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Research Article

Association of a 16SrIX-C phytoplasma with eggplant phyllody in Iran

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Abstract: In 2011-2012 surveys for phytoplasma diseases, eggplant (*Solanum melongena* L.) plants with phyllody symptoms were observed in eggplant fields of Roodan (Hormozgan province of Iran). Agent of Roodan Eggplant Phyllody (REP) was transmitted from phyllody affected eggplant to eggplant and tomato by grafting and to periwinkle via dodder inoculation inducing phytoplasma-type symptoms. Phytoplasmal infection also was demonstrated by positive direct PCR reaction with phytoplasma universal primer pair P_1/P_7 and nested PCR using P1/P7 and R16F2n/R16R2 primer pairs. A P_1/P_7 primed PCR product from a naturally phyllody affected eggplant was cloned and sequenced and submitted to GenBank under accession number JX464669. Restriction fragment length polymorphism (RFLP) analysis of P1/P7-primed PCR product indicated the presence of a pigeon pea witches'-broom (16SrIX) group related phytoplasma in naturally phyllody affected eggplants. Using 16S rRNA and SR sequences, Blast search, phylogenetic and virtual RFLP analyses and nucleotide homology percent revealed that REP associated phytoplasma is classified with members of 16SrIX-C subgroup. To our knowledge eggplant is reported for the first time as a host for a 16SrIX group related phytoplasma.

Keywords: eggplant phyllody, phytoplasma, dodder and graft transmission, 16SrIX-C subgroup, Roodan

Introduction

Phytoplasmas are a group of plant pathogenic wall-less, phloem inhabiting bacteria in the class Mollicutes that cause devastating damage to plants by loss in biomass and quality of products including flowers. These pathogens are known to affect approximately 1000 plant species worldwide including fruit trees, vegetables, ornamental plants, cereals, and legumes (Lee *et al.* 2000; Bertaccini and Duduk, 2009). Phytoplasmas belonging to different 16Sr groups including 16SrI (Okuda *et al*., 1997; Lee *et al*., 1998; Kelly *et al*., 2009; Kumar *et al.*, 2012), 16SrII (Al-Subhi *et al*., 2011; Omar and Foissac, 2012), 16SrIII (Amaral Mello *et al*., 2011), 16SrVI (Sertkaya *et al*., 2007; Siddique *et al.*, 2001) and 16SrXII (Ember *et al.*, 2011) have been identified in eggplant (*Solanum melongena* L.). Eggplant is a popular vegetable crop grown in different parts of Iran and is a common vegetable on Iranian diet. With an annual output of 1,300,000 tons, Iran ranks third in eggplant production in the world (FAO, 2012). Eggplant

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can be cultivated and grown round the year but the productivity and quality of this crop suffers due to its susceptibility to a number of diseases and insect-pests. In 2011-2012 surveys for phytoplasma diseases, phyllody disease was observed in eggplant fields of Roodan (Hormozgan province of Iran). Collectively, Roodan Eggplant Phyllody (REP) disease symptoms closely resembled those described for phytoplasma diseases. Disease incidence was up to 10%.

The present work reports on the partial biological and molecular characterization of a phytoplasma strain associated with REP. A preliminary report has been published recently (Salehi *et al.*, 2005).

Materials and Methods

Source of the disease

An eggplant with symptoms of phyllody disease was selected in an eggplant field in Roodan (a region 100 km west of Bandarabbas, in the Hormozghan province) transferred to greenhouse and used as the source of the phyllody agent for transmission and molecular studies. For long-term maintenance the disease agent was transmitted to a red line of Madagascar periwinkle (*Catharanthus roseus* (L.) G. Don) via dodder transmission.

Dodder transmission

Seeds of dodder (*Cuscuta campestris* Yunk.) were germinated on moist filter paper and transferred to healthy sugar beets. After 4 weeks, connection was established between dodder strands from healthy sugar beet and a phyllody-affected eggplant. Twenty days later, newly developed dodder strands from eggplant plant was connected to 10 healthy seed-grown periwinkle plants. Connections were maintained for 4 weeks, after which test plants were freed of dodder strands and kept in an insect free greenhouse. Controls were exposed to dodder grown on healthy sugar beet.

Graft inoculation

For side grafting, healthy seed-grown eggplants and tomato plants were used as rootstock and small auxiliary shoots from a naturally infected eggplant as scions. The graft unions were wrapped with parafilm and grafted plants were covered with plastic bags for two weeks to maintain humidity. Graft inoculated plants were transferred to an insect -free greenhouse.

