PLANT BIOLOGY

Coordinating the overall stomatal response of plants: Rapid leaf-to-leaf communication during light stress

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The plant canopy functions as an aerial array of light-harvesting antennas. To achieve maximal yield, each leaf within this array and the array as a whole need to rapidly adjust to naturally occurring fluctuations in light intensity and quality. Excessive light stress triggers the closing of pores in leaves called stomata to minimize moisture loss. We found that different leaves within the canopy of an *Arabidopsis thaliana* plant, including leaves not directly exposed to light, coordinated stomatal closure in response to light stress by sending and receiving rapid systemic signals. This response required the plant hormones abscisic acid and jasmonic acid and was mediated by a rapid autopropagating wave of reactive oxygen species (ROS) production. Furthermore, this response depended on the function of genes encoding the ROS-generating NADPH oxidase RBOHD and various stomatal regulators, such as the anion channel SLAC1, GHR1 (guard cell hydrogen peroxide resistant 1), and lipoxygenase 1 (LOX1). Our findings reveal that plants function as highly dynamic and coordinated organisms, optimizing the overall response of their canopies to fluctuating light intensities.

INTRODUCTION

Plants are the primary solar energy converter sustaining life on Earth. To achieve optimal photosynthetic productivity, the different leaves of a plant, and the plant as a whole, must rapidly acclimate to fluctuating changes in ambient light intensity and quality (*1*–*5*). Because under natural conditions not all parts of the plant are subjected to the same light intensity or quality, an ability to rapidly transmit signals from one leaf to another is thought to play a key role in optimizing the plant's overall photosynthetic activity (*6*–*12*). Such an ability is also thought to play a key role in the acclimation and survival of plants during different abiotic stresses (*11*, *12*). Optimizing the overall response of plants to abrupt changes in light conditions could require rapid changes in the size of stomatal aperture to manage the availability of CO_2 for photosynthesis and match this availability to changes in light quality and intensity, individual leaf temperature, leaf-to-air vapor pressure deficit, and overall plant transpiration (*13*, *14*). For a shade-adapted plant, for example, a sudden increase in light intensity caused by a light fleck (sun fleck) could result in enhanced photosynthesis or photoinhibition and induce rapid stomatal responses (*3*, *4*, *13*–*15*). Depending on the light intensity and relative humidity, sun flecks could induce rapid stomatal opening (to enhance photosynthesis and/or photorespiration), or in extreme conditions, during photoinhibition, rapid stomatal closure to prevent catastrophic xylem failure by restricting transpiration (*13*, *15*). Stomatal closure in response to light stress and changes in relative humidity is also found in field-grown plants during midday (also known as "midday stomatal closure") (*16*–*18*). Because light stress is accompanied by an increase in the concentration of the plant hormone abscisic acid (ABA) in leaves, and ABA is required for the accumulation of transcripts encoding several light stress–response factors in plants, the light stress–induced stomatal closure response of plants could be mediated through the ABA canonical pathway (*3*, *4*, *19*). Nevertheless, how this acclimation response to light stress is coordinated between the different leaves of a plant is not clear. To address this question, we studied the local and systemic responses of low light–adapted *Arabidopsis thaliana* plants to light stress. We found that the application of light stress to a single *Arabidopsis* rosette leaf resulted in a plant-wide coordinated stomatal closure and acclimation responses to light stress. This response required ABA for its initiation and was mediated by a rapid autopropagating wave of reactive oxygen species (ROS) production (the ROS/Ca^{2+} wave) (*11*, *12*), which depended on the function of the respiratory burst oxidase homolog D (RBOHD). In addition, it required the function of slow anion channel–associated 1 (SLAC1), guard cell hydrogen peroxide resistant 1 (GHR1), and lipoxygenase 1 (LOX1) for regulating stomatal closure. Our findings reveal that plants function in a highly dynamic and coordinated manner, optimizing the overall response of their canopy to fluctuating light intensities.

