DE GRUYTER OPEN

Agriculture (Poľnohospodárstvo), 63, 2017 (3): 112−119

Original paper DOI: 10.1515/agri-2017-0011

MOLECULAR DETECTION OF '*CANDIDATUS* PHYTOPLASMA AUSTRALASIA' AND '*CA.* P. CYNODONTIS' IN IRAQ

NAWRES ABDULELAH SADEQ ALKUWAITI*, TARIQ ABDULSADA KAREEM, LAYLA JABAR SABIER

University of Baghdad, Baghdad, Iraq

ALKUWAITI, N. – KAREEM, T.A. – SABIER, L.J.: Molecular detection of ´*Candidatus* Phytoplasma australasia´ and ´*Ca.* P. cynodontis´ in Iraq. Agriculture (Poľnohospodárstvo), vol. *63*, 2017, no. 3, p. 112–119.

The association of phytoplasma was investigated in symptomatic tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongen* L.), mallow (*Malva* spp.) and Bermuda grass (*Cynodon dactylon* L.) plants exhibiting witches' broom and white leaf diseases, respectively. Total DNA was extracted from tomato (n=3), eggplant (n=2), mallow (n=2) and Bermuda grass (n=8) samples. Direct polymerase chain reaction (PCR) was performed using P1/P7 primer set, then PCR products were sequenced. Sequences obtained from tomato, eggplant and mallow shared 99% maximum nucleotide identity with phytoplasma belonging to subgroup 16SrII-D, and resulted therefore *'Candidatus* Phytoplasma australasia'-related. Sequences obtained from Bermuda grass showed 100% maximum nucleotide identity to 16SrXIV-A subgroup and were '*Ca*. P. cynodontis'-related. The study presents the first molecular confirmation and sequence data of presence of *'Ca.* P. australasia' and '*Ca*. P. cynodontis' in Iraq.

Key words: witches' broom, white leaf, tomato, eggplant, Bermuda grass

Phytoplasms impact a wide range of economically important plant species causing serious losses worldwide (Bertaccini & Duduk 2009). They affect the quality and quantity of products resulted from infected crops including vegetables, field crops, fruit trees and ornaments (Chaturvedi *et al.* 2010; Bertaccini *et al.* 2014; Maejima *et al.* 2014). An extra damage may occur due to the cost paid for phytoplasma indexing procedures, quarantine regulations and disease control. Phytoplasms are wall- less prokaryotic plant pathogens belonging to the class Mollicutes (IRPCM 2004). They are phloem limited and insect transmitted by *Cicadellidae* (leafhoppers), *Fulgoridae* (planthoppers), *Cercopidae* (spittlebugs or froghoppers), *Cixiidae* (Cixiid planthoppers), *Derbidae* (Derbid planthoppers), *Delphacidae* (Delphacid planthoppers) and *Psyllidae* (Psyllid bugs) (Jarausch & Weintraub 2013). Moreover phytoplasms have been found to be transmitted by dodder, grafting (Bertaccini & Duduk 2009; Bertaccini *et al.* 2014) and through seeds (Bertaccini & Duduk 2009; Calari *et al.* 2011). Taxonomically, phytoplasma strains have been classified into 116 subgroups within 34 groups based on 16S ribosomal gene restriction fragment length polymorphism (RFLP) analyses (Duduk & Bertaccini 2011; Bertaccini *et al.* 2014; Fránová *et al.* 2014).

Phytoplasmas classified in 16SrII group are often associated with typical witches` broom symptoms including virescence, phyllody, proliferation and stunting (Bertaccini *et al.* 2014), while those belonging to 16SrXIV group are usually associated with white leaf symptoms (Marcone *et al.* 2004; Salehi *et al.* 2009). Papaya yellow crinkle disease associat-

Nawres Abdulelah Sadeq Alkuwaiti, PhD., Lecturer (*Corresponding author), Tariq Abdulsada Kareem, PhD., Assistant Professor, Layla Jabar Sabier, PhD., Lecturer, Plant Protection Department, College of Agriculture, University of Baghdad, Baghdad, Iraq. E-mail: N.Alkuwaiti@coagri.uobaghdad.edu.iq

ed phytoplasma (16SrII-D subgroup) was identified in Australia for the first time in 1996 (Gibbs *et al.* 1996). In 1998, this phytoplasma was assigned into the new taxon *'Candidatus* Phytoplasma australasia' (White *et al.* 1998). Phytoplasmas associated with white leaf diseases were reported for the first time on Bermuda grass since 1972 in Taiwan (Marcone *et al.* 1997), and were assigned into a separated taxon '*Ca. P. cynodontis*' in 2004 (Marcone *et al.* 2004). PCR technique is a powerful tool for phytoplasma characterization especially when combined with RFLP and sequence analyses (Bertaccini & Duduk 2009; Delić 2012). PCR approaches using universal primers designed to target conserved sequences (e.g. 16S rDNA) enable detection of phytoplasmas in both plants and insects (Bertaccini & Duduk 2009; Delić 2012). Then, phytoplasma sequences resulting from universal primers amplification can easily be identified up to group/sub group level using virtual RFLP digestion (Wei *et al.* 2007; Zhao *et al.* 2009).