DNA extraction and polymerase chain reaction

Total DNA was extracted from approximately 200 mg midrib tissue of 12 naturally phyllody affected eggplants and three of each experimentally graft and dodder inoculated plants including periwinkle, eggplant and tomato using Zhang *et al*. (1998) procedure with minor modifications as reported by Abou-Jawdah *et al*. (2002). Total DNA extracted from symptom-less eggplant and a symptomatic periwinkle plant infected with witches'-broom of lime phytoplasma were used as negative and positive controls, respectively. DNA pellets were suspended in 100 µL sterile water and were kept at–20 °C as template DNA in polymerase chain reaction (PCR) assays. The phytoplasma universal primer pair P1/P7 (Schneider *et al*., 1995) was used in a direct PCR to amplify a 1.8 kbp fragment of ribosomal operon consisting of the 16S rRNA gene, 16S-23S intergenic spacer region (SR) and a portion of the 5′ region of the 23S rRNA gene. Further 30-fold diluted PCR products from the first amplifications were used in nested PCR using the R16F2n/R16R2 primer pair which amplifies approximately 1.2 kbp fragment of 16Sr DNA (Gundersen and Lee, 1996).

PCR was performed in a 25 µl reaction volume containing 50 ng DNA, 0.4 mM of each primer, 0.25 mM of each dNTP, 1.5 mM $MgCl_2$ and 0.5 units of *Taq* DNA polymerase (CinnaGen, Iran) in the buffer supplied by the manufacturer. Amplification was carried out in a thermal cycler (Bio-Rad, USA**)** for 35 cycles as follows: 45 sec denaturation at 94 °C (3 min for the first cycle), 45 sec annealing at 55°C and 2 min of extension at 72 °C. In the final cycle the extension step was extended to 10 min. PCR conditions for the second round (nested) PCR were the same except that the annealing temperature was 58 °C. PCR products

were separated in 1% agarose gels in 1X TBE buffer (108 g Tris-HCl, 55 g boric acid, 40 ml EDTA (0.5M), pH 8.0). DNA bands were stained with 0.5 mg/ml ethidium bromide and visualized with a UV transilluminator. The molecular weight of the PCR products was estimated by comparison with 100 bp DNA ladder (Fermentas, Vilnius, Lithuania).

RFLP analysis of PCR products

A rDNA fragment (1·8 kbp) from REP phytoplasma amplified in direct PCR using P1/P7 primer pair was analyzed by restriction endonuclease digestion. Eight microliters of PCR product was separately digested at 37 °C with *Alu*I, *Hae*III, *Hinf* I and *Rsa*I enzymes according to the manufacturer's instructions (Fermentas, Vilnius, Lithuania). The restriction products were separated by electrophoresis through a 2% agarose gel and stained with ethidium bromide. DNA bands were visualized with a UV transilluminator.

Cloning and sequencing of PCR product

The P1/P7 primed rDNA product from REP phytoplasma was ligated onto pTZ57R/T vector and cloned into *Escherichia coli* DH5α cells using InsT/A clone™ PCR Product Cloning Kit (Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. After bacteria cells were cultured on solid LB culture medium, plasmid DNA from cultures of recombinant colonies was purified using a High Pure Isolation Kit (Roche, Germany) according to the manufacturer's instructions. Sequencing was performed by BioNeer (Southern Korea). M13 forward and reverse primers were used as sequencing primers. Internal primers were designed and used by the sequencing company. The whole lengths of P1/P7 primed rDNA product were sequenced and used for further analyses. Nucleotide Basic Local Alignment Search Tool (BLAST) was performed to determine the closest phytoplasma relatives of the REP isolate.

Virtual RFLP analysis of sequences

R16F2n/R16R2 region sequence (1.2 kbp) of REP and selected members of 16SrIX subgroups (A, B, C, D and E) were exported to pDRAW32 software (Wei *et al.*, 2007) for *in silico* restriction digestion by *Alu*I, *Bam*HI, *Bfa*I, *Tha*I (*Bst*UI), *Dra*I, *Eco*RI, *Hae*III, *Hha*I, *Hin*fI, *Hpa*I, *Hpa*II, *Kpn*I, *Sau3*AI (*Mbo*I), *Mse*I, *Rsa*I, *Ssp*I, *Taq*I enzymes and virtual gel plotting.

Sequence homology and phylogenetic analysis

Full-length 16S rDNA sequences of 28 phytoplasmas including REP phytoplasma and SR sequences of REP phytoplasma isolate with twelve reference phytoplasmas were separately aligned and related phylogenetic trees and sequence homologies were generated using MEGA5 software (Tamura *et al.*, 2011). *Acholeplasma laidlawii* was used as an outgroup to root the tree. Bootstrapping was performed 100 times to estimate the stability and support for the branches.

Results

Symptomatology

The main symptoms of the REP disease were foliar chlorosis, stem and flower proliferation, virescence, phyllody, shortened internodes, stunting, and fruit of reduced size (Fig. 1).

