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Full Length Article

Detection and Molecular Characterization of Alfalfa Witches'-Broom Phytoplasma and its Leafhopper Vector in Riyadh region of Saudi Arabia

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Abstract

This study was conducted to detect for the first time the occurrence of phytoplasma in symptomatic alfalfa plants as well as in its insect vectors in Riyadh region of Saudi Arabia together with its characterization by comparing it with isolates detected elsewhere in the world. Disease symptoms similar to those described for phytoplasma diseases were observed on alfalfa plants growing in the Riyadh region (Wadi aldawasir, Sagir and AlZulfi), Saudi Arabia. Three representative alfalfa samples and two groups of leafhopper samples out of the total samples collected from the fields in the visited areas tested positive for plant pathogenic phytoplasmas using P1/P7 primer pair in the first round of PCR test. An additional PCR was conducted using the nested R16F2n/R16R2 primer pair, which yielded fragments of approximately 1.2 kb. The rest of the collected samples were tested using a synthesized cDNA probe for phytoplasma detection by dot blot hybridization. Fifty four out of 84 alfalfa samples and 65 out of 94 leafhopper samples collected from the abovementioned locations hybridized with the cDNA probe, representing 64.3% and 69.1%, respectively. The nucleotide sequences for the five positive samples were determined and were found to have 99.3%-100% nucleotide sequences identity among them, and share 97.3-98.8% sequence similarity with seven phytoplasma isolates belonging to the 16S rII (peanut witches'-broom group) obtained from the GenBank database. The nucleotide sequences of the five isolates detected in this study were published in the GenBank with the following accession numbers: JQ808130, JQ818819, JQ818820, JX646694 and JX646695. © 2014 Friends Science Publishers

Keywords: Alfalfa; Phytoplasma; 16SrII; Leafhopper; PCR; Sequence; Hybridization

Introduction

Phytoplasmas are non-helical wall-less prokaryotes that are pleomorphic in shape, have a low G+C content, and colonize the plant phloem. The first report of phytoplasma was almost 40 years ago and currently is classified in the class Mollicutes (McCoy *et al.*, 1989; Firrao *et al.*, 2005). Phytoplasmas which are currently classified into phylogenetic groups and Candidatus species are known to severely affect the plants they infect resulting in several important diseases throughout the world, and are transmitted from plant to plant by sap-feeding insect vectors (Weintraub and Beanland, 2006; Zhao *et al.*, 2010). Alfalfa (*Medicago sativa* L.) is one of the major forage crops in Saudi Arabia. Average annual cultivation exceeds 122563 thousand hectares, and the productivity from this area is more than 252844 thousand tons (Agricultural Statistical Year Book, 2010). Symptoms such as those produced by phytoplasma are currently spreading extensively on alfalfa in Saudi Arabia and seem to reduce the crop yield, limit the life span of the crop in the field, and decrease the market and nutritional values of the crop. The diagnosis of phytoplasma diseases is dependant to a large extent on molecular techniques (Gundersen *et al.,* 1994; Lee *et al*., 1995, 1998; Gungoosingh-Bunwaree *et al.*, 2013). Phytoplasma diseases are spreading in many countries worldwide such as the United States (Peters *et al.*, 1999), Australia (Bowyer, 1969), Argentina (Conci *et al.*, 2005), Canada (Khadhair *et al.*, 1997), China (Chen, 1996, Li *et al.*, 2012), Iran (Esmailzadeh *et al.*, 2011; Raoofi and Salehi, 2012), Serbia (Starovic *et al.*, 2012), Oman (Khan *et al.*, 2002), Egypt (Omar *et al.*, 2008), Lebanon (Choueiri *et al.,* 2003), Mauritius (Gungoosingh-Bunwaree *et al.,* 2013), Kuwait (Al-Awadhi *et al.*, 2002) and Italy (Parrella *et al.*, 2008). In the eastern region of Saudi Arabia, phytoplasma agents have been detected from different crops such as tomato (Alhudaib and Rezk, 2011), alfalfa (Alhudaib, 2009), and date palm (El-Zayat *et al.*, 2002). This study was conducted to detect and characterize, for the first time, the agent associated with phytoplasma-like symptoms in alfalfa fields in the Riyadh region of Saudi Arabia and to determine whether the leafhopper vector Empoasca decipiens (Paoli), found in the field carries this disease agent as well.

