Additional File 6  The predicted amino acid sequence of HaDREB2 shows unique features that are conserved in some DREB2 factors, but not in AtDREB2A.

Alignment, using the Clustal X program [Larkin et al., Bioinformatics 23(21): 2947-2948 (2007)], of the predicted HaDREB2 protein (Ha) with other subgroup A-2 DREB proteins [9]: DvDREB2A (ABR23508, Dv), LeDREB1 (AAN77051, Le), CrORCA1 (CAB93939, Cr), and AtDREB2A (At). The highly conserved AP2 domain is underlined. Within the AP2 domain, the Valine-14 and Aspartic acid-19 residues characteristic of DREB proteins [9] are indicated by dots. Some AP2 domain residues conserved in the HaDREB2, DvDREB2A, LeDREB1, and CrORCA1 proteins, but not in AtDREB2A, are indicated by filled triangles. On the AtDREB2A protein, and next to AP2 domain, the sequences (amino acids 136 to 165 [18]) involved in its instability are boxed. These sequences are not present in HaDREB2, DvDREB2A, LeDREB1, or CrORCA1 proteins. The carboxyl-
terminal region is less conserved and enriched in acidic residues that could participate in transcriptional activation [10, 18]. On the alignment we indicate a carboxyl-terminal motif that is conserved in all five proteins (box with thin line). This motif is included in the sequences required for transcriptional activation by AtDREB2A in protoplast assays; the 81-amino acid, terminal, fragment between positions 254 and 335 in AtDREB2A [18]. Similarly, transcription activation by ZmDREB2A required the carboxyl-terminal 63 amino acids. In this case the necessary fragment included amino acid motifs specific for mono-cot DREB2 proteins [14]. We also indicate a second motif conserved in the HaDREB2, DvDREB2A, LeDREB1, and CrORCA1 proteins, but not in AtDREB2A (box with thick line). Therefore, DREB factors from different plant groups could use dissimilar motifs for transcriptional activation; these motifs would be located in the carboxyl-terminal 80-90 amino acids. The conserved amino acid residues are highlighted with black (when identical) or gray (when similar) background. Dashes show gaps in the amino acid sequences introduced to optimize alignment.