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Detection and characterization of phytoplasmas infecting tomato plants in Greece

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Abstract

Over the course of the 2005 and 2006 growing seasons, surveys were carried out in tomato fields in different parts of Greece in order to determine the occurrence and distribution of phytoplasma diseases of tomato, and to identify and classify the phytoplasmas involved. In all the tomato cultivating areas, the percentage of plants with symptoms resembling phytoplasma infection ranged usually between 1-2%, although in some cases it reached 70-80%. The presence of phytoplasmas in these plants was verified by PCR analysis with universal primers. Further analysis using group specific primers showed that mixed infections with phytoplasmas belonging to the aster yellows (16SrI) or the stolbur (16SrXII-A) group were very common, even in plants showing symptoms typical to either big bud or stolbur disease.

Key words: tomato big bud; phytoplasma infection; tomato stolbur; PCR.

Introduction

Phytoplasma diseases of tomato (tomato stolbur and tomato big-bud), though probably occurring in Greece since the 1960's, were not described until 1985, when mycoplasma-like organisms were first observed by thin-section electron microscopy in tissues from plants showing typical stolbur symptoms (Alivizatos, 1989). Symptoms for both diseases include malformed flowers, fruit size reduction and reduced yield (Alivizatos, 1989, 1993; del Serrone *et al.*, 2001; Anfoka *et al.*, 2003). Although, when first reported, infected plants occurred only in certain parts of Greece, lately the number of farmers reporting the existence of infected plants in their fields, (called "male tomatoes" because the infected plants do not produce normal fruit) has increased.

The aim of the present study is to investigate the distribution of phytoplasma diseases in tomato growing areas in Greece and to identify and classify the pathogens involved.

Materials and methods

Field surveys were made in tomato growing areas of Northern, Central and Southern Greece, during the summer of 2005 and 2006, from late April until mid September. From each area, at least one field with plants showing symptoms similar to those caused by phytoplasma infection was surveyed. The percentage of symptomatic plants in each field was calculated by measuring the number of symptomatic tomato plants per 100 plants, and up to 10 samples were randomly collected for further analysis.

DNA was extracted from plant tissue as described by Ahrens and Seemüller (1992). Universal primer pair P1/P7 (Deng and Hiruki, 1991; Smart *et al.*, 1996) were used in PCR for phytoplasma detection, as well as group specific primer pairs R16(I)F1/R1 (Lee *et al.*, 1994) and fStol/rStol (Maixner *et al.*, 1995).

Results

Phytoplasma infected plants were observed in all tomato cultivating areas that were surveyed, and in almost every field, starting from mid-July. The disease frequency in most cases ranged between 1-2%, but in some fields this percentage reached 15%. In very few cases a percentage around 70-80% was observed. Fields with no infected plants were seldom observed.

In most of the plants that were collected, detection of phytoplasma was obtained by PCR amplification using universal primers P1/P7, though in some plants, despite the presence of characteristic symptoms, it was not possible to detect phytoplasmatic DNA.

PCR analysis using group-specific primers revealed that the majority of the plants tested had mixed infections by phytoplasmas from the 16SrI or the 16SrXII group, although in some cases the symptoms were typical of one of the two diseases.

Discussion

Although 20 years ago tomato plants, naturally infected by phytoplasma, were only observed in certain parts of Greece, today they are quite widespread. Disease incidence was usually around 1-2%, which does not affect the economical value of the crop. This was not the case when this percentage rose up to 80%.

The plants that were sampled showed symptoms typical to tomato big bud, or tomato stolbur, or, in most cases, a mixture of both. PCR using group-specific primers showed that most plants that were tested had mixed infections by phytoplasmas from the aster yellows (16SrI) and the stolbur (16SrXII-A) group, which did not always correlate with the symptoms they showed.

The inability to detect phytoplasmatic DNA from symptomatic plants has been reported before, and could be a result of low titre of the pathogen (Del Serrone *et al.*, 2001).

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