SI MATERIALS and METHODS

Proteomics

Approximately 2 grams frozen tissue was ground for 15 minutes to a fine powder in liquid nitrogen by mortar/pestle and then transferred to a 50ml conical tube. Proteins were precipitated and washed with 50ml -20 °C methanol containing 0.2mM Na_3VO_4 three times, then 50ml -20 °C acetone three times. Protein pellets were aliquoted into four 2ml Eppendorf tubes and dried in a vacuum concentrator at 4 °C.

Protein pellets were suspended in 1 ml extraction buffer (0.1% SDS, 1mM EDTA, 50mMHepes buffer, pH7). Cysteines were reduced and alkylated using 1 mM Tris (2-carboxyethyl)phosphine (Fisher, AC36383) at 95 °C for 5 minutes, then 2.5 mM iodoacetamide (Fisher, AC12227) at 37°C in the dark for 15 minutes, respectively. Protein was quantified using a Bradford assay (Pierce). Proteins were digested with trypsin overnight (Roche, 03 708 969 001, enzyme:substrate w:w ratio = 1:100). A second digestion was perform the next day for 4 hours (enzyme:substrate w:w ratio = 1:100). Digested peptides were purified on a 500 mg Waters Oasis MCX cartridge to remove SDS. Peptides were eluted from the MCX column with 4 ml 50% isopropyl alcohol and 400mM NH4HCO3 (pH 9.5) and then dried in a vacuum concentrator at 4 °C. Peptides were resuspended in 0.1% formic acid and further purified on a 500 mg Sep-Pak C18 columns (Waters). Peptide amount was quantified using the Pierce BCA Protein assay kit.

We obtained higher than 95% iTRAQ labeling efficiency by treating100 µg of non-modified peptides with one tube of iTRAQ reagent for 2 hours at room temperature. Mock, 100 µM HC-toxin, Tox-, or Tox+ samples were individually labeled with iTRAQ tags 114, 115, 116, and 117, respectively. Labeled samples were dried down in a vacuum concentrator and resuspended in H_2O. Samples tagged with the 4 different iTRAQ reagents were pooled together.

Acetylated peptides were enriched using 2 mg of acetyl lysine antibody immobilized on agarose beads (ImmuneChem-ICP0388), added to ~10 mg of maize peptides in 50 mM Tris-HCL pH 7.4. Samples for each biological replicate series (ex. Mock_Rep1, HC-toxin_Rep1, Tox-_Rep1, and Tox+_Rep1) were processed in parallel. The antibody-peptide mixture was incubated for 1 hr with rotation at 4 °C on a 0.2 µM centrifugal device (Microsep MCPM02C68). Following incubation the beads were washed 3 times with 50 mM Tris-HCL pH 7.4 and then acetylated peptides were eluted using 1.5 ml of 0.1% trifluoracetic acid. The antibody-conjugated beads were washed 2 times with 50 mM Tris-HCL pH 7.4 and then used for a second round of immunoprecipitation of the same sample (i.e. the original flow-through). The 2 enrichments for a given sample were then pooled and passed over a Sep-Pak C18 column (Waters WAT054960).

Chromatography and electrospray ionization was done using an Agilent 1200 HPLC system (Agilent Technologies) which delivered a flow rate of 600 nL min^{-1} to a 3-phase capillary chromatography column through a splitter. Using a custom pressure cell, 5 µm

Zorbax SB-C18 (Agilent) was packed into fused silica capillary tubing (250 µm ID, 360 µm OD, 30 cm long) to form the first dimension reverse-phase column (RP1). A 5 cm long strong cation exchange (SCX) column packed with 5 µm PolySulfoethyl (PolyLC) was connected to RP1 using a zero dead volume 1 µm filter (Upchurch, M548) attached to the exit of the RP1 column. A fused silica capillary (250 µm ID, 360 µm OD, 20 cm long) packed with 2.5 µM C18 (Waters) was connected to SCX as the analytical column (RP2). The electrospray tip of the fused silica tubing was pulled to a sharp tip with the inner diameter smaller than 1 µm using a laser puller (Sutter P-2000). The peptide mixtures were loaded onto the RP1 column using the custom pressure cell. A new set of columns was used for each LC-MS/MS analysis. Peptides were first eluted from the RP1 column to the SCX column using a 0 to 80% acetonitrile gradient for 150 minutes. The peptides were then fractionated by the SCX column using a series of 28 step salt gradients for non-modified iTRAQ profiling (0, 20, 40, 50 55, 60, 62.5, 65, 67.5, 70, 72.5, 75, 77.5, 80, 82.5, 85, 87.5, 90, 92.5, 95, 97.5, 100, 120, 150, 180, 200, 500, 1000 mM ammonium acetate) or 19 steps for acetylome profiling (0, 10, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 1000 mM ammonium acetate for 20 minutes), followed by high-resolution reverse phase separation using an acetonitrile gradient of 0 to 80% for 120 minutes.

