**PROCEDURE:**

I. DNA Extraction

Extract total plant DNA from infected seeds using DNeasy plant kit (Qiagen, USA).

II. Real Time PCR

The authors used a SmartCycler II (Cepheid, USA) with a commercial PCR master-mix (iQ Supermix; Bio-Rad, USA) using primer and probe concentrations determined previously (Ha et al., 2009).

To determine the standard curve

1. Adjust a sample of fungal genomic DNA to 500 pg/μl
2. Dilute at 10-fold intervals to 10⁻⁶ (50 pg/μl – 0.5 fg/μl)
3. Use the mean Ct values to generate a standard curve

PCR conditions: initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 95°C for 15 s and a combined step for annealing and elongation at 60°C for 40 s.

Forward primer: GTCCAGAGA TGAGGA TGGAGT
Reverse primer: GCTTGTAGGCGAATAATGAGCC

Probe: Texas Red-CGAAGGATATTGATCAGACCGCACTTTC-BHQ2

REFERENCES:
