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# Systemic and Intracellular Responses to Photooxidative Stress in *Arabidopsis* [W]

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**As the sun tracks daily through the sky from east to west, different parts of the canopy are exposed to high light (HL). The extent of and mechanisms by which a systemic acquired acclimation (SAA) response might preacclimate shaded leaves that will be subsequently exposed to full sunlight is largely undefined. We investigated the role of an *Arabidopsis thaliana* zinc finger transcription factor, *ZAT10*, in SAA. *ZAT10* overexpression resulted in enhanced tolerance to photoinhibitory light and exogenous $H_2O_2$, increased expression of antioxidative genes whose products are targeted to multiple subcellular compartments. Partial HL exposure of a leaf or leaves rapidly induced *ZAT10* mRNA in distal, shaded photosynthetic tissues, including the floral stem, cauline leaves, and rosette, but not in roots. Fully 86% of fivefold HL-upregulated and 71% of HL-downregulated genes were induced and repressed, respectively, in distal, shaded leaves. Between 15 and 23% of genes whose expression changed in the HL and/or distal tissues were coexpressed in the *ZAT10* overexpression plants, implicating *ZAT10* in modulating the expression of SAA-regulated genes. The SAA response was detectable in plants with mutations in abscisic acid, methyl jasmonate, or salicylic acid synthesis or perception, and systemic $H_2O_2$ diffusion was not detected. Hence, SAA is distinct from pathogen-stimulated systemic acquired resistance and apparently involves a novel signal or combination of signals that preacclimate photosynthetic tissues to HL.**

## INTRODUCTION

Increasingly, the commonality and differences between stress networks are being investigated (Chinnusamy et al., 2004; Mittler, 2006). Managing the response to two stresses that typically occur concurrently, such as drought and light, requires mechanisms to maximize synergistic and limit antagonistic responses. With respect to synergistic responses, exposure of a plant to high light (HL) or drought leads to inactivation of photosynthetic functions, namely photoinhibition, and the production of reactive oxygen species (ROS), such as hydrogen peroxide ($H_2O_2$), superoxide ($O_2^-$), singlet oxygen ($^1O_2$), and hydroxyl radicals that can both signal and cause damage (Niyogi, 1999; Noctor et al., 2002). Since plants are unable to avoid oxidative damage caused by light or drought stress, they employ a broad repertoire of protective measures, including minimization of light absorption, avoidance of ROS overaccumulation, and repair of damaged proteins, lipids, and photosystems (Bennet, 1977; Kao and Forseth, 1991; Allen, 1992; Demmig-Adams and Adams, 1996; Asada, 1999; Niyogi, 1999; Pfannschmidt et al., 1999; Kasahara et al., 2002). The detoxification of excess ROS relies on both antioxidants and antioxidant enzymes. Antioxidants include ascorbate, tocopherols, carotenoids, and anthocyanins (Foyer, 2001; DellaPenna and Pogson, 2006). Key examples of antioxidant enzymes are superoxide dismutase (SOD) and ascorbate peroxidase (APX) that disproportionate $O_2^-$ radicals and catalyze the conversion of $H_2O_2$ to water, respectively (Asada, 1999). SOD and APX exist in multiple isoforms throughout the chloroplast, mitochondrion, peroxisome, and cytosol and are differentially regulated in a tissue-dependent manner during oxidative stress responses (Karpinski et al., 1997; Mittler et al., 2004).

A large suite of genes regulated by HL encode proteins whose functions include ROS detoxification, synthesis of antioxidants, adjustment of light-harvesting antenna size, maintenance and restoration of the photosynthetic apparatus, and protein protection and repair (Rossel et al., 2002; Kimura et al., 2003; Mittler et al., 2004). Similarly, there are changes in the thylakoid proteome of *Arabidopsis thaliana* (Giacomelli et al., 2006) and total soluble proteome of *Chlamydomonas reinhardtii* (Förster et al., 2006) in response to HL and changes to the transcriptome and metabolome in light-stressed grapevines (Cramer et al., 2007). Typical examples of the transcriptional response include the induction of heat shock proteins (HSPs) and the cytosolic

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isoforms of APX, *APX1* and *APX2* (Mullineaux et al., 2000; Rossel et al., 2002). ROS accumulation and a change in the redox poise of the plastoquinone pool influence *APX2* gene expression, as does the *regulator of APX2* (*rax1-1*) mutation in glutathione metabolism, demonstrating the involvement of glutathione biosynthesis and redox balance in oxidative stress signaling (Karpinski et al., 1999; Fryer et al., 2003; Ball et al., 2004). At present though, the extent to which transcriptional factors function in modulating *APX2* mRNA levels and photoprotective and photosynthetic capacity are inadequately understood.

Identifying and manipulating the key transcription factors that affect transcriptional responses across multiple cellular compartments would allow for an integrative protective approach compared with transgenic studies in which the expression of individual antioxidant enzymes is modified. While *cis*-acting ROS-responsive motifs have recently been identified in plants (Geisler et al., 2006), there are few examples, if any, of transcription factors that coordinately regulate photosynthesis, photoprotection, and ROS-detoxifying enzymes. One such report shows the link between a transcription factor and photosynthetic rates (Savitch et al., 2005), while other recent studies have identified a family of transcription factors that are proposed to repress gene expression via the ERF-associated amphiphilic repression (EAR) motif and that play key roles in biotic and abiotic stress resistance (Kazan, 2006). Research into a member of the EAR family, the $Cys_2/His_2$-type zinc finger transcription factor, *ZAT12* (At5g59820), has shown that tolerance to ROS is altered due to changes in the induction of *APX1* and other genes in response to $H_2O_2$ (Rizhsky et al., 2004; Davletova et al., 2005). However, although $H_2O_2$-dependent induction of *APX1* was suppressed in *ZAT12-KO* plants, it remained inducible by HL, indicating that a primary HL pathway was unaffected (Iida et al., 2000; Rizhsky et al., 2004). Furthermore, a substantial proportion of the HL-responsive transcriptome was not altered by *ZAT12* overexpression (Rossel et al., 2002; Kimura et al., 2003; Davletova et al., 2005).

Another member of the *Arabidopsis* ZAT gene family, *ZAT10* or *STZ* (At1g27730), has been shown to respond to drought, salt, and cold (Sakamoto et al., 2000, 2004; Gong et al., 2001; Lee et al., 2002) and appears to increase drought tolerance when overexpressed in transgenic plants (Sakamoto et al., 2004). ZAT10 negatively regulates gene expression (Sakamoto et al., 2000, 2004; Gong et al., 2001; Lee et al., 2002) and was placed on signaling pathways developed by Zhang et al. (2004), yet the extent of its function in photosynthesis and photoprotection is poorly understood (Kazan, 2006). In this article, we report on the overexpression and knockdown by RNA interference (RNAi) of *ZAT10* and how it affects intracellular and intercellular responses to HL-induced oxidative stress.

The first evidence for systemic signaling of HL-induced oxidative stress to distal leaves was presented in 1999 (Karpinski et al., 1999), in which it was demonstrated that *APX2* is induced in distal leaves to 11% of levels in exposed leaves. In addition, there were changes to photochemistry in shaded leaves that were consistent with a preacclimation response to excess light. That is, photosynthetic efficiency ($F_v/F_m$) and photochemical quenching ($q_p$) were marginally but significantly higher in a distal leaf subsequently exposed to HL compared with leaf treated with HL without preacclimation (0.72 versus 0.62 and 0.78 versus 0.70, respectively), and it was suggested that $H_2O_2$ may play a role in this SAA. Since this key study, only a few reports have considered this distal response further. It has been shown that two to three other genes are systemically inducible and that HL results in the accumulation of $H_2O_2$ in the vasculature of exposed but not shaded areas of detached, HL-treated leaves (Fryer et al., 2003; Ball et al., 2004). As such, many unknown factors of SAA remain, including the distance and rate it can travel, the transcriptional changes induced in distal tissues versus those occurring in HL-exposed leaves, the transcription factors that may be involved and the nature of the inducing signal(s) and the extent of preacclimation to HL.

## RESULTS

### Silencing and Overexpression of *ZAT10* Alters Photoprotection and Photosynthesis

*ZAT10 promoter:LUCIFERASE* (*ZAT10:LUC*) fusions were created and transformed into plants. In leaves, the majority of *ZAT10* expression was detected in the vascular tissue by CCD imaging of luminescence emitted as a product of luciferase activity (see Supplemental Figure 1A online). The induction of *ZAT10* mRNA by abiotic stresses is well established, and in this study, we demonstrated that it occurred very rapidly in response to HL. Within 5 min of HL exposure (1000 $\mu$mol $m^{-2}$ $s^{-1}$), a rapid 10-fold rise in *ZAT10* mRNA abundance was observed (see Supplemental Figure 1B online). Levels peaked at 10 min, after which mRNA levels slowly subsided, reaching basal levels after a period of hours. A number of stimuli contribute to the HL response, including ROS, light quality, the redox status of the cytosol and chloroplast (Foyer and Noctor, 2003), and abscisic acid (ABA) (Rossel et al., 2006), all of which alter *ZAT10* mRNA abundance (data not shown). The HL induction of *ZAT10* was eliminated by spraying wild-type plants with the antioxidant glutathione or with DCMU, an herbicide that inhibits photosystem II (PSII) (data not shown).

To examine the role of ZAT10 in photoprotection, *ZAT10* mRNA was either silenced using RNAi (*zat10(i)*) or constitutively overexpressed using the cauliflower mosaic virus 35S promoter (*35S:ZAT10*). A number of transgenic lines were collected and analyzed (Figure 1). Most RNAi lines had severely reduced *ZAT10* expression, ranging from 10 to 30% of wild-type levels (Figure 1A). Off-target effects of RNAi in plants have been reported for fragments of 21 to 23 nucleotides or more (Thomas et al., 2001; Watson et al., 2005; Xu et al., 2006); thus, several checks were performed to confirm that nonspecific gene silencing had not occurred. First, five RNAi lines had heritably reduced *ZAT10* expression and exhibited comparable phenotypic traits. Second, a report published since submission showed similar changes in RNAi and *ZAT10* knockouts (Mittler et al., 2006). Third, the fragment used for RNAi was designed to have minimal complementarity with other genes: a BLAST query against the National Center for Biotechnology Information *Arabidopsis* database revealed two regions of homology $\geq$21 nucleotides of 24 and 25 nucleotides, respectively, without mismatch in *ZAT6* (At5g04340), the most closely related gene to *ZAT10*. Another gene, *AZF3* (At5g43170), contained a homologous sequence 26 nucleotides in

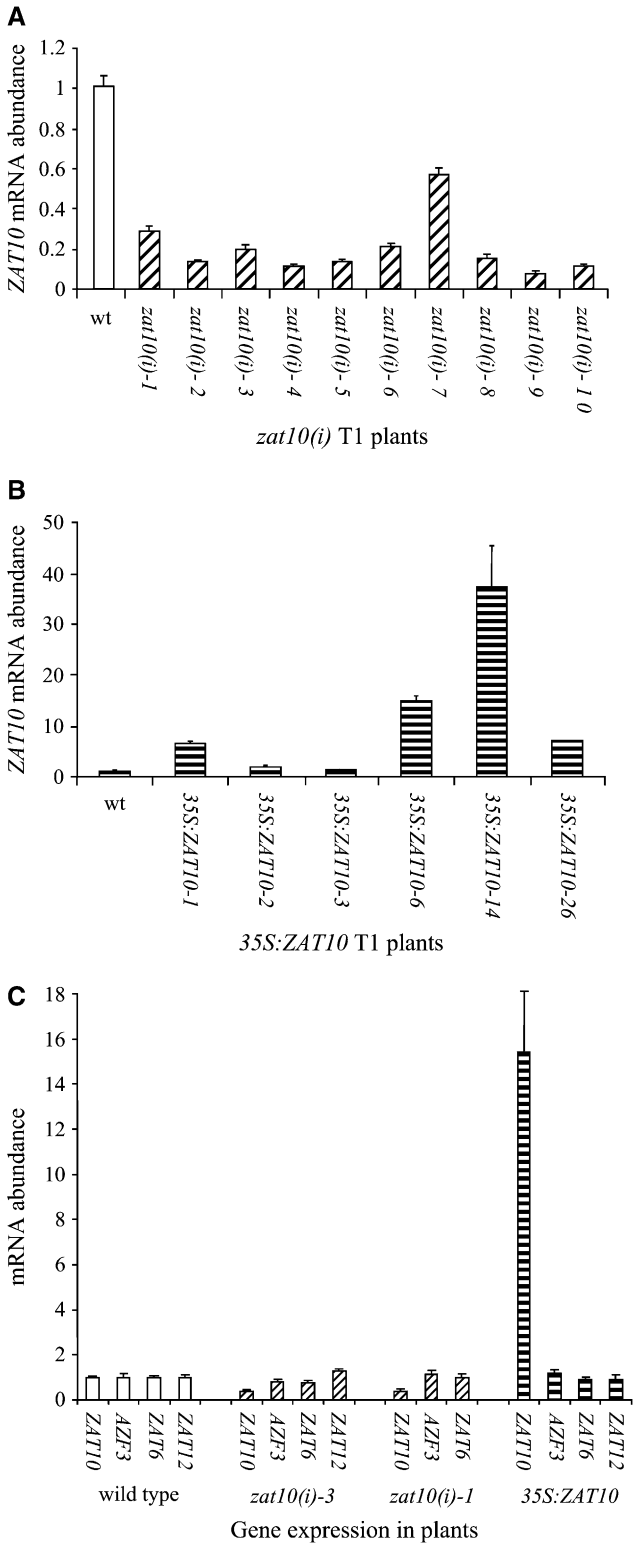



**Figure 1.** Analysis of *ZAT10* Silenced and Overexpressing Transgenic Plants.

**(A)** *ZAT10* mRNA abundance in 10 individual transformants of *ZAT10* RNAi lines [*zat10(i)*].

**(B)** *ZAT10* mRNA abundance in six individual lines constitutively expressing *ZAT10* (*35S:ZAT10*).

**(C)** mRNA abundance of *ZAT10*, *AZF3*, *ZAT6*, and *ZAT12* in wild-type, two *ZAT10* RNAi lines, and a *35S:ZAT10* line. Transcript abundance was measured by real-time RT-PCR and normalized against wild-type leaves of the same age. Each point is the mean ± SE of three to five leaves from at least three different plants.

length. There were no regions of homology to any other member of the *ZAT* family or, indeed, any other transcription factor that met this constraint. Real-time RT-PCR was undertaken for *ZAT6*, *AZF3*, and *ZAT12* in two *zat10(i)* lines and one *35S:ZAT10* line (Figure 1C) and for another experiment measuring *ZAT6*, *ZAT7*, and *AZF3* mRNA levels in one RNAi line (data not shown). These assays revealed no significant changes in the expression of *ZAT6*, *ZAT7*, *AZF3*, or *ZAT12* that could suggest off-target silencing due to the *ZAT10* RNAi construct.