Transmission of disease agent

Agent of REP was transmitted from naturally phyllody affected eggplant to 5/5 eggplant and 5/5 tomato plants by grafting inducing flower virescence, phyllody, severe stem proliferation, little leaf and short internodes (Fig. 2). Inoculated plants reacted positively in PCR assay. Eight of 10 healthy plants of periwinkle parasitized by dodder from phyllody affected eggplant developed phytoplasma-type symptoms after two months post inoculation. The characteristic symptoms in dodder inoculated periwinkle plants were severe yellowing, little leaf and stunting (Fig. 3). Symptomatic periwinkles inoculated with REP agent reacted positively in PCR assay.

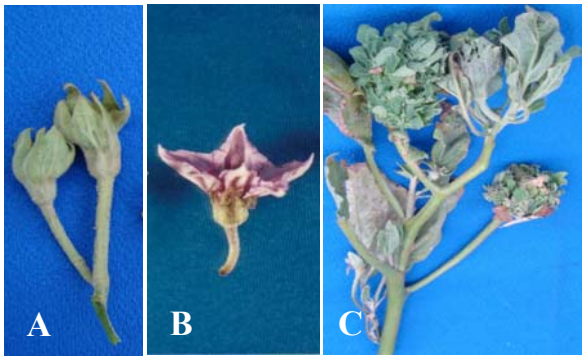

Figure 1 Symptoms of Roodan eggplant phyllody disease: Flower virescence and phyllody (A) compared to a normal flower (B) and flower proliferation and little leaf (C).

Figure 2 Severe stem proliferation, little leaf and short internode in eggplant (A) and tomato(B) plants graft inoculated with Roodan eggplant phyllody agent.

Figure 3 Severe yellowing, little leaf, internode shortening and stunting in a periwinkle plant dodder inoculated with Roodan eggplant phyllody agent (A) compared to a healthy periwinkle plant (B).

PCR amplification

After 35 cycles of direct PCR using P1/P7 primer pair and nested PCR, DNA fragments of expected size (approximately 1.8 and 1.2 kbp, respectively) were amplified from 12 of 12 naturally phyllody affected eggplants, positive control and all experimentally inoculated plants. Under the same conditions no amplification occurred with the symptomless samples (Fig. 4).

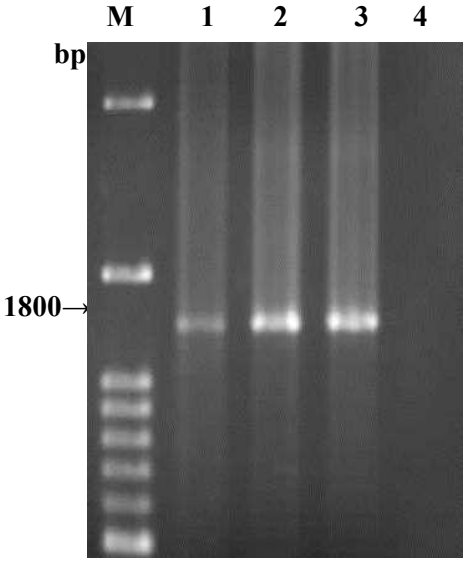

Figure 4 Agarose gel electrophoresis of polymerase chain reaction (PCR) products (1.8kbp) amplified by P1/P7 primer pair. Lanes 1, 2, 3 and 4, naturally phyllody affected eggplant, periwinkle and tomato plants inoculated with Roodan eggplant phyllody agent and healthy eggplant, respectively. Lane M, 100 bp DNA marker.

RFLP analysis

P1/P7 product (1.8 kbp) amplified from a naturally phyllody affected eggplant was analyzed by digestion with *Alu*I, *Hae*III, *Hin*fI and *Rsa*I enzymes (Fig. 5). Collective RFLP patterns of REP phytoplasma was most similar to those previously published for 16S rDNAs of members of the pigeon pea witches'-broom (PPWB)(16SrIX) phytoplasma group (Verdin *et al.*, 2003)**.**

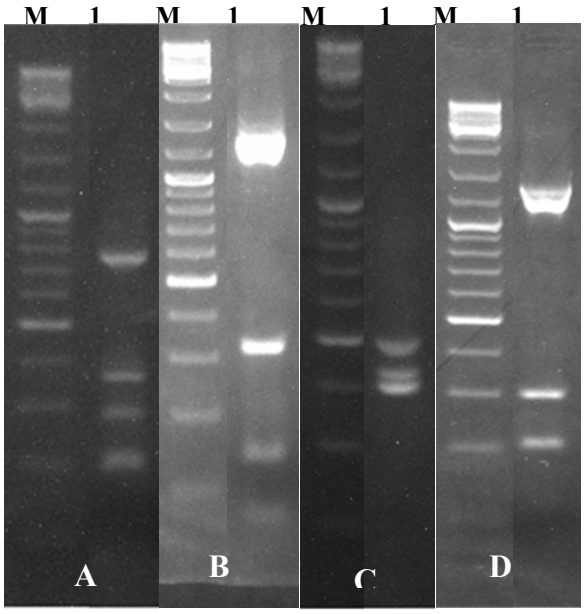

Figure 5 Restriction fragment length polymorphism (RFLP) profiles of P1/P7 primed PCR product (1.8kbp) from the Roodan eggplant phyllody phytoplasma. DNA product was digested with *Alu*I (A), *Hae*III (B), *Rsa*I (C) and *Hin*fI (D). M, 100 bp DNA ladder. Digested products were electrophoresed through a 2% agarose gel and stained in ethidium bromide.

