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RESEARCH PAPER

Arabidopsis HEAT SHOCK TRANSCRIPTION FACTORA1b overexpression enhances water productivity, resistance to drought, and infection

Ulrike Bechtold[1], Waleed S. Albihlal[1], Tracy Lawson[1], Michael J. Fryer[1], Penelope A.C. Sparrow[2], François Richard[1,*], Ramona Persad[1], Laura Bowden[3,†], Richard Hickman[3], Cathie Martin[2], Jim L. Beynon[3], Vicky Buchanan-Wollaston[3], Neil R. Baker[1], James I.L. Morison[1,‡], Friedrich Schöffl[4], Sascha Ott[3] and Philip M. Mullineaux[1,§]

[1] School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, UK
[2] John Innes Centre, Colney, Norwich NR4 7UH, UK
[3] School of Life Sciences and Systems Biology Centre, University of Warwick, Coventry CV4 7AL, UK
[4] Zentrum für Molekularbiologie der Pflanzen–Allgemeine Genetik, Eberhard-Karls-Universität Tübingen, D-72076 Tübingen, Germany
* Present address: Equipe: Bioinformatique structurale et modélisation moléculaire, Centre de Recherche de Biochimie Macromoléculaire (CRBM), UMR 5237 CNRS, Université Montpellier 1 et 2, 1919 route de Mende, 34293 Montpellier Cedex 5, France.
† Present address: Science and Advice for Scottish Agriculture, Roddinglaw Road, Edinburgh EH12 9FJ, UK.
‡ Present address: Centre for Forestry and Climate Change, Forest Research, Alice Holt Lodge, Farnham GU10 4LH, UK.

§ To whom correspondence should be addressed. E-mail: mullin@essex.ac.uk

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Abstract

Heat-stressed crops suffer dehydration, depressed growth, and a consequent decline in water productivity, which is the yield of harvestable product as a function of lifetime water consumption and is a trait associated with plant growth and development. Heat shock transcription factor (HSF) genes have been implicated not only in thermotolerance but also in plant growth and development, and therefore could influence water productivity. Here it is demonstrated that *Arabidopsis thaliana* plants with increased *HSFA1b* expression showed increased water productivity and harvest index under water-replete and water-limiting conditions. In non-stressed *HSFA1b*-overexpressing (*HSFA1bOx*) plants, 509 genes showed altered expression, and these genes were not over-represented for development-associated genes but were for response to biotic stress. This confirmed an additional role for *HSFA1b* in maintaining basal disease resistance, which was stress hormone independent but involved H_2O_2 signalling. Fifty-five of the 509 genes harbour a variant of the heat shock element (HSE) in their promoters, here named HSE1b. Chromatin immunoprecipitation-PCR confirmed binding of HSFA1b to HSE1b *in vivo*, including in seven transcription factor genes. One of these is *MULTIPROTEIN BRIDGING FACTOR1c* (*MBF1c*). Plants overexpressing *MBF1c* showed enhanced basal resistance but not water productivity, thus partially phenocopying *HSFA1bOx* plants. A comparison of genes responsive to *HSFA1b* and *MBF1c* overexpression revealed a common group, none of which harbours a HSE1b motif. From this example, it is suggested that HSFA1b directly regulates 55 HSE1b-containing genes, which control the remaining 454 genes, collectively accounting for the stress defence and developmental phenotypes of *HSFA1bOx*.

Key words: *Arabidopsis thaliana*, basal resistance, biotic and abiotic stress, *Brassica napus*, drought stress, heat stress, *Hyaloperonospora parasitica*, hydrogen peroxide, *Pseudomonas syringae*, transcription factors, water productivity.

Abbreviations: ABA, abscisic acid; HSF, heat shock transcription factor; JA, jasmonic acid; ROS, reactive oxygen species; SA, salicylic acid; TF, transcription factor.

Introduction

Water limitation is experienced by all terrestrial plants and is a major evolutionary force within plant populations (Heschel *et al.*, 2002; Morison *et al.*, 2008). Moderate limitation of water availability diverts resources away from growth into protective responses restricting stomatal conductance and CO_2 uptake, thus limiting photosynthesis and plant growth (Schulze, 1986*a*, *b*; Boyer, 1970; Condon *et al.*, 2004; Morison *et al.*, 2008). Molecular genetic studies using *Arabidopsis thaliana* often define the survival of dehydration stress as drought tolerance (Liu *et al.*, 1998; Passioura, 2007), but generally do not address its effects on plant productivity. The term water productivity describes the relationship between yield of the harvestable product and water loss, and is important when looking at plant productivity in water-limiting environments (Passioura, 1977; Monteith, 1984, 1993; Condon *et al.*, 2004; Steduto *et al.*, 2007; Morison *et al.*, 2008; Bechtold *et al.*, 2010).

Drought is often accompanied by elevated air and leaf temperatures; therefore, leaves experience additional evaporative demand due to an increase in leaf to air vapour pressure difference (VPD; Turner, 2004). Consequently, there may be cross-talk between heat and dehydration stress signalling networks. For example DREB2A, a dehydration-responsive transcription factor (TF), has been shown to have a dual function in *Arabidopsis*, regulating the responses to dehydration and heat stress (Sakuma *et al.*, 2006). The signalling by *DREB2A* is routed through activation of a heat shock TF gene, *HSFA3*, leading to the expression of heat shock protein genes (Schramm *et al.*, 2008; Yoshida *et al.*, 2008). Similarly, *MULTIPROTEIN BRIDGING FACTOR1c* (*MBF1c*) has been proposed to regulate the response to temperature stress in *Arabidopsis* (Suzuki *et al.*, 2008). *MBF1c* overexpression leads to improved tolerance to heat, osmotic, and biotic stress (Suzuki *et al.*, 2005), and its regulon includes *DREB2A* and two class B *HSF* genes (Suzuki *et al.*, 2011).

In all eukaryotes, HSFs are the central component of the cellular heat stress response, with their basic structure and the *cis* regulatory heat shock elements (HSEs) of their target genes being highly conserved (Wu, 1995; Morimoto, 1998; Nover *et al.*, 2001; Baniwal *et al.*, 2004). Plants, compared with all other eukaryotes, have large *HSF* families (Nover *et al.*, 2001; Czarnecka-Verner *et al.*, 2004). For example, the *Arabidopsis HSF* family consists of 21 genes, with the proteins they encode being divided into three structural classes (A, B, and C; Nover *et al.*, 2001; Kotak *et al.*, 2004, 2007*a*). There are 15 class A HSFs, which act as transcription activators. The B class HSFs may be transcriptional repressors and/or co-activators by interacting with class A *HSF* genes (Czarnecka-Verner *et al.*, 2004; Hahn *et al.*, 2011; Ikeda *et al.*, 2011). No function has been ascribed to the single *HSFC1* in *Arabidopsis* (Nover *et al.*, 2001).

The four clade A1 members in *Arabidopsis* (*HSFA1a* *HSFA1b*, *HSFA1d*, and *HSFA1e*) are key regulators in the early response to heat (Lee *et al.*, 1995; Prändl *et al.*, 1998; Panchuk *et al.*, 2002; Busch *et al.*, 2005; Yamada *et al.*, 2007; Liu *et al.*, 2011). When *HSFA1b* is overexpressed in *Arabidopsis* and tomato (*Solanum lycopersicum*) it improves thermotolerance (Prändl *et al.*, 1998) and heat-shock induced chilling tolerance, respectively (Li *et al.*, 2003). Similarly in tomato, *SlHSFA1* is a regulator of thermotolerance, and reduced expression of *SlHSFA1* leads to heat stress sensitivity (Mishra *et al.*, 2002). However, the size of plant HSF gene families has prompted the suggestion that some HSFs have evolved to regulate responses to other stresses (Kotak *et al.*, 2004). Many HSF genes are induced in response to environmental stresses other than heat (Miller and Mittler, 2006; Swindell *et al.*, 2007). For example, *HSFA2* shows strong transcriptional activation during high light, anoxia, salinity, and bacterial infection (Panchuk *et al.*, 2002; Rizhsky *et al.*, 2002; Miller and Mittler, 2006; Nishizawa *et al.*, 2006; Schramm *et al.*, 2006; Banti *et al.*, 2010). *HSFA9* has a key role in seed maturation (Kotak *et al.*, 2007*b*), and rice *OsHSFA4a* is implicated in tolerance to cadmium toxicity (Shim *et al.*, 2009). It has been demonstrated that *HSFB1* and *HSFB2b* are negative regulators of resistance to *Alternaria brassicicola* (Kumar *et al.*, 2009). This agrees with observations that induced thermotolerance can have negative effects on basal and R gene-mediated resistance (Noël *et al.*, 2007; Wang *et al.*, 2009). Thus, a role for HSFs in plants' responses to a range of environmental stresses appears likely.

