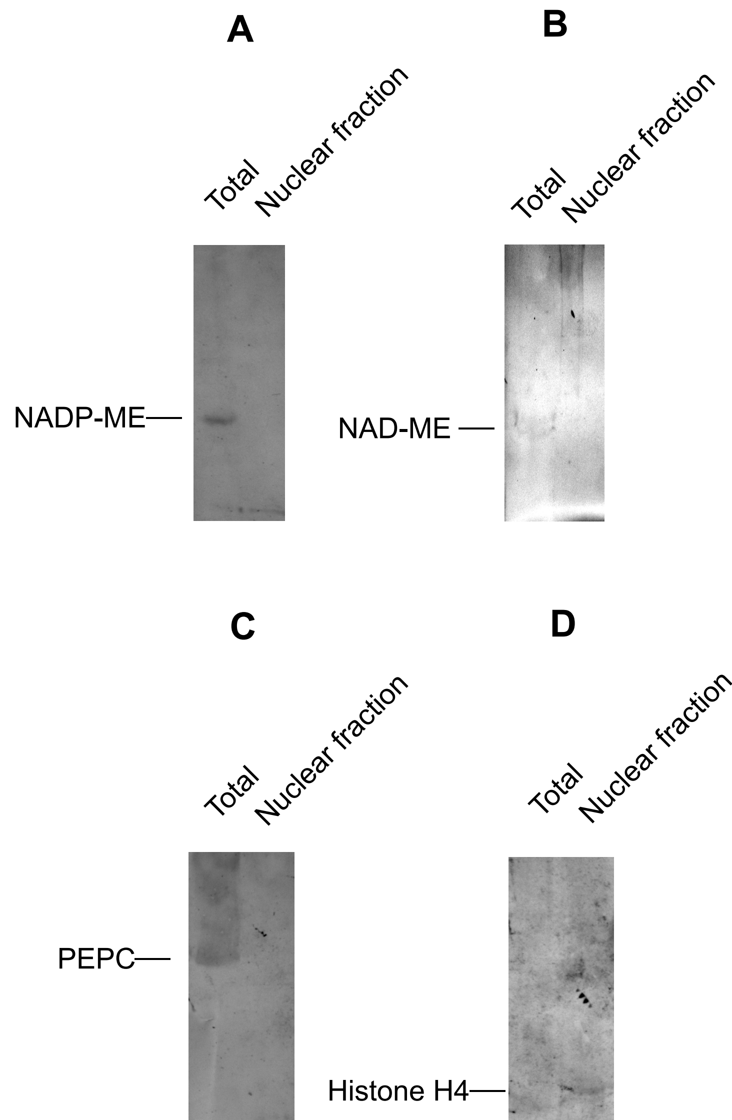
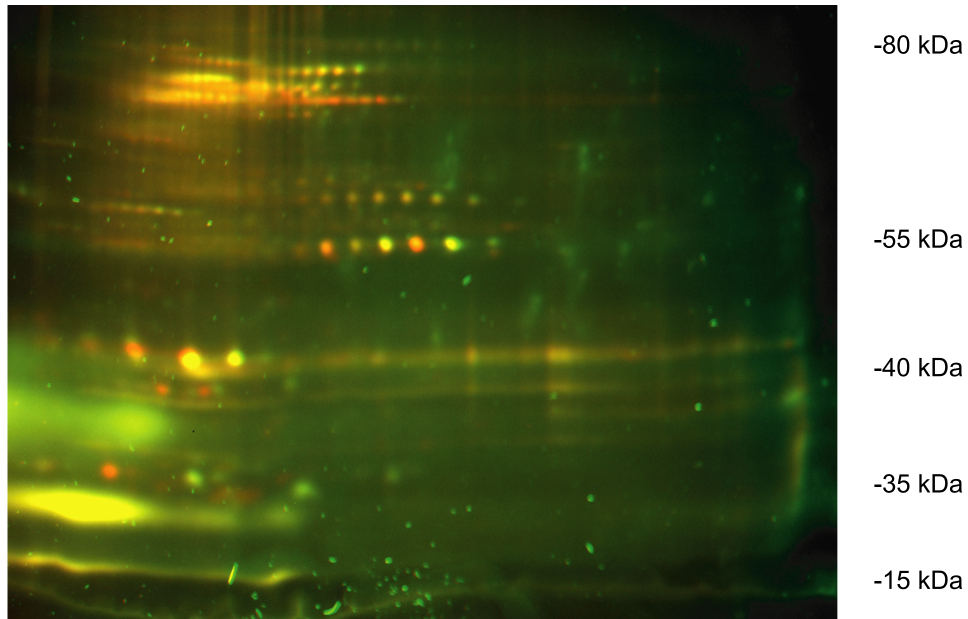
Supplemental Data. Casati et al. (2008). Histone Acetylation and Chromatin Remodeling are Required for UV-B Dependent Transcriptional Activation of Regulated Genes in Maize.