Spectra were acquired using an LTQ Velos linear ion trap tandem mass spectrometer (Thermo Electron Corporation, San Jose, CA) employing automated, data-dependent acquisition. The mass spectrometer was operated in positive ion mode with a source temperature of 250 °C. The full MS scan range of 400-2000 m/z was divided into 3 smaller scan ranges (400-800, 800-1050, 1050-2000) to improve the dynamic range. For iTRAQ (non-modifed peptides) runs both CID (Collision Induced Dissociation) and PQD (Pulsed-Q Dissociation) scans of the same parent ion were collected for protein identification and quantitation. Each MS scan was followed by 4 pairs of CID-PQD MS/MS scans of the most intense ions from the parent MS scan. For acetylated peptide samples only CID was used. A dynamic exclusion of 1 minute was used to improve the duty cycle of MS/MS scans. About 20,000 MS/MS spectra were collected for each salt step fractionation.

The raw data were extracted and searched using Spectrum Mill v3.03 (Agilent). The CID and PQD scans from the same parent ion were merged together. MS/MS spectra with a sequence tag length of 1 or less were considered to be poor spectra and were discarded. The remaining MS/MS spectra were searched against the maize B73 RefGen_v2 5b Filtered Gene Set. The enzyme parameter was limited to fully tryptic peptides with a maximum miscleavage of 1. All other search parameters were set to default settings of Spectrum Mill (carbamidomethylation of cysteines, iTRAQ modification, or K-Ac). A concatenated forward-reverse database was constructed to calculate the in-situ false discovery rate (FDR). Cutoff scores were dynamically assigned to each dataset to maintain the false discovery rate less than 0.1% at the peptide level. Proteins that share common peptides were grouped to address the database redundancy issue. The proteins within the same group shared the same set or subset of unique peptides.

iTRAQ intensities were calculated by summing the peptide iTRAQ intensities from each

protein group. Peptides shared among different protein groups were removed before quantitation. Isotope impurities of iTRAQ reagents were corrected using correction factors provided by the manufacturer (Applied Biosystems). Median normalization was performed to normalize the protein iTRAQ reporter intensities in which the log ratios between different iTRAQ tags (115/114, 116/114, 117/114) are adjusted globally such that the median log ratio is zero. Quantitative analysis was performed on the normalized protein iTRAQ intensities. Protein ratios between the mock and each treatment were calculated by taking the ratios of the total iTRAQ intensities from the corresponding iTRAQ reporter. Protein ratios were then $\log_2$ converted. Proteins that significantly changed in each treatment, relative to mock, were determined using t-tests (two tailed, paired). Proteins with more than 1.5-fold change and P-value less than 0.05 were considered significantly changed in abundance. For acetylation experiments spectral counting was used for quantification as previously described (2, 3). Acetylation sites and peptides were considered differentially abundant if they changed 2-fold and had a P-value relative to mock of 0.05 or less.

SUPPLEMENTAL REFERENCES

1. Chintamanani S, Multani DS, Ruess H, Johal GS (2008) Distinct Mechanisms Govern the Dosage-Dependent and Developmentally Regulated Resistance Conferred by the Maize Hm2 Gene. *Mol Plant-Microbe Interact* 21(1):79–86.
2. Liu H, Sadygov RG, Yates JR (2004) A Model for Random Sampling and Estimation of Relative Protein Abundance in Shotgun Proteomics. *Anal Chem* 76(14):4193–4201.
3. Walley JW, et al. (2013) Reconstruction of protein networks from an atlas of maize seed proteotypes. *Proc Natl Acad Sci U S A* 110(49):E4808–4817.

SUPPLEMENTAL FIGURE and DATASET LEGENDS

Figure S1. GO overrepresentation analysis. GO Biological Process terms overrepresented ($P < 0.05$) among proteins induced by HC-toxin, Tox-, or Tox+ treatment. White boxes represent terms that are not overrepresented.

Dataset S1. Non-modified Proteome

Dataset S2. Acetylome

Dataset S3. GO Biological Process terms overrepresentation among acetylated peptides altered by HC-toxin or pathogen treatment.

Figure S1. GO overrepresentation analysis. GO Biological Process terms overrepresented ($P < 0.05$) among proteins induced by HC-toxin, Tox-, or Tox+ treatment. White boxes represent terms that are not overrepresented.