the plant journal

SEB Society for Experimental Biology

The Plant Journal (2011) **66**, 844–851 doi: 10.1111/j.1365-313X.2011.04550.x

Identification of the MBF1 heat-response regulon of *Arabidopsis thaliana*

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Received 29 June 2010; revised 10 February 2011; accepted 14 February 2011; published online 4 April 2011.

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SUMMARY

Brief periods of heat stress of even a few days can have a detrimental effect on yield production worldwide, causing devastating economic and societal impacts. Here we report on the identification of a new heat-response regulon in plants controlled by the multiprotein bridging factor 1c (MBF1c) protein of *Arabidopsis thaliana*. Members of the highly conserved MBF1 protein family function as non-DNA-binding transcriptional co-activators involved in regulating metabolic and development pathways in different organisms from yeast to humans. Nonetheless, our studies suggest that MBF1c from Arabidopsis functions as a transcriptional regulator which binds DNA and controls the expression of 36 different transcripts during heat stress, including the important transcriptional regulator DRE-binding protein 2A (DREB2A), two heat shock transcription factors (HSFs), and several zinc finger proteins. We further identify CTAGA as a putative response element for MBF1c, demonstrate that the DNA-binding domain of MBF1c has a dominant-negative effect on heat tolerance when constitutively expressed in plants, and show that constitutive expression of MBF1c in soybean enhances yield production in plants grown under controlled growth conditions without causing adverse effects on growth. Our findings could have a significant impact on improving heat tolerance and yield of different crops subjected to heat stress.

Keywords: heat stress, transcription, regulon, multiprotein bridging factor 1, heat shock transcription factor, heat shock protein.

INTRODUCTION

The heat stress response is a highly conserved response involving multiple pathways, regulatory networks, and cellular compartments (Kotak *et al.*, 2007). At least two key components coordinating this response in plants have been identified. They include a network of heat shock transcription factors (HSFs), and the recently identified multiprotein bridging factor 1c (MBF1c) protein (von Koskull-Döring *et al.*, 2007; Suzuki *et al.*, 2008). The function of HSFs as DNA-binding transcriptional regulators that control the heat stress response by binding to a defined heat shock response element (HSE) has been established in multiple biological systems (Shamovsky and Nudler, 2008). In contrast, MBF1 was only recently identified as a key regulator of thermotolerance in plants, and its mode of action is largely unknown (Suzuki *et al.*, 2008). MBF1 is a highly conserved protein thought to function as a non-DNA-binding transcriptional co-activator involved in different developmental and metabolic pathways in different organisms, ranging from yeast to humans (Takemaru *et al.*, 1998; Kabe *et al.*, 1999; Liu *et al.*, 2003). In the model plant *Arabidopsis thaliana* MBF1 is encoded by three different genes (*Mbf1a*, *b*, and *c*; Tsuda and Yamazaki, 2004) of which *Mbf1c* (*At3g24500*) is required for thermotolerance (Suzuki *et al.*, 2008). Genetic analysis of MBF1c in Arabidopsis using gain- and loss-of-function mutants demonstrated that MBF1c functions upstream to salicylic acid, ethylene, and trehalose signaling during heat stress (Suzuki *et al.*, 2005, 2008). MBF1c is not, however, required for the expression of many heat shock proteins (HSPs) or ascorbate peroxidase 1 during heat stress (Suzuki *et al.*, 2005, 2008). Nonetheless, its mode of action during heat stress remains unknown. Here we show that MBF1c functions as a transcriptional regulator which most

likely binds DNA and controls a regulon of 36 different transcripts during heat stress, including the important transcriptional regulator DRE-binding protein 2A (DREB2A; Schramm *et al.*, 2007), two HSFs, and several zinc finger proteins. We further identify a putative response element for MBF1 and test it using gel-shift and yeast one-hybrid assays, as well as demonstrate that ectopic expression of the DNA-binding domain of MBF1c in transgenic plants has a dominant-negative effect on heat tolerance. Our study identifies a new heat stress-response regulon in plants and opens the way for the development of new strategies to enhance thermotolerance in different crops.

RESULTS

Identification of the MBF1c regulon

To identify transcripts that function downstream to MBF1c during heat stress we subjected wild type and *mbf1c* mutants (Suzuki *et al.*, 2008) to heat stress and analyzed their transcriptome using ATH1® DNA chips at 0, 20, and 60 min following the application of heat stress. For each time point we used three technical repeats (three different chips each representing 60 different plants), and the entire experiment was conducted with three biological repeats. Statistical analysis identified 36 different transcripts significantly less elevated in *mbf1c* compared with wild type during heat stress (Tables 1, S1, S3 and S4 in Supporting Information), and several of these transcripts were confirmed by quantitative real-time PCR (qPCR) analysis in wild-type and *mbf1c* plants subjected to heat stress (Figure 1), and in wild-type and *mbf1c* plants complemented by expression of MBF1c under the control of the CaMV35S promoter (Suzuki *et al.*, 2008) subjected to heat stress (Figure S1).

