Short title: MYB30 regulates systemic ROS signaling

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Article Title: MYB30 orchestrates systemic reactive oxygen signaling and plant acclimation

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One-sentence summary: The transcriptional regulator MYB30 links systemic reactive oxygen species signaling with systemic acquired acclimation during the response of Arabidopsis thaliana to excess light stress.

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Abstract

Systemic acquired acclimation (SAA) is a key biological process essential for plant survival under conditions of abiotic stress. SAA was recently shown to be controlled by a rapid systemic signaling mechanism termed the reactive oxygen species (ROS) wave in Arabidopsis (Arabidopsis thaliana). MYB30 is a key transcriptional regulator mediating many different biological processes. MYB30 was found to act downstream of the ROS wave in systemic tissues of Arabidopsis in response to local high light (HL) stress treatment. However, the function of MYB30 in systemic signaling and SAA is unknown. To determine the relationship between MYB30, the ROS wave, and systemic acclimation in Arabidopsis, the SAA response to HL stress of myb30 mutants and wild-type plants was determined. Although myb30 plants were found to display enhanced rates of ROS wave propagation and their local tissues acclimated to the HL stress, they were deficient in SAA to HL stress. Compared to wild type, the systemic transcriptomic response of myb30 plants was also deficient, lacking in the expression of over 3,500 transcripts. A putative set of 150 core transcripts directly associated with MYB30 function during HL stress was determined. Our study identifies MYB30 as a key regulator that links systemic ROS signaling with systemic transcriptomic responses, SAA, and plant acclimation to HL stress. In addition, it demonstrates that plant acclimation and systemic ROS signaling are interlinked, and that the lack of systemic acclimation drives systemic ROS signaling to occur at faster rates, suggesting a feedback mechanism (potentially involving MYB30) between these two processes.

Key words: Light stress, MYB30, Reactive oxygen species, Systemic acquired acclimation, Gene co-expression network.

Abbreviations: HL, high light; RBOHD, respiratory burst oxidase homolog D; ROS, reactive oxygen species; SAA, Systemic acquired acclimation; WGCNA, Weighted correlation network analysis.
**Introduction**

Plants acclimate to changes in their environment to survive episodes of abiotic stress. Rapid systemic signaling between different parts of the plant during stress is an important factor governing this process (Kollist et al., 2019). A systemic response that induces acclimation mechanisms in remote tissues is termed systemic acquired acclimation (SAA; Karpinski *et al.*, 1999), and this process was found to be essential for plant survival under different abiotic stress conditions (Suzuki *et al.*, 2013; Devireddy *et al.*, 2018; Zandalinas *et al.*, 2019, 2020). Systemic acquired acclimation is controlled by the integration of rapidly propagating systemic signals such as the reactive oxygen species (ROS), calcium, and hydraulic waves, as well as electric signals (Suzuki *et al.*, 2013; Fichman and Mittler, 2020b). The ROS wave is a cell-to-cell process of ROS-induced ROS-production that propagates from the particular tissue that initially senses the stress to the entire plant (Fichman *et al.*, 2019). It is activated in response to various stimuli, such as pathogen infection, wounding, heat, and high light (HL) stresses, and is dependent on the function of the respiratory burst oxidase homolog D (RBOHD) protein (Fichman and Mittler, 2020b).

In a previous study we demonstrated that the systemic response of plants to a local treatment of HL stress requires the ROS wave, is accompanied by rapid local and systemic transcriptional responses, and results in SAA (Zandalinas *et al.*, 2019). We further identified many RBOHD-dependent systemic transcripts and defined a core set of 82 ROS wave-associated transcripts (Zandalinas *et al.*, 2019). One of these 82 core ROS wave-associated transcripts is MYB30 (AT3G73810). MYB30 is a transcriptional regulator belonging to the R2R3-MYB gene family. The Arabidopsis (*Arabidopsis thaliana*) genome contains ~200 genes with a conserved MYB domain, involved in the regulation of a variety of functions, such as growth and development, metabolism, hormone signaling, and responses to biotic and abiotic conditions (Ogata *et al.*, 1994; Stracke *et al.*, 2001; Yanhui *et al.*, 2006; Dubos *et al.*, 2010; Ambawat *et al.*, 2013). Previous studies identified a role for MYB30 in mediating brassinosteroid responses in roots (Li *et al.*, 2009), calcium signaling in response to heat stress (Liao *et al.*, 2017), and root growth and development (Mabuchi *et al.*, 2018; Maki *et al.*, 2019).
Although we previously identified MYB30 as a core ROS wave-associated transcript (Zandalinas et al., 2019), its function in the regulation of the ROS wave and/or SAA was not experimentally defined. In the current study, we used a genetic approach to study the role of MYB30 in the ROS wave and SAA responses of Arabidopsis to HL stress.

