Transmission tests of « *Candidatus* Liberibacter solanacearum » by carrot seeds

Source : DRAAF-SRAL Centre

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Seed transmission trial : Main targets

1) Contamination rate of seed lots,

2) In-situ localization of the bacteria in seed tissues,

3) Evaluation of the transmission rate of the bacteria to seedlings,

4) Evaluation of the transmission rate of the bacteria to the progeny.

3 seed lots coming from infected mother plants. All materials detected positive for LSO.

Seed transmission trial: Materials

Type	Lot n°	Cultivar denomination	Origin
Seed	13/444-1S	R12 male	Parents of 12/434P
Seed	13/444-2S	R12 female	Parents of 12/434P
Seed	13/305-1S	120047	Parents of 12/464-1P
Seed	13/305-2S	211998B	Parents of 12/464-2P
Seed	13/305-3S	211998A	Parents of 12/464-2P
Plant	12/434P	CAC 3075	Mother seed plant, France
Plant	12/464-1P	WRT00427	Mother seed plant, France
Plant	12/464-2P	CAC 3075	Mother seed plant, France
Seed	**12/434S**	**CAC 3075**	**Mother seed plant, France**
Seed	**12/464-1S**	**WRT00427**	**Mother seed plant, France**
Seed	**12/464-2S**	**CAC 3075**	**Mother seed plant, France**
seed	2013S	‘Touchon’	commercial

Seed transmission trial: Methods

<u>Obj. 1 - Contamination rate of seed lots</u> : Methode MNP (most probable number – Swaroop, 1951)

<u>Obj. 2 - In-situ localization of the bacteria in seed tissues</u> : 50 g of seeds were germinated on moist paper at +20°C in the laboratory. After 7 days, on germinated seeds, seed coat tissue, cotyledons and radicle were separated. Rapid DNA extraction (spot method; Bertolini *et al*., 2014). Specific detection of LSO (Li *et al.*, 2009).

<u>Obj. 3 - Evaluation of the transmission rate of the bacteria to seedlings:</u>
Sowing of 3 x 100g seeds in greenhouses (NS2) for 3 months. The seedlings were collected after 30, 60 and 90 days (destructive test). DNA extraction (CTAB method). Specific detection of LSO (Li *et al*., 2009).

<u>Obj. 4 - Evaluation of the transmission rate of the bacteria to the progeny:</u>
Seedlings taking into account the germination rate of each batch to collect 100 samples each month after the 4th month until the next generation. Every month, 1g of leaves on each plant was collected. DNA extraction (CTAB method). Specific detection of LSO (Li *et al*., 2009).

Results (1):
The rate of contamination of seeds

Type	Lot n°	Cultivar denomination	Origin	Average Ct value
Plant	12/434P	CAC 3075	Mother seed plant, France	19.9
Plant	12/464-1P	WRT00427	Mother seed plant, France	22.8
Plant	12/464-2P	CAC 3075	Mother seed plant, France	20.9
Seed	**12/434S**	**CAC 3075**	**Mother seed plant, France**	**26.2**
Seed	**12/464-1S**	**WRT00427**	**Mother seed plant, France**	**24.6**
Seed	**12/464-2S**	**CAC 3075**	**Mother seed plant, France**	**23.3**
seed	2013S	‘Touchon’	commercial	No amplification

➔ 3 seed lots from plants infected by *Ca.* L. solanacearum.

Results (1):
The rate of contamination of seeds

Lot	Sampling[a]	No. of positive samples	MPN in 100 seeds[b]	Confidence interval[b]
12/434	1 batch of 500 5 batches of 100 5 batches of 10	1 5 5	> 16.1	3.9 to >45
12/464-1	1 batch of 500 5 batches of 100 5 batches of 10	1 5 5	> 16.1	3.9 to >45
12/464-2	1 batch of 500 5 batches of 100 5 batches of 10	1 5 5	> 16.1	3.9 to >45

[a]Number of batches each containing a defined number of seeds
[b]Most probable number (MNP) and associated confidence interval were obtained from reference tables.

➔High level of contamination for each lot. In Bertolini *et al*. (2014), by testing individually the seeds, infection rates ranged from 47-93%.

Results (2):
Localization of LSO in seed tissues

Lot	Individual sample			Pooled samples		
	Nb of positive on cotyledon tissue	Nb of positive on root tissue	Nb of positive on coat tissu	Cotyledon tissue	Root tissue	Coat tissue
12/434	0/10	0/10	2/10	negative	negative	negative
12/464-1	0/10	0/10	0/10	negative	negative	negative
12/464-2	0/10	0/10	2/10	negative	negative	negative

➔ Reduced sensitivity due to the DNA method of extraction.

➔ In Bertolini *et al.* (2014), following analysis by electron microscopy, the bacteria was found in the coat tissues.

Results (3):
Transmission of LSO: From seed to seedling

Period	1st month	2nd month	3rd month	4th month	5th to 7th month	8thand 9th months
Conditions	Greenhouse Destructive individual tests			Overwintering Individual tests (1g)		Greenhouse Individual tests (1g)
12/434	0/10	0/19	0/11	0/100	0/100 (x3)	0/100 (x2)
12/464-1	0/66	0/59	0/31	1/100	0/100 (x3)	0/100 (x2)
12/464-2	0/25	0/47	0/41	0/100	0/100 (x3)	0/100 (x2)

➔ Only 1 non-reproducible positive result;

➔ Different results from those published by Bertolini (1st positive at 1 month and 4 to 10% positive seedlings after 5 months)

<u>Hypothesis :</u>

- agronomical conditions;
- overwintering period of sowings...

Results (4):

Transmission of LSO : From one generation to another

Denomination F1 Seeds	Denomination F2 seeds	LSO detection on F1
13/444-1 (R12 male)	12/434	negative
13/444-2 (R12 female)		negative
13/305-1 (120047)	12/464-1	negative
13/305-2 (211998B)	12/464-2	negative
13/305-3 (211998A)		negative

➔ "Parents" of carrots found infected in 2012 were not contaminated.

➔ The trial was stopped due to agronomical problems for growing carrots in greenhouse.

Conclusions

1) Seedlots collected from plots infected by *Ca.* L. solanacearum are infected by the bacteria at high levels.

2) The bacterium is located in the seed coat of carrot.

3) The results of seed transmission obtained by Bertolini *et al* (2014) was not reproduced under our conditions.

4) The previous generation (mother plants) was not infected by the bacteria.

Prospects

1) Reproduce the test of seed transmission in the same agronomical conditions than those used in Spain, with a “freshly” infected seed lot.

2) Test the impact of a cold period on the viability of LSO on seeds and its transmission rate by seed.

3) In France, find the possible inoculum sources: epidemiological studies.

Thank you for your attention…