The majority of transcripts identified by our analysis were also identified in transcriptome databases as elevated during heat or salinity stresses (Table 1; Horan *et al.*, 2008), demonstrating that they belong to the plant abiotic stress-response network. Interestingly, MBF1c was required for the elevated expression of two HSFs (B2B and B2A), the transcriptional regulator DREB2A (that functions upstream to HSFA3; Schramm *et al.*, 2007), and a component of the RNA polymerase basal apparatus (TBP-Associated Factor 7). Because MBF1 proteins were postulated to associate with the TBP complex (Takemaru *et al.*, 1998; Kabe *et al.*, 1999; Liu *et al.*, 2003), the latter could represent a protein involved in the association of MBF1c with the RNA polymerase complex.

Genetic analysis of selected transcripts

To confirm that some of the transcripts identified by our analysis are involved in the heat stress response of plants we chose three genes encoding these transcripts – *Dreb2A* (*At5g05410*), *Sen1* (*At4g35770*), and the zinc finger protein *At3g21890* – and obtained two independent homozygous knockout lines for each gene. We then subjected these lines to heat stress and measured their survival. The three genes encoding the MBF1c-dependent transcripts were required for heat tolerance in Arabidopsis, demonstrating that they are an integral part of the plant thermotolerance pathway (Figure 2).

Identification of a putative MBF1c DNA-binding element

MBF1 proteins are not thought to be DNA-binding proteins (Takemaru *et al.*, 1998; Kabe *et al.*, 1999; Liu *et al.*, 2003). Nevertheless, they contain a putative helix-turn-helix domain that may bind DNA (de Koning *et al.*, 2009). Bioinformatics analysis of the promoter regions of the 36 MBF1c-dependent transcripts (Table 1) identified two DNA sequences that appeared at high frequencies in these promoters (Table 1 and Figure 3). Systematic evolution of ligands by exponential enrichment (SELEX) analysis (Bouvet, 2001; Clouaire *et al.*, 2005) using purified MBF1c protein identified two additional DNA sequences that showed binding to MBF1c (Table 1 and Figure S2). All four sequences identified by our analysis (Table 1) showed binding to the purified MBF1c protein in gel mobility shift assays (Figure S3). Nonetheless, only one sequence identified by our transcriptome analysis (CTAGA) showed specific MBF1c-dependent transcriptional activation in yeast (Figure 3), with another sequence identified by our SELEX assay (GGAGG) showing weaker activity in this system (Figure S4).

When the frequency of appearance of the different DNA elements in the 500-bp region of promoters from all heat stress response genes identified in this study (60-min heat stress) was compared with that of all Arabidopsis genes (Table 2), it was found that the CTAGA element was disproportionately more represented in the promoters of heat response genes. Based on this analysis (Table 2), the yeast one-hybrid assays (Figure 3) and the gel mobility shift assays (Figure S3), CTAGA is a good candidate for a putative MBF1c DNA-binding element.

Dominant-negative function of the DNA-binding domain of MBF1c

A dominant-negative effect can occur when the DNA-binding domain of a transcription factor is expressed in cells and competes with the endogenous protein without activating transcription. We therefore fused the putative DNA-binding domain of MBF1c [67 amino acids (aa) at the C-terminal of MBF1c; HTH-GFP], or the rest of the MBF1c protein (90 aa at the N-terminal of MBF1c; N-term-GFP) to GFP and expressed these fusion proteins in wild-type Arabidopsis plants (Figure 4a). The GFP-fusion protein containing the C-terminal DNA-binding domain of MBF1c, suppressed thermotolerance to levels similar to that of the null *mbf1c* mutant when expressed in transgenic plants (Figure 4b). In contrast, the

Table 1 MBF1c-dependent heat stress-response transcripts in Arabidopsis. Transcripts significantly less elevated in *mbf1c* compared with the wild type during heat stress (20 and 60 min; 40°C) are shown. Locus identifier, gene name, and response to drought, heat, salt, or cold as found in microarray databases (Horan *et al.*, 2008) are indicated for each transcript (red, elevated; blue, suppressed; white, unchanged). The frequency of putative MBF1c DNA-binding sites is indicated in the 1000-bp promoter region of each gene [left two binding elements are from bioinformatics analysis of microarray data; right two binding elements are from a systematic evolution of ligands by exponential enrichment (SELEX) assay]. Fold change (log_2) is also shown for each gene in wild-type and *mbf1c* plants at 60-min heat stress

