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Identification of phytoplasma associated with witches' broom and virescence diseases of sesame in Oman

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Abstract

Sesame (*Sesamum indicum* L.) is an important field crop grown in the northern and interior regions of Oman for oil and animal feed. Phyllody, virescence and witches' broom symptoms, resembling those caused by phytoplasma infection were observed during 2004 - 2006. A nested PCR assay using primer pair P1/16S-Sr followed by R16F2n/R16R2n was used to amplify a 1.53 kb and 1.2 kb fragment of the phytoplasma 16S rRNA gene, respectively. Restriction fragment length polymorphism (RFLP) analysis of 16S rDNA of sesame witches' broom phytoplasma strains singly digested with *Alu*I, *Mse*I, *Taq*I, and *Tsp509*I yielded patterns similar to those of alfalfa witches' broom phytoplasma belonging to subgroup 16SrII-D. The RFLP profiles of sesame witches' broom phytoplasma strains SIL and SIF were indistinguishable. Phylogenetic analysis showed that the sesame witches' broom phytoplasma strains (SIL, SIF) clustered with Omani alfalfa witches' broom forming a distinct lineage separate from peanut witches' broom and sesame phyllody (Thailand) phytoplasma strains.

Key words: 16S rRNA gene, phytoplasma, phyllody, RFLP, sesame, virescence, witches' broom.

Introduction

Sesame is cultivated in the Sultanate of Oman mainly in the Dakhliya region for seed and animal feed. Recently, sesame crops have been found associated with witches' broom disease phytoplasmas were associated with the disease. Omani farmers grow several local and exotic cultivars of sesame. During a field survey in November and December of 2004 - 2006 severe symptoms of phyllody and virescence usually associated with phytoplasma were observed. Variations in symptoms and disease severity in different varieties were evident. Some exhibited typical witches' broom symptoms (SIL) and others exhibited only virescence (SIF).

Materials and methods

Symptomatic and asymptomatic plants were sampled from various sesame fields. Total nucleic acid was extracted from sesame samples according to Doyle and Doyle (1990). A nested PCR using primer pair P1/16S-Sr in the first amplification followed by R16F2n/R2 (Lee *et al*., 2006) in the second amplification was performed to detect phytoplasmas in infected sesame samples. The P1/16S-Sr PCR yields an amplicon that includes near full length 16S rDNA (about 1.53 kb). A negative control, devoid of DNA templates in the reaction mix, was included in all PCR assays. For PCR amplification, 38 cycles were conducted in an automated thermal cycler (MJ Research DNA Thermal Cycler PTC-200) with AmpliTaq Gold polymerase. PCR was carried out in mixtures containing 1 µl of a 1: 10 DNA dilution, 200 µM of each dNTP and 0.4 µM of each primer. The following conditions were used: denaturation at 94 ºC for one minute (11 minutes for the first cycle), annealing for 2 min at 55 ºC, and primer extension for 3 min (7 min in the final cycle) at 72 ºC. One microliter of diluted (1: 30) PCR product from the first amplification was used as the template in the nested PCR. The PCR products (5 µl) were electrophoresed through a 1% agarose gel, stained in ethidium bromide, and visualized with a UV transilluminator.

Restriction fragment length polymerphism (RFLP) analysis of nested PCR products (1.2 kb 16S rDNA fragments) from two representative sesame samples (SIL and SIF) and several reference phytoplasma strains, including two described Omani phytoplasma strains, lime witches' broom (LWB) and alfalfa witches' broom (AlfWB), and three 16SrII representative phytoplasma strains, sesame phyllody Thailand (SEPT), peanut witches' broom (PnWB), and soybean phyllody (SOYP) was performed. Comparison of RFLP profiles with published profiles (Lee *et al*., 1998) was used for identification of the phytoplasmas detected. The PCR products (6 µl) were digested singly with the restriction enzymes *Alu*I, *Mse*I, *Taq*I, and *Tsp509*I. The restriction products were then separated by electrophoresis through a 5% polyacrylamide gel for one hour at 150 V, stained with ethidium bromide, and visualized with a UV transilluminator.

