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A diagnostic assay to detect the Phomopsis stem canker pathogens

Introduction:

Phomopsis stem canker is a disease that has been affecting sunflower production in the United States since it was first identified in Ohio in 1983 (Herr et al. 1983) and a year later in Minnesota and North Dakota (Hadju et al. 1984). However, the disease didn't gain much attention until the 2010 epidemic that occurred in Minnesota, North Dakota and South Dakota when disease incidence of more than 50% and yield losses of 40% were recorded (Mathew et al. 2010). The 2010 epidemic fueled research efforts and led to the identification of two causal agents of the disease, *Diaporthe helianthi* and *Diaporthe gulyae* (Mathew et al. 2015). Phomopsis stem canker is still a problem for the sunflower farmers, and was among the top three diseases in 2015 (Kandel and Gulya 2016) The disease was found in 61% of surveyed fields and disease incidence ranged from 40% in Texas up to 100% in Manitoba, Canada (Kandel and Gulya 2016). Stem symptoms of Phomopsis stem canker are often not seen until flowering. Characteristic symptoms include a light brown to tan lesion on the stem, pith degradation and lodging (Masirevic and Gulya 1992). Management practices for Phomopsis stem canker are limited. Currently, no commercial sunflower varieties offer complete resistance to the disease (Talukder et al. 2014). The primary objectives of this study was to (1) develop and validate a diagnostic assay to detect the Phomopsis stem canker pathogens *Diaporthe helianthi* and *Diaporthe gulyae* in sunflower plants, and (2) to screen sunflower germplasm for resistance to *D. helianthi* and *D. gulyae*.

Materials and Methods:

In order to develop the diagnostic assay (quantitative polymerase chain reaction (qPCR) assays), eight primer probe combinations specific to *Diaporthe helianthi*, and five primer probe combinations specific to *Diaporthe gulyae* were designed based on the translation elongation factor (EF1-α) region of *Diaporthe helianthi* and *Diaporthe gulyae* DNA sequences. The best fit primer/probe set for each *Diaporthe* species was chosen based on the free energy (ΔG) values, which was used to evaluate the stability of the amplicon secondary structure. The PCR product with a higher ΔG for the formation of Hairpins, Homo-dimers, and Hetero-dimers was selected as test candidate for further evaluation.

To validate the specificity of the *D. helianthi* and *D. gulyae* qPCR assays, genomic DNA template of target species has been tested against the two assays. DNA was extracted from pure fungal cultures of the two pathogens using the Wizard® Genomic DNA Purification Kit (Promega

Corporation, Madison WI). The qPCR was performed in a reaction volume of 20 µL. Each qPCR reaction consisted of 1.0 µL of template DNA, 0.4 µL of 10 µM forward primers, 0.4 µL of 10 µM reverse primers, 0.2 µL of 100 nM of the respective probe, 8.0 µL of sterile distilled water, and 10 µL of Taqman Environmental Master Mix (Applied Biosystems) in 20 µL of total volume. The C_t values were determined at a threshold of 0.05.

To determine the applicability of the qPCR assay to identify genetic resistance to *D. helianthi* and *D. gulyae* in the sunflower germplasm, a trial containing 288 sunflower Plant Introduction lines (representing 90% of commercial germplasm used today) were screened for resistance to Phomopsis stem canker in a field plot in Brookings, South Dakota. From the initial field screening, 54 out of 288 lines appeared to have resistance to the disease. These 54 lines are composed of both oilseed and non-oilseed sunflower types, and contain germplasm from nine different countries. Secondary screening for stem resistance to the Phomopsis stem canker pathogens was done in the greenhouse on the 54 lines. Resistance screening to both pathogens was conducted separately. The stem wound method developed by Mathew et al. (2015) was adopted for this study. Sunflower cv. 'HA 288' as used as the susceptible check. Six seeds were planted in moist potting mix (ProMix® HP Mycorrhizae, Premiere Horticulture Inc, Quakertown, PA) in 7.5 liter circular plastic pots. The pots were placed in a greenhouse kept between 22 and 25^{o}C with a 16 hour light and dark cycle. Plants were watered on alternate days. When the sunflower plants reached the V-4 to V-6 development stage (4 to 6 true leaves developed, Berglund 2007), the stems were wounded on the second internode with a micropipette tip (1000 µl) and a mycelial plug of a SD isolate of *Diaporthe* spp. was placed inside the puncture. Plants were kept in the same greenhouse conditions during, and after inoculation. Disease severity was evaluated 14 days after inoculation using a modified scale from Thompson et al. (2011) where 0= no discoloration; 1= low level discoloration at the site of inoculation; 2= slight discoloration or lesion 1 to 2 mm in length; 3= necrotic lesions 2 to 5 mm in length, some colored stem streaking leaf wilting, and twisting; 4= lesions 5 to 10 mm in length, significant necrosis and dark-colored stem streaking, leaf and plant wilting, twisting, stunting, and some lodging; and 5= lesions exceeding 10mm in length, severe leaf necrosis, lodging, or plant death. The experiment was set up in a completely randomized design with six plants per PI line (per experiment) and each plant was regarded as a replication for each of the PI lines. This experiment was repeated for a total of three times.

