Ref:	2008 175 Liberibacter sp.
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Subject:	**Liberibacter sp. graft transmission experiments – final results**

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1. PURPOSE

This document reports the final results of an experiment designed to investigate whether the '*Candidatus* Liberibacter' sp. identified in New Zealand solanaceous plants is transmitted by grafting. The purpose of this investigation is to provide information on the epidemiology of the disease caused by the liberibacter and thus assist in its management.

2. METHOD

Detection method

Total DNA was extracted from leaf midribs and petioles using the InviMag® Plant DNA Mini Kit (Invitek GmbH, Berlin, Germany) according to the manufacturer's instructions using a Thermo KingFisher mL automated nucleic acid extraction workstation.

A forward primer OA2 (5′-GCGCTTATTTTTAATAGGAGCGGCA-3′) was designed from the 16S rRNA sequence of the liberibacter (Liefting *et al.*, 2008). In combination with primer OI2c (5′-GCCTCGCGACTTCGCAACCCAT-3′) (Jagoeix *et al.*, 1996), this primer is expected to amplify an 1160-bp product. Amplification was performed in 20 µl reactions containing 2 × GoTaq Green Master Mix (Promega Corporation, Madison, WI), 0.25 µM of each primer, and 2 µl of template. The PCR conditions were: an initial cycle at 94°C for 5 min, then 40 cycles of 94°C for 30 s, 60°C (tomato) or 66°C (capsicum) for 30 s, and 72°C for 1 min, plus an additional cycle of 7 min at 72°C.

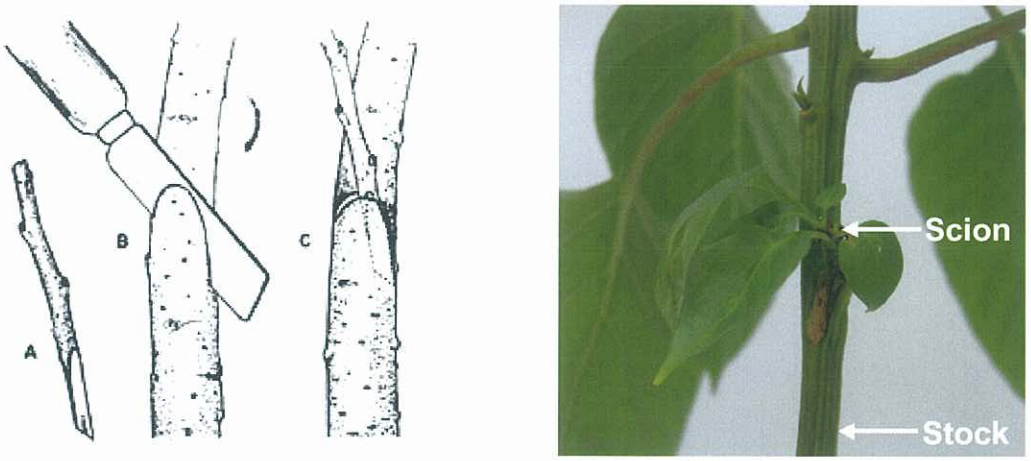
The PCR competency of all the DNA extractions was determined by an internal control assay using the general eukaryotic 28S rDNA primers 28Sf (5′-CCCTGTTGAGCTTGACTCTAGTCTGGC-3′) and 28Sr (5′-AAGAGCCGACATCGAAGGATC-3′) described by Werren *et al.* (1995). These primers yield a 500-600-bp product, depending upon the presence of expansion domains. The internal control PCR was performed simultaneously in separate tubes with the liberibacter-specific PCR using the same reaction and cycling conditions.

All amplifications were performed in a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA). The amplified DNA was analysed by agarose gel electrophoresis and bands were visualised with SYBR Safe (Invitrogen).

Grafting and testing of plants

Capsicum and tomato stock plants were grown from seed in pasteurised planting media (1:1 [v/v] peat:pumice supplemented with 6 kg/1 m^3 of Osmocote® Exact® 8-9 month slow-release fertiliser) and maintained in an insect-proof glasshouse at 23°C (± 2°C). Grafting was performed using the side-wedge technique (Figure 1) when the stock plants were approximately 15-20 cm high. Before grafting the stock plants were tested by PCR to confirm that they were negative for liberibacter. The scion was either from liberibacter-negative or liberibacter-positive plants. The grafts were wrapped with Parafilm to hold them in place and to prevent desiccation. The entire plant was covered with a plastic bag for 5 days after grafting. Approximately 4-6 weeks after grafting, leaf samples were taken from the stock plants above the graft union and tested by PCR as described above.

Figure 1: A schematic diagram of the side-wedge grafting technique (left) and a grafted capsicum plant (right). The left-hand image is taken from the University of Minnesota Extension Service Bulletin No. 532 (http://counties.cce.cornell.edu/Wyoming/agriculture/resources/ipd/graftingandbuddingfrutitrees.htm).



3. RESULTS

Capsicum and tomato plants were tested 36 and 38 days after grafting, respectively. The stocks of all five plants grafted with liberibacter-infected material tested positive (Table 1). The plants that remained ungrafted and those grafted with liberibacter-negative material all tested negative for the presence of the liberibacter (Table 1).

Table 1: Results of graft transmission studies.

Host	Treatment	No. positive/ No. tested
Tomato	Ungrafted	0/5
	Grafted with liberibacter-negative material	0/5
	Grafted with liberibacter-positive material	5/5
Capsicum	Ungrafted	0/5
	Grafted with liberibacter-negative material	0/5
	Grafted with liberibacter-positive material	5/5

4. DISCUSSION

Pioneering work on huanglongbing carried out from 1941 to 1955 determined that liberibacter is graft-transmissible (reviewed by Bové, 2006) and was studied more recently by Lopes and Frare (2008). Liberibacters only inhabit the phloem tissue and the pathogen systematically moves from the inoculation site to different parts of the plant along with the photosynthate flow.

5. CONCLUSION

The results of these experiments indicate that the liberibacter species identified in New Zealand solanaceous crops is graft-transmissible. These results support the conclusion that the disease observed in New Zealand is caused by a graft-transmissible liberibacter, rather than resulting from abiotic stress. Grafting is a common production practice in commercial tomato crops. These results underlie the importance for growers to ensure stock plants are free of the disease prior to grafting.

6. REFERENCES

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