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RESULTS

Local application of light stress triggers a local and systemic stomatal response in *Arabidopsis*

Fully expanded rosette leaves of *A. thaliana* (Col) plants, grown under low-light conditions (50 μmol m^{-2} s^{-1}) and subjected to different light intensities (200, 500, 1000, and 2000 μmol m^{-2} s^{-1}) for 10 min, exhibited a stomatal closure response that reached a maximum of about 70 to 75% stomatal closure at 2000 μmol m^{-2} s^{-1} (fig. S1). To study leaf-to-leaf communication during light stress, we subjected a single fully expanded rosette leaf of an *Arabidopsis* plant grown under low-light conditions (50 μmol m^{-2} s^{-1}) to light stress (2000 μmol m^{-2} s^{-1}) and measured the stomatal closure response of the treated (local) leaf, as well as of a nontreated distant (systemic) leaf, over time (Fig. 1A). Local and distant (systemic) leaves that were 3 to 4 cm apart responded almost in unison, closing their stomata by about 70% after 1 min of light stress application (Fig. 1B). Because stomatal closure leads to an increase in leaf temperature due to suppressed transpiration (*20*, *21*), we measured changes in leaf temperature of the local and systemic leaves of control- and light stress–treated plants using an infrared camera (fig. S2A). This method was chosen as opposed to gas exchange because clamping individual leaves, such as a local and/or a systemic

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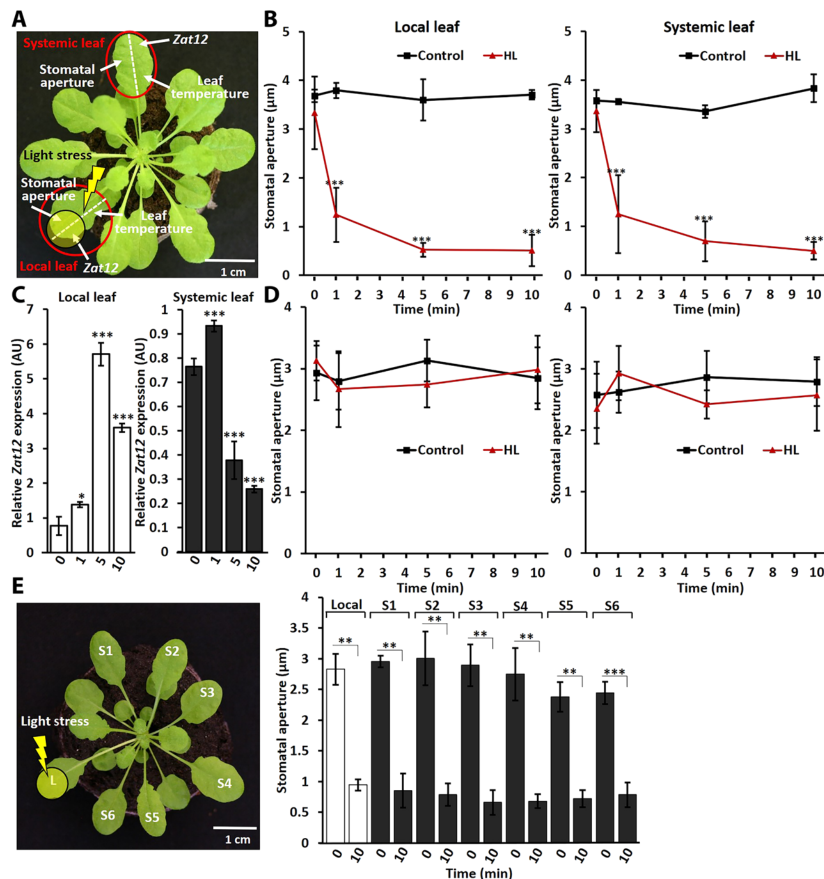

