Plant-fungal communication via lipid signals

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Abstract

Mycotoxins are metabolites produced by some fungi which have negative effects on human and animal health. The focus of this research was on *Aspergillus flavus,* a fungal plant pathogen that produces a highly carcinogenic mycotoxin, aflatoxin. After infection, corn seed accumulates this metabolite, making it unfit for human consumption or animal feed. Plant-fungal interactions are hypothesized to be regulated by numerous lipid signals produced by both the host and the pathogen. Growing evidence suggests that the lipid signals produced by host plant or pathogen have an effect on the growth and development of both organisms. The roles of one class of lipid signals, oxylipins, produced by lipoxygenase (LOX) pathway in plants and Psi Producing Oxygenase (Ppo) in fungi are explored in the current study. Recent studies have supported the lipid-based signal communication hypothesis but the exact roles of specific genes remain elusive. Deciphering cross-kingdom oxylipin communication can be done by studying interactions between oxylipin-deficient mutants of both host and pathogen. In this study, kernels of maize lipoxygenase mutant *lox3-4* and wild-type kernels were infected with *A.flavus* mutant strains *Δlox*, *ΔppoA*, *ΔppoC*, and *ΔppoD* along with the wild-type strain, and incubated for 3 and 5 days. Conidiation and ergosterol content were quantified to determine fungal reproduction and colonization, respectively. Results indicate that genotype of both organisms determine outcome of fungal parameters, suggesting the oxylipin blend produced by host and pathogen contributes to fungal responses.

KEY WORDS- *Aspergillus flavus*, maize, lipid signal exchange, mycotoxins, oxylipins.

Introduction and Objectives

Seed contamination by mycotoxigenic fungi is a threat to food security, human food, and animal feed. Aspergillus flavus is a fungal plant pathogen that infects and contaminates maize, a staple food globally, with mycotoxins such as aflatoxin causing significant economic losses and food safety concerns. Aflatoxin is among the most carcinogenic compound naturally found and when ingested it has acute toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic effects, mainly on the liver (Peraica, 1999). Molecular signaling during plant-pathogen interactions must be understood to create and apply better forms of disease resistance in staple crops. Oxylipins are the molecular signals that are little understood but recently gaining attention as potential signals regulating plant fungal interactions. They are oxygenated lipids made from polyunsaturated fatty acids and oxygenated through dioxygenase activity. Lipoxygenase or LOX, in plants, initiates biosynthesis of most oxylipins some of which are shown to regulate growth and development, biotic and abiotic stress defense, sex determination, programmed cell death, and senescence (Feussner and Wasternack, 2002). In addition to LOX, fungi have another family of dioxygenases, psi-producing oxygenase (PPO) that incorporate molecular oxygen into fatty acids to produce Psi (precocious sexual inducer) factors; they stimulate asexual or sexual spore development. *A.flavus* contains four Ppo and one Lox genes which have been characterized to contribute to mycotoxin production, sporulation, and density-dependent development (Tsitsigiannis, Dimitrios, and Keller, 2012). The maize lipoxygenase isoform LOX3, has been previously shown to be required for resistance to *Aspergilli* spececies. (Gao, 2009). Plant- and fungal-derived oxylipins are structurally and functionally similar to one another (Tsitsigiannis, Dimitrios and Keller, 2012). This prompted the hypothesis that both host- and parasite-derived oxylipins might play a role in plant-pathogen interactions. To test this hypothesis and elucidate the precise role of specific fungal and maize dioxygenases during seed infection, maize wild-type and oxylipin-mutant *lox3-4* was infected with Aspergillus flavus wild-type and oxylipin-mutants Δ*lox*, Δ*ppoA*, Δ*ppoC*, and Δ*ppoD.* Fungal parameters of sporulation and fungal biomass were quantified to asses effect on reproduction and colonization.

Methods

Kernel bioassays (Christensen et al. 2012) were performed on maize 9-LOX mutant *lox3-4* and wild-type line, B73. *Aspergillus flavus* oxylipin-deficient mutants utilized in this study were *ΔppoA*, *ΔppoC*, *ΔppoD*, and *Δlox* (Brown et al., 2009). The kernels were surface sterilized with 70% ethanol for 5 minutes, sterilized water for 1 minute, 6% sodium hypochlorite for 10 minutes, and rinsed three times for 5 minutes each time, in sterilized water. For infection to occur, kernels were wounded at the embryo-side with an 18 G needle to the depth of 0.5 cm. Four kernels were placed into each scintillation vial and their mass was determined. Inoculation consisted of 200 µl per vial of [10^6 conidia / ml] suspended in 0.01% Tween-20. The vials were incubated at 28C in a humidity chamber under a 12-h-light/12-h-dark photoperiod. After incubation, sporulation was determined (Gao et al. 2009) with 5 ml of 100% methanol being added to each vial and vortexed for 15 seconds, 20 µl of suspension was removed and diluted 1:1 with sterilized water before enumerating conidia with a hemocytometer. For fungal biomass, ergosterol was extracted by adding 10 ml of chloroform to the vials which were then incubated in darkness overnight. Ergosterol was quantified by directly injecting a 20 µl aliquot from each vial into a High Performance Liquid Chromatography (4.6 U ODS-C18 column) with a UV/VIS detection at 282 nm and comparing peak against a standard.

