Functional Study of Lipoxygenase-Mediated Resistance to Fungal Pathogens in Maize and Grapevine.

Mikias Damtew Guche[1,2,3], Stefania Pilati[3], Lorenza Dalla Costa[3], Claudio Moser[3], Graziano Guella[4], Francesco Trenti[4], Alessandra Lanubile[1], Adriano Marocco[1]

1. Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122, Piacenza, Italy.
2. C3A - Centro Agricoltura Alimenti Ambiente, Via Edmund Mach, 1 - 38010 San Michele all'Adige, Italy.
3. Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach 1, San Michele all'Adige, 38098, Italy.
4. Department of Physics University of Trento, Via Sommarive,14 - 38123 Povo, Italy.

BACKGROUND

Mazie is challenged by mycotoxigenic fungi *Aspergillus flavus* and *Fusarium verticillioides*, while powdery mildew caused by *Erysiphe necator* affects grapevine production. Plant lipoxygenase genes (*LOXs*) synthesize oxylipins which play a crucial role in the regulation of defense mechanisms against pathogens through peroxidation of plant membrane lipids. Lipoxygenase pathway mediated plant–pathogen interactions is reported in maize for different pathosystems, while the specific role of the isoforms and their role in grapevine is largely unexplored (Lanubile et al., 2021; Battilani et al., 2018; Gao et al., 2007).

OBJECTIVES

- To study transcriptional and metabolic LOX signatures in resistant and susceptible maize and grapevine genotypes infected by *A. flavus*, *F. verticillioides* and *E. necator* for the identification of LOX isoforms involved in plant defense and the resistance mechanism.
- To perform the functional characterization of promising LOX isoforms by means of CRISPR/Cas9 system.

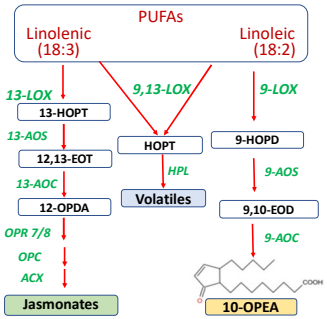
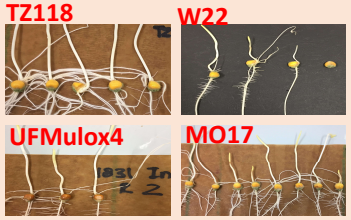


Fig 1. Key oxylipin compounds in the lipoxygenase pathways.

RESULTS

➢ Phenotyping and Transcriptional Expression Analysis in Maize

Roll towel assay (RTA) infection experiment of *A. flavus* showed increased fungal susceptibility in maize inbreds, Mo17 and W22 coupled with higher levels of aflatoxin contamination as compared to Tzi18 and UFMulox4.



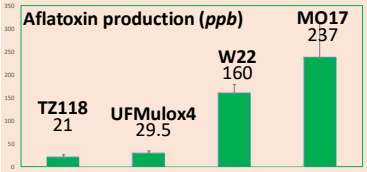
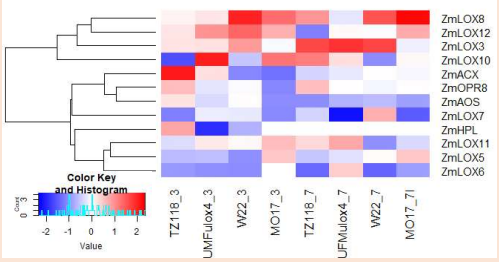
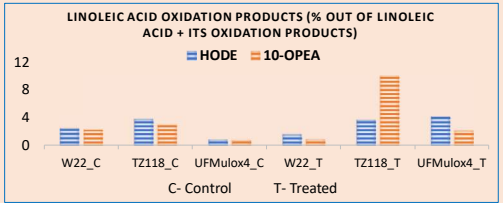


Fig 2. Disease severity (above) and total aflatoxin levels (below) against *A. flavus* infection in the studied maize lines.



Expression Analysis Profile of Maize inbred lines against *A. flavus* infection

Fig 3. Hydroperoxide lyase 1 and acyl-coenzyme A oxidase were induced earlier in Tzi18, whereas 12-oxo-phytodienoic acid (12-OPDA) reductase (*ZmOPR8*) and *ZmLOX10* were upregulated at 7 dpi in infected samples.



Accumulation of the 10-oxo-11-phytoenoic acid (10-OPEA) in the Tzi18 ears after *F. verticillioides* infection in field.

