Disease Note

Diseases Caused by Bacteria and Phytoplasmas

First Report of *Xanthomonas euvesicatoria* Causing Bacterial Leaf Spot of Pepper (*Capsicum annuum*) in Montenegro

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Leaf spot of pepper was observed on different pepper cultivars in central Montenegro during summer and early autumn in three consecutive growing seasons (2017 to 2019). Necrotic spots were numerous, varying in size, irregular in shape, brown, and surrounded by a weak halo. The most intensive symptoms were observed on lower leaves. In conditions conducive for the infection, the lesions merged, resulting in leaf drop. Symptoms were not observed on pepper stems and fruits. A total of 17 bacterial strains were isolated from infected pepper leaves collected in seven different localities from 2017 to 2019. They formed yellow, convex, and mucoid colonies on yeast extract–dextrose–$CaCO_3$ (YDC) medium and induced hypersensitive reaction in tobacco leaves. They were gram-negative, strictly aerobic, oxidase-negative, catalase-positive, hydrolyzed gelatin and esculin, did not reduce nitrate, and did not grow on 0.1% triphenyl tetrazolium chloride and at 37°C. Out of the 17 tested strains, eight hydrolyzed starch and three showed pectolytic activity, thus differing in these biochemical traits from *Xanthomonas euvesicatoria* (Xe) reference strain KFB 1 (KFB, Collection of Phytopathogenic Bacteria, University of Belgrade, Faculty of Agriculture, Serbia; Obradović et al. 2004) used in all tests as a positive control. PCR analysis, with primer pair XeF/XeR, produced a single characteristic band of 173 bp in all 17 strains (Koenraadt et al. 2009). Additionally, the BOX-PCR profile of all the strains produced with the BOX A1R primer (Schaad et al. 2001) showed 100% homology with KFB 1. Based on the locality and year of isolation, nine strains were selected for amplification and partial sequencing of the *gyrB* gene using sets of primers described by Parkinson et al. (2007). Obtained partial DNA sequences showed that all nine strains (GenBank nos. MZ569011, MZ574079, MZ574080, MZ574081, MZ574082, MZ574083, MZ574084, MZ574085, and MZ574086) share 99.86 to 100% identity of the *gyrB* sequence with Xe type strain ICPM:109 and 98.71 to 100% *gyrB* sequence identity with Xe strain LMG930 isolated from pepper in the United States. Pathogenicity of all strains was confirmed by spraying young pepper plants (cv. 'Slonovo uvo') using a handheld sprayer with the bacterial suspension (10^8 CFU/ml of sterile tap water), in three replicates. Sterile distilled water (SDW) and KFB 1 were used as negative and positive controls, respectively. The inoculated plants were incubated under plastic bags in the greenhouse providing high humidity conditions for 48 h. Symptoms were monitored for 2 weeks after inoculation. Lesions surrounded by a halo appeared on leaves of all inoculated plants within 10 to 15 days after inoculation, while plants inoculated with SDW remained symptomless. Koch's postulates were confirmed by reisolation of the pathogen from necrotic tissue and identity check by PCR using the primer set of Koenraadt et al. (2009). The pathogen race was determined according to the reaction of cultivar 'Early Calwonder' (ECW) and its isogenic lines (ECW-10R, ECW-20R, and ECW-30R) (Stall et al. 2009). Results indicated that all tested strains and KFB 1 belong to the pepper race P8. Based on pathogenic, biochemical, and molecular characteristics, the strains isolated from pepper leaves in Montenegro were identified as *X. euvesicatoria.* Pepper production is particularly significant for small farmers in Montenegro. Favorable climate, use of noncertified seed, and lack of crop rotation contributes to the disease occurrence and severity. The disease has probably been around for years, but the etiology was not previously confirmed. This is the first report of *X. euvesicatoria* affecting pepper in this country.

References:

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