

Talk

A new method for the identification of phytoplasmas in strawberries (*Fragaria fragaria*)



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ABSTRACT

Motivation: Phytoplasmas are plant pathogens that generally inhabit the phloem and are transmitted from plant to plant by vector insects that feed on phloem. Their genome is small, with a high content of genes (1). The presence of extrachromosomal DNA has also been detected (2). Serological and molecular studies have shown that phytoplasmas contain a gene encoding a membrane protein and this is unique for each specie and helps to identify what type of phytoplasma is, according to its taxonomic classification (3). Different phytoplasmas had been detected and identified in strawberries plants (*Fragaria fragaria*). Symptoms observed on strawberries were yellow coloration of leaves, plant stunting, and reduced leaf size and the virescence were observed in the inflorescence. PCR analyses as well as sequencing of 16S ribosomal gene enabled the identification of phytoplasmas belonging to two ribosomal groups, namely stolbur and aster yellows, but the efficiency for this detection is not reliable. Here we are working to develop a new method to easily detect and identify this pathogen in plant samples before symptoms appear.

Methods: 70 samples of strawberries infected with phytoplasma were tested, by analysing leaves, crown and root in the strawberry plant to determine the most optimal zone to be sampled. The identification of phytoplasmas required a double PCRs using phytoplasma universal primer pair P1/P7, followed by nested PCR with Fu5/Ru3 primers. Further nested PCR reactions on R16mF2/R1 amplicons were also performed with R16F2N/R2 primer pair, specific for phytoplasma belonging to aster yellow to determine their class. Asymptomatic samples were also analysed as negative controls in each PCR reaction. The amplified DNA was sequenced and the sequence analysed by Blast.

Results: With this PCR method, the presence of phytoplasma was mainly detected in strawberries roots but not in leaves and crowns. This technique allowed us to detect presence of high amount of phytoplasma because a positive result in the first PCR, low amount with a positive result only in the second one, or no presence because negative result in both. Finally,, the sequencing of the amplified DNA, allowed us to determine if they belong to aster yellow or stolbur because they DNA identity.

Conclusions: This new phytoplasma identification method is effective. It would be interesting to obtain a specific primer for each specie of phytoplasma and thus have a more precise method.

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