Fig 4. MS/MS quantification of 10-OPEA and HODE in maize kernel samples

Maize and Grapevine LOXs Belong to 9-S and 13-S Lipoxygenase sub-groups

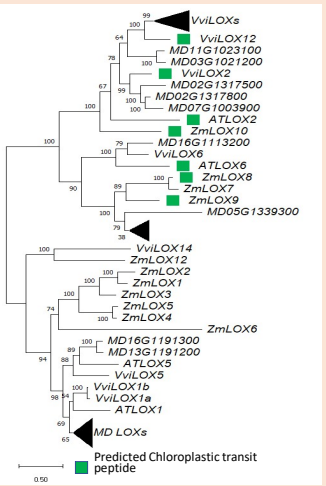


Fig 5. Phylogenetic analysis of grapevine, maize, apple and Arabidopsis LOXs with Maximum likelihood method using 500 bootstrap replication .

➢ Phenotyping and Transcriptional Expression Analysis in Grapevine

***E. necator* infection on grapevine genotypes:**

The pathogen penetrated at 24 hours post inoculation (hpi) in Teroldego (S) with a secondary hyphal growth at 72 hpi. In contrast it's establishment and growth was hampered in NY5XEger99_39 (R) with reactive oxygen species (ROS) accumulation at the infection site

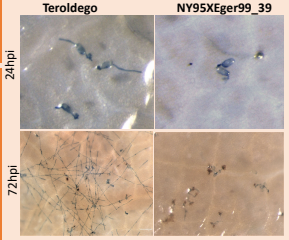


Fig 6. Development of *E. necator* on a (S) and (R) leaves using aniline blue staining with 11.5 times magnification

Grapevine LOXs show different expression profiles in the R and S varieties inoculated with *E. necator:*

VviLOX2 and *VviLOX12* were significantly upregulated at 12 hpi in the infected leaves of the (R) genotype, while upregulation of *VviLOX7* and *VviLOX9* occurred at 48 hpi. Conversely, *VviLOX13* was upregulated in infected leaves of the (S) genotype at 24 hpi.

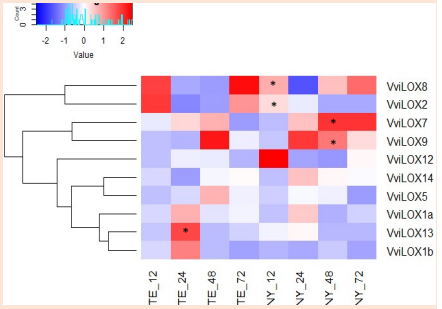


Fig 7. Grapevine LOXs expression analysis at 12,24,48 and 72 hpi in (R) and (S) genotypes leaf infection of *E. necator.* Significant fold changes of infected leaves over the control are indicated in *

MATERIALS AND METHODS

Plant Materials

Maize inbred lines: TZ118, W22, MO17 and UFMulox4

Grapevine: Teroldego (S) and NY95XEger39-99 (R)

Phenotyping

Maize: Seedling rot severity indexing and Aflatoxin analysis with monoclonal antibodies.

Grapevine: Microscopic analysis of stained infected leaves under stereomicroscope

- **Lipid Analysis:** total lipid was extracted with Bligh and Dyer method followed by MS/MS quantification
- **Expression analysis:** was carried with SYBR green based qPCR and delta-delta Ct quantification method

SUMMARY AND CONCLUSIONS

- The phylogenetic analysis revealed a distinct classification of maize and grapevine in to 13S and 9S-LOXs with grapevine LOXs showing a more close relationship with their *Arabidopsis* and apple counterparts.
- The investigated genotypes showed contrasting resistance patterns coupled with different transcriptional modulation trends. Preliminary evidences support the involvement of lipoxygenase derived compound 10-OPEA showing increased accumulation in a resistant maize genotype.

Further field infection experiments are being carried out in maize against *A. flavus* and *F. verticilliodes* to assess *ZmLOXs* expression patterns and associated lipid changes, while gene knock-out (CRISPR/Cas9 system) and over-expression experiments are underway for in-depth functional characterization of promising grapevine LOXs.

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- Battilani P, Lanubile A, Scala V, et al. Oxylipins from both pathogen and host antagonize jasmonic acid-mediated defence via the 9-lipoxygenase pathway in Fusarium verticillioides infection of maize. *Mol Plant Pathol*. 2018;19(9):2162-2176. doi:10.1111/mpp.12690
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