To study the extent to which ZAT10 mediates the induction of HL-responsive genes and alters stress response mechanisms in multiple cellular compartments, gene expression was examined in the transgenic lines (Figure 2). APX1, APX2, and sHSP are targeted to the cytosol, and Fe-superoxide dismutase (FSD1), Cu-superoxide disumutase (CSD2), and protochlorophyllide oxidoreductase B (PORB) are targeted to the chloroplast. The patterns of expression in response to low-light (LL) and HL treatments were not markedly altered in transgenic plants for *APX1*, *CSD2*, and *sHSP* mRNA (Figures 2A, 2C, and 2E). By contrast, the HL-inducible response of *APX2* was severely reduced by approximately fivefold in *zat10(i)* plants and elevated 20-fold in *35S:ZAT10* leaves compared with the wild type. In fact, *APX2* mRNA abundance was >30-fold higher in unstressed *35S:ZAT10* leaves than in wild-type leaves (Figure 2B). Total APX activity was measured for protein extracts from leaves of the wild type (0.125 ± 0.037 μmol $min^{-1}$ mg $protein^{-1}$), *zat10(i)-1* (0.154 ± 0.049), *zat10(i)-3* (0.154 ± 0.049), *35S:ZAT10-6* (0.137 ± 0.0004), and *35S:ZAT10-14* (0.123 ± 0.033). The *t* tests showed no significant difference between each transgenic line and wild-type APX activity. This result may reflect a lack of change in protein for APX2 or the masking of any change by the multiple isoforms of APX, such as APX1, which is the most abundant isoform in nonstressed leaves (Panchuk et al., 2002). *FSD1* and *PORB* mRNA levels were constitutively lower in *zat10(i)* than in the wild type and much higher in *35S:ZAT10*, although there was a decline under HL in *35S:ZAT10* plants (Figures 2D and 2F).

Whole plants were infused with stains for detection of endogenous ROS prior to LL and HL treatments (Figure 3A). The leaves of mature *zat10(i)* plants accumulated more $H_2O_2$ in both LL-grown and HL-exposed treatments than wild-type plants. Consistent with this observation, *35S:ZAT10* leaves accumulated less $H_2O_2$ following HL treatment than wild-type or *zat10(i)* leaves. Additionally, the cotyledons of *zat10(i)* seedlings were chlorotic and accumulated substantially more $O_2^-$ than wild-type seedlings, suggesting that ROS-induced photobleaching was taking place (see Supplemental Figure 1C online). Leaves of *zat10(i)* plants were paler green than the wild type, an observation reflected in reduced chlorophyll per gram of fresh weight (Figure 3B); this was observed in five different RNAi lines, with chlorophyll levels

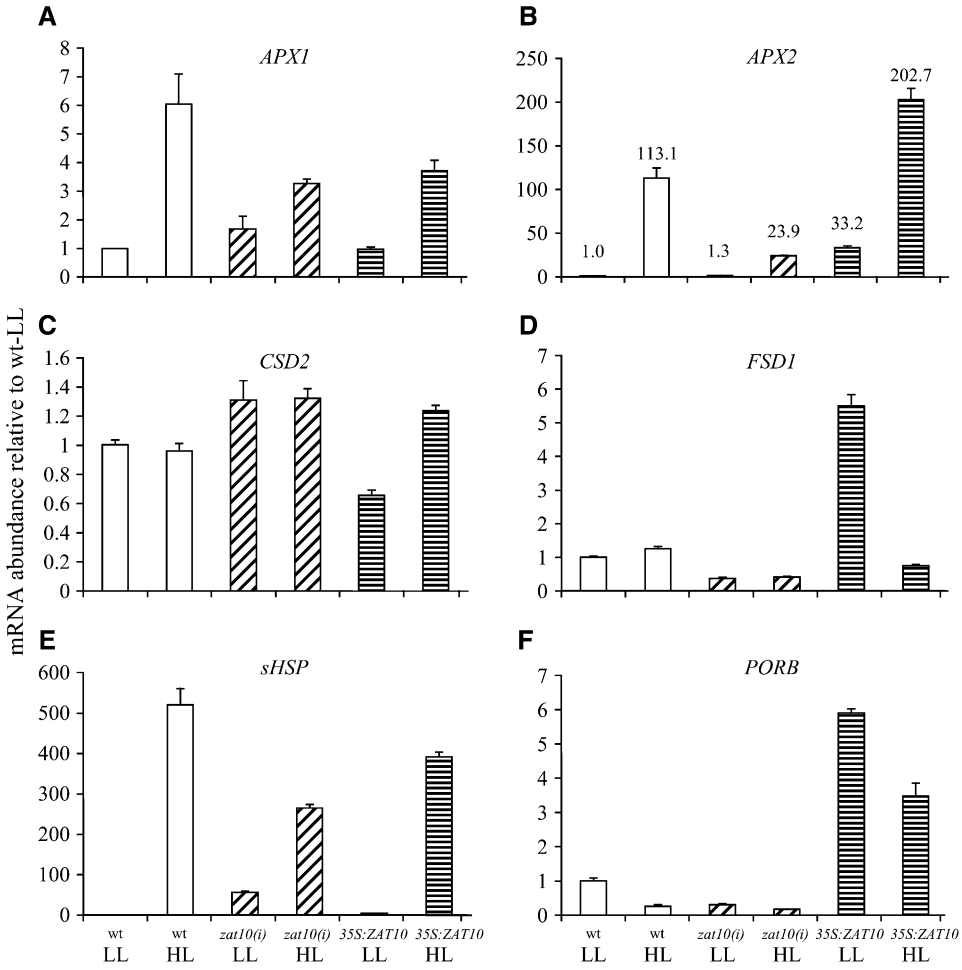



**Figure 2.** Real-time RT-PCR Analyses of HL-Responsive Genes in Wild-Type, *zat10(i)*, and *35S:ZAT10* Plants.

mRNA abundance after LL and HL treatments for 1 h. **(A)** *APX1*, **(B)** *APX2*, **(C)** *CSD2*, **(D)** *FSD1*, **(E)** *sHSP*, and **(F)** *PORB*. mRNA values were normalized against the wild type in LL. Each experiment was repeated at least three times, and all values are an average of three samples per time point. The mean and SD are plotted. Open bars, the wild type; diagonally hatched bars, *zat10(i)*; horizontally hatched bars, *35S:ZAT10*.

ranging from 40 to 65% of normal. The reduction in chlorophyll was more pronounced in younger plants than older, in which chlorophyll content was ~80% of wild-type levels. The average chlorophyll *a*/*b* ratio was marginally higher for mature *zat10(i)* plants (Figure 3B), but this was not significant and is not indicative of major changes to the photosystems (Pogson et al., 1998). There was no change in the proportion of individual carotenoids (Figure 3C). Anthocyanins are predominantly vacuolar localized and have been implicated as antioxidants (Yamasaki et al., 1996). After HL exposure, *zat10(i)* plants accumulated less anthocyanins than wild-type plants, while *35S:ZAT10* plants accumulated more (Figure 3D). The rosette size of the RNAi plants was significantly smaller compared with the wild type, but there was no such difference in the size of *35S:ZAT10* plants (Figure 3E) This is in contrast with previous reports of growth retardation in *ZAT10*-overexpressing plants (Sakamoto et al., 2004).

Leaves were subjected to exogenous application of $H_2O_2$ to determine if the *35S:ZAT10* plants were more resistant to oxidative damage. To determine the optimal protocol, a range of $H_2O_2$ concentrations were tested, as was preillumination of specimens under LL and HL. The protocol adopted involved pretreatment of intact plants with 30 min of HL prior to incubation in $H_2O_2$ overnight at LL. In three replicate experiments, *35S:ZAT10* leaves were observed to be more resistant to $H_2O_2$-induced bleaching (Figure 3F).

The genetic manipulation of *ZAT10* mRNA abundance altered $CO_2$ fixation (*A*) and linear electron transport rate through PSII (*ETR*) (Figures 4A and 4B). Under a range of elevated light regimes, photosynthetic rates were significantly increased in *35S:ZAT10* and significantly reduced in *zat10(i)* plants compared with wild-type plants. At the latter stage of development used for Li-COR analyses, the *zat10(i)* plants were only mildly chlorotic. Statistical analysis was undertaken by two-way analyses of variance of the data sets between 300 and1200 μmol photons $m^{-2}$ $s^{-1}$. The three genotypes differed significantly in rates of $CO_2$ fixation ($P < 0.0001$) and *ETR* ($P < 0.0001$). The *t* tests of the

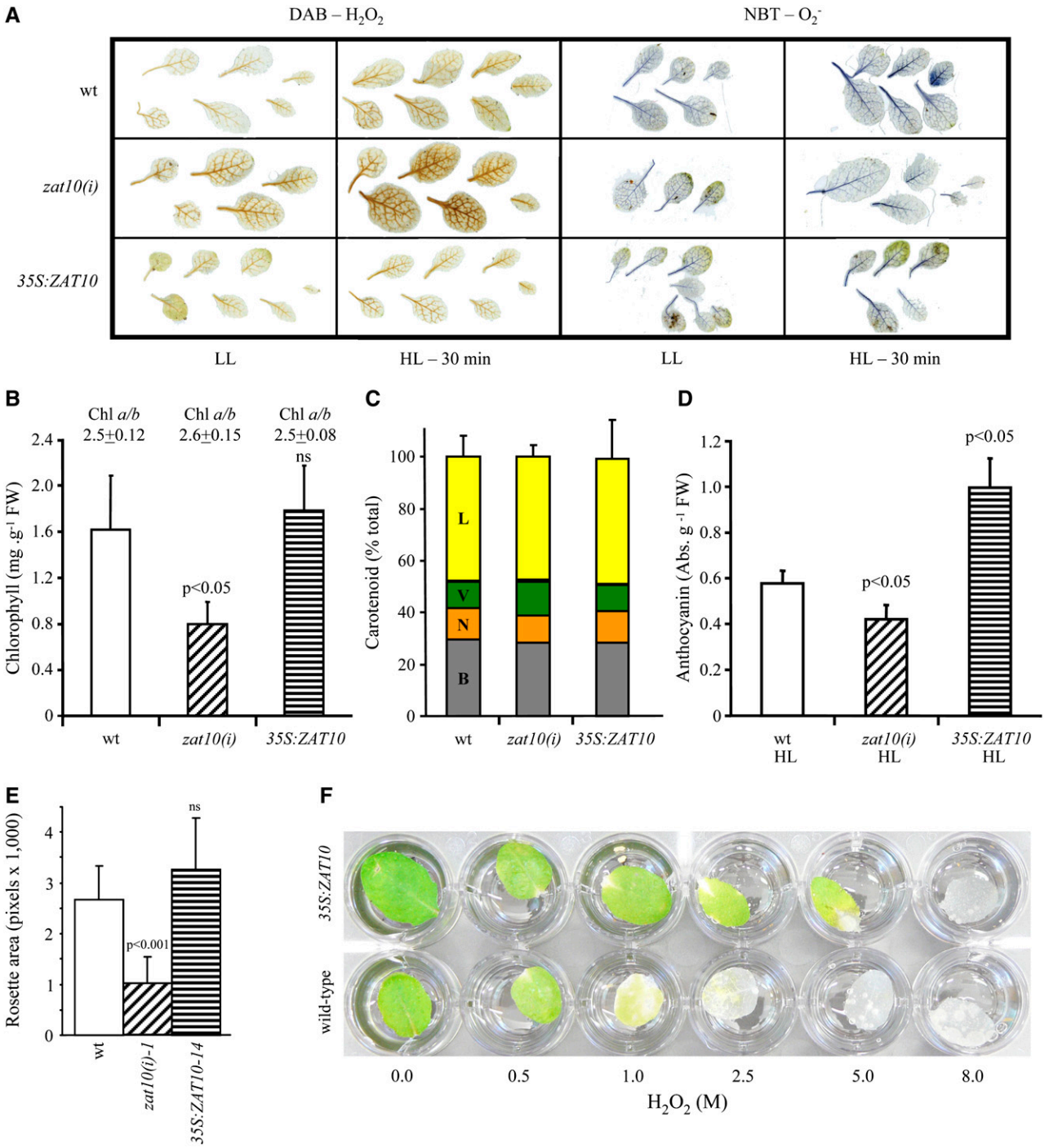



**Figure 3.** Analysis of *ZAT10* Silenced and Overexpressing Transgenic Plants.

**(A)** Representative images illustrating $H_2O_2$ and $O_2^-$ accumulation in wild-type, *zat10(i)*, and *35S:ZAT10* leaves, visualized by prestaining with 3,3′-diaminobenzidine (DAB) and nitro blue tetrazolium (NBT), respectively.