Nucleotide sequence analyses

The complete nucleotide sequence of P1/P7 amplified fragment of the Roodan isolate was sequenced and submitted to GenBank database under the accession number JX464669. BLAST search using 1.8 kbp of ribosomal RNA operon showed that REP isolate had the highest homology with phytoplasma sequences belonging to members of the 16SrIX phytoplasma group. Among selected members of 16SrIX group, REP phytoplasma had maximum homology (100%) with *'Brassica rapa'* phyllody phytoplasma isolate Bharatpur (HM559246), a 16SrIX-C subgroup related phytoplasma (Azadvar and Baranwal, 2010).

Phylogenetic analysis of 16S rDNA sequences of 22 phytoplasma strains and *A. laidlawii* yielded a tree (Fig. 6) whose branching order was in general agreement with previous findings (Verdin *et al*., 2003). The tree clearly showed that the REP phytoplasma clustered with '*Ca*. Phytoplasma phoenicium' strain A4 (AF515636), representative of 16SrIX group. Phylogenetic tree constructed with SR sequences (Fig. 7) also showed that REP isolate clustered with 16SrIX group phytoplasmas. This analysis revealed that REP phytoplasma is closer to *Knautia arvensis* phyllody (Y18052) and Khafr almond witches'-broom (KAWB) (DQ195209) phytoplasmas, members of 16SrIX-C subgroup (Khan *et al*., 2007; Molino Lova *et al*., 2011) than to other 16SrIX subgroups. The percentage homology between SR sequences was determined and the result is presented as a matrix (Table 1) which shows REP isolate shares highest homology with *Knautia arvensis* phyllody and KAWB phytoplasmas, members of 16SrIX-C subgroup.

Virtual RFLP analysis

Visualization and comparison of virtual gel plotted images revealed that RFLP patterns of REP phytoplasma was similar to those of *Picris echioides* yellows phytoplasma (Y16389) a 16SrIX-C subgroup related phytoplasma (Molino Lova *et al*., 2011). Among 17 enzymes used for virtual RFLP analysis, eight enzymes including *Alu*I, *Bfa*I, *Dra*I, *Hae*III, *Hha*I, *Hin*fI, *Rsa*I and*Taq*I were unique for identification of 16SrIX subgroups (Fig. 8).

Table 1 Percent homology among Roodan eggplant phyllody phytoplasma and other reference phytoplasmas as determined by 16S-23S rDNA spacer region sequences.

	1	2	3	4	5	6	7	8
1		9.2	9.1	9.2	8.3	8.3	8.4	9.2
2			99.2	99.1	96.6	96.6	97.4	99.2
3				99.8	96.3	96.3	97.1	99.9
4					96.2	96.2	97.0	99.8
5						100	98.2	96.4
6							98.2	96.4
7								97.2

1. *Acholeplama laidawii* (D13260); 2. Juniperus witches'-broom (GQ925918); 3. Khafr almond witches-broom (DQ195209); 4. *Knautia arvensis* phyllody (Y18052); 5. *Candidatus* Phytoplasma phoenicium strain A4 (AF515636); 6. *Candidatus* Phytoplasma phoenicium strain 21(AF515637); 7. Pigeon pea witches-broom (AF24895); 8. Roodan eggplant phyllody (JX464669).