Due to the connection between heat and drought stress, the hypothesis was tested that among the *Arabidopsis* class A *HSF* family, some could play a direct role in a drought response independent of heat shock, and display dual functions similar to *DREB2A*. Furthermore, given the influence some HSF A class genes have on growth and development (Kotak *et al.*, 2007*b*; Liu *et al.*, 2011), their influence might extend to effects on lifetime traits such as water productivity (Morison *et al.*, 2008). To test this hypothesis, the focus of this study was on two *HSF* genes, *HSFA1b* and *HSFA2*, whose constitutive overexpression promotes thermotolerance and enhances expression of genes responsive to leaf water status such as *DREB2A* and *ASCORBATE PEROXIDASE2* (*APX2*; Panchuk *et al.*, 2002; Nishizawa *et al.*, 2006; Ogawa *et al.*, 2007; Galvez-Valdivieso *et al.*, 2009).

Material and methods

Plant material and growth conditions

Plants were grown in both controlled-environment and glasshouse conditions exactly as described by Bechtold *et al.* (2010). All transgenic 35S:*HSFA1bOx* lines were produced by *Agrobacterium*-mediated transformation using the floral dip method (Clough and Bent, 1998) in three different laboratories: Tübingen (*HSFA1bOx1*/Ws-2), Essex (*HSFA1bOx2* and *-3*/Col-0), and Warwick (*HSFA1b::mRFP_B*/Col-0). The transgenic and mutant genotypes *hsfA1a*/*hsfA1b*, *HSFA2Ox*, *hsfA2-1*, *MBF1cOx*, and *mbf1c-1* have been described previously (Busch *et al.*, 2005; Suzuki *et al.*, 2005, 2008; Nishizawa *et al.*, 2006).

Oil seed rape transformation

A doubled haploid *Brassica napus* L. cv. Q6 was transformed using an *Agrobacterium*-mediated tissue culture approach as previously

described (Sparrow *et al*., 2004) using a pGreen Ti vector harbouring a *Cauliflower mosaic virus* (CaMV) 35S: *nptII* coding sequence (Supplementary Fig. S3A available at *JXB* online; Hellens *et al*., 2000) and a 35S:*AtHSFA1b* cDNA fusion from pJIT30 (Guerineau *et al*., 1988). Segregation of T-DNA loci in progeny from the primary transformant was determined by PCR of genomic DNA using primers for the *nptII* gene (forward, 5′-TGAATGAACTGCAGGACGAG-3′; reverse, 5′-AGCCAACGCTATGTCCTGAT-3′). The two transgenic lines generated in this study were confirmed to be independent by DNA gel blotting (Fig. S3B) using standard procedures described previously (Hellens *et al*., 2000). The blot was probed with a ^{32}P-labelled *nptII* DNA fragment amplified by PCR from pGreen0029 (Hellens *et al*., 2000) and washed at 0.1× SSC (65 °C).

Application of stresses

Five-week-old plants were subjected to a 15 min heat stress at 36 °C, 78% relative humidity maintaining VPD at 1 kPa. Drought stress and determination of water productivity, rosette biomass, and harvest index (HI) were carried out exactly as described previously (Bechtold *et al*., 2010). Virulent *Pseudomonas syringae* pv *tomato DC3000* (*Pst*) infection were carried out on 4-week-old plants by vacuum infiltration, or dipping in cultures of the bacteria at a density of 10^5 colony-forming units (cfu) ml^{-1} in 10 mM $MgCl_2$ buffer containing 1% (v/v) Silwett L-77. Bacterial growth in leaves was monitored at 0, 2, and 4 d post-infection as described by Innes *et al*. (1993). For oomycete infection, *Hyaloperonospora arabidopsidis* strain WAC09 (*Hpa*) was used. Spores were extracted in 10 ml of water from plant leaves prior to infection, and 2-week-old seedlings were inoculated and counted as described by Muskett *et al*. (2002).

Gas exchange measurements

Transpiration and photosynthesis parameters were measured as previously described (Lawson and Weyers, 1999). Briefly, the response of A to changes in the intercellular CO_2 concentration (c_i), and the response of A to changes in photosynthetic photon flux density (PPFD) from saturating to subsaturating levels was measured using a combination of red and white LEDs (PP Systems, Amesbury, MA, USA) at ambient CO_2 concentration (390 µmol mol^{-1}), leaf temperature of 20 (±1) °C, and a VPD of 1 (±0.2) kPa. Snapshot measurements were carried out in the glasshouse, and readings were taken at steady-state rates of A and stomatal conductance (g_s) at current atmospheric [CO_2].

Hormone, hydrogen peroxide (H_2O_2), and glutathione (GSH) determinations

H_2O_2 and GSH measurements were carried out as described previously (Bechtold *et al*., 2010). Hormone measurements were analysed using an adapted method described in Forcat *et al*. (2008). A 20 mg aliquot of freeze-dried leaf samples was extracted three times for 1 h in a rotary extractor using 10% (v/v) methanol, 1% (v/v) acetic acid in a total volume of 1100 µl. After the third extraction step 200 µl of the samples were used to analyse salicylic acid (SA) and jasmonic acid (JA) levels as described in Bechtold *et al*. (2010).

Transcriptomics, gene expression, and microarray data analysis

RNA was extracted from fully expanded leaves of stressed and non-stressed plants exactly as described by Bechtold *et al*. (2010). The comparison of Ws-2 versus *HSFA1bOx1* was carried out using Agilent Arabidopsis 3 Oligo Microarrays, and the data were processed as previously described (Bechtold *et al*., 2010). Three independent experiments using individual rosettes per experiment and one dye swap were carried out. Up-regulated genes were determined as >2-fold in *HSFA1bOx1* at a 5% false discovery rate (FDR). Raw data from these experiments and for those from *HSFA2Ox1* plants can be found on the ArrayExpress database at http://www.ebi.ac.uk/microarray-as/ae. Quantitative real-time reverse transcription–PCR (qRT–PCR) of cDNA using the SYBR Green (Sigma Ltd, UK) chemistry was carried out as described previously (Bechtold *et al*., 2010) using the primers listed in Supplementary Table S10 at *JXB* online. The data were normalized against cyclophilin (Rossel *et al*., 2006).

CATMA arrays

RNA extraction from rosettes of four pooled plants, and the comparison of Col-0 versus *HSFA2Ox* was carried out using the CATMA (version 3) microarray (Allemeersch *et al*., 2005). Normalization and analysis of the array was carried out using LimmaGUI, a graphical front end for the limma (Linear Models for MicroArray; Wettenhall and Smyth, 2004) package for R available from Bioconductor (http://www.bioconductor.org).

Bioinformatics

Public data sets The publicly available microarray data sets accessed for this study (see legend of Fig. 4) can be found at ArrayExpress (http://www.ebi.ac.uk/arrayexpress). Raw fluorescence data from *35S:MBF1c* (ID: E-GEOD-5539) arrays were downloaded from the Array Express database and analysed as previously described for Affymetrix microarrays (Bechtold *et al*., 2010).

Analysis of Gene Ontology (GO) This was done using the software packages in the Database for Annotation, Visualization and Integrated Discovery v6.7 (DAVID; http://david.abcc.ncifcrf.gov/; Huang *et al*., 2008).

Promoter analysis using MEME Sequences 500 bp upstream of the predicted transcription start site were retrieved from TAIR (version 8; www.arabidopsis.org). Promoters of the top 50 expressed genes were searched using MEME (3.5.7). A position-specific scoring matrix (PSSM) was generated and used to scan the promoters of all 352 up-regulated genes to identify those enriched for occurrences of the motif (Supplementary Table S7 at *JXB* online). To identify putative direct targets of *HSFA1b*, each promoter sequence from genes up-regulated in *HSFA1bOx1* was scored for over-representation of the HSE1b motif detected by MEME. For each promoter, the matrix similarity score (Kel *et al*., 2003) was computed at each position in the sequence. A P-value for each score was computed from a score distribution by applying the HSE1b PSSM to a random sequence 100 million bases in length, which was generated by a third-order Markov model learned from the whole *Arabidopsis* genome. A score for potential multiplicity was calculated by taking the top k non-overlapping hits and computing the binomial probability for the presence of k sites within the sequence of length n. Genes that had a binomial P-value ≤0.05 were classed as over-represented for the motif and therefore putative direct targets of HSFA1b. Regulatory sequence analysis was performed using the APPLES software framework to scan for hits of the MEME motif in promoters using binomial testing.

