REAL-TIME PCR-BASED DETECTION OF *RHIZOCTONIA* LEVELS IN SOIL

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Rhizoctonia damping-off and crown and root rot (RCRR) caused by *Rhizoctonia solani* AG 2-2 have been the most common root diseases on sugarbeet in Minnesota and North Dakota for several years (1-3,6). Disease can occur throughout the growing season and reduces plant stand, root yield, and quality. Warm and wet soil conditions favor infection. Control options include rotating with non-host crops (cereals), planting partially resistant varieties, planting early when soil temperatures are cool, improving soil drainage, and applying fungicides as seed treatments, in-furrow (IF), or postemergence. An integrated management strategy should take advantage of multiple control options to reduce Rhizoctonia crown and root rot (5).

OBJECTIVE

To develop a real-time PCR assay for detection and quantification of DNA of *R. solani* AG 2-2 directly from soil samples for use in predicting inoculum potential.

MATERIALS AND METHODS

Soil sample collection. In 2016 we located 16 fields with a history of Rhizoctonia root rot based on the best knowledge of the agriculturists from ACSC (8 fields), MDFC (4 fields), and SMBSC (4 fields). From each field, 5 soil cores were taken at a depth of 6 inches representing approximately 1 acre area. Each soil core was divided in to 0-2 inch, 2-4 inch and 4-6 inch sub-samples. In total, we collected 240 soil samples from all 16 fields (16 x 15). Total soil DNA was isolated from all 240 samples. At each sampling point (16 fields x 5 sites per field = 80 samples) where we collected soil cores, we also collected approximately 1 gallon of soil to determine Rhizoctonia root rot index (RRI) values using a growth chamber assay.

Growth chamber assay. For each of the 80 samples, soil was added to four 10 x 10 x 10 cm pots (~350 cc soil/pot). Seed was sown (25 seed/pot, 4 replicate pots/infested soil treatment) and then another 250 cc of soil was added over the seed to each pot. Pots were arranged in a randomized block design and incubated in a growth chamber at 77°F with a 14-hour photoperiod for 4 weeks. Pots were watered once or twice daily to keep soil moisture high to favor infection by *R. solani*. Seedlings were counted three times per week. Dying seedlings were removed and assayed in the laboratory to verify presence of *R. solani*. Four weeks after planting, remaining seedlings were removed from soil, washed, and rated on a 0-3 scale (0 = no disease, 3 = hypocotyl completely necrotic/plant dead). The number of seedlings that died during the 4-week assay along with the ratings after 4 weeks were used to calculate a root rot index (RRI, 0 = no disease, 100 = all plants died during the 4-week assay).

Root rot rating. In each of the 16 fields, 10 sugarbeet roots adjacent to the soil sampling site were rated for root rot severity using a 0-7 scale (10 roots x 5 spots = 50 roots per field).

Soil DNA isolation. PowerMax® soil DNA isolation kits from MO BIO Laboratories Inc. (Carlsbad, CA) were used for DNA isolation. Manufacturer's protocols were followed, using 5 g (dry wt.) of soil as starting material. Final DNA was eluted in 5 mL of Solution C6, concentrated and stored at -20 °C until downstream PCR application.

Real-time PCR. Primers and probe specific for internal transcribed spacer (ITS) region of *R. solani* anastomosis group (AG) 2-2 used in this study were developed by Budge et al. (4). All real-time PCR assays were set up as duplicate 20 µL reactions using LightCycler® 480 Probes Master (Roche Life Science) following manufacturer's protocols. 20x Custom TaqMan® Gene Expression Assay (contains 18 µM each primer and 5 µM 6-FAM™ dye-

labeled TaqMan® MGB probe) was obtained from Life Technologies (Carlsbad, CA) and 1 µL of DNA template was used in the assay. Thermal cycling parameters were 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. *R. solani* AG 2-2 IIIB and IV DNA (10ng/ µL) as positive control and no template control was included in each run. Real-time PCR assays were performed using Roche LightCycler® 480 System.

