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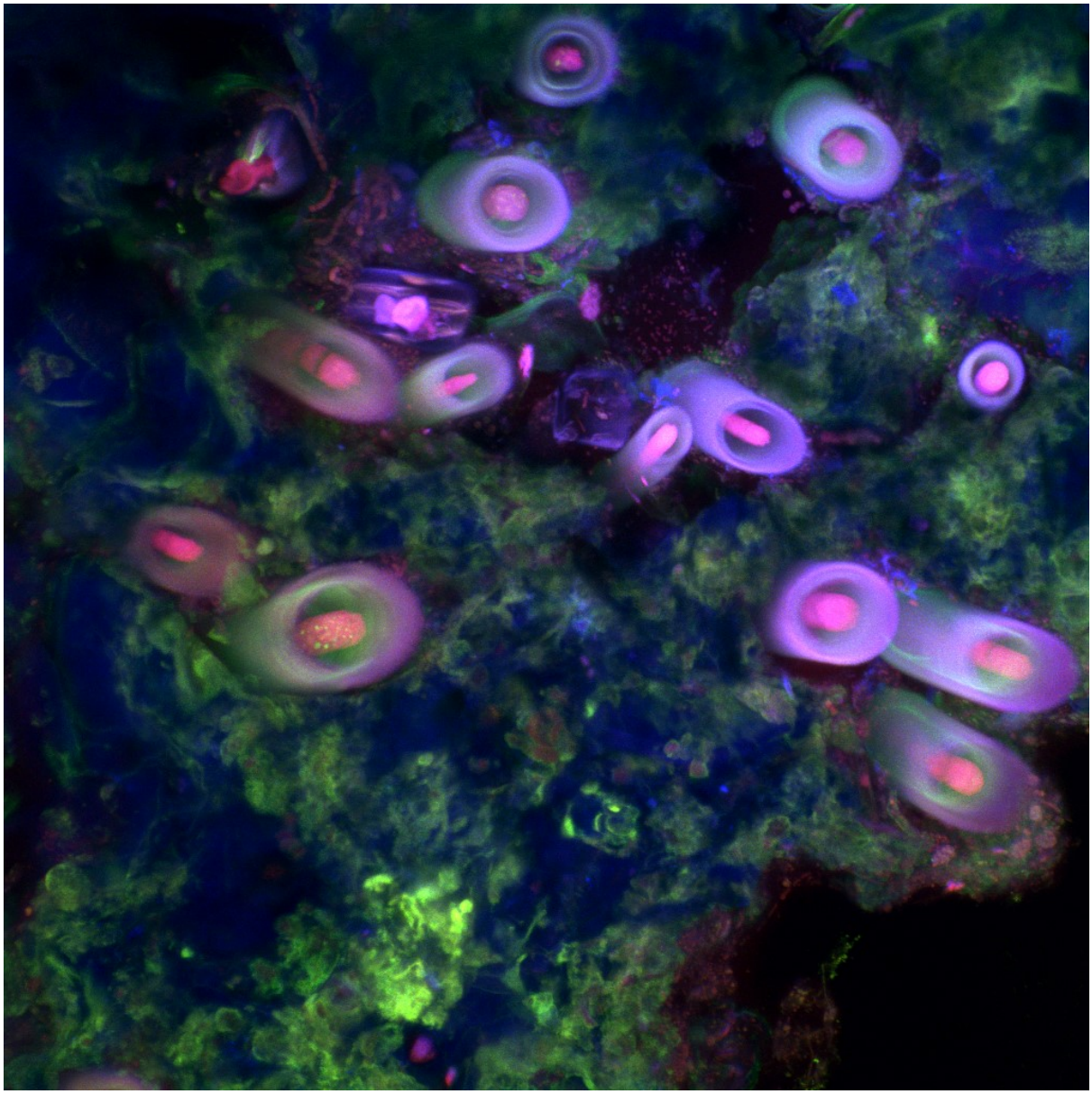

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PL-P02-49
CIS-ELEMENTS ENRICHMENT ANALYSIS ON *Prunus persica* COREGULATED GENES DURING INFECTION WITH *Taphrina deformans*