**(B)** Chlorophyll content in wild-type (open bars), *zat10(i)* (diagonally hatched bars), and *35S:ZAT10* (horizontally hatched bars) leaves. Chlorophyll *a*/*b* ratios plus SD are shown. P values from *t* tests compared with wild-type levels are shown. ns, not significant; FW, fresh weight. For each line, $n = 5$.

**(C)** Carotenoid profile of wild-type, *zat10(i)-1*, and *35S:ZAT10-14* leaves as a percentage of total carotenoids. The data are the average values of three independent experiments. The mean and SD are plotted; for each line, $n = 9$. N, neoxanthin; V, violaxanthin; B, β-carotene; L, lutein.

**(D)** Anthocyanin content of leaves after 24 h of exposure to 700 μmol photons $m^{-2}$ $s^{-1}$. The data are the average values of three independent experiments. The mean and SD are plotted; for each line, $n = 9$.

**(E)** Rosette leaf surface area of wild-type and *ZAT10* transgenic plants after 6 weeks of growth. Average rosette area and SD are depicted; for each line, $n = 14$ to 19. The *t* tests comparing wild-type and transgenic lines were performed, and the resulting P values are shown.

**(F)** Effect of exogenous $H_2O_2$ on wild-type and 35S:*ZAT10* leaves pretreated with HL for 30 min and then floated on a solution of $H_2O_2$ at the indicated concentration. The experiment was repeated three times.

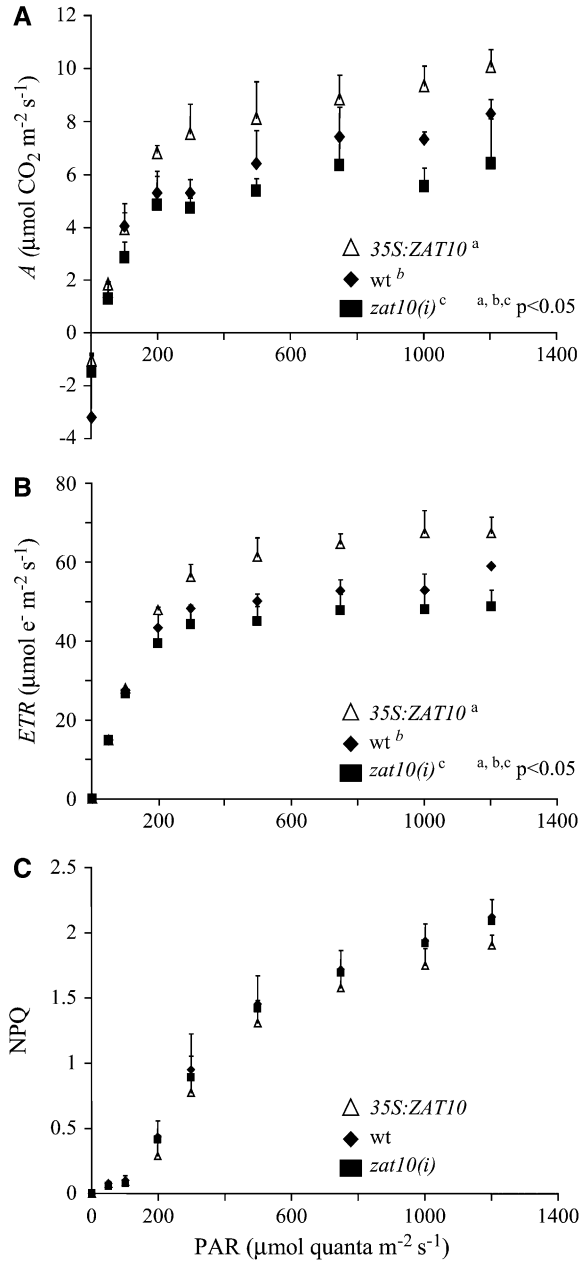



**Figure 4.** Photosynthetic Rates and Nonphotochemical Quenching for the Wild Type, *zat10(i)*, and *35S:ZAT10*.

**(A)** Net $CO_2$ fixation (*A*).

**(B)** Linear electron transport rate through PSII (*ETR*).

**(C)** Nonphotochemical quenching (NPQ).

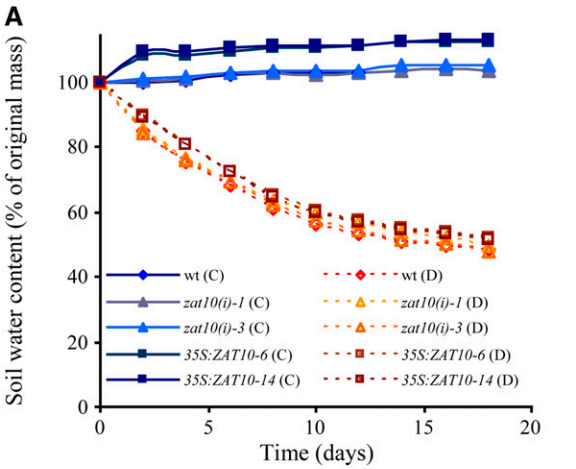
Parameters were measured by gas exchange analysis over increasing light intensities (photosynthetically active radiance [PAR]). Each point is the mean ± SE of three to five leaves from at least three different plants. P values from *t* tests of the means of values for 500 to 1200 μmol photons $m^{-2}$ $s^{-1}$ are shown.

means of values undertaken for *A* and *ETR* at photosynthetically active radiances between 500 and 1200 μmol photons $m^{-2}$ $s^{-1}$, at which intensities the photosynthetic parameters were at saturation levels. There were significant differences for each set of pairwise comparisons between the wild type, *zat10(i)*, and *35S:ZAT10* for *A* and *ETR* ($P < 0.05$). Additionally, there was also a small but significant decrease in the oxidation state of the plastoquinone pool in *35S:ZAT10*, as indicated by measurements of photochemical quenching (data not shown). Nonphotochemical quenching was unaltered in RNAi plants, and the marginally lower levels observed in *35S:ZAT10* specimens are unlikely to have any physiological effect (Figure 4C).

Overexpression of *ZAT10* was previously shown to increase drought tolerance (Sakamoto et al., 2004), although no mechanism was presented. We undertook an analysis of drought tolerance by withholding water from the wild type, two RNAi lines, and two overexpression lines (Figure 5). The onset and progression of visible symptoms (wilting, anythocyanin accumulation, and chlorosis) and the rate of water loss from the pots were similar for all lines examined (Figure 5A; see Supplemental Figure 2D online). After 20 d of drought, all plants were wilted and appeared to have lost viability; at this time, the specimens were rewatered to confirm survival. No wild-type or *35S:ZAT10* plants recovered from drought treatment, and only a few individuals from the RNAi lines survived (Figure 5B). Gas exchange analyses showed that *35S:ZAT10* plants exhibited increased stomatal conductance but similar $CO_2$ partial pressure to *zat10(i)* and wild-type plants (see Supplemental Figures 2A and 2B online). Thus, although *35S:ZAT10* plants exhibited increased net $CO_2$ fixation, the increased stomatal conductance led to an increased transpiration rate (*E*), resulting in a similar water use efficiency (*WUE*, *A*/*E*) for *35S:ZAT10*, *zat10(i)*, and wild-type plants (see Supplemental Figure 2C online). The experiment was repeated with a larger population using the decline in $F_v/F_m$ to determine the onset of damage and the day at which plants became inviable (Figure 5C). The association of loss of viability with a threshold $F_v/F_m$ value of 1/3 of initial levels has been validated on a range of drought-tolerant and wild-type lines in multiple experiments (N.S. Woo and B.J. Pogson, unpublished data). In this experiment, plants were rewatered when their $F_v/F_m$ parameter fell below the threshold value; no plants recovered following rewatering, confirming loss of viability. The survival times of the RNAi lines and *35S:ZAT10-6* were not significantly different to the wild type, whereas *35S:ZAT10-14* plants endured drought marginally longer, with a P value of 0.05 (Figure 5D). Anthocyanins were measured during drought, and significantly higher levels were detected in the *35S:ZAT10* lines compared with the wild type (Figure 5E), reminiscent of the changes observed in response to HL (Figure 3D).

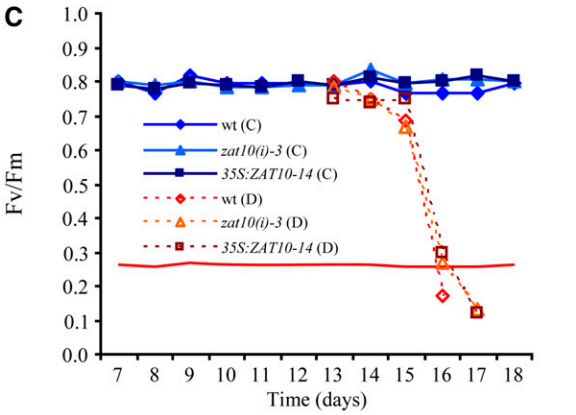
### Systemic Acquired Acclimation and HL Induction of *ZAT10*

The vascular localization of luciferase in *ZAT10:LUC* transgenic plants (Figure 6B; see Supplemental Figure 1A online) was similar to studies on *APX2* (Fryer et al., 2003); thus, we hypothesized that *ZAT10* may also respond to systemic acquired acclimation (SAA) as *APX2* does (Karpinski et al., 1999). Indeed, *ZAT10* expression was systemically induced to high levels in distal, shaded leaves of plants partially exposed to HL stress as determined by luciferase




B

| # of viable plants | wt | zat10(i) | | 35S:ZAT10 | |
|---|---|---|---|---|---|
| | | #1 | #3 | #6 | #14 |
| Day 0 | 7 | 7 | 7 | 7 | 7 |
| Post-rehydration | 0 | 2 | 1 | 0 | 0 |




D

| | wt | zat10(i) | | 35S:ZAT10 | |
|---|---|---|---|---|---|
| | | #1 | #3 | #6 | #14 |
| Survival time (days) ± sd. | 15.2 ± 1.5 | 15.3 ± 0.9 | 15.8 ± 2.5 | 15.5 ± 1.2 | 16.2 ± 1.1 |
| *t*-test | - | ns | ns | ns | ~0.05 |

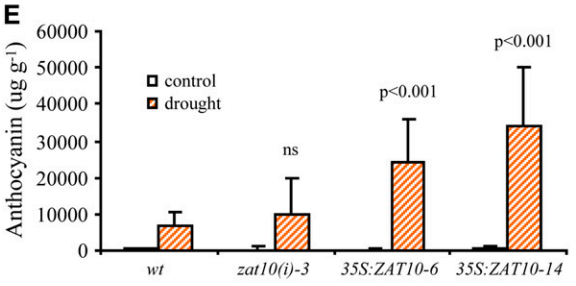



**Figure 5.** Response of *ZAT10* Transgenics to Drought.

**(A)** and **(B)** Results of drought survival experiments performed using traditional methods. For each of the wild-type, *zat10(i)-1*, *zat10(i)-3*, *35S:ZAT10-6*, and *35SZAT10-14* lines, $n = 7$.

**(A)** The rate of water loss from the plants and soil subjected to drought (D) was estimated by recording pot weight. The weights of watered control specimens (C) were also monitored.

**(B)** At 20 d the plants were rewatered and the numbers that were viable stated.

**(C)** and **(D)** Results of drought survival experiments as assessed through monitoring of photosynthetic changes.

**(C)** Change in Fv/Fm during progression of drought. Representative measurements are shown of one control and one drought-treated specimen each of the wild-type, *zat10(i)-3*, and *35S:ZAT10* lines. The 33% threshold value of the wild-type population, calculated daily from the mean $F_v/F_m$ values of wild-type control plants, is depicted as a solid line. Loss of viability was confirmed by rehydration of all plants that yielded $F_v/F_m$ values below their respective thresholds; no specimens showed signs of recovery after rehydration in this experiment.

**(D)** Survival of drought-treated plants, as evaluated through monitoring of the decline in $F_v/F_m$. Survival time is expressed as the day on which the measured $F_v/F_m$ fell below the threshold value of that line. Threshold values were calculated daily for each line as 33% of the mean $F_v/F_m$ of two watered control specimens. For each line, $n = 13$, with SD shown. *t* tests were undertaken to compare drought-affected transgenic plants to the wild type. ns, not significant.