RESULTS AND DISCUSSION

The mean root rot ratings ranged from 1.12 to 3.72 and root rot incidence values ranged from 18 to 68 % for 16 fields. The mean root rot index (RRI) ranged from 0 to 100% for 16 fields. The lowest Ct value of 27.02 (highest Rhizoctonia DNA) was found in one field in MDFC area (MDFC4). There was a significant correlation at the field level between RRI and root rot ratings (r = 0.59 and r^2 = 0.34), and RRI and root rot incidence values (r = 0.56 and r^2 = 0.32). There was a significant correlation between RRI and DNA of *R. solani* (r= 0.24; r^2= 0.06) (Fig. 1). However, some soil samples with higher RRI had lower DNA of *R. solani* Ag 2-2. This could be explained by the presence of other AG groups such as AG 4 in soil which can cause seedling damping off.
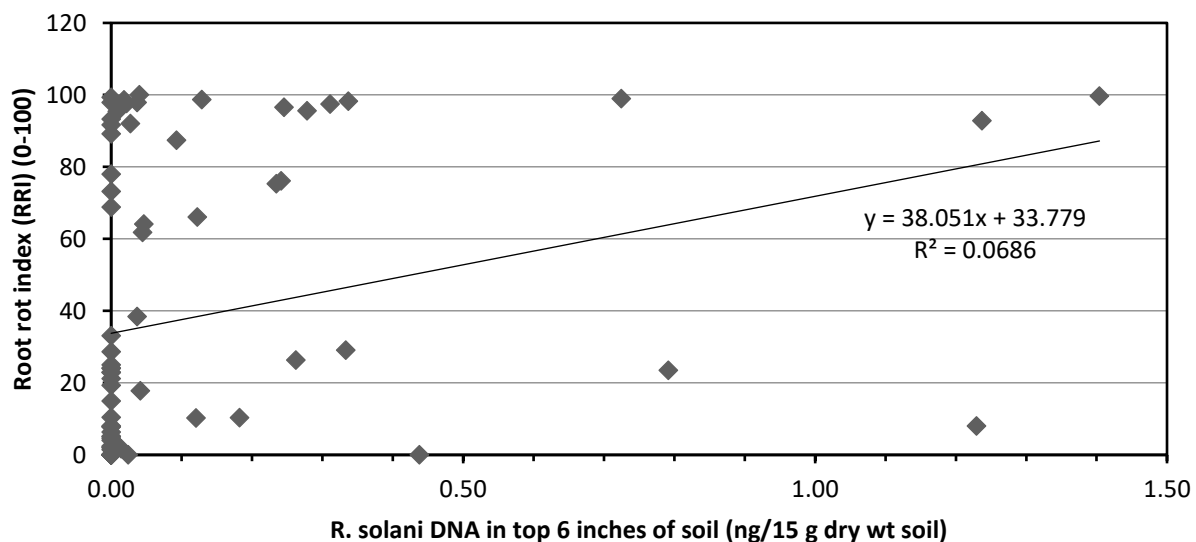

Figure 1. Relationship between Rhizoctonia root rot index and amount of DNA of *Rhizoctonia solani* AG 2-2 in soil

There was also a significant correlation between root rot rating and DNA of *R. solani* (r = 0.31; r^2= 0.11) (Fig. 2). However, soil samples from some fields with higher root rot ratings had lower amounts of DNA of AG 2-2. This could be explained by the non-uniform distribution of Rhizoctonia inoculum in the soil. We also observed Aphanomyces in some of the soil samples corroborating the evidence that mixed infestation of soil with Rhizoctonia and Aphanomyces is gradually increasing in our growing region. Out of 80 samples, DNA of Rhizoctonia was detected in twenty nine 0-2 inch samples, fifteen 2-4 inch samples, and eleven 4-6 inch samples. In only 6 out of 80 samples, DNA of Rhizoctonia was detected in all 0-2, 2-4 and 4-6 inches depths.
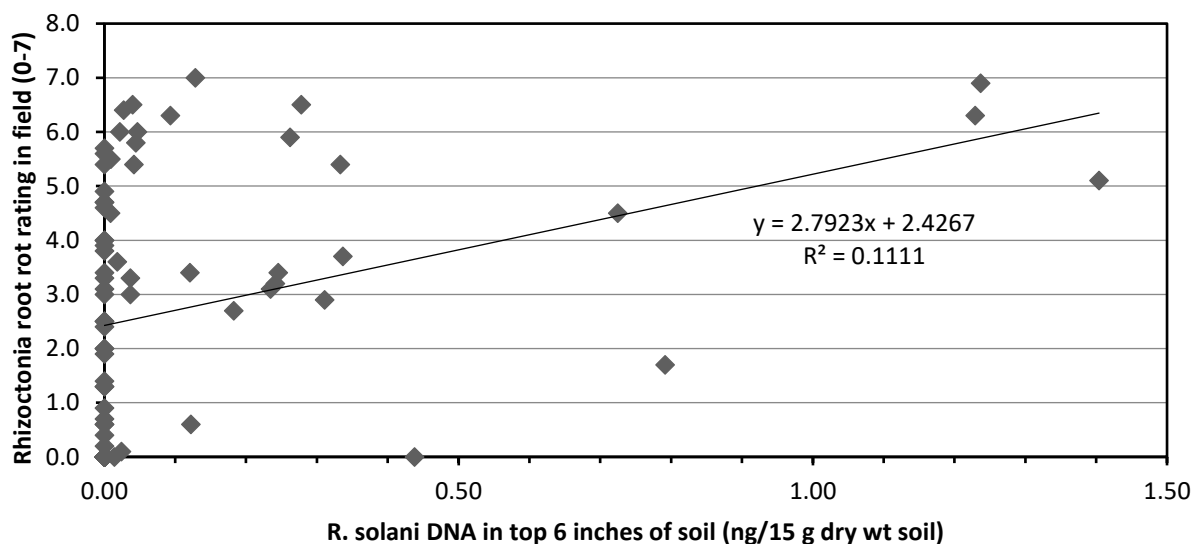
Figure 2. Relationship between Rhizoctonia root rot rating and amount of DNA of *Rhizoctonia solani* AG 2-2 in soil.

ACKNOWLEDGEMENTS

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