Materials and Methods

Source of Samples

Eighty seven alfalfa samples exhibiting typical phytoplasma

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symptoms, which included stunting, rosette, discoloration of leaves, witches'-broom and early senescence (Fig. 1) were collected from plants growing under field conditions in Riyadh region. The samples were wrapped in plastic bags and carefully transported to the laboratory. Three of these samples were selected as representative to three different locations in the Riyadh region (Wadi aldawasir, Sagir and Alzulfi locations), Saudi Arabia during 2012. Ninety six Leafhopper (*E. decipiens*) samples (5 insects each) were collected from Wadi aldawasir and Sagir areas in a suction trap from the same alfalfa fields. These insect samples were placed in eppendorf tubes and kept at -20°C. The three selected alfalfa samples in addition to two selected leafhopper samples were analyzed for the presence of phytoplasma using PCR assays whereas the rest of the samples were tested by dot blot hybridization.

DNA Extraction and PCR Analysis

Extraction of DNA from the selected alfalfa and leafhopper samples was performed according to the protocol described by Arismendi *et al.* (2010). The primer base pair P1 and P7 were used to prime the amplification of a 1.8 kb product as mentioned by Schneider *et al.* (1995), Deng and Hiruki (1991). The extracted DNAs from symptomatic alfalfa samples and leafhoppers were used as templates for PCR analysis using KAPA™ LongRange DNA Polymerase kit (KAPA Biosystems). For amplification, 1 µL of template DNA was used. Fifty microliters of PCR reaction mixture were added to each PCR tube containing the following reaction mixture: 1.25 units of the KAPA Long Range HotStart DNA Polymerase, (2.5 u/µL), 0.3 mM dNTPs (10 mM each dNTP), 10 µL of 5X KAPA Long Range Buffer (without $MgCl_2$), 1.75 mM $MgCl_2$ (25 mM), 10 µM of each primer P1/P7, and PCR grade water up to 50 µL. The DNA amplification program used was according to (Khan *et al.*, 2002) in a thermocycler (PeQLab, Primus 96). The R16F2n and R16R2 as a nested primer pair (Lee *et al.,* 1995; Gundersen and Lee, 1996) was used to amplify the phytoplasma 16S rRNA gene. The reaction mixture with template DNA extracted from healthy alfalfa was used as a negative control. The method described by Sambrook and Russell (2001) was employed in the gel electrophoresis step. The amplified fragments of the expected size for the three alfalfa samples obtained from Wadi aldawasir, Sagir and Alzulfi locations and the two leafhoppers samples from Wadi aldawasir and Sagir locations were excised from agarose gel and purified with Agarose Gel Extract Mini kit (Promega).

Generation of Digoxigenin cDNA Probe for Detection of Phytoplasma

The target fragment obtained from Wadi aldawasir isolate (Alf-SA-1), which was purified was labeled by PCR using nested primer pair (R16F2n and R16R2) with digoxigenin-dUTP according to the manufacturer's instructions (Roche) for synthesis of the digoxigenin cDNA probe. In the dot blot hybridization assay, sap extraction was performed by grinding 30 mg of fresh alfalfa plant and leafhopper samples as described by Arismendi *et al.* (2010). Five µL of the DNA extract were spotted onto a nitrocellulose membrane, air dried, and irradiated with a UV cross linker. Pre-hybridization and hybridization were carried out at 68°C (under conditions of high stringency) essentially as described by Pallas *et al.* (1998). Binding of the probe with the DNA samples was immunologically detected using 200 µl Nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3'-indolyphosphate (BCIB) stock solution (Roche). The results were documented by wet filter photography.