**(E)** Anthocyanin content of watered and water-stressed plants after 15 d of drought treatment. Error bars indicate SD; for each line and treatment, $n = 9$, comprising three leaves from each of three separate specimens. *t* tests were undertaken to compare drought-affected transgenic plants to the wild type.

imaging (Figures 6A to 6C) and real-time RT-PCR (Figure 7D). Multiple independent transgenics with both 1.8- (Figure 6) and 0.9-kb *ZAT10:LUC* fusions demonstrated similar responses to SAA. The signal moved rapidly, within 15 to 30 min, presumably via the vasculature as exposure of leaves lowest on the rosette increased *ZAT10:LUC* activity in nonexposed younger rosette leaves, cauline leaves, and in the stem of the floral bolt (Figures 6B and 6C). *sHSP* and *APX2* also increased (Figures 6E and 6G), albeit with small increases akin to that previously observed for *APX2* during SAA (Karpinski et al., 1999). *APX1* and *HSP70* were not systemically inducible (Figure 6F; data not shown). The conditions for SAA were similar to that used in other HL experiments (Rossel et al., 2002) such that: temperatures in the shaded and exposed leaves were similar (28°C) and at levels that do not induce *HSP70*; filtered light was used to screen out the majority of the infrared and UV spectrums (Rossel et al., 2002); the plants were well hydrated and not wounded; and placing a plant fully under the shade did not induce *ZAT10*. Thus, the changes in gene expression reflect a response to HL-induced SAA.

To investigate whether the HL response is dependent on the area of tissue irradiated, an alternative method of HL treatment was developed (Figure 7). A fiber optic cable produced irradiance of similar intensity to that achieved using direct illumination from a halide light source, but without transmission of infrared radiation and illuminating only a small section of leaf (Figure 7A). Using this HL spot treatment, the SAA response was investigated in nine replicate experiments. *ZAT10* was induced to similar levels in the HL-exposed and distal tissues of treated leaves and was also upregulated in distal rosette leaves (Figure 7B). However, there

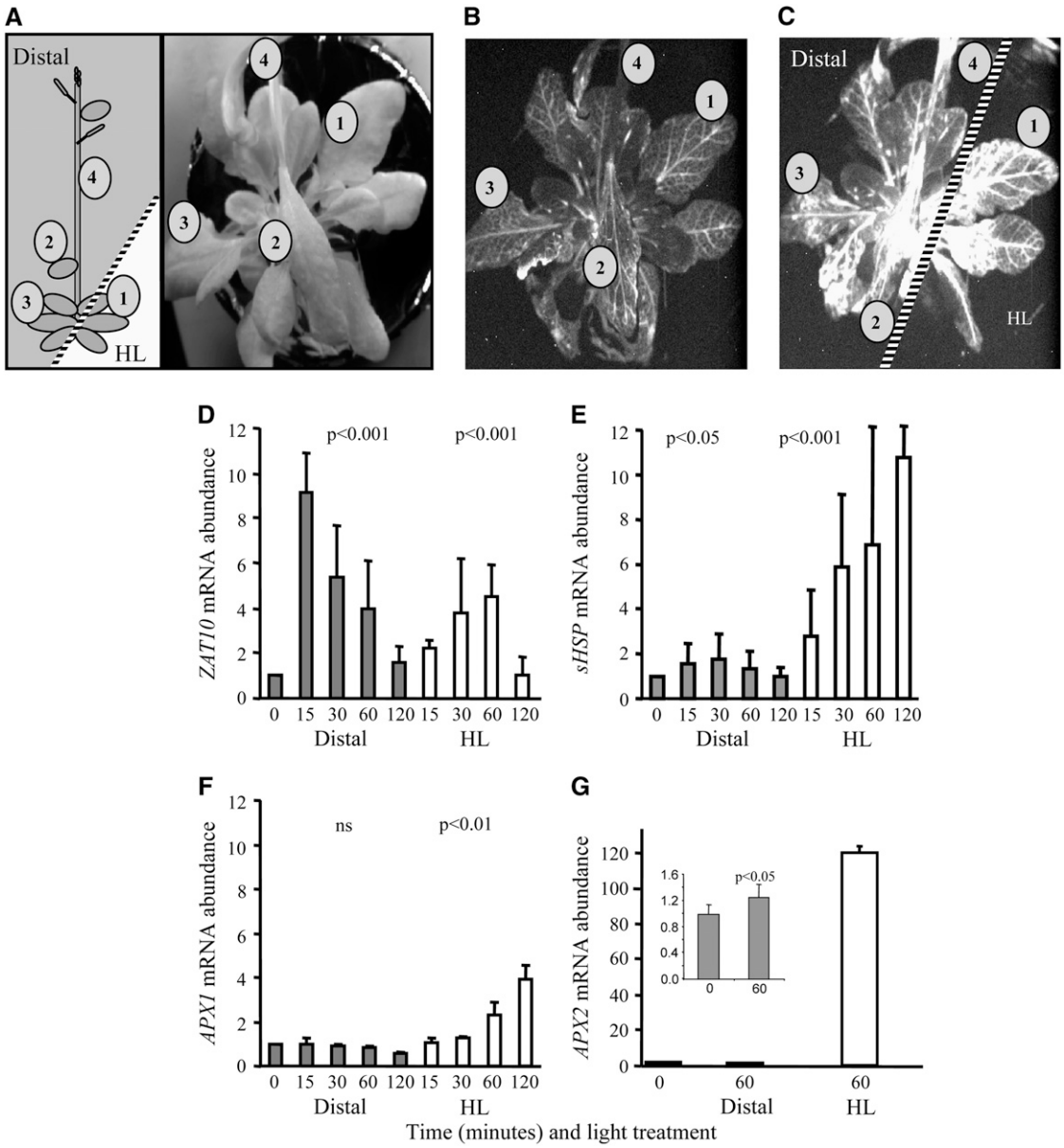



**Figure 6.** Systemic Induction of HL-Responsive Genes.

**(A)** Illustration and photograph of a representative 1.8-kb *ZAT10:LUC* plant imaged for luciferase activity. The hatched line shows the boundary between HL-exposed and shaded distal sections of the plant. (1) Leaf exposed to HL; (2) shaded cauline leaf; (3) shaded rosette leaf; (4) shaded stem.
**(B)** *ZAT10:LUC* activity at time zero (prior to HL treatment).
**(C)** *ZAT10:LUC* activity in the same plant after exposure to HL (1 h, 1500 μmol photons $m^{-2}$ $s^{-1}$) of the rosette leaves indicated.
**(D)** to **(G)** mRNA abundance in HL-exposed (open bars) and shaded distal (closed bars) tissues after the given time. Abundance was determined by real time RT-PCR and was normalized against control tissue taken at time zero in each experiment. **(D)** *ZAT10*, **(E)** *sHSP*, **(F)** *APX1*, and **(G)** *APX2* mRNA abundance, with an inset expanding the scale for control and distal. Each experiment was repeated three to six times, and all values are an average of at least three samples per time point. The mean and SD are plotted. *t* tests were performed comparing distal and HL-exposed samples with control tissues; P values for each sample type are indicated.

was no upregulation of *ZAT10* mRNA in the roots following HL spot treatment.

There was a marked increase in $H_2O_2$ in the irradiated section of the HL-exposed leaf, but there was no detectable diffusion of, or increase in, $H_2O_2$ in the surrounding areas of the treated leaf or in any other part of the rosette (Figure 7C). Furthermore, the preinfiltration of intact plants with DAB did not generate wound-induced staining as is routinely observed in experiments involving detached leaves. The effect of SAA on acclimation of leaves to oxidative stress was assayed by subjecting leaf discs to HL

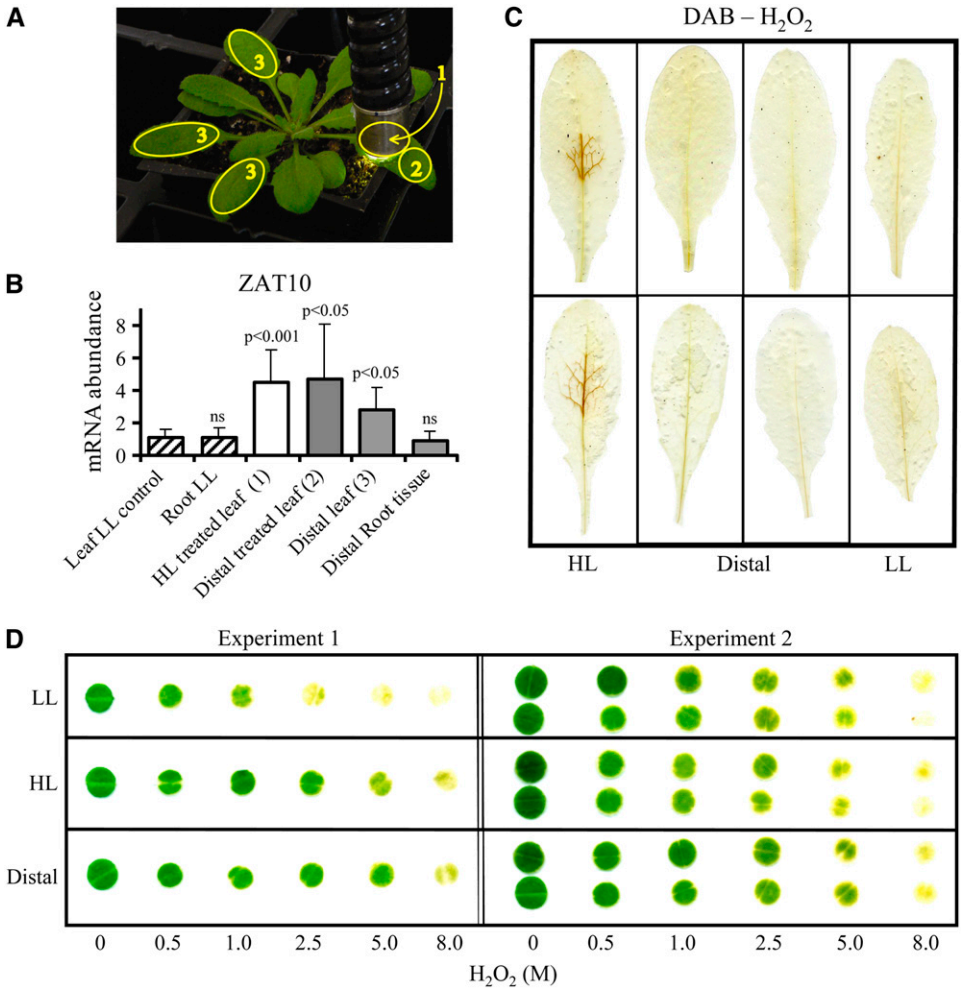



**Figure 7.** Investigation of the Role of ZAT10 and $H_2O_2$ in SAA to Oxidative Stress.

**(A)** Representative photograph of the HL spot treatment procedure. A single leaf section was exposed to a HL spot as shown for 1 h, after which the indicated plant tissues were collected for real-time RT-PCR analysis or visualization of $H_2O_2$ accumulation. (1) HL-exposed leaf tissue; (2) distal leaf tissue from HL-treated leaves; (3) distal leaf tissue from non-HL-treated leaves.

**(B)** Real-time RT-PCR analysis of *ZAT10* mRNA abundance in HL spot-treated tissue and distal leaf and root tissues, normalized by comparison to non-HL-treated control leaf samples. For all sample types, $n = 9$.

**(C)** $H_2O_2$ accumulation in HL spot-treated wild-type plants, as visualized through infiltration of DAB. Plants were prestained with 25 mM DAB prior to HL spot treatment for 1 h. HL, leaves subjected to HL spot treatment; distal, distal leaves from HL-treated plants; LL, leaves from control plants not exposed to HL. Images are representative of six separate experimental replicates.

**(D)** Induction of oxidative stress tolerance through HL pretreatment and SAA. Leaf discs were taken from appropriate tissues of HL-exposed plants and nontreated controls; sample types are as described in **(C)**. Discs were floated on $H_2O_2$ (concentrations as indicated) and incubated under HL conditions for 1.5 to 2 h, LL for 3 to 4 h, and then in darkness overnight. Two experimental replicates were performed; $n = 3$ per experiment.

plus increasing concentrations of $H_2O_2$. Distal and HL leaves from preacclimated plants were more resistant to oxidative damage than LL controls as the concentration of $H_2O_2$ required to bleach discs from distal and HL-exposed leaves was greater than discs from control, LL-treated plants (Figure 7D).

