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Biotic and Abiotic Stress Tolerance in Plants: the Challenge for the 21st Century

BOOK OF ABSTRACTS

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Workshop on Biotic and Abiotic Stress Tolerance in Plants: the Challenge for the 21st Century

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Solanum lycopersicum, *Oryza sativa*, *Sorghum bicolor* and *Theobroma cacao* bZIP homologs. Analysis of protein domains, search for conserved motifs and alignment of the bZIP proteins were conducted using the PFAM, MEME, CLUSTALW, respectively. The distribution of the bZIP sequences on cacao chromosomes was obtained on the CacaoGenDB site (<http://cocoagendb.cirad.fr>) using the Interpro number. Protein sequences of the species under study were subjected to a multiple alignment using the software MEGA v5.0, and a Neighbor-joining tree was constructed based on the genetic distance matrix JTT. A classification of the *A. thaliana* bZIP TFs according to biological function was obtained using the TAIR site (<http://www.arabidopsis.org/>). We identified 65 bZIPs in cacao, 75 in tomato, 90 in rice and 89 in sorghum. The bZIPs found in cacao were distributed across all 10 chromosomes (Chr), except on chromosome 6, with higher abundance in Chr 01, 02 and 09. According to the study, some region of QTLs related to cacao resistance to witches' broom was located on Chr 2 and 9, may seek markers within genes in this region and use in plant breeding. The bZIP family proteins were grouped into 13 possible orthologous groups based on the classification performed in *Arabidopsis thaliana*. Five of the cacao bZIP were clustered with proteins highly related to defense to pathogens, being promising candidates for functional study in cacao plants, especially against the witches' broom disease.

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Expression analysis of *Mildew Resistance Locus O* of cacao in resistant and susceptible plants infected by *Moniliophthora perniciosa*

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A *Mildew Resistance Locus O* (*MLO*) cDNA was identified from a library of *Theobroma cacao* L. meristems (Catongo varieties) infected by *Moniliophthora perniciosa*, the fungus responsible for the witches' broom disease. In other plants, the *MLO* gene is characterized as a defense and programmed cell death (PCD) modulator, and for this reason may be a good candidate for functional studies aiming the increase of plant resistance. An *in silico* analysis of the cacao *MLO* (*TcMLO*) using the BLAST, Pfam, InterProScan and ORF-Finder programs, as well as a search on CacaoGenDB databank were performed. *TcMLO* belongs to a multigene family of proteins containing 19 sequences present in the cacao genome: 12, 5 and 2 of them showed one, two and three *MLO* domains, respectively. The complete *TcMLO* sequence (including UTRs and ORF) is 5712 bp in length with 13 exons and 12 introns, and is located on the chromosome 5. The *TcMLO* ORF is 1629 bp in length and encodes a protein with 542 amino acids containing 2 *MLO* domains. The expression of *TcMLO* was analyzed by quantitative PCR (qPCR) in resistant (TSH1188) and susceptible (Catongo) cacao varieties infected or not by *Moniliophthora perniciosa*. Plantlets of cacao were inoculated by the droplet method with a basidiospore suspension of *M. perniciosa*. After inoculation, the plantlets were kept for 24h at 25±2°C and 100% humidity. Apical meristems were harvested in triplicates at 24, 48 and 72 hours after inoculation (hai), and 8, 15, 30, 45, 60 and 90 days after inoculation (dai). Non-inoculated plants (controls) were kept and harvested in the same conditions. The qPCR of *Tc MLO* was obtained using the standard settings of the ABI PRISM 7500 and using the System of Sequence Detection software. The *TcMLO* expression was analyzed with the comparative Ct method ($2^{-\Delta\Delta Ct}$) using malate dehydrogenase and actin as endogenous reference genes, and non-inoculated plants (control) as calibrator. The results showed that *TcMLO* was more expressed in Catongo than in TSH1188 at the early and final stages of disease. In TSH1188, the highest expression of *MLO* was observed at 15 dai. The expression of *TcMLO* at the final stage of the disease in the susceptible infected plants may be related to the PCD events occurring in this variety as a signal for the finalization of the fungus life cycle.

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