In Iraq, phytoplasma diseases have been investigated based on biology, serology and microscopy (Al-Rawi *et al.* 2001). PCR amplification was applied to detect the association of phytoplasmas with a phyllody disease of Arabic jasmine (Al-Kuwaiti *et al.* 2015) and sesame (Mohammed *et al.* 2016). Witches' broom symptoms, suspected to be a phytoplasma disease, were observed in tomato (*Solanum lycopersicum* L.) and eggplant (*Solanum melongena* L.) in Basra province. This disease caused serious economic losses on tomato and eggplant crops to producers as most fruits failed to set. White leaf was another phytoplasma disease observed in Bermuda grass (*Cynodon dactylon* L.) in Baghdad. Two mallow (*Malva* spp.) samples exhibiting witches' broom symptoms were found in Al-Nassiriya province. This study aimed at the molecular investigation of identity of the phytoplasma associated with witches' broom and white leaf diseases in Iraq.

MATERIAL AND METHODS

Leaf samples were collected from symptomatic tomato (n=3) and eggplant (n=2), grown under tunnels at Al-Zubair region in Basra province. Mallow (n=2) samples were collected from a field in Al-Nassiriya province. Bermuda grass (n=8) samples were collected from Baghdad province. Total DNA was extracted from samples using AccuPrep® Plant DNA Extraction Kit from (Bioneer, S. Korea) following the manufacturer instructions. Direct PCR was performed using AccuPower PCR PreMix kit from (Bioneer, S. Korea) and P1/P7 primer set (Deng & Hiruki 1991; Smart *et al.* 1996). PCR reaction was prepared by adding 1µl (50 ng) of extracted DNA and 1 µl of each primer (10 picomole) to PCR Premix then reaction volume was adjusted to 20 µl. PCR amplification was preformed using 1 cycle of pre-denaturation for 2 min. at 94°C, 35 cycles of denaturation for 30 s min at 94°C, annealing for 2 min at 55C° and extension for 3 min. at 72°C. The Final extension step was set for 15 min at 72°C. PCR products were analysed by ethidium bromide gel electrophoresis using 2.5% agarose for 15 min at 125 mAmp (Sambrook & Russell 2006). PCR products of expected size were sent to (Bioneer, S. Korea) for sequencing in both directions, to generate consensus sequences. Sequences obtained were compared to equivalent GenBank sequences using MEGA BLAST analysis. Phylogenetic tree was constructed using MEGA6 software package (Tamura *et al.* 2013). Virtual RFLP was performed to resolve group/subgroup of phytoplasma sequences isolated using *i*PhyClassifier (Zhao *et al.* 2009). Phytoplasma sequences obtained were deposited in GenBank database with accession codes (KU724309), (KX008307- KX008310) and (KY284836- KY284845).

RESULTS AND DISCUSSION

During December 2015, an outbreak of witches' broom disease occurred in tomato and eggplant grown under tunnels in Al-Zubair region at Basra province in Iraq. About 80% of tomato and eggplant exhibited typical symptoms of phytoplasma disease including phyllody, proliferation and stunting (Figure 1 A-D). In the same time a white leaf disease was observed in Bermuda grass grown in public and private gardens in Baghdad (Figure 1 E-F). PCR amplification using P1/P7 phytoplasma specific primers amplified ~1.8 kb DNA fragment size (Smart *et al.* 1996) from symptomatic tomato, eggplant, mallow and Bermuda grass (Figure 2). Se-

quence analyses confirmed all sequences obtained from symptomatic plants were from 16S rRNA region of phytoplasma genome (Smart *et al.* 1996), when compared to equivalent GenBank sequences. Sequences from tomato, eggplant and mallow plants, infected with witches' broom disease, shared 99.8–99.9% nucleotide identities to 16SrII-D subgroup phytoplasmas from Egypt (GenBank accession number KU056919), Iran (GenBank accession numbers KP869129, JX441321, KR706443 and KJ016231), Oman (GenBank accession number AB257291), Australia (GenBank accession numbers Y10097 and JQ868446) and South Korea (GenBank accession number AB690307) (Tables 1 and 2). The sequences obtained from Bermuda grass showing white leaf disease shared 99.7–100% identities