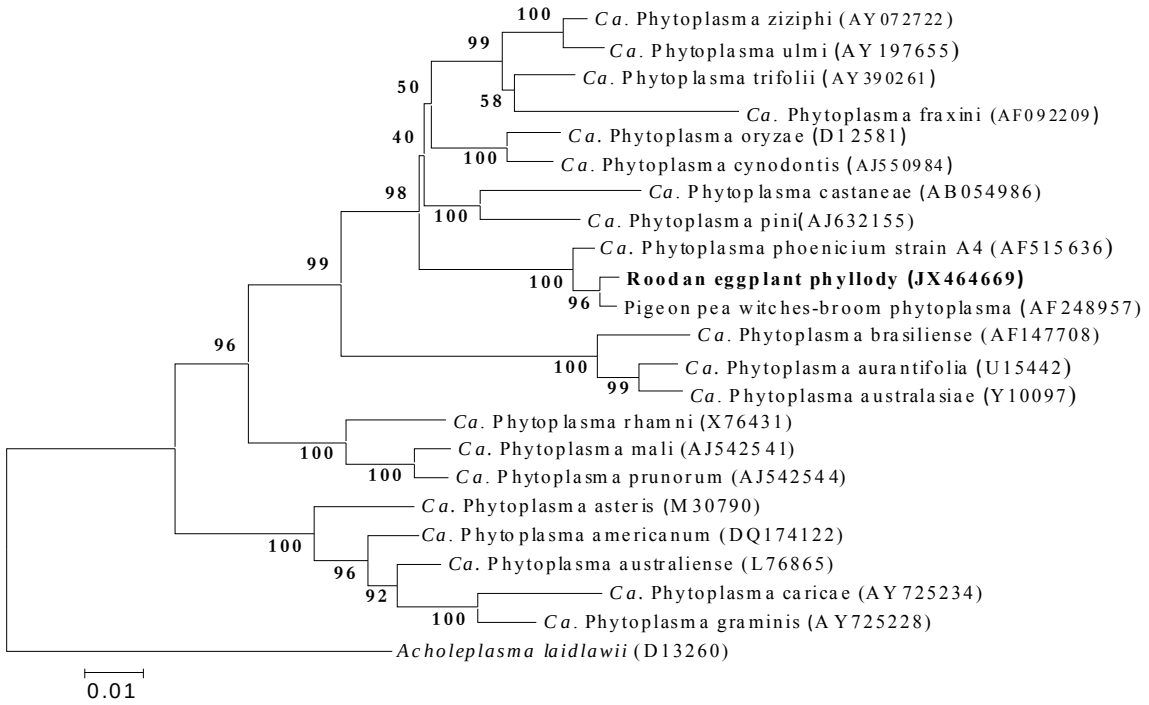

Figure 6 Phylogram of full-length 16S rDNA sequences of 22 phytoplasmas and *Acholeplasma laidlawii* as outgroup and position of Roodan eggplant phyllody phytoplasma (bold) in the phylogram. The tree was constructed using Neighbor-Joining method using MEGA5 software. GenBank accession numbers are in parentheses to the right of phytoplasma names. Bootstrap values are from 100 bootstrap replications. Bar = one nucleotide substitution per hundred nucleotides.

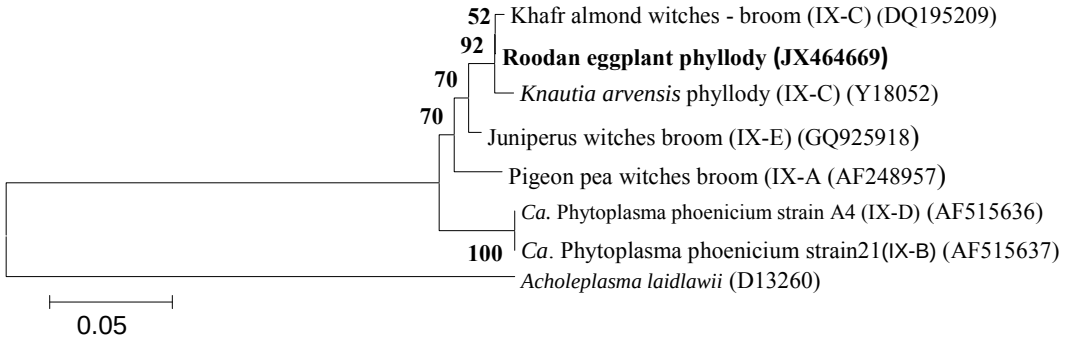

Figure 7 Phylogram of 16S-23S rDNA spacer region (SR) sequences of seven phytoplasmas related to seven pigeon pea witches'-broom group (16SrIX) subgroups including Roodan eggplant phyllody phytoplasma (bold). *Acholeplasma laidlawii* was used as outgroup. The tree was constructed by Neighbor-Joining method using MEGA5 software. GenBank accession numbers are in parentheses to the right of phytoplasma names. Bootstrap values are from 100 bootstrap replications. Bar = five nucleotide substitution per hundred nucleotides.