DNA Sequencing and Phylogenetic Analysis

The nucleotide sequences of the 16S rRNA gene of these phytoplasma isolates detected in the three alfalfa (Alf SA-1, Alf-SA-2, Alf-SA-3) and the two leafhopper samples (C5 and E6) were determined through bidirectional sequencing with the R16F2n/R16R2 primers using AB3730xl DNA Analyzer model HITACHI. Analysis of the obtained sequences was carried out using the DNAMAN software trial version 5.2.10 program (Lynnon BioSoft, Canada). To achieve a valid comparison, 36 phytoplasma isolate sequences belonging to different groups obtained from GenBank database (Table 1) were reduced to the longitude of the isolated sequences in this study.

Results

Detection of Phytoplasma in Alfalfa Plants and Leafhopper using PCR

Too weak fragments (1.8 kb) were observed on 1% agarose gel for the DNA extracted from the three alfalfa samples showing phytoplasma-like symptoms and the two leafhopper samples using P1/P7 primer pair in the PCR assay. A readily visible PCR products (1.2 kb) were amplified however using the weak products obtained previously as a template with the nested primer pair (R16F2n/R16R2) through 1% agarose gel electrophoresis (Fig. 2A: lanes 2-4) and (Fig. 2 B: lanes 1-2). HyperLadder^{TM} II, (Bioline) as a molecular weight standard (lane M) was used for estimating fragments sizes. No PCR product was obtained from healthy alfalfa plants as a negative control (lane A: 1 and B: H). These results confirmed the occurrence of a phytoplasma in the alfalfa plants and the leafhopper insects collected from Riyadh region, Saudi Arabia.

Digoxigenin cDNA Probe for Detection of Alfalfa Phytoplasma

The cDNA probe synthesized from Wadi aldwasir isolate of alfalfa Phytoplasma (Alf-SA-1) was used for detection of phytoplasma in the rest of the plant and leafhopper samples. This probe hybridized with the total DNA

Table 1: Percentage of nucleotide sequences similarity for Saudi isolates of alfalfa witches'-broom phytoplasma and 36 different phytoplasma isolates obtained from the GenBank using DNAMAN software analysis