Chromatin immunoprecipitation followed by PCR (ChIP-PCR)

ChIP was carried out using fully expanded leaves of 5-week-old *HSFA1b:mRFP_B* plants according to Saleh *et al*. (2008). A red fluorescent protein (RFP)-specific antibody (anti-RFP, AB62341; Abcam, Oxford, UK) was used to precipitate the HSFA1b:mRFP–DNA complexes from chromatin. The primers used were promoter specific, spanning the HSE1b or canonical HSE elements in the respective genes (Supplementary Table S10 at *JXB* online). PCR was carried out on the immunoprecipitated DNA, input DNA (before precipitation), and on no antibody control-precipitated DNA. Products were separated on 1.5% (w/v) TAE agarose gels and visualized under UV light after staining with ethidium bromide.

Cycloheximide treatments

Plants were grown aseptically on half-strength Murashige and Skoog (MS) medium containing 3% (w/v) sucrose and incubated under the short-day growth conditions described above. Plates of 10-day-old *AtHSFA1bOx-1*/Ws-2 and Ws-2 seedlings were sprayed with 10 mM cycloheximide or water and after 4 h their RNA was extracted and used to determine gene expression. Expression of selected genes was determined by qRT–PCR as described above.

Results

Initially, three transgenic *Arabidopsis* lines were used for this study, *HSFA1bOx-1*/*Ws-2*, *HSFA1bOx-2*/*Col-0*, and *HSFA2Ox*/*Col-0*. These lines showed 50- to 160-fold, overexpression compared with their wild-type controls (Supplementary Fig. S1A–C at *JXB* online). A *HSFA1bOx-3*/*Col-0* line with 34-fold induction of *HSFA1b* was also included in part of the study (Supplementary Fig. S1A), as was a line overexpressing an *HSFA1b-RFP* fusion (Supplementary Fig. S2A). *HSFA2* expression was not affected in *HSFA1bOx* and vice versa (Supplementary Fig. S1B, C). An *hsfA1a*/*hsfA1b* double mutant was also used because single mutants do not have diminished responses to heat stress (Busch *et al.*, 2005). *HSFA1b* is expressed in all organs under a range of environmental conditions (Supplementary Fig. S1D; Miller and Mittler, 2006). Consequently, expression of the CaMV 35S:*HSFA1b* transgene was enhanced but not ectopic.

HSFA1b is involved in dehydration and drought stress responses

Detached rosettes of *HSFA1bOx* plants dehydrated more slowly, while *hsfA1a*/*hsfA1b* plants showed the opposite phenotype (Fig. 1A). At 20% relative soil water content (rSWC), intact *HSFA1bOx* plants did not wilt, unlike wild-type controls (Fig. 1B). Increased dehydration tolerance may divert resources away from growth and limit photosynthesis (Passioura, 2007; Morison *et al.*, 2008). Therefore, seed yield was measured in these plants when well watered and after exposure to progressive drought stress to 20% rSWC, followed by re-watering. *HSFA1bOx* plants showed reduced soil drying rates compared with controls (Fig. 1C). In contrast, *hsfA1a*/*hsfA1b* plants had significantly faster drying rates (Fig. 1C). Seed yield was elevated in both watered and droughted *HSFA1bOx* lines compared with the wild type (Fig. 1D), although no reciprocal difference was observed in *hsfA1a*/*hsfA1b* plants.

HSFA1b overexpression influences seed yield under limited watering conditions

The robustness of the seed yield phenotype was tested in either well-watered (80% rSWC) or water-limited (40% rSWC) growth regimes. The ratio of seed yield to total above-ground biomass (HI) is a component of water productivity (Passioura, 2007; Morison *et al.*, 2008; Bechtold *et al.*, 2010). At 40% rSWC, *HSFA1bOx* plants showed significant increases in water productivity and HI compared with their wild-type controls (Fig. 1E, F; see also Fig. 6F, G). Conversely, *hsfA1a*/*hsfA1b* plants showed lowered water productivity and HI at 40% rSWC (Fig. 1E, F). Significant differences were also detected in the 80% rSWC treatment of *HSFA1bOx2* plants (Fig. 1E, F; see also Fig. 6F, G). No consistently significant effects of *HSFA1b* overexpression were observed for seed weight or viability (Supplementary Table S1 at *JXB* online). There was no effect of altered *HSFA1b* expression on the capacity for photosynthetic carbon assimilation, stomatal conductance, or instantaneous transpiration efficiency (Supplementary Table S2).

Overexpression of HSFA1b in oil seed rape increases harvest index and seed yield

To establish whether the effects of *HSFA1b* overexpression are conserved in other *Brassicaceae*, the 35S:*HSFA1b* chimaeric gene was transformed into *B. napus* (oil seed rape). Two independent single locus transgenic oil seed rape lines (*BnHSFA1bOx#1* and *BnHSFA1bOx#3*) overexpressing *Arabidopsis HSFA1b* 109-fold (SD±33; n=4) and 59-fold (SD±32; n=4), respectively, showed the same improved productivity traits of seed yield and HI (Supplementary Fig. S3C, D at *JXB* online). *BnHSFA1bOx* plants had a bushier flowering phenotype than their azygous siblings (Supplementary Fig. S3E).

HSFA1b overexpression influences basal resistance to two pathogens

While *HSFA1b* overexpression positively influences plant productivity, drought tolerance, and thermotolerance, such plants could be more susceptible to pathogens (see Introduction). However, *HSFA1bOx* plants showed increased resistance to the bacterial pathogen *Pst* after inoculation either by vacuum infiltration (Fig. 2A) or by dipping (Fig. 2B), and *Hpa* (Fig. 2D). Conversely, *hsfA1a*/*hsfA1b* plants showed decreased resistance to these pathogens (Fig. 2C, D).

HSFA1b overexpression effects the expression of >500 genes

A pair-wise comparison of the transcriptome of *HSFA1bOx1* with Ws-2 under non-stress conditions revealed 352 and 157 differentially expressed genes (DEGs) with >2-fold and <0.5-fold altered expression, respectively (P <0.05; FDR q <0.05; Supplementary Table S3 at *JXB* online). Many of these DEGs were classed as heat stress responsive (Supplementary Tables S3, S4) and highly significantly overlapped with microarray data from heat-stressed plants (Table 1). The overlap with microarray data sets from plants infected with virulent *Pst* and with microarray data collated from infection of *Arabidopsis* with three different isolates of *Hpa* (Eulgem *et al.*, 2004; Supplementary Table S5) was also highly significant (Table 1; Supplementary Table S3). However, the significance of the overlap between *HSFA1b*-regulated genes and drought-responsive genes was much lower in comparison with those affected by heat or pathogen infection (Table 1; Supplementary Tables S3, S4). While for heat, *Pst*, and *Hpa*,