Results

Figure 1: Colonization and sporulation of wild-type and oxylipin-deficient *Aspergillus flavus* strains on wild-type and *lox3* maize seeds at 3 days after infection.

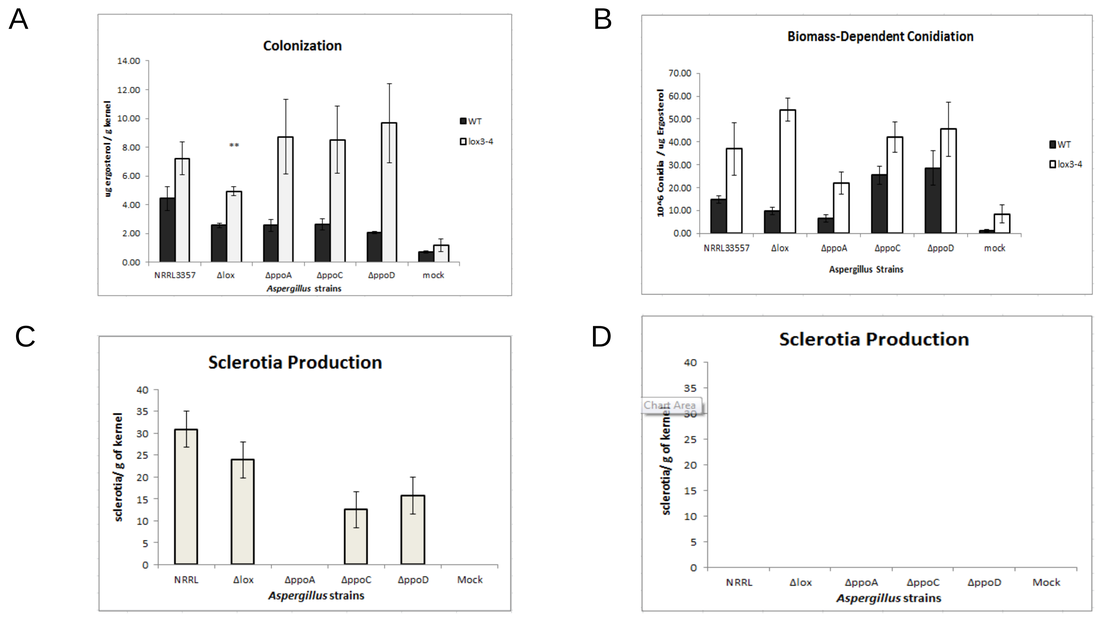
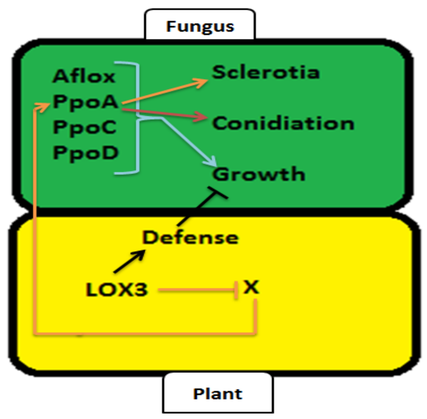


Fig. 1: Colonization **(A)** and conidiation **(B)** of kernel assay taken three **(A, B)** days after infection. Three days after infection, *lox3-4* mutant kernels supported increased colonization and conidiation than wild-type. *Δlox* mutant displayed decreased colonization but increased conidiation on *lox3-4 kernels* compared with other strains. *ΔppoA* displayed decreased conidiation on *lox3-4* mutant kernels. *ΔppoA and ΔppoA* displayed increased conidiation on wild-type kernels. Day five **(C,D)** after infection showed Sclerotia in the *lox 3-4* mutant kernels **(C)** and no sclerotia on wild-type kernels **(D)**.

Conclusion

- LOX3 plays a role in defense against colonization by *Aspergillus flavus*.
- During pathogenic phase, Aflox, PpoA, PpoC, and PpoD may promote growth of *A. flavus*.
- PpoA appears to be required for normal colonization on *lox3-4* mutant kernels.
- LOX3 is a Sclerotia inhibitor
- PpoA is needed for Sclerotia production



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