Accession No.	Phytoplasma name	Group	Saudi Isolates of phytoplasma				
			JQ808130 (Alf-SA-1)	JQ818819 (Alf-SA-2)	JQ818820 (Alf-SA-3)	JX646694 (C 5)	JX646695 (E 6)
U15442	Candidatus Phytoplasma aurantifolia	16SrII	98.0%	97.5%	98.0%	97.5%	97.6%
EF193157	Pear decline phytoplasma (Taiwan II)	16SrII	98.7%	98.3%	98.7%	98.3%	99.4%
EF656453	Crotalaria witches'-broom	16SrII	98.7%	98.3%	98.7%	98.3%	99.4%
HM584815	Tomato witches'-broom phytoplasma	16SrII	98.9%	98.6%	98.9%	98.5%	99.4%
JQ808130	Alfalfa witches'-broom phytoplasma	16SrII	100%	99.5%	99.3%	99.7%	99.8%
JQ818819	Alfalfa witches'-broom phytoplasma	16SrII	99.5%	100%	99.5%	99.4%	99.5%
JQ818820	Alfalfa witches'-broom phytoplasma	16SrII	99.3%	99.5%	100%	99.3%	99.8%
JX646694	'Empoasca decipiens' phytoplasma	16SrII	99.7%	99.4%	99.7%	100%	99.5%
JX646695	'Empoasca decipiens' phytoplasma	16SrII	99.8%	99.5%	99.8%	99.5%	100%
AF028813	Chinese pigeon pea witches'-broom	16SrII	98.7%	98.3%	98.7%	98.3%	99.4%
L33765	Peanut witches'-broom phytoplasma	16SrII	98.7%	98.3%	98.7%	98.3%	99.4%
DQ452417	Sweet potato witches'-broom	16SrII	98.7%	98.3%	98.7%	98.3%	99.4%
AY734453	Barley deformation	16SrI	87.9%	86.2%	87.9%	86.6%	87.8%
D12569	Onion yellows	16SrI	87.9%	86.2%	87.9%	86.6%	87.8%
DQ913090	Date palm phytoplasma	16SrI	87.8%	86.0%	87.8%	98.7%	87.6%
M30790	*Candidatus* Phytoplasma asteris	16SrI	87.8%	86.2%	87.8%	86.6%	87.6%
X76430	Vaccinia witches'-broom	16SrIII	90.5%	88.3%	90.5%	88.5%	90.3%
AY072722	Candidatus Phytoplasma ziziphi	16SrV	86.9%	85.0%	86.9%	85.4%	86.9%
U43569	Phormium yellow leaf'	16SrXII	88.5%	86.4%	88.5%	86.8%	88.2%
X76427	Stolbur transmitted from C. anuum	16SrXII	88.0%	86.4%	88.0%	86.8%	87.7%
Y10095	Paoaya dieback	16SrXII	88.7%	86.7%	88.7%	87.1%	88.4%
Y10095	Paoaya dieback	16SrXII	88.7%	86.7%	88.7%	87.1%	88.4%
AJ243044	Strawberry green petal	16SrXII	88.7%	86.7%	88.7%	87.1%	88.4%
AJ243045	Strawberry lethal yellows	16SrXII	88.7%	86.7%	88.7%	87.1%	88.4%
DQ222972	Corn-reddening phytoplasma 2005/2	16SrXII	88.1%	86.4%	88.1%	86.8%	87.8%
DQ913092	A. decedens phytoplasma	16SrXII	88.1%	86.4%	88.1%	88.7%	87.8%
L76865	Candidatus Phvtoplasma australiense	16SrXII	88.5%	86.7%	88.5%	87.1%	88.4%
D12581	Candidatus Phytoplasma oryzae	16SrXI	89.2%	86.9%	89.2%	87.1%	89.3%
X76432	Sugarcane white leaf	16SrXI	88.7%	86.2%	88.7%	86.4%	88.6%
AF248960	Periwinkle virescence	16SrXIII	88.1%	86.4%	88.1%	86.6%	87.8%
AJ542544	*Candidatus* Phytoplasma prunorum	L6SrX	88.5%	86.7%	88.5%	86.8%	88.4%
X76431	*Candidatus* Phytoplasma rhamni	16SrX	88.6%	87.6%	88.6%	87.8%	88.8%
X92869	*Candidatus* Phytoplasma spartii	16Sr X	88.5%	86.9%	88.5%	82.4%	88.4%
AF092209	Candidatus Phytoplasma fraxini	16SrVII	88.3%	86.7%	88.3%	86.9%	88.4%
AF147708	Candidatus Phytoplasma brasiliense	16SrXV	94.9%	94.5%	94.9%	94.3%	95.7%
AF176319	Candidatus Phytoplasma vitis	16SrV	87.6%	85.5%	87.6%	85.9%	87.6%
AY390261	Candidatus Phytoplasma trifolii	16SrVI	87.8%	86.2%	87.8%	86.4%	87.8%
AY725228	Candidatus Phytoplasma gaminis	16SrXVI	86.1%	83.8%	86.1%	84.1%	85.4%
AY725234	Candidatus Phytoplasma caricae	16SrXVII	87.2%	85.2%	87.2%	85.6%	86.7%
L33764	Loofah witches'-broom phytoplasma	16SrVIII	88.6%	87.4%	88.6%	87.6%	88.8%
U18747	Coconut lethal yellowing phytoplasma	16SrIV	89.6%	88.6%	89.6%	89.1%	90.1%

extracted from symptomatic alfalfa plants and the leafhopper insects collected from fields in the surveyed locations using dot blot hybridization (Fig. 3A and B). Positive hybridization reaction indicated by formation of purple color on the nitrocellulose membrane was observed in DNA extracts of 54 out of 84 alfalfa samples collected, representing 64.3%, and 65 out of 94 leafhopper collected samples representing 69.1%, but was not observed with DNA extracts from healthy alfalfa plants (A: F2 and G2) and (B: K12 and L12) as a negative control.

