Research

Diaporthe gulyae: The New Pathogen on Common Buckwheat (*Fagopyrum esculentum*)

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Abstract

A fungus associated with stem disease on common buckwheat (*Fagopyrum esculentum* Moench) was shown to be pathogenic on this crop, with differences in reaction of buckwheat varieties to causal agent. Sequence analyses support identification of the pathogen as *Diaporthe gulyae*, which has also been shown to be a

pathogen on soybean and sunflower. As a result, the use of buckwheat in rotations with sunflower or soybean may need to be reconsidered.

Keywords: *Diaporthe*, *Phomopsis*, buckwheat, sunflower, soybean

Common buckwheat (*Fagopyrum esculentum* Moench) is a low-acreage specialty crop in North Dakota, with between 12,000 and 27,000 acres sown annually (United States Department of Agriculture Farm Service Agency 2013). In 2008, stem lesions, discolored pith, defoliation, and premature senescence were observed for the first time on plants in common buckwheat variety trials at the North Dakota State University North Central Research Extension Center in Minot, ND. Samples were sent to the NDSU Plant Diagnostic Lab for diagnosis, but symptoms observed in the field and laboratory were not consistent with any known disease that has been described on buckwheat previously (Berglund 2003). Symptoms included lesions on stems with underlying light brown to gray discolored pith or no pith. Symptomatic tissue was surface disinfested and plated on half-strength acidified potato dextrose agar (PDA), and a *Diaporthe*-like fungus was consistently isolated from symptomatic tissue. Hyphal tips from an isolate originating from the buckwheat cultivar Mancan were transferred to PDA to obtain a pure culture. Because little information on diseases of buckwheat is available, and species of *Diaporthe* have not been previously reported as a pathogen on common buckwheat, Koch's postulates were undertaken.

Identification of the Isolated Fungus

The isolated *Diaporthe*-like fungus, designated AR 4603, first appeared tan-colored when grown on PDA (Fig. 1). By

approximately 4 weeks of growth under ambient conditions and 12-h dark and light cycle, the colony had become darker brown and pycnidia with α -conidia had formed. Culture characteristics of the pathogen were similar to those of *Diaporthe* species (Mathew et al. 2015; Thompson et al. 2011). A portion of the internal transcribed spacer (ITS) gene region was sequenced (GenBank accession MK010815) using universal primers ITS5 and ITS4 under the standard amplification conditions (White et al. 1990). A portion of the elongation factor 1- α (EF1- α) gene was also sequenced (GenBank accession MK012555) using primers EF1-728F and EF1-986R (Carbone and Kohn 1999).

The BLAST (Altschul et al. 1990) search restricted to sequences from type material revealed a 100% match (516/516 identities) with *Diaporthe gulyae* BRIP 54025 (sequence ID NR_116151.1) based on the ITS5/ITS4 sequence. The sequence obtained from the EF1- α gene identified a 99% match (366/367 identities) with *D. gulyae* BRIP 54025 (JN645803.1). Between the ITS and EF1- α gene regions, EF1- α is a better phylogenetic marker for defining species of *Diaporthe* (Udayanga et al. 2014). Hence, the EF1- α sequences

FIGURE 1

Diaporthe gulyae first appears cream to tan on potato dextrose agar (left, after 2 weeks). Pycnidia form within 2 to 3 weeks, and the colony darkens in 4 weeks (right).

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were used for performing neighbor-joining analysis in Molecular Evolutionary Genetics Analysis (MEGA) software (version 7; Kumar et al. 2016). The phylogeny based on EF1- α grouped AR 4603 in the *D. gulyae* clade (Fig. 2; bootstrap value = 98%).

Pathogenicity of *D. gulyae* on Buckwheat

An experiment to evaluate pathogenicity of *D. gulyae* was conducted twice, in a randomized complete block design with four replications in a greenhouse. Five varieties of common buckwheat were used: Mancan, Manor, Kona, and two unnamed genotypes, 26 and 29. For each genotype and replication, three seeds were sown to a single Cone-tainer pot, and emerged seedlings were thinned to one plant per pot after emergence. Plants were maintained on greenhouse benches at ~25°C, with a 16-h photoperiod. Seventeen-day-old seedlings were clipped above the cotyledon leaves (12 plants in the first experiment, one plant in the second), above the first true node (12 plants in the first experiment, 16 in the second experiment), or above the second true node (no plants in the first experiment; seven plants in the second experiment), depending on plant growth. Clipped plants were inoculated with the isolate of *D. gulyae* by inverting 200- μ l pipette tips containing a plug of the fungus (grown on PDA) over the cut end of the stem (Fig. 3). A total of 20 plants were inoculated per experiment (one plant per genotype per replicate). In addition, the susceptible cultivar Mancan served as a noninoculated control by inverting a 200- μ l pipette tip that contained only a plug of sterile PDA over clipped plants (one plant per replicate).