Results

To determine the involvement of MYB30 in the systemic response of Arabidopsis to HL stress, two independent T-DNA insertion mutants of MYB30 (myb30-1 and myb30-2) were studied (Fig. 1A). Wild-type (WT) and mutant plants were exposed to a local HL stress treatment and whole-plant ROS accumulation was imaged. Compared to WT, ROS accumulation was found to be faster and more intense in the MYB30 mutants (Fig. 1B; Supplemental Fig. S1). This finding was in contrast to the systemic ROS accumulation results of the rbohD mutant that is impaired in MYB30 expression and SAA to HL stress and displays a suppressed systemic ROS signal (Fichman et al., 2019; Zandalinas et al., 2019). In contrast to a local treatment of HL stress, the rate of systemic ROS accumulation in the myb30-1 and myb30-2 mutants was similar to WT in response to a local treatment of wounding or heat stress (Supplemental Fig. S2), suggesting that the role of MYB30 could be specific to HL stress. To determine the relationship between MYB30, the ROS wave, and plant acclimation, we compared the SAA of WT plants to that of the myb30 mutants. It was shown earlier that SAA to HL stress is dependent on ROS wave signaling (Suzuki et al., 2013; Devireddy et al., 2018; Zandalinas et al., 2019, 2020). Wild-type plants, showing normal levels of the ROS wave, displayed local and systemic acclimation to HL stress following a 10 min application of HL stress to a single local leaf (Fig. 1C). In contrast, following the same treatment, myb30 mutants displayed local acclimation, but were deficient in SAA (Fig. 1C). These findings revealed a surprising effect in the myb30 mutants in which the ROS wave and SAA were uncoupled. Although the myb30 mutants displayed a stronger and faster ROS wave response, this response was not accompanied by SAA, suggesting that MYB30 may be required to couple these two processes.

To determine the underlying cause of the decoupling between the ROS wave and SAA in the myb30 mutants, transcriptomic analysis was performed. Changes in transcript steady-state levels in WT and the myb30-1 (Fig. 1A; Li et al., 2009; Liao et al., 2017) mutant in local and systemic tissues were quantified in response to 2, 8, and 30 min of HL stress applied to a single local leaf.
(Fig. 2; Supplemental Figs. S3, S4; Supplemental Tables S1-S9). Similar to Zandalinas et al., (2019, 2020), large sets of transcripts responded within this time frame in the local and systemic leaves of WT plants to the local application of light stress (Fig. 2, Supplemental Fig. S4). Comparing these sets of transcripts between WT and the myb30-1 mutant revealed that the systemic response of WT to HL stress that included over 4,200 upregulated transcripts, was much more comprehensive than that of the myb30-1 mutant that included over 1,400 upregulated transcripts (Fig. 2A). Similar differences were found between WT and myb30 for downregulated transcripts in systemic leaves in response to the same treatment (Fig. 2A; Supplemental Fig. S4).

In contrast to the differences in the extent of the systemic transcriptomic response between WT and myb30-1 to the local application of light stress (Figs. 2A, 2B), the amount of transcripts upregulated or downregulated in the local leaves of WT and the myb30-1 mutant was about the same (Fig. 2A, Supplemental Fig. S4). The differences observed between the systemic transcriptomic response of the myb30-1 mutant and that of WT could be a potential underlying cause for the differences observed between the local and systemic acclimation of WT and the myb30 mutants (Figs. 1C, 2A). Local leaves of both WT and myb30 plants, directly subjected to light stress, displayed therefore a strong transcriptomic response and were able to acclimate (Figs. 1C, 2A). In contrast, systemic leaves of WT that displayed a strong transcriptomic response were able to acclimate, whereas systemic leaves of myb30-1 that displayed a weak transcriptomic response were unable to acclimate (Figs. 1C, 2A).

