Plant Pathology An International Journal edited by the British Society for Plant Pathology

Grapevine yellows in Jordan: associated phytoplasmas, putative insect vectors and reservoir plants

Journal:	*Plant Pathology*
Manuscript ID	PP-23-041.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Abu Alloush, Asem; Università degli Studi di Milano, Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia (DiSAA); National Agricultural Research Center (NARC) Bianco, Piero; Università degli Studi di Milano, Dipartimento di Scienze agrarie e ambientali - Produzione, Territorio, Agroenergia (DiSAA) Busato, Enrico; Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA) Alkhawaldeh, Yousef; National Agricultural Research Center (NARC) Alma, Alberto; Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA) Tedeschi, Rosemarie; Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA) Quaglino, Fabio; Università degli Studi di Milano, Dipartimento di Scienze agrarie e ambientali - Produzione, Territorio, Agroenergia (DiSAA)
Topics:	aetiology, epidemiology
Organisms:	phytoplasmas & relatives
Other Keywords:	'Candidatus Phytoplasma solani', 'Candidatus Phytoplasma omanense', 'Candidatus Phytoplasma asteris', 'Candidatus Phytoplasma aurantifolia', Orosius cellulosus

SCHOLARONE™
Manuscripts

Grapevine yellows in Jordan: associated phytoplasmas, putative insect vectors, and reservoir plants

Asem Habes Abu Alloush[1,2], Piero Attilio Bianco[1], Enrico Busato[3], Yousef Alkhawaldeh[2], Alberto Alma[3], Rosemarie Tedeschi[3], Fabio Quaglino[1]

[1]Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Milano, Italy;
[2]National Agricultural Research Center (NARC), Amman, Jordan;
[3]Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Italy

Correspondence to: Fabio Quaglino (fabio.quaglino@unimi.it)

For Peer Review

Abstract

During field surveys conducted from June to October 2020 in 13 locations belonging to five governorates in North and South Jordan, typical grapevine yellows symptoms, including leaf reddening/yellowing and rolling, were observed in wine and table grape cultivar vineyards. ~~Disease incidence in the investigated vineyards~~Percentage of symptomatic vines ranged from 10 to 55%. Nested PCR-based amplification of 16S rRNA gene detected phytoplasmas in 22% and 15.7% of the analyzed symptomatic wine and table grape cultivar plants, respectively. Amplicon nucleotide sequence analyses allowed attributing the detected phytoplasmas to '*Candidatus* Phytoplasma solani' (taxonomic subgroup 16SrXII-A), '*Ca*. P. omanense' (16SrXXIX-A and -B), '*Ca*. P. aurantifolia' (16SrII-C), and '*Ca*. P. asteris' (16SrI-R) in 72.4%, 17.2%, 6.9%, 3.4% of infected plants, respectively. Such phytoplasmas were found differentially distributed in wine and table grape cultivar vineyards in the considered locations. Further investigation allowed identifying '*Ca*. P. solani' in the putative insect vectors *Orosius cellulosus* (firstly reported in Jordan), *Euscelidius mundus, Laodelphax striatellus*, and *Circulifer* sp., and in bindweed ~~*Convolvulus arvensis*~~; '*Ca*. P. aurantifolia' in the insect *O. cellulosus* and in bindweed; '*Ca*. P. omanense' in the insect *Psammotettix striatus*; '*Ca*. P. asteris' in the insects *Arboridia adanae*, *Cicadulina bipunctata*, *Circulifer* sp., *L. striatellus*, *Hyalesthes obsoletus*, and *P. striatus*. Based on this preliminary data, ecological cycles of such phytoplasmas are discussed. Obtained results suggest that grapevine yellows phytoplasma diversity and ecology in Jordan are more complex than previously known, leading to a potential risk of disease outbreaks.

Key words: '*Candidatus* Phytoplasma solani'; '*Candidatus* Phytoplasma omanense'; '*Candidatus* Phytoplasma asteris'; '*Candidatus* Phytoplasma aurantifolia'; *Orosius cellulosus*

For Peer Review

1. INTRODUCTION

Grapevine yellows (GY) are a complex of diseases associated with genetically distinct phytoplasmas causing undistinguishable symptoms on *Vitis vinifera* L., including desiccation of inflorescences, berry shrivel, leaf discolorations, reduction of growth, and irregular ripening of wood (Belli et al., 2010). Phytoplasmas are cell wall-less plant pathogenic bacteria localized in the phloem sieve elements, transmitted by phloem-feeding insects, and classified into 49 species and 37 taxonomic ribosomal groups based on molecular analysis of the gene 16S rRNA, as well as other conserved genes (Bertaccini et al., 2022). The main GY diseases are: (i) Flavescence dorée (FD), present mainly in Europe, associated with ~~with 16SrV-C and -D~~ phytoplasmas belonging to ribosomal subgroups 16SrV-C and -D and vine-to-vine transmitted ~~mainly~~ by the vector *Scaphoideus titanus* Ball (Malembic-Maher et al., 2020); (ii) Bois noir (BN), widespread throughout Europe, Asia, South America, and South Africa, associated with '*Candidatus* Phytoplasma solani' (ribosomal subgroup 16SrXII-A) and transmitted to vine mainly by *Hyalesthes obsoletus* Signoret and *Reptalus panzeri* Loëew (Quaglino et al., 2013); (iii) Palatinate grapevine yellows (PGY), present in Germany and in other European countries, associated with ~~16SrV-C~~ phytoplasmas belonging to subgroup 16SrV-C and transmitted to vine by *Oncopsis alni* Schrank (Angelini et al., 2001); (iv) Australian grapevine yellows (AGY), present in Australia ~~and New Zealand~~ and, associated with '*Ca*. P. australiense' (subgroup 16SrXII-B) ~~, transmitted by *Oliarus atkinsoni* Meyers~~ (Liefting et al., 1997); (v) North America grapevine yellows (NAGY), associated with '*Ca.* P. pruni' (subgroup 16SrIII-A) ~~and ,~~ transmitted ~~to vine~~ by *Jikradia olitoria* Say, and '*Ca*. P. asteris' (subgroup 16SrI-A) (Lenzi et al., 2019); (vi) South Africa grapevine yellows, associated with '*Ca*. P. asteris' (subgroup 16SrI-B) and transmitted to vine by *Mgenia fuscovaria* Stål (Pietersen et al., 2018). In MENA (North Africa and Middle East) region, ~~grapevine is very commonly cultivated, with an ancient history back to 6,000 years and still possesses very rich potential of both wild (*Vitis*~~

plantpath@bspp.org.uk

~~*vinifera* ssp. *sylvestris)* and cultivated (*Vitis vinifera* ssp. *sativa*) germplasm (Bayram et al., 2014). D~~different ~~studies reported the presence of GY diseases in MENA countries. In detail, BN (‘*Ca*. P. solani’) was found in Lebanon, Turkey, Syria, and Iran (Pierro et al., 2019; Hemmati et al., 2021). Moreover,~~ GY were reported in Iran, Lebanon, Syria, and Turkey in association ~~found associated~~ with ‘*Ca*. P. solani’ (16SrXII-A), ‘*Ca*. P. trifolii’ (16SrVI), ‘*Ca*. P. asteris’ (16SrI-B), ‘*Ca*. P. phoenicium’ (16SrIX-C), ‘*Ca*. P. aurantifolia’ (16SrII-B), ‘*Ca*. P. fraxini’ (16SrVII-A), and ‘*Ca*. P. omanense’ (16SrXXIX-A) ~~in Syria, Turkey, Lebanon, and Iran~~ (Pierro et al., 2019; Hemmati et al., 2021).

Noteworthy, most GY studies were focused on wine grape cultivars, and a poor knowledge is available for table grape cultivars. Up to now, studies on these latter cultivars reported infections by ‘*Ca*. P. solani’ in Chile and Syria, and by ‘*Ca*. P. asteris’, ‘*Ca*. P. phoenicium’, ‘*Ca*. P. fraxini’, ‘*Ca*. P. aurantifolia’, and ‘*Ca*. P. trifolii’ in Iran (Gajardo et al., 2009; Hemmati et al., 2021). In Jordan, grapevine (both wine and table grape cultivars) is a key commercial fruit crop ~~with two farming styles: family and commercial farms. More~~cultivated in more than 8,960 ha and a ~~are cultivated with~~ total production estimated by 56,000 ton in 2019 (MAO STAT, 2021). ~~Table grape cultivars, very popular and cultivated in the whole Country, are characterized by a long harvesting season extending from May (for the early seedless cultivar growing in Jordan valley) to October.~~ Concerning GY in Jordan, BN associated with ‘*Ca.* P. solani’ (16SrXII-A) was reported in wine grape cultivars and bindweed in one vineyard in North Padia (Salem et al., 2013). No further studies were conducted in the Country.

GY epidemiology is still poorly understood in MENA region; therefore, further studies concerning the identification of GY phytoplasma insect vectors and non-crop plant hosts are crucial steps in forecasting GY outbreaks and designing efficient control measures. Following the first report of BN and based on the recent observation of phytoplasma-like symptoms in several viticultural areas in Jordan, the present study aimed to survey the diffusion of GY

For Peer Review

symptoms throughout the Country focusing on both table grape and wine grape cultivars, detect and identify the GY-associated phytoplasmas in grapevines, potential insect vectors, and reservoir plants.

