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Overexpression of *Arabidopsis HsfA1a* enhances diverse stress tolerance by promoting stress-induced *Hsp* expression

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ABSTRACT. *Arabidopsis* (*Arabidopsis thaliana*) group A1 heat shock factors (Hsfs), including HsfA1a, are important regulators in the heat shock response. Previous studies have revealed that genetically engineered *HsfA1* members result in constitutive Hsf activation and heat shock protein gene (*Hsp*) expression under normal conditions, eventually enhancing basic thermotolerance in transgenic plants. In this study, we generated transgenic *Arabidopsis* plants overexpressing *HsfA1a*. One transgenic line showed a 94-fold increase in the level of *HsfA1a* mRNA (OE line 1). Overexpressing *HsfA1a* in OE line 1 plants resulted in higher levels of the inducible expression of *Hsp18.2* and *Hsp70* genes in response to heat stress, low/high pH changes, and hydrogen peroxide. Analysis of *in vivo* HsfA1a-promoter binding

suggested that the higher level of inducible *Hsp* expression was mediated by stress-induced activation of elevated levels of HsfA1a in the OE plants. The OE plants showed an increase in tolerance to low/high pH changes and hydrogen peroxide, in addition to heat shock. These results revealed that overexpressing *HsfA1a* had positive effects on tolerance to diverse stressors by promoting inducible *Hsp* expression following stress-induced HsfA1a activation. This study suggests a different mechanism for the activation of genetically engineered Hsfs from that suggested in previous reports, thus providing new insight into complex mechanisms used for achieving stress tolerance by genetic engineering.

Key words: *Arabidopsis thaliana*; Heat shock factor; Heat shock protein; Stress tolerance; Transgenic plants

INTRODUCTION

Plants respond to environmental and physiological stressors by the rapid expression of heat shock proteins (Hsps), which primarily act as molecular chaperones. Hsps bind to the hydrophobic surfaces of unfolded proteins, preventing their aggregation, and allowing the proper folding of stress damaged proteins. An increased accumulation of Hsps is essential for cell survival under exposure to stressors. In general, the expression of *Hsp* genes is genetically controlled at the transcriptional level by the binding of heat shock transcription factors (Hsfs) to heat shock elements (HSEs; consensus: nGAAnnTTCnnGAAn), which are present in the promoter regions of *Hsp* genes. The plant Hsf family is large; genome sequence databases contain 21 *Hsf* genes in *Arabidopsis*, 25 in soybean (*Glycine max*), and 26 in rice (*Oryza sativa*) (Nover et al., 2001; Mittal et al., 2009; Soares-Cavalcanti et al., 2012). Based on structural peculiarities, 3 classes (A, B, and C) and 14 groups of plant Hsfs are defined. Class A Hsfs have aromatic, large hydrophobic, and acidic peptide motifs (AHA) at the C-terminus and function as transcriptional activators, whereas class B and C Hsfs lack AHA motifs and have no activator functions of their own (Döring et al., 2000; Nover et al., 2001). A number of Hsfs have been characterized in *Arabidopsis*, revealing that HsfA1 (group 1 in class A, including members a, b, c, and d) are important transcriptional activators of the heat shock response (Lee et al., 1995; Prändl et al., 1998; Lohmann et al. 2004). In addition, recent evidence points to the involvement of HsfA1 in response to other environmental stressors. Although it is not yet possible to generate a single *HsfA1* knock-out mutant phenotype in *Arabidopsis* because of genetic redundancy in this group (Lohmann et al., 2004), analyses of *HsfA1a/b/d/e* quadruple knockout mutants indicate that these HsfA1 members engage in responses to hydrogen peroxide (H_2O_2), salt, and mannitol stress (Liu et al., 2011; Yoshida et al., 2011). Unlike HsfA2 (group 2 in class A), whose expression is heat induced (Nishizawa et al., 2006), HsfA1 members are constitutively expressed at low levels, whereas in wild-type (WT) plants, DNA binding activities and the transcriptional activation of *Hsp* genes are not constitutive. These activities are repressed under normal conditions. In the presence of heat stress, Hsf molecules form homotrimers from monomers, bind to HSEs, and gain transcriptional competence for target gene expression (Zhang et al., 2003).