DNA Sequencing and Phylogenetic Analysis

Nucleotide sequences for the three phytoplasma isolates detected in alfalfa plants in Wadi aldawasir, Sagir and Alzulfi locations, designated (Alf-SA-1, Alf-SA-2 and Alf-SA-3) and the two phytoplasma isolates detected in leafhopper samples from Wadi aldawasir and Sagir locations, which were designated (C5 and E6) were determined and compared with each other. The percentage of sequence identity between these five Saudi isolates ranged between 99.3-100%. A multiple sequence alignment was done between sequences of the five Saudi phytoplasma isolates and 36 sequences obtained from the GenBank database for phytoplasma isolates reported in different countries and used as reference sequences in other studies to determine their phylogenetic relationship with the Saudi isolates. The percentage of sequence identity of these five Saudi isolates and the 36 GenBank isolates ranged between 82.4%-99.4% (Fig. 4; Table 1). Seven 16S rRNA phytoplasma isolates belonging to the peanut witches'-broom group out of the 36 isolates shared 97.5%-99.4% of their nucleotides [(AF028813 (98.7%, 98.3, 98.7%, 98.3%

Fig. 1A, B and C: Alfalfa plants exhibiting stunting, rosette, discoloration of leaves, witches'-broom and early senescence as typical phytoplasma symptoms. D: healthy alfalfa plants

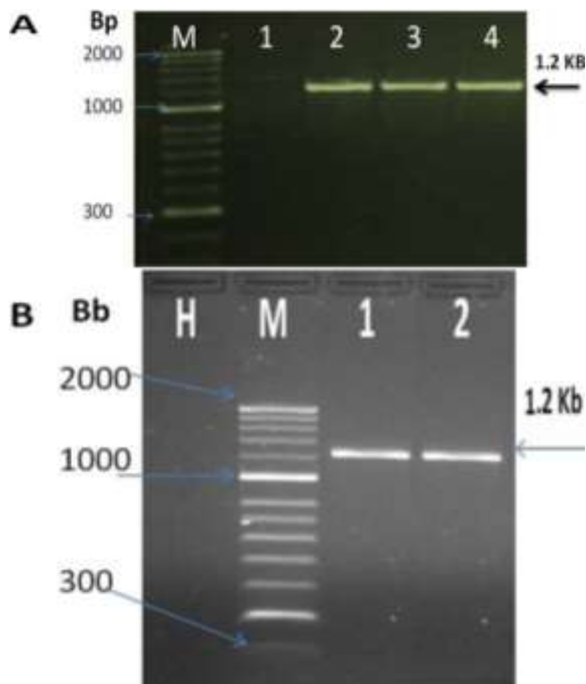

Fig. 2A: Agarose gel electrophoresis (1%) of nested PCR products amplified from **A**: symptomatic alfalfa plants collected from alfalfa fields in Wadi aldawasir, Sagir and Alzulfi locations (lanes 2-4). **B**: leafhoppers collected from alfalfa fields in Wadi aldawasir and Sagir (lanes 1-2), with the specific primer pair R16F2n and R16R2). Arrow on the right indicates the expected fragment size of nested PCR products (1.2 KB). DNA extract from healthy alfalfa plants (lane A: 1 and B: H). HyperLadder^{TM} II (DNA marker), as a molecular weight standard (lane M)

and 99.4%); DQ452417 (98.7%, 98.3%, 98.7%, 98.3% and 99.4%); EF193157 (98.7%, 98.3%, 98.7%, 98.3% and 99.4%; EF656453 (98.7%, 98.3%, 98.7%, 98.3% and 99.4%); HM584815 (98.9%, 98.6%, 98.9%, 98.5% and