2. MATERIALS AND METHODS

2.1 Field surveys, plant sampling, and insect collection

Field surveys on grapevine yellows-like symptoms were conducted from June to October 2020 in 13 locations belonging to five governorates in North (12) and South (1) Jordan, including vineyards with table grape cultivars (11 locations) and wine grape cultivars (2 locations) (Figure 1). In each location, ~~phytoplasma-like disease incidence was calculated as~~ the percentage of symptomatic trees out of the observed ones was calculated. Leaf samples were collected from 50 plants of wine grape cultivars, and 102 plants of table grape cultivars exhibiting ~~grapevine yellows~~GY-like symptoms, and from 25 symptomless plants (five from wine grape cultivars; 20 from table grape cultivars). Moreover, leaf samples were collected from 22 ~~symptomatic and 8 symptomless~~ bindweed (*Convolvulus arvensis* L.) plants showing phytoplasma-like symptoms and from 8 symptomless bindweed plants ~~observed~~ within and around the surveyed vineyards (Table 1). Leaf samples were maintained at 4°C at National Agricultural Research Center (NARC), Baqaà, Jordan, until total nucleic acids extraction. Insects collected by entomological sweeping net within the examined vineyards were observed by stereomicroscope for preliminary selection of phloem feeders at NARC. The selected phloem feeders, maintained in 99% ethanol, were recognized at genus/species level by stereomicroscope observation of phenotypic characters and male genitalia, conducted after body dissection and clarification in a 10% potassium hydroxide solution, at the Department of Agriculture, Forest, and Food Science (DISAFA), University of Turin, Italy. Insects were kept in 99% ethanol at -20°C until total nucleic acids extraction.

For Peer Review

111

112 **2.2 Phytoplasma detection**

113 Total nucleic acids (TNA) were extracted from the collected plant and insect samples as
114 previously described by Angelini et al. (2001) and Marzachì et al. (1998), respectively.
115 Concerning plant samples, leaf midribs and petioles (0.5 g) were grounded in 3 ml of
116 prewarmed 2.5% CTAB-based buffer in sterile mortars. Concerning insects, single or pooled
117 (2 to 5) individuals were crashed by sterile pestles in a 1.5 ml tube containing sand and 0.5 ml
118 of prewarmed 2% CTAB-based buffer. Extracted TNA were dissolved in 40 (insects) or 100
119 (plants) µl of TE-based buffer, measured for quantity and quality by Nanodrop system, and
120 stored at -20°C until molecular analyses.
121 TNA extracted from plants and insects were used as templates in nested PCR reactions to detect
122 the presence of phytoplasmas. Nested PCRs were carried out to amplify the 16S rRNA gene
123 using the primer pairs P1/P7 and R16F1/R16R0. Reaction mixtures, including GoTaq® G2
124 DNA Polymerase (Promega, Italy), and conditions were as previously described (Lee et al.,
125 1995). No positive controls (TNA extracted from phytoplasma-infected plants) were utilized
126 to avoid reaction contamination. Reaction mixtures devoid of TNA were used as negative
127 controls. PCR products (6 µl) were electrophorized on 1% (w/v) agarose gels in 1X TBE buffer,
128 stained with Midori Green, and visualized on UV transilluminator. Phytoplasma infection rate
129 was estimated as the percentage of infected plants/insects out of the examined ones.

130

131 **2.3 Phytoplasma identification**

132 For each sample (plant and insect) found infected by phytoplasmas, three ~~N~~nested PCR
133 products (F1/R0 fragment) were sequenced in both strands (3X coverage per base position) by
134 a commercial service (Eurofins Genomics, Germany), ~~. Nucleotide sequences were~~ assembled
135 by the Contig Assembling Program, ~~and~~ trimmed to the annealing sites of the primers R16F1

For Peer Review

and R16R0, and aligned to obtain a consensus sequence ~~using the Contig Assembling Program~~ in the sofware BioEdit, version 7.1.3.0 (Hall, 1999). ~~Trimmed nucleotide~~Obtained consensus sequences were aligned using the ClustalW Multiple Alignment program and analyzed by Sequence Identity Matrix in the sofware BioEdit to estimate their genetic diversity. For attribution to '*Ca*. Phytoplasma' species, 16S rDNA nucleotide sequences, representative of the phytoplasma populations detected in this study, were aligned with those of representative strains of the 49 '*Ca*. Phytoplasma' species described in literature (Bertaccini et al., 2022) and checked for their sequence identity in the software ~~Bioedit~~BioEdit. Species attribution was confirmed searching the species-specific signatures within the 16S rDNA nucleotide sequences. Group/subgroup attribution was determined by *in silico* RFLP analysis of 16S rDNA nucleotide sequences using the online tool *i*PhyClassifier (Zhao et al., 2009).

Nucleotide sequences of 16S rRNA gene of phytoplasmas, identified in the present study, and reference strains of '*Ca*. Phytoplasma' species were employed for phylogenetic analyses. The Minimum-Evolution method was employed using the Neighbor-Joining algorithm and bootstrap replicated 1,000 times with the software MEGAX to obtain a phylogenetic tree (Kumar et al., 2018). *Acholeplasma palmae* (GenBank Acc. No. L33734) was used for rooting the tree.

3. RESULTS

3.1 Grapevine yellows symptoms observed in vineyards

During field surveys, undistinguishable leaf reddening/yellowing and rolling symptoms (Figure 2 A, B, C), typical of grapevine yellows (GY) disease complex, were observed in wine and table grape cultivars in vineyards localized in all the 13 considered locations. The ~~disease incidence (~~percentage of symptomatic trees out of the observed ones~~)~~ was ranging from 10 to 55%. The highest ~~incidence~~ percentage (55%) was observed in wine grape cultivar vineyards

For Peer Review

in Alsalhieh, followed by table grape cultivar vineyards in Jaber Alsarhan (25%), both in Almafraq governorate. The lowest ~~incidence~~ percentage (10%) was reported in table grape cultivar vineyards situated in Aldisi (Aqaba governorate) and in the three locations of Ajloun governorate. The remnant seven locations had a ~~disease incidence~~percentage ranging from 12 to 15%. Within and around surveyed vineyards, 22 bindweed plants ~~exhibited~~ exhibiting little leaf and reddening were observed and collected (Figure 2 D).

3.2 Molecular detection and identification of phytoplasmas in plants

According to results of universal nested PCR-based amplification, phytoplasmas were detected in 29 out of 152 symptomatic grapevines (infection rate 19.1%), and 12 out of 22 symptomatic bindweeds (infection rate 54.5%). In detail, F1/R0 amplicons of the expected size (around 1370 bp) were obtained in 16 out of 102 symptomatic table grape cultivars (15.7%), and in 11 out of 50 symptomatic wine grape cultivars (22%). No amplification was obtained in symptomless wine and table grape cultivars, in symptomless bindweed (Table 1), and in negative controls. Concerning wine grape cultivars, infection rate ranged from 20 to 27.5% in SamaAlSarhan and Alsalhieh locations, respectively. Concerning table grape cultivars, the highest infection rates were reported in plant samples collected in vineyards located in Jaber AlSarhan (AlMafraq governorate) (33%) and UmAlyanabee (Ajloun) (30%). Samples from Alfuhaisnad (AlBalqa) and Alboedah (Irbid) shared the same infection rate (20%). The lowest infection rate (8.3%) was found in Kufranjeh (Ajloun). No phytoplasma-infected table grape samples were identified in Ain Jana, Alkom Alahmar, and Thagrat AlJob. Regarding *C. arvensis*, the infection rates were 45% (9 plants out of 20) in AlSalhieh, 40% (2 plants of 5) in Sama AlSarhan and AlDisi (Table 1).

Based on 16S rDNA sequence identity versus the reference strains of '*Ca*. Phytoplasma' species and on the presence of species-specific signature sequences, the phytoplasma strains

For Peer Review

detected in the present study in 29 symptomatic grapevine plants were attributed to the species '*Ca*. P. solani' (72.4%; 21 strains out of 29), '*Ca*. P. omanense' (17.2%; 5 out of 29), '*Ca*. P. aurantifolia' (6.9%; 2 out of 29), and '*Ca*. P. asteris' (3.4%; 1 out of 29) (Table 2). '*Ca*. P. solani' and '*Ca*. P. aurantifolia' strains were found in both table and wine grape cultivars, while '*Ca*. P. omanense' and '*Ca*. P. asteris' were detected exclusively in table and wine grape cultivars, respectively. In detail, '*Ca*. P. solani' strains have identical 16S rDNA nucleotide sequence (GenBank Acc. No. OL873119), distinct from the reference strain STOL by four single nucleotide polymorphisms (SNPs) at positions 504 (T/A), 595 (A/G), 888 (C/T), and 1084 (T/C) from the annealing site of the primer R16F1. '*Ca*. P. aurantifolia' strains have identical 16S rDNA nucleotide sequence (GenBank Acc. No. OL873116), distinct from the reference strain WBDL by six SNPs at positions 62 (T/A), 83 (G/A), 285 (C/T), 559 (-/G), 793 (-/C), and 1032 (T/C) from the annealing site of the primer R16F1. '*Ca*. P. asteris' strain VV112 (GenBank Acc. No. OL873120) is distinct from the reference strain OAY by seven [323 (G/-), 346 (G/-), 488 (C/T), 539 (C/T), 698 (C/T), 984 (A/G), 1122 (G/A)] SNPs. Within '*Ca*. P. omanense', the identified strains have diverse 16S rDNA nucleotide sequences. Sequences of strains VV95, VV103, VV1007, and VV1034 identical between them (GenBank Acc. No. OL873118) are distinct from the reference strain IM-1 by SNPs at positions 152 (G/A), 274 (T/C), 331 (C/T), 344 (G/A), and 712 (G/A), while the sequence of strain VV1259 (GenBank Acc. No. OL873117) is identical to the reference strain IM-1. Based on similarity coefficient obtained by comparison of virtual RFLP patterns, '*Ca*. P. solani' strains were attributed to taxonomic subgroup 16SrXII-A, '*Ca*. P. aurantifolia' strains VV162 and VV632 to a variant of subgroup 16SrII-C, '*Ca*. P. omanense' strain VV1259 to subgroup 16SrXXIX-A and strains VV95, VV103, VV1007, and VV1034 to subgroup 16SrXXIX-B, and '*Ca*. P. asteris' strain VV112 to a variant of subgroup 16SrI-R (Figure 3). Phytoplasma clustering in phylogenetic tree confirmed the attribution to phytoplasma species and taxonomic subgroups (Figure 4).

