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Phytoplasma detected in reverted black currants in Finland

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Abstract

Preliminary results of nested PCR and RFLP analysis indicated that phytoplasmas were detected in old reverted black currant plants. RFLP analysis showed that the phytoplasmas belonged to the aster yellows (AY) group (16SrI).

Key words: *Ribes*, aster yellows, PCR detection, RFLP.

Introduction

Until recently phytoplasma associated diseases had not been identified or considered important in Finland. Some plant species have shown symptoms typical of phytoplasma associated diseases, such as yellowing and proliferation in woody plants and leaf-like structures in berries of strawberry or flower abnormalities in ornamentals and weeds. These were sporadic cases and there were no economic losses in cultivated plants.

Global warming (Christensen *et al*., 2007) and increasing import of plant material increase the risk of phytoplasma occurring in Finland. Furthermore, some phytoplasma-transmitting insect species, e.g. leafhopper, *Macrosteles laevis* (Ribaut), and psyllid, *Cacopsylla picta* (Förster), do occur in Finland.

The first goal of the present study was to apply direct and nested PCRs with different primer sets to detect phytoplasma 16S rDNA and intergenic spacer region to determine whether old reverted black currants plants harbour phytoplasmas. The results presented here are preliminary and the work is ongoing.

Materials and methods

Samples were collected from 16-year-old reverted black currant plants, cv. Brödtorp, with severe symptoms of proliferation. The plants also had malformed flowers and they were infected with Black currant reversion virus. Nucleic acid was isolated from 3 g of a mixture of freshly cut midribs, petioles, malformed flowers and phloem scrapes from symptomatic black currant samples and in some cases frozen samples. Samples were collected from several plants. Positive phytoplasma control (aster yellows, AY, 16SrI-C) was maintained in periwinkle (*Catharanthus roseus*). Periwinkle and black currant cv. Baldwin plants both raised from seeds were used as healthy controls. Nucleic acid extraction was performed as described by Lee *et al*. (1993a). The sequence of 16S rDNA and 16S-23S rDNA was first amplified using the primer pair P1/P7 (Deng and Hiruki 1991, Schneider *et al.,* 1995), followed by primer pair R16F1 and B6 (Davis and Lee 1993, Padovan *et al.* 1995). The last nested PCR was done with the primer pair R16F2n/R2 (Gundersen *et al*., 1996, Lee *et al*., 1993b). Diluted (1: 30) PCR product from the first amplification (1 µl) was used as template in the nested PCR. PCR products were separated by electrophoresis through 1% agarose gel.

Products of double-nested PCR primed by primer pair R16F2n/R2 were subjected to enzymatic restriction fragment length polymorphism (RFLP) analysis. Phytoplasmas were classified in groups by comparison of RFLP patterns in accordance with the classification scheme of Lee *et al*., (1998a).

Results

A phytoplasma-characteristic fragment (1.2 kb) of 16S rDNA was amplified in double-nested PCR from 10 out of 14 samples of black currant showing proliferation and from infected periwinkle control. No product was amplified from healthy control plants. RFLP analysis of amplified 16S rDNA from one sample indicated that old black currants were positive for phytoplasma belonging to the group 16SrI (aster yellows, AY). The other samples from black currant showed non-specific RFLP patterns.

Discussion

The occurrence of phytoplasmas in the nordic countries is uncommon. However, the situation can change if global warming will expand the area of distribution of phytoplasma vectors. Furthermore, in the 1960s Murtomaa (1966) described in Finland a disease in cereals and grasses caused by an aster yellows-type virus transmitted by *M. laevis*. Earlier the aetiological agent was believed to be a virus instead of phytoplasma (Lee *et al*., 1998b). This indicates that the causal agent has existed in Finland for some time and perhaps *M. laevis* species also visit black currants among other plants in the field. In addition, black currants have become weaker by heavy infestation of gall mites and reversion disease. This results agrees with the first detection of phytoplasma in black currant in the Czech Republic (Spak *et al*., 2004).

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