Because plants are sessile, they often encounter a wide array of environmental stressors. Transgenic technology has long been a focus of research aiming to develop plant lines with increased adaptations to respond to various environmental changes, such as improving stress tolerance. Various strategies and transgenes employed in transgenic technology involve diverse physiological processes; however, their detailed mechanisms remain largely unclear. To date, 2 transgenic approaches have been adopted in studies of genetically engineered HsfA1 members: 1) expression of HsfA1a or HsfA1b fusion proteins (previously designated as HSF1 and HSF3, respectively) with glucuronidase (GUS) (Lee et al., 1995; Prändl et al., 1998) and 2) overexpression of *HsfA1b* (Prändl et al., 1998). In these cases, genetic engineering results in the derepression of Hsfs, i.e., constitutive Hsf activation and *Hsp* expression under normal conditions eventually increases basic thermotolerance. In this study, we generated transgenic *Arabidopsis* plants overexpressing *HsfA1a* that showed positive effects on stress tolerance to low/high pH changes, H_2O_2, and heat shock. Interestingly, when overexpressed in transgenic plants, HsfA1a could not sufficiently derepress Hsf activity, as does HsfA1b. Stress tolerance was instead achieved by promoting inducible *Hsp* expression, following stress-induced HsfA1a activation.

MATERIAL AND METHODS

pB7WG2/HsfA1a construction and generation of transgenic plants

Genomic DNA fragments of the gene *HsfA1a* (GenBank accession No. X76167), spanning the region from the start codon ATG to the stop codon TAA, were synthesized by polymerase chain reaction (PCR), and cloned into the binary vector pB7WG2 (Plant System Biology Department, Flanders Interuniversity Institute for Biotechnology, 9052 Gent, Belgium) using the recombination-based Gateway™ system (Invitrogen). The resulting binary construct, pB7WG2/HsfA1a, contained a *BAR* selection marker gene and *HsfA1a*, whose expression is under control of the *CaMV 35S*-promoter and the *CaMV 35S*-terminator in the T-DNA region (Figure 1A). Finally, the binary construct was introduced from the DH5α strain of *Escherichia coli* into the *Agrobacterium tumefaciens* strain LBA4404 by triparental mating. The resulting bacterial culture was used to transform *Arabidopsis*, ecotype Columbia, using the flower dip method (Clough and Bent, 1998). Seeds of the transformed plants were selected by germination on MS medium (Murashige and Skoog, 1962) containing 10 mg/L glufosinate ammonium (BASTA). The T_3 generation of the selfed plants was used for further analyses.

Semi-quantitative reverse transcription PCR (RT-PCR), real-time PCR, and Western blot analysis

Arabidopsis plants were grown in soil in a growth chamber under a 16-h light/8-h dark cycle at 25°C (60% humidity, 5000-lux light intensity). Whole plants of 4-week-old plantlets were harvested, immersed in section incubation buffer [SIB: 0.5 mM K_2HPO_4, 0.5 mM KH_2PO_4, 1% (w/v) sucrose, pH 6.0], and subjected to the following stress treatments:

1) Heat stress: The incubation was conducted at 37°C for 0 to 10 min.

2) Low pH change: Succinic acid was added to the SIB buffer to a final concentration of 0.05 M from the stock solution (0.5 M, titrated to pH 3.0 with Tris base), which changed the pH from 6.0 to 3.5, followed by incubation at room temperature for 0 to 10 min under vacuum.

3) High pH change: Tris was added to a final concentration of 0.1 M from the stock solution (2 M, pH 9.0), which changed the pH from 6.0 to 8.0, followed by incubation at room temperature for 0 to 10 min under vacuum.

4) H_2O_2 treatment: H_2O_2 was added to the SIB buffer to a final concentration of 0.4 mM, followed by incubation at room temperature for 0 to 10 min under vacuum.

5) Non-stress treatment: The incubation was performed at room temperature for 0 to 10 min.

