The Role of OPR2 in Maize- Insect Interactions

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Introduction

Lipid derivatives of oxo-phytodienoic acid reductases (OPRs) play a central role in plant immunity against insects. OPRs are commonly known as enzymes in the octadecanoid pathway that reduce the double bond in the cyclopentanone ring of OPDA, an important intermediate in the jasmonic acid (JA) pathway. JA is a known defensive hormone used by plants to ward off necrotrophic pathogens and insects. Previous studies have shown the maize OPRs, ZmOPR7, and ZmOPR8, to be involved in the JA biosynthesis pathway (Yan et al., *submitted*), however the role of OPR2 remains unknown. This study aims to elucidate the function of OPR2 in JA production during maize defensive responses.

Original Hypotheses

- OPR2 competes with OPR7 for the important JA biosynthesis intermediate OPDA. Therefore, upon wounding, the *opr2* knockout mutants will produce higher levels of JA than wild type plants.
- Higher levels of JA in *opr2* mutants will make them less attractive and more resistant to insects such as *Spodoptera frugiperda*. In choice assays, *S. frugiperda* will prefer wild type seedlings over *opr2* mutants.

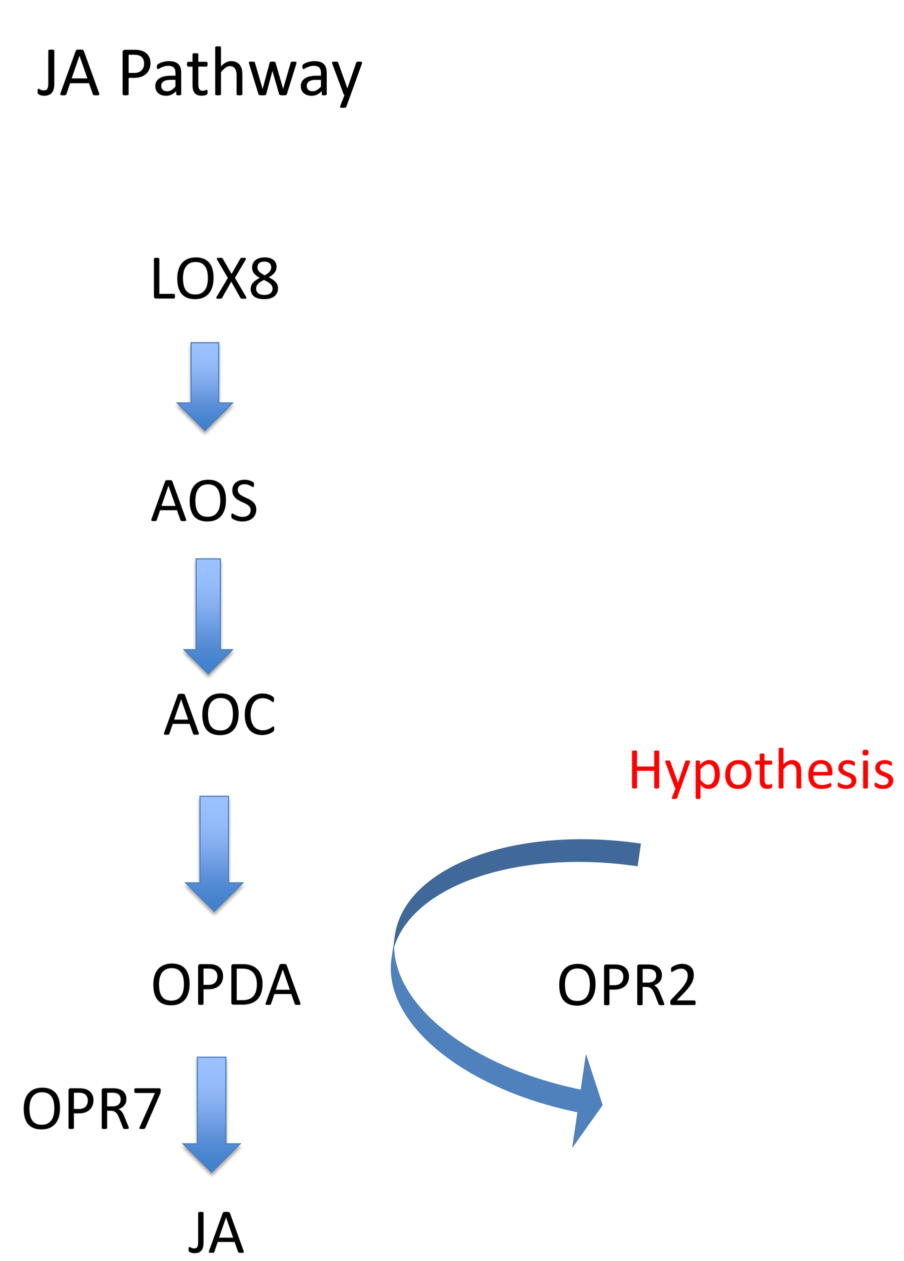

Figure 1. Jasmonic acid pathway and OPR2 taking substrate from the pathway

Methods

V3 stage maize plants were wounded by gently scraping the underside of the leaf with a scalpel. Four of the plants, for both genotypes, were treated with an insect elicitor. Four of the plants, for both genotypes, were treated with H_2O. Four of the plants, for both genotypes, were left untreated as basal controls. One gram