### Microarrays of HL, Distal, and *35S:ZAT10* Leaves

To determine what other genes are induced by SAA, we performed microarray analysis of HL-exposed (30 min) and shaded, distal leaves and leaves from LL-exposed control plants. Data presented are for those that are significantly different ($P < 0.05$, $q \leq 0.1$) with a greater than twofold difference in signal intensity compared with the control LL treatment (see Table 1 for a subset and Supplemental Table 1 online for the complete data set). Exposure to HL increased the expression of 360 genes and decreased 247 in distal leaves after 30 min (Figure 8A). Approximately 70% of the genes up- and downregulated in HL-exposed leaves were similarly altered in distal leaves. A more stringent analysis comprising genes exhibiting a fivefold or

**Table 1.** Subset of Genes Upregulated in Distal and Exposed HL-Treated Tissues

| | | Distal versus Control | | | Exposed versus Control | | | |
|---|---|---|---|---|---|---|---|---|
| AGI | Gene Title (Gene Symbol) | Fold Change | P Value | q Value | Fold Change | P Value | q Value | Ref |
| AT1G17420 | Lipoxygenase (LOX3) | 102.62 | 0.000 | 0.022 | 100.85 | 0.000 | 0.029 | V, R |
| AT1G61340 | F-box family protein | 98.94 | 0.000 | 0.014 | 88.12 | 0.004 | 0.090 | V |
| AT4G31800 | WRKY18 transcription factor | 98.49 | 0.000 | 0.019 | 74.93 | 0.000 | 0.051 | D |
| AT1G27730 | C2H2-type zinc finger (ZAT10) | 52.18 | 0.001 | 0.039 | 44.22 | 0.005 | 0.099 | |
| AT2G34930 | Disease resistance family protein | 39.19 | 0.002 | 0.042 | 43.39 | 0.000 | 0.024 | |
| AT1G43160 | AP2 domain–containing protein (RAP2.6) | 38.16 | 0.002 | 0.045 | 7.45 | 0.059 | **0.259** | K |
| AT5G52310 | Desiccation-responsive protein 29A (RD29A) | 30.09 | 0.001 | 0.039 | 14.57 | 0.005 | 0.098 | R |
| AT1G11960 | Early responsive to dehydration (ERD-like) | 29.24 | 0.000 | 0.025 | 23.85 | 0.000 | 0.042 | |
| AT2G30360 | CBL-interacting protein kinase 11 (CIPK11) | 22.15 | 0.000 | 0.020 | 13.89 | 0.005 | 0.098 | |
| AT3G14440 | 9-*cis*-epoxycarotenoid dioxygenase (NCED3) | 16.86 | 0.000 | 0.014 | 17.69 | 0.000 | 0.030 | K |
| AT5G66210 | Calcium-dependent protein kinase (CDPK) | 16.56 | 0.000 | 0.024 | 11.70 | 0.002 | 0.072 | D |
| AT1G21550 | Calcium binding protein, putative | 16.04 | 0.006 | 0.066 | 14.08 | **0.154** | **0.317** | |
| AT5G04340 | Zinc finger (C2H2-type) family protein (ZAT6) | 16.03 | 0.010 | 0.080 | 6.54 | **0.151** | **0.315** | |
| AT2G29440 | Glutathione *S*-transferase (GST), putative | 13.27 | 0.001 | 0.038 | 9.79 | 0.001 | 0.054 | |
| AT1G32640 | Basic helix-loop-helix protein (RAP-1) | 12.08 | 0.011 | 0.080 | 16.46 | 0.005 | 0.100 | |
| AT2G42540 | Cold-regulated protein (COR15A) | 11.09 | 0.005 | 0.062 | 10.10 | 0.003 | 0.074 | K |
| AT1G20440 | Dehydrin (COR47) | 11.03 | 0.000 | 0.026 | 7.56 | 0.000 | 0.048 | K |
| AT4G36900 | AP2 domain–containing protein (RAP2.10) | 10.96 | 0.002 | 0.045 | 11.28 | 0.003 | 0.081 | |
| AT1G76650 | Calcium binding EF hand family protein | 10.75 | 0.002 | 0.042 | 12.04 | 0.003 | 0.073 | D |
| AT1G49450 | WD-40 repeat family protein | 10.32 | 0.004 | 0.058 | 4.46 | 0.012 | **0.144** | |
| AT3G05640 | Protein phosphatase 2C (PP2C), putative | 9.55 | 0.000 | 0.022 | 7.06 | 0.006 | **0.106** | |
| AT4G08170 | Inositol 1,3,4-trisphosphate 5/6-kinase | 9.10 | 0.002 | 0.047 | 8.77 | 0.002 | 0.072 | |
| AT1G20450 | Dehydrin (ERD10) | 8.30 | 0.004 | 0.056 | 9.72 | 0.000 | 0.051 | |
| AT5G05410 | DRE binding protein 2A (DREB2A) | 7.81 | 0.034 | **0.123** | 4.10 | **0.349** | **0.391** | V |
| AT3G55980 | CCCH-type zinc finger family protein | 7.10 | 0.007 | 0.069 | 13.41 | 0.017 | **0.162** | D |
| AT5G59820 | C2H2-type zinc finger family protein (ZAT12) | 7.03 | 0.011 | 0.082 | 9.97 | 0.012 | **0.145** | V |
| AT2G20560 | DNAJ heat shock family protein | 6.65 | 0.013 | 0.086 | 5.20 | **0.349** | **0.391** | |

Gene induced by HL and/or oxidative stress in D (Davletova et al., 2005), K (Kimura et al., 2003), R (Rossel et al., 2002), and V (Vanderauwera et al., 2005). Genes whose expression was not significantly co-upregulated are indicated in bold.

greater change in expression revealed that 86% of genes upregulated in HL-exposed leaves were also induced in distal leaves, while 71% of genes downregulated in HL were likewise suppressed in distal leaves (Figure 8A). The induced genes in distal tissues included many known HL-, ROS-, and drought-responsive genes, including *ZAT12*, *ERD10*, *RAP2.6*, *COR47*, and an *IP3 KINASE*, in addition to *ZAT10*, as expected (Table 1). To validate the arrays, a subset of genes was tested for the systemic response, including *DREB2A* (At5g05410; 2.5 ± 1.6-fold increase in distal leaves versus LL control), *IP3 KINASE* (At4g08170; 7.9 ± 4.5-fold), *ZAT12* (At5g59820; 4.9 ± 1.0-fold), and a calcium binding protein (At1g76550; 9.5-fold). With respect to genes assayed in Figure 6, *APX2* was not detected in the arrays, *sHSP* was induced by 3.3-fold in distal tissue but the P value was not significant, and *APX1* was unchanged (1.1-fold).

To investigate the role of ZAT10 in the SAA response, microarrays were undertaken using *35S:ZAT10* plants and correlated with HL and distal arrays. RNA from leaves of LL, 4-week-old wild-type, and *35S:ZAT10-14* individuals were probed for global changes in gene expression. The averages of three biological replicates are presented in the supplemental data online for genes whose expression changed more than twofold and were significantly different from the wild type (see Supplemental Table 2 online). Using these criteria, 615 genes were identified as upregulated in *35S:ZAT10* plants, while 565 were downregulated. The upregulated genes included an osmotic stress–responsive proline dehydrogenase (ERD5), members of the *WRKY* transcription factor family, trehalose-6-phosphate synthase, calmodulin-related proteins, chitinase, a mildew resistance *RPW8* family protein, disease resistance proteins, wound-induced proteins, and a few known HL-responsive proteins, such as *VSP2* (see Supplemental Table 2 online). *APX2* was called absent, and there was no change in *FSD* and *PORB*. Among the downregulated genes were one member of the *DREB* family, early response to dehydration proteins (*ERD*), and HSPs such as the HL-inducible HSP70-3 (Rossel et al., 2002).

The arrays were cross-compared to determine the percentage of genes coexpressed in HL, distal, and *35S:ZAT10* leaves (Table 2; see Supplemental Table 3 online). Of the genes upregulated in HL, 20% were upregulated and 21% were downregulated in *35S:ZAT10*. With respect to SAA, for genes upregulated in distal and HL + distal subsets, 15 and 16%, respectively, were also

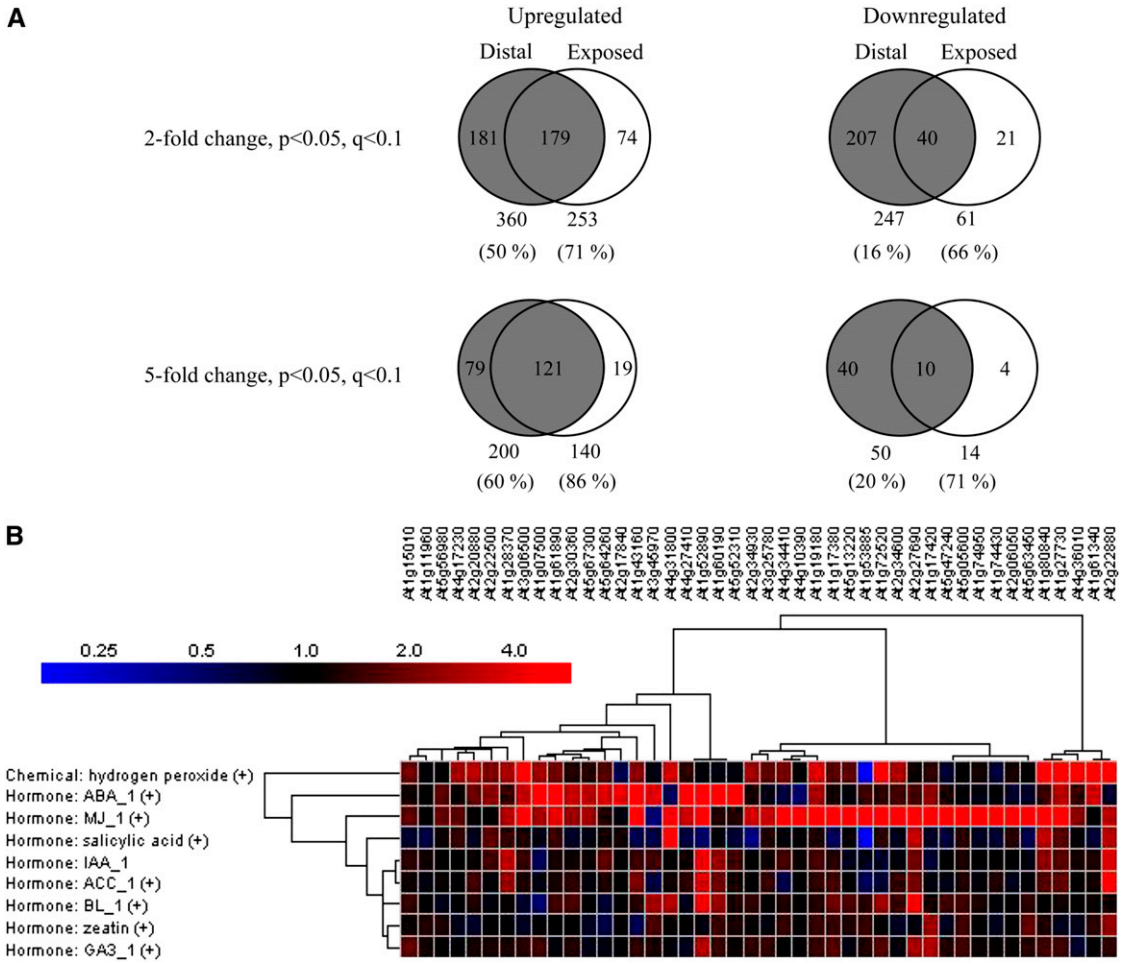



**Figure 8.** Global Gene Expression Profiling of SAA.

**(A)** Venn diagrams of genes significantly changed by two- or fivefold in distal and HL-exposed leaves compared with the control treatment.
**(B)** Genes significantly upregulated 20-fold or more by SAA were hierarchically clustered using Pearson's correlation (Genevestigator v3.0) with gene expression changes in response to hormone and $H_2O_2$ treatments. Red indicates upregulation and blue downregulation (see inset for scale).

upregulated in *35S:ZAT10* leaves. Of the genes downregulated in distal and HL + distal, 18 and 23% were similarly repressed in *35S:ZAT10*. Only 1 to 3% of genes upregulated in the HL and HL + distal subsets were oppositely regulated in *35S:ZAT10*, while 5 to 7% of genes downregulated in HL and HL + distal were conversely upregulated in *35S:ZAT10*. By contrast, the only gene coexpressed between the HL, distal, and HL + distal arrays with *35S:ZAT12* arrays (Davletova et al., 2005) was *ZAT12* itself.

### Potential SAA Signals

To identify candidates for putative SAA signals, genes showing changed expression in distal tissues were compared with microarray data sets available at Genevestigator (Figure 8B). First, with respect to shading, there was little to no correlation between the SAA data set and an early response to dark experiment (Kim and von Arnim, 2006). Second, 20-fold and 2-fold upregulated genes were compared with hormone, pathogen, and abiotic stress treatments (Figure 8B; data not shown). Few of the distally upregulated genes were increased by brassinosteroid (BL), zeatin (cytokinin), gibberellin (GA3), ethylene precursor (1-aminocyclopropane-1-carboxylic acid), auxin (indole-3-acetic acid [IAA]), or salicylic acid (SA); however, the majority of the strongly upregulated genes were responsive to one or more of ABA, methyl jasmonate (JA), and $H_2O_2$ (Figure 8B). The same hormone response data set has been analyzed to identify genes that are induced by a single hormone (Nemhauser et al., 2006). The twofold upregulated distal gene list contained no ACC-, zeatin-, or BL-specific genes, but many ABA and a number of IAA- and JA-specific genes were present (see Supplemental Table 1 online). Furthermore, petiole feeding of 0, 0.1, 1, 50, and 100 μM ABA demonstrated that *ZAT10* expression increased in distal intact leaves with increasing ABA concentrations, while *APX1* did not change (data not shown).

The subset of 29 genes co-upregulated in distal, HL, and *35S:ZAT10* arrays (Table 2) was analyzed, and the majority was

**Table 2.** Coexpressed Genes in *35S:ZAT10*, HL, and Distal Experiments

| Upregulated | HL | Distal | HL + Distal |
|---|---|---|---|
| Up in *35S:ZAT10* | 46/253 (18%) | 55/360 (15%) | 29/179 (16%) |
| Down in *35S:ZAT10* | 4/253 (1%) | 10/360 (3%) | 4/179 (2%) |
| **Downregulated** | **HL** | **Distal** | **HL + Distal** |
| Down in *35S:ZAT10* | 12/61 (21%) | 56/247 (23%) | 7/40 (18%) |
| Up in *35S:ZAT10* | 3/61 (5%) | 14/247 (6%) | 3/40 (7%) |

Coexpression of genes in HL, distal, and *35S:ZAT10* microarrays with a greater than twofold change ($P < 0.05$, $q \leq 0.1$).

responsive to either $H_2O_2$, JA, or ABA (Figure 9). Many were upregulated in leaves during the early stages of drought and by cold, but fewer were represented in a late drought of green tissue and only one in roots during late drought. In fact, ~55 to 60% of the SAA-induced and HL-induced genes are upregulated in the early responses to drought in shoots (data not shown). Also, the percentage of genes that are associated with response to stress and response to abiotic or biotic stimulus classification by Gene Ontology at The Arabidopsis Information Resource increased from 4 and 5% of the whole genome, respectively, to 10 and 15% for HL, 13 and 18% for distal, and 6 and 10% for *35S:ZAT10*, respectively, of significantly upregulated genes (see Supplemental Table 4 online).