Following stress treatments, total RNA was extracted from 200 mg whole plant materials using the AxyPrep™ Multisource Total RNA Miniprep Kit (TaKaRa, Dalian, China). Poly(A)-mRNA was isolated from total RNA using the E.Z.N.A.™ mRNA-Enrichment kit (TaKaRa). One hundred and fifty nanograms of mRNA was converted into cDNA using the PrimeScript® 1st Strand cDNA Synthesis Kit (TaKaRa). Ten nanograms of cDNA was used as a template in subsequent amplification for semi-quantitative RT-PCR. PCR was performed in a volume of 20 µL for 30 cycles under the following program: 94°C, 20 s; 59°C, 40 s; 72°C, 1 min. Gene-specific primer pairs used for amplifying *HsfA1a*, *Hsp18.2*, *Hsp70*, or *Actin2* are described in Lohmann et al. (2004). Real-time PCR was performed following methods described in Lohmann et al. (2004), following the same protocols for mRNA isolation and cDNA preparation as in semi-quantitative RT-PCR.

For the Western blot analysis, crude protein was extracted, following methods described in Lohmann et al. (2004), from *Arabidopsis* leaves of stressed plantlets as described above. Samples (15 µg per lane) were subjected to SDS-PAGE and electroblotted onto a polyvinylidene difluoride membrane (PVDF, Solarbio, China). Hsp70 was detected with an Anti-Hsp70 polyclonal antibody, which is specific to the inducible form of Hsp70 (Stressgen, USA). Actin was detected using an actin (26F7) monoclonal antibody against *Arabidopsis* actin 11 (Abmart, China). After incubation with an alkaline phosphatase-conjugated secondary antibody, bands were visualized with a combination of nitro-blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3'-indolyphosphate p-toluidine salt (BCIP) (Thermo, USA).

Chromatin immunoprecipitation

Transgenic OE line 1 plants were stressed by heat, low/high pH changes, and H_2O_2, essentially as described above. Since the threshold for detection of *in vivo* HsfA1a-promoter binding is very low, probability of detection is low early after the onset of stress (Zhang et al., 2003). Therefore, the duration of stress treatments was adjusted to a longer time of 20 min. For the sorbitol treatment, sorbitol was added into the SIB buffer to a final concentration of 1 M, followed by incubation at room temperature for 20 min under vacuum. The stressed plants were subjected to formaldehyde crosslinking/chromatin immunoprecipitation (X-Chip) using HsfA1a antibodies, and the immunoprecipitated DNA was analyzed by PCR using primer pairs specific to *HSP18.2* and *HSP70* gene promoters, according to Guo et al. (2008). A primer pair amplifying a far upstream sequence of the *HSP18.2* promoter was used as a negative control. The primer sequences used are described in Zhang et al. (2003).

Stress tolerance tests of transgenic plants

Seeds were disinfected in 1% HgCl (w/v). Sterile seeds were sown on filter paper

moistened using sterile 1/2 MS medium, pH 6.0, on Petri dishes. The Petri dishes were kept in a climate incubator (RXZ-380D, Ningbo Jiangnan Instrument Factory, Ningbo, China) under normal growth conditions: 16-h light/8-h dark cycle at 25°C, 60% humidity, and 5000-lux light intensity provided by fluorescent lamps. Stress tolerance was tested on 10-day-old seedlings.

For the thermotolerance analysis, Petri dishes were placed on a thermostatic heating block at 37°C, and incubated for 0, 0.5, or 1 h. After pretreatments at 37°C, the Petri dishes were transferred to the heating block at 42°C for 2 h. Following heat treatments, the Petri dishes were returned to normal growth conditions for 1 week, and the seedlings were photographed.

To test tolerance to low/high pH changes, the seedlings were transferred to filter paper on Petri dishes, which were wetted daily using 1/2 MS medium at pH 4, 6, or 8. The Petri dishes were maintained under normal growth conditions, and seedlings were photographed after 1 week.

Tolerance to H_2O_2 was assayed by transferring the seedlings to filter paper on Petri dishes, which were wetted daily using 1/2 MS medium, pH 6.0, containing 0, 2, or 5 mM H_2O_2. The Petri dishes were maintained under normal growth conditions, and seedlings were photographed after 12 days.