Figure 1. Naturally infected tomato (A,B), eggplant (C, D) plants exhibiting phyllody (white arrows) and proliferation symptoms. (E) and (F) Bermuda grass exhibiting white leaf symptoms

T a b l e 1

Nucleotide sequence identities representing partial16S rDNA region of *'Candidatus* Phytoplasma australasia' and *'Ca*. P. cynodontis' (Bold letters) from Iraq (marked with *) and other GenBank strains. Sequence identities were calculated using MEGA6 software.

	Isolate/Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1.	Al-Zubair*																												
2.	Al-Zubair1*	99.1																											
3.	Al-Zubair2*	99.9	99.2																										
4.	Al-Zubair3*	99.6	99.3	99.8																									
5.	Al-Zubair4*	99.8	99	99.6	99.6																								
6.	Malva1*	98.9	98.4	99.1	99	98.9																							
7.	Malva2*	99.5	99	99.6	99.6	99.4	98.9																						
8.	(KU056919)Egypt	99.8	99.3	99.9	99.9	99.7	99.2	99.7																					
9.	(KP869129)Iran	99.8	99.3	99.9	99.9	99.7	99.2	99.7	100																				
10.	(JX441321)Iran	99.8	99.3	99.9	99.9	99.7	99.2	99.7	100	100																			
11.	(KR706443)Iran	99.8	99.3	99.9	99.9	99.7	99.2	99.7	100	100	100																		
12.	(KJ016231)Iran	99.7	99.2	99.9	99.8	99.6	99.1	99.6	99.9	99.9	99.9	99.9																	
13.	(Y10097)Australia	99.8	99.3	99.9	99.9	99.7	99.2	99.7	100	100	100	100	99.9																
14.	(AB690307)_S._Korea	99.6	99.1	99.7	99.6	99.5	98.9	99.5	99.8	99.8	99.8	99.8	99.7	99.8															
15.	(JQ868446)Australia	99.6	99.2	99.8	99.7	99.6	99	99.6	99.9	99.9	99.9	99.9	99.8	99.9	99.9														
16.	(AB257291)Oman	99.8	99.3	99.9	99.9	99.7	99.2	99.7	100	100	100	100	99.9	100	99.8	99.9													
17.	**WF1***	**90.7**	**90.2**	**90.7**	**90.7**	**90.8**	**90.2**	**90.7**	**90.8**	**90.8**	**90.8**	**90.8**	**90.7**	**90.8**	**90.9**	**90.8**	**90.8**												
18.	**WF2***	**91.1**	**90.6**	**91.1**	**91**	**91.2**	**90.5**	**91**	**91.2**	**91.2**	**91.2**	**91.2**	**91.1**	**91.2**	**91.2**	**91.2**	**91.2**	**99.4**											
19.	**WF3***	**91.4**	**90.9**	**91.4**	**91.3**	**91.4**	**90.8**	**91.3**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.5**	**91.4**	**91.4**	**99.2**	**99.7**										
20.	**WF4***	**91.4**	**90.9**	**91.4**	**91.3**	**91.4**	**90.8**	**91.3**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.5**	**91.4**	**91.4**	**99.2**	**99.7**	**100**									
21.	**WF5***	**91.2**	**90.7**	**91.2**	**91.2**	**91.3**	**90.7**	**91.2**	**91.3**	**91.3**	**91.3**	**91.3**	**91.2**	**91.3**	**91.4**	**91.3**	**91.3**	**99.4**	**99.7**	**99.9**	**99.9**								
22.	**WF6***	**91.2**	**90.7**	**91.2**	**91.1**	**91.2**	**90.6**	**91.1**	**91.2**	**91.2**	**91.2**	**91.2**	**91.2**	**91.2**	**91.3**	**91.2**	**91.2**	**99.3**	**99.6**	**99.8**	**99.8**	**99.9**							
23.	**WF7***	**90.4**	**89.9**	**90.4**	**90.3**	**90.4**	**89.8**	**90.3**	**90.4**	**90.4**	**90.4**	**90.4**	**90.4**	**90.4**	**90.5**	**90.4**	**90.4**	**98.9**	**99.1**	**98.8**	**98.8**	**98.9**	**99**						
24.	**WF8***	**91.1**	**90.6**	**91.1**	**91**	**91.2**	**90.5**	**91**	**91.2**	**91.2**	**91.2**	**91.2**	**91.1**	**91.2**	**91.2**	**91.2**	**91.2**	**99.4**	**99.9**	**99.7**	**99.7**	**99.9**	**99.8**	**99.1**					
25.	**(KF383980)Albania**	**91.4**	**90.9**	**91.4**	**91.3**	**91.4**	**90.8**	**91.3**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.5**	**91.4**	**91.4**	**99.2**	**99.7**	**100**	**100**	**99.9**	**99.8**	**98.8**	**99.7**				
26.	**(AJ550984)Italy**	**91.4**	**90.9**	**91.4**	**91.3**	**91.4**	**90.8**	**91.3**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.5**	**91.4**	**91.4**	**99.2**	**99.7**	**100**	**100**	**99.9**	**99.8**	**98.8**	**99.7**	**100**			
27.	**(AB741630)Myanmar**	**91.2**	**90.7**	**91.2**	**91.2**	**91.3**	**90.7**	**91.2**	**91.3**	**91.3**	**91.3**	**91.3**	**91.2**	**91.3**	**91.4**	**91.3**	**91.3**	**99**	**99.5**	**99.8**	**99.8**	**99.6**	**99.6**	**98.7**	**99.5**	**99.8**	**99.8**		
28.	**(AF248961)Thailand**	**91.2**	**90.7**	**91.2**	**91.1**	**91.2**	**90.6**	**91.1**	**91.2**	**91.2**	**91.2**	**91.2**	**91.2**	**91.2**	**91.3**	**91.2**	**91.2**	**98.9**	**99.4**	**99.7**	**99.7**	**99.6**	**99.5**	**98.7**	**99.4**	**99.7**	**99.7**	**99.9**	
29.	Acholeplasma palmae (L33734)	87.3	87	87.3	87.3	87.3	86.8	87.3	87.4	87.4	87.4	87.4	87.3	87.4	87.3	87.4	87.4	88.6	89	89.2	89.2	89.2	89.1	88.3	89	89.2	89.2	89.2	89.2