		Response to abiotic stress				Putative DNA element				Fold change (log2)	
Locus	Gene name	Heat	Drought	Salt	Cold	CTAGA	CTTTA	GGAGG	ACGGAA	WT	*mbf1c*
20 min											
AT3G24500	MBF1C					0	1	0	0	3.535	−0.738
AT2G16720	MYB7					1	2	0	0	1.119	0.247
AT3G21890	Zinc finger (B-box type)					2	2	1	0	1.042	0.197
60 min											
AT3G24500	MBF1C					0	1	0	0	3.535	−0.738
AT5G11110	Sucrose-phosphate synthase					2	3	0	0	0.83	−0.085
AT5G07330	Unknown protein					2	4	0	0	4.386	1.573
AT3G21890	Zinc finger (B-box type)					2	2	1	0	1.042	0.197
AT1G14200	Zinc finger (C3HC4-type RING)					2	3	0	0	3.615	2.077
AT1G55300	TBP-associated factor 7					1	3	1	0	2.114	1.236
AT1G79160	Unknown protein					2	2	1	1	0.782	−0.31
AT1G55530	Zinc finger (C3HC4-type RING)					1	1	1	0	2.891	2.208
AT4G34680	GATA transcription factor 3					0	4	0	0	1.454	0.874
AT4G30460	Glycine-rich protein					1	1	2	0	1.664	0.812
AT3G13310	DNAJ heat shock protein					3	7	0	0	1.867	0.084
AT5G05410	DREB2A					2	4	1	1	5.833	3.982
AT1G07500	Unknown protein					1	2	0	0	3.982	1.998
AT2G22240	Inositol-3-phosphate synthase					1	0	0	0	3.069	2.321
AT4G05070	Unknown protein					3	1	0	1	1.398	−0.276
AT5G03380	Heavy-metal-associated protein					2	1	0	0	1.454	0.725
AT5G35320	Hypothetical protein					2	0	1	0	5.639	4.7
AT1G78080	RAP2.4 (related to AP2 4)					0	1	0	1	1.422	0.866
AT1G62760	Invertase/pectin methylesterase inhibitor					0	2	0	0	3.214	2.201
AT4G11660	Heat shock transcription factor B2B					1	3	0	0	3.7	3.084
AT5G62020	Heat shock transcription factor B2A					0	1	0	0	4.175	3.275
AT1G55620	CLC-F voltage-gated chloride channel					0	2	0	0	0.735	−0.131
AT1G59980	ARL2/ATDJC39 heat shock protein					1	2	0	0	1.834	1.248
AT5G47610	Zinc finger (C3HC4-type RING)					2	1	3	0	4.638	3.36
AT3G12320	Unknown protein					2	3	1	0	1.637	1.551
AT1G13600	ATBZIP58 (Basic Leucine Zipper)					1	1	1	0	1.221	0.447
AT2G34590	Transketolase					0	2	0	0	0.732	0.308
AT1G26800	Zinc finger (C3HC4-type RING)					3	0	0	0	4.557	4.054
AT5G37710	Lipase/calmodulin-binding heat-shock					0	5	1	1	1.482	0.868
AT2G44130	Kelch repeat F-box protein					3	2	0	0	0.993	−1.14
AT1G56610	Syntaxin					0	2	0	0	0.621	0.126
AT5G66090	Adenine nucleotide alpha hydrolases-like					0	0	1	0	1.587	1.195
AT2G27580	Zinc finger (AN1-like)					0	1	1	0	3.559	2.886
AT5G52570	Beta-carotene hydroxylase 2					2	5	0	0	2.256	0.893
AT5G06280	Hypothetical protein					2	2	0	0	1.477	1.013
AT5G25280	Serine-rich protein					0	3	0	0	1.285	0.584

GFP-fusion protein containing the N-terminal of MBF1c without the DNA-binding domain, did not significantly suppress thermotolerance when expressed in transgenic plants (Figure 4c). Although the two fusion proteins were localized to the nuclei of transgenic plants following heat stress, as is the full-length MBF1c (Suzuki *et al.*, 2008), the