Supplemental Figure 1. Immunoblot analysis of total cellular and nuclear proteins resolved by 10% SDS-PAGE.

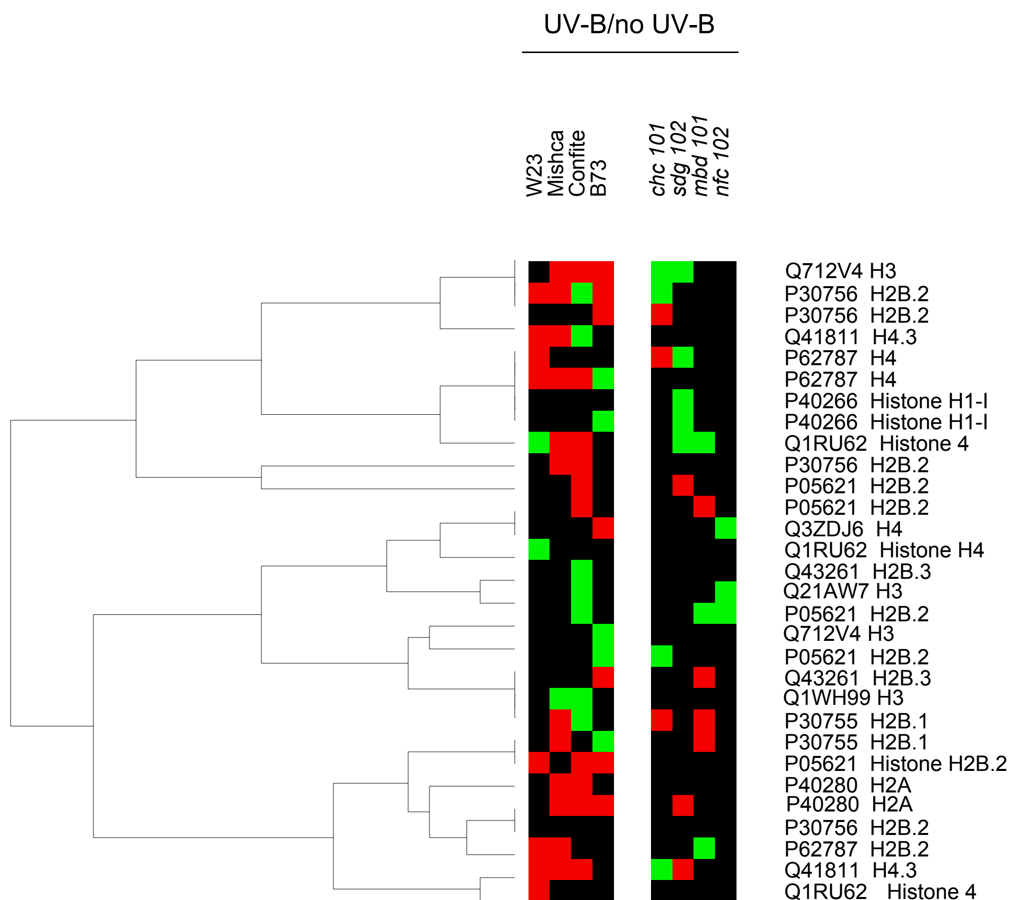
Analysis was performed using antibodies against NADP-malic enzyme (NADP-ME, A); NAD-malic enzyme (NAD-ME, B); phosphoenolpyruvate carboxylase (PEPC, C); and Histone H4 (D). Thirty micrograms of total soluble proteins or 5 μg of nuclear proteins were loaded in the designated lanes. Both NADP-ME and PEPC are highly expressed leaf proteins, but neither antibody reacted with nuclear fractions. Antibodies against histone H4 reacted only with the nuclear fractions. Although H4 was not detected in 30 μg total protein extracts, when 60 μg were used, a faint reactive band was observed (not shown), demonstrating the low abundance of H4 in total protein compared to photosynthetic proteins such as NADP-ME and PEPC.

