

Rachdawong, Sansanalak. 1999. PCR-based test for differentiating varieties of *Gaeumannomyces graminis*, the take-all pathogens. Dissertation presented in partial fulfillment of the requirements for a Ph.D. in Plant Pathology. Virginia Polytechnic Institute and State University, Blacksburg, VA 24060, 184 pp.

Portions of dissertation noting results with *Ggg* isolates not isolated from wheat:

**CHAPTER 3: A PCR-BASED TEST FOR DIFFERENTIATING VARIETIES OF
Gaeumannomyces graminis, THE TAKE-ALL PATHOGENS**

3.3 MATERIALS AND METHODS

Table 3.1. Fungal isolates used in this study.

Isolate	Host	Source Location	Source/reference
<i>Gaeumannomyces</i>			
<i>graminis</i> var. <i>graminis</i>			
ATCC 12761	<i>T. aestivum</i>	United Kingdom	³ ATCC
FL-19	<i>Cyanodon dactylon</i> (L.) Pers.	Florida, USA	⁴ M. Elliott
FL-39	<i>Stenotaphrum secundatum</i> (Walt.) Kuntze	Florida, USA	M. Elliott
FL-175	<i>Oryza sativa</i> L.	Florida, USA	M. Elliott
2033	<i>Glycine max</i> (L.) Merr.	Florida, USA	M. Elliott

³ American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209

⁴ Dr. Monica L. Elliott, Fort Lauderdale Research and Education Center, University of Florida, Ft. Lauderdale, FL 33314-7799.

3.3.3 PCR and PCR conditions.

See file for “PCR *Ggt* Protocol”

3.4 RESULTS

Ggg isolates other than those isolated from wheat did not amplify efficiently with Ggg-specific primers (**Fig. 3.12, panel a**). Ggg isolates FL-39, FL-175 and 2033 produced little Ggg-specific product when amplified with both single Ggg-specific primer and all of the three variety-specific primers (**Fig. 3.12, panel b**). Other non-specific products were visualized from all of them. Ggg isolate FL-19, which was isolated from bermudagrass generated a PCR product of 300 bp that was not related to any of variety-specific fragments. In this case, more Ggg isolates from wheat and other host plants are needed before conclusions can be made.

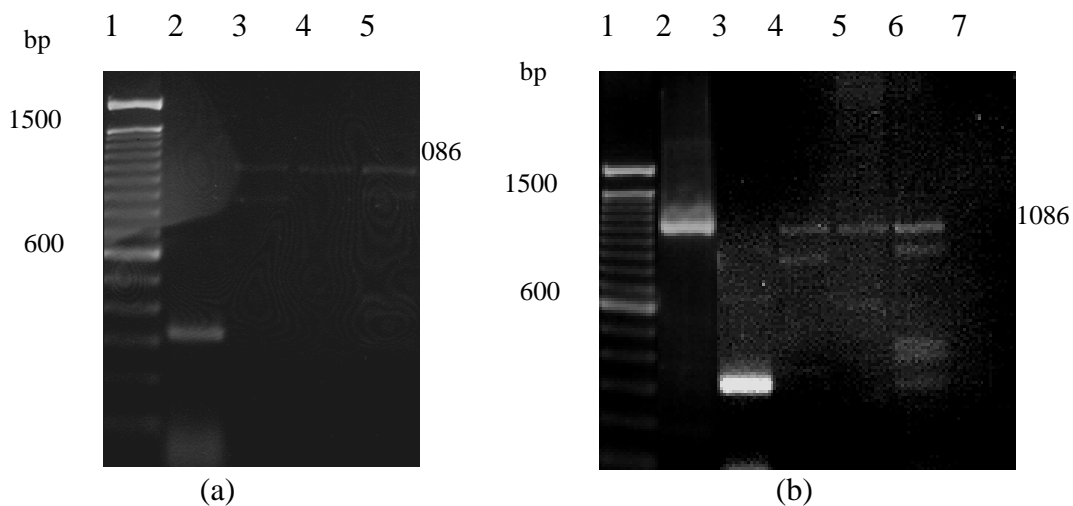


Fig. 3.12. PCR-based differentiation test for *Gaeumannomyces graminis* var. *graminis* (Ggg) isolates with Ggg-specific primer (panel a) and all three varieties-specific primers (panel b). PCR products were electrophoresed on 1.8% agarose gel, stained with ethidium bromide, visualized and photographed with 302nm UV light using a Wratten 22A filter. In both panels, lane 1 contains 100 bp DNA ladder as molecular weight standards. In panel (a), lanes 2-5 contain Ggg isolates FL-19, FL-39, FL-175, and 2033, respectively. In panel (b), lanes 2-7 contain Ggg isolates ATCC 12761, FL-19, FL-39, FL-175, 2033 and no DNA, respectively.

3.5 DISCUSSION

Results with Ggg-specific primers indicated a significant amount of variation among the PCR products obtained from diverse Ggg isolates. Three possibilities exist for this result. First, the variation may mirror genetic variation in the avenacinase-like genes of Ggg. Perhaps, this gene is either inactive or not conserved by functions in host pathogenicity in Ggg. Second, because Ggg may cause pathogenesis on several grassy weeds, the variation may reflect co-evolution with different compatible hosts. Third, the variation may indicate sub-optimal priming from other parts of the genome. DNA sequencing of the atypical PCR products will confirm if they have similarity to avenacinase-like genes.