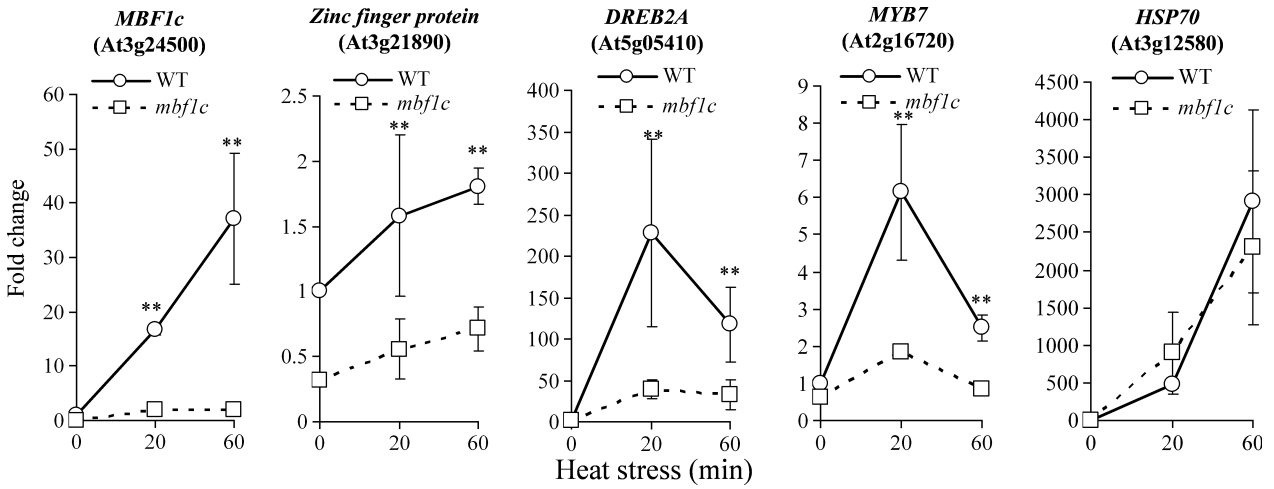

Figure 1. Expression of heat-response transcripts in wild-type and *mbf1c* plants.
The expression of *MBF1c*, three transcripts identified by our microarray analysis as dependent on MBF1c for expression during heat (*DREB2A*, *MYB7*, and *At3g21890*), and one MBF1c-independent transcript (*HSP70*) was determined using quantitative (q)PCR in wild-type and *mbf1c* plants subjected to heat stress (40°C) for 0, 20, and 60 min. Results are average and SD of three independent experiments, each performed with five technical repeats.

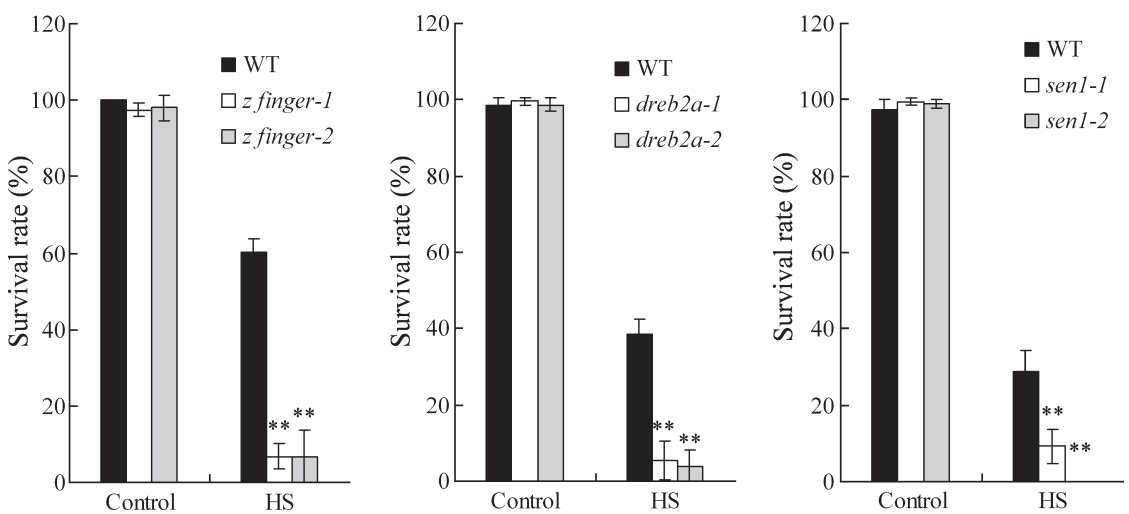

Figure 2. Heat stress (HS) survival of Arabidopsis seedlings deficient in different genes encoding MBF1c-dependent transcripts.
Survival assays were performed with two independent knockout mutants for the zinc finger protein *At3g21890* (*z-finger*), *Dreb2A*, and *Sen1*. Results are average and SD of three independent experiments, each performed with five technical repeats. WT, wild type.

HTH-GFP protein appeared to do so more efficiently than the N-term-GFP protein (Figure 4b,c).

Ectopic expression of MBF1c in soybean improves yield in plants grown under controlled growth conditions

Our previous studies demonstrated that ectopic expression of MBF1c in Arabidopsis enhances tolerance to heat and osmotic stress without a negative effect on plant growth and yield (Suzuki *et al.*, 2005, 2008). Moreover, ectopic expression of MBF1c was found to improve the growth and yield of transgenic Arabidopsis plants grown under controlled growth conditions (Suzuki *et al.*, 2005). To test whether MBF1c has a similar effect on a crop plant we transformed soybean with Arabidopsis MBF1c. Ectopic expression of MBF1c in soybean resulted in enhanced yield in plants grown under controlled growth conditions in a greenhouse without any adverse effects on the growth of plants (Figure 5).