RESULTS

Effect of overexpressing *HsfA1a* on *Hsp* expression

The *CaMV 35S* promoter-driven *HsfA1a* transgenic plants that were generated exhibited visibly WT-like growth and development. Five different transgenic lines (T_3) were assayed for *HsfA1a* mRNA expression by semi-quantitative RT-PCR (Figure 1B) using plant materials without prior stress treatments, as both native *HsfA1a* and the transgene are constitutively expressed. Transgenic lines 1, 2, and 3 showed strong *HsfA1a* mRNA expression, with significantly higher levels than those of WT plants. The *HsfA1a* mRNA level of line 4 was lower, whereas the level of line 5 was similar to that of WT plants. We further quantified the *HsfA1a* mRNA level of 1 strong overexpression line (OE line 1) using real-time PCR. The data were normalized with respect to the mRNA level of *Actin2*, a housekeeping gene. The relative *HsfA1a* mRNA levels of OE line 1 and WT plants were 19.0853 ± 0.9200 and 0.2033 ± 0.0287, respectively. OE line 1 showed a 94-fold increase in the level of *HsfA1a* mRNA relative to the WT. This transgenic line was chosen for further analyses.

In order to test the effect of overexpressing *HsfA1a* on *Hsp* gene expression, OE line 1 plants were incubated following heat stress, low/high pH changes, H_2O_2, or no stress treatment. *Hsp18.2* and *Hsp70* mRNA levels were assayed by semi-quantitative RT-PCR (Figure 2). Without stress application, the OE plants showed weak expression of *Hsp18.2* and *Hsp70* genes. In contrast, expression of these *Hsp* genes was not detectable in WT plants. Heat stress, low/high pH changes, and H_2O_2 treatments all markedly increased the mRNA levels of *Hsp18.2* and *Hsp70* in both WT and OE plants, compared to plants without stress application. Time course analyses further showed that short-term stress treatments of 2 min resulted in higher levels of stress-induced *Hsp18.2* and *Hsp70* mRNAs in the OE plants compared to WT plants, although these differences disappeared after extended stress treatments for 10 min. Similarly, Western blot analysis revealed that the levels of the stress-induced Hsp70 protein were higher in the OE plants than in the WT plants (Figure 3). The timing of protein accumulation showed

that these differences were maintained under stress treatments for 10 min. This might be due to the increased stability of the protein compared to the mRNA. In certain experiments, we also noted that Hsp70 protein expression was not visualizable with NBT/BCIP under the non-stress condition in the OE plants, as this method is not highly sensitive. Overall, the elevated level of *HsfA1a* expression in the OE plants correlated with increased levels of induced *Hsp18.2* and *Hsp70* expression in the early stage of the stress response.

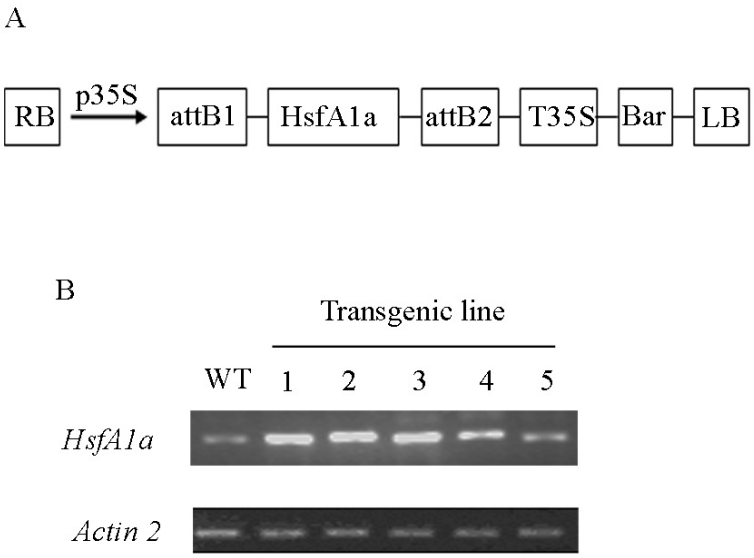

Figure 1. mRNA levels of *HsfA1a* in different transgenic lines. **A.** Block diagram illustrating transgene cassette in transgenic *Arabidopsis*. **B.** mRNA levels of *HsfA1a* in different transgenic lines. Total RNAs were isolated from wild-type *Arabidopsis* plants (WT) and five independent transgenic lines grown under normal growth conditions, respectively, reverse transcribed into cDNA and used as templates for semi-quantative RT-PCR. *Actin 2* was used as an internal control.

