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Proteomic profiling of maize chromatin-associated proteins modulated by pathogen attack

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The genomic DNA of eukaryotes is packaged into chromatin, which controls access of DNA to transcriptional machinery as well as serving as a binding scaffold for chromatin-associated proteins. The differential recognition and subsequent binding of these chromatin-associated or "reader" proteins to various states of post-translationally modified histones has been linked to many key developmental and physiological processes. HC-toxin is a cyclic tetra-peptide effector molecule secreted by *Cochliobolus carbonum* that functions as a histone deacetylase inhibitor (HDACi) and is the virulence factor which enables successful infection of susceptible host plants. Using mass spectrometry-based acetylome profiling we have determined that treatment of susceptible corn plants with exogenous HC-toxin or virulent *C. carbonum* results in hyper-acetylation of maize histone H4 at lysine residues 5, 8, 12, and 16 (K5/8/12/16). We hypothesize that HC-toxin-induced hyper-acetylation of K5/8/12/16 on histone H4 leads to recruitment of specific host reader(s) that act to potentiate pathogen virulence. To identify these differential proteins, we are employing a peptide pull-down screen using synthesized peptides of non-acetylated and K5/8/12/16 histone tails to probe and capture reader proteins from maize nuclear extract. Once the differential reader proteins from the peptide baits have been obtained, we will utilize mass spectrometry-based proteomic profiling to identify and characterize the sets of readers that have been recruited to bind our synthesized histone baits. This approach will identify potentially novel reader proteins and set up future experiments examining downstream targets of these reader proteins through various protein and DNA interaction assays. The elucidation of both direct and downstream components of histone mark targeting by reader proteins will provide knowledge of how pathogens can modulate their host in an epigenetic matter thus possibly leading to the creation of molecular tools to counteract this virulence mechanism.

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Rice *Phosphorus Starvation Tolerance 1* gene and its sorghum and maize homologs improve root and vegetative growth in transgenic tobacco

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Low phosphorus (P) availability in soil is a major constraint for crop production in tropical regions. PHOSPHORUS-STARVATION TOLERANCE1 (OsPSTOL1) is a protein kinase that enhances root surface, P acquisition and grain yield in rice under P deficiency. Sorghum homologs of *OsPstol1* were identified by association mapping in two sorghum association panels phenotyped for P uptake, root system morphology and architecture in hydroponics and grain yield and biomass accumulation under low-P conditions, in Brazil and/or in Mali. Maize and sorghum candidate genes co-localized with quantitative trait loci (QTL) for traits underlying root morphology and dry weight accumulation under low P via QTL mapping. In order to validate the function of these genes, rice *OsPstol1* (control) and its maize (ZmPSTOL3.06, ZmPSTOL8.02 and ZmPSTOL8.05_1) and sorghum (Sb07g002840, Sb03g031690 and Sb03g006765) homologs were cloned downstream of ubiquitin promoter in pMCG1005 vector, using *Bar* gene as a selective marker. Tobacco *Petit havana* plants were genetically transformed via *Agrobacterium tumefaciens* EHA101 strain and regenerated from selected callus in shooting and rooting medium supplemented with 100 mg/ml of Tioxin and 1 mg/L of Phosphinothricin. PCR with gene specific (~700 bp) and Bar (~400 bp) primers confirmed the presence of *Pstol1* genes in tobacco plants. Several plants presented one copy of the trangene, and those that also showed overexpression of the transgene were selected for evaluation under low P conditions. Overexpression of *Pstol1* genes significantly enhanced vegetative plant growth and root surface area on low P, indicating that these genes act in a similar manner to *osPstol1* gene in rice plants.

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