PCR amplified products of 16S rRNA genes were cloned and sequenced. To obtain near full-length 16S rDNA, P1A/P7A PCR products (about 1.8 kb and extends from the 5'-end of 16S rRNA gene, through the intergenic spacer region, to the 3'-end of the 23S rRNA) were cloned. P1A/P7A DNA fragments were amplified using diluted P1/P7 PCR products as templates in nested PCR (Lee *et al*., 2004). The P1A/P7A PCR products were purified using PCR Kleen Spin Columns (Bio-Rad) and cloned into *Escherichia coli* by using the TOPO TA Cloning Kit (Invitrogen) according to the manufacturers' instructions. Sequencing was performed with an automated DNA sequencer

(ABI Prism Model 3730) at the Center for Biosystems Research, University of Maryland, College Park, MD. The cloned nucleotide sequences were deposited in GenBank.

Phylogenetic interrelationships among sesame witches' broom phytoplasma strains and representative phytoplasma strains in each phytoplasma group were assessed based on 16S rRNA gene sequences. Partial sequences of 16S rDNA (1.5 kb) available in GenBank were aligned by using CLUSTAL, version 5, and DNASTAR's Laser Gene software (DNASTAR, Madison, WI, USA). Cladistic analyses were performed with PAUP (phylogenetic analysis using parsimony), version 4.0 (Swofford 1998), on a Power Mac G4. Uninformative characters were excluded from analyses. A phylogenetic tree was constructed by a heuristic search via random stepwise addition implementing the tree bisection and reconnection branch-swapping algorithm to find the optimal tree(s) (Gundersen *et al.,* 1994). *Acholeplasma palmae* was selected as the out-group to root the tree. The analysis was replicated 1000 times. Bootstrapping was performed to estimate the stability and support for the inferred clades.

Results

An amplicon of approximately 1.2 kb was amplified from all symptomatic sesame plants, but not from asymptomatic plants or the negative control. RFLP profiles of 16S rDNA digested singly with four restriction enzymes indicated that sesame witches' broom phytoplasma strains were most closely related to Omani alfalfa witches' broom phytoplasma strain belonging to subgroup 16SrII-D. Two strains, SIL and SIF, had identical RFLP profiles (figure 1). Nucleotide sequence analysis of cloned 16S rDNA (GenBank accession numbers: EU072505, EU072504) confirmed the results based on RFLP analyses. Phylogenetic analysis showed that the sesame witches' broom phytoplasma strains in Oman (SIL, SIF) clustered with Omani alfalfa witches' broom forming a distinct lineage separate from PnWB and SEPT phytoplasma strains.

Discussion

Group 16SrII phytoplasma strains associated with sesame phyllody have been reported in Thailand (strain SEPT). Based on RFLP and sequence analyses of 16S rDNA, the phytoplasma strains associated with sesame witches' broom in Oman are distinct from strain SEPT, which is closely related to PnWB phytoplasma. The remarkable sequence similarity between the sesame witches' broom phytoplasma strains and the alfalfa witches' broom strains previously reported in Oman (Khan *et al*., 2002) implies that these phytoplasma strains may share similar ecological niches.

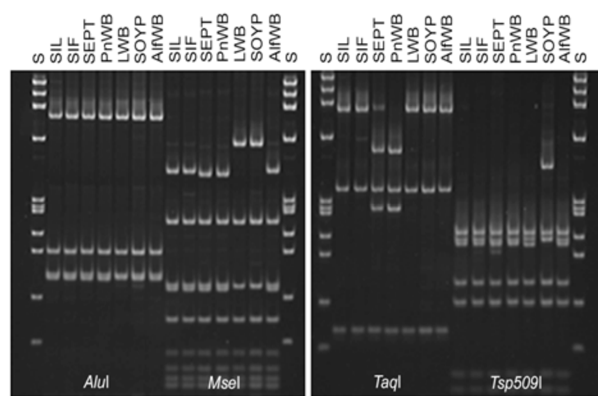

Figure 1. Polyacrylamide gel showing the restriction fragment length polymorphism of sesame phyllody phytoplasmas from Oman (SIL and SIF strains) and 16SrII representative phytoplasma strains amplified with primers R16F2n/R2 in nested PCR and digested with *Alu*I, *Mse*I, *Taq*I, and *Tsp509*I.

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