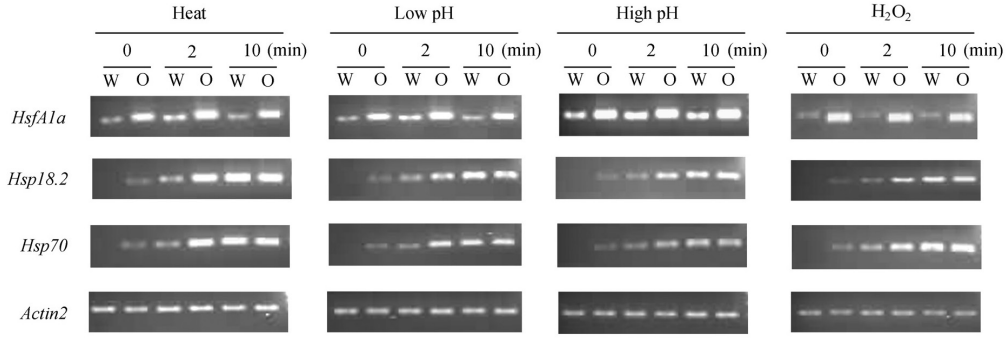

Figure 2. Analysis of *Hsp* mRNA levels in transgenic plants overexpressing *HsfA1a* under different stress conditions. *Arabidopsis* plants were incubated in the absence of stress, or stressed by heat shock, pH changes, and H_2O_2, respectively, for the time as indicated. Total RNAs were isolated, reverse transcribed into cDNA and used as templates for semi-quantative RT-PCR. *Actin 2* was used as an internal control. W = wild-type plants. O = OE plants.

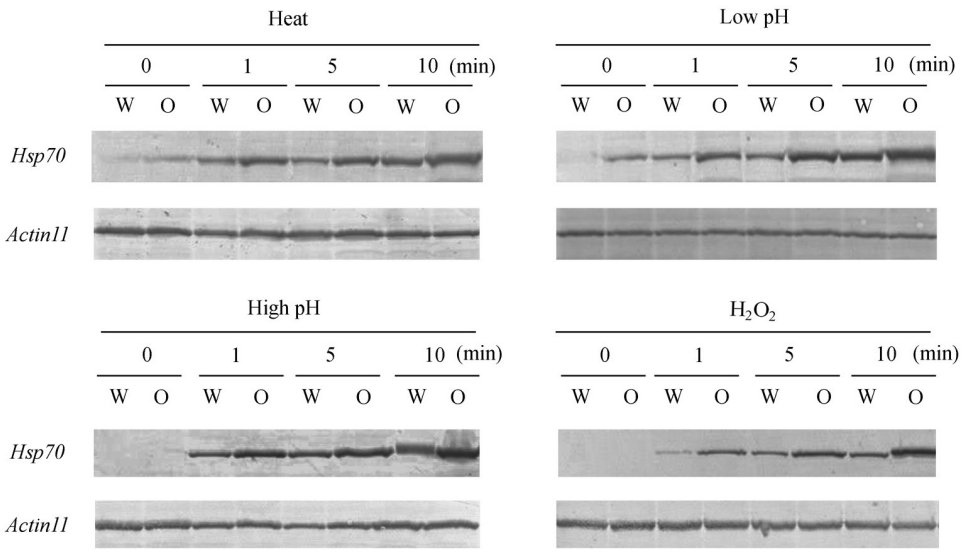

Figure 3. Immuno-detection of the Hsp70 protein expressed in transgenic plants overexpressing *HsfA1a* under different stress conditions. *Arabidopsis* plants were incubated in the absence of stress, or stressed by heat shock, pH changes, and H_2O_2, respectively, for the time as indicated. Crude protein samples were subjected to SDS-PAGE, electroblotted onto a PVDF membrane, detected by anti-Hsp70 antibody, and anti-Actin 11 antibody, respectively, followed by visualization with NBT/BCIP. W = wild-type plants. O = OE plants.

