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DISEASE NOTES

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First Report of Bacterial Leaf Blight of Rice Caused by *Xanthomonas oryzae* pv. *oryzae* in Benin

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In May 2013, bacterial leaf blight (BLB)-like symptoms were observed on wild rice *Oryza longistaminata* plants near Tanguiéta town (10°38.721′ N; 1°17.323′ E), near Pendjari National Park, northwest Benin. Symptoms included water-soaked stripes below the leaf tip and on leaf margins. The stripes occasionally enlarged and turned yellow and the most severely affected leaves became greyish-brown with yellow margins on one or both sides of leaves. For diagnosis, symptomatic 1- to 2-cm^2 leaf pieces were surface-sterilized and macerated in sterile water. The resulting sap was subjected to a multiplex PCR assay that is diagnostic for *Xanthomonas oryzae* pathovars (Lang et al. 2010). A sample yielding the two *X. oryzae* pv. *oryzae*-specific DNA fragments (162 and 331 bp) was used to isolate single colonies on semiselective peptone-sucrose-agar (PSA) medium (peptone 10 g/liter, sucrose 10 g/liter, glutamic acid 1 g/liter, bacto agar 16 g/liter, actidione 50 mg/liter,

cephalexin, 40 mg/liter, and kasugamycin 20 mg/liter) (Poulin et al. 2014). Incubation at 28°C for 3 to 4 days yielded yellow-pigmented *Xanthomonas*-like colonies that were mucoid, convex and straw-colored to yellow, like those of the reference strain BAI3 (Gonzalez et al. 2007). One isolate named ABB1 was obtained. Partial DNA sequence analysis of the *gyrB* gene was conducted upon amplification using the primers XgyrB1F and XgyrB1R (Young et al. 2008). The obtained 645-bp sequence was identical to that of 12 other African *X. oryzae* pv. *oryzae* strains (Hajri et al. 2012). The partial nucleotide sequence of the *gyrB* gene of ABB1 was submitted to GenBank (Accession No. KT325857) and ABB1 was deposited in the Collection Française de Bactéries Phytopathogènes as strain CFBP8172 (http://www6.inra.fr/cirm/CFBP-Bacteries-associees-aux-Plantes). Pathogenicity assays were conducted on 10 *O. sativa* rice cultivars as follows: ABB1 was grown overnight in PSA broth and adjusted to 1×10^8 CFU/ml in sterile distilled water. Leaves of 35-day-old rice plants were clipped 2 to 3 cm below the leaf tip with sterile scissors dipped in the bacterial suspension (Gonzalez et al. 2007). Twelve leaves per seedling and 3 to 4 seedlings per accession were inoculated. Control plants were inoculated with sterile distilled water. Fifteen days after incubation in the greenhouse (27 ± 5°C), inoculated leaves exhibited typical bacterial leaf blight lesions, whereas control plants remained symptomless. The reisolated bacteria from diseased leaves yielded colonies that had the same morphology as ABB1, and were confirmed to be *X. oryzae* pv. *oryzae* via multiplex PCR and sequence analysis of the *gyrB* gene portion, fulfilling Koch's postulates. In 2013, new samples were collected during the rainy (July) and dry (November) seasons from different host plants including rice (*O. sativa* and *O. glaberrima*) and wild rice (*O. longistaminata*) around the site where ABB1 originated. While all samples collected in July were negative in multiplex PCR assays, those collected in November from wild rice (*O. longistaminata*) led to the isolation of ABB40 and ABB41 that were confirmed as *X. oryzae* pv. *oryzae* by multiplex PCR assay and *gyrB* sequencing. Further studies are needed to clarify the importance of the disease in Benin. If spread from wild to cultivated rice, serious damage can be caused as several farmer-adopted cultivars were found to be highly susceptible to these 3 isolates.