An update on phytoplasma diseases in New Zealand

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Abstract

‘Candidatus Phytoplasma australiense’ occurs in New Zealand and Australia where it is associated with plant diseases in native, weed and crop plants. Between January 2009 and July 2010, four new diverse hosts of ‘Ca. P. australiense’ have been identified in New Zealand: potato, Jerusalem cherry, swan plant and celery, as well as a new disease association in boysenberry. A 1.2 kb region of the 16S rRNA gene of the phytoplasma amplified from the new hosts were identical to each other. Partial tuf gene sequence analysis of 29 strains from the new plant hosts revealed that they belong to two separate subgroups, tuf variant VII and tuf variant IX. Two of the strains, one from potato and the other from celery, contained a mixed infection of both phytoplasma subgroups.

Key words: Mollicute, PCR, detection, epidemiology.

Introduction

‘Candidatus Phytoplasma australiense’ (16SrXII-B) is found in New Zealand and Australia where it is associated with a range of host plants. In New Zealand, the phytoplasma is historically associated with the diseases, Phormium yellow leaf, strawberry lethal yellows, Cordonine sudden decline, and Coprosma lethal decline (Liefting et al., 2007). In Australia, this phytoplasma species has been associated with Australian grapevine yellows, Papaya dieback, as well as diseases in a range of other hosts including strawberry, pumpkin and bean (Streten and Gibb, 2006).

Sequence analysis of the tuf gene of different strains of ‘Ca. P. australiense’ in New Zealand determined that there are nine different tuf variant groups (I-IX) that cluster into two distinct clades (Andersen et al., 2006). Andersen et al. (2006) also analysed the available tuf gene sequences from Australian strains and determined that some strains formed a third distinct tuf gene clade while other strains clustered into one of the clades formed by the New Zealand isolates.

Here we provide an update on four new diverse hosts of ‘Ca. P. australiense’ in New Zealand: potato (Solanum tuberosum), Jerusalem cherry (Solanum pseudocapsicum), swan plant (Gomphocarpus fruticosus) and celery (Apium graveolens), as well as a new association of ‘Ca. P. australiense’ in boysenberry (Rubus hybrid). This phytoplasma was previously detected in boysenberry exhibiting different symptoms that were attributed to the fungus Cercospora rubi (Wood et al., 1999). The tuf gene sequences of ‘Ca. P. australiense’ from these new hosts were analysed extending the work of Andersen et al. (2006).

Materials and methods

Total plant DNA was extracted from leaf midribs and petioles, stems or tubers using an InvitMag Plant DNA Mini Kit (Invitek, Berlin, Germany) and a KingFisher mL workstation (Thermo Scientific, Waltham, MA, USA) according to the manufacturer’s instructions.

Initial screening of samples for phytoplasma infection was performed using the TaqMan real-time PCR assay of Christensen et al. (2004). For sequence analysis of the 16S rRNA gene, conventional nested-PCR was performed with the primer pairs, P1 (Deng and Hiruki, 1991)/P7 (Schneider et al., 1995) followed by R16F2/R16R2 (Lee et al., 1995). The tuf gene was amplified using the fTufAY/rTufAY primer pair (Schneider et al., 1997).

Conventional PCR products were either sequenced directly or cloned into the pCR 4-TOPO vector (Invitrogen) according to the manufacturer’s instructions.

The sequences were assembled and edited using Geneious Pro (Drummond et al., 2010). Searches of the GenBank database for homologous sequences were performed using the BLASTn network service available at the National Centre for Biotechnology Information (Bethesda, MD, USA).

Results

Potato (Solanum tuberosum) plants exhibited upward rolling and purpling of the leaves. Symptoms in Jerusalem cherry (Solanum pseudocapsicum) included witches’ broom, foliar yellowing and reduced leaf size. Swan plant, also known as milkweed (Gomphocarpus fruticosus), showed abnormal foliar yellowing and slight upward rolling of the leaves that resulted eventually in plant death. Celery (Apium graveolens) plants were observed to be showing unusual symptoms of pink and yellow foliage and leaf deformation. Symptoms in boysenberry plants become obvious close to flowering when the lateral branches become stunted and young leaves are chlorotic and smaller than normal. As the disease progresses, the older leaves become purple-bronze in colour, particularly towards the margin.

All symptomatic plants described above produced positive real-time PCR results. In order to identify the...
phytoplasma present in these samples, a 1.2 kb region of the 16S rRNA gene generated from the R16F2/R16R2 primers was sequenced directly. BLAST analysis of the 1.2 kb region of the 16S rRNA gene amplified from symptomatic Jerusalem cherry, swan plant, celery and boysenberry showed 100% identity to ‘Ca. P. australiensis’ 16S rRNA gene sequences in the GenBank database.

Partial sequences (~800-840 bp) of the tuf gene of ‘Ca. P. australiensis’ amplified from 29 strains from the symptomatic plants described above were determined. Twenty-two of these sequences were identical to tuf variant VII and five sequences were identical to tuf variant IX of Andersen et al. (2006). Interestingly, these two tuf variants belong to distinct subgroups: tuf clade 1 (includes variants VIII and IX) isolates originate from both New Zealand and Australia whereas tuf clade 2 (includes variants I to VII) consists exclusively of isolates from New Zealand (Andersen et al., 2006). None of the isolates in this study belonged to tuf variants I to VI and VIII and no new variants were discovered.

The sequences of the PCR products from two strains, one from celery and the other from potato indicated that these hosts contained a mixed phytoplasma population due to the presence of ambiguous bases. The tuf gene PCR amplicon from these two samples were cloned and the sequence was determined from 10 resulting clones. Sequence analysis of the clones from celery revealed that 7 were identical to variant VII and 3 were identical to variant IX, and for potato, 2 clones were of variant VII and 8 were of variant IX, thereby confirming the presence of two tuf gene types in these samples.

Discussion

Between January 2009 and July 2010, four new hosts (potato, Jerusalem cherry, swan plant and celery) of ‘Ca. P. australiensis’ have been identified in New Zealand, as well as a new disease association in boysenberry. These are the first new hosts of ‘Ca. P. australiensis’ to be recognised in New Zealand since Coprosma in 1998 (Beever et al., 2004), although they may have gone unnoticed for several years.

The economic impact that ‘Ca. P. australiensis’ will have on these newly identified hosts is potentially significant. Already, phytoplasma-infected potato tubers have failed quality control checks at the processing factory. In boysenberry, fruit from infected plants are unmarketable and the plants die within a year from when the symptoms are first noticeable. Although there have been no reports of the phytoplasma in commercially grown celery, the discolouration of the foliage that the phytoplasma induces in this crop would also render it unmarketable.

The polyphagous feeding behaviour of Zeolarius oppositus, the insect responsible for transmitting the phytoplasma into Cordyline and Coprosma (Beever et al., 2008), suggests that it may also be moving the phytoplasma into the new hosts described here. Z. oppositus is especially abundant in grasses and sedges that commonly grow around crops in New Zealand. These hosts may act as symptomless reservoirs of the phytoplasma and along with weed hosts such as Jerusalem cherry, play an important role in the spread of the phytoplasma. The diversity of the new hosts described in this paper emphasises the potential that ‘Ca. P. australiensis’ has to spread into additional native plants and horticultural crops.

References


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