Effect of different stresses on HsfA1a-promoter binding in the OE plants

The correlation between elevated levels of *HsfA1a* expression and induced *Hsp18.2* and *Hsp70* expression in response to the stressors applied to the OE plants suggested that the transcriptional competency of HsfA1a was induced by the tested stressors. Overexpression resulted in elevated levels of active HsfA1a, elevating *Hsp* promoter occupation, and thus increasing transcription. However, previous studies have shown that overexpression of *HsfA1b* in transgenic plants and expression of fusion proteins of HsfA1a or HsfA1b with GUS result in constitutive Hsf activation (Lee et al., 1995; Prändl et al., 1998). This discrepancy motivated us to further examine whether *in vivo* HsfA1a-promoter binding, a key step in its activation, is stress inducible or constitutive in our OE plants. We employed an *in vivo* X-ChIP approach. OE line 1 plants were subject to different stress treatments, followed by formaldehyde cross-linking and immunoprecipitation by an anti-HsfA1a antibody. The immunoprecipitated DNA was analyzed by PCR using primer pairs specific to the *Hsp18.2* and *Hsp70* promoters. Input DNA, prepared from the same plant materials used in X-ChIP, was used as templates for control amplification. Results are shown in Figure 4. All input DNA samples gave strong PCR bands, indicating that the different stress treatments did not affect DNA quality for PCR amplification. The immunoprecipitated DNAs from samples without stress treatment or treated by sorbitol resulted in only weak bands, which may reflect the low level of constitutive binding. In contrast, samples stressed by heat shock, low/high pH changes, and H_2O_2 gave clear PCR signals, indicating that the high levels of HsfA1a binding to the respective *Hsp* promoters in the OE plants were not constitutive, but were induced by these stressors, leading to increased

Hsp expression. As a control of promoter specificity of HsfA1a binding, we amplified immunoprecipitates using a primer pair located far upstream of the *Hsp18.2* promoter. In this case, no PCR band was detected, and HsfA1a did not bind to non-target DNA.

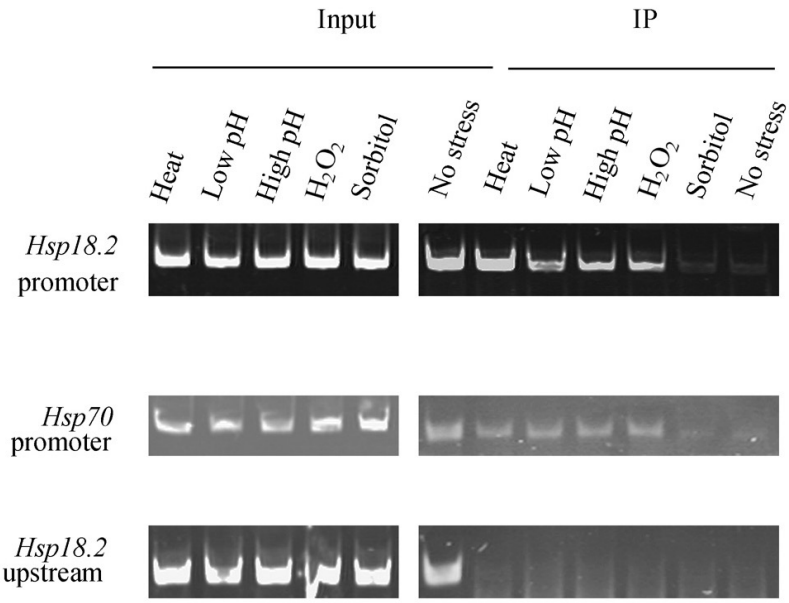

Figure 4. Effect of different stresses on HsfA1a-promoter binding in transgenic plants overexpressing *HsfA1a*. OE line 1 plants were subjected to different stress treatments as indicated, followed by X-ChIP with HsfA1a antibody. The immunoprecipitated DNAs (IP) were analyzed by PCR using primer pairs specific for *Hsp18.2* and *Hsp70* promoters. A primer pair amplifying a far upstream sequence of *HSP18.2* promoter was used as negative control. Input DNAs prepared from the same plant materials as in X-ChIP were used as templates for control PCR amplification.

Overexpressing *HsfA1a* in transgenic plants enhances tolerance to diverse stressors

We next investigated the effect of *HsfA1a* overexpression on stress tolerance. For the thermotolerance analysis, seedlings were subjected to a lethal dose of heat stress at 42°C for 2 h, either without or following mild sublethal doses at 37°C for 0.5 or 1 h, in order to induce *Hsp* gene expression. Without pretreatment at 37°C, both OE line 1 and WT plants were bleached, indicating that overexpressing *HsfA1a* was not sufficient for increasing basic thermotolerance. An increase in acquired thermotolerance in the OE plants was clearly revealed by pretreatments at 37°C for 0.5 or 1 h; OE plants survived while WT plants were severely bleached (Figure 5A).

