Microarray analysis of oxalate oxidase transgenic soybean challenged with *Sclerotinia sclerotiorum*

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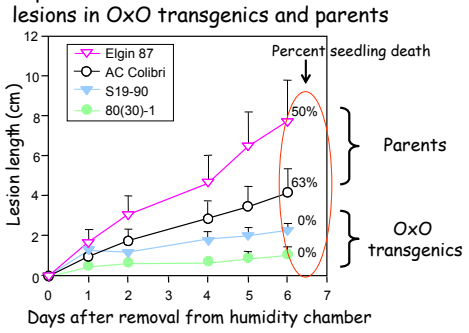
Oxalate is a major virulence factor of Sclerotinia sclerotiorum. Research involving fungal mutants as well as transgenic plants, has clearly shown that virulence of *S. sclerotiorum* is substantially reduced if oxalate is removed from the interaction. We are utilizing a transgenic soybean plant that constitutively produces the oxalate-degrading enzyme, oxalate oxidase (OxO), to study how soybean plants respond to oxalate and *S. sclerotiorum*.
Freshly opened flowers, inoculated between the standard and wing petals with 10 μL of ascospores (5000 ascospores/10 μL of 0.006% Triton X-100 in water), were incubated for 3 days in humid petri dishes and used for inoculum. The central leaflet of V4 leaves of the transgenic line 80(30)-1 and its parent AC Colibri were inoculated behind the first lateral vein with infected flowers.
Leaves were collected rapidly and frozen in liquid nitrogen within 30 seconds after removal from the plant. The lateral two leaflets were removed from the leaf, the remaining leaflet was removed from the plant by cutting the petiole close to the main stem, the infected flower was removed and the stage of invasion determined under a dissecting microscope. The leaflet was cut transversely 3 cm beyond its base and together with the petiole was frozen in liquid nitrogen. Two sample times were taken: 1. early appressorial formation (10-12 hour post inoculation) and 2. early vascular entry (18-20 hour post inoculation).
RNA from harvested tissue is being analyzed with soybean microarrays to determine how approximately 36,000 genes are responding to inoculation in the OxO transgenic line 80(30)-1 and the parent AC Colibri. ANOVA analysis of the microarray data will assign statistical significance to each gene as to whether or not its expression is differentially changing, and to what degree. Such knowledge promises to identify genes responding to *S. sclerotiorum* under normal and very low oxalate [in 80(30)-1] environment. This project promises to advance our understanding of the basic biology behind soybean's response to this serious pathogen and to contribute to our goal of developing crops with resistance to *Sclerotinia*.

OxO transgenics reduce severity of disease

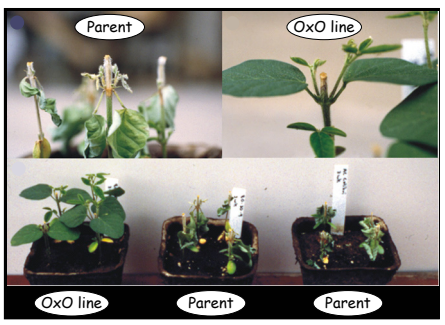
Oxalate Oxidase (OxO, or Germin) catalyzes the oxidation of oxalic acid

$(COOH)_2 + O_2 \longrightarrow H_2O_2 + CO_2$

Comparison of *Sclerotinia*-induced disease lesions in OxO transgenics and parents

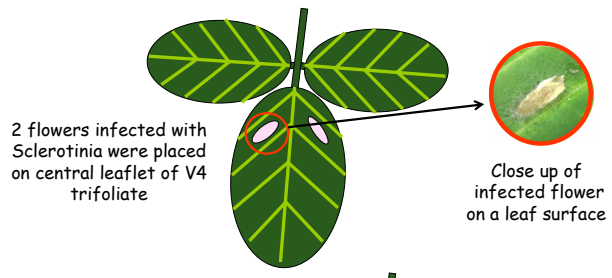


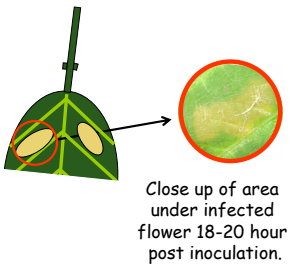
Seedling assay of OxO transgenic vs parent

