

FUNCTIONAL STUDY OF LIPOXYGENASE-MEDIATED RESISTANCE AGAINST ASPERGILLUS FLAVUS AND FUSARIUM VERTICILLIOIDES INFECTION IN MAIZE

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Mycotoxin contamination of maize kernels by fungal pathogens *Aspergillus flavus* (Af) and *Fusarium verticillioides* (Fv) is a chronic global challenge impacting food security, health and trade. Current disease management practices are proven inadequate and strategies gearing towards host-mediated resistance can be an effective and sustainable approach to strengthen efforts to control the pathogens. Lipid modification into a diverse array of compounds, oxylipins, are recognized in response of plants to fungal pathogens. *Lipoxygenase* genes (*LOXs*) play a crucial role in the enzymatic oxidation of polyunsaturated fatty acids (PUFAs) into 9(S)- and 13(S)-hydroperoxides that are further metabolized into different oxylipins including jasmonates, oxo- and keto-fatty acids and volatiles. The involvement of maize *LOXs* (*ZmLOXs*) in this respect has been a subject of studies and their genetic manipulation resulted in the alteration of resistance or susceptibility to fungal pathogens in maize. However, the maize genome encodes six 9-LOX and seven 13-LOX isoforms and the specific role of each isoform remains elusive. The current study investigated the role of *ZmLOXs* in host resistance against the fungi Af and Fv using *in silico* and *in planta* approaches. The phylogenetic relationship, sequence

similarity, protein domain structures, and transcript level structural variations were explored by comparing publicly available maize pan-genomes. Furthermore, the role of *ZmLOXs* against *Af* and *Fv* infection was investigated through their expression analysis along with further key genes involved in oxylipin biosynthesis, mycotoxin accumulation and lipid profiles in a *ZmLOX4* mutant line (UFMu*lox4*) together with W22, Mo17 and Tzi18 inbred lines at 3- and 7-days post-inoculation (dpi). *ZmLOX* proteins showed considerable variations in their sequences, functional domain structure and transcript structural variations among the pan-genome members. Among the studied lines, Tzi18 showed the highest resistance to the pathogens coupled with the lowest mycotoxin accumulation, while the mutant, UFMu*lox4*, was highly susceptible to both pathogens with the most elevated content of mycotoxins. *Fv* inoculation determined a stronger induction of *ZmLOXs* and maize *allene oxide synthase* genes as compared to *Af*. Higher constitutive levels of 9-LOXs genes, *ZmLOX1* and *ZmLOX2* and an induction of *ZmLOX4* were recorded in Tzi18, while the upregulation of *ZmLOX1* and *ZmLOX4* observed in Mo17 might have larger active role in resistance against *Fv*. Liquid chromatography-mass spectrometry further revealed an increased accumulation of the linoleic (18:2) derived 9-cyclopentenone, 10-oxo-11-phytoenoic acid (10-OPEA), in *Fv* inoculated kernels of Tzi18 and Mo17, which was previously identified to inhibit fungal growth *in vitro*. Hence, the results confirm the pivotal role of *ZmLOXs* in controlling the resistance mechanisms against these two pathogens.