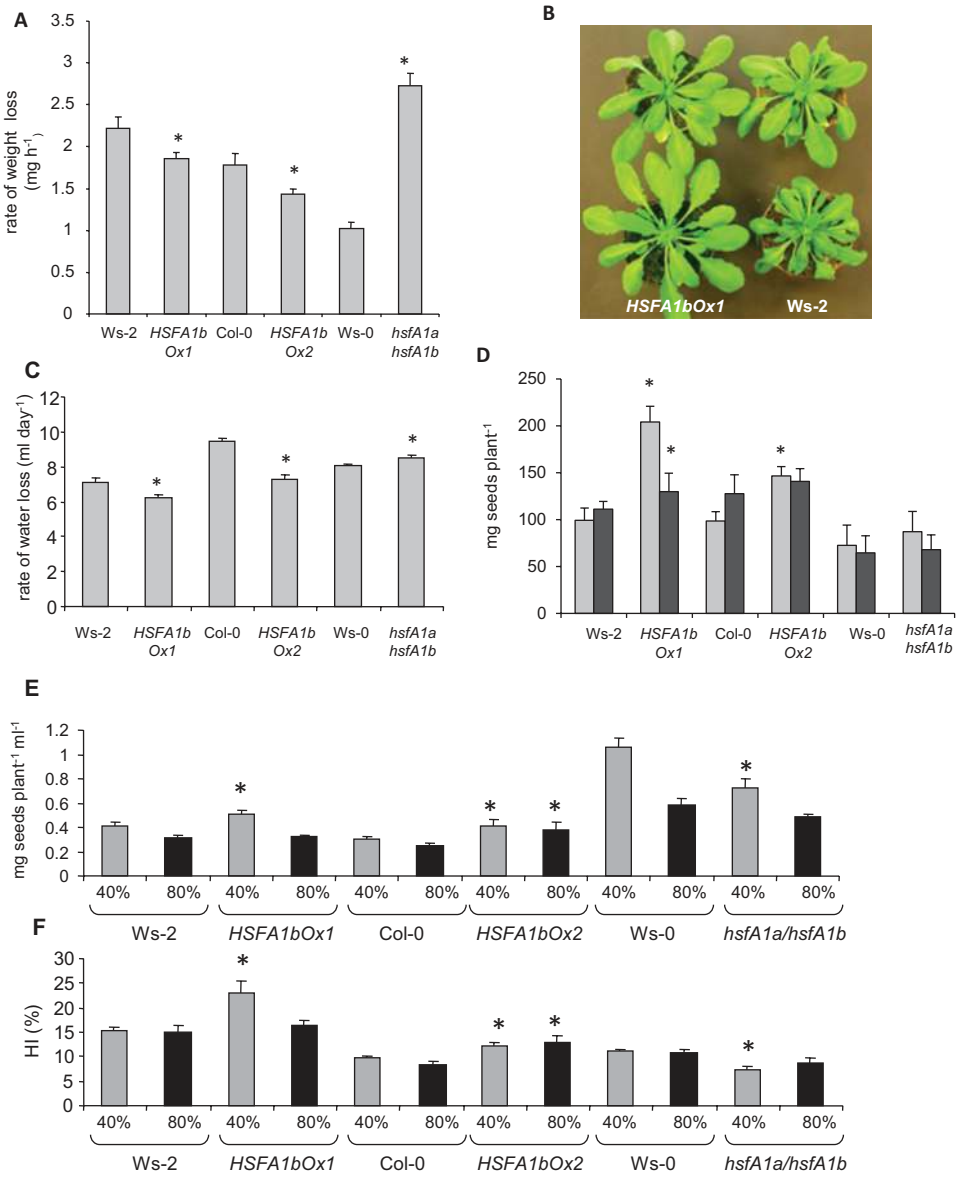

Fig. 1. *HSFA1b* regulates drought tolerance, seed yield, and water productivity. (A) Rate of weight loss of detached 5-week-old rosettes of *HSFA1b*-overexpressing lines (*HSFA1bOx1* and *HSFA1bOx2*) and the *hsfA1a/hsfA1b* double null mutant, and their wild-type controls Ws-2, Col-0, and Ws-0, respectively (n=6). (B) Typical phenotype of *HSFA1b*-overexpressing plants compared with their controls after 14 d without water. (C) Rate of water loss in *HSFA1bOx* and *hsfA1a/hsfA1b* plants compared with their wild-type controls averaged over 13 d with no watering (n=8). (D) Seed yield in *HSFA1b*-overexpressing plants and their wild-type controls after water withdrawal to 25% relative soil water content (rSWC) followed by re-watering to seed set (grey bars; n=8) or watered controls throughout this period (black bars; n=8). (E) Water productivity in *HSFA1bOx* and *hsfA1a/hsfA1b* plants in well-watered (80% rSWC, n=10; black bars) and water-limited (40% rSWC, n=10; grey bars) conditions. (F) Harvest index from the plants in E. All data are presented as means (±SEM). The asterisks (*) denote significant differences ($P \leq 0.05$; Student's t-test) between the overexpressing or mutant lines and their controls.

there were many *HSFA1bOx1*-responsive genes common to two or all stresses (Fig. 3A), 63, 53 and 30%, respectively, were responsive only to a single stress (Fig. 3A).

H_2O_2 signalling but not stress hormone signalling is stimulated in HSFA1bOx plants

GO analysis revealed no significant enrichment of abscisic acid (ABA)- and SA-responsive DEGs in the *HSFA1bOx1*/Ws-2 microarray data set (Supplementary Table S6 at *JXB* online). There was significant enrichment of JA-responsive genes (Supplementary Table S6), but their increased expression in *HSFA1bOx2* or *-3* plants could not be verified. The levels of SA, JA, and ABA were not consistently affected by *HSFA1b* overexpression in the different *HSFA1bOx* lines (Supplementary Table S7).

From the same GO analysis, there was enrichment of H_2O_2-responsive genes (Supplementary Table S4 at *JXB* online). A more specific group of H_2O_2-responsive genes, based on expression patterns in mutants with altered

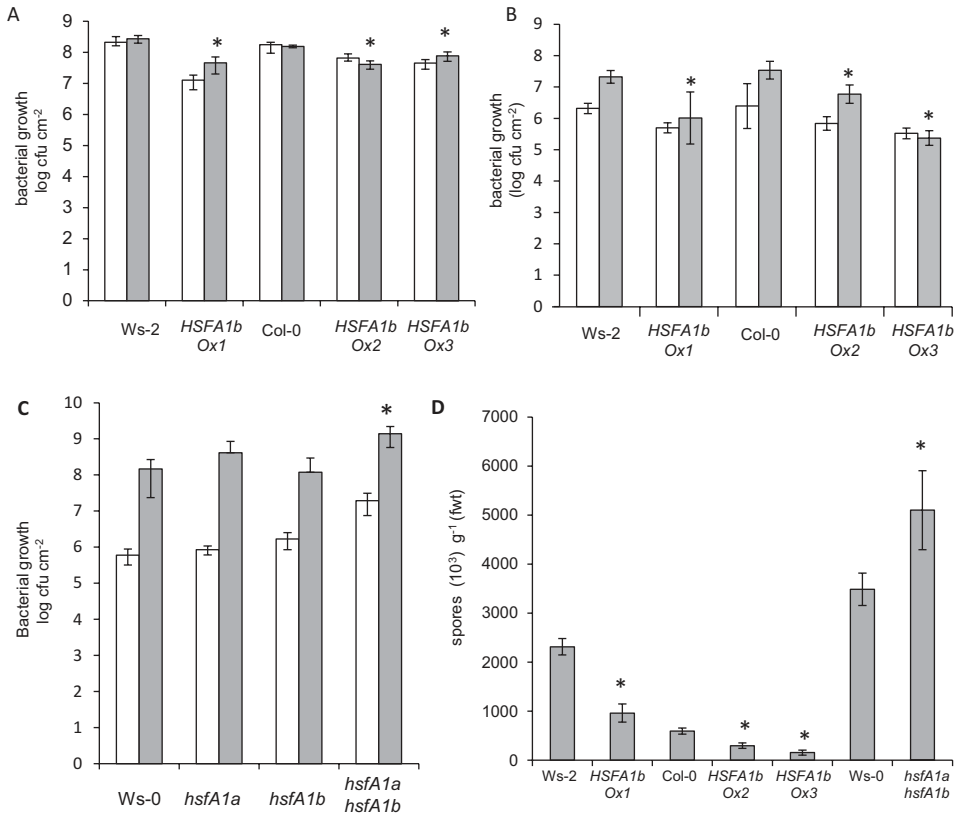

Fig. 2. *HSFA1b* regulates basal resistance to a bacterial and an oomycete pathogen. Colonization of virulent *Pst* on *HSFA1bOx* plants (A, B) or *hsfaA1a*, *hsfA1b*, *hsfA1a/hsfA1b* knockout mutants (C) compared with wild-type controls at 2 d (white bars) and 4 d (grey bars) post-inoculation. Bacteria were inoculated by vacuum infiltration (A, C) or by dipping (B). Data are representative of at least two independent experiments for each method (n=6). The inocula recovered from leaves at day 0 were 2.37 log cfu ml^{-1} (± 0.43). (D) Spore yields from 12-day-old *HSFA1bOx* and *hsfA1a/hsfA1b* plants ($n \geq 7$) inoculated 5 d previously with 5×10^4 spores of *Hyaloperonospora arabidopsidis* pv. WACO9. The asterisks (*) denote significant differences ($P \leq 0.05$; Student's t-test) between the overexpressing or mutant lines and their controls.

H_2O_2 levels (Gadjev *et al*., 2006) also revealed a significant overlap ($P \leq 0.0001$; hypergeometric distribution test; Supplementary Table S4). Six of these genes, chosen as the most differentially expressed in *HSFA1bOx1* plants (Supplementary Tables S3, S4), were confirmed as such in the Col-0 *HSFA1bOx* lines (Fig. 3B). *HSFA1bOx* and *hsfA1a/hsfA1b* plants had higher and lower foliar levels of H_2O_2, respectively (Fig. 3C). Foliar H_2O_2 levels are often associated with increased levels of the thiol antioxidant GSH (see Discussion), and these were significantly elevated in the *HSFA1bOx* lines (Fig. 3D).

