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First Report of Alfalfa Leaf Curl Virus Affecting Alfalfa (*Medicago sativa*) in Jordan, Lebanon, Syria, and Tunisia

S. G. Kumari, A. R. Moukahel, Cecile Richet, Serge Galzi, Denis Filloux,
Philippe Roumagnac, N. Asaad, J. Hijazi, S. Mghandef

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1 **First report of *Alfalfa leaf curl virus* affecting alfalfa (*Medicago sativa* L.) in Jordan,**
2 **Lebanon, Syria and Tunisia**

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4 **Safaa G. Kumari** and **Abdul Rahman Moukahel**, International Center for Agricultural
5 Research in the Dry Areas (ICARDA), Terbol Station, Beqa'a, Zahle, Lebanon; **Cécile Richet**,
6 **Serge Galzi**, **Denis Filloux** and **Philippe Roumagnac**, CIRAD, BGPI, Montpellier, France and
7 BGPI, INRA, CIRAD, SupAgro, Univ Montpellier, Montpellier, France; **Nader Asaad**, General
8 Commission for Scientific Agricultural Research (GCSAR), Al-Ghab, Hama, Syria; **Jomana**
9 **Hijazi**, National Center for Agricultural Research and Extension (NCARE), Jordan; and **Samia**
10 **Mghandef**, Virology Lab, ICARDA, Tunisia

11
12 Corresponding author: P. Roumagnac, E-mail address: philippe.roumagnac@cirad.fr

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14 The genus *Capulavirus* (family *Geminiviridae*) includes plant-infecting single-stranded DNA
15 viruses with circular genomes that have been characterized in Africa, Europe and Asia (Bernardo
16 et al. 2016; Susi et al. 2017). Among the capulaviruses, alfalfa leaf curl virus (ALCV) is
17 transmitted by the aphid species *Aphis craccivora* Koch (Roumagnac et al. 2015). While initially
18 isolated from France and Spain (Bernardo et al. 2016), ALCV was recently reported from
19 Argentina (Bejerman et al. 2018). In 2017, leaves of 184 alfalfa (*Medicago sativa* L.) plants with
20 symptoms suggestive of virus infection (leaf roll, stunting, mottling, leaf thickening) were
21 collected from Jordan (57 samples from Jordan valley and Ar Ramtha), Lebanon (50 samples
22 from West and Middle Beka'a valley), Syria (40 samples from Hama Governorate) and Tunisia
23 (37 samples from Beja, Bizerte, Ariana and Manouba Governorates). Total DNA was extracted

24 for all collected samples using DNeasy Plant Mini Kit (Qiagen). PCR-mediated detection of
25 ALCV was performed using My TaqTM Red DNA Polymerase (Bioline) according to the
26 manufacturer's instructions and capulavirus-specific primers (Capula2F:
27 GAGRAABTCGGACTTGGAKGT and Capula4R: CAYCTYCACTGYCTYGTCCA) designed
28 to alignments of the 47 available whole genome sequences of capulaviruses to amplify a 267-bp
29 fragment of the coat protein gene. Amplification conditions consisted of: 95°C for 5 min,
30 followed by 35 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C, and a final extension
31 for 10 min at 72°C. PCR results revealed that 86 samples (37 from Jordan, 7 from Lebanon, 22
32 from Syria and 20 from Tunisia) generated expected 267 nt amplicons. DNA of four samples that
33 tested positive for ALCV (one from each country) were then used as a template for PCR
34 amplification of the complete genome using the HotStarTaq Plus Master Mix Kit (Qiagen)
35 following the manufacturer's protocol and the pair of abutting primers (Cap-ncolF and Cap-
36 ncolR) with a *NcoI* overlapping site as described in Bernardo et al. (2016). Amplification
37 conditions consisted of: 95°C for 5 min, 35 cycles at 94°C for 20 s, 60°C for 30 s, 68°C for 165
38 s, and 72°C for 3 min. The amplicons were gel purified using the PCR Clean-Up System
39 (Promega), cloned into pGEM-T Easy (Promega) and Sanger sequenced by primer walking at
40 Genewiz (South Plainfield, USA). The four ALCV complete genome sequences that were
41 obtained (GenBank accession numbers: MH020803, isolate SyAl37-17 from Syria; MH020804,
42 isolate Tua16-17 from Tunisia; MH020805, isolate JoAl28-17 from Jordan and MH020806,
43 isolate Lal22-17 from Lebanon) ranged in size from 2726 nt to 2745 nt in length and shared
44 93.9-98.5%, 82.2-85.4% and 83.4-84.1% genome-wide pairwise identity with ALCV strain A
45 isolates from France, ALCV strain B isolates from France and ALCV isolate Manfredi from
46 Argentina, respectively (Bejerman et al. 2018; Bernardo et al. 2016). In addition, the four

47 isolates from Jordan, Lebanon, Syria and Tunisia shared 94-97.1% genome-wide pairwise
48 identity with each other. The genomes of these four isolates harbor a typical organization of
49 ALCV isolates, including seven open reading frames and the nonanucleotide stem-loop sequence
50 “TAATATTAC” in the intergenic region. These results indicate that ALCV is widely present in
51 alfalfa in Lebanon, Tunisia, Jordan and Syria. This is to our knowledge the first report of ALCV
52 in these four countries.

54 **References:**

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