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

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

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

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

References:

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