



Final report

Laboratory tests during the greenhouse trial

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Final report, laboratory tests.

This report describes the outcome of two laboratory tests performed on material from the greenhouse trial performed at FC Aarslev. This report is part of the fulfillment of the contract between CPVO and RC Flakkebjerg of 21. august 2003.

The two laboratory tests consists of:

Test 1: Analysis for the presence of phytoplasmas in phytoplasma-free varieties

Test 2: Test of transmission of phytoplasma after grafting.

The report contains the following items:

- Outcome of the analysis
- A full protocol of the methods used for the sampling, methods/equipment used, method of analysis of the results
- Annex with detailed description of detection method.

Mogens

15/11-04 Mogens Nicolaisen

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Material:

Leaf samples was sent from Lars Jacobsen, FC Aarslev from putatively phytoplasma-free plants (KLEW 01071, KLEW 01073, Cortez/Fiscor, Freedom, C-27 Celebrate) and from plants after grafting onto phytoplasma-infected rootstocks to check for phytoplasma transmission (samples 1-30, in total there were 24 scion/rootstock combinations but in some cases 2 samples were taken from one combination).

Method:

DNA from samples was extracted using the DNeasy method according to the manufacturers protocol (QIAGEN, Hilden, Germany).

Phytoplasma content was tested by the TaqMan method (see annexe for details). This method is very sensitive and semiquantitative.

Results:

Test 1 (test for freedom of phytoplasma):

Using the TaqMan method no phytoplasma was detected in leaf samples from KLEW 01071, KLEW 01073, Cortez/Fiscor, Freedom, C-27 Celebrate.

Test 2 (test for transmission of phytoplasma):

TaqMan assay for presence of phytoplasma in leaves from scions of poinsettia

| Positive | negative | doubtful |
|-------------------------------------|-------------------------------------|------------|
| First test, april 14, 2004: | | |
| 3, 5, 6, 7, 8, 12, 14, 15, 20 | 1, 4, 9, 10, 11, 13, 16, 17, 19, 21 | 2, 18 |
| Second test, may, 11, 2004 | | |
| 2, 9, 10, 18, 21, 23 | | 11, 16, 22 |
| Third test, june 8, 2004 | | |
| 11, 16, 22, 24, 26, 29, 30 | | 25, 27, 28 |
| Fourth test, august 23, 2004 | | |
| 27, 28 | 25 | |

The test shows, that over time most samples have become infected by phytoplasma.

Positive samples include:

2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 21, 22, 23, 24, 26, 27, 28, 29, 30.

Negative samples include:

1, 3, 4, 13, 17, 19, 25.

The identity of individual samples is not known by FC Flakkebjerg (Mogens Nicolaisen), but Lars Jacobsen knows which samples corresponds to which rootstock/ scion combinations.

Annex 1

TaqMan detection of phytoplasma

Use 1 μ l of DNeasy isolated DNA

TaqMan assay (example):

| | | |
|--|--|--|
| 1 well: 12.5 μ l TaqMan Universal PCR Master Mix (4304437) 1.5 μ l Phytoplasma forward primer (5 μ M stock) 4.5 μ l Phytoplasma reverse primer (5 μ M stock) 1 μ l Phytoplasma probe (5 μ M stock) 4.5 μ l water 1 μ l template | Mastermix (13 wells) 162.5 μ l PCR master mix 19.5 μ l forward primer 58.5 μ l reverse primer 13 μ l probe 58.5 μ l H ₂ O aliquot 24 μ l | Mastermix (50 wells): 625 μ l PCR master mix 75 μ l Forward primer (5 μ M) 225 μ l Reverse primer (5 μ M) 50 μ l probe 225 μ l water aliquot 24 μ l |
|--|--|--|

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|--|---|---|---|---|---|---|---|---|---|----|----|----|
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Set the TaqMan machine to run 25 μ l reactions.

Phytoplasma 16S primers/probe:

Forward: 5' CGTACGCAAGTATGAAACTTAAAGGA

Probe: 5' TGACGGGACTCCGCACAAGCG FAM TAMRA

Reverse: 5' TCTTCGAATTAACAACATGATCCA