Fig. 1. Local application of light stress triggers a local and systemic stomatal closure response in *Arabidopsis*. (**A**) The experimental design used to measure stomatal responses and changes in steady-state transcript levels in response to local application of light stress. Each sampled leaf was divided along its mid vain and used for stomatal aperture measurements and determination of *Zat12* expression. (**B**) Local and systemic stomatal aperture response of *Arabidopsis* to local application of light stress (high light; HL). n = 500 stomata from 30 different plants for each group. (**C**) Changes in the steady-state levels of the rapid systemic response transcript *Zat12* in local and systemic leaves after application of light stress to a local leaf. n = 30 plants for each group. (**D**) Local and systemic stomatal aperture response of *Arabidopsis* mutants lacking the respiratory burst oxidase homolog D enzyme (*rbohD*) to local application of light stress (high light; HL). n = 500 stomata from 30 different plants for each group. (**E**) The experimental design used to measure canopy-wide systemic stomatal responses to local application of light stress (left) and bar graphs showing the systemic stomatal aperture response of six different systemic leaves (S1 to S6) in response to light stress application to a local leaf (local, right). n = 500 stomata from 30 different plants for each group. Statistical significance was determined by a one- or two-tailed Student's t test as described in (*25*). Results are presented as the means ± SE, ***$P < 0.005$, **$P < 0.01$, and *$P < 0.05$.

leaf within the same plant to measure local and systemic gas exchange, would induce mechanical pressure and injury responses that would affect rapid systemic signaling (*12*, *22*, *23*). In agreement with the light stress–induced changes in stomatal aperture measurements (Fig. 1B), leaf temperature increased in the local and systemic leaves of light stress–treated plants (fig. S2A). The rapid stomatal response of local and systemic leaves to light stress (Fig. 1B and fig. S2A) was accompanied by alterations in the steady-state level of *Zat12*, a key marker for rapid systemic signaling and the propagation of the ROS/Ca^{2+} wave (*12*, *22*), demonstrating that this rapid physiological response is also accompanied by alterations in transcript expression (Fig. 1C).

Because stomatal responses and the propagation of the ROS/Ca^{2+} wave require the function of the RBOHD protein (*12*, *22*–*29*), we tested the local and systemic responses of *rbohD* mutants to light stress. In contrast to the rapid stomatal closure response of wild-type plants to light stress (Fig. 1B and fig. S2A), *rbohD* mutants failed to induce local or systemic stomatal responses to light stress (Fig. 1D and fig. S2B). This finding demonstrated that the local and systemic stomatal closure responses to light stress in *Arabidopsis* required the function of the ROS-producing protein RBOHD.

The rapid stomatal response shown in Fig. 1B was not restricted to a single systemic leaf and occurred simultaneously in many other leaves comprising the plant canopy (Fig. 1E). The rapid rate of this systemic response (Fig. 1B and fig. S2A), its occurrence in almost all leaves of the plant canopy (Fig. 1F), and its suppression in *rbohD* mutants (Fig. 1D and fig. S2B) suggest that it is mediated by the autopropagating ROS/Ca^{2+} wave, which travels at a maximal rate of 8.4 cm min^{-1}, depends on calcium signaling, and can potentially reach almost all parts of the plant (*12*, *22*–*28*).

The ROS/Ca^{2+} wave is required to mediate the rapid systemic stomatal closure response of *Arabidopsis* plants to light stress

To test whether the ROS/Ca^{2+} wave is involved in mediating the rapid systemic signal that triggers stomatal closure in distant leaves of light stress–treated plants, we used the ROS/Ca^{2+} wave inhibitor diphenyleneiodonium (DPI) (*12*, *22*). The application of DPI at the midpoint between the local and the systemic leaf (Fig. 2A) prevented the closure of stomata in the systemic leaf without affecting the stomatal closure response of the local leaf (Fig. 2B). The application of DPI also prevented the accumulation of H_2O_2 in the systemic leaf without affecting the accumulation of H_2O_2 in the local leaf (Fig. 2C). Because the ROS/Ca^{2+} wave depends on calcium signaling (*22*–*28*), we also tested the effect of applying the calcium wave inhibitor $LaCl_3$ (*23*–*26*) at the midpoint between the local and the systemic leaf (Fig. 2A). As with DPI, $LaCl_3$ application prevented the closure of stomata in the systemic leaf without affecting the stomatal closure response of the local leaf (Fig. 2D). Measurements of the ROS/Ca^{2+} wave signal in local and systemic leaves using *Zat12::Luciferase* reporter plants (*22*) further supported the involvement of the ROS/Ca^{2+} wave in the systemic response of plants to light stress (Fig. 2E). Collectively, the findings shown in Figs. 1 and 2 and fig. S2 support a model in which different leaves within the plant use the ROS/Ca^{2+} wave to coordinate their rapid stomatal responses following a local application of light stress.