A shift from a sublethal to a lethal dose of heat stress is a common method used to study the role of Hsfs in heat shock responses. However, such programmed shifts have not yet been established for tests of other stressors. In order to test tolerance to low/high pH changes, we placed seedlings on filter paper, which were wetted daily using 1/2 MS medium at pH 4, 6, or 8 for 1 week. Note that pH changes from 6 to 4 or 8 were not immediately lethal and could induce *Hsp* expression in the early stage after the onset of the stress treatment, as revealed by

the gene expression analysis described above. However, after a longer duration of 1 week, impairment of plant growth was evident. WT plants turned yellow at pH 4 and 8, but OE plants were less impaired, remaining green (Figure 5B). H_2O_2 tolerance was assayed in a similar way, treating seedlings on Petri dishes with medium containing 0, 2, or 5 mM H_2O_2 for 12 days (Figure 5C). The OE plants were tolerant up to the 5 mM H_2O_2 treatment level, whereas WT plants were more sensitive and were bleached at this treatment level. These results indicate that overexpressing *HsfA1a* in transgenic plants had positive effects on stress tolerance to low/high pH changes and to H_2O_2, in addition to heat stress. The increased stress tolerance in OE plants coincided with higher levels of *Hsp* expression induced by short periods of stress exposure, suggesting that early induction of *Hsp* expression was important for the development of stress tolerance.

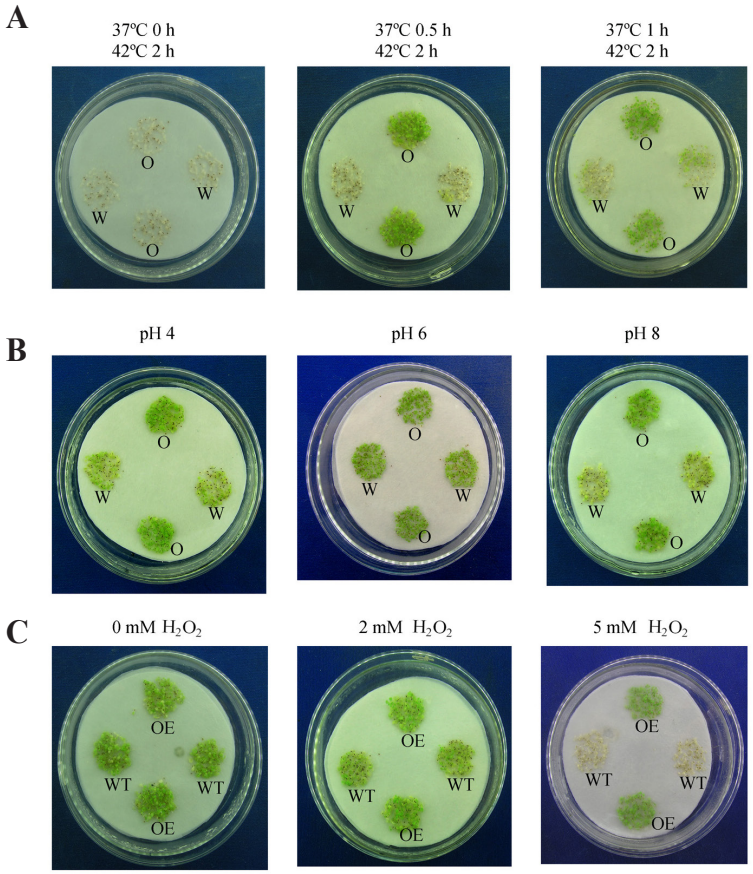

Figure 5. Stress tolerance of transgenic plants overexpressing *HsfA1a*. **A.** *Arabidopsis* seedlings were pretreated at 37°C for 0, 0.5, or 1 h, followed by heat stress at 42°C for 2 h. After the heat stress, the seedlings were returned to normal growth conditions for 1 week. **B.** *Arabidopsis* seedlings were transferred to filter paper, daily wetted using 1/2 MS medium at pH 4, 6, and 8, respectively, maintained under normal growth conditions for 1 week. **C.** *Arabidopsis* seedlings were transferred to filter paper, daily wetted using 1/2 MS medium, pH 6.0, with addition of 0, 2, and 5 mM H_2O_2, respectively, maintained under normal growth conditions for 12 days. W = wild-type plants. O = OE plants.