3 10



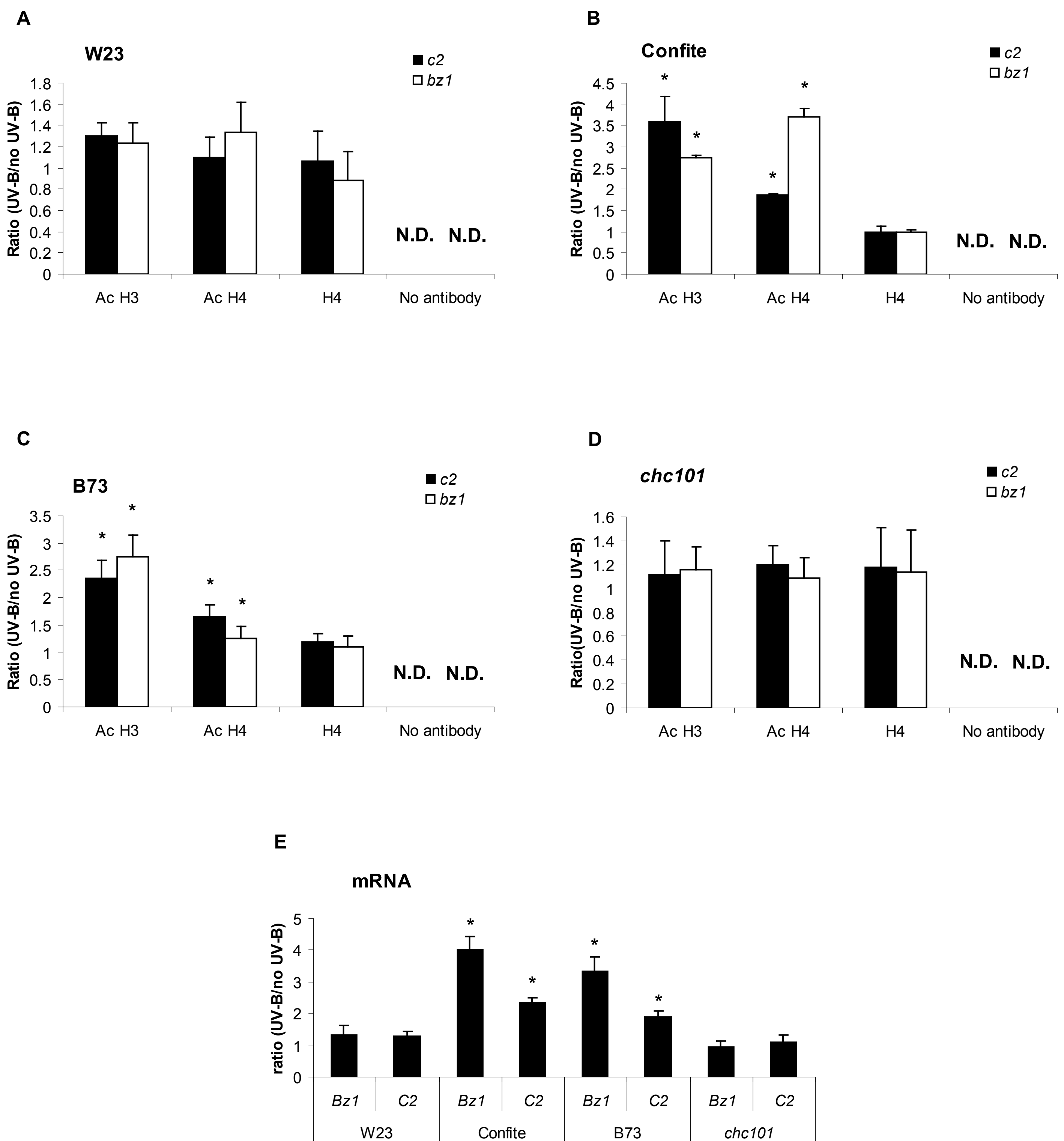

Supplemental Figure 2. Analytical 2D gel of Confite leaf nuclei from control plants (no UV-B) or plants supplemented with UV-B for 8 h.

