Exploiting Genetic Traits of Plant Defense Mechanisms Against Phytoparasitic Nematodes

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Phytoparasitic nematodes (PPN) cause extensive damages to essential food crops. These pests have been estimated to cause the annual loss of 173 billion U.S. dollars, accounting around 12.3% of global food production (Gamalero and Glick, 2020). Nematicides offer some relief, but their economic costs greatly exceed their utility, as well as concerns over toxicity to helpful organisms, insects, and mammals (Gamalero and Glick, 2020). In this study, we examine and characterize plant defense responses to PPN, in order to develop inexpensive and effective genetically resistant cultivars.

Salicylic acid (SA) has long been considered as a major plant defense hormone against PPN. SA signaling activates hypersensitive response (HR) that is effective at suppressing the spread of pathogenic microorganisms, and presumably PPN (Branch et al, 2004). Earlier, it was suggested that reactive oxygen species (ROS) production is an indication of HR which stems from SA signaling. In line with this scenario, previous studies showed some accumulations of ROS at the infection site of PPN (Branch et al, 2004). However, in our recent study, cotton roots inoculated with *Rotylenchulus reniformis* did not show any HR nor even localized cell death. While ROS accumulations were induced in high amounts, HR characteristic of SA signaling did not occur. Previous studies may have mischaracterized cell death from PPN feeding as HR.

Oxo-phytodienoic acid (OPDA) signaling may play a role in plant defense responses against PPN due to its roles for signaling redox reactions and root hair growth, which our recent study found that the amount of root hairs may play a role in tolerance to PPN (Liu and Park, 2021). OPDA is a precursor to jasmonic acid (JA), which antagonizes SA signaling. In order to assess OPDA's role in plant defense response, transfer DNA insertion knockout (KO) mutant Arabidopsis plants disrupting OPDA, SA, JA signaling and biosynthesis were grown in MS Gelrite plates and inoculated with PPN. These mutants include *cyp20-3*, the loss-of-function of *CYP20-3*, a small plastid protein that interacts with OPDA and intricately coordinate defense gene expressions. Interestingly, *cyp20-3* display severe inhibition of root hair growth (Liu and Park 2021) and impairs the activation of plant defense response against a range of pathogens and pests. Another mutant grown was *2cpa*, which disrupts *2CPA* (*2-cysteine peroxiredoxin A*), which in turn, reduces toxic byproducts of photosynthesis like H_2O_2. A recent study in our lab found H_2O_2 in high amounts when PPN were inoculated. *NPR1* (*Nonexpressor of PR1*) KO and *JAR1* KO were also grown to test SA signaling and JA isoleucine biosynthesis in a plant defense response to PPN. Wild type (WT), the genotype characterizing what is "normal" for *Arabidopsis*, was grown as a control. Seeds were sterilized and pipetted onto plates. MS Gelrite plates with five sterile seeds each were kept at 22°C for two days and were then grown for two weeks in a 12-hour light/dark growth chamber.

Meloidogyne hapla, a root-knot nematode, were harvested at the Auburn University Center for Advanced Science, Innovation and Commerce (CASIC) in Dr. Lawrence's lab. Eggs were then kept in a nematode hatchery, a container with a mesh strainer, at slightly above room temperature on a heating pad for two weeks so that stage two juvenile (J2) nematodes could hatch and develop. J2 *M. hapla* nematodes will seek a host and infect it more commonly at this stage. After the two-week period, *M. hapla* would be strained and sterilized. *M. hapla* would be sterilized by filtering through a vacuum flask with a mercury chloride solution and streptomycin solution. Autoclaved water would be filtered down as well, and

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sterile *M. hapla* were then inoculated onto M.S. Gelrite plates containing aforementioned genotypes. Plates were then wrapped with tinfoil to simulate dark light conditions of roots suitable for nematode growth and kept in the growth chamber for fifteen days.

M. hapla creates root galls/knots, or deformations in roots, and these root galls were counted for each plant inoculated under a microscope. Figure 1 shows root galls on *2cpa*, where large abnormal bumps on roots (root galls) exemplify where a female nematode has penetrated the root system and has begun laying eggs. After root galls were counted, roots were then weighed in mg. Roots were weighed after counting, and then were weighed after being dried. Root gall to root weight measurements were used to assess the level of infection of *Arabidopsis thaliana*.

Fig. 1 Root galls were counted and then compared to root weight. Root galls are abnormal bumps on roots and in this context, are caused by the root-knot nematode *M. hapla*.

While our study is not complete, our preliminary results (see Figure 2) show that *2cpa* seems to have more root galls and more average root galls per mg than WT. Root hairs on *2cpa* seem to be a little closer to root galls than on WT. Although, *cyp20-3* was found to have similar amounts of root hairs before inoculation while having a lack of root hairs after inoculation.

Once more results come in, implications can be justified more strongly. For now, *2CPA*, which reduces H_2O_2 into H_2O, can be deemed important for a plant defense response to PPN such as M. hapla. As *2CPA* is related to *CYP20-3*, and *CYP20-3* has roles in plant root hair signaling, *CYP20-3* will likely be found to be important in a plant defense response, but more data from this ongoing study is needed to derive such a conclusion. Finally, if both *CYP20-3* and *2CPA* are considered to be important in tolerance against PPN, improvements to OPDA signaling will be necessary for a genetically modified resistant cultivar.

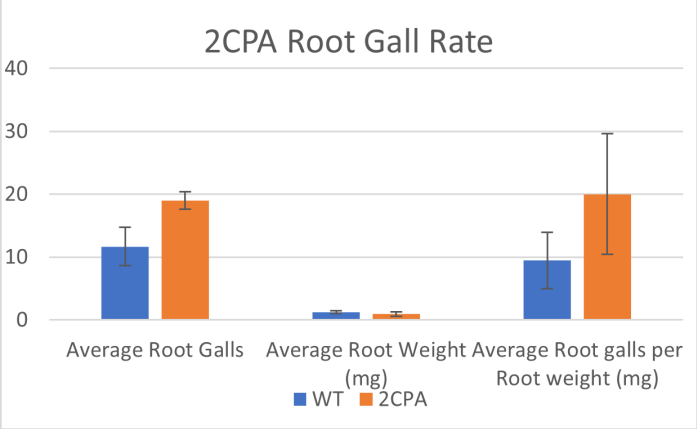

Fig. 2 Above shows our current data on *2cpa* KO that was inoculated with *M. hapla*. Blue bars show WT's root galls, weight, and root gall per root weight ratio, while orange shows *2cpa* KO's.

Statement of Research Advisor

Ben Welsh has done a great work in developing and establishing a standard pathosystem and assay protocol of plant parasitic nematode pests using a model plant, Arabidopsis thaliana. Based on this tool, we are now able to explore molecular details in plant and nematode interactions, which will provide noble insights on how to develop new crop cultivars that upgrade their own defense capacity against the pest infestations.

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Authors Biography

Benjamin Welsh is a sophomore-year student pursuing a B.S. degree in Applied Biotechnology at Auburn University. He has been working on this project since early spring. He has a passion for studying potential cases for genetic modification for increased crop yields.

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