To further study the role of MYB30 in the systemic response of plants to light stress we focused on transcripts altered in their abundance in systemic leaves of WT plants, but not in myb30-1. These systemic MYB30-dependent transcripts included 2,989 upregulated and 2,976 downregulated transcripts (Fig. 2A, Supplemental Tables S1, S2). Interestingly, although MYB30 is a single transcriptional regulator, these transcripts could be segregated into three similar size clusters, representing fast and transiently responsive transcripts, fast responsive transcripts that are altered in abundance but thereafter are maintained at their new level, and slower response transcripts (Fig. 2B). This finding suggests that MYB30 could function during early stages of the systemic response and that its disruption affects multiple responses that span different groups of transcripts and pathways. In support of such a central role of MYB30 is also the sheer number of transcripts affected by the lack of MYB30 (over 5,000 transcripts that are altered in abundance in WT but not in myb30-1; Fig. 2A; Supplemental Tables S1, S2). MYB30-
dependent upregulated transcripts include a high representation of protein transport, proteolysis, ubiquitination, and stress-response pathways; while MYB30-dependent downregulated transcripts include mostly translation related pathways (Fig. 2B). Comparing the MYB30-dependent transcripts identified in this study (Supplemental Tables S1, S2) with groups of transcripts associated with different abiotic stresses (drought, cold, heat, high light, salt, and ozone), response to wounding, pathogen, and hormones, different types of ROS, or overexpression of MYB30 in the presence or absence of ROS (Mabuchi et al., 2018; Zandalinas et al., 2019; Willems et al., 2016) revealed that they contain a high proportion of high light-, wounding-, H2O2-, MYB30-associated, and ABA-response transcripts (Supplemental Table S3, Supplemental Fig. S5).

We previously identified a core set of 82 transcripts associated with the systemic propagation of the ROS wave in response to a local HL stress in Arabidopsis (Zandalinas et al., 2019). Although this list included MYB30, it was not known whether MYB30 regulates the expression of any of the other genes in this list. To determine the relationship between MYB30 and some of the other genes in the list, we compared the expression of the 82 core ROS wave-associated transcripts between WT and myb30-1 in systemic leaves in response to the local HL treatment. As shown in Fig. 2C, under the conditions tested in this work, the expression of 73% of the transcripts included in the list was elevated in systemic leaves of WT plants. In contrast, the expression of only 51% of the transcripts included in the list was elevated in systemic leaves of myb30-1 plants. We therefore identified 27 putative MYB30-dependent ROS wave-associated core transcripts (Supplemental Table S4). Because the ROS wave is enhanced in myb30 mutants but SAA does not occur, it is possible that these transcripts are required for SAA. Another possibility is that these MYB30-dependent transcripts are involved in suppressing the ROS wave and their absence enables the ROS wave to propagate faster. Two of the ROS wave-associated transcripts induced in systemic leaves of WT, but not myb30, GATA8, and the GDSL esterase/lipase, were previously shown to be essential for SAA to HL stress in Arabidopsis (Zandalinas et al., 2019). In addition, the promoters of these genes contain putative MYB binding motifs, suggesting that they could be under the control of MYB30 (Supplemental Tables S5-S7 and text below). We therefore tested whether mutants of these genes display a ROS wave phenotype similar to the myb30 mutants. As shown in Figure 3, compared to WT, the gata8-1, gata8-2, gdsl-1, and gdsl-2 mutants displayed an enhanced ROS wave phenotype, similar to the myb30 mutants (Fig. 1), in
response to a local treatment of HL stress. In addition, the expression of MYB30 was found to be delayed in local and systemic leaves the *gdsl-1* mutant, and suppressed in systemic leaves of the *gata8-1* mutant in response to a local application of HL stress (Supplemental Fig. S6), suggesting that the function of MYB30, GATA8, and GDSL esterase/lipase is interlinked during responses to HL stress. The findings described above support the proposed role of MYB30 in coupling between the ROS wave and SAA, and demonstrate that MYB30 function and regulation are interlinked with two other genes (GATA8, and GDSL esterase/lipase) that are required for this process.

