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Molecular characterization of phytoplasma associated with *Echinops* witches' broom disease

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Abstract

Echinops spinosissimus plants in Al-Sharqiya region were found having excessive leaf and shoot growth and stunting reminiscent of phytoplasma symptoms. Polymerase chain reaction (PCR) was used to amplify 16S ribosomal gene of phytoplasma using universal primers P1/P7. The PCR amplifications from all infected plants yielded a product of 1.8 kb by P1/P7. Restriction fragment length polymorphism showed that *E. spinosissimus* phytoplasma is different from alfalfa and lime witches' broom phytoplasmas from Oman. Sequence homology results on BLAST and phylogenetic analysis of 16S rRNA gene sequences confirms that the closest phytoplasma relatives of Echinops witches' broom phytoplasma are members of pigeon pea witches' broom phytoplasma ribosomal group (16SrIX) that share more then 98% 16S rDNA sequence similarity.

Key words: *Echinops spinosissimus,* phytoplasma, ribosomal gene, PCR, RFLP

Introduction

Echinops spinosissimus Turra (known as hink or Shook Jamal in Arabic) is a shrub belonging to the family Asteraceae that thrives well in semi-arid habitats from the Mediterranean region to the Arabian Peninsula. *E. spinosissimus* is characterized by having the flowers reduced and organized into an involucrate pseudanthium in the form of a head or capitulum. The plant is commonly known as Egyptian medicinal plant and possesses antiinflamatory properties.

During fall 2006, *E. spinosissimus* plants in several parts of Al-Sharqiya region (300 km south of Muscat) were found with symptoms of shoot proliferation, reduction in leaf size and general stunting, typicals of a phytoplasma infection.

Materials and methods

E. spinosissimus samples from plants showing witches' broom symptoms and from asymtpomatic plants were collected from five different locations in Al-Sharqiya region in Oman. For PCR analysis, total nucleic acid extraction was carried according to Doyle and Doyle (1990). Sequence and RFLP analyses of ribosomal fragments were performed by using ribosomal DNA that was amplified with the primer pair P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) and extended from the 5' end of the 16S rRNA gene to the 5' region of the 23S rRNA gene, thus including the 16S–23S rDNA spacer region.

PCR products were digested by RFLP with *Rsa*I, *Alu*I, *THB*81 and *Hpa*II endonucleases. The resulting RFLP patterns were compared with those of the alfalfa and lime witches' broom phytoplasma from Oman (Khan *et al*., 2002; Zreik *et al*., 1995).

P1/P7 product from symptomatic *E. spinosissimus* sample was purified using Qiagen PCR spin columns and cloned into *Escherichia coli* using the pGEM-Easy-T (Promega Corp.) cloning kit according to the manufacturers' instructions.

Partial sequences (ca. 1.5 kb) of 16S ribosomal DNA of Echinops witches' broom phytoplasma and phytoplasmas belonging to different groups, and *Acholeplasma laidlawii* (available in GenBank) were aligned by Clustal W method using Lasergene 6.1 program (DNA Star). Cladistic analysis and phylogenetic tree construction were performed with PAUP (Phylogenetic Analysis Using Parsimony), version 4b10 (Swofford, 2002). A phylogenetic tree was constructed using a heuristic search with random stepwise addition, implementing the tree bisection and reconnection branch-swapping algorithm to find the optimum arrangement. Bootstrap analyses (500 replicate) were performed to estimate the stability and support for inferred clades. *A. laidlawii* was selected as the out-group taxon to root the tree.

Results and discussion

A product of 1.8 kb DNA fragment from 16S ribosomal gene was consistently amplified by PCR from all infected samples using P1/P7 primers. No amplification was obtained from asymptomatic *E. spinosissimus* samples and water sample by direct or nested PCR. RFLP analyses of direct PCR products with *Rsa*I, *Alu*I, *T.HB*81 and *Hpa*II yielded patterns different to those of alfalfa and lime witches' broom phytoplasmas (figure 1).

A 1,726 bp sequence was obtained from PCR amplified rDNA by P1/P7 primers. Sequence homology results on BLAST search (Altschul *et al*., 1990) revealed that Echinops witches' broom (EWB) phytoplasma shares 98% similarity with pigeon pea witches' broom (EF186825), *Lactuca serriola* phytoplasma from Iran (DQ889749), *Knautia arvensis* phyllody phytoplasma (Y18052), Iranian almond witches' broom (DQ195209) and *Picris echioides* yellows (Y16389) phytoplasma

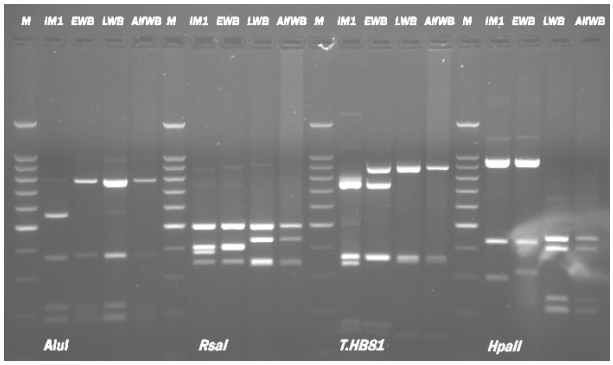

Figure 1. 2.5% Agarose gel showing the restriction fragment length polymorphism patterns of strain IM-1 (from Oman), *Echinops* witches' broom phytoplasma (EWB) alfalfa witches' broom (AlfWB) and lime-witches' broom (LWB). M, 100 bp DNA ladder.

