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First report of witches' broom disease of tomato associated with phytoplasmas in Saudi Arabia

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Abstract

Tomato is a very important crop in Saudi Arabia grown in both open fields and in greenhouses. Leaf samples of tomato plants showing witches' broom symptoms were tested by nested PCR assays followed by sequencing. A DNA fragment of the expected size was amplified from symptomatic and not from symptomless plants. Phylogenetic analysis of the 16S rDNA sequence clustered the tomato witches' broom phytoplasma detected in Saudi Arabia within the peanut witches' broom (16SrII) group ('*Candidatus* Phytoplasma aurantifolia'). To our knowledge, this is first detection of a witches' broom phytoplasma associated with tomato in Saudi Arabia.

Key words: Tomato, phytoplasmas, witches' broom, sequencing.

Introduction

Tomato (*Solanum lycopersicum*) is a very important crop in Saudi Arabia; grown in open fields and greenhouses. The cultivated area is estimated about 15,127 ha, with a year production in 2009 of more than 0.5 million tons. Recently witches' broom and stunting symptoms disease were observed in tomato growing areas, therefore molecular analyses were carried out in order to identify the phytoplasma possibily associated with the disease.

Materials and methods

In 2010 leaf samples of tomato plants showing witches' broom symptoms were collected from different locations in Alhasa, Saudi Arabia. Samples with and without symptoms were analyzed for the presence of phytoplasmas using 16SrDNA PCR assays. Total nucleic acids were extracted from leaves using the DNeasy Plant Mini kit (QIAGEN) and were used as template in a nested PCR reaction using primers P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) followed by primers R16F2/R16R2 (Lee *et al*., 1995).

A PCR product obtained from P1/P7 primers was purified and directly sequenced.

Results and discussion

A DNA fragment of the expected size (1.2 kb) was amplified by nested PCR from all the symptomatic plants tested. No amplification was obtained from symptomless plants.

The 1,753 bp sequence obtained from the sequencing of a P1/P7 amplicon was deposited in GenBank (accession no. HM584815). Phylogenetic analysis of this 16S rDNA sequence showed that the tomato witches' broom phytoplasma clustered with the peanut witches' broom (16SrII) group members ('*Candidatus* Phytoplasma aurantifolia'). In particular the strain showed a significant sequence identity with the *Catharanthus roseus* phytoplasma II strain, a member of the 16SrII-E (EU096500) collected in United Arab Emirates.

The 99% homology was found with scaevola witches' broom, tomato big bud, and alfalfa witches' broom phytoplasmas described in Oman (Khan *et al*., 2002; Al-Zadjali *et al*., 2007).). The 99% similarity with the obtained sequence was also found with peanut witches' broom (EF193356), subgroup 16SrII-A (Lee *et al*., 1998) and sweet potato witches' broom (DQ452417) subgroup 16SrII-D; a 98% homology was obtained with faba been phyllody phytoplasma (HQ589188).

To our knowledge, this is first detection of witches' broom phytoplasma associated with tomato in Saudi Arabia and also the first detection of 16SrII phytoplasmas in tomato indicating the wide ability of this phytoplasmas to infect both herbaceous and woody host also in Arabian countries.

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