of tissue was collected from each plant. JA was extracted and analyzed using liquid chromatography mass spectrometry (LCMS). The second experiment was done with *S. frugiperda* which are natural maize herbivores. *S. frugiperda* were given the choice between wild type and opr2 mutants. In this experiment sixty plants were grown. One *opr2* mutant and one wild type were grown simultaneously in insect isolating cylinders. Five *S. frugiperda* larvae were placed in the whirl of each plant. After three days of infestation the leaves were harvested and the damage area was analyzed using ImageJ. The final experiment was run simultaneously with the choice experiment. In this experiment 60 plants were grown, 30 were *opr2* mutants, and 30 were wild type. All of the plants were grown in separate insect isolating cylinders. Five larvae were placed in the whirl of each plant. After three days of infestation, the leaves were harvested and the damage area was analyzed using ImageJ.

Wounding experiment

Wounding

Figure 2. Leaf wounding

Adding treatment

Figure 3. Treatment application

S. frugiperda Experiment

Choice

Figure 4. Choice experimental set up

No Choice

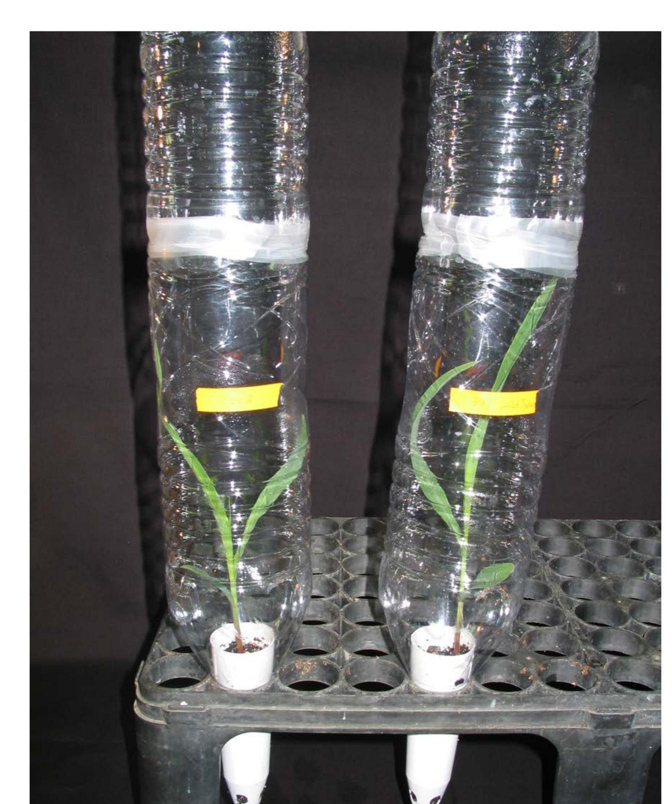

Figure 5. No Choice experimental set up

Results

Wounding Experiment

Local JA

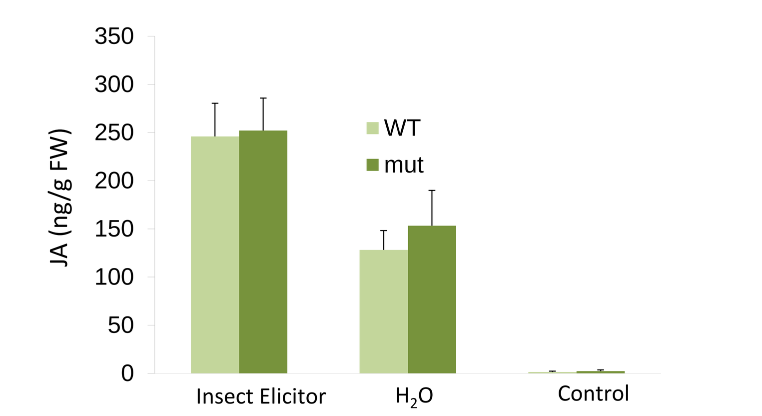

Figure 6. Local JA levels were not higher for *opr2* mutants at the local sight of insect elicitor treatment. The insect elicitor in combination with wounding incited the highest jump in JA. H_2O and wounding makes up the second highest spike. Finally the control was the lowest spike and acted as the base line for measuring JA levels.

