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MPSG ANNUAL EXTENSION REPORT

PROJECT TITLE: Root rot of pea in Manitoba

PROJECT START DATE: 1 April 2018 **PROJECT END DATE: 31 March 2023**

DATE SUBMITTED: 31 January 2020

PART 1: PRINCIPAL RESEARCHER

PRINCIPAL

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PART 2: EXECUTIVE SUMMARY

Outline the project objectives, their relevancy to pulse and soybean farmers, and a summary of the project to date, including methods and preliminary results.

Root rots have become a major factor limiting the yield of pulse crops in Manitoba as well as western Canada. In Manitoba, recent surveys of field pea demonstrated that Fusarium root rot was present in all fields. *Aphanomyces euteiches* was also identified in Manitoba pea fields. At present, there are no effective management strategies for root rot; no resistant cultivars are available, seed treatments do not provide full-season protection and there are no in-crop fungicide products. One potential management strategy is to develop resistant pea lines. Resistance has been identified but markers associated with resistance are needed, so that resistance can be readily incorporated into new cultivars. Screening recombinant inbred lines carrying QTLs for resistance to *A. euteiches* and *Fusarium* spp. against the pathogens alone and in combination to identify the best sources of resistance in the field is in progress. The cultivation of field pea cultivars with resistance to the common root diseases in Manitoba and western Canada will result in better economic returns to the producers, higher seed yields, and greater inclusion of field pea in crop rotations. Another management strategy for root rot is to avoid planting pea crops in heavily infested soils. Development of a soil test to categorize fields as low, medium or high risk based on levels of Aphanomyces inoculum is in progress. Refinement of this test to include multiple pathogens and improve the sensitivity of detection is ongoing and will benefit the pulse industry by ensuring that pea crops are planted in low risk fields, thus minimizing the possibility of yield loss.

PART 3: PROJECT ACTIVITIES AND PRELIMINARY RESULTS

Outline project activities, preliminary results, any deviations from the original project and communication activities. You may include graphs/tables/pictures in the Appendix.

Objective 1. Molecular techniques for detection and quantification of *Aphanomyes euteiches* and *Fusarium* spp. A molecular protocol to improve detection and quantification of pathogen spores in soil (*A. euteiches* and selected *Fusarium* spp.) will be developed. The initial version of a root rot risk assessment tool will be refined and validated using samples from producer fields in Manitoba and across the prairie region. Targeted surveys focused on isolate collection and detection of new/emerging pathogens will be conducted as required.

Activities and preliminary results: One strategy to reduce impact of Fusarium and Aphanomyces root rot is disease avoidance based on calculating the inoculum potential of field soil. Real-time PCR (qPCR) or droplet digital PCR (ddPCR) are both quantitative molecular techniques and are efficient methods for determining the presence and quantity of *A. euteiches* oospores and other pathogens propagules (chlamydospores or mycelia) in soil. The ability to accurately quantify soil inoculum potential is a prerequisite for the development of a decision support system for soil-borne pathogens, as there will be different management strategies depending on the risk associated with different levels of inoculum.

Soil samples and roots were collected from 10 sites in each of 45 Manitoba fields in June of 2019. Fusarium root rot was the most prevalent root disease and was present in 98% of fields. Roots from each field were rated for disease severity, which ranged from 1.2 to 5.7 with a mean of 2.9. Roots were then tested for the presence of *Fusarium avenaceum, F. solani, F. redolens* and *A. euteiches* using ddPCR. *Fusarium avenaceum* was the most predominant *Fusarium* species. *Aphanomyces euteiches* was present in 56% of the pea fields surveyed in 2018. Assessment of 2019 samples is ongoing. Soils from these fields will be tested to determine the association between observed root rot levels and soil pathogen levels.

Tests are currently being performed (Dr. Chatterton's lab) to validate an oospore extraction protocol from larger soil samples (10 or 100 g compared with 250 mg using commercial kits), to determine whether this protocol has a lower detection limit accuracy than the commercially available kit. In addition, it is important to determine whether this protocol can reduce the cost per sample for quantifying *A. euteiches* inoculum in soil samples as compared to the commercial kit. Once this protocol has been tested, it will be shared with specific AAFC labs, including those in Manitoba, for cross-lab validations.

Objective 2. Screening for resistance to the root pathogens *Aphanomyces euteiches* and *Fusarium* species. Recombinant inbred lines (RILs) carrying QTLs linked to resistant genes to important root pathogens will be screened against the pathogens alone and in combination to identify the best sources of resistance in the field. Markers for marker-assisted selection (MAS) in field peas will be developed.

Activities and preliminary results: In 2019, 141 recombinant inbred lines from a cross of the two partially resistant germplasm lines 00-2067 x Carman were evaluated for their root rot reactions and different measures of plant growth in the Aphanomyces root rot (ARR) nursery at Morden. The RILs and three check cultivars were arranged in a partially balanced lattice with three replications. Two weeks after seeding, emergence counts were made in each plot. At the end of July, each plot was assessed for seedling vigor, root rot severity, root nodulation and the weights of the dried roots and top growth (continued on page 3).

APPENDIX

Despite two irrigations early in the growing season, disease development initially was poor because of the dry growing conditions. However, at the end of the growing season, consistent differences in disease symptoms and plant growth were apparent. This enabled the identification of susceptible and tolerant RILs and that information was used together with similar tests in 2018 in molecular research to identify markers for tolerance. A series of greenhouse experiments at the University of Alberta verified the RILs with tolerance to ARR. Close correlations were detected among all the variables within and between the field and greenhouse experiments.

The genomic regions associated with tolerance to Aphanomyces root rot was investigated by genotyping 135 of the 141 RILs of the cross '00-2067' × 'Reward' with 13204 SNP (Single Nucleotide Markers Polymorphisms). Filtering was carried out to remove markers that failed to amplify genomic DNA and monomorphic markers. Based on Chi square tests, markers that did not fit a 1R:1S segregation ratio expected for an F8 population were also discarded. Overall, 4174 high quality polymorphic SNP markers were used for linkage analysis and the QTL mapping. The molecular analysis identified 11 QTLs on four (LGII, LGIII, LGIV and LGVII) of the seven chromosomes of pea to be associated with root rot severity caused by *A. euteiches* isolate Aph2. These QTLs explained 16.82-49.95% of total variance. This study also identified QTLs associated with plant vigor, dry root and foliar weight and plant height in both the greenhouse and the field experiments. Some of the QTLs for tolerance to ARR were also associated with the different measures of plant growth. The consistent QTLs are highly stable and holds much promise for identifying markers for marker-assisted selection.