Tag-Profiling of Differentially Expressed cDNA for Developing Phytophthora Root Rot Resistant Raspberry Cultivars

Progress Report submitted to the North American Bramble Growers Association Research Committee regarding 2009 Funding

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The objective of the project in the first year was to use a short run DNA sequencing technique called Tag-profiling (Vega-Sanchez et al., 2007) as a tool to identify genes associated with resistance to *Phytophthora* root rot in red raspberry. This method is powered by the Illumina Genome Analyzer in a process called massively parallel signature sequencing (MPSS) (Brenner et al., 2000) and is available for low cost use (by sequencing standards) to Cornell Researchers through the Cornell University Life Sciences Core Laboratories Center in Ithaca, NY. Using this machine with a "Paired End Module" both ends of RNA transcript fragments from 'Latham' root tissues before, during and after infection with *Phytophthora fragariae* var. *rubi* were targeted for sequencing. The specific objectives within this framework are listed below.

Specific Objectives:

1) Grow 'Latham' raspberry plants in the hydroponic screening system.
2) Isolate RNA from 'Latham' roots prior to infection and at 4 time points post exposure to *Phytophthora fragaria* var. *rubi* isolates.
3) Produce cDNA from the RNA for MPSS sequencing
4) Perform MPSS on cDNA from 5 time points in the PRR disease progression
5) Analyze data to identify target genes for development of DNA markers for mapping and marker assisted selection.

The project is on track to sequence the cDNAs from the 'Latham' RNA in January 2010. During 2009, final isolation of 2 new isolates of *P. fragariae* var. *rubi* from fields near Geneva, NY were completed with 2 cultures established from a single zoospore each. These cultures were tested for virulence in the hydroponic system on replicates of the susceptible variety 'Titan' and the resistant variety 'Latham'. The response to the pathogen was consistent with previous reports with the 'Titan' plants being killed and the 'Latham' plants being infected and showing early root symptoms and later growing out of the disease and remaining healthy.

Tissue culture propagules of 'Latham' and 'Titan' were established in the hydroponic system for challenge with the pathogen and isolation of mRNA for sequencing and analysis. The plants were inoculated on November 23. The first RNA sample was isolated prior to inoculation with the subsequent isolations on Nov. 25, Dec. 3 and Dec. 14. All the samples were stored at –80F until the last sample was taken at which time RNA was isolated from all samples. The RNA samples were submitted to the Cornell University Life Sciences Core Laboratories Center in Ithaca, NY on December

21 for sequencing. Pre-sequencing processing to produce the cDNA for sequencing was completed on December 23 and sequencing is ongoing. Sequencing results are expected prior to January 15, 2010 with annotation and analysis of the sequencing data to follow to identify differentially expressed genes associated with infection and resistance to *Phytophthora* root rot.