Disease Note

Diseases Caused by Bacteria and Phytoplasmas

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

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In Madagascar, rice is the staple food, and rice production represents a major challenge for food security. Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a devastating disease of rice crops in many countries. BLB was first reported in Japan in 1884 and is now prevalent in most of the rice-producing countries of Asia. In Africa, *Xoo* was reported for the first time in Mali and Cameroon (Buddenhagen et al. 1979) and later in many West African countries. In contrast, in East Africa, BLB seems less prominent and has only been reported in Tanzania and Uganda (Oliva et al. 2019). To our knowledge, in Madagascar, despite continuous monitoring for rice diseases since the 1980s, BLB had never been reported until this study. In December 2019, BLB-like symptoms were observed in two fields in Vakinankaratra, the Central Highlands of Madagascar. In December, most rice fields in Vakinankaratra were at the seedling stage where typical symptoms are often not visible, making it impossible to assess disease severity and incidence. Typical symptoms were characterized by yellow to grayish, water-soaked lesions that start from the leaf tip and progress along the central vein or leaf margin. At an advanced stage of the disease, the leaves were completely desiccated sometimes with droplets of yellow exudate at the leaf margin. Four symptomatic leaf fragments from two fields were successively disinfected, rinsed in sterile water, and ground. Leaf powder was resuspended in 1.5 ml of sterile water, of which 10 µl was plated on semiselective PSA medium and incubated at 28°C for 3 to 7 days. Typical *Xoo*-like single colonies were purified from four individual leaf samples from two distinct sites. The multiplex PCR developed for the identification of *X. oryzae* pathovars (Lang et al. 2010) produced, for eight isolates (two per leaf), the diagnostic two-band pattern characteristic of *Xoo*. Five-week-old plants of *Oryza sativa* cultivar IR24 were subjected to a pathogenicity test performed with four strains. Leaves were clipped with scissors and dipped in a bacterial suspension at an optical density of 0.2 as previously described (Kauffman et al. 1973). Fourteen days after incubation of rice plants in a greenhouse (27 ± 1°C with a 12-h photoperiod), the inoculated leaves (n = 8) developed typical BLB lesions identical to those challenged with the *Xoo* reference strain PXO99^{A}. In contrast, water-inoculated leaves remained asymptomatic. The lesions were subjected to strain isolation, and purified strains were validated as *Xoo* by multiplex PCR as described above, thus fulfilling Koch's postulates. Finally, we amplified by PCR and sequenced the housekeeping gene *gyrB* for two of the isolates using the primers XgyrB1F and XgyrB1R (Young et al. 2008). Analysis of 825 bp of the *gyrB* sequence of the strains Md5 and Md11 revealed 100% identity with that of the Indian *Xoo* strain BXO1 (accession no. CP033201) and highlighted 10 and 12 polymorphic nucleotides compared with the *gyrB* sequence of the *Xoo* reference strains PXO99^{A} from the Philippines (accession no. CP000967) and BAI3 from West Africa (accession no. CP025610). To our knowledge, this is the first report of BLB in Madagascar. Surveys conducted in the Vakinankaratra and Menabe regions between 2020 and 2022 confirmed the presence of BLB in Madagascar and a sharp increase in disease incidence. Further surveys will be necessary to evaluate if the disease has already spread over the island, and further analysis will be needed to understand the origin of the outbreak and identify strategies to control BLB in Madagascar.

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