To further identify transcripts associated with MYB30 in response to HL, we performed additional comparisons using different bioinformatics tools. First, a weighted correlation network analysis (WGCNA) tool was employed to construct a gene co-expression network using Arabidopsis RNA-Seq datasets obtained from NCBI GEO repository. This analysis revealed 2,625 genes that appeared as immediate neighbors to MYB30 in the network (their expression correlation with MYB30 was above the established threshold), representing 11 modules (Fig. 4A and Supplemental Table S5). Next, a screen for the following MYB cis-binding elements, (A/C)ACC(A/T)A(A/C)C, (A/C)TCC(A/T)ACC, TAACT(G/C)GTT, TACTAACC, and A(A/C)C(A/T)A(A/C)C, in the Arabidopsis genome (Supplemental Table S6) was conducted to identify potential genes regulated by MYBs. This analysis unveiled 10,682 genes that contain a potential MYB binding domain in their promoters. In addition, a search for MYB30-associated genes was conducted using the TF2Network tool (Kulkarni et al., 2018), which encompasses several different data sources including MYB30 cis element binding data from the plant cistrome database (O'Malley et al., 2016). This analysis revealed 3,063 MYB30-associated genes (Supplemental Table S7). Lastly, a list of systemic transcripts dependent on RBOHD function was imported from the study of Zandalinas et al., (2019). This list includes many of the systemic transcripts that are dependent on the ROS wave during the systemic response of plants to HL stress. We then compared these different datasets and identified 51 (Supplemental Fig. S7; Supplemental Table S8) and 150 transcripts (Fig. 4B; Supplemental Table S9) common to systemic MYB30-dependent transcripts (5,965; Figs. 2A, 2B, 4B and Supplemental Tables S1, S2), WGCNA MYB30 associated transcripts (2,625; Figs. 4A, 4B and Supplemental Table S5), MYB30 cis-elements containing (10,682, Supplemental Fig. S7; Supplemental Table S6), or MYB30-TF2Network-associated (3,062; Fig. 4B, Supplemental Table S7) genes, and
upregulated systemic RBOHD-dependent transcripts (3,447; Zandalinas et al., 2019; Fig 4B). These transcripts may represent direct targets of MYB30 in systemic leaves of Arabidopsis in response to light stress and set the stage for follow-up studies of MYB30 function.

Discussion

The systemic accumulation of MYB30 in response to a local HL stress treatment was previously found to require the function of RBOHD, suggesting that MYB30 operates downstream of RBOHD in systemic tissues (Zandalinas et al., 2019). While the rbohD mutant displays a suppressed rate of the ROS wave response and does not accumulate ROS or MYB30 in systemic leaves in response to a local HL stress treatment (Zandalinas et al., 2019), the null mutants of myb30 displayed a stronger and faster ROS wave signal, compared to wild type, in response to the same local HL stress treatment (Fig. 1B). This unexpected finding could suggest that MYB30 is a repressor of the ROS wave that could function as part of a feedback loop that regulates and coordinates systemic responses during light stress. Contrary to RBOHD that is required for both local and systemic acquired acclimation (Suzuki et al., 2013; Devireddy et al., 2018; Zandalinas et al., 2019), myb30 mutants displayed local acquired acclimation, but not SAA, suggesting that MYB30 is required for SAA. Taken together, the observations described above suggest that MYB30 functions as a conduit between the spread of the ROS signal to systemic tissues and SAA (Fig. 4C). As depicted in the model shown in Figure 4C, MYB30 could function as a central regulator that suppresses the ROS wave signal on the one hand and enhances acclimation on the other, balancing these two responses and resulting in tolerance to excess light at the whole plant level. In its absence, the coupling of these two processes is lost and acclimation does not occur even though the ROS wave occurs at high levels (Fig. 1). MYB30 could also be required for acclimation, without directly impacting the ROS wave, and in its absence the ROS wave could be turned on even faster and stronger in an attempt to compensate for the lack of SAA (Fig. 4C).