Alexa 532 image of samples without UV-B (green), Alexa 610 image of samples supplemented with UV-B for 8 h (red). 75 µg of each labeled protein sample was loaded onto the gels. The molecular masses of marker proteins are indicated on the right of the gel, and pH values are indicated on the top.



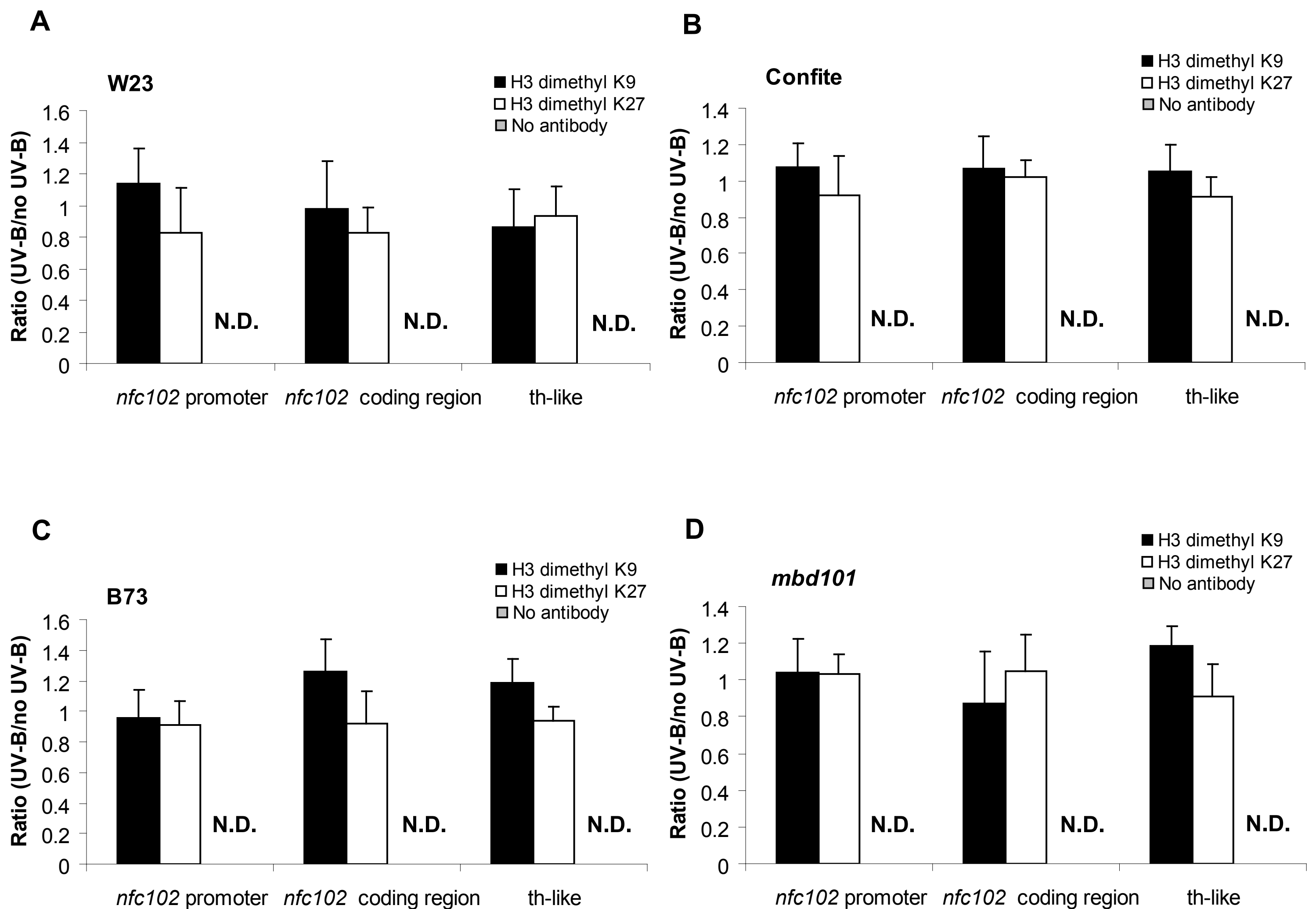

Supplemental Figure 3. Hierarchical cluster analysis of histones after 8 h of UV-B exposure.