Fifty-five promoters of HSFA1bOx DEGs contain a novel HSE variant

HSFs interact with HSEs [(TTCnn)GAAnnTTC] in the promoters of target genes (Nishizawa *et al*., 2006; Larkindale and Vierling, 2008; Kumar *et al*., 2009). Using MEME, a motif searching algorithm (see the Materials and methods), a novel version of HSE (here called HSE1b; Fig. 4A) was identified in the promoter regions of 55 *HSFA1bOx1* DEGs (Supplementary Tables S3, S7 at *JXB* online). It was hypothesized that these genes could constitute an *HSFA1b* regulon in *HSFA1bOx* plants. To test this hypothesis, the focus was on seven HSE1b-containing

Table 1. *Hypergeometric distribution test for commonality of DEGs from publicly available microarrays of stress-exposed Arabidopsis plants and the HSFA1bOx1/Ws-2 comparison (Supplementary Table S3 at JXB online)*

	DEGs from the publically available stress microarray data (*n*)	Genes from the stress microarrays present in the *HSFA1bOx1*/Ws-2 data set (*n*)	Overlapping DEGs (*n*)	*P*-value
Heat	815	397	161	1.4×10^{-133}
Drought	4407	397	94	0.003
Pst	1314	397	124	6.5×10^{-61}
Hpa	224	147	33	7.7×10^{-38}

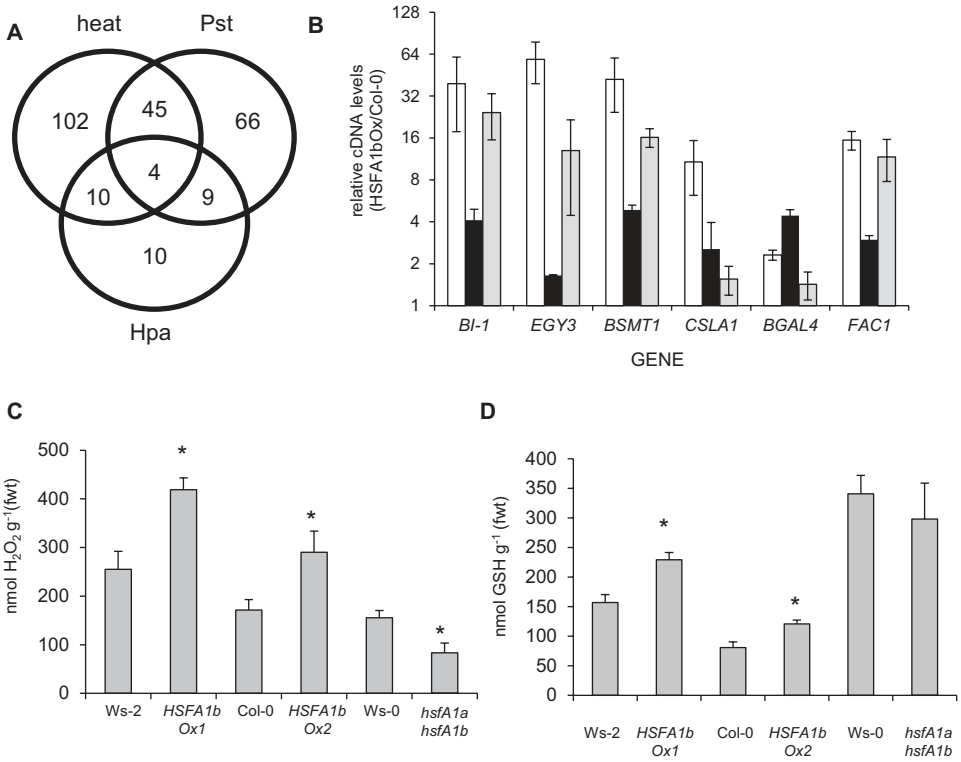

Fig. 3. *HSFA1b*-responsive genes are also responsive to heat stress, H_2O_2, and infection by *Hpa* and *Pst*. (A) The Venn diagram shows the overlap of *HSFA1bOx1*-responsive genes with those responsive to heat stress (database ID, E-GEOD-5628), infection with virulent *Pst* (E-GEOD-5520), or *Hpa* (up to three isolates; Supplementary Table S5 at *JXB* online; Eulgem *et al*., 2004). The significances of the overlaps can be found in Table 1. (B) Expression of six *HSFA1b*-responsive genes (mean ±SEM; $n \geq 4$) classified as controlled by H_2O_2-mediated signalling (Supplementary Table S4 at *JXB* online; Gadjev *et al*., 2006). The data are from *HSFA1bOx2* (white bars), *HSFA1bOx3* (black bars), and *HSFA1b:mRFP_B* (grey bars) 5-week-old non-stressed plants and Col-0 controls using real-time qRT–PCR. Values are significant between transgenic lines and Col-0. (C and D) Foliar levels of H_2O_2 (C) and GSH (D) for *HSFA1bOx*, *hsfA1a/hsfA1b*, and wild-type 5-week-old non-stressed plants (n=6). The differences marked with an asterisk (*) are significant at $P \leq 0.05$ (Student's t-test).

TF genes (*HSFA7A*, *HSFB2b*, *HSFB2a*, *MBF1c*, *MYB*, *TFIIS*, and *ZAT6*; Supplementary Tables S3, S10 at *JXB* online). With the possible exception of *ZAT6*, the induction in expression of these HSE1b-TF genes was inhibited in heat-stressed *hsfA1a/hsfA1b* plants (Fig. 4B), suggesting that they are regulated by clade A1HSFs in wild-type plants.

To discriminate between genes harbouring or lacking HSE1b motifs, *HSFA1bOx1* and Ws-2 plants were treated with cycloheximide, an inhibitor of protein synthesis. In cycloheximide-treated *HSFA1bOx1* plants, the transcription of genes directly regulated by *HSFA1b* would be unaffected since the level of HSFA1b protein would be high enough to persist and exert control in the absence of its synthesis (Yamada *et al*., 2007). In contrast, the expression of genes indirectly affected by *HSFA1b* overexpression would be inhibited, since the levels of their transcripts would be dependent on synthesis of the TFs regulating their expression. The transcript levels of the seven HSE1b-TF genes were unaffected by cycloheximide (Fig. 4C), whereas the transcript levels of seven DEGs not harbouring a HSE1b promoter motif were lowered by the treatment (Fig. 4C).

To establish in *HSFA1bOx* plants whether HSFA1b interacts with promoters harbouring the HSE1b element, ChIP followed by PCR was carried out using a 35S::*HSFA1b:RFP* fusion line (*HSFA1bOx-mRFP_B*). C-terminal fusions of proteins do not affect HSFA1b function (Prändl *et al*., 1998). The *HSFA1bOx-mRFP_B* line showed 165-fold induction of *HSFA1b* expression (Supplementary Fig. S2A at *JXB* online), 1.5- to 3-fold overexpression of the seven HSE1b-TF genes (Supplementary Fig. S2B), and enhanced resistance to *Hpa* and *Pst* (Supplementary Fig. S2C, D). To demonstrate the specificity *in vivo* of HSFA1b, three genes were selected (Fig. 4D) which harbour a single HSE1b element in their promoters (Supplementary Table S7). These genes do not have any other HSE-like motif present in their promoter regions (Supplementary data). A further three genes were selected (Fig. 4D) that harbour only a core HSE (GAAnnTTC; Larkindale and Vierling, 2008), and no match to the consensus HSE1b sequence (see Supplementary data). The promoter segments for the three HSE1b-containing genes showed amplification of DNA recovered after precipitation with the anti-RFP antibody (Fig. 4D). In contrast, the three genes which only harbour a core HSE consistently failed to give a PCR amplicon from the same ChIP preparations (Fig. 4D). Therefore, in non-stressed *HSFA1bOx* plants, promoters harbouring the HSE1b element can be specifically recognized *in vivo* by HSFA1b at least when overexpressed. As with most of the 55 HSE1b-containing genes (Supplementary Table S3), the seven HSE1b-containing TF genes contain both core HSE