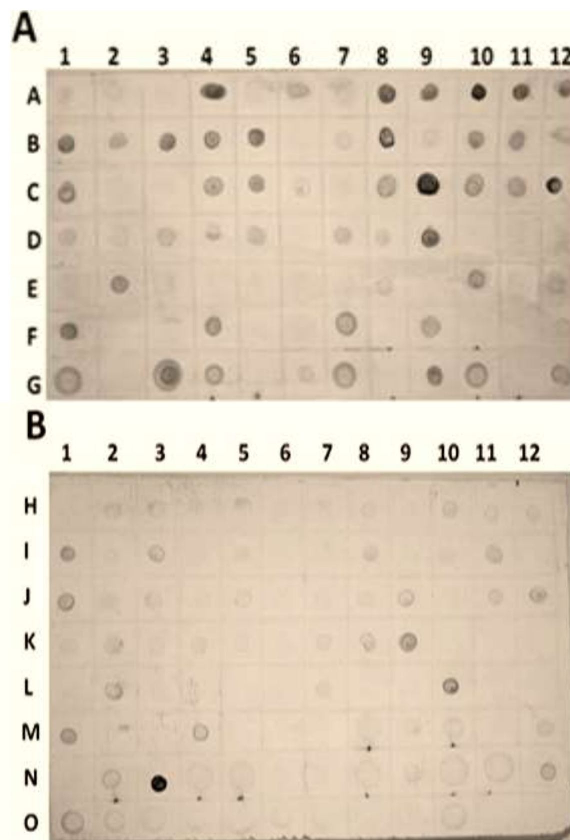

Fig. 3: Dot blot hybridization of DNA extracted from symptomatic alfalfa plants **(A)**: samples collected from Wadi aldawasir (row: A, B and C), Sagir (row: D and E) and AlZulfi (row: F and G), and **(B)**: leafhopper samples collected from alfalfa fields in Wadi aldawasir (row: H, I, J and K), Sagir (row: L, M, N and O) using a DIG-labeled phytoplasma cDNA probe. Purple spots indicate positive signals for phytoplasma infection. No hybridization reaction was observed with DNA extracts from healthy alfalfa plants (**A:** F-2 and G-2 and **B:** K-12 and L-12)

99.4%); L33765 (98.7%, 98.3%, 98.7%, 98.3% and 99.4%) and U15442 (98.0%, 97.5%, 98.0%, 97.5% and 97.6%)] with the five Saudi isolates (Alf-SA-1, Alf-SA-2, Alf-SA-3, C5 and E6), respectively. This close relationship in nucleotide similarity suggests that the Saudi isolates belong to the peanut witches'-broom group of phytoplasma. The nucleotide sequences for the five Saudi isolates were published in the GenBank with the accession numbers JQ808130, JQ818819, JQ818820, JX646694 and JX646695.

Discussion

More than 300 phytoplasma agents causing diseases in different plant species belonging to field crops, vegetables, trees and weeds as well as in their insect vectors have been reported (Parrella *et al.*, 2008).