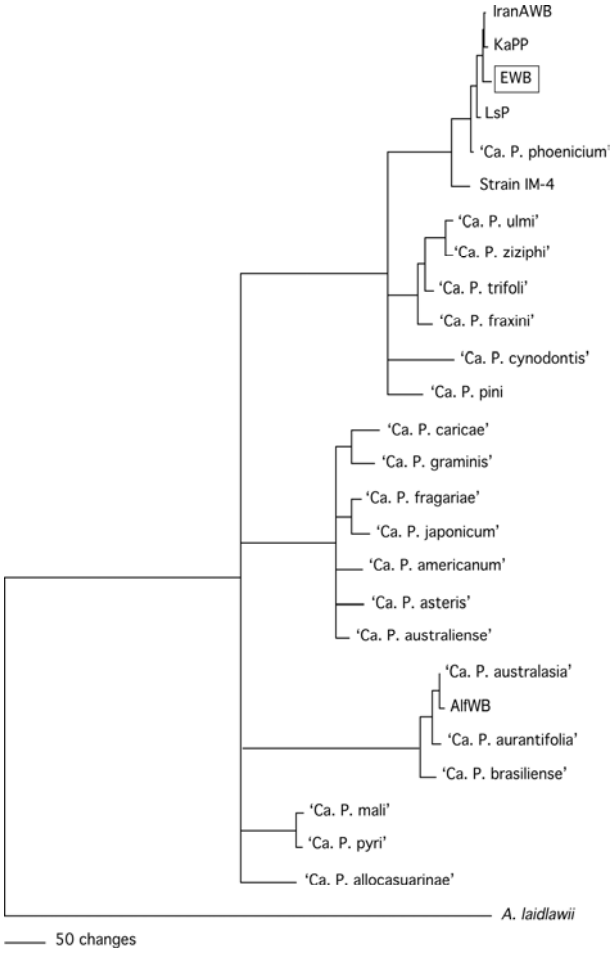

Figure 2. Phylogenetic tree constructed by parsimony analysis of nearly full-length of 16S rRNA sequences from described '*Ca*. Phytoplasma' species and *Echinops* witches' broom phytoplasma (EWB), Iranian almond witches' broom (Iranian AWB), *Knautia arvensis* phyllody (KaPP), Iranian lactuca serriola (Iranian LsP) and *Echinops* witches' broom (EWB), strain IM-4 (from Oman). Branch lengths are proportional to the number of inferred character state transformations. Bootstrap values > 50% are shown on branches.

and 98% with '*Ca*. Phytoplasma phoenicium' (AF515636) and Honduran *Gliricidia* little leaf phytoplasma (AF361017).

RNA gene sequence of EWB phytoplasma and 25 other phytoplasmas from GenBank is presented in figure 2. It is evident that EWB phytoplasma from Oman clustered with *Lactuca serriola* phytoplasma from Iran witch is member of pigeon pea witches' broom phytoplasma ribosomal group (16SrIX). Based on RFLP data and phylogenetic analyses, the phytoplasma identified from *E. spinosissimus* in Oman appears to belong to pigeon pea witches' broom phytoplasma group (16SrIX). No report is available on the presence of a phytoplasma belonging to 16SrIX subgroup from Oman and hence, it appears to be the first report.

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References

ALTSCHUL S. F., GISH W., MILLER W., MYERS E. W., LIPMAN D. J., 1990.- Basic local alignment search tool.- *Journal of Molecular Biology*, 215: 403-410.

DENG S. J., HIRUKI C., 1991.- Genetic relatedness between two non-culturable mycoplasma-like organisms revealed by nucleic acid hybridization and polymerase chain reaction.- *Phytopathology*, 81: 1475-1479.

DOYLE J. J., DOYLE J. L., 1990.- Isolation of plant DNA from fresh tissue.- *Focus*, 12: 13-15.

KHAN A. J., BOTTI S., AL-SUBHI A. M., GUNDERSEN-RINDAL D. E., BERTACCINI A., 2002.- Molecular identification of a new phytoplasma associated with alfalfa witches' broom in Oman.- *Phytopathology*, 92: 1038-1047.

SCHNEIDER B., SEEMÜLLER E., SMART C. D., KIRKPATRICK B. C., 1995.- Phylogenetic classification of plant pathogenic mycoplasmalike organisms or phytoplasmas, pp. 369-380. In: *Molecular and Diagnostic Procedures in Mycoplasmology, vol. 2* (RAZIN S., TULLY J. G., Eds). Academic Press, San Diego, CA, USA.

SWOFFORD D. L., 2002.- PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods) Version 4 b10.- Sinauer Associates, Sunderland, MS, USA.

ZREIK L., CARLE P., BOVE J. M., GARNIER M., 1995.- Characterization of the mycoplasma-like organism associated with witches' broom disease of lime, and proposition of a *Candidatus* taxon for the organism `*Candidatus* Phytoplasma aurantifolia'.- *International Journal of Systematic Bacteriology*, 45: 449-453.

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