The two plausible scenarios described above could be reflected in the transcriptomic analysis conducted, as well as in the response of the 82 ROS wave-associated transcripts. For example, two of the ROS wave-associated and RBOHD-dependent transcripts significantly altered in systemic leaves of WT, but not in myb30, GATA8, and the GDSL esterase/lipase, also accumulate high systemic levels of ROS (Fig. 3), but are unable to mount a SAA to light stress.
Many of the other RBOHD-dependent transcripts that were previously found to be involved in SAA to light stress (list of 3,447 transcripts; Fig. 4B; Zandalinas et al., 2019), were also among the MYB30-dependent systemic response transcripts identified in this study (1,166 transcripts; Fig. 4B), and 399 (Supplemental Fig. S7) or 902 (Fig. 4B) of them contained a putative MYB binding domain in their promoters (including GATA8 and the GDSL esterase/lipase). Such a high number of potential MYB30-dependent transcripts, with some already shown to be directly required for systemic plant acclimation to light stress (Zandalinas et al., 2019), supports a proposed role for MYB30 in regulating the acclimation of systemic tissues to light stress. Nevertheless, the question of whether MYB30 is a negative regulator of the ROS wave remains to be answered. A recent study found that MYB30 interacts with phytochrome-interacting factor 4 (PIF4) to regulate light responses in Arabidopsis (Yan et al., 2020). Interestingly, PIF4 was found by our analysis to be a MYB30-dependent light stress response systemic transcript (Supplemental Table S1), suggesting that MYB30 could, at least partially, regulate systemic light responses via PIF4 (Fig. 4C). In addition to regulating light responses, PIF4 was also found by another recent study to regulate ROS homeostasis (Sun et al., 2020). This finding could further suggest that an interaction between MYB30 and PIF4 is involved in the regulation of systemic ROS responses (Fig. 4C).

To relate MYB30 expression in systemic tissues in response to light stress in the current study with MYB30 overall function in plants, we conducted a transcript co-expression analysis using WGCNA (Fig. 4A). This analysis considered a large number of experimental datasets and identified transcripts that co-express with MYB30 under a large number of experimental and developmental conditions. Although the overlap between all transcripts identified by the WGCNA analysis (2,625; Fig. 4B) and the transcripts identified as MYB30-dependent in our current analysis was high (775; Fig. 4B), the number of WGCNA-identified transcripts that contained a putative MYB binding domain in their promoter, and were RBOHD-dependent was rather small (51; Supplemental Table S8, or 150, Fig. 4B; Supplemental Table S9). This finding supports previous findings and suggests that MYB30 has a vast array of overlapping and non-overlapping functions in cells that may or may not be linked to ROS, HL stress, and/or SAA (Ogata et al., 1994; Stracke et al., 2001; Yanhui et al., 2006; Dubos et al., 2010; Ambawat et al., 2013; Li et al., 2009; Liao et al., 2017; Mabuchi et al., 2018; Maki et al., 2019). Because light plays such a pivotal role in plants, the triggering of SAA by light stress could impact many
different physiological, metabolic, and molecular processes in systemic tissues and these could be directly or indirectly mediated by MYB30.

Conclusions

Taken together, our findings highlight an important function for MYB30 as a master regulator that couples the ROS wave with SAA to light stress (Fig. 4C). When MYB30 is triggered by the RBOHD-driven ROS wave in systemic tissues, its function is to induce SAA, as well as to decrease the ROS signal, allowing the plant to acclimate to the HL stress.