Potential SAA signals were investigated by assaying *ZAT10* induction in a range of ABA, JA, and SA mutants and the APX2 signaling mutant, *rax1-1* (Tables 3 and 4). *abi1-1* and *abi2-1* are mutations in PP2C proteins involved in ABA signaling (Leung et al., 1994, 1997; Meyer et al., 1994), while *aba2-3* affects a short-chain alcohol dehydrogenase crucial in ABA synthesis (Leon-Kloosterziel et al., 1996; González-Guzmán et al., 2002). *rax1-1* is defective in γ-glutamylcysteine synthetase 1, the rate-determining enzyme in glutathione biosynthesis, and shows constitutive upregulation of *APX2* (Ball et al., 2004). In each experiment, a wild-type control was used as there is variability in the absolute level of *ZAT10* expression between plants, treatments, and leaves. For ABA and *rax1* mutants, the average of all wild-type experiments is given.

*ZAT10* was induced in HL-exposed and distal leaves of the ABA-insensitive mutants *abi1-1* and *abi2-1* as well as in the ABA-deficient mutant *aba2-3* and the regulator of APX2 mutant *rax1-1* (Table 3). Among the combined wild-type samples, distal expression of *ZAT10* was significantly different from LL but not to HL levels. A *t* test combining values for all *abi* and *aba* mutants had a marginal P value of 0.049 for HL versus distal leaves. *ZAT12*, *DREB2A*, and *IP3KINASE* were also induced in *abi1-1* (data not shown). *ZAT10* expression was not affected by the *rax1-1* mutation in either LL- or HL-treated specimens.

Mutants that affect jasmonate synthesis or perception, SA accumulation, and aspects of the pathogen-mediated systemic acquired resistance (SAR) response were also analyzed for SAA induction of *ZAT10*. *jasmonate resistant1* (*jar1-1*) is defective in a variety of responses to JA, in particular the NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1)-independent systemic resistance response (Clarke et al., 2000) and in the SA-independent, NPR1-dependent induced systemic resistance response (Pieterse et al., 1998). *jasmonate-insensitive1* (*jin1*/*myc2*) is impaired in the JA-dependent wounding response (Rojo et al., 1998) and the development of systemic immunity (Truman et al., 2007). *suppressor of G two allele of skp1b*/*jasmonic acid-insensitive4* (*sgt1b-3*/*jai4*) is also inhibited in the systemic response to DC3000 (*avrRpm1*) (Truman et al., 2007). The *aos* mutant is a lesion in *ALLENE OXIDE SYNTHASE* and is defective in JA biosynthesis (Park et al., 2002). The *npr1* mutant is a lesion in *NPR1*, which is a key regulator of SAR that is essential for transducing the SA signal to activate PATHOGENESIS-RELATED gene expression (Cao et al., 1994; Delaney et al., 1995; Spoel et al., 2003). *NahG* is a salicylate hydroxylase that converts SA to catechol, resulting in transgenic *NahG Arabidopsis* plants that are defective in resistance to *Pseudomonas syringae* (van Wees and

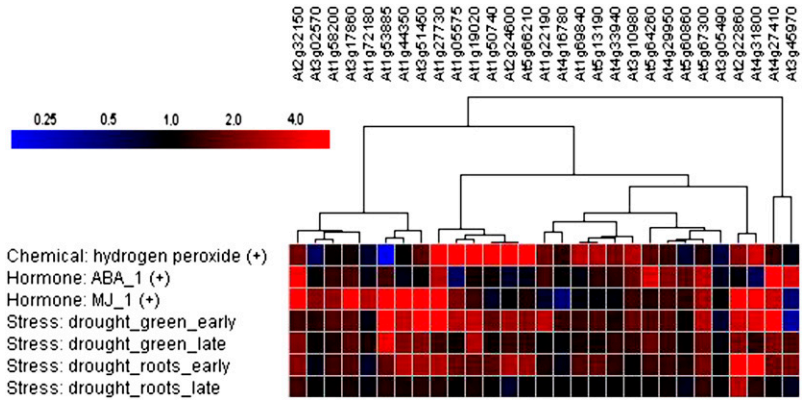



**Figure 9.** Coregulated genes in HL, Distal, and *35S:ZAT10* Leaves.

Genes significantly upregulated in HL, distal, and 35S:ZAT10 arrays were hierarchically clustered using Pearson's correlation (Genevestigator v3.0) with gene expression changes in response to hormone and selected stress treatments. Red indicates upregulation and blue downregulation (see inset for scale).

**Table 3.** SAA Induction of *ZAT10* in *aba*, *abi*, and *rax* Mutants

| | Wild Type | *abi1-1* | *abi2-1* | *aba2-3* | *rax1-1* |
|---|---|---|---|---|---|
| LL | 1 | 1 | 1 | 1 | 1 |
| HL | 4.6 ± 2.4 | 10.1 ± 4.6 | 5.4 ± 1.3 | 3.6 ± 1.6 | 5.1 ± 4.0 |
| D | 4.1 ± 3.1 | 6.4 ± 5.6 | 2.3 ± 0.8 | 2.1 ± 0.8 | 5.9 ± 3.2 |

The average fold changes in *ZAT10* mRNA abundance ± SD in various ABA mutants and the APX2 signaling mutant *rax1-1* are shown, as measured by real-time RT-PCR. For the wild type, $n = 8$; for each of the mutant lines, $n = 3$.

Glazebrook, 2003). *ZAT10* expression was also measured in the respective parent backgrounds, Columbia and Landsberg *erecta*; the SAA response was found to be similar in the different ecotypes (Table 4). In each JA, SA, or SAR mutant or transgenic, the distal SAA induction of *ZAT10* was detected, although the extent of induction did vary between different lines (Table 4).

## DISCUSSION

### ZAT10- and HL-Inducible Genes

*ZAT10* is induced by all signals that alter *APX2*, and its expression in vascular tissue of HL-exposed and distal, shaded leaves and in *alx8* mutants is also similar to *APX2* (Karpinski et al., 1997, 1999; Fryer et al., 2003; Rossel et al., 2006) (Figures 6 and 7). Altering the levels of *ZAT10* in the overexpression and RNAi transgenics correspondingly alters the level of *APX2*, *FSD1*, and *PORB* mRNA, but not *APX1* or *CSD2*. Interestingly, while *rax1-1* did not alter *ZAT10* expression (Table 3), it does alter *APX2* and *FSD1*, but not *CSD2* and *APX1* (Ball et al., 2004), which leads to speculation as to whether *rax1-1* and *ZAT10* affect similar stress response pathways.

We found little functional redundancy between ZAT10 and ZAT12 when comparing our data with the literature (Iida et al., 2000; Rizhsky et al., 2004; Davletova et al., 2005; Vogel et al., 2005). This includes both the subsets of genes each regulates and to some extent the abiotic stresses to which they confer tolerance. Temporal responses of *ZAT10* and *ZAT12* to excess light differ, with the induction of *ZAT10* peaking after 10 min of HL exposure (see Supplemental Figure 1 online), whereas *ZAT12* reaches maximum induction after up to 5 h at HL (Iida et al., 2000). Constitutive overexpression or knockout of *ZAT12* led to altered expression of a number of genes (Iida et al., 2000; Rizhsky et al., 2004; Davletova et al., 2005), but this included just a subset of HL-inducible genes, such as *APX1*, *sHSP*, and *CSD2*; notably, the expression of these genes was unchanged in *ZAT10* transgenics (Figure 2). Furthermore, just one gene from the *35S:ZAT12* arrays was represented in our HL arrays, that being *ZAT12* itself (Table 1). Likewise, a different subset of stress-responsive genes was induced by overexpression of *ZAT10*, including *APX2*, *FSD1*, and *PORB* (Figure 2; see Supplemental Table 2 online). The microarrays of *35S:ZAT10* revealed that both up- and downregulated genes account for ~20% of the genes that are altered in the HL microarray (Table 2), and a high percentage of these coexpressed genes are also induced in the early stages of drought. Thus, within stress signaling networks, both ZAT10 and ZAT12 are necessary and appear to function autonomously.

Consistent with the observed downregulation of hundreds of genes are reports indicating that *ZAT10* functions as a negative regulator of transcription (Ohta et al., 2001; Sakamoto et al., 2004). It was previously proposed that genes negatively regulated by ZAT10 may include photosynthesis-related genes and that this suppression may account for increased stress tolerance in *35S:ZAT10* plants (Sakamoto et al., 2004). However, we conclude that photosynthetic machinery-related genes are largely excluded from this negative regulation (see Supplemental Table 2 online). Also, in *35S:ZAT10* plants, photosynthetic rates were actually increased and there was no detectable change in chlorophyll, ribulose-1,5-bis-phosphate carboxylase/oxygenase, or D1 protein levels (Figure 3B; data not shown). Whether ZAT10 functions as a positive regulator of the upregulated genes or the change in expression is a consequence of its action as a negative regulator (Sakamoto et al., 2004) remains to be determined.

### ZAT10 Modulates Photoprotection in Different Organelles

Elevated levels of $H_2O_2$ were detected in *zat10(i)* leaves, while *35S:ZAT10* exhibited lower than normal $H_2O_2$ accumulation (Figure 3A). Thus, a key question arising from this study is whether the modulation of ROS levels and resistance to oxidative stress in *ZAT10* transgenics reflects changes in ROS detoxification or production. First, with respect to detoxification, *ZAT10*

**Table 4.** SAA Induction of ZAT10 in JA- and SA-Deficient or -Insensitive Mutants

| | Col | L*er* | *jar1-1* | *jin1* | *sgt1b-3* | *aos* | *npr1* | *NahG* |
|---|---|---|---|---|---|---|---|---|
| LL | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| HL | 7.6 ± 3.8 | 4.7 ± 2.3 | 5.8 ± 1.5 | 4.1 ± 1.7 | 12.7 ± 2.5 | 2.6 ± 1.4 | 3.0 ± 1.7 | 5.5 ± 2.4 |
| D | 4.9 ± 2.1 | 3.4 ± 1.5 | 4.1 ± 1.8 | 2.3 ± 0.9 | 5.5 ± 0.9 | 4.3 ± 1.5 | 2.4 ± 1.9 | 3.2 ± 1.3 |

The average fold changes in *ZAT10* mRNA abundance ± SD in various mutants defective in JA or SA biosynthesis or signaling, as measured by real-time RT-PCR. Col represents the wild type for *jar1-1*, *jin1*, *aos*, and *NahG*; L*er* is the wild type for *sgt1b-3*. For Col, $n = 8$; for all other lines, $n = 3$.

transgenics exhibited reciprocal changes in levels of anthocyanins in response to HL (Figure 3D) and drought (Figure 5E). Anthocyanins are induced by a range of abiotic stresses, including transcriptional changes in response to HL (Vanderauwera et al., 2005; Giacomelli et al., 2006), are predominantly vacuolar localized, and may also be in the nucleus (Wagner, 1979). They have been implicated as antioxidants that can scavenge $H_2O_2$ (Yamasaki et al., 1996; Gould et al., 2002) and therefore could contribute to photoprotection (Merzlyak and Chivkunova, 2000; Havaux and Kloppstech, 2001; Steyn et al., 2002). Second, there were reciprocal changes in cytosolic *APX2* and chloroplastic *FSD1* mRNA in parallel with the altered levels of ROS. Overproduction of *Arabidopsis* Fe-SOD was sufficient to confer oxidative stress tolerance in maize (*Zea mays*; Van Breusegem et al., 1999); likewise, reducing the expression of genes encoding SOD and thylakoidal APX was sufficient to impair chlorophyll accumulation and photosynthetic electron transport (Danna et al., 2003; Rizhsky et al., 2003). Both of these latter effects were observed in *zat10(i)* plants, and it is tempting to attribute their occurrence to the reduced expression of antioxidant enzyme genes and subsequent ROS overaccumulation. However, the lower *PORB* mRNA levels in the RNAi plants may also have contributed to the reduced chlorophyll content. Finally, the tolerance to light plus $H_2O_2$ in *35S:ZAT10* leaves (Figure 3F) is consistent with an increased capacity to detoxify $H_2O_2$. Thus, overexpressing *ZAT10* confers enhancements to the ROS detoxification processes in the chloroplast, cytosol, and vacuole.

The reduced $H_2O_2$ levels observed in *35S:ZAT10* plants may also be related to the observations of increased photochemical quenching during HL due to enhanced *ETR* and carbon fixation. Elevated photochemical quenching could reduce excitation pressure on PSII and thereby reduce the formation of excited singlet state chlorophylls and thus $^1O_2$. At photosystem I, the increase in photochemical quenching could reduce the formation of $O_2^-$ and $H_2O_2$. Combined with elevated mRNA levels of chloroplast-localized ROS detoxification enzymes, such as Fe-SOD, the observed reduction in ROS in *35S:ZAT10* plants is consistent with an hypothesis that increasing ZAT10 enhances photosynthetic rates and photoprotection in the chloroplast.