DISCUSSION

Current and predicted climatic conditions, such as prolonged drought and heat episodes, pose a serious challenge to agricultural production worldwide, affecting plant growth and yield, and causing annual losses estimated at billions of dollars (Mittler and Blumwald, 2010). Dissecting the heat stress response of plants could therefore lead to the

(a)

	CTAGA	
	Word	Count
20 min HS	------T CTAGA A------	4
	-----TT CTAGA A------	4
60 min HS	------ CTAGA ------	91
	-----T CTAGA ------	48
	------ CTAGA A-----	37
	-----T CTAGA A-----	22
	------ CTAGA AG----	17
	----TT CTAGA A-----	17
	---CTT CTAGA ------	12
	---AAT CTAGA ------	8
	------ CTAGA AGC---	7
	------ CTAGA TAT---	6

(b)

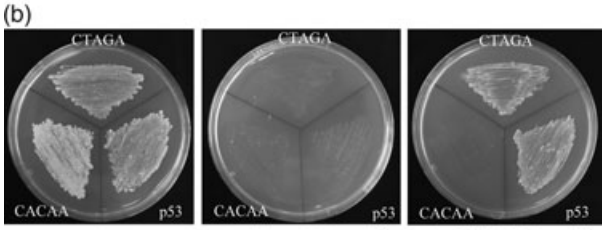

Figure 3. Identification of a putative DNA-binding element for MBF1c.
(a) Alignment of sequences from the 1000-bp promoter region of genes encoding MBF1c-dependent transcripts (Table 1) identifying the CTAGA consensus. HS, heat stress.
(b) Yeast one-hydrid assay showing specific CTAGA transcriptional activation by MBF1c.

Table 2 Frequency of appearance of DNA elements in the promoter region of Arabidopsis genes. The frequency of DNA element appearance in the 500- or 1000-bp region of promoters from all heat response genes (60-min heat stress) from this study is compared with that of all Arabidopsis genes

	In 60 min heat responsive genes (1039 genes)		In all the genes (33 518 genes)		
Sequence	Number of genes	Ratio (%)	Number of genes	Ratio (%)	*P*-value
500 bp					
CTAGA	224	21.56	3989	11.90	5.96E-19
CTTTA	270	25.99	7859	23.45	4.56E-03
GGAGG	65	6.26	1870	5.58	3.30E-02
ACGGAA	148	14.23	3980	11.87	2.52E-03
1000 bp					
CTAGA	287	27.68	7506	22.39	1.01E-05
CTTTA	417	40.21	12 807	38.21	1.05E-02
GGAGG	120	11.57	3980	11.87	3.70E-02
ACGGAA	264	25.46	7498	22.37	1.80E-03

development of crops with enhanced thermotolerance (Mittler, 2006; von Koskull-Döring *et al.*, 2007; Kotak *et al.*, 2007; Mittler and Blumwald, 2010). Here we report on the identification of a new heat stress-response regulon in plants controlled by MBF1c. This regulon includes important transcriptional regulators such as DREB2A (that controls HSFA3; Schramm *et al.*, 2007), two HSFs (B2A, B2B), and at least two other proteins required for thermotolerance in Arabidopsis (Figure 2). We further demonstrate that MBF1c is likely to function as a DNA-binding transcriptional regulator and identify at least one putative DNA-binding element for MBF1c (CTAGA; Table 2, Figure 3). The finding that MBF1c could function as a DNA-binding protein is new because this protein was previously proposed to function as a non-DNA-binding transcriptional co-activator in different systems (Takemaru *et al.*, 1998; Kabe *et al.*, 1999; Liu *et al.*, 2003; Tsuda *et al.*, 2004; de Koning *et al.*, 2009). Unlike the MBF1a and MBF1b proteins of Arabidopsis, MBF1c is highly responsive to abiotic stress and shows the least homology to MBF1 proteins from other sources (Tsuda and Yamazaki, 2004; Tsuda *et al.*, 2004). It is therefore possible that MBF1c has undergone significant changes during evolution making it a DNA-binding protein involved in the abiotic stress response of plants. This possibility is in agreement with the unique function of MBF1c as a stress-response protein, which is different from the function proposed for many MBF1 proteins from other sources generally thought to be involved in regulating development and metabolism (Takemaru *et al.*, 1998; Kabe *et al.*, 1999; Liu *et al.*, 2003). Moreover, MBF1c shares only 54–55% identity in its putative DNA-binding domain with MBF1a or MBF1b, whereas MBF1a and MBF1b share 93% identity within their putative DNA-binding domains. MBF1c might therefore represent a newer branching of the MBF1 protein family involved in stress regulation.

