



First report of *Peanut stunt virus* on beans in Bulgaria

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Common bean (*Phaseolus vulgaris*) is a traditional vegetable in Bulgaria. The area grown in for 2013 was estimated to be 344 ha which increased gradually to reach 708 ha in 2016. During the summer of 2013, leaf samples (n=76) with virus-like symptoms were collected from bean plants in fields around the Maritsa Vegetable Crops Research Institute and in private gardens in regions near Plovdiv, Bulgaria. Disease incidence varied from 10 to 50%. Symptoms consisted of green or yellow mosaic, blisters, and leaf distortion (Fig. 1). In some locations the prevalent disease was associated with severe stunting.

To identify the casual agent(s) of these symptoms DAS/TAS-ELISA tests were performed using eleven antisera, specific to *Alfalfa mosaic virus*, *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus*, *Bean yellow mosaic virus* (BYMV), *Broad bean wilt virus 1*, *Broad bean wilt virus 2*, *Clover yellow vein virus* (CIYVV), *Cucumber mosaic virus* (CMV), *Tobacco necrosis virus*, *Watermelon mosaic virus* (Leibniz-Institute DSMZ, Germany) and *Peanut stunt virus* (PSV) (Agdia, USA). Results revealed the presence of common viruses, BCMV, CMV, BYMV and CIYVV, in the majority of the samples. However, three samples were positive for PSV. The PSV samples were traced to fields in which stunting symptoms were prevalent.

Sap inoculation of the PSV-infected samples to *Chenopodium amaranticolor* and *Nicotiana benthamiana* resulted in local chlorotic lesions and leaf distortion, respectively. A single lesion isolate of each virus was gradually propagated in tobacco plants (cv. White Burley) and used for further characterisation. Mechanical inoculation of these isolates caused systemic symptoms with large chlorotic lesions and rings in bean plants (cv. Pinto) (Fig. 2) and local chlorotic and necrotic lesions in *C. quinoa* and *C.*

murale, respectively, followed by systemic chlorotic lesions in the upper leaves in *C. quinoa* (Fig. 3). Electron microscopy of bean extracts revealed isometric particles approximately 28 nm in diameter. RT-PCR of bean extracts was performed using specific primers (PSV-CP-For, 5'-ATGGCATCTAGATCTGGTAA-3' and PSV-CP-Rev 5'-GACCGGAGCTTGGAAAGCGGAA-3'), that were designed using a reference sequence from GenBank (Accession No. U31366.1). Amplicons of the expected size (651 bp) were obtained from the coat protein region for all three PSV CP isolates. Sequencing of these amplicons confirmed the identification of PSV. These sequences were deposited in the NCBI database (MG256178 - MG256180). Phylogenetic analysis (Fig. 4) of all PSV isolates currently published in the NCBI database placed all three Bulgarian isolates in the Western subgroup (II), typified by the American PSV-W isolate (U31366).

PSV is known to cause severe infections on legumes worldwide. As a non-persistently transmitted aphid-borne cucumovirus, it might spread to other legumes including forage crops in Bulgaria. Monitoring infection of natural hosts would provide additional information about the epidemiology of the virus in Eastern Europe. To our knowledge this is the first report of PSV in the country and the second report of the occurrence of the Western strain of PSV in Europe (Diaz-Ruiz *et al.*, 1979).

References

Diaz-Ruiz JR, Kaper JM, Waterworth HE, Devergne JC, 1979. Isolation and characterization of peanut stunt virus from alfalfa in Spain. *Phytopathology* 69, 504-509. <http://dx.doi.org/10.1094/Phyto-69-504>



Figure 1



Figure 2



Figure 3

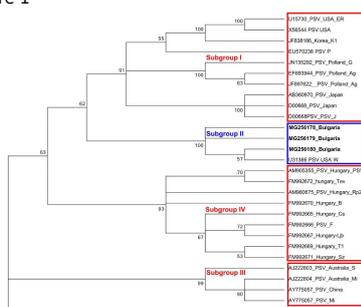


Figure 4

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