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Poster Communication Abstract – 6.12

THE CRISPR/CAS9 EDITING OF A WRKY GENE AND THE OVEREXPRESSION OF A LIPOXYGENASE GENE FOR IMPROVING PATHOGEN RESISTANCE IN MAIZE

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Fusarium verticillioides (*Fv*) is a major cereal pathogen causing stalk rot and ear rot in maize, negatively affecting crop productivity, and compromising food safety by producing the secondary metabolites fumonisins. Several studies were conducted to identify maize genes associated with host plant resistance to *Fv* infection and fumonisin accumulation. The maize WRKY transcription factors and the lipoxygenases (ZmLOXs) are well recognized as important players in plant defense against pathogens, and it is known that the host-pathogen lipid cross-talk influences the pathogenesis. In this regard, previous RNA-seq experiments reported the enhanced expression of *ZmLOX* genes in maize resistant genotypes and GWAS resulted in one SNP significantly associated with *ZmWRKY125*.

The Clustered Regularly Interspaced Short Palindromic Repeat/associated Cas9 (CRISPR/Cas9) editing of *ZmWRKY125* and the transgenic overexpression of *ZmLOX4* genes were carried out to investigate the possible implication of these two genes in the resistance mechanisms against *Fv*. Before cloning experiments, protein domain conservation and different splicing products have been analyzed comparing homologues and orthologues for both genes.

As regards *ZmWRKY125*, the CRISPR cloning was based on a double cloning using two different guides (sgRNA) for one gene target. *Agrobacterium tumefaciens* mediated transformation was used for introducing the construct under the maize promoter ZmpUBI in the binary vector p1609 in maize A188

line. Mutants from three different transformation events were obtained. For each event, T2 plants will be genotyped to find homozygous for the mutation that in turn will be phenotyped for *Fv* resistance and fumonisin content.

As regards *ZmLOX4*, the gene was cloned under an overexpressed promoter involved in kernel development in the vector L1781, and the same transformation conditions adopted for the CRISPR/Cas9 editing of *ZmWRKY125* were used. Mutants from two different transformation events were obtained. For each event, T2 plants were genotyped in order to find homozygous for the mutation. Homozygous plants will be further evaluated for *Fv* resistance, fumonisin accumulation, oxylipin content as well as for the expression analysis of the main genes involved in the jasmonic acid pathway.