The same dataset described in Figure 1B was employed, selecting the identified histones. Proteins from leaves under no UV-B were used as references. Clustering was performed according to Eisen et al. (1998). Red color indicates higher protein levels than the reference, green indicates lower protein levels than the reference, and black indicates no significant change.



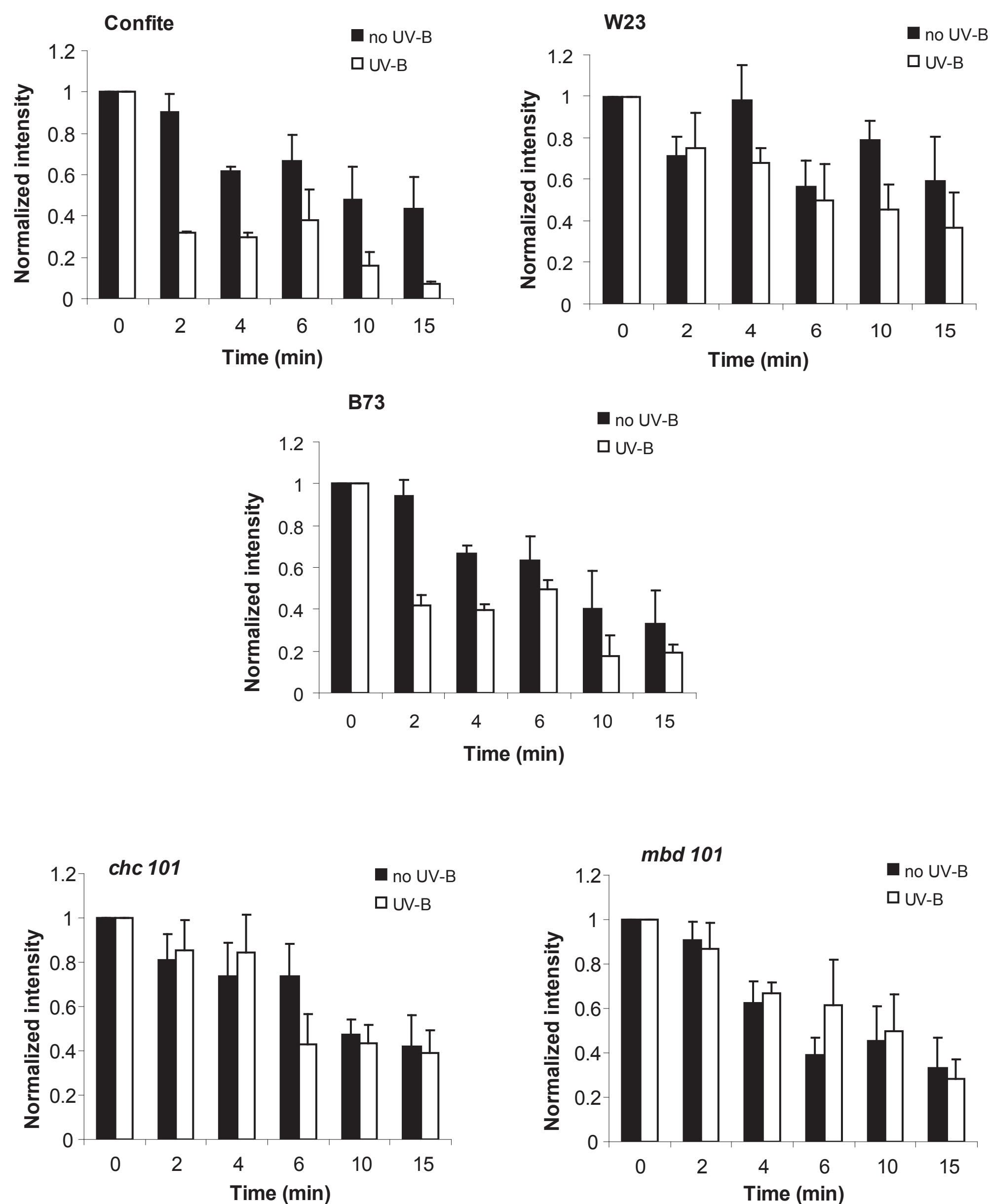

Supplemental Figure 4. Acetylation state of histones H3 and H4 associated with the c2 and bz1 loci.

