

UNIVERSITÀ CATTOLICA DEL SACRO CUORE
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Dottorato per il Sistema Agro-alimentare

Ph.D. in Agro-Food System

cycle XXX

S.S.D: AGR/07

LIPOXYGENASE 4 characterization and CRISPR-Cas9
approach to enhance *Fusarium verticillioides* (*Fv*) resistance in
Zea mays

Candidate: Virginia Maria Grazia Borrelli
Matr. n.: 4411877

Academic Year 2016/2017



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Abstract

Fusarium verticillioides (*Fv*) causes ear rot in maize and contaminates the kernels with fumonisins, a family of mycotoxins that affects feed and food and considered carcinogenic for humans and animals. Several studies were conducted to identify maize genes associated with host plant resistance to *Fv* infection and fumonisin accumulation. It is known that plant lipoxygenase (LOX)-derived oxylipins regulate defense against pathogens and that the host-pathogen lipid cross-talk influences the pathogenesis. In this regard, maize mutants carrying Mu insertions in the *ZmLOX4* gene, the susceptible W22 and the resistant TZI18 lines were tested for *Fv* resistance by the screening method rolled towel assay (RTA). Additionally, the expression profiles of 16 genes involved in the LOX and green leaves volatiles (GLV) pathway were studied and the lipoxygenase activity was investigated in the same lines as well.

Furthermore, the genome editing technology of Clustered Regularly Interspaced Short Palindromic Repeat/associated Cas9 (CRISPR/Cas9) was applied in order to investigate the possible implication of the lipoxygenase gene *ZmLOX6* and the transcription factor *ZmWRKY125* in the resistance mechanisms against *Fv*. The enhanced expression of these genes was previously observed by RNA - Seq experiments in maize resistant genotypes and Genome Wide Association Studies (GWAS) resulted in one SNP significantly associated with *ZmWRKY125*. Moreover, the gene *ZmLOX4* was over-expressed in the line A188 for evaluating a possible improvement of the disease resistance towards *Fv*. The CRISPR cloning was based on a double cloning using two different guides (sgRNA) for one gene target. The constructs under the maize promoter *ZmpUBI* in the binary vector p1609 were transformed into the maize A188 line using *Agrobacterium tumefaciens* mediated transformation. Maize plants edited in the genes *ZmLOX6* and *ZmWRKY125*, and over-expressing *ZmLOX4* will be characterized for *Fv* resistance using rolled towel assay, field assay and for their fumonisin content. Furthermore, the content of jasmonic acid, its derivative metabolites, and oxylipins will be tested, as well as the expression analysis of the main genes involved in the jasmonic acid pathway will be performed.

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