Materials and Methods

Plant material, growth conditions and stress treatments

Homoygous Arabidopsis (Arabidopsis thaliana) knockout mutants of MYB30, myb30-1 and myb30-2 (SALK_122884 and SALK_027644, respectively; Fig. 1A), GATA8 (AT3G54810), gata8-1 and gata8-2 (SALK_091040C and SALK_148073C, respectively), and GDSL esterase/lipase (AT1G29670), gdsl-1 and gdsl-2 (SALK_005724C and SALK_025240C, respectively), and control WT Col-0 plants were grown on peat pellets (Jiffy International, Kristiansand, Norway) under controlled conditions of 10hr/14hr light/dark regime, 50 µmol photons s\(^{-1}\)m\(^{-2}\) and 21°C for 4 weeks. Homozygosity was determined by PCR according to O’Malley et al., (2015). T-DNA and flanking sequences were amplified using the primers described in Supplemental Table S10. PCR cycles included 5 min denaturation at 95°C, 5 min annealing at 60°C, and 1 min elongation at 72°C. The cycles were repeated 30 times and then followed by 5 min of 72°C. PCR products were separated on a 1.2% Tris-Acetic acid-EDTA agarose gel with 0.5µg/ml ethidium bromide and visualized using Uvidoc HD6 gel documentation system (Uvitec Cambridge, UK). High light (HL) stress was applied by illuminating a single leaf with 1,700 µmol photons s\(^{-1}\)m\(^{-2}\) using a ColdVision fiber optic LED light source (Schott, Southbridge, MA, USA) for 2, 8, or 30 min, as previously described (Fichman et al., 2019; Zandalinas et al., 2019, 2020). Due to the use of an LED light source, leaf temperature was not significantly altered due to a 2, 8, or 30 min illumination as published previously (Devireddy et al., 2018; Zandalinas et al., 2020), and shown by thermal camera measurements (FLIR systems, Wilsonville, OR, USA) in Supplemental Fig. S8. A local
treatment of heat stress was applied as described in Zandalinas et al., (2020), and a local wounding treatment was applied as described by Fichman et al., (2019).

**Imaging of ROS accumulation**

Based on our established whole-plant imaging protocol (Fichman et al., 2019, Fichman and Mittler, 2020a; Zandalinas et al., 2020), 50 µM H$_2$DCFDA (Millipore-Sigma, St. Louis, MO, USA) in 0.05 M phosphate buffer, pH 7.4, 0.01% (v/v) Silwet L-77, was applied to plants using fumigation for 30 min in a glass container using a nebulizer (Punasi Direct, Hong Kong, China). H$_2$DCFDA is nonfluorescent, however, once deacetylated upon entering cells, DCF fluoresces at Ex./Em. 490nm/520nm. Following the fumigation, a local HL stress treatment was applied to a single leaf for 2 min. Images of DCF fluorescence were acquired using IVIS Lumina S5 (PerkinElmer, Waltham, MA, USA). ROS accumulation was analyzed using Living Image 4.7.2 software (PerkinElmer). Time-course images were generated and radiant efficiency of regions of interest (ROI) was calculated. Each data set includes standard error for 8-12 biological repeats and was assigned a Student t-test score. Local leaf (L) is the leaf subjected to the HL stress and systemic leaves (S1, S2, S3) are three systemic younger leaves positioned approximately 137.5° from the local leaf. Dye penetration controls shown in Supplemental Fig. S1 were performed by fumigation of plants with 0.3% (v/v) hydrogen peroxide for 10 min following the H$_2$DCFDA fumigation and acquisition of images in the IVIS Lumina S5 (Fichman et al., 2019, Fichman and Mittler, 2020a; Zandalinas et al., 2020). The live whole-plant imaging method was already validated by three different studies using different inhibitors and ROS metabolizing mutants (Fichman et al., 2019, Fichman and Mittler, 2020a; Zandalinas et al., 2020). In addition, in the study of Zandalinas et al., (2020), the ROS imaging method was followed by transcriptomic analysis that demonstrated a tight association between ROS imaging and the expression of ROS-response and ROS wave- associated (Zandalinas et al., 2019) transcripts. Further, a considerable overlap was found between transcripts identified by our study as under the control of MYB30 and ROS-associated transcripts defined by (Willems et al., 2016), as well as transcripts altered due to MYB30 overexpression in the presence of absence of ROS (Mabuchi et al., 2018; Supplemental Fig. S5; Supplemental Tables S11 and S12).

**Measurements of SAA to HL stress**
Leaf injury following light stress was measured using the electrolyte leakage assay, as previously described (Suzuki et al., 2013; Devireddy et al., 2018; Zandalinas et al., 2019). In short, a single leaf from a 4-week-old plant was illuminated with 1,700 µmol photons s\(^{-1}\)m\(^{-2}\) for 45 min and sampled for electrolyte leakage. Systemic acclimation to HL stress was examined by exposing a local leaf to light stress for 10 min, incubating the plant under controlled conditions for 50 min and then exposing the same leaf (local) or another younger leaf (systemic) to HL stress for 45 min. The experiment consisted of 5 biological repeats for each condition in each line. Standard error and Student t-test score were calculated.