Systemic JA

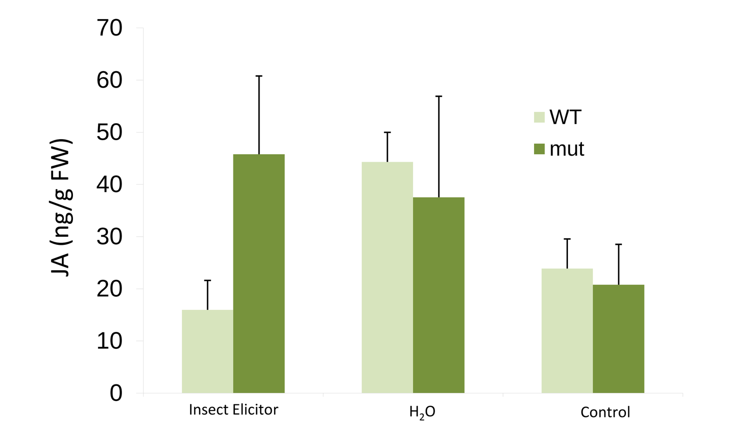

Figure 7. Systemic JA spiked the highest in opr2 when the insect elicitor was added, suggesting that OPR2 hijacks OPDA from the JA pathway in systemic tissues. Wild type did not show a similar spike.

S. frugiperda Experiment

Choice

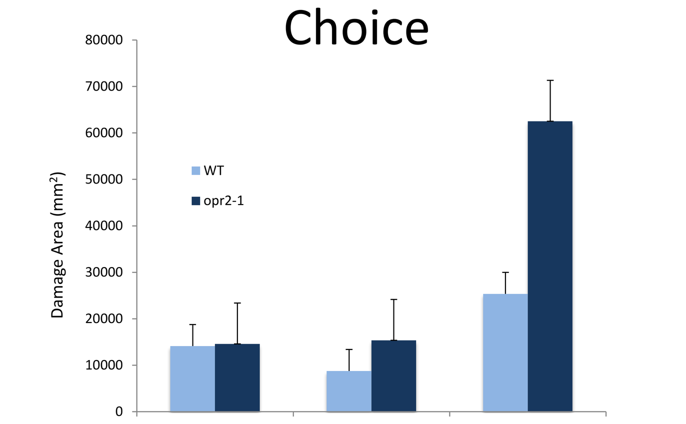

Figure 9. The graph above indicates that *opr2* mutants were more damaged than wild type. The systemic leaf showed the most damage.

No Choice

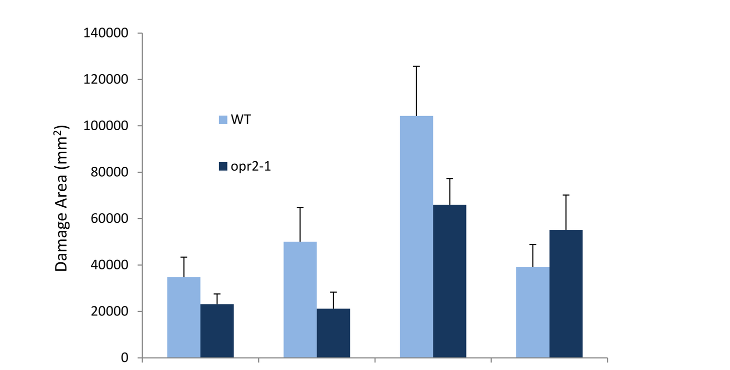

Figure 10. The graph shows that wild type plants have a greater damage area on the first three leaves, than *opr2* mutants. This supports the original hypothesis. The systemic leaf contradicts that hypothesis. It shows *opr2* mutants having a slightly higher damage area than wild type maize.

Conclusions

According to wounding experiment *opr2* mutants produce more JA. The choice experiment showed that *opr2* mutants seem to be preferred by *S. frugiperda* however this could be due to volatile composition. It could also be due to an allopathic interaction between the wild type and the opr2 mutants. The third experiment supported the hypothesis that wild type (B73) plants would be preferred by *S. frugiperda opr2* plants were more resistant on all of the leaves except for the systemic leaf. The systemic leaf may have a different composition since it is the youngest leaf and therefore the least developed. The OPR2 gene may benefit insects by removing some of the JA intermediate OPDA, thus reducing the amount of JA produced. Insects may have evolved to manipulate OPR2 expression in order to improve their odds of survival. Insects are more likely to thrive on plants that are infected with a pathogen that induces OPR2.