ABA is required to trigger the systemic stomatal closure response of *Arabidopsis* to local application of light stress

Stomatal closure in response to abiotic stress is thought to be primarily mediated by the plant hormone ABA (*29*–*31*). Moreover, ABA accumulates in leaves in response to light stress (*3*) and could mediate the stomatal closure response of plants to this stress (Fig. 1). To study the role of ABA in mediating the local and systemic stomatal closure responses of *Arabidopsis* to light stress, we tested whether ABA application to a

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local leaf would result in a systemic stomatal response, similar to that induced by light stress (Figs. 1 and 3A). As expected, application of ABA (50 μM) to a local leaf resulted in a rapid stomatal closure response (Fig. 3B). Moreover, it also induced a rapid systemic stomatal response (Fig. 3B). Similar to the application of light stress (Fig. 2B), the ABA-induced rapid systemic stomatal response was also blocked by the ROS/Ca^{2+} wave inhibitor DPI (Fig. 3C), suggesting that ABA did not relocate from the local to the systemic leaf per se but rather triggered the ROS/Ca^{2+} wave that induced stomatal closure in systemic leaves. The application of ABA (50 μM) to a local leaf of *Zat12::Luciferase* (Fig. 3D and fig. S3A) (*22*) or *WRKY40::Luciferase* (fig. S3B) (*32*) ROS/Ca^{2+} wave reporter plants revealed that ABA triggered the ROS/Ca^{2+} wave in local and systemic leaves. No other plant hormone or treatment with H_2O_2 or calcium has been previously shown to trigger this signal (*12*, *22*, *23*).

To further study the role of ABA in local and systemic leaves of plants subjected to light stress, we used high humidity to suppress ABA levels in wild-type plants. High humidity enhances ABA degradation through the activation of ABA 8′-hydroxylase (*33*). ABA concentrations in local or systemic leaves of *Arabidopsis* plants subjected to high humidity (80 to 85% relative humidity) decreased by about 65 to 70% (Fig. 4A). When plants were subjected to high humidity, local or systemic stomatal closure (Fig. 4B) and H_2O_2 accumulation (Fig. 4C) responses to a local application of light stress were suppressed. Because high humidity may also affect the stomatal closure response of plants through other routes that are unrelated to ABA, we measured stomatal aperture in response to the local application of light stress in wild-type *Arabidopsis* plants (Ler and Col) and mutants impaired in ABA biosynthesis (*aba1-1*, *aba2-1*, and *aba3-1*) (*21*) or ABA perception (*abi1-1*) (*34*, *35*). Compared to wild-type plants (Fig. 4D), all mutants impaired in ABA biosynthesis failed to display a local or systemic stomatal closure response after the application of light stress to a local leaf (Fig. 4E). This finding supported the results obtained with high humidity (Fig. 4, A to C). The *abi1-1* mutant is impaired in ABA perception because of a constitutively active ABI1 protein (which is a type 2C protein phosphatase) (*34*) that cannot interact with and be inhibited by the ABA receptor complex, which would lead to stomatal closure. In contrast to the mutants impaired in ABA biosynthesis (Fig. 4E), the *abi1-1* mutant displayed stomatal aperture closure in local and systemic leaves in response to a local application of light stress (Fig. 4F). Similar findings were obtained when the stomatal closure response of wild-type (Ler; fig. S4A), *aba1-1* (fig. S4B), and *abi1-1* (fig. S4C) mutants was studied using an infrared camera. These findings suggest that although ABA is required to trigger the local and systemic stomatal responses to light stress (Figs. 3 and 4, A to E) and to trigger the ROS/Ca^{2+} wave that links between the local and systemic leaves (Fig. 3), other plant hormones involved in stomatal regulation,