with 16SrXIV-A subgroup phytoplasmas detected in Bermudagrass from Albania (KF383980), Italy (AJ550984), Myanmar (AB741630) and Thailand (AF248961) (Tables 1 and 2). Phylogenetic analysis supported this grouping of all the Iraqi sequences from witches' broom diseased plants with phytoplasmas enclosed in 16SrII-D subgroup (Figure 3). Moreover in the phylogenetic tree all sequences obtained from white leaf diseased Bermuda grass and those of phytoplasmas enclosed in 16SrXIV-A subgroup groupend in a single clade (Figure 3).The two phylogenetic groups were separated from each other's and the relatedness was supported by 99% boots trap value. Data obtained from *i*PhyClassifier analyses confirmed that all sequences isolated from witches' broom diseased plants belong to 16SrII-D. The virtual RFLP patterns derived from Al-Zubair, Al-Zubair(1–4) and Malva1 16S rDNA

T a b l e 2

Phytoplasma sequences obtained in this study. Identity percentages were calculated based on ''*Candidatus* Phytoplasma australasiae'' reference strain (GenBank accession: Y10097) ''*Ca.* P. cynodontis'' reference strain (GenBank accession: AJ550984) using iPhyClassifier

GenBank acc. No.	Isolate/ sequence name	Source	Location	Group/sub group	Ident. [%]	Similarity coefficient
KU724309	Al-Zubair	tomato	Basra	16SrII-D	99.0	1.00
KX008307	Al-Zubair1	eggplant			99.3	
KX008308	Al-Zubair2				99.9	
KX008309	Al-Zubair3				99.7	
KX008310	Al-Zubair4				99.6	
KY284836	Malva1	mallow	Al-Nassiriya		98.6	
KY284837	Malva2				99.1	0.98
KY284838	WF1	Bermuda grass	Baghdad	16SrXIV-A	99.0	0.98
KY284839	WF2				99.7	0.98
KY284840	WF3				99.9	1.00
KY284841	WF4				99.9	1.00
KY284842	WF5				99.8	1.00
KY284843	WF6				99.7	1.00
KY284844	WF7				99.7	0.98
KY284845	WF8				99.6	0.98

