Bacterial Blight of Lentil (*Lens culinaris*) Caused by *Pseudomonas syringae* pv. *syringae*

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Accepted for publication 30 January 2018.

In June 2017, lentil (*Lens culinaris* Medikus) plants were observed in a commercial field in Buffalo County, South Dakota (44°03'39"N; 99°25'45"W) with brown, circular lesions on the foliage leaves that had dried, torn centers resembling “shot holes.” Disease incidence was observed to be 5%, and the affected plants were found in 0.01 ha of the total field (1 ha) surveyed.

Identification of Causal Bacterium

Symptomatic leaves were rinsed in autoclaved distilled water for 10 min, air-dried at 25°C, macerated into ~2 mm sections, and streaked onto nutrient agar plates. White to cream-colored colonies (2 mm diameter) formed on nutrient (NA) and nutrient broth yeast extract medium (NBY) agars after 72 h of incubation. Single colonies were picked and transferred onto King’s medium B plates, of which only one colony (LL1) produced green fluorescence after 48 h of incubation. In LOPAT tests (levan production, oxidase production, pectinolytic activity, arginine dihydrolase production, and tobacco hypersensitivity) (Lelliott et al. 1966), LL1 was positive for levan and gelatin liquefaction but oxidase negative and pectolytic activity negative on potato tuber slices. Based on these LOPAT results, LL1 was classified in the Ia group, which includes pathovars of *Pseudomonas syringae*.

Molecular identification of LL1 was performed by amplifying and sequencing the 16S rRNA (Weisburg et al. 1991) and *gyrB* genes (Ferrante and Scortichini 2010). A BLASTn analysis (MG388253 and MG425968) showed the best match was *P. syringae* pv. *syringae* HS191, complete genome (CP006256) for 16S rRNA (99% similarity) and *P. syringae* pv. *syringae* strain ITACyL P106 (HE604388) for *gyrB* genes (100% similarity).

Pathogenicity Experiment

Pathogenicity tests were performed on 3-week-old lentil (cultivar ‘Crimson’) and pea (*Pisum sativum* L. ‘Admiral’) plants in the greenhouse (23 ± 3°C and ~80% relative humidity). Six expanded leaves of 10 lentil and pea plants were abraded with 3M Sandblaster sandpaper (220 grit). A suspension of LL1 (10⁶ CFU/ml; OD₆₀₀ of 0.002) was prepared from a 72-h-old NA culture by diluting in autoclaved distilled water (1:100), which was spread on the abraded leaves with a toothpick. Ten control plants of lentil and pea were inoculated similarly with

autoclaved distilled water. After inoculation, all plants were misted for 10 s every 1 h for 4 days and 10 s every 3 h for the next 3 days. At 7 days after inoculation, brown lesions were observed on the inoculated lentil (Fig. 1) and pea leaves. The host response to the bacteria was scored using a 0 to 4 rating scale (Martín-Sanz et al. 2013). The experiment was performed twice and combined after verifying homogeneity of variance ($P > 0.05$). Across 10 inoculated plants, the median disease scores on lentil and pea were 2 (= necrotic lesions of 0.5 to 1.5 cm around the inoculation point) and 3 (= necrotic lesions longer than 1.5 cm), respectively. On control plants, no lesions developed on the leaves and the bacterium was not isolated. *P. syringae* pv. *syringae* was reisolated from the leaves of the inoculated lentil plants and confirmed by LOPAT tests and *gyrB* gene sequencing, thus fulfilling Koch’s postulates.

Significance

Lentil is planted in the cooler regions of the Great Plains (Nebraska, North Dakota, and South Dakota) and the Palouse (Idaho, Oregon, and Washington) in the United States. In 2016, the lentil production in the United States totaled to about 0.34 million metric tons (USDA National Agricultural Statistics Service 2017). Although it is not certain that *P. syringae* pv. *syringae* caused yield loss in this field, the bacterium is known to reduce yield in other leguminous crops. For example, yield loss of 94% owing to *P. syringae* pv. *syringae* was reported in a susceptible pea cultivar by Richardson and Hollaway (2011), and it is an endemic problem in dry edible beans (*Phaseolus*

FIGURE 1

Brown lesions caused by *Pseudomonas syringae* pv. *syringae* isolate LL1 on the leaves of lentil plant cultivar Crimson upon inoculation in the greenhouse.

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vulgaris L.) causing bacterial brown spot in the central High Plains (western Nebraska and northeast Colorado). Because the bacterium is generally known to survive on seed and/or crop debris (Richardson and Hollaway 2011), we suspect that in lentil production regions where frost days are above average during planting, *P. syringae* pv. *syringae* may become a newly emerging pathogen. This bacterium is reported to cause disease in more than 200 plant species (Bradbury 1986); however, to the best of our knowledge, this is the first report of *L. culinaris* (lentil) as a host of *P. syringae* pv. *syringae*.

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