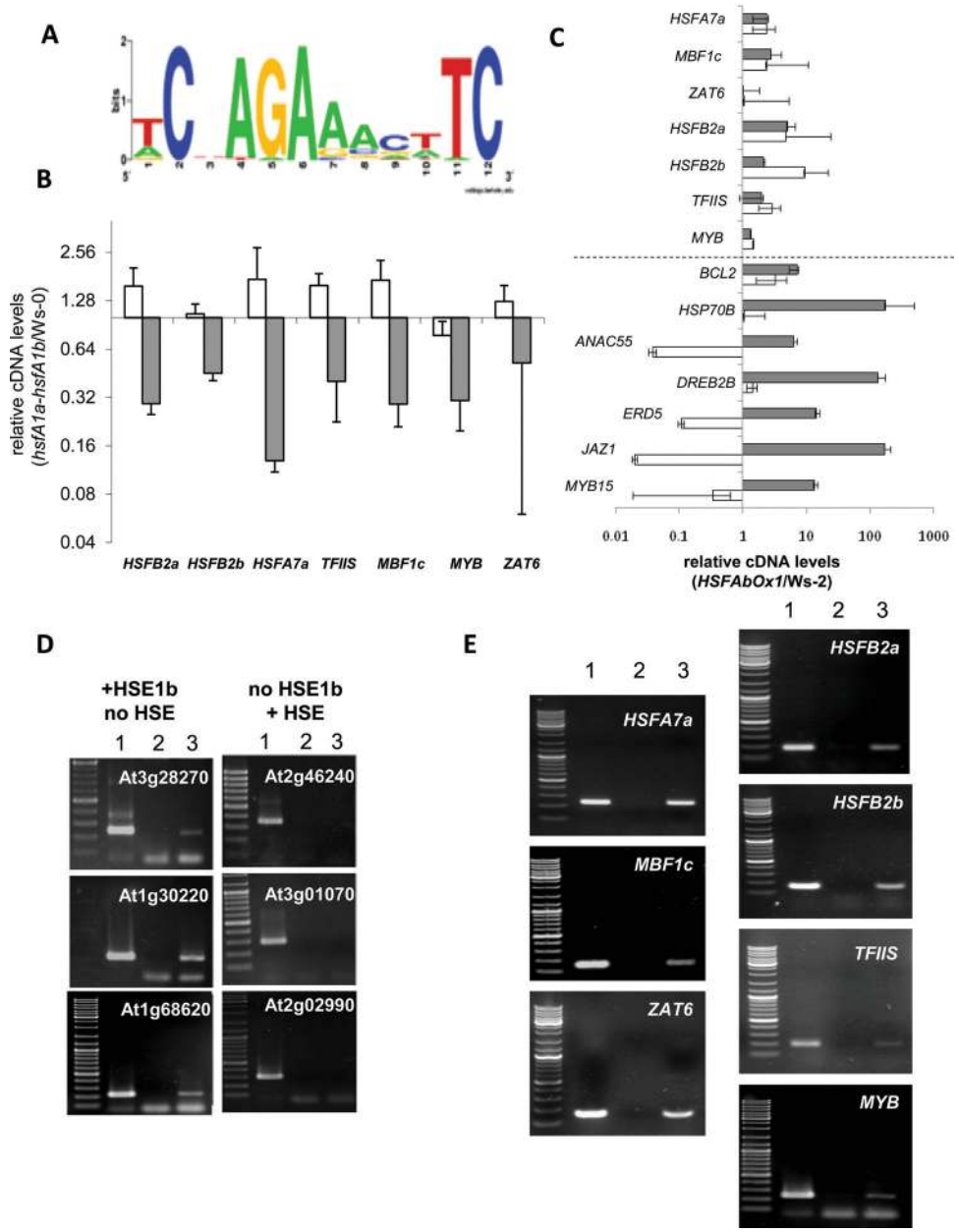

Fig. 4. The HSE1b motif is recognized *in vivo* in the promoters of TF genes regulated by *HSFA1b* overexpression. (A) The coloured letters show the consensus sequence, generated by MEME (see the Materials and methods), for the HSE1b motif present in the promoter regions of 55 *HSFA1bOx1*-up-regulated genes. (B) Expression, determined by qRT–PCR of HSE1b-containing TF genes in *hsfA1a/hsfA1b* plants subjected to 15 min at 22 °C (white bars) or 37 °C (grey bars). VPD was maintained at 1 kPa. All differences in the heat-stressed samples are significant ($P < 0.05$; Student's *t*-test) except for *ZAT6*. (C) Expression of the HSE1b-TF genes and *HSFA1b*-responsive genes without the HSE1b element (below the dotted line) in the presence (white bars) and absence (grey bars) of the protein synthesis inhibitor cycloheximide in plate-grown seedlings. The data are the means (±SEM) of two separate experiments, totalling six plates per treatment and three technical replicates per assay. (D) PCR amplification of ChIP promoter fragments of three genes containing a single HSE1b element (see Supplementary data) but no canonical HSE element (+HSE1b no HSE). The same procedure was carried out on three genes containing canonical HSE motif(s) but no HSE1b motif (no HSE1b +HSE; see Supplementary data). Gels showing PCR amplicons from positive control, input DNA (lane 1); negative control, no antibody control precipitation (lane 2); and ChIP DNA (lane 3). The result presented here is one of four representative experiments. The chromatin was immune-precipitated from fully expanded leaves of non-stressed 5-week-old *HSFA1b-mRFP_B* plants with anti-RFP antibody. (E) ChIP-PCR of the promoter regions of the seven HSE1b-TF genes from the same immune-precipitated samples as in D.

and HSE1b motifs in their promoter regions (Supplementary data). ChIP-PCR experiments revealed that HSFA1b binds *in vivo* to the promoters of the TF genes (Fig. 4E). Based on the analysis of the 'positive' and 'negative' control promoters in these experiments (Fig. 4D), it was concluded that HSFA1b most probably recognizes the HSE1b element in each TF gene.

MBF1c is part of the HSFA1b regulon and controls resistance to Pst and Hpa

Of the seven HSE1b-containing TF genes regulated by *HSFA1b* (Figs 5B, D; Supplementary Table S3 at *JXB* online), *MULTIPROTEIN BRIDGING FACTOR1c* (*MBF1c*) has already been studied extensively in the context of tolerance to heat and osmotic stress and resistance to pathogen infection (Suzuki *et al.*, 2005, 2008, 2011). The overexpression of HSE1b-containing genes, and especially the seven TF genes, could be responsible for the phenotypes observed in *HSFA1bOx* plants (Figs 1, 2). A corollary of this is that overexpression of some HSE1b-containing genes would reproduce all or part of the phenotypes observed in *HSFA1bOx* plants. Microarray data from *35S:MBF1c* plants (Suzuki *et al.*, 2005; here called