Overexpression of *ZAT10* was previously shown to increase drought tolerance (Sakamoto et al., 2004). In this study, however, no consistent increase in drought tolerance or change in *WUE* was observed, although elevated anthocyanin levels were noted in *35S:ZAT10* lines and may contribute to tolerance of drought-induced oxidative stress. Since submission, another group has reported no change in drought tolerance in different *35S:ZAT10* transgenics, although they did observe a change in osmotic tolerance to sorbitol (Mittler et al., 2006). In fact, both knockout and overexpression of *ZAT10* produced tolerance to osmotic shock (Mittler et al., 2006). The varying results for the different *35S:ZAT10* lines may reflect differences in the methods of measuring and applying drought or the degree of overexpression, as it seems only lines with high levels of overexpression exhibit a growth penalty and drought tolerance (Sakamoto et al., 2004; Mittler et al., 2006) (Figure 5D). Alternatively, overexpression of *ZAT10* induced the expression of some oxidative stress-responsive genes and repressed others (see Supplemental Table 2 online), particularly *ERD*s and *DREB*s. This could lead to a complex interplay of stress-responsive genes that in some *35S:ZAT10* lines promoted drought tolerance (Sakamoto et al., 2004) and in others promoted oxidative and osmotic stress tolerance without a change in *WUE* (Figure 3; see Supplemental Figure 2 online).

### SAA and ZAT10

Until now, the evidence for SAA had been largely limited to a seminal study reporting that one gene, *APX2*, was induced in distal leaves to just 11% of levels in HL-treated leaves concomitant with small changes in photochemistry (Karpinski et al., 1999); this is in contrast with the extensive evidence for SAR of pathogen or biotic stress. However, the rapid 15-min induction of *ZAT10* and hundreds of other HL-inducible genes in nonstressed tissues observed in this study provides conclusive evidence for the existence of SAA (Table 1). Additionally, distal leaves subjected to HL plus $H_2O_2$ were more resistant to oxidative damage in a similar manner to leaves preacclimated to HL directly (Figure 7D). Furthermore, we have shown that the SAA signal can be transmitted to rosette leaves, cauline leaves, and floral stems through monitoring of *ZAT10* induction in distal tissues. SAA is apparently limited to photosynthetic tissues as there was no change in *ZAT10* expression in roots, which is consistent with SAA being an adaptive response for the preacclimation of photosynthetic tissues to excess light.

It could be argued that subjecting one-third of the plant to HL and shading other sections could generate experimental artifacts, such as shade-induced responses, temperature variations, or even accidental exposure of meristematic tissue to HL. The HL spot experiments effectively discount temperature, shade, and accidental exposure as potential activators of SAA (Figures 7A to 7C). It is possible that excessive light stress on approximately one-third of the plant may induce a global effect on the physiology of the rosette that activates SAA; for example, changes in the net production and translocation of photosynthates such as sugars during photoinhibitory HL treatment could be hypothesized to cause SAA. However, as the HL spot treatment of a section of one leaf was shown to be sufficient to induce a measurable response in both that leaf and across the rosette, SAA is consistent with a hormonal-like signal rather than a consequential change in overall fitness of the rosette.

*ZAT10* expression is primarily localized to the vascular tissue and is induced within 5 min in exposed tissues and 15 min in distal tissues, which is a requirement for a role in a rapid SAA response that induces hundreds of genes within 30 min. *ZAT10* modulates the expression of key acclimatory genes, such as *APX2*, and 15% of the SAA-upregulated genes, and 23% of those downregulated are coexpressed through constitutive overexpression of *ZAT10* (Table 2). That this coexpression is indicative of a role for *ZAT10* in SAA and is not coincidental with it being a stress-responsive gene is supported by observations that few SAA-regulated genes were oppositely regulated in *35S:ZAT10* plants (Table 2) and that no SAA-inducible genes (other than *ZAT12*) were coexpressed in *35S:ZAT12* arrays (Davletova et al., 2005). Interestingly, the 29 genes upregulated in HL, distal, and *35S:ZAT10* arrays are responsive to a number of abiotic stresses and hormones. Furthermore, the

coexpression of this set of 29 genes with early leaf drought arrays, but not late leaf or root drought arrays, is indicative of a role for these genes in the early response to abiotic stresses (Figure 9). In fact, classification of the genes induced by Gene Ontology demonstrated that the percentage of genes that were involved in stress, signal transduction, or transcription were two- to threefold overrepresented in each of the HL, SAA, or *35S:ZAT10* arrays versus the whole-genome composition (see Supplemental Table 4 online). Finally, both SAA and *ZAT10* overexpression increase tolerance to oxidative damage. Thus, *ZAT10* is both induced by SAA and could modulate a significant part of the SAA response.

### Communication of SAA

Despite extensive investigation in this study, the identity of the SAA signal remains unknown. Hierarchical clustering of the distal arrays with a seven-plant hormone experiment at AtGenExpress largely eliminated ethylene, SA, BL, zeatin, GA3, and IAA as candidates for SAA signaling but implicated ABA, JA, and $H_2O_2$ as potential signals (Figure 8).

SAR could be largely discounted due to the limited number of SAA-induced genes coexpressed in SA and pathogen experiments (Figure 8; data not shown). Furthermore, of the genes inducible by SA, nearly all were also responsive to $H_2O_2$, ABA, and/or JA. However, the potential for some interaction between biotic and abiotic stress signaling within a cell has been considered, most recently by de Torres-Zabala et al. (2007), who demonstrated that pathogens can stimulate ABA biosynthesis, thus resulting in increased susceptibility to the pathogen.

Recently, JA has been implicated in SAR, not as the actual signal, but instead responding to the signal in the distal tissues: almost all JA biosynthetic transcripts are induced, JA is synthesized, and JA-responsive genes are upregulated in distal regions of pathogen-treated plants (Truman et al., 2007). We observed that three JA biosynthetic enzymes were induced by SAA, namely, LOX3, allene oxide cyclase, and 12-oxophytodienoate reductase (see Supplemental Table 1 online). However, the timing of SAR at 4 h to days after infection (Truman et al., 2007) rather than 15 to 30 min after HL for SAA suggests a different process is at work (Figure 6). Thus, we investigated a range of mutants defective in the accumulation and perception of JA and SA, together with alterations in the aspects of the SAR response (Table 4). In all mutants and transgenics, *ZAT10* was induced in HL and distal tissues, indicating SAA was functional despite changes to SAR, jasmonates, and/or SA content or perception. So, while a degree of commonality between perception of SAA and SAR in distal tissues should not be discounted, we could find no conclusive evidence for a direct link.

ABA would be a candidate systemic signal as both *ZAT10* and *APX2* can be induced by introduction of ABA into intact plants via petiole feeding; furthermore, one-third of the 20-fold upregulated genes in distal tissues are ABA responsive. However, the SAA signal was transmitted and perceived in distal leaves of ABA biosynthesis and perception mutants, albeit marginally attenuated (Table 3), eliminating ABA from consideration as the primary signal. We also tested the *rax1-1* mutation that regulates *APX2* levels within a cell by altering glutathione metabolism (Ball et al., 2004), but it had no impact on systemic signaling or on the induction of *ZAT10* in HL.

$H_2O_2$ was hypothesized to be a component of SAA (Karpinski et al., 1999) as it was colocalized with APX2 in the vasculature of HL-treated, but not distal tissue, in detached leaves (Fryer et al., 2003). However, if the SAA signal was the result of $H_2O_2$ diffusion, diminishing luciferase activity with distance and no apparent tissue specificity would be expected in *ZAT10:LUC* plants exposed to HL, unless $H_2O_2$ was being produced in the distal vasculature. In this study, we used intact plants to avoid any effects of wounding and demonstrated SAA between leaves without any detectable change in $H_2O_2$. We observed no diffusion of $H_2O_2$ nor could we detect it in distal tissues, and there was no decline in induction of *ZAT10:LUC*, even if just a portion of one leaf was treated with HL (Figures 6 and 7).

In conclusion, intracellular responses to genetic manipulation of *ZAT10* altered rates of photosynthesis under HL and the levels of protective pigments, antioxidants, and mRNA of 20% of HL-inducible genes whose products are targeted to the chloroplast, cytosol, and vacuole. Overexpression of *ZAT10* improved ROS detoxification and production in different subcellular compartments, resulting in enhanced tolerance to photoinhibitory light and exogenous $H_2O_2$. Within 15 to 30 min, the HL systemic response induced a strikingly similar set of genes (up to 86%) in distal leaves. Approximately 20% of genes affected by SAA are responsive to overexpression of *ZAT10*. As such, it appears that *ZAT10* is both induced by SAA and in turn is implicated in mediating a significant proportion of the SAA response. Although genes involved in JA biosynthesis were upregulated in distal tissues and a large percentage of the SAA genes are inducible by ABA, JA, and/or $H_2O_2$, SAA is transmitted in mutants that perturb ABA, JA, SA, and SAR signaling. Thus, evidence to date indicate that SAA is likely to be a rapid, novel signal or, less likely, a complex interaction between known signals. Once perceived in a distal cell, SAA appears to activate similar signaling cascades to those activated in directly exposed tissues, inducing 86% of the HL-inducible genes that may facilitate acclimation to oxidative damage (Figure 8) and photoinhibition (Karpinski et al., 1999) of a shaded part of the canopy prior to exposure to the sun.

## METHODS

### Plant Growth and Stress Treatments

All plants were in the *Arabidopsis thaliana* ecotype Columbia (Col-0) background unless otherwise noted. For growth in soil, a mix of three parts soil and one part vermiculite was soaked with 0.5× Hoagland fertilizer (Hoagland and Arnon, 1950); seeds were then sprinkled onto the surface of the soil and vernalized for 3 d in darkness at 4°C before transfer to growth chambers. For most experiments, growth conditions were 16-h days with 100 to 150 μmol photons $m^{-2}$ $s^{-1}$ and 21°C. For microarrays, SAA, growth rate, and drought experiments, the growth conditions were 12-h days with 90 to 160 μmol photons $m^{-2}$ $s^{-1}$ and 24°C. Plants were watered every 2 to 3 d and fertilized with 0.5× Hoagland fertilizer every fortnight. For growth in tissue culture, seeds were surface sterilized and plated on Murashige and Skoog plates containing 1% agar and 2% sucrose. The plates were vernalized as described above prior to transfer to growth at 21°C under continuous illumination (100 μmol photons $m^{-2}$ $s^{-1}$).

HL treatment of whole or partially shaded rosettes was performed using metal halide mercury vapor lamps (Sunmaster) at intensities indicated (Forster et al., 2005; Rossel et al., 2006). For glutathione, DCMU and $H_2O_2$ treatments, 2- to 3-week-old plants were sprayed with 10 μM DCMU, 5 mM glutathione, or 10 mM $H_2O_2$. At different time intervals after spraying, leaf tissue was snap-frozen in liquid nitrogen for subsequent RNA extraction. To perform HL spot treatments, mature wild-type plants were exposed to 1400 to 1600 μmol photons $m^{-2}$ $s^{-1}$ for 1 h via a fiber optic cable. A 0.8-$cm^2$ area of a single leaf encompassing the midvein was illuminated in this manner without generation of radiant heat; the distal and LL intensity was 40 to 70 μmol photons $m^{-2}$ $s^{-1}$, and the ambient temperature was 20°C. After treatment, leaf tissues were isolated and immediately frozen for RNA extraction as described.

Drought treatments were applied by first providing specimens with a sufficiency of water and then withholding further water for the duration of the treatment. Survival of drought-affected plants was assessed using both traditional rehydration methods and also by monitoring the photosynthetic efficiency of the individual specimens. Traditional drought treatments were initiated at 4 weeks of age; rates of water loss were monitored by measuring total pot weight every 48 h. After 20 d of drought, all pots were rehydrated and viable plants were identified as those that evidenced physical signs of recovery within 3 d. For drought treatments involving photosynthetic measurements, treatments were initiated at 6 weeks of age. Prior to the light period (after a minimum of 7 h of dark adaptation), the maximum efficiency of PSII ($F_v/F_m$) was determined by subjecting individual specimens to a saturating pulse (>1800 μmol photons $m^{-2}$ $s^{-1}$). Drought-affected plants were deemed to have lost viability when their $F_v/F_m$ parameter declined to <33% of that of watered control plants of that line. Loss of viability was confirmed through rehydration as described above. Photosynthetic measurements were performed using a MAXI-PAM series IMAGING-PAM chlorophyll fluorometer (Walz) and the ImagingWin software application (Walz; version 2.10d).

For measurements of vegetative growth, 6-week-old wild-type, *zat10(i)*, and *35S:ZAT10* plants were used. The leaf area of whole plants was calculated in pixels using an image analyzing system employing a CCD camera and software to record the area of the plant (LemnaTec Scanalyzer; LemnaTec). For the SAA microarray experiments, 4-week-old plants were exposed to 30 min of HL stress using UV- and infrared-filtered light at 1300 to 1600 μmol photons $m^{-2}$ $s^{-1}$; the temperature at the plant surface was 28°C and plants were well hydrated before and during the experiment. Shades were used to cover approximately two-thirds of the rosette such that the light intensity under the shade was 40 to 70 μmol photons $m^{-2}$ $s^{-1}$. Some germplasm used for the experiments were kindly provided by P. Mullineaux (University of Essex) (*rax1-1*) and The Arabidopsis Information Resource (*abi1-1*, *abi2-1*, and *aba2-3*).