For Peer Review

Concerning the phytoplasma distribution, '*Ca*. P. solani' was found in table grape cultivars (10 out of 16 plants: 62.5%) from 5 out of 11 considered locations, and in wine grape cultivars (11 out of 13 plants: 84.6%) from both AlSalhieh and SamaAlSarahn. '*Ca*. P. omanense' was found exclusively in table grape cultivars (5 out of 16 plants: 31.3%) from Kufranjeh (subgroup 16SrXXIX-A), UmAlYanabee and Aldisi (subgroup 16SrXXIX-B). '*Ca*. P. aurantifolia' was found in both table grape (1 out of 16 plants: 6.3%) and wine grape (1 out of 13 plants: 7.7%) cultivars from Javer AlSarahan and AlSalhieh, respectively. '*Ca*. P. asteris' was found only in one wine grape cultivar plant (1 out of 13 plants: 7.7%) from AlSalhieh.

Regarding the non-crop weeds, the phytoplasma strains detected in the present study in 12 symptomatic bindweed plants were attributed to the species '*Ca*. P. solani' (83.3%; 10 strains out of 12 identified in SamaAlSarahn and AlSalhieh), and '*Ca*. P. aurantifolia' (16.7%; 2 out of 12 identified in Aldisi) (Table 2). '*Ca*. P. solani (16SrXII-A) and '*Ca*. P. aurantifolia' (variant of 16SrII-C) strains, found in bindweed, shared identical 16S rDNA sequence with strains of the same species found in *V. vinifera* plants.

3.3 Molecular detection and identification of phytoplasmas in insects

During the field survey carried out in the localities AlSalhieh, Alkom AlAhmar, Sabha (AlMafraq governorate), Kufranjeh and Aain Jana (Ajloun governorate) in August, September and November 2020, 1173 Auchenorryncha adult insects (557 and 616 from wine and table grape cultivar vineyards, respectively) were collected and distinguished, based on stereomicroscope analyses, in 11 taxonomic groups defined at species (8) and genus (3) level. Most of such insects belong to the family Cicadellidae (1133 specimens), while the remnant 40 collected specimens belong to the families Delphacidae (33) and Cixiidae (7). Within Cicadellidae, the more abundant insect taxa were *Cicadulina bipunctata* (Melichar), (393 specimens), *Empoasca* sp. (350 specimens), *Arboridia adanae* (Dlabola) (271 specimens),

For Peer Review

Circulifer sp. (49 specimens), and *Orosius cellulosus* (Lindberg) (28 specimens) (firstly reported in Jordan). *C. bipunctata*, *Laodelphax striatellus* (Fallén), and *Psammotettix striatus* (Linnaeus) were the only species captured in both wine and table grape vineyards. *Empoasca* sp., *Balclutha* sp., *O. cellulosus*, *Toya propinqua* Fieber, *Circulifer* sp., and *Euscelidius mundus* (Haupt) were collected exclusively in wine grape vinayards in AlSalhieh, while *Hyalesthes obsoletus* Signoret and *A. adanae* exclusively in table grape vineyards in Sabha and Ain Jana (Table 3). Molecular analyses for phytoplasma detection and identification were conducted on 112 insect pools (54 from wine grape vineyards located in AlSalhieh; 58 from table grape vineyards located in Alkom AlAhmar, Sabha, Kufranjeh, and Ain Jana) representative of the observed diversity. Nested PCR allowed detecting phytoplasmas in 34 insect pools (infection rate 34.8%), belonging to 10 different insect taxa. Infection rate in insect pools from wine and table grape vineyards was 38.9% (21 out of 54 pools) and 22.4% (13 out of 58 pools), respectively. No positive insect pools were found in *Balclutha* sp. and *T. propinqua*. Among insects captured in wine grape vineyards, infectioned rates were 100% in *E. mundus*, 83.3% in *P. striatus*, 50% in *Circulifer* sp., 40% in *O. cellulosus*, 33.3% in *C. bipunctata*, and 11.1% in *Empoasca* sp. Concerning the insects captured in table grape vineyards, ~~infected~~ infection rates were 100% in *P. striatus*, 60% in *L. striatellus*, 50% in *H. obsoletus*, 19% in *C. bipunctata*, and 10.7% in *A. adanae* (Table 3).

Analyses of 16S rDNA nucleotide sequences allowed attributing the phytoplasma strains detected in insects to the species '*Ca*. P. asteris' (23 pools out of 34), '*Ca*. P. solani' (8 pools), '*Ca*. P. aurantifolia', '*Ca*. P. omanense', and '*Ca*. P. pyri' each in one pool (Table 4).

In detail, 22 out of 23 '*Ca*. P. asteris' strains were found in *P. striatus* and *C. bipunctata* in both wine and table grape vineyards, in *Circulifer* sp. in wine grape vineyards, in *L. striatellus*, *H. obsoletus*, and *A. adanae* in table grape vineyards. Such strains share identical 16S rDNA nucleotide sequence with the wine grape-infecting strain VV112, attributed to a variant of

For Peer Review

taxonomic subgroup 16SrI-R. The '*Ca*. P. asteris' strain SUF5-4, identified in one pool of *P. striatus* from table grape vineyard in Alkom AlAhmar, is distinct from the reference strain OAY by three [323 (G/-), 346 (G/-), 539 (C/T)] SNPs. This strain was attributed to taxonomic subgroup 16SrI-B. Concerning '*Ca*. P. solani', its strains were identified in *O. cellulosus* (three strains), *E. mundus* (three strains), and *Circulifer* sp. (one strain) from wine grape vineyards, and in *L. striatellus* (one strain) from table grape vineyard in Alkom AlAhmar. Such strains share identical 16S rDNA nucleotide sequence with the ones infecting wine and table grape cultivars and bindweed, attributed to taxonomic subgroup 16SrXII-A. '*Ca*. P. aurantifolia' strain MH8-16 and '*Ca*. P. omanense' strain MH5-11, found respectively in *O. cellulosus* and *P. striatus* in wine grape vineyards, share identical 16S rDNA sequences respectively with '*Ca*. P. aurantifolia' (variant of 16SrII-C) strains identified in grapevine and bindweed and '*Ca*. P. omanense' (16SrXXIX-A) strain identified in grapevine. Moreover, a '*Ca*. P. pyri' strain, sharing identical 16S rDNA sequence with the reference strain PD1 (subgroup 16SrX-C), was found in one *Empoasca* sp. pool in AlSalhieh. Phytoplasma clustering in phylogenetic tree confirmed the attribution to phytoplasma species and taxonomic subgroups (Figure 4).

4. DISCUSSION

This study provided new insights on GY diffusion, associated phytoplasmas and their putative vectors and reservoir plants in Jordan. Obtained results confirmed the large diffusion of Bois noir (BN), associated with '*Ca*. P. solani' (16SrXII-A), in wine grape cultivars, as previously reported by Salem and colleagues (2013). Moreover, '*Ca.* P. asteris' (variant of 16SrI-R) and '*Ca*. P. aurantifolia' (variant of 16SrII-C) were firstly reported in the Country in association with GY-affected wine grape cultivars. In previous studies, '*Ca.* P. asteris'- and '*Ca*. P. aurantifolia'-related strains were found in association with peach and potato diseases, respectively, in Jordan (Anfoka & Fatash, 2004; Salem et al., 2019). In MENA region, '*Ca.* P.

For Peer Review

asteris' (16SrI-B) and '*Ca*. P. aurantifolia' (16SrII-B) were reported in GY affected grapevines (Hemmati et al., 2021). Thus, this is the first report in MENA region of '*Ca.* P. asteris' (variant of 16SrI-R) and '*Ca*. P. aurantifolia' (variant of 16SrII-C) in association with GY. Moreover, '*Ca*. P. solani' (16SrXII-A) was found as prevalent also in table grape cultivars in different locations, confirming its previous identification in table grapes in Chile and Syria (Gajardo et al., 2009; Hemmati et al., 2021). Also '*Ca*. P. aurantifolia' (variant of 16SrII-C) was found in table grape, as previously reported in Iran (subgroup 16SrII-B) (Hemmati et al., 2021). Furthermore, '*Ca*. P. omanense' subgroup 16SrXXIX-A, previously reported in association with wine grape cultivars in Lebanon (Foissac et al., 2019), and subgroup 16SrXXIX-B, recently reported in almond in Jordan (Abu Alloush et al., 2023), were found for the first time in association with GY-affected table grape cultivars in Jordan.