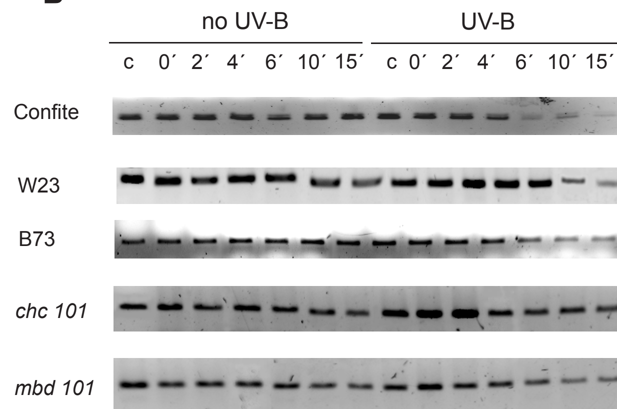
ChIP assays utilized antibodies specific for acetylated histone H3 (Ac H3), acetylated H4 (Ac H4), or total histone H4 (H4) in nuclei prepared from W23 (A), Confite (B), B73 (C), or chc101 (D) after UV-B treatment (UV-B) and control conditions (no UV-B). The immunoprecipitates were analyzed for the presence of promoter sequences of the target genes c2 and bz1 by qPCR. ChIP data was normalized to input DNA before immunoprecipitation. The PCR was also performed with samples that were incubated in the absence of any antibody as a control (No antibody). Real-time RT PCR analysis (E) assessed transcript levels (mRNA) in control and UV-B treated plants. 50 ng of cDNA after reverse transcription of RNA were used. All measurements were done at least in triplicate using each of three independent preparations. Error bars are standard errors. Statistically significant differences between the UV-B and control treatments (t-Student test, significance level P=0.05) are labeled with *. N.D.: not detected.




Supplemental Figure 5. Methylation state of histone H3 associated with the nfc102 loci in K9 and K27 residues.

ChIP assays utilized antibodies specific for dimethyl K9 and dimethyl K27 histone H3 in nuclei prepared from W23 (A), Confite (B), B73 (C), or mbd101 (D) after UV-B treatment (UV-B) and control conditions (no UV-B). The immunoprecipitates were analyzed for the presence of promoter and transcribed sequences of nfc102 and a transcribed sequence of a control gene that is not UV-B-regulated (th-like) by qPCR. ChIP data was normalized to input DNA before immunoprecipitation. The PCR was also performed with samples that were incubated in the absence of any antibody (No antibody) as a control. All measurements were done at least in triplicate using each of three independent preparations. Error bars are standard errors. There were not statistically significant differences between the UV-B and control treatments (t-Student test, significance level P=0.05). N.D.: not detected.




Supplemental Figure 6. Accessibility of the promoter regions of the nfc102 gene to micrococcal nuclease.

Nuclei from UV-B treated (UV-B) and control (no UV-B) plants of Confite, W23, B73 plus the chc101 and mbd101 RNAi transgenic lines were incubated with nuclease for 2, 4, 6, 10, or 15 min. The amount of DNA sequence remaining intact at each time was determined by quantitative (A) and standard PCR (B). (A) Amplicons were normalized to time zero and plotted against time to compare degradation rates in the samples listed. (B) PCR products were separated in 2% agarose gels. Control (c) represents a reaction without added nuclease.

**Supplemental Table 1.** Samples compared directly in the proteomic analysis in the same or different gels.

| Comparison | Details of comparison | Samples |
|---|---|---|
| UV-B Treatment | Control vs UV-B treated samples | -W23 *b, pl*<br>-Confite<br>-Mishca<br>-B73<br>-B73-RNAi *chc101*<br>-B73-RNAi *mbd101*<br>-B73-RNAi *sdg102*<br>-B73-RNAi *nfc102* |
| Lines | Genotype vs W23 | -Confite vs W23<br>-Mishca vs W23<br>-B73 vs W23 |
| | RNAi line vs B73 | -B73 vs RNAi *chc101* siblings<br>-B73 vs RNAi *mbd101* siblings<br>-B73 vs RNAi *sdg102* siblings<br>-B73 vs RNAi *nfc102* siblings |