Our functional characterization of MBF1c adds to our knowledge of the plant thermotolerance network, demonstrating that MBF1c functions by regulating a new heat response regulon that is intertwined with the important HSF network. Our demonstration that MBF1c can enhance thermotolerance in Arabidopsis, as well as yield of Arabidopsis and soybean plants grown under controlled growth conditions (Suzuki *et al.*, 2005; Figure S5), could open the way for the testing of this gene in laboratory and field studies in different crops. These studies could have a significant impact on improving heat tolerance and yield in regions subjected to the detrimental effects of global warming.

EXPERIMENTAL PROCEDURES

Plant material, growth conditions and stress treatment

Arabidopsis thaliana plants (cv. Columbia) were grown on peat pellets (Jiffy-7, http://www.jiffygroup.com/), under controlled conditions: 21°C, 14-h light cycle, 100 μmol m^{-2} sec^{-1} and a relative humidity of 70% (Suzuki *et al.*, 2005). Null mutants for the zinc finger protein At3g21890 (CS27058 and CS25831), DREB2A (Salk_016495 and Salk_084889), and SEN1 (Salk_116080 and Salk_151963) were obtained, backcrossed, and homozygoused as described in Rizhsky

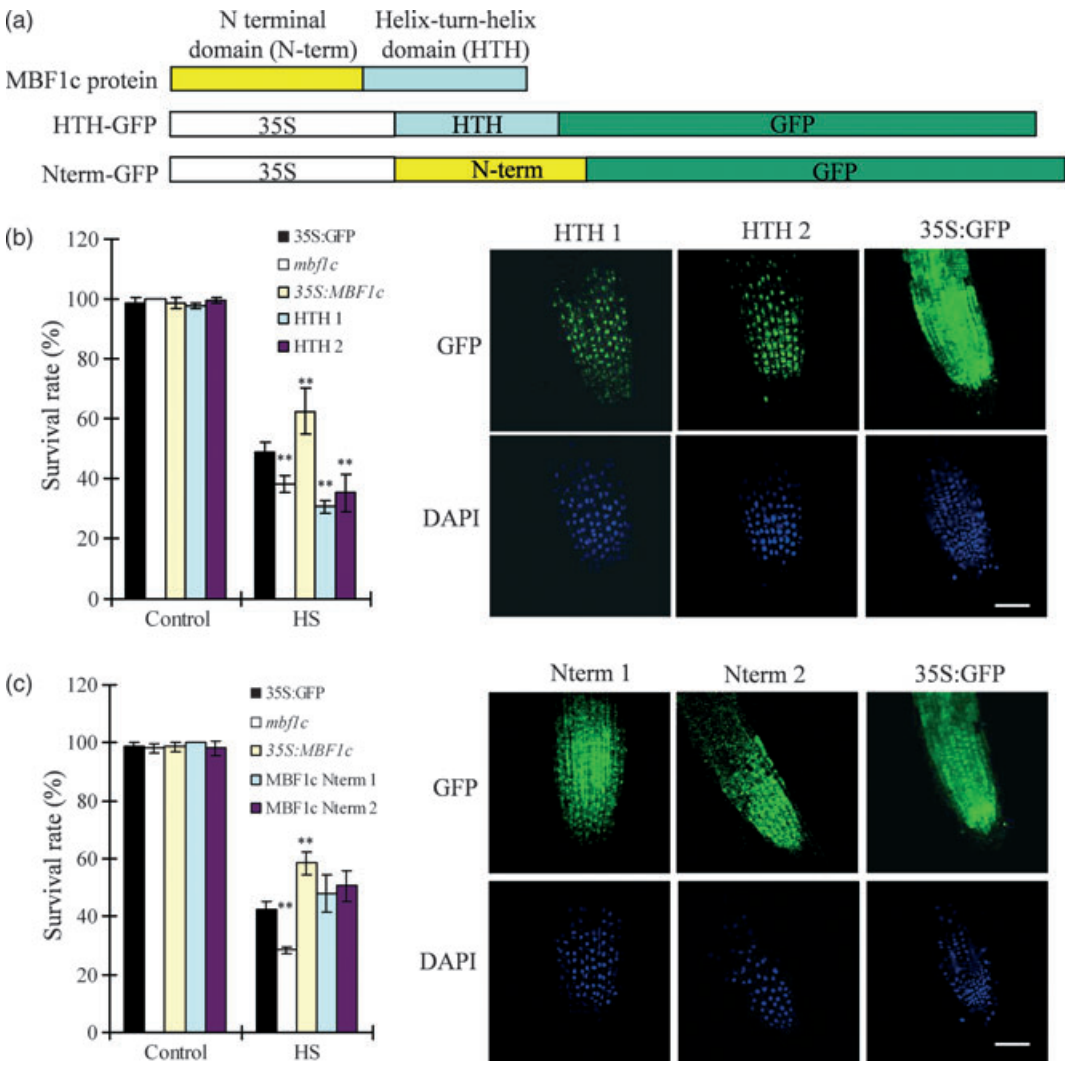

Figure 4. Dominant-negative function of the DNA-binding domain of MBF1c.
(a) The different DNA constructs used for ectopic expression of the DNA-binding (HTH), or N-terminal (N-term) domains of MBF1c in transgenic plants.
(b) Left panel: heat stress (HS) survival of Arabidopsis seedlings expressing the DNA-binding (HTH) GFP-fusion (HTH 1 and 2 are two independent homozygous transgenic lines). Right panel: localization of the HTH-GFP fusion in root cells of seedlings subjected to heat stress (40°C) for 1 h.
(c) Same as (b), but for the N-terminal of MBF1c fused to GFP (Nterm). Results are average and SD of three independent experiments, each performed with five technical repeats.

