A Rapid Method for Identification of *Aphanomyces euteiches* Race 2 Using DNA Markers

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Aphanomyces euteiches Drechs. is an Oomycete that causes seedling root rot and poor stand establishments in alfalfa and results in stand decline of established fields with a heavy soil structure. Hence, management strategies of *A. euteiches* include planting disease resistant cultivars and avoiding poorly drained soils (Grau 199). Within the alfalfa pathotype, there have been two races identified, namely race 1 (R1) and race 2 (R2), based on the disease response of standard resistant and susceptible check cultivars (Fitzpatrick et al. 1998). Abundant alfalfa cultivars are available with resistance to R1 but fewer are currently resistant to R2. At present, 57.7% of the available certified alfalfa cultivars are rated as resistant or highly resistant to R1 of *A. euteiches*, with only 7.5% of these varieties rated as resistant or highly resistant to R2 of *A. euteiches* (NAFA 2010). At present, the only method to identify the two races is through virulence testing which requires greenhouse evaluation over time after applying R1 and R2 inoculum to test plants (Fitzpatrick et al. 1998). As such, DNA markers that can rapidly and unambiguously distinguish the two races would be highly useful.

Nine isolates of R1 and five isolates of R2 were obtained from alfalfa fields in Illinois, Washington, and Wisconsin. Each isolate was grown from hyphal plugs at 22˚C for 7 to 14 days in a potato dextrose broth on a rotary shaker. DNA was then extracted from mycelia mats. The mats were washed in TES, lyophilized overnight, and the DNA extracted following the manufacturers instructions for the FastDNA® kit (Q-Biogene, Carlsbad, CA) as described by Larsen et al. (2007). DNA from R1 and R2 isolates were screened using 150 different sequence related amplified polymorphism (SRAP) primer combinations and 250 random amplified polymorphic DNA (RAPD) primers.

A single SRAP marker ca 190 bp in length was identified across all five R2 isolates that did not occur in DNAs of R1. No polymorphisms were detected in R1 or R2 using RAPD primers. The SRAP marker is being developed into a sequence-characterized amplified region (SCAR) that will be useful for rapid identification of race 2. Additional SRAP and RAPD markers are being screened to identify markers specific to R1 and to obtain additional markers specific to R2.

Race 2 of *A. euteiches* represents a widespread risk to alfalfa cultivars having resistance only to R1 in fields with varied cropping histories (Malvick and Grau, 2001). It is anticipated that development of robust DNA markers specific to R1 and R2 will also be useful in identifying the two races in soil samples, thus avoiding the time-consuming practice of pathogen isolation or identification using differential cultivars. It will be possible to use the markers that were specific to one race, but not present in all isolates, for a multiplex polymerase chain reaction (PCR) to identify R1 and R2 In addition, quantifying pathogen DNA using real-time PCR in alfalfa breeding programs can assist in the identification of resistant lines through marker-assisted selection.

References

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