

Development of a Pcr-Based diagnostic assay for detecting *Bipolaris oryzae*, the brown spot fungus in rice

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Bipolaris oryzae is the common causal agent of brown spot in rice (*Oryza sativa* L.), and infection by this fungal pathogen results in severe yield losses. A polymerase chain reaction (PCR) based diagnostic assay was developed to detect *Bipolaris oryzae*, the brown spot fungus in rice. Universal primers ITS1 and ITS4 were used to obtain the rDNA sequence of the available *B. oryzae* isolates of diverse geographic origins of India. Species-specific primers were designed based on *B. oryzae* ITS sequence data obtained in the present study and in comparison with other *Bipolaris* spp., *Curvularia* spp., *Drechslera* spp. and *Exserohilum* spp. in NCBI database. The specific primers designed

were employed to amplify DNA in PCR from a total of 171 isolates of *Alternaria* spp., *Bipolaris* spp., *Curvularia* spp., *Drechslera* spp., *Exserohilum* spp. and *Magnaporthe oryzae*. The species-specific primers BoVf and BoVr were used in the detection of *B. oryzae* that amplified a 275 bp fragment from the DNA of all *B. oryzae* isolates but not the DNA from other species. The primer was also used to detect *B. oryzae* in rice seed and successfully amplified the predicted size of the DNA fragment in the infected material. PCR detection of as little as 100 ng μl^{-1} to 1 fg μl^{-1} of *B. oryzae* DNA was possible. The method described here requires one day for completion, compared to 10 days required for the cultural method.