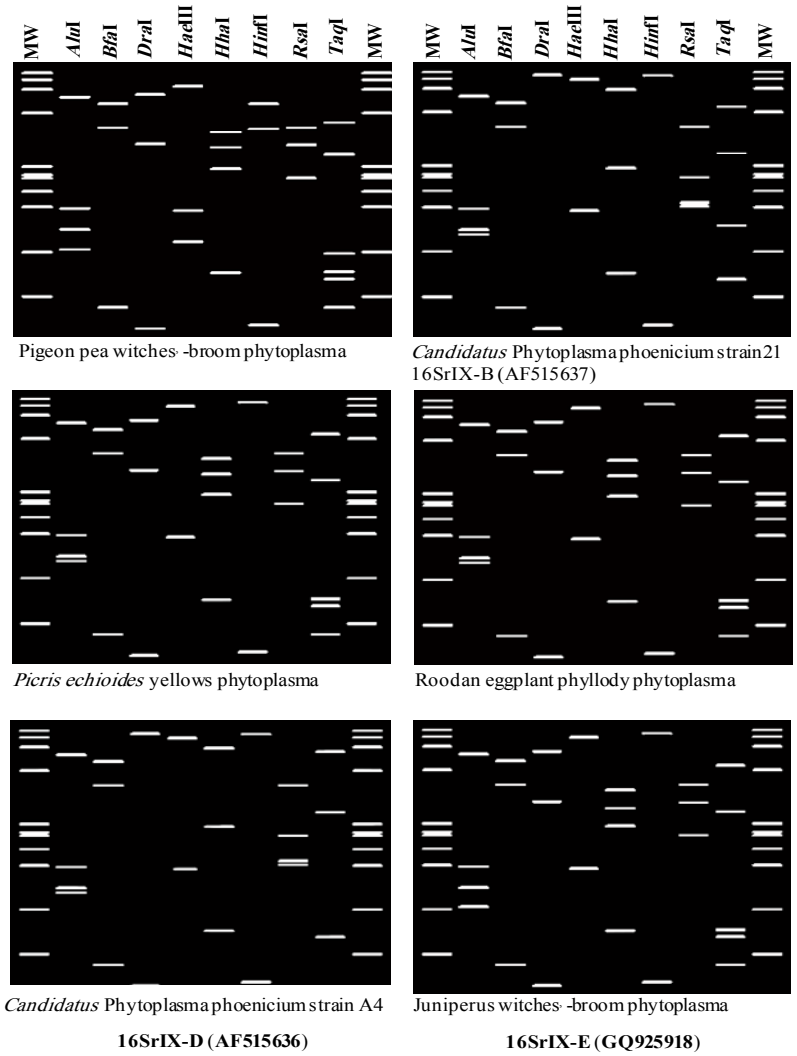

Figure 8 Virtual RFLP patterns of 1.2 kb 16S rRNA gene fragments of representative strains of 16SrIX subgroups and REP phytoplasma using pDRAW32 software. Recognition sites for the following eight restriction enzymes were used in the simulated digestions: *Alu*I, *Bfa*I, *Dra*I, *Hae*III, *Hha*I, *Hin*fI, *Rsa*I and *Taq*I. MW, DNA molecular weight marker.

Discussion

In the present study presence of a phytoplasma in phyllody affected eggplant plants was confirmed by direct and nested-PCR assays using universal phytoplasma primers. Dodder transmission of REP agent to healthy seed-grown periwinkle seedlings and production of characteristic symptoms of phytoplasma diseases is another strong evidence for association of phytoplasma with the disease. Graft inoculation showed that REP phytoplasma is transmissible to tomato plant. Hormozgan province is one of the main tomato growing area in Iran and phytoplasmal big bud disease of tomato have been previously reported from this province (Akbarpour *et al.*, 2012). It is yet to be determined whether REP agent occurs naturally in tomato fields of Hormozgan province. RFLP analysis of PCR product amplified by the primer pair P1/P7 and phylogenetic analysis of full-length 16S rRNA gene sequence showed that the detected phytoplasma in eggplant plants from Roodan is molecularly

classified as a member of the pigeon pea witches'-broom (16SrIX) group. Sequence homology and phylogenetic analysis of SR sequence indicated that as a 16SrIX related phytoplasma, REP phytoplasma is placed closer to the members of the subgroup 16SrIX-C than to selected members of the other 16SrIX subgroups. The virtual RFLP analysis also confirmed this conclusion. The pigeon pea witches'-broom phytoplasma group consists of diverse phytoplasma strains that cause numerous diseases in leguminous trees and herbaceous plants (family Fabaceae), vegetable crops (Brassicaceae and Dipsacaceae), a nut crop (almond), herbs and weeds (Asteraceae) and, recently, a forest tree (juniper) and a fruit crop (blueberry) in various geographical regions including North and South America, Europe, Asia and the Middle East (Lee *et al.*, 2012). To our knowledge, eggplant is reported for the first time as a host for 16SrIX phytoplasma group. Sesame (Salehi *et al.*, 2005) lettuce and wild lettuce (Salehi *etal*., 2007) are other herbaceous hosts of 16SrIX phytoplasma group in Iran. 16SrIX related phytoplasmas also have been previously detected in witches'-broom affected almonds in Iran (Salehi *et al*., 2006). Significance of phytoplasmas associated with phyllody diseases of eggplant, sesame, lettuce and wild lettuce as sources of almond witches'-broom disease remains to be determined.

References

Abou-Jawdah, Y., Karakashina, A., Sobh, H., Martini, M. and Lee, I. M. 2002. An epidemic of almond witches-broom in Lebanon: classification and phylogenetic relationships of the associated phytoplasma. Plant Disease, 86: 477-484.