Although the high incidence of phytoplasma-like symptoms in the examined vineyards, only 19.1% of samples collected from symptomatic wine and table grapes were found infected by phytoplasmas. This result can be explained by different hypotheses: (i) the phytoplasmas are unevenly distributed in phloem tissues of infected plants (Constable et al., 2003); (ii) the phytoplasma concentration in plant tissues in the different sampling periods (from July to November) can be extremely low (Martini et al., 2011); (iii) the persons involved in the surveys could have not enough expertise to clearly distinguish symptoms associated with phytoplasmas from those caused by other etiological agents or nutritional disorders ~~could be associated with the observed symptoms; (iv) PCR inhibitors could be present in the extracted TNAs~~. Moreover, it should be considered that surveys and sample collection were carried out during a limited period throughout the season.

Interestingly, obtained results evidenced the presence of '*Ca*. P. solani' and '*Ca*. P. aurantifolia' strains, undistinguishable from those found in wine and table grape cultivars, in the putative insect vector *Orosius cellulosus* (firstly reported in Jordan) and in symptomatic

For Peer Review

bindweed, a non-crop plant known for its epidemiological role in BN diffusion (Quaglino et al., 2021). No studies are available about *O. cellulolus* and its vectoring activity of phytoplasmas, but other *Orosius* species, such as *O. albicinctus* and *O. orientalis*, living on different plant hosts (Rao et al., 2018), were reported as vectors of phytoplasmas belonging to groups 16SrIX and 16SrII in Turkey and Iran (Ikten et al., 2014; Salehi et al., 2017). Based on this evidence, it is reasonable to hypothesize that diffusion of '*Ca*. P. solani' and '*Ca*. P. aurantifolia' in Jordan couldan involve *O. cellulolus* and bindweed.

Moreover, '*Ca*. P. solani' strains, undistinguishable from those found in wine and table grape cultivars, were found also in *Euscelidius mundus*, *Laodelphax striatellus*, and *Circulifer* sp. *E. uscelidius mundus* was reported as putative vector of '*Ca*. P. phoenicium' in Lebanon (Dakhil et al., 2011), but other species of the genus *Euscelidius*, such as *E. variegatus*, are known as vector of '*Ca*. P. solani' to grapevine (Quaglino et al., 2019). *L. aodelphax striatellus* has tipically grasses as primary hosts to which it transmits virus of great economic importance (Achon et al., 2013), anyhow it was found positive to grapevine phytoplasmas (16SrI, 16SrXII-A) (Orságova et al., 2011) and reported as a vector of '*Ca*. P. solani' to grapevine (Quaglino et al., 2019). In light of these evidence and our results, certainly new investigations are needed to understand the actual interactions between *L. striatellus*, grasses or weeds (located within or surrounding around vineyards) and grapevine in the GY epidemiology of phytoplasmosis. Concerning *Circulifer* sp., different species, such as *C. tenellus* and *C. haematoceps*, were described as vectors of phytoplasmas belonging to taxonomic groups 16SrI, 16SrII, 16SrVI, and 16SrIX (Salehi et al., 2017; Aslam et al., 2021). Thus, '*Ca*. P. solani' transmission to grapevine in Jordan could involve also *Circulifer* sp., *E. mundus*, and *L. striatellus*. Further investigation should be carried out in Jordan to investigate more accurately the spread and epidemiological role of *Hyalesthes obsoletus*, the main vector of '*Ca*. P. solani' to grapevine in Europe (Maixner et al., 1994).

For Peer Review

Concerning '*Ca*. P. omanense' (subgroup 16SrXXIX-A), found exclusively in table grape cultivars, it was identified (subgroup 16SrXXIX-A) also in *Psammotettix. striatus*, known as vector of '*Ca*. P. tritici' (16SrI) (Wu et al., 2010). No insects were found infected by 16SrXXIX-B phytoplasma strains. Recent studies reported *H. obsoletus* as putative vector of '*Ca.* P. omanense' in Lebanon (Foissac et al., 2019), but in the present work it was found not infected by this phytoplasma. Due to the association of the subgroup 16SrXXIX-B to almond (Abou Alloush et al., 2023) and grapevine (this study) diseases in Jordan, it will be useful to focus further studies on improving the knowledge on its epidemiology throughout the Country, in different agroecosystems.

'*Ca*. P. asteris' (variant of subgroup 16SrI-R), identified only in one plant of wine grape cultivar, was prevalent in the examined insects vectors. It was found in *Circulifer* sp., *P. striatus*, *Cicadulina. bipunctata, L. striatellus*, *H. obsoletus*, and *Arboridia. adanae*. As reported above, *Circulifer* sp. and *P. striatus* are known as vectors of 16SrI phytoplasmas, and *L. striatellus* as vector of 16SrXII phytoplasmas. *C. bipunctata* is a potential vector of '*Ca*. P. asteris'-related strain to date palm (Alhudaib et al., 2007); *H. obsoletus* is known as '*Ca*. P. solani' vector and putative vector of '*Ca*. P. asteris' (Maixner et al., 1994; Zambon et al., 2018). *Arboridia A. adanae*, a typical mesophyll-feeder as most of Typhlocibinae, is considered a serious pest of grapevine in Eastern Mediterranean Regions and Europe (Yigit and Erckle 1992; Olivier et al., 2012) emptying mesophyll cell content or by cell rupture feeding. Anyhow, we detected few phytoplasma-positive individuals collected in vineyards as similarly reported for the very close mesophyll-feeder genus *Erytroneura* (Olivier et al., 2014). Although phytoplasmas usually reside in the phloem sieve elements, they have been occasionally observed in parenchyma cells (Siller et al., 1987), intercellular spaces of bundle sheath cells (Fontaniella et al., 2003), and companion cells (Sears & Klomparens, 1989). That could explain why phytoplasmas are occasionally found in mesophyll-feeders and in our case in *A. adanae*

For Peer Review

that may have ingested cell fluids infested by phytoplasmas transmitted by other leafhopper species.

Based on these findings, it is reasonable to suggest that diffusion of '*Ca*. P. asteris' subgroup 16SrI-R to grapevine in Jordan could~~an~~ involve multiple insect species. Upscaling the surveyed vineyards and surroundings could provide better insights on the '*Ca*. P asteris' diffusion in grapevine.

The epidemiology of phytoplasma-associated diseases is determined by the interactions between host plants, pathogen, and environmental conditions (Rotter et al., 2018). Further studies in terms of transmission trials and upscaling the surveying orchards and non-crop plant hosts will be crucial to profound the knowledge about GY etiology and epidemiology in Jordan. Outbreak of GY epidemics could be a concrete risk in the vineyard agroecosystems in all viticultural areas. Monitoring and control strategies against GY are essential to prevent epidemic phytoplasma spread (Pierro et al., 2019).

Most phytoplasmas identified in *Vitis vinifera* in this study were detected also in almond and pomegranate in different areas of the Country, suggesting that phytoplasma diversity and distribution in Jordan are more complex than previously known, leading to a potential risk of disease outbreaks. Studies and knowledge about the insect vectors including their identification, distribution, and population dynamics are essential for proper management measures and mitigation of the risk of disease outbreaks. Considering the preliminary results~~,~~ obtained in the present study~~,~~ about the GY epidemiology in Jordan, further studies covering more areas throughout the full vegetative season of grapevine and non-crop reservoir plants are essential and will provide comprehensive insights about the GY phytoplasma diversity, ecological complexity, and epidemiology.

ACKNOWLEDGMENTS

For Peer Review

We thank Wafaa Abu Hammour for technical assistance in preparing map of GY surveys in Jordan, Dr. Jafar AlWidyan, Eng. Nizar Obeidat and Sadeer Amashah for their assistance during insect collection and lab activities, and Jordanian farmers met during field surveys.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Abu Alloush, A.H., Bianco, P.A., Busato, E., AlMahasneh, A., Alma, A., Tedeschi, R., et al. (2023) Association of seven '*Candidatus* Phytoplasma' species to an almond disease complex in Jordan, and preliminary information on their putative insect vectors. *Crop Protection*, 164, 106147.

Achon, M. A., Subira, J. & Sin, E. (2013) Seasonal occurrence of *Laodelphax striatellus* in Spain: effect on the incidence of Maize rough dwarf virus. *Crop Protection*, 47, 1–5.

Alhudaib, K., Arocha, Y., Wilson, M. & Jones, P. (2007) Identification and molecular characterization of a phytoplasma associated with Al-Wijam disease of date palm in Saudi Arabia. *Arab Journal of Plant Protection*, 25, 116–122.

Anfoka, G.H. & Fattash, I. (2004) Detection and identification of aster yellows (16SrI) phytoplasma in peach trees in Jordan by RFLP analysis of PCR-amplified products (16S rDNAs). *Journal of Phytopathology*, 152, 210–214.