et al. (2004) and Davletova *et al.* (2005a,b). Green fluorescent protein, or GFP fused in-frame to the C- or N-terminal domains of MBF1c, was expressed in plants under the control of the CaMV35S promoter, using a pGreen-based vector, and visualized using an Olympus, IX 81 FV 1000 confocal microscope (Olympus, http://www.olympus-global.com/), as described in Luhua *et al.* (2008) and Suzuki *et al.* (2008). Two T_3 homozygous lines obtained from two individual transformation events were used in these studies. The survival rate of seedlings subjected to heat stress was determined as described in Suzuki *et al.* (2005). Two independent *mbf1c* mutants expressing MBF1c under the control of the CaMV35S promoter were obtained as described in Suzuki *et al.* (2008). Transgenic soybean (*Glycine max* cv. Thorne) plants constitutively expressing MBF1c under the control of the CaMV35S promoter were generated at the Iowa State Plant Transformation Lab (Paz *et al.*, 2006). Six homozygous transgenic lines were tested by RNA blots for MBF1c expression and three independent homozygous lines were grown in a greenhouse under controlled growth conditions and assayed for growth and yield as described in Suzuki *et al.* (2005).

DNA chip analysis

For microarray analysis, 11-day-old wild-type and *mbf1c* plants grown on peat pellets as described above were heat stressed at 40°C, 100 μmol m^{-2} sec^{-1}, and sampled at 0, 20, and 60 min (each time point had three technical repeats and the entire experiment had three biological repeats). RNA samples were used to perform chip hybridization analyses (Arabidopsis ATH1® chips; Affymetrix, http://www.affymetrix.com/) at the Nevada Genomics Center in University of Nevada, Reno. Conditions for RNA isolation, labeling, and hybridization are described in Davletova *et al.* (2005a). All GeneChip® arrays were processed first by robust multi-array averaging

