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FUNCTIONAL STUDY OF LIPOXYGENASE-MEDIATED RESISTANCE TO FUNGAL PATHOGENS IN MAIZE AND GRAPEVINE

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Fungal pathogens represent a big concern in maize and grapevine production, two economically relevant crops for Italian agriculture. Maize is challenged by the mycotoxigenic fungi *Aspergillus flavus* and *Fusarium verticillioides*, while grapevine production is affected by *Erysiphe necator*, the causal agent of powdery mildew disease. Plant lipoxygenases genes (*LOXs*) synthesize oxylipins that play a crucial role in the regulation of defense mechanisms against pathogens and influence the outcome of pathogenesis. Their genetic manipulation results in the alteration of plant resistance or susceptibility to certain pathogens. The role of *LOX* in host resistance against these fungi was investigated using *in silico* and *in planta* approaches. The phylogenetic analysis of grapevine and maize *LOXs* including well-characterized *Arabidopsis* and apple homologs showed the separation of 9-*LOX* and 13-*LOX* and several orthologous with a stronger clustering tendency in dicot species. Moreover, several duplication events leading to paralogous groups were inferred. In addition, *in silico* analysis of grapevine transcriptomic data on VESPUCCI platform was carried out to examine grapevine *LOXs* expression pattern against multiple fungal infections. As regards maize, a mutant carrying transposon insertion in the *ZmLOX4* gene (UFMu*lox4*) together with inbred lines W22, Mo17 and Tzi18 were tested for the resistance to Aspergillus seedling rot (ASR) caused by *A. flavus* as well as for the aflatoxin production at 3- and 7-days post inoculation (dpi). Mo17 and W22 showed increased fungal susceptibility and higher levels of aflatoxin contamination compared to Tzi18 and UFMu*lox4*. Moreover, the expression of genes involved in the *LOX* and jasmonic acid pathways (as *ZmLOX4*, hydroperoxide lyase 1 and acyl-coenzyme A oxidase) were induced earlier in Tzi18, whereas the genes 12-oxo-phytodienoic acid (12-OPDA) reductase (*ZmOPR8*) and *ZmLOX10* were upregulated at 7 dpi in infected samples. Interestingly, from lipid analysis an increased accumulation of the compound 10-oxo-11-phytoenoic acid was observed in the Tzi18 ears after *F. verticillioides* infection in field. In grapevine, controlled infection experiment of *E. necator* was performed using a susceptible and a resistant genotype (Teroldego and NY95 x Eger99_39, respectively) and gene expression pattern was analysed in treated and control leaves. Grapevine *VviLOX2* and *VviLOX12* were upregulated at 12 hours post inoculation (hpi), while upregulation of *VviLOX7* and *VviLOX9* occurred at 48 hpi in infected samples of the resistant genotype. Conversely, *VviLOX13* was upregulated in infected samples of the susceptible genotype. Currently, field infection experiments are being

carried out in maize against *A. flavus* and *F. vorticellids* 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*.