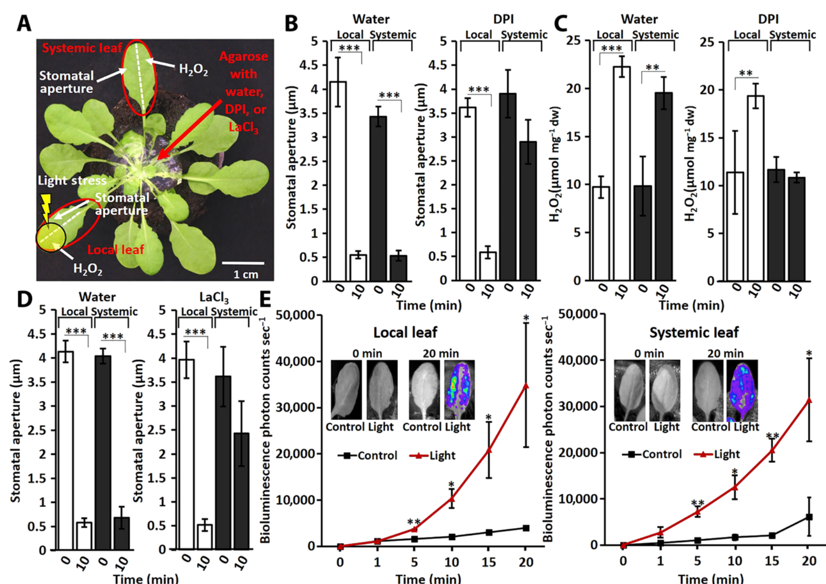

Fig. 2. Inhibition of reactive oxygen or calcium signaling blocks the signal that mediates the rapid systemic stomatal closure response in *Arabidopsis*. (**A**) The experimental procedure used to block the systemic signal using different inhibitors and to measure stomatal responses and reactive oxygen species (ROS) accumulation. (**B**) Effect of diphenyleneiodonium (DPI; 50 μM) application on the systemic stomatal aperture response to local application of light stress. *n* = 500 stomata from 30 different plants for each group. (**C**) Effect of DPI (50 μM) application on the systemic H_2O_2 accumulation response of *Arabidopsis* to local application of light stress. *n* = 30 plants for each group. (**D**) Effect of $LaCl_3$ application on the systemic stomatal aperture response of *Arabidopsis* to local application of light stress. *n* = 500 stomata from 30 different plants for each group. (**E**) Response of *Zat12::Luciferase* reporter plants to local application of light stress. *n* = 30 plants for each group. Statistical significance was determined by a one- or two-tailed Student's *t* test as described in (*25*). Results are presented as the means ± SE. ***$P < 0.005$, **$P < 0.01$, *$P < 0.05$.