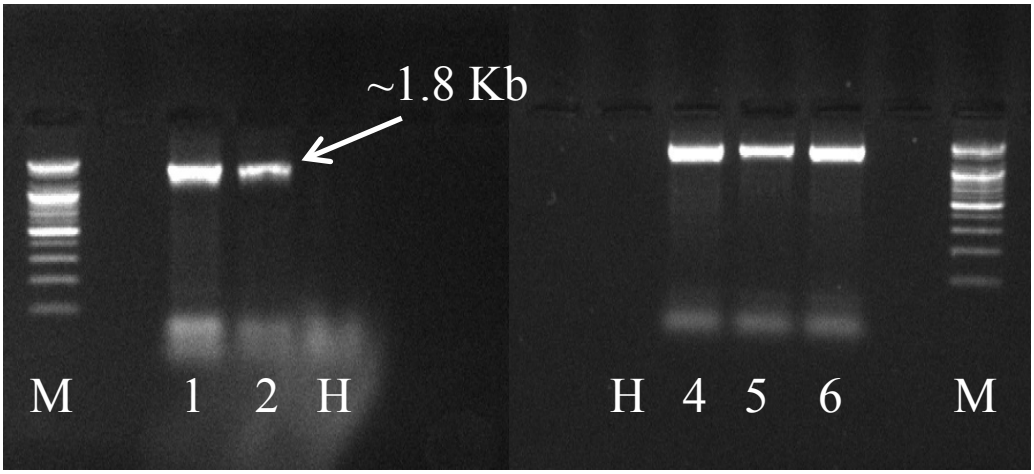

Figure 2. Gel electrophoresis pattern of ~1.8 Kb DNA fragments amplified by P1/P7 primers set. M: 100bp DNA marker, H: healthy plant, 1 & 2 lanes: witches' broom infected tomato, 4–6 lanes: white leaf infected Bermuda grass

F2n/R2 fragments were identical to the reference pattern of 16SrII-D (GenBank accession number Y10097) with similarity coefficient of 1.00 (Table 2). Malva2 shared low similarity coefficient (0.98) with 16SrII-D phytoplasmas showing a slightly different profile in virtual restriction pattern with *Mse*I. Thus, Malva2 could be a variant of 16SrII-D. *In silico* RFLP performed confirmed that all sequences obtained from white leaf diseased Bermuda grass were members of subgroup 16SrXIV-A. The virtual RFLP patterns derived from WF (1–8) 16S rDNA F2n/R2 fragment shared similarity coefficient ranging between 0.98 and 1.00 with the reference pattern of 16SrXIV-A (GenBank accession number AJ550984) (Table 2). WF(1, 2, 7 and 8) showed also a slightly different RFLP pattern when virtually cleaved with *Bst*UI. Thus, WF(1, 2, 7 and 8) may represent variants of 16SrXIV-A. Based on molecular and *in silico* approaches, the association of '*Ca.* P. australasiae', subgroup 16SrII-D with witches' broom disease has been reported in tomato and eggplant worldwide including Oman (Al-Subhi *et al.* 2011) Egypt (Omar & Foissac 2012), Iran (Salehi *et al.* 2014), and India (Singh *et al.* 2012; Yadav *et al.* 2016). Whereas, '*Ca.* P. cynodontis' subgroup 16SrXIV-A associated with Bermuda grass white leaf disease has been investigated based on molecular and *in silico* analyses in Iran (Salehi *et al.* 2009),