Results/Summary:

- Two diagnostic qPCR assays have been developed and validated for quantification and detection of the two causal agents of Phomopsis stem canker, *D. helianthi* and *D. gulyae*.

- Preliminary results suggest significant differences between sunflower germplasm in their resistance to *D. helianthi* and *D. gulyae.* Sunflower cv. HA 378 (PI 561918) showed resistance to *D. gulyae* and *D. helianthi* when compared to susceptible check, 'HA 288.'

(Figure 1). Differentiation of reactions to *Diaporthe* spp. among the remaining PI lines appears to be minimal.

Future Work:

- The diagnostic qPCR will be used to identify and quantify the causal agent in sunflower field samples infected with Phomopsis stem canker from Minnesota, North Dakota and South Dakota.

- The diagnostic qPCR assay will be used to confirm resistance to *D. helianthi* and *D. gulyae* in the sunflower germplasm by quantifying the amount of DNA present in the tissue 14 days after inoculation.

- The diagnostic qPCR assay will be used in the evaluation of fungicide efficacy for the management of Phomopsis stem canker.

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References:

Hajdu, F., Baumer, J. S., and Gulya, T. 1984. Occurrence of Phomopsis stem canker in Minnesota and North Dakota. Proc. Sunflower Research Workshop, Bismarck, North Dakota, p. 15.

Herr, L. J., Lipps, P. E., and Watters, B. L. 1983. Diaporthe stem canker of sunflower. Plant Dis. 67: 911-913.

Kandel, H., and Gulya, T., 2016. 2015 National Sunflower Crop Survey. The Sunflower (January, 2016). National Sunflower Association, p. 14-17.

Mathew, F. M., Alananbeh, K. M., Jordahl, J. G., Meyer, S. M., Castlebury, L. A., Gulya, T. J., and Markell, S. G. 2015. Phomopsis stem canker: A reemerging threat to sunflower (*Helianthus annuus*) in the United States. Phytopathology 105:990-997.

Talukder, Z. I., Hulke, B. S., Marek, L. F., and Gulya, T. J. 2014. Sources of resistance to sunflower diseases in a global collection of domesticated USDA Plant Introductions. Crop Sci. 54:694-705.

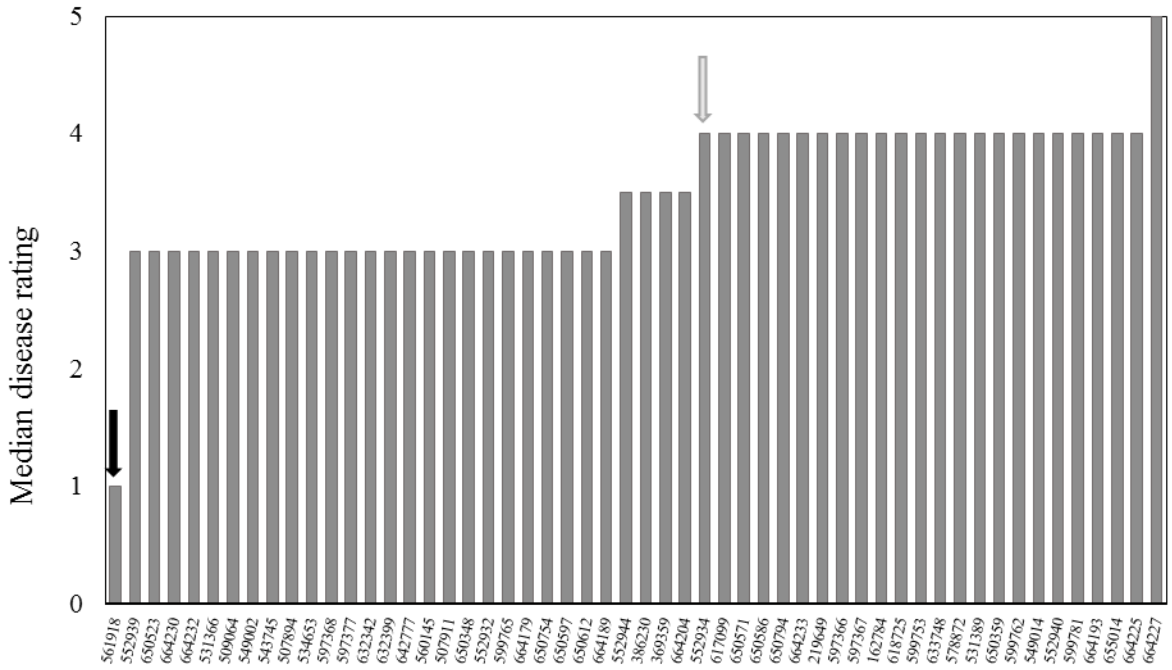

Figure 1. Median disease severity ratings of sunflower PI lines in their response to *Diaporthe* spp. The PI line that showed consistent resistance is indicated by black colored arrow (PI 561918, HA 378), with a median disease severity rating of 1; while the susceptible check is circled by grey colored arrow (PI 552934, HA 288), with a median disease severity rating of 4.