Symptoms developed on 18 plants of 20 that were inoculated in the first experiment and on all 20 inoculated plants in the second experiment. No symptoms developed on the noninoculated control in either experiment. The pathogen was successfully reisolated from selected symptomatic plants in each experiment. Symptoms caused

by the pathogen included a tan lesion that extended below the point of inoculation. Darkly pigmented pycnidia of the pathogen formed within lesions after about 2 weeks (Fig. 4). Lesion length was measured 1 week after inoculation and compared among buckwheat genotypes using PROC GLM in SAS (Table 1). Despite the low numbers of plants tested, significantly shorter lesions developed on genotype 29, compared with Mancan and Manor, suggesting that a varietal response to infection exists (Table 1). However, more work in the area of screening buckwheat genotypes for possible resistance to infection by *D. gulyae* is needed.

Broader Impact and Conclusion

In 2009, disease was not observed in buckwheat variety trials or on volunteer buckwheat from the 2008 location in North Dakota. However, in 2010, a suspected isolate of *D. gulyae* (based on plant symptoms and morphology of the fungus) was recovered from a symptomatic common buckwheat sample from the same region. Interestingly, in 2010, *D. gulyae* was isolated from sunflower (*Helianthus annuus* L.) in the United States showing symptoms consistent with Phomopsis stem canker, and pathogenicity was demonstrated (Mathew et al. 2015). In 2012, soybean plants with reddish-brown stem cankers in North Dakota were shown to be infected by *D. gulyae* (Mathew et al. 2018). Although buckwheat is a relatively minor crop in the United States, with less than 73,000 ha harvested in 2017, it is globally important, with over 5.6 million hectares harvested (Food and Agriculture Organization of the United Nations 2019). China and Russia lead the world in buckwheat production: 1.7 million and 1.5 million hectares, respectively, were harvested in that year. In these same regions, soybean and sunflower are also prevalent. It is not known whether this disease is present in those regions. The ability to use buckwheat in soybean or sunflower rotations may be limited in fields where stem diseases caused by *D. gulyae* are problematic.

FIGURE 2

The evolutionary history was inferred using the neighbor-joining method in Molecular Evolutionary Genetics Analysis (MEGA) software (version 7; Kumar et al. 2016). The optimal tree with the sum of branch length = 1.64 is shown. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The evolutionary distances were computed using the Jukes–Cantor method (Jukes and Cantor 1969), and the analysis involved 10 nucleotide sequences. All positions containing gaps and missing data were eliminated.

FIGURE 3

For inoculations, a stem was snipped above the cotyledon or above the first or second true node. A pipette tip (200- μ l capacity) was then inverted over a colony of the pathogen growing on potato dextrose agar to collect a pathogen-and-agar plug, which remained in the wider portion of the pipette tip. The tip was then placed snugly over the cut end of the stem.

FIGURE 4

A diseased stem (left) shows a typical lesion with black pycnidia forming within (noninoculated control at right).

TABLE 1
Reaction of common buckwheat varieties to *Diaporthe gulyae*

Variety	Average lesion length (cm)
Mancan	4.06 a
Manor	4.44 a
Koma	3.33 ab
26	3.84 ab
29	2.89 b
Control	0.00 c
Least significant difference ($P = 0.05$)	1.17
Coefficient of variation	37.35

Further study of this pathogen may be warranted, particularly because it has been confirmed on at least two other crops (soybean and sunflower) in the United States. Furthermore, a varietal response in buckwheat exists, suggesting that breeding for resistance in buckwheat to this stem canker disease may be possible. This work describes the first time that *D. gulyae* has been reported as a pathogen on common buckwheat. Yield impact of *D. gulyae* on buckwheat is not known, and its host range has not been fully described. Such information may influence future management strategies if this disease becomes a recurring problem on common buckwheat, soybean, and sunflower.

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