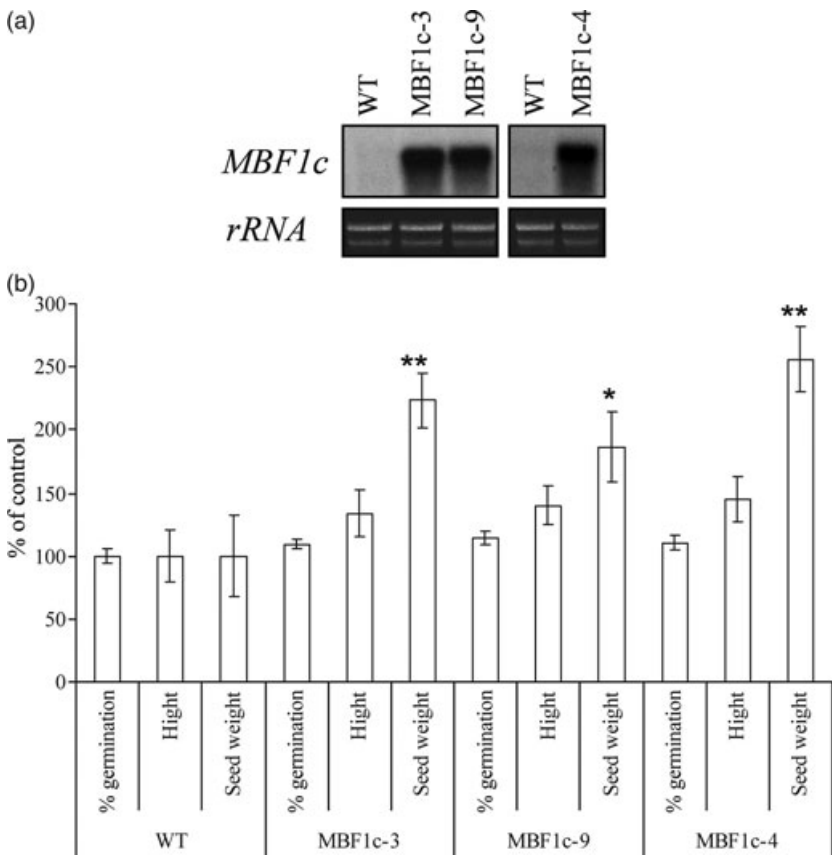

Figure 5. Ectopic expression of MBF1c in soybean improves yield in plants grown under controlled growth conditions.
(a) RNA gel blot analysis showing expression of MBF1c in three independent transgenic lines of soybean. WT, wild type.
(b) Percent germination, plant height, and weight of seeds per plant of three independent homozygous lines ectopically expressing MBF1c grown in a greenhouse to maturity at 25–28°C. No differences in individual seed weight were found between the different plants. Results are average and SD of three independent experiments, each performed with 12 technical repeats arranged in a mixed plot setting.

(RMA) (Irizarry *et al.*, 2003) using the R package affy (Gautier *et al.*, 2004). Expression values were computed from raw CEL files by first applying the RMA model of probe-specific correction of perfect match (PM) probes. These corrected probe values were then normalized via quantile normalization, and a median polish was applied to compute one expression measure from all probe values. Resulting RMA expression values were $\log_2$-transformed (Davletova *et al.*, 2005a). Statistical analysis (ANOVA) was performed as described in Davletova *et al.* (2005a). Microarray data from this experiment were deposited in Array Express (E-MEXP-2760).

Complementary DNA synthesis and quantitative (q)PCR analysis

Complementary DNA synthesis, qPCR, and RT-PCR data analysis were performed as described in Miller *et al.* (2009). First-strand cDNAs were produced from 1 μg of total RNA that was used for the microarray analysis. Threshold cycle values for MBF1c (At3g24500), zinc finger protein (At3g21890), DREB2A (At5g05410), MYB7 (At2g16720), and HSP70 (At3g12580) were calculated with the C_T of EF1-α (At5g60390) as an internal control. Primer pairs used for amplifications are shown in Table S2.

SELEX assay

The DNA-binding specificity of the MBF1c was determined by SELEX as described in Bouvet (2001) and Clouaire *et al.* (2005) with minor modifications (Figure S1).

In silico analysis

Promoters of the genes identified by the microarray analysis (Table 1) were assembled by ELEMENT (http://element.cgrb.oregonstate.edu/) as described in Nemhauser *et al.* (2004) and Koussevitzky *et al.* (2007). Oligonucleotide sequences recovered by SELEX were analyzed by MEME (http://meme.sdsc.edu/meme4_6_0/introhtml). Genes containing the identified consensus sequences in their 1000-bp promoter regions were identified by Patmatch (http://www.arabidopsis.org/cgi-bin/patmatch/nph-patmatch.pl). The DNA element frequency analysis was performed using Motif Analysis from The Arabidopsis Information Resource (TAIR, http://www.arabidopsis.org/tools/bulk/motiffinder/index.jsp).

Yeast one-hybrid

Yeast one-hybrid assay was performed using the Matchmaker® Gold Yeast One-Hybrid Library Screening System (Clontech, http://www.clontech.com/) as described in the manufacturer's instructions. Thirty-six base pair oligonucleotides with three or five tandem repeats of consensus sequences or two mutated forms were cloned into the pAbAi vector, carrying the AUR1-C gene. The plasmid was linearized and integrated into the genome of yeast (Y1H Gold). Yeast cells were then transformed with pGADT7-AD plasmid (Invitrogen, http://www.invitrogen.com/) containing MBF1c (Suzuki *et al.*, 2008). Interaction was determined based on the ability of transformed yeast to grow on -Leu medium in the presence of 200–1000 ng ml^{-1} of aureobasidin A.

ACKNOWLEDGEMENTS

Supported by funding from The National Science Foundation (IBN-0420033, NSF-0431327, IOS-0639964, and IOS-0743954), The Nevada Agricultural Experimental Station, NIH grant number P20 RR-016464 from the INBRE Program of the National Center for Research Resources, and EU grant FP7 – MARIE CURIE 447.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Expression of heat-response transcripts in wild-type, *mbf1c*, and *mbf1c* plants expressing MBF1c under the control of the CaMV35S promoter.

Figure S2. Putative MBF1c-binding elements identified by systematic evolution of ligands by exponential enrichment (SELEX).

Figure S3. Gel-mobility shift assay for the four different MBF1c putative binding elements.

Figure S4. Yeast one-hybrid assay.

Table S1. Transcripts with significantly altered expression in *mbf1c* compared with wild type during heat stress.

Table S2. Primers used for quantitative (q)PCR analysis.

Table S3. Fold change in log 2 of transcripts elevated in wild-type plants at 60-min heat stress.

Table S4. Fold change in log 2 of transcripts elevated in *mbf1c* plants at 60-min heat stress.

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