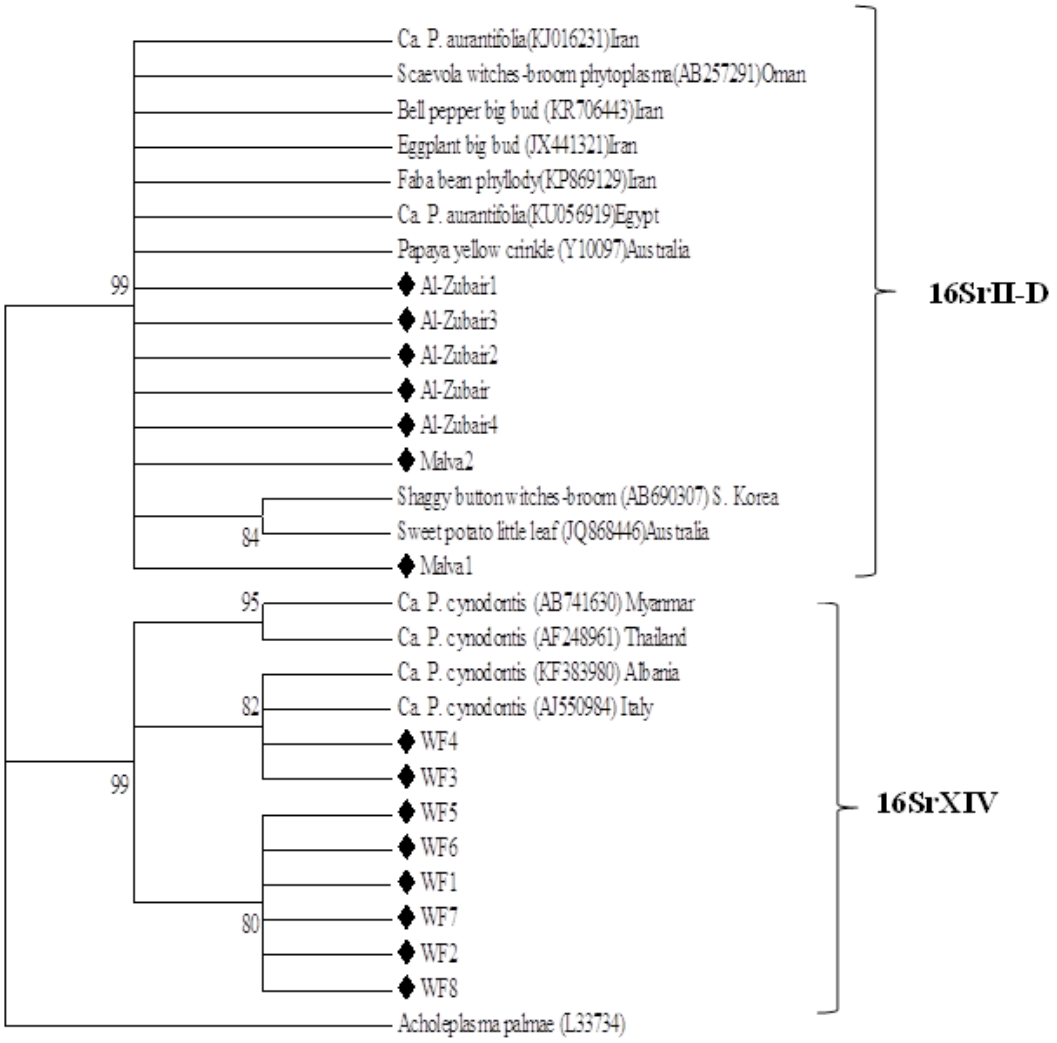

Figure 3. Neighbor-Joining phylogenetic tree of '*Candidatus* Phytoplasma australasia' from tomato, eggplant and mallow and '*Ca.* P. cynodontis' from Bermuda grass. This tree was constructed from partial 16S rDNA sequences (including 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S ribosomal RNA gene, partial sequence) from Iraq (marked with•) and selected GenBank sequences. *Acholeplasma palmae* (L33734) used as an out group comparison. This tree was constructed by MEGA6 software.

Kenya (Obura *et al.* 2010) Italy, Albania and Serbia (Mitrović *et al.* 2015) and Saudi Arabia (Omar 2016) .

CONCLUSIONS

This study presents the first report of '*Ca.* P. australasiae' and '*Ca.* P. cynodontis' association with witches' broom and white leaf diseases in Iraq, respectively. Moreover it confirms the presence of phytoplasmas in tomato, eggplant, mallow and Bermuda grass based on molecular and *in silico* analyses since previous studies conducted (Al-Kuwaiti *et al.* 2015; Mohammed *et al.* 2016) did not present sequence information regarding phytoplasmas in Iraq. Further molecular based studies, however, are required to investigate phytoplasma diseases and their epidemiology in Iraq.

Acknowledgements. The authors would like to thank Agronomist Majid Jabber Khudhair (Municipality of AL-Zubair), Miss Noor Raad Khuder and Mr. Ali Majid Jawad (Almusaib Bridge for Scientific and Lab Equipment) for their technical assistance.

REFERENCES

AL-KUWAITI, N.A.S. – KAREEM, T.A. – JAMEEL, D.S. 2015. The molecular investigation of phytoplasma '*Candidatus* Phytoplasma' infecting Arabic jasmine *Jasminum sambac* in Iraq. In *Iraqi Journal of Science*, vol. *56*, pp. 2787–2904.

AL-RAWI, A.F. – AL-FADHIL, F.K. – AL-ANI, R.A. 2001. Histological methods for detection and monitoring distribution of phytoplasmas in some crops and weed plants in central Iraq. In *Arab Journal of Plant Protection*, vol. *19*, pp. 3–11.

AL-SUBHI, A.M. – AL-SAADY, N.A. – KHAN, A.J. – DEADMAN, M.L. 2011. First report of a group 16SrII Phytoplasma associated with witches'-broom of eggplant in Oman. In *Plant Diseases*, vol. *95*, pp. 360.

