Detection of Pantoea agglomerans in Germplasm Rice Accessions (Oryza sativa) in Brazil

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DISEASE NOTES

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The cultivated area devoted to rice crops (*Oryza sativa*) in Brazil has expanded in recent years, along with improvements in yield. This has prompted the continuous exchange of material from active germplasm banks between government breeding programs. When placed in a greenhouse for multiplication purposes, rice accessions resulting from exchanges between USDA-ARS (United States Department of Agriculture – Agricultural Research Service) showed little or no germination. The accessions were sent to the tissue culture laboratory of BAG-CNPAF (Embrapa National Rice and Bean Research Center) to recover embryos by aseptically removing the seed coat of 10 seeds from each accession. Bacterial colony growth concomitant to germination inhibition was observed in some accessions, from which four bacterial isolates were obtained. The isolates and their respective associated rice accessions are: Bac 1887 from accession Chikanari 2 (CIor 12454), Bac 2821 from Fujisaka 2 (PI 184496), Bac 2926 from Ehime Suito (PI 202954), and Bac 2935 from Kinai No. 37 (PI 202968). Hypersensitive response (HR) in tobacco plants and pathogenicity by inoculation in 'Caloro' rice plants were positive for two bacterial isolates (Bac 2821 and Bac 2926). Both isolates produced symptoms of light brown leaf spots, progressing to leaf browning and drying. The pathogens were reisolated from the symptomatic tissues and the process was repeated, fulfilling Koch's postulates. The genus and species were identified according to Schaad et al. (2001). The gram-negative bacterial isolates exhibit yellow nonmucoid colonies in yeast extract-dextrose-calcium carbonate agar medium (YDC), nonsporogenic, nonfluorescent in King's B medium, with growth at 37°C, facultative anaerobes, and negative for pectolytic activity on potato tuber slices. The biochemical, physiological, and staining tests indicated that the isolates belong to the genus *Pantoea*. Tests suggested the isolates were more closely related to *P. agglomerans*. Additionally, the cultures were submitted to analysis of fatty acid methyl esters by gas chromatography (GC-FAME), using a Sherlock microbial identification system (TSBA40 library; MIDI Inc., Newark, DE), which classified the isolates as *P. agglomerans*. Since the isolates displayed the same characteristics, only one (Bac 2926) was used in the following phases. Two complementary tests were conducted to confirm the identity of the bacterial isolate, namely amplification of molecular markers linked to specific genes for the detection of *Pantoea* sp. and sequencing of the 16S rDNA region. The PCR reactions employed primers to amplify the *P. agglomerans*-specific cytokinin biosynthesis gene (gene *etz*) (Manulis et al. 1998), the *P. ananatis*-specific ice nucleation active (*ina*) gene (Kido et al. 2008), and the *P. stewartii*-specific *pstS-glmS* region (Wensing et al. 2010). The resulting profiles produced only 600 pb amplicons, obtained by using etz primers that amplified the *etz* gene, specific to the identification of *P. agglomerans*. The remaining reactions, which amplified the *ina* and *pstS-glmS* genes, produced no amplicons. At the same time, the isolate was submitted to partial sequencing of the 16S rDNA gene, amplified by PCR using the primers fD1/rD1 (Zahid et al. 2015). The sequence was analyzed using BLASTn (GenBank MF040189) and showed 97.0% identity with *P. agglomerans* (HM854285.1) (Lee et al. 2010). This is the first report of *P. agglomerans* in germplasm of rice seeds in Brazil. The importance of this pathogen in rice production in Brazil is unknown.

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