### Construction of *ZAT10* Transgenics

For generating a hairpin *ZAT10* transgene, a purified *ZAT10* PCR product, corresponding to a region +118 bp to 592 bp relative to the transcriptional start site, was introduced by a BP reaction into the entry vector pDONR201 (Invitrogen) and transferred to the binary hairpin-RNA generating vector pHELLSGATE8 by an LR recombination reaction (Wesley et al., 2001). The resulting binary vector was transferred to *Agrobacterium tumefaciens* LBA4404 via heat shock, which was then used for floral dip transformation of wild-type plants (Clough and Bent, 1998). Kanamycin-resistant transformants were selected on Murashige and Skoog medium and transferred to soil for further characterization.

The *35S:ZAT10* construct was made by cloning the PCR-amplified wild-type *ZAT10* coding sequence into a *Hin*dIII-digested and blunt-ended binary vector containing the double cauliflower mosaic virus 35S promoter. The resulting binary vectors in *A. tumefaciens* were used for floral dip transformation of wild-type plants.

For generating a *ZAT10:LUC* reporter construct, a PCR fragment corresponding to 2 kb upstream of the *ZAT10* transcriptional start site was amplified from genomic DNA and subcloned into pGEM-Teasy (Promega). A 1.8-kb *Nco*I/*Bam*HI and a 0.9-kb restriction fragment were each cloned in frame into the binary vector pHYGRO (Invitrogen), which contained the *LUC+* reporter gene. *Arabidopsis* plants were transformed as described above. For each construct, at least two independent transformants were generated and analyzed by luciferase imaging.

### Gene Expression Analyses

Real-time RT-PCR analyses were performed on the RotorGene 2000 (Corbett Research). RNA was extracted with the Qiagen RNeasy plant kit, including an on-column DNase step using the Qiagen RNase-free DNase kit. Real-time RT-PCR was performed using the Qiagen QuantiTect SYBR Green real-time RT-PCR kit. Primers used for real-time RT-PCR were as follows: *APX2* (At3g09640), 5′-GGCTGGGACATTTGATGTG-3′ and 5′-AGGGAACAGCTCCTTGATAGG-3′; *APX1* (At1g07890), 5′-CCACTCG-CATTTCTCCAGAT-3′ and 5′-TCGAAAGTTCCAGCAGAGTG-3′; *PORB*, (At4g27440) 5′- TGATAATGGCGTGCAGAGAC-3′ and 5′-GCTCTTTAGC-TGTCGGGAAA-3′; *CSD2* (At2g28190), 5′-ACACAATCCTCGCATTC-TCA-3′ and 5′-AAGCACTGCAACAGCCTTCT-3′; *FSD1* (At4g25100), 5′-AATGAAACCAGGTGGTGGAG-3′ and 5′-GGATTCACAGCATTGGG-AGT-3′; *sHSP* (At2g29500), 5′-CCTGGATTGAAGAAGGAGGAAG-3′and 5′-TAGGCACCGTAACAGTCAACAC-3′; *ZAT10* (At1g27730), 5′-AGGC-TCTTACATCACCAAGATTAG-3′ and 5′-TACACTTGTAGCTCAACTTC-TCCA-3′; *cyclophilin* (At2g29960), 5′-TCTTCCTCTTCGGAGCCATA-3′ and 5′-AAGCTGGGAATGATTCGATG-3′; *AZF3* (At5g43170), 5′-GGA-GCTTATACGGTGGA-3′ and 5′-TCAAATCCACGGTGGCTACT-3′; *ZAT6* (At5g04340), 5′-ACCTCCTCTGCTTCCTCCTC-3′ and 5′-GGTGGCGAAC-GATTTATGAC-3′; *ZAT7* (At3g46070), 5′-ACTACGACGACGGGCTCATA-CTT-3′ and 5′-CACCGAAGTTAAGTCGAAATCC-3′; *ZAT12* (At5g59820), 5′-TGTCCCATATGTGGAGTGGA-3′ and 5′-ATTGTCCACCATCCCTAG-ACT-3′; $IP_3$ Kinase (At4g08170), 5′-GACGAGAACTCCCCAAATCA-3′ and 5′-CTGGGAAATAGTTGATGTCGATC-3′; Ca binding protein (At1g76650), 5′-GGAGATGGGATGTTGGATTTT-3′ and 5′-CGCATCATAAGAGCAAAC-TCA-3′; DREB2A (At5g05410), 5′-AGACTATGGTTGGCCCAATG-3′ and 5′-TCGAGCTGAAACGGAGGTAT-3′; HSP70 (At3g09440), 5′- GCTGCT-ATTGCTTACGGTCTTG-3′ and 5′- CTCTCGGGTTTCCACTAATGTC-3′.

Real-time RT-PCR data were analyzed using the relative quantification method or the comparative $C_t$ method ($\Delta\Delta C_t$) (Pfaffl, 2001), allowing for the quantification of a gene of interest relative to its expression in control plants as previously described (Rossel et al., 2006). To account for experimental variation, assays were referenced to an internal control gene (Schmittgen and Zakrajsek, 2000), *cyclophilin*, using the primers described above. Real-time RT-PCR reactions were performed as three technical and three biological replicates unless otherwise stated.

For luciferase imaging, the plants were sprayed with a 1 mM D-luciferin solution (Biosynth) containing a few drops of Tween 80 and then left in growth light conditions for 5 min and imaged using a cooled CCD camera (Model DV 435; Andor Technology) and Image-Pro software (Media Cybernetics).

### Microarray Analyses

Transcriptomic analysis was performed using Affymetrix GeneChip *Arabidopsis* genome ATH1 arrays. Three biological replicates were analyzed for each treatment with each array representing a single biological replicate. The SAA arrays performed included the wild type under LL (control; three arrays), wild-type leaves subjected to 30 min of HL (exposed; three arrays), and shaded leaves from the same plant (distal; three arrays). For distal and exposed arrays, samples bearing the same number are from the same plant. The arrays on *ZAT10* transgenics were performed on three wild-type plants and three *35S:ZAT10-14* overexpressing plants. For all arrays,

mature, fully expanded, green rosette leaves were harvested and immediately frozen in liquid nitrogen before total RNA was isolated from leaves pooled from individual plants as described above. The quality of the RNA was verified using an Agilent Bioanalyzer (Agilent Technologies) and spectrophotometric analysis to determine concentration and the $A_{260}$-to-$A_{280}$ ratio. Preparation of labeled cRNA from 1 to 5 μg of total RNA and target hybridization as well as washing, staining, and scanning of the arrays were performed exactly as described in the Affymetrix GeneChip expression analysis technical manual using the Affymetrix one-cycle target labeling and control reagents, an Affymetrix GeneChip Hybridization Oven 640, an Affymetrix Fluidics Station 450, and an Affymetrix GeneChip Scanner 3000 7G at the appropriate steps. Data quality was assessed using GCOS 1.4 (Affymetrix) before CEL files were imported into Avadis 4.3 (Strand Genomics) for further analysis. Raw intensity data were initially normalized using the MAS5 algorithm allowing probe IDs called present in at least two replicates for each treatment to be identified. Probe intensities were then analyzed using the GC-RMA algorithm and log transformed, and lists of differentially expressed genes were generated by performing *t* tests (unpaired with asymptotic P value computation). These gene sets were further reduced by filtering for fold changes >2, and P values were converted to q values to correct for multiple testing false discovery rate as described (Storey and Tibshirani, 2003). All microarray data have been deposited in the ArrayExpress database (http://www.ebi.ac.uk/arrayexpress/) under the accession numbers E-ATMX-19 and E-ATMX-20. A subset of genes upregulated in the distal arrays was compared with publicly available microarray data sets available at Genevestigator (https://www.genevestigator.ethz.ch) as represented in Figure 8B, where the numbers shown are the average linear fold change for all replicates in each experiment compared with the appropriate control.

### Measurement of Photosynthetic and Photoprotective Parameters

Leaf photosynthetic parameters were measured using an open circuit, LI-6400 infrared gas exchange system with the leaf chamber chlorophyll fluorometer attachment (LI-6400-40 LCF; Li-COR Biosciences) and calculated as previously described (Rossel et al., 2006). Concurrent measurements of leaf carbon assimilation (*A*), stomatal conductance ($g_s$), linear electron transport rate through PSII (*ETR*), intercellular airspace $CO_2$ concentration ($C_i$), leaf temperature, and fluorescence yields were monitored in this system. Artificial illumination was supplied to the leaf from a red–blue LED light source attached to the sensor head. Gas exchange parameters were calculated as described (von Caemmerer and Farquhar, 1981) with revisions (von Caemmerer and Quick, 2000). Fluorescence and gas exchange measurements were taken at fully saturating light intensities as determined by multiple flashes after the plant had been dark acclimated for at least an hour.

### Measurement of ROS, APX Activity, and Oxidative Stress Tolerance

To visualize $O_2^-$, 4-week-old whole plants were removed from soil and the roots carefully washed before being placed in a 6 mM nitro blue tetrazolium solution prior to LL or HL exposure. After 30 min of LL or HL, leaves were boiled in ethanol to remove chlorophyll. After this, the leaves were rehydrated in 40% glycerol and mounted on glass slides. The same procedure was undertaken to visualize $H_2O_2$, except 5 to 25 mM DAB solution, pH 3.8, was used. Each experiment was repeated on at least three different plants, and multiple leaves per plant were examined. Representative images are shown.

The ability of plants to resist different reactive oxygen sensitizers was determined by adapting the method developed for *Chlamydomonas reinhardtii* (Forster et al., 2005). Different dilutions of $H_2O_2$ and different light regimes were trialed to develop an assay that bleached leaves at higher $H_2O_2$ concentrations. Three-week-old plants were exposed to HL (1000 μmol photons $m^{-2}$ $s^{-1}$) for 30 min. Leaves were then detached, floated on solutions of $H_2O_2$ diluted in water, and incubated for 3 to 4 h at 100 to 150 μmol photons $m^{-2}$ $s^{-1}$ and then overnight in the dark. For testing the tolerance of distal leaves to oxidative stress, plants were subjected for 2 h of HL or LL as described above. Leaf discs including the midvein were isolated from LL, HL, and distal leaves. The leaf discs were floated on solutions of $H_2O_2$ for 2 h in HL for experiment 1 and 1.5 h of HL for experiment 2 and then LL for 3 to 4 h followed by dark overnight. A total of three biological replicates were undertaken in two separate experiments.

APX activity assays were performed as described (Panchuk et al., 2002). First, 100 mg of leaf tissue was frozen and ground in liquid $N_2$, and 0.5 mL of 50 mM sodium phosphate, pH 7.0, 0.25 mM EDTA, 2% (w/v) polyvinylpyrrolidone-25, 10% (w/v) glycerol, and 1 mM ascorbate was added. The homogenate was centrifuged at 0°C at 14,000*g* for 10 min. The supernatant was immediately added to a reaction mixture consisting of 25 mM sodium phosphate, pH 7.0, 0.1 mM EDTA, 1 mM $H_2O_2$, and 0.25 mM ascorbate, with the extract added last to initiate the reaction. Ascorbate oxidation was monitored as the decline in $A_{290}$ over 1 min. The concentration of protein in the extract was determined using the Bio-Rad protein assay system (Bio-Rad).

### Pigment Quantification

For carotenoid, chlorophyll, and anthocyanin analyses, pigments were extracted from leaves of 3-week-old plants under normal growth conditions (control) or after 1 h of exposure to HL (1000 μmol photons $m^{-2}$ $s^{-1}$) and analyzed by HPLC or spectrometry (Pogson et al., 1998). Chlorophyll was extracted and total amount expressed as micrograms of chlorophyll per milligram of fresh weight and the ratio of chlorophyll *a*/*b* calculated as previously described (Porra et al., 1989; Rissler et al., 2002). Anthocyanin extraction and quantification was performed as previously described (Neff and Chory, 1998). Carotenoids were assayed using an Agilent HPLC and photodiode array detector as described (Pogson et al., 1998; Rissler and Pogson, 2001). Carotenoids were identified by comparison of their spectra and retention times to standards and the peak areas recorded for quantification using molar extinction coefficients.

### Accession Numbers

Arabidopsis Genome Initiative locus identifiers for the genes mentioned in this article are as follows: At1g07890 (*APX1*), At3g09640 (*APX2*), At5g43170 (*AZF3*), At1g76550 (calcium binding protein), At2g28190 (*CSD2*), At2g29960 (*cyclophilin*), At5g05410 (*DREB2A*), At4g25100 (*FSD1*), At3g09440 (*HSP70*), At2g29500 (*sHSP*), At4g08170 (*IP3 KINASE*), At4g27440 (*PORB*), At5g04340 (*ZAT6*), At3g46070 (*ZAT7*), At1g27730 (*ZAT10/STZ*), and At5g59820 (*ZAT12*),

### Supplemental Data

The following materials are available in the online version of this article.

**Supplemental Figure 1.** Localization of *ZAT10:LUC* in Vasculature, HL Induction of *ZAT10*, and Photographs of *ZAT10* RNAi Plants.

**Supplemental Figure 2.** Stomatal Conductance, Water Use Efficiency, and Images of Wild-Type and *35S:ZAT10* Plants during Drought.

**Supplemental Table 1.** Arrays of Systemic Acquired Acclimation and List of genes Significantly Changed in HL versus LL, Distal versus LL, and *35S:ZAT10*.

**Supplemental Table 2.** Arrays of Wild-Type versus *35S:ZAT10* and List of Genes Significantly Changed.

**Supplemental Table 3.** Subset of Genes Coexpressed in HL, Distal, and *35S:ZAT10* Arrays.

**Supplemental Table 4.** Gene Ontology Classification of Array Data.

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