BERTACCINI, A. – DUDUK, B. 2009. Phytoplasma and phytoplasma diseases: a review of recent research. In *Phytopathology Mediterranean*, vol. *48*, pp. 355–378.

BERTACCINI, A. – DUDUK, B. – PALTRINIERI, S. – CONTALDO, N. 2014. Phytoplasmas and Phytoplasma Diseases: A Severe Threat to Agriculture. In *American Journal of Plant Sciences*, vol. *5*, pp. 1763–1788.

CALARI, A. – PALTRINIERI, S. – CONTALDO, N. – SAKALIEVA, D. – MORI, N. – DUDUK, B. – BERTACCINI, A. 2011. Molecular evidence of phytoplasmas in winter oilseed rape, tomato and corn seedlings. In *Bulletin of Insectology*, vol. *64* (Supplement), pp. S157–S158.

CHATURVEDI, Y. – RAO, G.P. – TIWARI, A.K. – DUDUK, B. – BERTACCINI, A. 2010. Phytoplasma on Ornamentals: detection, diversity and management. In *Acta Phytopathologica et Entomologica Hungarica*, vol. *45*, pp. 31–69.

DELIĆ, D. 2012. Polymerase chain reaction for phytoplasmas detection, polymerase chain reaction. In HERNANDEZ-RODRIGUEZ, P. (Ed.) *Polymerase chain reaction.* Rijeka : InTech, pp. 91–118. ISBN: 978-953-51-0612-8

DENG, S. – HIRUKI, C. 1991. Amplification of 16S rRNA genes from culturable and nonculturable mollicutes. In *Journal of Microbiological Methods*, vol. *14*, pp. 53–61.

DUDUK, B. – BERTACCINI, A. 2011. Phytoplasma classification: Taxonomy based on 16S ribosomal gene, is it enough? In *Phytopathogenic Mollicutes*, vol. *1*, pp. 3–13.

IRPCM. 2004. 'Candidatus Phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. In *International Journal of Systematic and Evolutionary Microbiology*, vol. *54*, pp. 1243–1255.

FRÁNOVÁ, J. – BERTACCINI, A. – DUDUK, B. 2014. Molecular tools in COST FA0807 Action. Phytoplasmas and phytoplasma disease management: how to reduce their economic impact. In BERTACCINI, A. (Ed.) *Phytoplasmas and phytoplasma disease management: how to reduce their economic impact.* Bologna : IPWG – International Phytoplasmologist Working Group, pp. 179–185. ISBN: 978-88-909922-0-9

GIBBS, K.S. – PERSLEY, D.M. – SCHNEIDER, B. – THOMAS, H.E. 1996. Phytoplasmas associated with papaya diseases in Australia. In *Plant Disease*, vol. *80*, pp. 174–178.

JARAUSCH, B. – WEINTRAUB, P. 2013. Spread of phytoplasmas by insect vectors: an introduction. In BERTACCINI, A. (Ed.) – LAVIÑA, A. –TORRES, E. *New Perspectives in Phytoplasma Disease Management*. COST action FA0807 Workshop, 22 March, Barcelona, Spain, Available at http://www.cost.eu/download/FAP_FA0807.

MAEJIMA, K. – OSHIMA, K. – NAMBA, S. 2014. Exploring the phytoplasmas, plant pathogenic bacteria. In *Journal of General Plant Pathology*, vol. *80*, pp. 210–221.

MARCONE, C. – SCHNEIDER, B. – SEEMÜLLER, E. 2004. '*Candidatus* Phytoplasma cynodontis', the phytoplasma associated with Bermuda grass white leaf disease. In *International Journal of Systematic and Evolutionary Microbiology*, vol. *54*, pp. 1077–1082.

MARCONE, C. – RAGOZZINO, A. – SEEMÜLLER, E. 1997. Detection of Bermuda grass white leaf disease in Italy and characterization of the associated phytoplasma by RFLP analysis. In *Plant Disease*, vol. *81*, pp. 862–866.

MITROVIĆ, J. – SMILJKOVIĆ, M. – SEEMÜLLER, E. – REINHARDT, R. – HUTTEL, B. – BERTACCINI, A. – KUBE, M. – DUDUK, B. 2015. Differentiation of 'Candidatus Phytoplasma cynodontis' based on 16S rRNA and groEL genes and identification of a new subgroup, 16SrXIV-C. In *Plant Disease*, vol. *99*, pp. 1578–1583.