For Peer Review

411 Angelini, E., Clair, M., Borgo, A., Bertaccini, A. & Boudon-Padieu, E. (2001) "Flavescence
412 dorée" in France and Italy: occurrence of closely related phytoplasma isolates and their
413 near relationships to palatinate grapevine yellows and an alder yellows phytoplasma.
414 *Vitis*, 40, 79–86.
415 Aslam, M., Tanwir, S., Akhtar, Z.R. & Ahmad, J.N. (2021) First report of 16SrII-D phyllody
416 phytoplasma and associated insect vectors infecting multi-flower inbred lines of
417 sunflower (*Helianthus annuus* L.) in Faisalabad, Pakistan. *Pakistan Journal of*
418 *Agricultural Sciences*, 58, 985–992.
419 ~~Bayram, S., Zeybekoglu, U., Soylemezoglu, G., Canik, D., Karavin, M., Cakir, A. et al. (2014)~~
420 ~~Presence of putative insect vectors of grapevine yellows phytoplasmas in Turkey.~~
421 ~~*Phytopathogenic Mollicutes*, 4, 22–26.~~
422 Belli, G., Bianco, P.A. & Conti, M. (2010) Grapevine yellows: past, present and future. *Journal*
423 *of Plant Pathology*, 92, 303–326.
424 Bertaccini, A., Arocha-Rosete, Y., Contaldo, N., Duduk, B., Fiore, N., Montano, H.G. et al.
425 (2022). Revision of the '*Candidatus* Phytoplasma' species description guidelines.
426 *International Journal of Systematic and Evolutionary Microbiology*, 72, 005353.
427 Constable, F.E., Gibb, K.S. & Symons, R.H. (2003) Seasonal distribution of phytoplasmas in
428 Australian grapevines. *Plant Pathology*, 52, 267–276.
429 Dakhil, H.A., Hammad, E.A., El-Mohtar, C. & Abou-Jawdah, Y. (2011) Survey of leafhopper
430 species in almond orchards infected with almond witches'-broom phytoplasma in
431 Lebanon. *Journal of Insect Science*, 11, 60.
432 Foissac, X., Jreijiri, F., Salar, P., Wakim, S., Danet, J.L. & Choueiri, E. (2019). A '*Candidatus*
433 Phytoplasma omanense'-related strain detected in yellowing grapevine, stunted
434 bindweed and Cixiidae planthoppers in Lebanon. *European Journal of Plant Pathology*,
435 153, 265–272.

For Peer Review

Fontaniella, B., Vicente, C., Legaz, M.E., de Armas, R., Rodrıguez, C.W., Martínez, M. et al. (2003). Yellow leaf syndrome modifies the composition of sugarcane juices in polysaccharides, phenols and polyamines. *Plant Physiology and Biochemistry*, 41, 1027–1036.

Gajardo, A., Fiore, N., Prodan, S., Paltrinieri, S., Botti, S., Pino, A.M. et al. (2009) Phytoplasmas associated with grapevine yellows disease in Chile. *Plant Disease*, 93, 789–796.

Hall, T.A. (1999) Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.

Hemmati, C., Nikooei, M., Al-Subhi, A.M. & Al-Sadi, A.M. (2021) History and Current Status of Phytoplasma Diseases in the Middle East. *Biology*, 10, 226.

Ikten, C., Catal, M., Yol, E., Ustun, R., Furat, S., Toker, C. et al. (2014) Molecular identification, characterization, and transmission of phytoplasmas associated with sesame phyllody in turkey. *European Journal of Plant Pathology*, 139, 217–229.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547–1549.

Lee, I.-M., Bertaccini, A., Vibio, M. & Gundersen, D.E. (1995) Detection of multiple phytoplasmas in perennial fruit trees with decline symptoms in Italy. ~~*Phytopathologia Mediterranea*~~ *Phytopathology*, 85, 728–735.

Lenzi, P., Stoepler, T.M., McHenry, D.J., Davis, R.E. & Wolf, T.K. (2019) *Jikradia olitoria* ([Hemiptera] [Cicadellidae]) transmits the sequevar NAGYIIIβ phytoplasma strain associated with north american grapevine yellows in artificial feeding assays. *Journal of Insect Science*, 19, 1.

For Peer Review

460 Liefting, L.W., Beever, R.E., Winks, C.J., Pearson, M.N. & Forster, R.L.S. (1997) Planthopper
461 transmission of Phormium yellow leaf phytoplasma. *Australasian Plant Pathology*, 26,
462 148–154.
463 Maixner, M. (1994) Transmission of German grapevine yellows (Vergilbungskrankheit) by the
464 planthopper *Hyalesthes obsoletus* (Auchenorrhyncha: Cixiidae). *Vitis*, 33, 103–104.
465 Malembic-Maher, S., Desqué, D., Khalil, D., Salar, P., Bergey, B., Danet, J.-L. et al. (2020)
466 When a palearctic bacterium meets a nearctic insect vector: genetic and ecological
467 insights into the emergence of the grapevine flavescence dorée epidemics in Europe.
468 *PLoS Pathogens*, 16, e1007967.
469 Martini, M., Ermacora, P., Magris, G., Ferrini, F. & Loi, N. (2011) Symptom expression and
470 '*Candidatus* Phytoplasma prunorum' concentration in different *Prunus* species.
471 *Bulletin of Insectology*, 64, S171–S172.
472 Marzachì, C., Veratti, F. & Bosco, D. (2008) Direct PCR detection of phytoplasmas in
473 experimentally infected insects. *Annals of Applied Biology*, 133, 45–54.
474 Ministry of Agriculture (MOA) in Jordan (2021) Annual statistical year. Available at:
475 http://www.moa.gov.jo/AR/ [Accessed December 5, 2021].
476 Olivier, C., Vincent, C., Saguez, J., Galka, B., Weintraub, P.G. & Maixner, M. (2012)
477 Leafhoppers and planthoppers: their bionomics, pathogen transmission and
478 management in vineyards. In: Arthropod Management in Vineyards. Dordrecht:
479 Springer, pp. 253–270.
480 Olivier, C., Saguez, J., Stobbs, L., Lowery, T., Galka, B., Whybourne, K. et al. (2014).
481 Occurrence of phytoplasmas in leafhoppers and cultivated grapevines in Canada.
482 *Agriculture, Ecosystems & Environment*, 195, 91–97.
483 Orságová, H., Březíková, M. & Schlesingerova, G. (2011) Presence of phytoplasmas in
484 hemipterans in Czech vineyards. *Bulletin of Insectology*, 64, S119–S120.

For Peer Review

485 Pierro, R., Semeraro, T., Luvisi, A., Garg, H., Vergine, M., De Bellis, L. et al. (2019) The
486 distribution of phytoplasmas in south and east Asia: an emerging threat to grapevine
487 cultivation. *Frontiers in Plant Science*, 10, 1108.
488 Pietersen, G., Pietersen, G., Pietersen, I. & Stiller, M. (2018) Identification of *Mgenia*
489 *fuscovaria* (Stål) (Hemiptera: Cicadellidae), a vector of aster yellows disease on
490 grapevines in South Africa, and differentiation from *Mgenia angusta* (Theron) by
491 nucleotide sequences of the mitochondrial cytochrome oxidase I (cox1) gene. *South*
492 *African Journal of Enology and Viticulture*, 39, 176–179.
493 Quaglino, F., Passera, A., Faccincani, M., Moussa, A., Pozzebon, A., Sanna, F. et al. (2021)
494 Molecular and spatial analyses reveal new insights on bois noir epidemiology in
495 Franciacorta vineyards. *Annals of Applied Biology*, 179, 151–168.
496 Quaglino, F., Sanna, F., Moussa, A., Faccincani, M., Passera, A., Casati, P. et al. (2019)
497 Identification and ecology of alternative insect vectors of '*Candidatus* Phytoplasma
498 solani' to grapevine. *Scientific Reports*, 9, 19522.
499 Quaglino, F., Zhao, Y., Casati, P., Bulgari, D., Bianco, P.A., Wei, W. et al. (2013) '*Candidatus*
500 Phytoplasma solani', a novel taxon associated with stolbur- and bois noir-related
501 diseases of plants. *International Journal of Systematic and Evolutionary Microbiology*,
502 63, 2879–2894.
503 Rao, G.P., Fiore, N., Bertaccini, A. & Liefting, L.W. (2018) Phytoplasmas: Plant pathogenic
504 bacteria - I: Characterisation and epidemiology of phytoplasma - associated diseases.
505 Singapore: Springer Nature.
506 Rotter, A., Nikolić, P., Turnšek, N., Kogovšek, P., Blejec, A., Gruden, K. et al. (2018)
507 Statistical modeling of long-term grapevine response to '*Candidatus* phytoplasma
508 solani' infection in the field. *European Journal of Plant Pathology*, 150, 653–668.

