

Final Report: A proposed study for the identification of differentially expressed genes associated with exposure to *Phytophthora cinnamomi* in *Castanea dentata* (susceptible) and *C. mollissima* (resistant)
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Objectives:

Our goal was to attempt to identify genes that are differentially expressed in the very early stages of *Phytophthora cinnamomi* infection by *Castanea dentata* (susceptible) and *C. mollissima* (resistant). The basic approach is analogous to previous studies in *C. sativa* (susceptible) and *C. crenata* (resistant); however, we planned to use Representational Difference Analysis (RDA), rather than whole transcriptome analysis, to identify genes of interest related to susceptibility/resistance. Our results were intended to complement and inform work by others in identifying and employing genes for Pc-PRR resistance in chestnuts, and assist in identifying the mechanisms by which such resistance is established in Asian *Castanea* species.

Methods of Monitoring, Evaluation:

Chinese chestnut (*Castanea mollissima*) and American chestnut (*Castanea dentate*) seedlings were exposed for 52hrs with *Phytophthora cinnamomi* spores (grown at UGA Tifton). Soil and root samples after exposure were analyzed for the presence of *P. cinnamomi* spores to verify the presence of an inoculated condition. Replicate root samples taken after exposure were flash frozen in liquid nitrogen or immersed in RNA Later. Total RNA was extracted and reverse-transcribed from four treatment groups: 1) *C. mollissima* exposed to *P. cinnamomi*, 2) *C. mollissima* unexposed, 3) *C. dentata* exposed to *P. cinnamomi*, and 4) *C. dentata* unexposed. Representational Difference Analysis (aka RDA), a form of subtractive hybridization, was used to generate cDNA fragments for the RDA analysis. Detecting *P. cinnamomi* transcripts in the RDA products of the exposed treatments would be evidence of a successful inoculation. Sequence information from RDA products were used for developing qPCR primers to quantify relative differences in expression between treatments.

Actual Results:

RDA hybridizations focused initially on transcriptional differences between *C. mollissima* exposed vs *C. dentata* exposed samples. 192 ESTs were cloned and sequenced which represented 118 RDA products. A preliminary bioinformatics analysis was performed on RDA products using BLASTX to putatively identify each expressed sequence tags (ESTs) (aka cDNA fragments) as well as the organism expressing the transcript. No *P. cinnamomi* transcripts were detected/represented in the RDA products. Replicate soil and root samples also did not show any evidence of *P. cinnamomi* spores after 52hrs of exposure. ESTs from five other plant pathogens were detected: *Fusarium fujikuroi*, *Melampsora larici-populina*, *Verticillium alfalfa*, *Sclerotinia sclerotiorum*, and *Valsa mali*. Sequences representative of a number of other soil fungi were also detected: *Pyronema omphalodes*, *Purpureocillium lilacinum*, *Tuber aestivum*, and *Sphaerospora brunnea*. In addition, there were also a variety of prokaryotic transcripts detected. In the RDA hybridization of *C. mollissima* exposed as the "tester" population, *C. mollissima* ESTs were identified. The qPCR analysis of samples was halted since there was no evidence of successful *P. cinnamomi* inoculation.

Differences from Objectives:

The lack of *P. cinnamomi* spores in soil samples suggests infection by *P. cinnamomi* spores had not occurred and was most likely a function of cooler overnight temperatures during the inoculation period plus the use of a particulate medium (peat/vermiculite mixture). The presence of Chinese chestnut ESTs in the RDA products suggests the RDA protocol is capable of selectively amplifying ESTs uniquely associated with one population. However, without confirmed *P. cinnamomi* inoculation it is most likely that isolated ESTs are a function of genomic differences between the species. Without confirmed *P. cinnamomi* infection, differential expression of *Castanea sp.* ESTs in response to a *P. cinnamomi* infection cannot be confirmed by qPCR. The presence of prokaryotic transcripts were most likely reverse-transcribed because random hexamers were used in the RT reactions. Once again without confirmed *P. cinnamomi* inoculation, differences in the composition of soil microbiomes may play a factor influencing differential expression of *Castanea sp.* ESTs. Recent experience in other labs using exposure of roots in a liquid medium will likely produce infection within the expected time window. We hope to collaborate with others undertaking such studies with the hopes of employing the RDA molecular approach to cDNA samples from exposed and unexposed root samples.

Published work and presentations: None

Press coverage: None

Final Financial Report:

\$2600.25 was spent on consumables for the project.

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