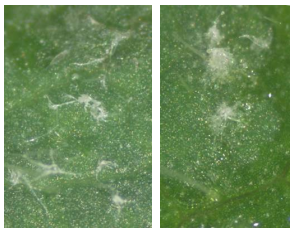


OxO transgenics grow fine and yield as well as parent in field studies. This field photo shows transgenic 80(30)-1 grown next to its parent, AC Colibri.

Inoculation and sample collection

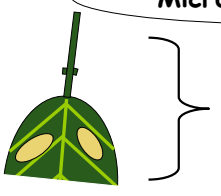




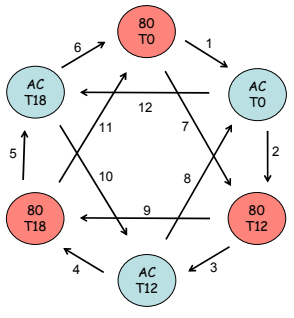


Examples of fungal growth under infected leaf petal within 10 hours inoculation. Note, appressoria are beginning to form.

Microarray design



Tissue collected at 12 and 18 hour post inoculation, microscopically verified for infection, frozen in liquid nitrogen within 30 seconds of removal from plant. RNA was extracted, fluorescently labelled and compared to other samples according to experimental design below.

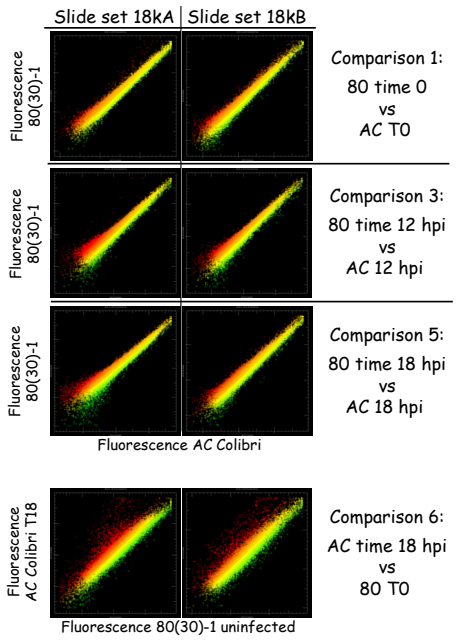


80 = oxo transgenic line 80(30)-1

AC = parental line AC Colibri

Each arrow represents one microarray comparison.
Two slide sets were used, each with approximately 18,000 different genes.

Preliminary microarray data



Scatter plots of individual slide hybridizations show most of the 18,000 genes per slide had little change in fluorescent intensity regardless of plant source, and that hundreds of genes appear to be differentially expressed based on these single reps. Comparison #6 illustrates that a much stronger degree of differential expression occurred when comparing an infected plant, AC Colibri at 18 hpi, versus an uninfected plant, 80(30)-1 at T0.

Summary

Previous work in the Simmonds lab and others have shown that oxalic acid is a major virulence factor of *Sclerotinia*. Soybean transgenic lines expressing oxalate oxidase (OxO or Germin) degrade fungal produced oxalic acid and provide significant levels of resistance to white mold disease. We are utilizing one of these OxO lines, 80(30)-1, to study *Sclerotinia* – soybean interaction and the role of oxalic acid. We have completed the first round of inoculations, sample collections, RNA extractions, and microarray hybridizations corresponding to all 12 sample comparisons illustrated in the microarray design to the left. We will repeat the entire experiment at least once more for a total of two or three biological repeats. The microarray expression data will be analyzed by ANOVA using the MAANOVA program to identify genes that are significantly, differentially expressed between these samples.

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