For Peer Review

509 Salehi, M., Esmailzadeh Hosseini, S. A., Salehi, E. & Bertaccini, A. (2017) Genetic diversity
510 and vector transmission of phytoplasmas associated with sesame phyllody in Iran. *Folia*
511 *Microbiologica*, 62, 99–109.
512 Salehi, M., Izadpanah, K. & Siampour, M. (2011). Occurrence, molecular characterization and
513 vector transmission of a phytoplasma associated with rapeseed phyllody in Iran.
514 *Journal of Phytopathology*, 159, 100–105.
515 Salem, N.M., Quaglino, F., Abdeen, A., Casati, P., Bulgari, D., Alma, A. et al. (2013) First
516 report of '*Candidatus* Phytoplasma solani' strains associated with grapevine bois noir
517 in Jordan. *Plant Disease*, 97, 1505.
518 Salem, N.M., Tahzima, R., Abdeen, A.O., Bianco, P.A., Massart, S., Goedefroit, T. et al. (2019)
519 First report of '*Candidatus* Phytoplasma aurantifolia'-related strains infecting potato
520 (*Solanum tuberosum*) in Jordan. *Plant Disease*, 103, 1406.
521 Sears, B.B. & Klomparens, K.L. (1989) Leaf tip cultures of the evening primrose allow stable,
522 aseptic culture of mycoplasmalike organism. *Canadian Journal of Plant Pathology*, 11,
523 343–348.
524 Siller, W., Kuhbandner, B., Marwitz, R., Petzold, H. & Seemuller, E. (1987) Occurrence of
525 Mycoplasma-like organisms in parenchyma cells of *Cuscuta odorata* (Ruiz et Pav.).
526 *Journal of Phytopathology*, 119, 147–159.
527 Wu, Y., Hao, X., Li, Z., Gu, P., An, F., Xiang, J. et al. (2010) Identification of the phytoplasma
528 associated with wheat blue dwarf disease in China. *Plant Disease*, 94, 977–985.
529 Yigit, A. & Erckle, L. (1992) Studies on bio-ecology and control of grape leafhopper
530 (*Arboridia* (= *Erythroneura*) *adanae* Dlab.) (Homoptera: Cicadellidae) in southern
531 Anatolia region. *Zirai Mücadele Arastirma Yilligi*, 22, 25–28.

For Peer Review

Zambon, Y., Canel, A., Bertaccini, A. & Contaldo, N. (2018) Molecular diversity of phytoplasmas associated with grapevine yellows disease in north-eastern Italy. *Phytopathology*, 108, 206–214.

Zhao, Y., Wei, W., Lee, I.-M., Shao, J., Suo, X. & Davis, R.E. (2009) Construction of an interactive online phytoplasma classification tool, *i*PhyClassifier, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). *International Journal of Systematic and Evolutionary Microbiology*, 59, 2582–2593.

Figure Legends

Figure 1. Maps of governorates and locations in Jordan where the surveys on GY diseases in vineyards were conducted.

Figure 2. Phytoplasma-like symptoms observed in *Vitis vinifera* L. and *Convolvulus arvensis* L. in Jordan. Leaf yellowing and rolling in wine grape cultivar (A); leaf reddening and rolling in wine grape cultivar (B); leaf yellowing and rolling in table grape cultivar (C); little leaf and reddening in bindweed (D).

Figure 3. Virtual RFLP profiles of 16S rDNA nucleotide sequences of phytoplasma strains identified in *Vitis vinifera* and insects in Jordan. One strain among those sharing identical 16S rDNA sequence (Tables 2 and 4) was selected as representative strain for *i*PhyClassifier analyses.

Figure 4. Phylogenetic tree based on the alignment of 16S rDNA nucleotide sequences of representative phytoplasma strains identified in *Vitis vinifera* L., putative insect vectors, and reservoir plants in Jordan (bold characters), and reference strains of previously described '*Candidatus* Phytoplasma' species. Regarding phytoplasmas identified in this study, one strain among those sharing identical 16S rDNA sequence (Tables 2 and 4) was selected as representative strain for phylogenetic analysis. The evolutionary history was inferred using the

For Peer Review

Minimum Evolution method. The optimal tree with the sum of branch length = 0.91088584 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm at a search level of 1. The Neighbor-joining algorithm was used to generate the initial tree. This analysis involved 52 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1424 positions in the final dataset.

For Peer Review

Table 1. Collected and phytoplasma-infected plant samples from surveyed locations in Jordan.

Governorate	Location	Plant	No. of collected samples	No. of phytoplasma-infected samples
Almafraq	AlSalhieh	Symptomatic wine grape	40	11
		Asymptomatic wine grape	3	0
		Convolvulus arvensis L.	20	9
	Sama-AlSarhan	Symptomatic wine grape	10	2
		Asymptomatic wine grape	2	0
		Convolvulus arvensis L.	5	1
	Sabha	Symptomatic table grape	10	1
		Asymptomatic table grape	2	0
	Thagrat Aljob	Symptomatic table grape	7	0
		Asymptomatic table grape	2	0
	Alkom AlAhmar	Symptomatic table grape	5	0
		Asymptomatic table grape	2	0
	Jaber Alsarhan	Symptomatic table grape	15	5
		Asymptomatic table grape	2	0
Irbid	Hofa	Symptomatic table grape	4	0
		Asymptomatic table grape	1	0
	Bouida	Symptomatic table grape	5	1
		Asymptomatic table grape	2	0
Ajloun	Kufranjeh	Symptomatic table grape	12	1
		Asymptomatic table grape	2	0
	Ain Jana	Symptomatic table grape	5	0
		Asymptomatic table grape	2	0
	UmALyanabee	Symptomatic table grape	10	3
		Asymptomatic table grape	1	0
Aqaba	Aldisi	Symptomatic table grape	14	2
		Asymptomatic table grape	2	0
		Convulvulus arvensis L.	5	2
AlBalga	Alfuhais	Symptomatic table grape	15	3
		Asymptomatic table grape	2	0
		Overall	207	41

For Peer Review

Table 2. Attribution to species and taxonomic subgroups of phytoplasmas detected in plants (part I)

Strain	Plant host	Grapevine cultivar	Location	Phytoplasma species	% id vs ref strain	16Sr subgroup	Acc. No.
VV1259	*Vitis vinifera*	table grape	Kufranjeh	'*Ca*. P. omanense'	100	XXIX-A (1.00)	OL873117 (b)
VV1003	*Vitis vinifera*	table grape	UmAlYanabee	'*Ca*. P. omanense'	99.6	XXIX-B (1.00~~new~~)	c
VV1007	*Vitis vinifera*	table grape	UmAlYanabee	'*Ca*. P. omanense'	99.6	XXIX-B (1.00~~new~~)	c
VV1034	*Vitis vinifera*	table grape	UmAlYanabee	'*Ca*. P. omanense'	99.6	XXIX-B (1.00~~new~~)	c
VV95	*Vitis vinifera*	table grape	Aldisi	'*Ca*. P. omanense'	99.6	XXIX-B (1.00~~new~~)	OL873118 (c)
VV37	*Vitis vinifera*	table grape	Aldisi	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV1395	*Vitis vinifera*	table grape	AlFuhais	'*Ca*. P. solani'	99.7	XII-A (1.00)	OL873119 (d)
VV1398	*Vitis vinifera*	table grape	AlFuhais	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV1399	*Vitis vinifera*	table grape	AlFuhais	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV636	*Vitis vinifera*	table grape	Bouida	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV1005	*Vitis vinifera*	table grape	UmAlYanabee	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV635	*Vitis vinifera*	table grape	Jaber AlSarahan	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV629	*Vitis vinifera*	table grape	Jaber AlSarahan	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV633	*Vitis vinifera*	table grape	Jaber AlSarahan	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV634	*Vitis vinifera*	table grape	Jaber AlSarahan	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV632	*Vitis vinifera*	table grape	Jaber AlSarahan	'*Ca*. P. aurantifolia'	99.7	II-C* (0.99)	a
VV162	*Vitis vinifera*	wine grape	AlSalhieh	'*Ca*. P. aurantifolia'	99.7	II-C* (0.99)	OL873116 (a)
VV112	*Vitis vinifera*	wine grape	AlSalhieh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	OL873120 (e)
VV134	*Vitis vinifera*	wine grape	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV131	*Vitis vinifera*	wine grape	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV110	*Vitis vinifera*	wine grape	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV157	*Vitis vinifera*	wine grape	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV158	*Vitis vinifera*	wine grape	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV159	*Vitis vinifera*	wine grape	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV163	*Vitis vinifera*	wine grape	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV1	*Vitis vinifera*	wine grape	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV2	*Vitis vinifera*	wine grape	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV3	*Vitis vinifera*	wine grape	SamaAlSarahn	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
VV4	*Vitis vinifera*	wine grape	SamaAlSarahn	'*Ca*. P. solani'	99.7	XII-A (1.00)	d

For Peer Review

4

Table 2. Attribution to species and taxonomic subgroups of phytoplasmas detected in plants (part II)

Strain	Plant host	Location	Phytoplasma species	% id vs ref strain	16Sr subgroup	Acc. No.
CAr25	*Convolvulus arvensis*	Aldisi	'*Ca*. P. aurantifolia'	99.7	II-C* (0.99)	a
CAr26	*Convolvulus arvensis*	Aldisi	'*Ca*. P. aurantifolia'	99.7	II-C* (0.99)	a
CAr6	*Convolvulus arvensis*	SamaAlSarahn	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
CAr108	*Convolvulus arvensis*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
CAr5	*Convolvulus arvensis*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
CAr65	*Convolvulus arvensis*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
CAr153	*Convolvulus arvensis*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
CAr165	*Convolvulus arvensis*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
CAr168	*Convolvulus arvensis*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
CAr360	*Convolvulus arvensis*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
CAr109	*Convolvulus arvensis*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
CAr112	*Convolvulus arvensis*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d

5

6

7

For Peer Review

Table 3. Collected and phytoplasma-infected insects from surveyed locations in northern Jordan.

