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

BOOK OF ABSTRACTS

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POSTER SESSION

S01P01

In silico characterization and expression analysis of a _Selenium-Binding Protein_ gene from cacao

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Witches' broom disease, caused by the fungus *Moniliophthora perniciosa*, is one of the main diseases of cacao (*Theobroma cacao* L.) and is responsible to severe economic losses in the production areas. Recently, expressed sequence tags (ESTs) from cacao-*M. perniciosa* interaction were obtained and differentially defense-related genes expressed during the cacao-*M. perniciosa* interaction were identified. Among them, a Selenium-Binding Protein (*TcSBP)* was found. In other organisms, *SBP* genes are related to the increase of plant defenses against abiotic and biotic stresses; in rice the SBP gene was successfully used to increase the plant resistance to *Magnaporthe grisea* by plant transformation. Here, *in silico* characterization and expression analysis of *TcSBP* were developed. Search on the Cacao Genome Database revealed the presence of only one SBP sequence of 4774 pb in length located on the chromosome 4. The *TcSBP* ORF is 1431 bp in length and encodes a protein of 476 amino acids which does not contain any signal peptide. Prediction of possible post-translational events allowed the identification of several glycosylation, phosphorylation and acetylation sites. The comparison of TcSBP sequence with SBP from other organisms using the BLASTP tool revealed identity from 62% to 91% and allowed the identification of specific conserved regions. The expression analysis of *TcSBP* in meristems of cacao plantlets varieties Catongo (susceptible) and TSH1188 (resistant to *M. perniciosa*), inoculated or not with *M. perniciosa*, was obtained by RT-qPCR using 3 biological and 3 experimental replicates. qPCR analysis of *TcSBP* gene was conducted using the standard settings of the ABI PRISM 7500 and the System of Sequence Detection software. The *TcSBP* relative 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 relative expression of *TcSBP* was significantly increased 8 and 15 days after inoculation in the resistant variety TSH1188 compared to susceptible Catongo. These data suggest the possible role of *TcSBP* in cacao resistance to *M. perniciosa*. This study is the first step to better understand the role of *TcSBP* in cacao resistance as well as for the development of control strategies of the witches' broom disease (e.g. using plant transformation).

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S01P02

Identification, classification and phylogenetic analysis of bZIP proteins from _Theobroma cacao_ for subsequent studies of resistance to witches' broom disease

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Biotic and abiotic stresses are a major factor in decreased production of various cultures around the world. The culture of cacao (*Theobroma cacao*) has been suffering for many years with one of the diseases that most affect their crops, the witches' broom caused by the fungus *Moniliophthora perniciosa.* Studies have identified several transcription factors as promising candidates for developed roles in the regulation and signaling via various stresses in plants. bZIP family proteins are transcription factors (TF) that regulate various physiological and development processes, such as seed maturation, vascular development, and responses to biotic and abiotic stresses. Here, we performed an *in silico* analysis of the bZIP family from *Theobroma cacao* to subsequently develop a comprehensive phylogenetic analysis in four angiosperms species. For this, bZIP protein sequences of *Arabidopsis thaliana* were downloaded from the Phytozome database. BLASTP search was performed to identify

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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S01P03

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 CocoaGenDB 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±2ºC 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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