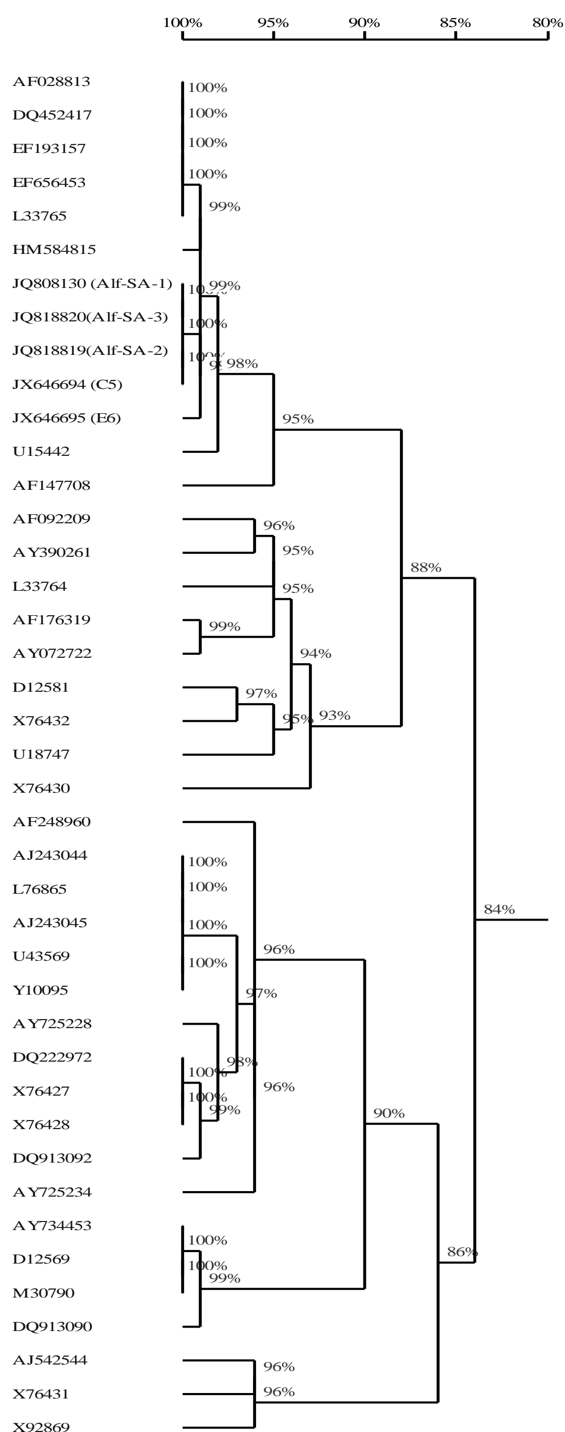

Fig. 4: A phylogenetic tree constructed from the multiple alignment of the nucleotide sequences of the 16S rRNA genes for three phytoplasma isolates from alfalfa (Alf-SA-1, Alf-SA-2 and Alf-SA-3) and two phytoplasma isolates from leafhoppers (C5 and E6) together with 36 different phytoplasma isolates obtained from the GenBank database using DNAMAN software analysis

The wide spreading phytoplasma-like disease symptoms that included stunting, rosette, discoloration of leaves, witches'-broom and early senescence in alfalfa (Conci *et al.*, 2005, Li *et al.*, 2012, Raoofi and Salehi, 2012) growing in the Riyadh region is the reason for the initiation of this investigation. PCR analysis was employed to detect phytoplasma in many of earlier investigations (Chang, 2004; Salehi *et al.*, 2006; Esmailzadeh *et al.*, 2011; Li *et al.*, 2012; Raoofi and Salehi, 2012). In the present study, an amplified PCR product (1.2 Kb) was obtained from the DNA extract of the samples collected from symptomatic alfalfa plants growing in different locations in the Riyadh region of Saudi Arabia using two pairs of universal primer sequences derived from phytoplasma 16S rRNA, but no such products were obtained from healthy alfalfa plants. The detection of phytoplasma by PCR in the leafhopper, which is an important phytoplasma vector, consistently found in alfalfa yields showing the abovementioned symptoms in the visited locations may be implicated in the current extensive spread and dissemination of this phytoplasma disease in Riyadh region of Saudi Arabia. However, the primary source of infection will remain to be determined. Although high quality of total nucleic acid extracted is a pre-requisite for success of detection of phytoplasma by PCR, that goal is usually practically difficult to achieve (Firrao *et al.*, 2007). Added to that the low amount of phytoplasma DNA in the total nucleic acid extracted from samples, which is estimated to be around 1% (Bertaccini, 2007) makes the success of PCR in detection of phytoplasma even more difficult. That could probably explain the low titer of phytoplasma obtained through direct PCR using P1/P7 primers in this study. These findings imposed the use of nested PCR assay, which is performed by preliminary amplification using a universal primer pair (P1/P7) followed by a second amplification using a second universal primer pair (R16F2n/R16R2) and the product of the first PCR as a template, which was reported to increase both sensitivity and a specificity of detection of phytoplasma from samples having unusually low titers (Gundersen *et al.*, 1994). The use of this technique has helped to get readily detectable fragments in the agarose gel in this study and to detect phytoplasma present in mixed infections in other studies (Lee *et al.*, 1995).