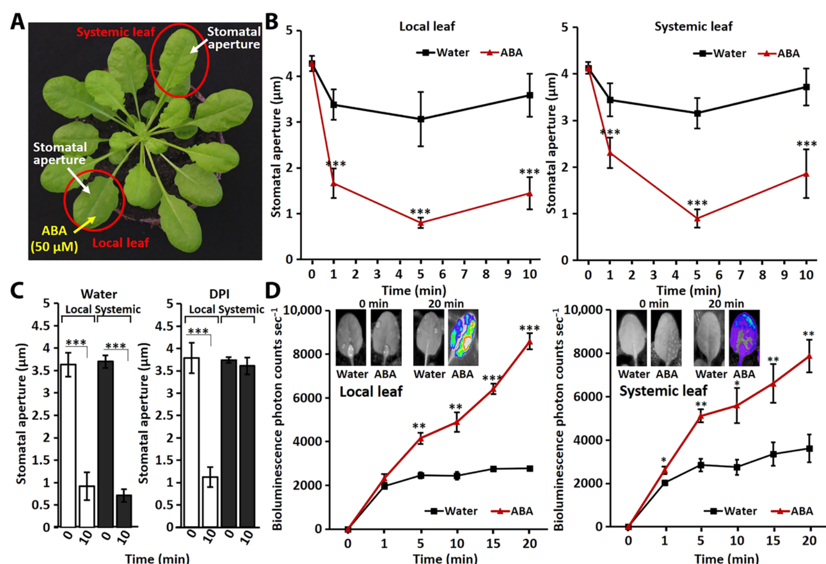

Fig. 3. Application of ABA to a local leaf triggers local and systemic stomatal closure responses. (**A**) The experimental procedure used to measure local and systemic stomatal aperture responses to local application of abscisic acid (ABA) (50 μM) in *Arabidopsis*. (**B**) Local and systemic stomatal aperture response to local application of ABA. *n* = 500 stomata from 30 different plants for each group. (**C**) Bar graphs showing the effect of DPI (50 μM) application (as in Fig.2A) on the systemic stomatal aperture response of *Arabidopsis* to local application of ABA. *n* = 500 stomata from 30 different plants for each group. (**D**) The response of *Zat12::Luciferase* reporter plants to local application of ABA (50 μM). *n* = 30 plants for each group. Statistical significance was determined by a one- or two-tailed Student's *t* test as described in (*25*). Results are presented as the means ± SE, ***$P < 0.005$, **$P < 0.01$, and *$P < 0.05$.

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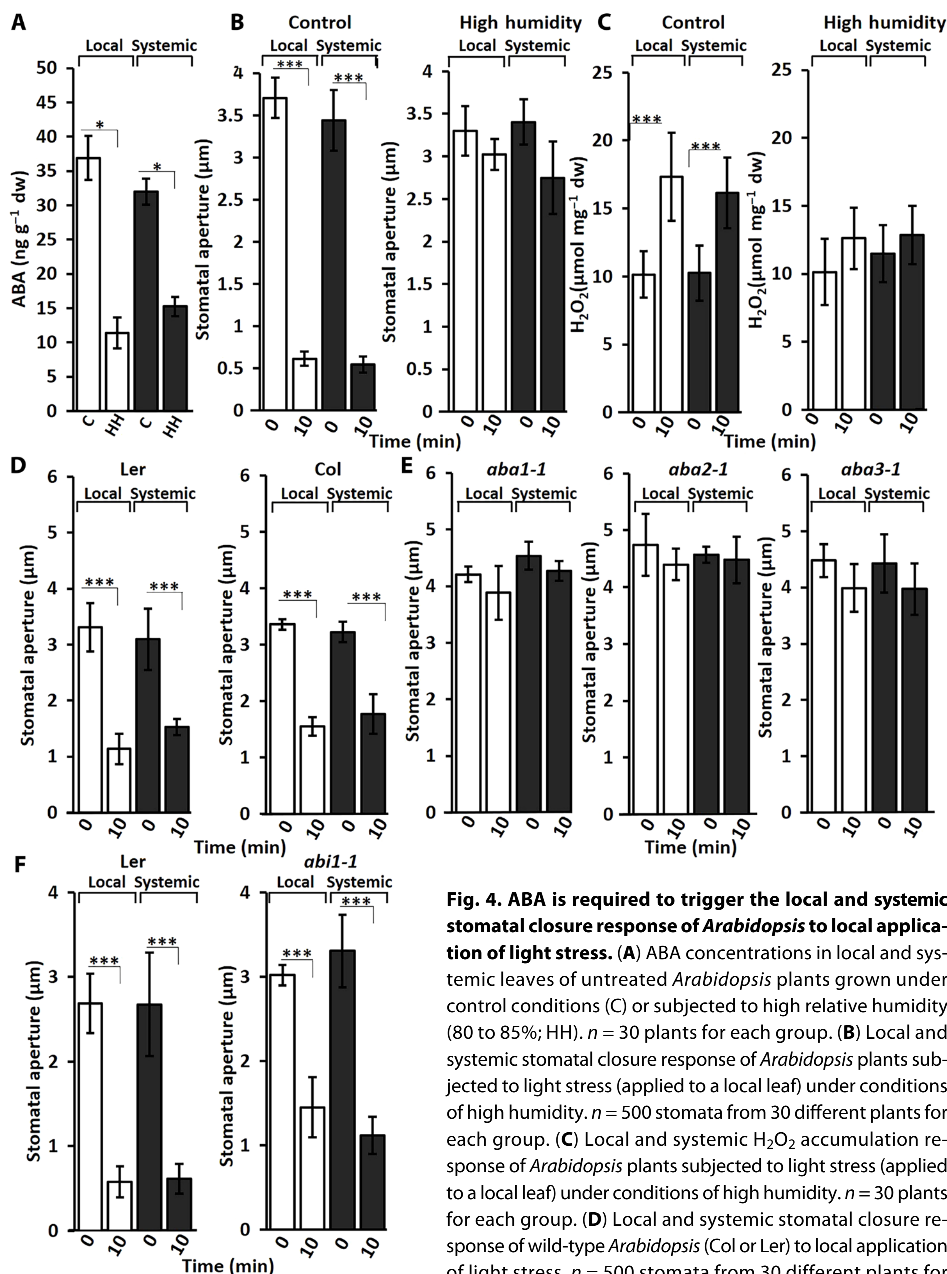

