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OPTIMIZATION OF A qPCR PROTOCOL FOR DETECTION OF *Stenocarpella macrospora* AND *S. maydis* IN CORN SEEDS / Otimização do protocolo de qPCR para detecção de *Stenocarpella macrospora* e *S. maydis* em sementes de milho. C.C.L. ANDRADE[1]; M. DALBOSCO[1]; J.G. ZANIN[1]; M.P. ROMERO[2]; J. FERNANDES[1]; B.R. FALKENBACH[1]; E. BERTOLINI[3]; P.S. TELÓ[1]; V. DUARTE[1]. [1]Agronômica - Laboratório de Diagnóstico Fitossanitário e Consultoria, Porto Alegre, RS, 91530-000, Brazil / [2]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907. / [3]Federal University of Rio Grande do Sul, Faculdade de Agronomia. Avenida Bento Gonçalves, Porto Alegre, RS, 91540000, Brazil. E-mail: camila.andrade@agronomicabr.com.br.

Corn seeds infected with *Stenocarpella macrospora* and *S. maydis* are the primary source of inoculum for stalk rot, ear rot, and Diplodia leaf streak. These diseases cause significant yield losses in corn production in Brazil. One of the challenges of identifying these two species is relying only on the visually identification of their symptoms and signs. Previous studies showed that the use of real-time quantitative PCR (qPCR) technique using SYBR Green is specific and sensitive to detect *Stenocarpella* spp. The aim of this study was to optimize a qPCR protocol for detection of *S. macrospora* and *S. maydis* in corn seeds. Individual isolates of each specie were used to inoculate corn seeds. In order to verify the reliability of this protocol, corn pathogens *Fusarium verticillioides* and *Aspergillus flavus* were included as non-target species. DNA concentrations were adjusted to 100-200 ng/µL. The annealing temperature for qPCR was optimized at 57ºC for both species. The detection limit of *S. macrospora* and *S. maydis* was 1 ng/µL for inoculated corn seeds. Positives *S. macrospora* and *S. maydis* strains from inoculated corn seeds had the range melting peaks of 90.4 to 91.6 ºC; 90.0 to 91.1 ºC and cycle threshold (C_t) values of 15.0 to 30.0, and 12.0 and 26.0, respectively. No cross-amplifications were obtained when DNA from *F. verticillioides*, *A. flavus* and non-inoculated corn seeds were tested. This optimized qPCR protocol was rapid, accurate, and specific for the detection of *S. macrospora* and *S. maydis* in corn seeds.

Keywords: Diplodia leaf streak; Fungal infection; Molecular detection; Stalk and ear rot.