Governorate	Location	Grapevine cultivar	Insect code	Family	Species	Collection date	No. of collected insects	No. of pools	No. of positive pools	Infection rate (%)
AlMafraq	AlSalhieh	wine grape	MH2	Cicadellidae	*Empoasca* sp.	Aug	350	9	1	11.1
AlMafraq	AlSalhieh	wine grape	MH3b	Cicadellidae	*Balclutha* sp.	Aug	12	1	0	0
AlMafraq	AlSalhieh	wine grape	MH5	Cicadellidae	*Psammotettix striatus*	Aug	15	6	5	83.3
AlMafraq	AlSalhieh	wine grape	MH7	Cicadellidae	*Cicadulina bipunctata*	Aug	65	9	3	33.3
AlMafraq	AlSalhieh	wine grape	MH8	Cicadellidae	*Orosius cellulosus*	Aug	28	10	4	40
AlMafraq	AlSalhieh	wine grape	MH11	Delphacidae	*Toya propinqua*	Aug	23	4	0	0
AlMafraq	AlSalhieh	wine grape	MH12	Delphacidae	*Laodelphax striatellus*	Aug	5	2	0	0
AlMafraq	AlSalhieh	wine grape	MH18	Cicadellidae	*Circulifer* sp.	Nov	49	10	5	50
AlMafraq	AlSalhieh	wine grape	MH19	Cicadellidae	*Euscelidius mundus*	Nov	10	3	3	100
AlMafraq	Alkom AlAhmar	table grape	SUF1	Cicadellidae	*Cicadulina bipunctata*	Aug	88	7	2	28.6
AlMafraq	Alkom AlAhmar	table grape	SUF3	Delphacidae	*Laodelphax striatellus*	Aug	5	5	3	60
AlMafraq	Alkom AlAhmar	table grape	SUF5	Cicadellidae	*Psammotettix striatus*	Aug	5	2	2	100
AlMafraq	Sabha	table grape	YM2	Cixiidae	*Hyalesthes obsoletus*	Aug	7	2	1	50
AlMafraq	Sabha	table grape	YM3	Cicadellidae	*Cicadulina bipunctata*	Aug	135	6	1	16.7
Ajloun	Kufranjeh	table grape	Z1	Cicadellidae	*Cicadulina bipunctata*	Sep	105	8	1	12.5
Ajloun	Ain Jana	table grape	G61	Cicadellidae	*Arboridia adanae*	Sep	271	28	3	10.7
						Overall	1173	112	34	34.8

For Peer Review

Table 4. Attribution to species and taxonomic subgroups of phytoplasmas detected in insects.

Strain	Species	Region	Phytoplasma species	% id vs ref strain	16Sr subgroup	Acc. No.
MH2-1	*Empoasca* sp.	AlSalhieh	'*Ca*. P. pyri'	100	X-C (1.00)	OL873122
MH18-3	*Circulifer* sp.	AlSalhieh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
MH18-4	*Circulifer* sp.	AlSalhieh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
MH18-5	*Circulifer* sp.	AlSalhieh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
MH18-6	*Circulifer* sp.	AlSalhieh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
MH18-23	*Circulifer* sp.	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
MH5-7	*Psammotettix striatus*	AlSalhieh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
MH5-8	*Psammotettix striatus*	AlSalhieh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
MH5-9	*Psammotettix striatus*	AlSalhieh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
MH5-10	*Psammotettix striatus*	AlSalhieh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
MH5-11	*Psammotettix striatus*	AlSalhieh	'*Ca*. P. omanense'	100	XXIX-A (1.00)	b
MH7-12	*Cicadulina bipunctata*	AlSalhieh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
MH7-13	*Cicadulina bipunctata*	AlSalhieh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
MH7-14	*Cicadulina bipunctata*	AlSalhieh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
MH8-16	*Orosius cellulosus*	AlSalhieh	'*Ca*. P. aurantifolia'	99.7	II-C* (0.99)	a
MH8-17	*Orosius cellulosus*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
MH8-18	*Orosius cellulosus*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
MH8-21	*Orosius cellulosus*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
MH19-24	*Euscelidius mundus*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
MH19-25	*Euscelidius mundus*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
MH19-26	*Euscelidius mundus*	AlSalhieh	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
SUF3-1	*Laodelphax striatellus*	Alkom AlAhmar	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
SUF3-2	*Laodelphax striatellus*	Alkom AlAhmar	'*Ca*. P. solani'	99.7	XII-A (1.00)	d
SUF3-3	*Laodelphax striatellus*	Alkom AlAhmar	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
SUF5-4	*Psammotettix striatus*	Alkom AlAhmar	'*Ca*. P. asteris'	99.9	I-B (1.00)	OL873121
SUF5-5	*Psammotettix striatus*	Alkom AlAhmar	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
SUF1-6	*Cicadulina bipunctata*	Alkom AlAhmar	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
SUF1-7	*Cicadulina bipunctata*	Alkom AlAhmar	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
YM2-9	*Hyalesthes obsoletus*	Sabha	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
Z1-20	*Cicadulina bipunctata*	Kufranjeh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
Z1-40	*Cicadulina bipunctata*	Kufranjeh	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
G61-1	*Arboridia adanae*	Ain Jana	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
G61-2	*Arboridia adanae*	Ain Jana	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e
G61-3	*Arboridia adanae*	Ain Jana	'*Ca*. P. asteris'	99.6	I-R* (0.98)	e

For Peer Review

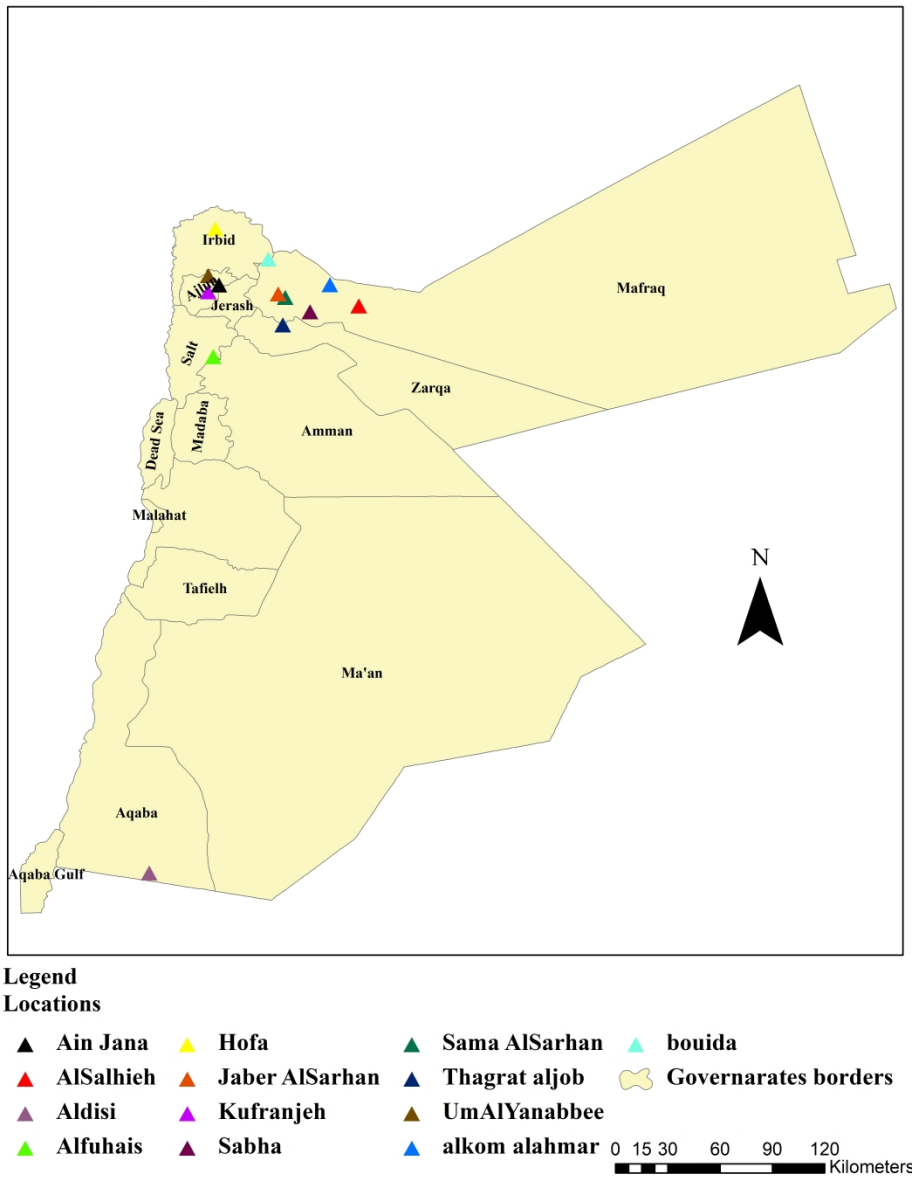

Figure 1. Maps of governorates and locations in Jordan where the surveys on GY diseases in vineyards were conducted.