Akbarpour, K., Salehi, E. and Salehi, M. 2012. Firs report of tomato big bud disease in Hormozgan province. In: (Ed.). Proceeding of the 20th Iranian Plant Protection Congress, Shiraz, Iran, p. 569.

Al-Subhi, A. M., Al-Saady, N. A., Khan, A. J. and Deadman, M. L. 2011. First report of a group 16srii phytoplasma associated with witches'-broom of eggplant in Oman. Plant Disease, 95: 360.

Amaral Mello, A. P. O., Ba´rbara, E., Daniela, F., Patrı´cia, F. K. and Bedendo, I. P. 2011. Identification by computer-simulated RFLP of phytoplasmas associated with eggplant giant calyx representative of two subgroups, a lineage of16SrIII-J and the new subgroup 16SrIII-U. International Journal of Systematic and Evolutionary Microbiology, 61: 1454-1461.

Azadvar, M. and Baranwal, V. K. 2010. Molecular Characterization and Phylogeny of a Phytoplasma Associated with Phyllody Disease of toria (*Brassica rapa* L. subsp. dichotoma (Roxb.) in India. Indian Journal of Virology**,** 21: 133-139.

Bertaccini, A. and Duduk, B. 2009. Phytoplasma and phytoplasma diseases: a review of recent research. Phytopathologia Mediterranea, 48: 355-378.

Ember I., A. C. Z., Munyaneza, J. E., Crosslin, J. M. and Kölber, M. 2011. Survey and molecular detection of phytoplasmas associated with potato in Romania and Southern Russia*. European* Journal of Plant Pathology*,* 130: 367-377.

FAO, 2012. Faostat data. Available on: http://www.fao.org Retrieved on 25 February, 2014.

Gundersen D. E. and Lee I. M., 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopathologia Mediterranea, 35: 144-151.

Kelly, P., Arocha, Y. and Dider, S. Z. 2009. First report of a 16SrI, *Candidatu* Phytoplasma asteris' isolate affecting eggplant and *Mikania* sp. in Bangladesh. Plant Pathology, 58: 789.

Khan, A. J., Al-Subhi, A. M., Calari, A., Al-Saady, N. A. and Bertaccin, A. 2007. A new phytoplasma associated with witches'-broom of Cassia italica in Oman. Bulletin of Insectology, 60: 269-270.

Kumar, J., Gunapati, S., Singh, S. P., Lalit, A., Sharma, N. C. and Tuli, R. 2012. First report of a *'Candidatus* Phytoplasma asteris' (16SrI group) associated with little leaf disease of *Solanum melongena* (accept) in India. New Disease Reports, 26: 21-21.

Lee I. M., Davis R. E. and Gundersen-Rindal D. E. 2000. Phytoplasma: phytopathogenic

mollicutes. Annual Review of Microbiology, 54: 221-255.

Lee, I. M., Botner-Parker, K. D., Zhao, Y., Bertaccini, A. and Davis, R. E. 2012. Differentiation and classification of phytoplasmas in the pigeon pea witches'-broom group (16SrIX): an update based on multiple gene sequence analysis. International Journal of Systematic and Evolutionary Microbiology, 62: 2279-2285.

Lee, I. M., Gundersen-Rindal, D. E., Davi, R. E. and Bartoszyk, I. M. 1998. Revised classification scheme of phytoplasma based on RFLP analyses of 16S rDNA and ribosomal protein gene sequences. International Journal of Systematic Bacteriology, 48:1153-1169.

Molino Lova, M., Quaglino, F., Abou-Jawdah, Y., Choueiri, E., Sobh, H., Alma, A., Tedeschi, R., Casati, P. and Bianco, P. A. 2011. '*Candidatus* Phytoplasma phoenicium'-related strains infecting almond, peach and nectarine in Lebanon. Bulletin of Insectology, 64: 267-268.

Okuda, S., Prince, J. P., Davis, R. E. Dally, E. L., Lee, I. M., Mogen, B. and Kato, S. 1997. Two groups of phytoplasma from Japan distinguished on the basis of amplification and restriction analysis of 16S rDNA. Plant Disease 81: 301-305.

Omar, A. F. and Foissac, X. 2012. Occurrence and incidence of phytoplasmas of the 16SrII-D subgroup on solanaceous and cucurbit crops in Egypt. European Journal of Plant Pathology, 133: 353-360.

Salehi, M., Heydarnejad, J. and Izadpanah, K. 2005. Molecular characterization and grouping of 35 phytoplasmas from central and southern provinces of Iran. Iranian Journal of Plant Pathology, 41: 62-64.

Salehi, M., Izadpanah, K. and Heydarnejad, J. 2006. Characterization of a new almond witches' broom phytoplasmas in Iran. Journal of Phytopathology, 154: 386-391.

