to dilute	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Carrot leaves	pur		+	+	+	+	+	+
	10^{-1}		+	+	+	+	-	+
	10^{-2}		+	+	+	+	-	-
	10^{-3}		+	+	+	+	-	+
	pur	Carrot leaves	+	+	+	+	+	+
	10^{-1}		+	+	+	+	-	+
	10^{-2}		+	+	+	+	-	-
	10^{-3}		+	+	+	+	-	+
Carrot seeds	pur		(+)	+	+	+	+	+
	10^{-1}		+	+	+	+	+	+
	10^{-2}		-	+	+	+	+	+
	10^{-3}		-	-	-	+	+	+
	pur	PBS	(+)	+	+	+	+	+
	10^{-1}		+	+	+	+	+	+
	10^{-2}		-	+	+	+	+	+
	10^{-3}		-	-	-	+	+	-
Celery	pur		+	+	+	+	+	+
	10^{-1}		+	-	+	+	+	+
	10^{-2}	PBS	-	-	-	-	+	-
	10^{-3}		-	-	-	-	-	-
Apiaceous	pur		+	+	+	+	+	+
	10^{-1}		+	+	+	+	+	+
	10^{-2}	PBS	-	+	+	+	+	+
	10^{-3}		-	-	-	+	+	+

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Results : DNA extraction methods

Sample contaminated by LSO		CTAB	Dneasy	QuickPick
Analytical sensitivity	Carrot leaves	10^{-3}	10^{-3}	pur
	Carrot seeds	10^{-1}	10^{-2}	10^{-2}
	Celery	pur	10^{-1}	10^{-1}
	Apiaceous	10^{-1}	10^{-2}	till 10^{-3}

➜ After dilution of the pur DNA extracts in DNase free (10^{-1}), QuickPick is as sensitive as the other methods.

Results : DNA amplification methods

Method	Li *et al.*, 2009	Teresani *et al.*, 2014	Ravindran *et al.*, 2011 (ITS 16S-23S)
NA	30	30	30
PA	30	24	20
ND	0	6	10
PD	0	0	0

	NA	PA	ND	PD
Expected result	-	+	+	-
Obtained result	-	+	-	+

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Results : DNA amplification methods

Type of sample contaminated by LSO	Dilution	Use to dilute	Li et al., 2009*	Teresani et al., 2014*	Ravindran et al., 2011*
DNA of carrot seeds	pur		6/6	6/6**	6/6
	10^{-1}	« Healthy » DNA of carrot seeds	6/6	6/6	6/6
	10^{-2}		6/6	6/6	6/6
	10^{-3}		6/6	5/6	1/6
	10^{-4}		3/6	3/6	0/6
DNA of carrot leaves	pur		6/6	6/6	6/6
	10^{-1}	« Healthy » DNA of carrot leaves	6/6	6/6	6/6
	10^{-2}		6/6	6/6	6/6
	10^{-3}		6/6	6/6	6/6
	10^{-4}		6/6	5/6	3/6

*: nb of positive results/nb of repetitions
**: obtained after a 10 fold dilution.

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Results : DNA amplification methods

Method	Li *et al.*, 2009	Teresani *et al.*, 2014	Ravindran *et al.*, 2011
Diagnostic sensitivity	100%	80%	67%
Diagnostic specificity	100%	100%	100%
Analytical specificity	100%	90%	83%
Analytical sensitivity (seeds)	10^{-3}	10^{-2}	10^{-2}
Analytical sensivity (leaves)	Till 10^{-4}	10^{-3}	10^{-3}
Repeatability	97%	97%	95%

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Conclusion

- DNA extraction methods:
  - Kits tested for DNA extraction are reliable.
  - Carefull : problems of inhibition !!

- DNA amplification methods:
  - Teresani *et al.*, 2014 and Ravindran *et al.*, 2011 need to be improved in order to increase sensitivity;
  - Li *et al.,* 2009 seems to be the method of choice to detect *Candidatus* Liberibacter solanacearum in our conditions.

Discussion

- Automatized extraction method should be tested in order to test large number of samples.

- Optimization of amplification methods targeting different parts of the genome in order to confirm results on 16S rDNA.

- A interlaboratory study is necessary to complete assessment datas.

- Multiplex real-time PCR targeting phytoplasmas, *Ca.* L. solanacearum and plant control will be assessed.

Thank you ….

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- Technicians colleagues in ANSES LSV UBVO : Pascaline Cousseau-Suhard and Isabelle Renaudin
- Student: Pierre-Marie Lucas

And thank you all for your attention….

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