215x279mm (500 x 500 DPI)

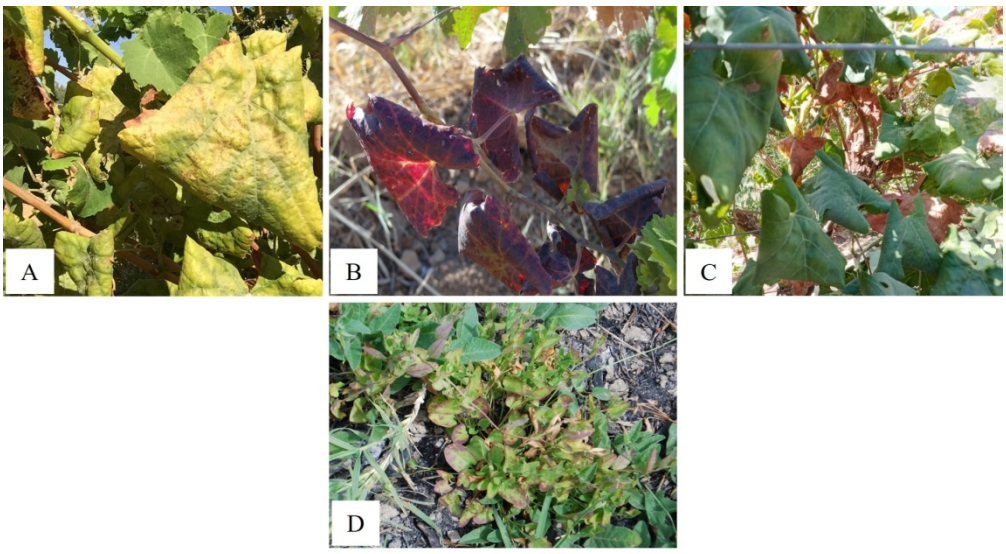

Figure 2. Phytoplasma-like symptoms observed in *Vitis vinifera* L. and *Convolvulus arvensis* L. in Jordan. Leaf yellowing and rolling in wine grape cultivar (A); leaf reddening and rolling in wine grape cultivar (B); leaf yellowing and rolling in table grape cultivar (C); little leaf and reddening in bindweed (D).

170x92mm (300 x 300 DPI)

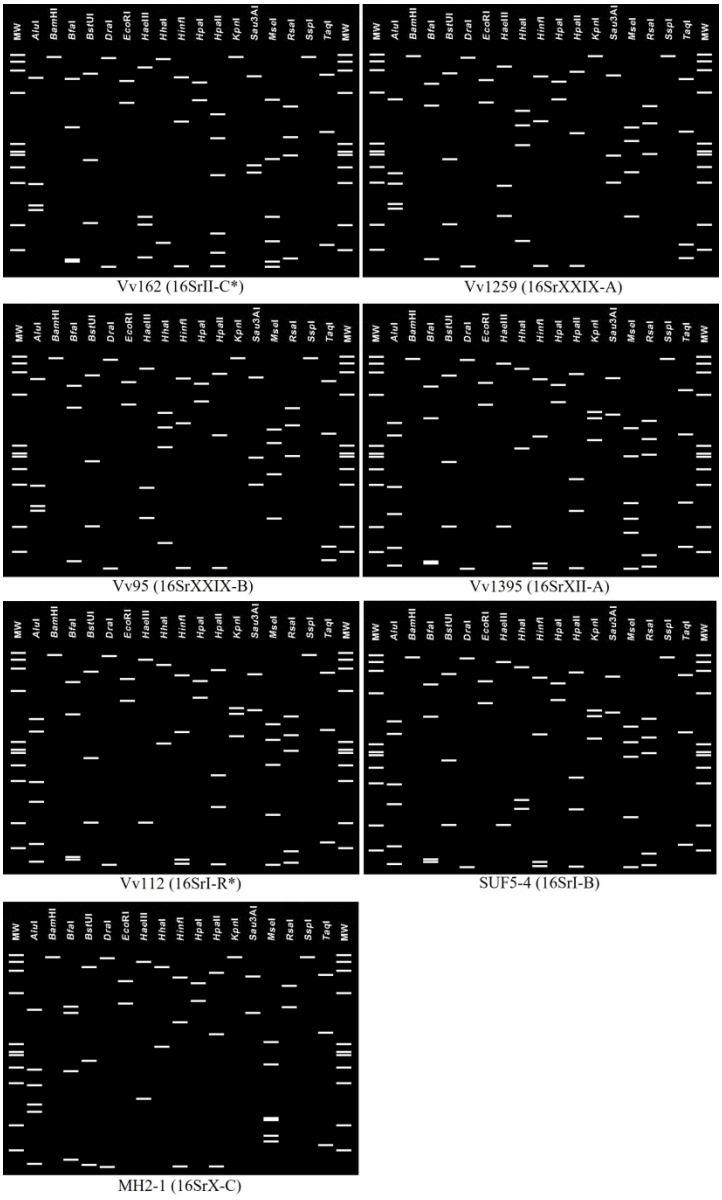

Figure 3. Virtual RFLP profiles of 16S rDNA nucleotide sequences of phytoplasma strains identified in *Vitis vinifera* and insects in Jordan. One strain among those sharing identical 16S rDNA sequence (Tables 2 and 4) was selected as representative strain for *i*PhyClassifier analyses.

153x256mm (330 x 330 DPI)

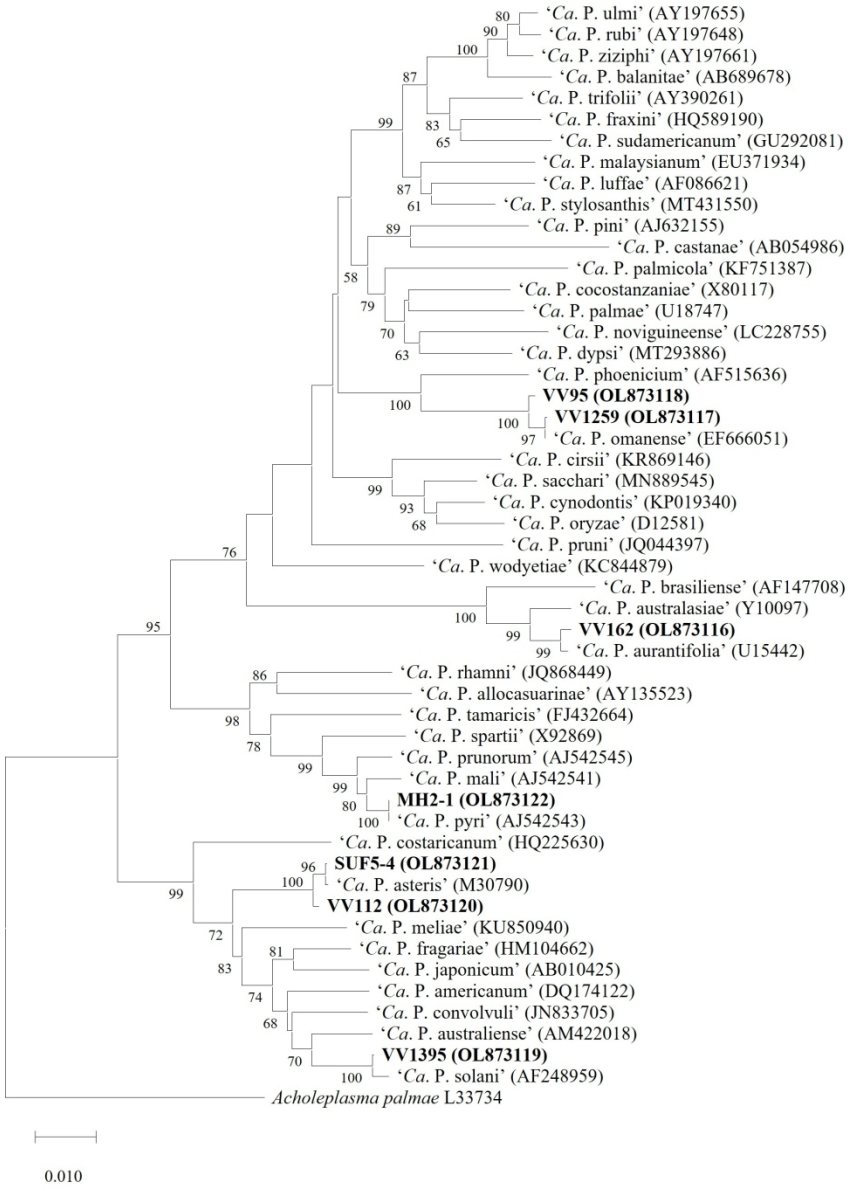

Figure 4. Phylogenetic tree based on the alignment of 16S rDNA nucleotide sequences of representative phytoplasma strains identified in *Vitis vinifera* L., putative insect vectors, and reservoir plants in Jordan (bold characters), and reference strains of previously described '*Candidatus* Phytoplasma' species. Regarding phytoplasmas identified in this study, one strain among those sharing identical 16S rDNA sequence (Tables 2 and 4) was selected as representative strain for phylogenetic analysis. The evolutionary history was inferred using the Minimum Evolution method. The optimal tree with the sum of branch length = 0.91088584 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm at a search level of 1. The Neighbor-joining algorithm was used to generate the initial tree. This analysis involved 52 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1424 positions in the final dataset.

143x202mm (330 x 330 DPI)