Salehi, M., Izadpanah, K., Nejat, N. and Siampour, M. 2007. Partial characterization of phytoplasmas associated with lettuce and wild lettuce phyllodies in Iran. Plant Pathology, 65: 669-670.

Schneider, B., Seemuller, E., Smart, C. D. and Kirkpatrick, B. C. 1995. Phylogenetic classification of plant pathogenetic mycoplasmalike organisms or phytoplasmas. In: Razin, S. and Tulley, J. G. (Eds), Molecular and Diagnostic Procedures in Mycoplasmology, Vol. I. New York, Academic Press. pp. 369-380.

Sertkaya, G., Martini, M., Musetti, R. and Osler, R. 2007. Detection and molecular characterization of phytoplasmas infecting sesame and solanaceous crops in Turkey. Bulletin of Insectology, 60: 141-142.

Siddique, A. B. M., Agrawal, G. K., Alam, N. and Krishina Reddy, M. 2001. Electron microscopy and molecular characterization of phytoplasmas associated with little leaf disease of Brinjal (*Solanum melongena* L.) and periwinkle (*Catharanthus roseus*) in Bangladesh. Journal of Phytopathology, 149: 237-244.

Tamura, K., Peterson, D., Peterson, N. Stecher, G., Nei, M. and Kumar, S. 2011. MEGA5: evolutionary genetics analysis using maximum likelihood, Evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28: 2731-2739.

Verdin, E., Salar, P., Danet, J.-L., Choueiri, E., Jreijiri, F., El Zammar, S., Gélie, B., Bové J. and Garnier, M. 2003. '*Candidatus* Phytoplasma phoenicium' sp. nov., a novel phytoplasma associated with an emerging lethal disease of almond trees in Lebanon and Iran. International Journal of Systematic and Evolutionary Microbiology, 53: 833-838.

Wei, W., Lee, I. M., Davis, R. E., Suo, X. and Zhao, Y. 2008. Automated RFLP pattern comparison and similarity coefficient calculation for rapid delineation of new and distinct phytoplasma 16Sr subgroup lineages. International Journal of Systematic and Evolutionary Microbiology, 58: 2368-2377.

Zhang, Y. P., Uyemoto, J. K. and Kirkpatrick, B. C. 1998. A small-scale pro-cedure for extracting nucleic acids from woody plants infected with various phytoplasmas for PCR assay. Journal of Virological Methods, 71: 45-50.

همراهی یک فیتوپلاسما از زیرگروه 16SrIX-C با بیماری فیلودی بادمجان در رودان (استان هرمزگان)

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چکیده: در بازدیدهایی که در سال‌های ۱۳۹۰ و ۱۳۹۱ برای ردیابی بیماری های فیتوپلاسمایی به عمل آمد در مزارع بادمجان (*Solanum melongena* L.) رودان (استان هرمزگان) علائم بیماری فیلودی مشاهده گردید. عامل فیلودی بادمجان در رودان به‌وسیله سس (*Cuscuta campestris* Yunck.) از بادمجان به پروانش و با روش پیوند از بادمجان به بادمجان و گوجه‌فرنگی انتقال داده شد. در گیاهان مایه‌زنی شده علائم بیماری‌های فیتوپلاسمایی مشاهده گردید. آلودگی به فیتوپلاسما در نمونه‌های بادمجان دارای علائم در طبیعت و نمونه‌های بادمجان،گوجه‌فرنگی و پروانش مایه‌زنی شده با عامل فیلودی بادمجان در آزمون واکنش زنجیره‌ای پلیمراز (PCR) مستقیم با استفاده از جفت آغازگر P1/P7 و آزمون PCR دو مرحله‌ای با استفاده از جفت آغازگرهای P1/P7 و R16F2n/R16R2 تأیید شد. محصول PCR با جفت آغازگر P1/P7 همسانه‌سازی و تعیین ترادف شد وتحت رس شمار JX464669 .در بانک جهانی ترادف‌ها ثبت گردید. مقایسه چندشکلی طولی قطعات برشی (RFLP) نشان داد که فیتوپلاسمای همراه با فیلودی بادمجان رودان متعلق به گروه جاروک نخود کبوتر (pigeon pea witches'-broom, 16SrIX) می‌باشد. جستجو با برنامه Blast، آنالیزهای فیلوزنتیکی، RFLP مجازی و مقایسه تشابه نوکلئوتیدی نشان داد که عامل فیلودی بادمجان رودان در بین اعضای گروه جاروک نخود کبوتر با اعضاء زیرگروه 16SrIX-C طبقه‌بندی می‌شود. براساس اطلاعات موجود، بادمجان برای اولین بار به‌عنوان میزبان یک فیتوپلاسما از گروه جاروک نخود کبوتر معرفی می‌شود.

واژگان کلیدی :فیلودی بادمجان، فیتوپلاسما، انتقال با پیوند و سس، زیرگروه 16SrIX-C، رودان