DISCUSSION

Previous analyses of transgenic plants with altered expression of *HsfA1* members have shown that differences in thermotolerance correlate with different levels of Hsf-regulated *Hsp* expression. Previous studies revealed that ectopic expression of HsfA1a- or HsfA1b-GUS fusion proteins and overexpression of *HsfA1b* in transgenic *Arabidopsis* plants result in the derepression of Hsfs, i.e., constitutive Hsf activation and synthesis of Hsps that confer basic thermotolerance (Lee et al., 1995; Prändl et al., 1998). In the present study, we found that overexpression of *Arabidopsis HsfA1a* caused only weak constitutive *Hsp* expression without stress treatment. In WT plants, HsfA1a activity is repressed by negative regulatory mechanisms, such as interaction with Hsp70, under normal conditions (Kim and Schöffl, 2002). Overexpression of *HsfA1a* may titrate the negative regulatory signal. Consequently, a substantial proportion of HsfA1a molecules was derepressed without stress treatment, causing weak constitutive stress-independent *Hsp* expression that may benefit plants exposed to stress. However, as demonstrated in our thermotolerance tests, both OE l and WT plants were bleached when subjected to heat stress at 42°C without a mild conditioning treatment at 37°C, indicating such weak stress-independent *Hsp* expression observed in our OE plants was not sufficient for increasing stress tolerance. Unlike HsfA1b, it appears that HsfA1a is not able to sufficiently derepress Hsf activity when overexpressed in transgenic plants. Our study revealed that higher levels of stress-inducible *Hsp* expression, through the activation of elevated levels of HsfA1a in OE plants induced by distinct stressors, are more essential to stress tolerance, thus providing new insight into the complex mechanisms underlying stress tolerance using genetically engineered Hsf.

In our experiments, the transgene *HsfA1a* was under the control of the *CaMV 35S*-promoter and the *CaMV 35S*-terminator, and we obtained OE line 1 plants showing 94-fold elevated levels of *HsfA1a* mRNA compared to WT plants. The *CaMV 35S*-terminator has been shown to be more effective than the *NOS* terminator for transgene expression (Mitsuhara et al., 1996). Furthermore, Wunderlich et al. (2003) studied a transgenic *Arabidopsis* line overexpressing *HsfA1a* (HsfA1a-42) that was under the control of the *CaMV 35S* promoter and the *NOS* terminator. This line showed only a 10-fold elevated level of *HsfA1a* mRNA, which was indistinguishable from that of WT with respect to its effect on *Hsp* induction. This different pattern may be due to lower expression of the transgene used in their OE line of HsfA1a-42. In the early stages of the stress response, the stress-induced *Hsp* expression levels in our OE plants were higher than those of WT plants, whereas extended stress treatments eliminated these differences. This result is in agreement with observations of Lohmann et al. (2004), who showed that HsfA1 members are regulators of immediate heat shock responses. In late stages, other factors will also be involved for mediating heat shock gene expression (Nishizawa et al., 2006).

The molecular basis underlying the role of Hsfs in stress tolerance is still not clear. Expression of Hsps as chaperones, including Hsp18.2 and Hsp70, protects cells against injuries associated with various stressors (Simões-Araújo et al., 2003; de Azevedo et al., 2012; Magdalena et al. 2012). On the other hand, HsfA1a can bind various promoter targets that are not limited to classical *Hsp* genes and also include other genes (Guo et al., 2008), implying a broad physiological basis for the stress tolerance role of HsfA1a. However, determining the exact genome-wide target genes of individual HsfA1 members, and whether target genes regulated by HsfA1a in response to distinct stressors are identical or only partially overlap require further investigation. Nevertheless, this study revealed that overexpressing *HsfA1a* had positive effects

on stress tolerance to low/high pH changes and to H_2O_2 in addition to heat stress. These results may be applied to other crops as a means to improve tolerance to diverse stressors.

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