**RNA sequencing and expression analysis**

Local and systemic transcriptional responses to light stress were studied by illuminating a local leaf for 2, 8 or 30 min and collecting local and systemic leaves from control and treated plants (20 plants for each group) in 3 biological repeats for total RNA purification (Supplemental Fig. S2; Systemic leaves were younger leaves positioned approximately 137.5° from the local leaf). Plant RNeasy kit (Qiagen, Hilden, Germany) was used for RNA purification. Reverse transcription quantitative PCR (RT-qPCR) analysis was conducted as described in (Balfagón et al., 2019). Primer sequences used for the RT-qPCR analysis and their efficiency values are listed in Supplemental Table S10. In accordance with efficiency values range, expression results are represented as percent of control which were calculated as \(2^{-\Delta\Delta C_t}\). RNA sequencing was performed using NovaSeq 6000 at the University of Missouri DNA Core Facility. RNA libraries were prepared for sequencing using standard Illumina protocols (Balfagón et al., 2019; Zandalinas et al., 2019, 2020). Read quality control was performed using FastQC v1.20.0 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), followed by alignment of reads onto the Arabidopsis reference genome (TAIR 10; https://www.arabidopsis.org/) using STAR aligner v2.4.0.1, and analysis of differential gene expression using DESeq2 v1.20.0 (Balfagón et al., 2019; Zandalinas et al., 2019, 2020). The genome index was built using the gene annotation file (gff file) downloaded from TAIR 10. Differences in expression were quantified as the logarithm of the ratio of mean normalized counts between two conditions (log fold change). Differentially abundant transcripts were defined as those that have a log fold change with an adjusted P-value < 0.05 (negative binomial Wald test followed by a Benjamini–Hochberg correction; both integral to the DESeq2 package). Differentially expressed genes were classified...
into upregulated or downregulated based on significant positive or negative log fold change values, respectively. Venn diagram overlaps were calculated using VIB Bioinformatics and Evolutionary Genomics web tool (http://bioinformatics.psb.ugent.be/webtools/Venn/). Clusters (k-means soft clustering) were generated with Mfuzz v2.40.0 package of R Bioconductor (Kumar and Futschik, 2007). Functional annotation and quantification of overrepresented GO terms (P<0.05) were conducted using DAVID 6.8 (Huang et al., 2009). Raw and processed RNA-Seq data files were deposited in GEO (https://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE141916.

**Weighted correlation network analysis (WGCNA)**

The entire collection of Arabidopsis RNA-Seq datasets available at the NCBI Sequence Read Archive (SRA) repository were downloaded and converted to FASTQ format using the prefetch and fastq-dump utilities of the sratools v2.92 package (Leinonen et al., 2011). Reads from each dataset, comprised of either single-end or paired-end reads, were aligned to the Arabidopsis TAIR 10 transcriptome (Rhee et al., 2003) using salmon v0.12.0 and an index was built for each dataset using the TAIR 10 cDNA FASTA from the Ensembl Plants release 46 FTP (Bolser et al., 2016; Patro et al., 2017). Alignment datasets were further examined and those with less than 80% alignment and 50% transcriptome representation were removed to minimize the effects of poor-quality or potentially misannotated data on the resultant network. Gene expression vectors, each representing the normalized gene expression (transcripts-per-million) values from an experiment (dataset), were imported into the WGCNA R package (Langfelder and Horvath, 2008). The blockwise Modules function of WGCNA was used to construct a weighted gene co-expression network using a soft threshold power of 5, a minimum module size of 30, and a cut height of 0.25. Module assignments and weighted correlation values for gene pairs were extracted from the topological overlap matrix using the “export Network To Cytoscape” function of WGCNA. Network figures were generated using Cytoscape v3.7.2 (Shannon et al., 2003).

**MYB cis-element analysis**

MYB cis-element motifs were identified based on AGRIS (https://agris-knowledgebase.org/; Yilmaz et al., 2011) and were searched within 1,000 bp regions upstream to the gene models of the Arabidopsis genome (TAIR 10; https://www.arabidopsis.org/) using an in-house Perl script.
In addition, a list of MYB30-associated genes was obtained using the TF2Network prediction tool (Kulkarni et al., 2016).