PCR analysis was employed to detect phytoplasma in many of previous investigations (Chang, 2004; Salehi *et al.*, 2006; Esmailzadeh *et al.*, 2011; Li *et al.*, 2012; Raoofi and Salehi, 2012). In fact it was not only used for detection of phytoplasma in symptomatic plants but it was also used to detect this agent in its insect vector (Khan *et al.*, 2002; Salehi *et al.*, 2006; Parrella *et al.*, 2008; Alhudaib, 2009; Raoofi and Salehi, 2012). Phytoplasma has also been detected in some weeds (Marcone *et al.*, 1997; Blanche *et al.*, 2003; Arocha *et al.*, 2005, Tolu *et al.*, 2006; Babaie *et al.*, 2007), a fact that completes the epidemiological cycle of this disease and encourages initiation of further epidemiological studies crucial for its management.

Detection of phytoplasma by dot blot hybridization (Marzachì *et al.,* 2000; Bertolini *et al.*, 2007) in 64.3% and

69.1%, of the collected alfalfa and leafhopper samples respectively suggest a probable role of this insect vector in the wide spread of this pathogenic agent which was reported to cause important diseases that significantly reduce the yield and the economic life expectancy of the crop in the field in the surveyed regions as reported early (Khan *et al*., 2002). It worth mentioning that noticeable differences were observed in phytoplasma concentration between the tested plant and insect samples (30 mg) and that phytoplasma titer in the plant samples was generally higher than that in the insects samples based on the purple color density observed on the nitrocellulose membrane.

Sequencing of the five detected phytoplasma isolates was performed to facilitate their comparison with other isolates detected in alfalfa and leafhopper. The high similarity observed in the Phylogenetic analyses among the three phytoplasma isolates detected in alfalfa (99.3-100%) and the two phytoplasma isolates detected in leafhoppers (99.4-100%) does not only suggest that theses five isolates are in fact the same isolate but it may also suggest the involvement of this insect vector in the dissemination of this phytoplasma isolate from plant to plant. The phylogenetic tree also indicated the close relationship between the phytoplasma isolates detected in Saudi Arabia with seven phytoplasma isolates belonging to the peanut witches'-broom group detected elsewhere in the world (more than 97.5% similarity). The sequence analysis also indicated that the Saudi isolates are however distinguishable from other 29 phytoplasma isolates, with similarity percentages of less than 97.5%, which belong to phytoplasma groups other than the peanut witches'-broom group (Table 1 and Fig. 4). The justification for the above grouping was based on the decisions of the International Organization for Mycoplasmology, which considered that a phytoplasma that shares more than 97.5% homology of its 16S rDNA can be classified as the same organism and not a different species, in its two meetings held at Fukuoka, Japan and Vienna, Austria, in 2000 and 2002, respectively. Our future research will consider the occurrence of phytoplasma in alfalfa, other plant species and weeds in the major agricultural regions in Saudi Arabia along with their impact on the infected plants and the relative significance of the diseases they cause in efforts to seek means of their management.

In conclusion, for the first time we report the association of the pathogenic agent with phytoplasma-like symptoms in alfalfa fields (three isolates) in Riyadh region together with its occurrence in its leafhopper vector (two isolates) found in the same fields using molecular techniques. This study also revealed that the five phytoplasma isolates detected in this investigation belong to peanut WB group since they share more than 97.5% similarity of their nucleotide sequences. The results of this study help to explain the wide spread of the phytoplasma-like symptoms that were encountered on alfalfa in the surveyed region and may suggest probable occurrence of that agent in other regions of Saudi Arabia.

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