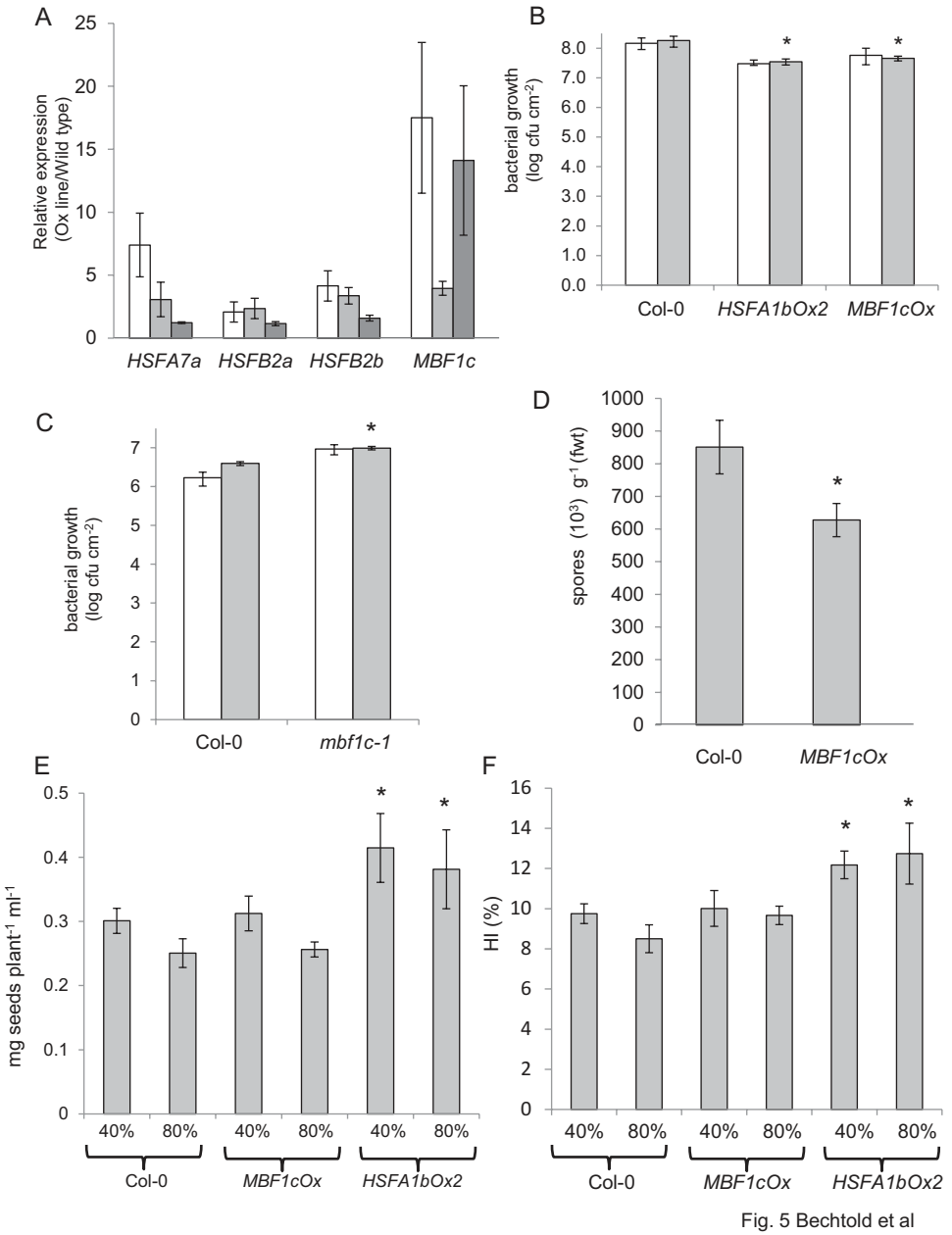

Fig. 5. Basal resistance but not water productivity is enhanced in *MBF1cOx* plants. (A) Analysis of gene expression of *HSFA1b*-responsive *HSF* genes and *MBF1c* in leaves of 5-week-old *HSFA1bOx* and *MBF1cOx* plants. White bars, *HSFA1bOx1*; light grey bars, *HSFA1bOx2*; dark grey bars, *MBF1cOx.* (B) Colonization of *Pst* in *MBF1cOx* and *HSFA1bOx2* compared with Col-0 at 2 d (white bars) and 4 d (grey bars) post-inoculation (n=6). The inocula recovered from leaves at day 0 was 2.3 log cfu ml^{-1} (±0.01). (C) Colonization of *Pst* in *mbf1c-1* plants compared with Col-0 at 2 d (white bars) and 4 d (grey bars) post-inoculation (n=6). (D) Spore yields from 12-day-old *MBF1cOx* and Col-0 plants ($n \geq 7$) inoculated 5 d previously with 5×10^4 spores of *Hpa*. Data for B–D are combined from two separate experiments. (E) Water productivity in *MBF1cOx*, *HSFA1bOx2*, and Col-0 plants (n=11) in well-watered (80% of maximum rSWC) and water-limited (40% rSWC) conditions; the data represent the mean (±SEM). (F) Harvest index from the plants and conditions in E. The differences marked with an asterisk (*) are significant at $P \leq 0.05$ (Student's t-test).

MBF1cOx) were compared with the microarray data set from *HSFA1bOx1* plants (Supplementary Table S8). There was a significant ($P < 0.0001$; hypergeometric distribution test) overlap of 24 genes between the 463 and 352 up-regulated genes of *MBF1cOx* and *HSFA1bOx1*, respectively (Supplementary Table S8). None of these 24 genes harbours a HSE1b element (Supplementary Table S3). *MBF1c* has been reported to regulate the expression of HSF genes (see Introduction), but the expression of *HSFA2a*, *HSFB2b*, and *HSFA7a* was unaffected in *MBF1cOx* plants (Fig. 5A; Supplementary Table S8). *MBF1cOx* and *mbf1c-1* plants (Suzuki *et al.*, 2008) were analysed for water productivity and resistance to pathogen infection. Significant resistance to *Pst* and *Hpa* was observed in *MBF1cOx* plants (Fig. 5B, D) and there was increased susceptibility of *mbf1c-1* to *Pst* infection (Fig. 5C). However, there were no significant increases in H_2O_2, GSH, and SA levels, HI, and water productivity of *MBF1cOx* plants in comparison with Col-0 (Supplementary Fig. S4A–C; Figs 5E, F).

Overexpression of HSFA2 does not result in phenotypes similar to HSFA1bOx plants

No improvements in water productivity, HI (Supplementary Fig. S3A, B at *JXB* online), and immunity to *Hpa* and *Pst* (Supplementary Fig. S4D–G), or increases in H_2O_2, GSH, and SA levels were observed in *HSFA2Ox* plants (Supplementary Fig. S4A–C). A microarray comparison between *HSFA2Ox* and Col-0 revealed only 43 DEGs (Supplementary Table S9) in contrast to the 509 for *HSFA1bOx1* (Supplementary Table S3). The overlap between data sets was 14 genes, of which 10 are heat stress responsive (Supplementary Table S9).

Discussion

HSFA1b controls a developmental component to drought tolerance and water productivity

The data presented show that *HSFA1b* is a determinant of drought/dehydration tolerance when overexpressed in *Arabidopsis* (Fig. 1A–C). In addition, *HSFA1b* fulfils the same role in wild-type plants since reciprocal effects on these parameters were observed in the *hsfA1a/hsfA1b* mutant (Fig. 1A, C). This effect of *HSFA1b* overexpression on drought/dehydration tolerance did not involve changes in the expression of *DREB2A* or many other ABA- or dehydration-responsive genes (Supplementary Tables S3, S4 at *JXB* online). Furthermore, *HSFA1b*-regulated genes were not as over-represented in microarray data sets from plants subjected to drought stress compared with those suffering infection or heat (Table 1). Instead, the enhanced drought tolerance and water productivity of *HSFA1bOx* plants (Figs 1A–E, 5E) are traits connected to the increase in HI (Figs 1F, 5F), revealing a developmental component to the *HSFA1bOx* water productivity phenotype. Overexpression of *Arabidopsis HSFA1b* in transgenic oil seed rape plants (Supplementary Fig. S3B) supports this interpretation since clear changes in seed yield and HI were observed in this species (Supplementary Fig. S3C–E). The drought response and water productivity phenotypes of *hsfA1a/hsfA1b* plants, in many cases, were the opposite of those of the *HSFA1bOx* plants (Fig. 1A, C, E, F). Thus the fecundity of both wild-type and *HSFA1bOx* plants under differing water regimes is influenced by the constitutive expression of *HSFA1b*, consistent with the properties of a robust water productivity trait (Morison *et al.*, 2008). To the authors' knowledge, there has been no single gene, when overexpressed in transgenic plants, specifically identified as influencing the HI component of water productivity (Passioura, 1977; Morison *et al.*, 2008). Biomass water ratio (BWR) is a component of water productivity (Morison *et al.*, 2008). In laboratory conditions, BWR is considered equivalent to water use efficiency (WUE; Morison *et al.*, 2008) and therefore single gene manipulations which influence WUE could also promote water productivity. These would include *ERECTA* (Masle *et al.*, 2005) and the TF genes *STRESS-RESPONSIVE NAC1* (Hu *et al.*, 2006), *HARDY* (Karaba *et al.*, 2007), and *NUCLEAR FACTOR-YB1* (Nelson *et al.*, 2007). A strong growth-defective phenotype has been observed in a quadruple knockout mutant of the clade A1 HSFs (Liu *et al.*, 2011), but the microarray analysis of *HSFA1bOx1* plants (Supplementary Tables S3, S4) did not reveal any enrichment of genes associated with development.

HSFA1b overexpression enhances basal resistance without compromising thermotolerance or yield

HSFA1bOx plants show enhanced resistance to virulent *Hpa* and *Pst* (Fig. 2A–D; Supplementary Fig. S2C, D at *JXB* online) while *hsfA1a/hsfA1b* plants show enhanced susceptibility to these pathogens (Fig. 2A–D). In general, there is much evidence of cross-talk between abiotic and biotic stress signalling (Fujita *et al.*, 2006; Miller and Mittler, 2006; Swindell *et al.*, 2007). Heat stress can induce programmed cell death which is associated with a burst of reactive oxygen speicies, which links biotic and heat stress signalling cascades (Vacca *et al.*, 2004; Larkindale and Vierling, 2008) with SA and ABA signalling (Dat *et al.*, 1998*a*, *b*; Larkindale and Knight, 2002; Larkindale and Huang, 2004; Larkindale *et al.*, 2005). This may explain the negative interaction between resistance to biotrophic pathogens and sudden exposure to high temperatures (Wang *et al.*, 2009). Furthermore, *HEAT SHOCK COGNATE70-1* (*HSC70-1*) overexpressing plants, which show enhanced thermotolerance, are negatively affected in basal and R gene-mediated resistance (Noël *et al.*, 2007). In contrast, *HSFA1bOx* plants reveal an important positive relationship in the signalling between heat and biotic stress responses.