**Accession numbers**

The RNA-seq data analyzed in this study were deposited in the Gene Expression Omnibus (GEO) database, https://www.ncbi.nlm.nih.gov/geo (accession no. GSE141916). Quantitative data of the described transcripts is provided in Supplemental Tables S1 and S2.

**Supplemental Data**

The following supplemental materials are available.

**Supplemental Figure S1.** Dye penetration controls for wild type and the two myb30 mutants.

**Supplemental Figure S2.** Null mutants of MYB30 accumulate ROS in a similar manner to wild type in response to a local application of heat stress or wounding.

**Supplemental Figure S3.** The experimental design used for comparing the local and systemic responses of wild type and the myb30-1 mutant to a local application of HL stress.

**Supplemental Figure S4.** Changes in local transcriptomics responses in the myb30-1 mutant compared to wild type in response to HL stress.

**Supplemental Figure S5.** Overlap between MYB30-dependent systemic HL-response transcripts and ROS-associated genes (Willems et al., 2016), or transcripts upregulated in MYB30 overexpressing plants in the presence or absence of ROS (Mabuchi et al., 2018).

**Supplemental Figure S6.** Expression of MYB30 in gata8-1 and gdsl-1 mutants in response to a 2, 8, and 30 min treatment of HL stress.

**Supplemental Figure S7.** Venn diagram showing the overlap between HL-response systemic MYB30-dependent transcripts, MYB30 WGCNA-associated transcripts, transcripts encoded by genes containing MYB30 cis-elements at their promoters, and upregulated systemic RBOHD-dependent transcripts.

**Supplemental Figure S8.** Thermal camera images and measurements of leaf temperature in HL-treated plants.
Supplemental Table S1. MYB30-dependent upregulated transcripts in systemic leaves in response to a local high light stress.

Supplemental Table S2. MYB30-dependent downregulated transcripts in systemic leaves in response to a local high light stress.

Supplemental Table S3. Overlap between MYB30-dependent transcripts and transcripts associated with stress responses and hormonal signaling.

Supplemental Table S4. MYB30-dependent ROS wave core transcripts.

Supplemental Table S5. List of MYB30-associated WGCNA genes.

Supplemental Table S6. List of MYB cis elements-containing genes in the Arabidopsis genome based on AGRIS.

Supplemental Table S7. List of MYB30 associated genes in the Arabidopsis genome based on TF2Network.

Supplemental Table S8. List of common MYB30-dependent, RBOHD-dependent, WGCNA and MYB cis elements (AGRIS) regulated transcripts.

Supplemental Table S9. List of common MYB30-dependent, RBOHD-dependent, WGCNA and MYB cis elements (TF2Network) regulated transcripts.

Supplemental Table S10. List of primers for determination of homozygosity and RT-qPCR.

Supplemental Table S11. List of overlapping transcripts between MYB30-dependent systemic HL-response transcripts and ROS-associated transcripts (Supplemental Fig. S5A; Willems et al., 2016).

Supplemental Table S12. List of overlapping transcripts between MYB30-dependent systemic HL-response transcripts and transcripts upregulated in MYB30 overexpressing plants in the presence or absence of ROS (Supplemental Fig. S5B; Mabuchi et al., 2018).

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**Figures legends**

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Supplemental Table S5), transcripts encoded by MYB30-regulated genes identified by the TF2Network prediction tool (Supplemental Table S7), and upregulated systemic RBOHD-dependent transcripts (Zandalinas et al., 2019). C, Model for MYB30 function. The expression of MYB30 is shown to be triggered by the ROS wave, and MYB30 is shown to be required for the induction of SAA. MYB30 could also be involved in directly suppressing the ROS wave, or indirectly affecting the initiation or strength of the ROS wave by inducing acclimation mechanisms that alleviate stress levels and reduce the necessity to trigger or amplify the ROS wave. Abbreviations used: HL, high light; ROS, reactive oxygen species; RBOHD, respiratory burst oxidase homolog; SAA, systemic acquired acclimation; WGCNA, Weighted correlation network analysis; WT, wild type.
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