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Peach Leaf Curl is a widely worldwide distributed disease that affects peach trees and generates millions of dollars losses each year. The causal agent is the dimorphic fungus *Taphrina deformans*. The disease is currently controlled by fungicides. Nevertheless, to reduce risks to health and the environment, the use of strategies based on genetically resistant materials is required. Here, to get insight into host responses against the pathogen, leaves of susceptible and resistant *P. persica* genotypes, DOFI-71.043.018 (DS) and DOFI-84.364.060 (DR), respectively, were inoculated with *T. deformans*. Samples were collected at 0, 12 and 96 hours post inoculation (hpi). RNAseq analysis was used to elucidate transcriptomic peach early responses to the infection with the pathogen. Comparisons between 0 and 12 hpi, and 0 and 96 hpi (DS12-DS0, DR12-DR0, DS96-DS0, DR96-DR0) were made in both genotypes and presented previously. Here, to identify transcription factors responsible for the expression behavior of responsive genes, as a first step, an analysis of their proximal regulatory regions was conducted. For this purpose, several clusters of co-regulated genes were generated. A 1500 bp of upstream sequence from the translational start site of each gene was downloaded from Phytozome database. A transcription factor binding sites enrichment analysis on the promoter regions of the different clusters was performed with cisAnalyzer. The statistical analysis was carried out both against a random control group and against the whole genome with the aid of Rstudio. By this approach, we selected 10 clusters that arranged 720 responsive genes. In each cluster, between 153 and 201 unique motifs related to abiotic and biotic stress, phyohormones, signaling, light and sugar response were identified. On one hand, 82% of the genes induced in all conditions presented JAI1/JIN1BS motif and a 70 % presented JARE motif in their promoters indicating that they may be regulated by jasmonic acid. On the other hand, only one motif, ABRE-RELATED was significantly enriched in the cluster of repressed genes in both genotypes and times. This cis element is known to respond to cold and dehydration stimuli. Moreover, 85% and 95% of up-regulated genes in DS presented motifs associated with auxins and cytokinins responses, respectively. In contrast, the majority of repressed genes showed in their sequence a PR-10ASEBFBS motif, related to biotic stress. In down-regulated DR genes, MYBC1BS (cold-stimulus related) and SRE (sugar-response element) were significantly enriched. In contrast, exclusively up-regulated genes in DR only displayed under-enriched cis-elements. Together, in both genotypes a general down regulation of abiotic stress responses and an induction of jasmonic responsive genes is observed when facing the pathogen. It is interesting the presence of auxin-responsive motifs in DS up-regulated genes since disease symptoms in infected leaves could be related to this hormone.

PL-P03-50
TYR-NITRATION IN MAIZE CDKA;1 RESULTS IN LOWER AFFINITY FOR ATP BINDING

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The cyclin-dependent kinase A (CDKA) is a protein closely involved in the control of eukaryotic cell cycle G1/S and G2/M phase transitions. As part of the protein kinase family, its structure is characterized for presenting highly conserved core sites among species. These sequences include the ATP binding site, the Ser/Thr kinase active site, the PSTAIRE cyclin binding motif and the T-loop or activation loop. Cell cycle arrest and plant growth impairment under abiotic stress have been linked to different molecular processes triggered by increased levels of reactive oxygen and nitrogen species (ROS and RNS). As part of a project to determine possible sites of CDKA protein nitration, embryo axes isolated from *Zea mays* L (maize) were subjected to sodium nitroprusside (SNP) as an inductor of nitrosative conditions. After detecting an increased level of whole protein nitrotyrosination, immunoprecipitation of the CDKA;1 protein revealed that the basal level of nitrotyrosine detected in endogenous CDKA;1 was increased, confirming that CDKA;1 protein is a target for RNS. To decipher specific Tyr-nitration sites, recombinant CDKA;1 was cloned, expressed in *E. coli*, and exposed *in vitro* to peroxynitrite ($ONOO^-$). As well as the endogenous CDKA;1, the recombinant maize *Zm*CDKA;1 was target of tyrosine nitration. The primary sequence of CDKA;1, identified as P23111 (CDC2_MAIZE, cell division control protein 2 homolog - *Zea mays*) in UniProt database, contains 294 amino acids, 12 of which are Tyr residues. Analysis using GPS-YNO2 software predicted Y4 and Y15 as target of nitration, both with high scores, but also predicted Y19 and Y270, with medium score. Mass spectrometry analysis of recombinant nitrated CDKA;1 (*Zm*CDKA;1-NO_2) showed Tyr 15 and Tyr 19, located at the ATP-binding site, as selective targets for nitration. Kinase-ATP interaction of nitrated and non-nitrated recombinant protein was analyzed by using TNT-ATP fluorescent analog. Comparative spectrofluorometric measurements demonstrated a reduction of *Zm*CDKA;1-NO_2 affinity for ATP. The difference in ATP affinity between the two forms -*Zm*CDKA;1 and *Zm*CDKA;1-NO_2- could be attributable to steric restrictions since nitrotyrosine is more spacious than tyrosine. Nonetheless, other physico-chemical properties of Tyr are modified by the introduction of the nitro group into its aromatic ring that can also contribute to the functional change of the CDKA;1. From these results, we conclude that Tyr-nitration in CDKA;1 could act as an active and rapid modulator of cell cycle progression during redox stress. In this sense, this mechanism could contribute to plant growth arrest caused by abiotic stress conditions, allowing them to trigger adaptive responses to cope with the stress.