The enhanced and diminished resistance to infection in *HSFA1bOx* and *hsfA1a/hsfA1b* plants, respectively (Fig. 2A–D), did not significantly involve SA-, JA-, and ABA-dependent signalling (Supplementary Table S4 at *JXB* online) or alterations in the levels of these hormones (Supplementary Table S6). *HSFA1b*-directed signalling could be mediated by H_2O_2 since genes responsive to it (Gadjev *et al.*, 2006) were significantly over-represented in the microarray data (Supplementary Table S4), selected genes from this group showed elevated expression in three *HSFA1bOx* lines (Fig. 3B),

and enhanced levels of H_2O_2 were detected in *HSFA1bOx* plants (Fig. 3C). GSH and H_2O_2 levels are often correlated with one another in plants showing altered basal resistance (de Gara *et al*., 2003; Mateo *et al* 2006; Bechtold *et al*., 2010; Dubreil-Maurizi *et al*., 2011). However, here, while GSH levels were enhanced in the *HSFA1bOx* lines (Fig. 3D), SA levels were not altered (Supplementary Table S6). Furthermore, the enhanced immunity of *HSFA1bOx* plants may also have been due to the overexpression of single genes such as *HSP90.1* and *LURP1* (*LATE UP-REGULATED IN RESPONSE TO H. PARASITICA RECOGNITION1*; Supplementary Table S3), which promote resistance to *Pst* and *Hpa*, respectively (Hubert *et al*., 2003; Knoth and Eulgem, 2008).

Many *Arabidopsis* mutants that constitutively express ABA and/or SA signalling pathways, or are primed for resistance to infection, show diminished fecundity (Dietrich *et al*., 2005; Heidel and Dong, 2006; Mateo *et al*., 2006; van Hulten *et al*., 2006; Bechtold *et al*., 2010). Clearly, by using SA- and ABA-independent basal disease resistance (Supplementary Tables S4, S6 at *JXB* online), *HSFA1bOx* plants were not compromised in seed yield or fitness (Fig. 1D–F; Supplementary Table S1) or thermotolerance (Prändl *et al*., 1998; Panchuk *et al*., 2002; Busch *et al*., 2005).

The HSE1b promoter motif suggests discrimination in HSFs binding to their cognate genes

Bioinformatics identified a modified HSE element associated with 55 genes up-regulated by *HSFA1b* overexpression (Fig 4A; Supplementary Table S7 at *JXB* online). Of the seven HSE1b-TF genes, six showed lowered expression in heat-stressed *hsfA1a/hsfA1b* plants (Fig. 4B) as well as all being overexpressed in non-stressed *HSFA1bOx* plants (Figs. 4C, 5B; Supplementary Table S3, Fig. S2B). From the cycloheximide experiments (Fig. 4C), it can be suggested that overexpressed *HSFA1b* directly regulates HSE1b-containing genes. The ChIP-PCR experiments (Fig. 4D) on genes containing either a single HSE1b or a single HSE showed that HSFA1b, at least when overexpressed under non-stressed conditions, specifically binds to the former. This suggests that HSFA1b recognizes HSE1b motif(s) in the promoters of the seven TF genes (Fig. 4E), supporting the conclusion from the cycloheximide experiments (Fig. 4C) that these genes are directly regulated by HSFA1b.

It must be emphasized that the functioning of the HSE1b element in wild-type plants remains to be established, but in support of the observations here a transcriptome analysis of *hsfA1a/hsfA1b* compared with wild-type plants showed that under heat stress, *HSFA1a* and *HSFA1b* could co-regulate the expression of >100 genes, most of which do not contain perfect HSEs (Busch *et al*., 2005).

MBF1c expression is regulated by HSFA1b in HSFA1bOx plants and contributes to the basal resistance phenotype

Direct regulation of HSE1b-TF genes in *HSFA1bOx* plants suggests that they could regulate in turn some of the remaining 454 genes (Supplementary Table S3 at *JXB* online), thus extending the *HSFA1bOx1* network to indirectly regulated genes. The example provided here comes from considering the interaction of HSFA1b with *MBF1c*. From the data presented (Figs 4B–E, 5A; Supplementary Table S3, Fig. S2B), it is concluded that under non-stressed conditions, overexpressed HSFA1b directly regulates *MBF1c* expression via its interaction with the HSE1b motif in the *MBF1c* promoter. *MBF1cOx* and *HSFA1bOx1* plants share altered expression of 24 genes (Supplementary Table S8), none of which containd a HSE1b motif (Supplementary Table S3). These genes would be classed as being indirectly regulated by *HSFA1b*. It is suggested that it is the combination of this direct and indirect regulation of the 509 genes (Supplementary Table S3) that determines the range of observed phenotypes of *HSFA1bOx* plants (Figs 1, 2; Supplementary S3; Prändl *et al*., 1998; Panchuk *et al*., 2002). *MBF1cOx* plants have enhanced basal resistance (Fig 6B, D), but did not show any enhancement of water productivity or HI (Fig. 6E, F). Thus the improved basal resistance of *HSFA1bOx* plants may be due to its direct control of *MBF1c* expression, in turn altering expression of downstream genes that contribute to the resistance phenotype. These observations contrast with recent studies which suggest that *MBF1c* acts upstream of an SA-dependent thermotolerance pathway, routed through *DREB2A*, *HSFB2b*, and *HSFB2a* (Suzuki *et al*., 2008, 2011). While up-regulation of *HSFB2a*, *HSFB2b* (and *HSFA7a*) expression in *HSFA1bOx* plants was readily measured (Figs. 4B, 5A; Supplementary Table S3), no effect of *MBF1c* overexpression was noted on the expression of these genes under non-stressed conditions (Fig. 5A). From the microarray data (Supplementary Table S3), no altered *DREB2A* expression was noted in *HSFA1bOx1* plants.

Overexpression of HSFA2 does not phenocopy HSFA1bOx plants

From the parallel studies on *HSFA2Ox* plants (Supplementary Figs S1, S4 at *JXB* online) it is evident that not all A-class HSFs control a broad spectrum of resistances to abiotic and biotic challenges or influence plant development. Although both *HSFA1b* and *HSFA2* are implicated in thermotolerance (Prändl *et al*; 1998; Nishizawa *et al*., 2006), they control early and late responses to heat stress, respectively (Li *et al*., 2010). In particular, it is suggested that early responding *HSF* genes such as *HSFA1b* appear to have developed as regulators of much larger gene networks in comparison with late responding *HSF* genes such as *HSFA2*, as evidenced from the present microarray analyses (Supplementary Tables S3, S9). It has been proposed that all clade A1 HSFs are regulators of the same environmental stress responses, which implies a high degree of redundancy (Liu *et al*. 2011). However, *HSFA1d* and *HSFA1e* directly regulate *HSFA2* expression during thermotolerance and high light responses (Nishizawa-Yokoi *et al*., 2011), while *HSFA1b* does not impact on *HSFA2* expression, and vice versa (Supplementary Fig. S1B, C; Busch *et al*. 2005). Rather, these observations suggest distinct but overlapping regulons for each of the clade A1 HSFs.

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. *HSFA1b* and *HSFA2* expressed in leaves of transgenic *Arabidopsis* plants and *HSFA1b* organ-specific expression in wild-type plants.

Figure S2. Phenotypes of *HSFA1bOx-mRFP_B* plants.

Figure S3. Overexpression of *HSFA1b* in oil seed rape improves seed yield and HI.

Figure S4. Foliar levels of SA, GSH, H_2O_2, HI, and water productivity in *HSFA2Ox* and *MBF1cOx* plants and response to *Pst* and *Hpa* of *HSFA2Ox* plants.

Table S1. Seed weight and viability of *HSFA1bOx* and *hsfA1a*/*hsfA1b* plants.

Table S2. Photosynthesis measurements.

Table S3. Microarray comparison of *HSFA1bOx1*/*Ws-2*, promoter analysis, and microarray data comparisons.

Table S4. GO analysis of *HSFA1bOx1*-responsive genes.

Table S5. Genes responsive to *Hpa* interaction with different *RPP* genes (Eulgem *et al*., 2004).

Table S6. Stress hormone levels.

Table S7. Occurrence of the HSE1b motif in putative direct target gene promoters.

Table S8. Microarray comparison of *MBF1cOx*/*HSFA1bOx1* and GO analysis of genes responsive to *MBF1cOx*.

Table S9. Microarray analysis of *HSFA2Ox*/*Col-0* and comparison with *HSFA1bOx1* genes.

Table S10. Primer sequences used in quantitative real-time PCR and ChIP-PCR.

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