MOHAMMED, M. – ALFADHA, F. – ALHATAMI, A. 2016. The relationship between sesame Phytoplasma (phyllody) and others plant Phytoplasma by using polymerase chain reaction (PCR). In *Kufa Journal for Agricultural Science*, vol. *8*, pp. 130–150.

OBURA, E. – MASIGA, D. – MIDEGA, C.A.O. – WACHIRA, F. – PICKETT, J.A. – DENG, A.L. – KHAN, Z.R. 2010. First report of a phytoplasma associated with Bermuda grass white leaf disease in Kenya. In *New Disease Reports*, vol. *21*, pp. 23.

OMAR, A.F. 2016. Association of '*Candidatus* Phytoplasma cynodontis' with Bermuda grass white leaf disease and its new hosts in Qassim province, Saudi Arabia. In *Journal of Plant Interactions*, vol. *11*, pp. 101–107.

OMAR, A.F. – FOISSAC, X. 2012. Occurrence and incidence of phytoplasmas of the 16SrII-D subgroup on solanaceous

and cucurbit crops in Egypt. In *European Journal of Plant Pathology*, vol. *133*, pp. 353–360.

SALEHI, E. – SALEHI, M. – TAGHAVI, S.M. – IZADPANAH, K. 2014. A 16SrII-D phytoplasma strain associated with tomato witches'-broom in Bushehr province. In *Iranian Journal of Crop Protection*, vol. *3*, pp. 377–388.

SALEHI, M. – IZADPANAH, K. – SIAMPOUR, M. – TAGHIZADEH, M. 2009. Molecular characterization and transmission of Bermuda grass with white leaf phytoplasma in Iran. In *Journal of Plant Pathology*, vol. *91*, pp. 655–661.

SAMBROOK, J.F. – RUSSELL, D. 2006. Condensed protocols: from molecular cloning: a laboratory manual. New York : Cold Spring Harbor Laboratory Press, pp. 800.

SINGH, J. – RANI, A. – KUMAR, P. – BARANWAL, V.K. – SAROJ, P.L. – SIROHI, A. 2012. First report of a 16SrII-D phytoplasma *'Candidatus* Phytoplasma australasia' associated with a tomato disease in India. In *New Disease Reports*, vol. *26*, pp. 14.

SMART, C.D. – SCHNEIDER, B. – BLOMQUIST, C.L. – GUERRA, L.J. – HARRISON, N.A.– AHRENS, U. – LORENZ, K.H. – SEEMÜLLER, E. – KIRKPATRICK, B.C. 1996. Phytoplasma-specific PCR primers based on sequences of the 16S-23S rRNA spacer region. In *Applied Environmental Microbiology*, vol. *62*, pp. 2988–2993.

TAMURA, K. – STECHER, G. – PETERSON, D. – FILIPSKI, A. – KUMAR, S. 2013. MEGA6:Molecular Evolutionary Genetics Analysis version 6.0. In *Molecular Biology and Evolution*, vol. *30*, pp. 2725–2729.

WEI, W. – DAVIS, R.E. – LEE, I. – M. – ZHAO, Y. 2007. Computer-simulated RFLP analysis of 16S rRNA genes: identification of ten new phytoplasma groups. In *International Journal of Systematic and Evolutionary Microbiology*, vol. *57*, pp. 1855–1867.

WHITE, D.T. – BLACKALL, L.L. – SCOTT, P.T. – WALSH, K.B. 1998. Phylogenetic positions of phytoplasmas associated with dieback, yellow crinkle and mosaic diseases of papaya, and their proposed inclusion in '*Candidatus* Phytoplasma australiense' and a new taxon, '*Candidatus* Phytoplasma Australasia'. In *International Journal of Systematic and Evolutionary Microbiology*, vol. *48*, pp. 941–951.

YADAV, V. – MAHADEVAKUMAR, S. – TEJASWINI, G.S. – SHILPA, N. – SREENIVASA, M.Y. – AMRUTHAVALLI, C. – JANARDHANA, G.R. 2016. First report of 16SrII-D phytoplasma associated with eggplant big bud (*Solanum melongena* L.) in India. In *Plant Disease*, vol. *100*, pp. 517.

ZHAO, Y. – WEI, W. – LEE, I.-M. – SHAO, J. – SUO, X. – DAVIS, R.E. 2009. Construction of an interactive online phytoplasma classification tool, iPhyClassifier, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). In *International Journal of Systematic and Evolutionary Microbiology*, vol. *59*, pp. 2582–2593.

Received: May 27, 2017