Fig. 4. ABA is required to trigger the local and systemic stomatal closure response of *Arabidopsis* to local application of light stress. (**A**) ABA concentrations in local and systemic leaves of untreated *Arabidopsis* plants grown under control conditions (C) or subjected to high relative humidity (80 to 85%; HH). $n = 30$ plants for each group. (**B**) Local and systemic stomatal closure response of *Arabidopsis* plants subjected to light stress (applied to a local leaf) under conditions of high humidity. $n = 500$ stomata from 30 different plants for each group. (**C**) Local and systemic H_2O_2 accumulation response of *Arabidopsis* plants subjected to light stress (applied to a local leaf) under conditions of high humidity. $n = 30$ plants for each group. (**D**) Local and systemic stomatal closure response of wild-type *Arabidopsis* (Col or Ler) to local application of light stress. $n = 500$ stomata from 30 different plants for each group. (**E**) Local and systemic stomatal closure response of *Arabidopsis* mutants deficient in ABA biosynthesis (*aba1-1*, Ler; *aba2-1* and *aba3-1*, Col) to local application of light stress. $n = 500$ stomata from 30 different plants for each group. (**F**) Local and systemic stomatal closure response of *Arabidopsis* mutants deficient in ABA sensing (*abi1-1*, Ler) to local application of light stress. $n = 500$ stomata from 30 different plants for each group. Statistical significance was determined by a one- or two-tailed Student's *t* test as described in (*25*). Results are presented as the means ± SE, $^*P < 0.05$, $^{***}P < 0.001$. dw, dry weight.

such as jasmonic acid (JA) (*11*, *31*), may also be involved in this response.

An interplay between ABA and JA could mediate the rapid systemic stomatal response of *Arabidopsis* to light stress

The possibility that other plant hormones may be involved in the rapid stomatal closure response of *Arabidopsis* to light stress (Fig. 4F and fig. S4C) prompted us to measure the concentrations of H_2O_2, JA, and salicylic acid (SA), also thought to be involved in stomatal regulation (*11*, *31*, *35*), in wild-type (Ler), *aba1-1*, and *abi1-1* plants in response to light stress (application of light stress to local leaf for 10 min). The concentrations of ABA (Fig. 5A), H_2O_2 (Fig. 5B), JA (Fig. 5C), and SA (Fig. 5D) increased in local leaves of wild-type plants subjected to light stress. In contrast, the concentrations of H_2O_2 (Fig. 5B), JA (Fig. 5C), and SA (Fig. 5D), but not ABA (Fig. 5A), increased in systemic leaves of wild-type plants subjected to the same local light stress treatment. The concentrations of ABA (Fig. 5A), SA (Fig. 5D), and H_2O_2 (Fig. 5B) did not increase in the local or systemic leaves of *aba1-1* plants subjected to light stress. In contrast, the concentration of JA (Fig. 5C) increased in local and systemic leaves of *aba1-1* plants subjected to a local light stress, albeit to lower concentrations than in wild-type plants. In the *abi1-1* mutant, SA (Fig. 5D) and JA (Fig. 5C) concentrations increased, but ABA (which was very high in the absence of any treatment; Fig. 5A) and ROS (Fig. 5B) did not significantly change in local or systemic leaves in response to light stress. It should be noted that the *abi1-1* mutant is tolerant to light stress and constitutively expresses transcripts encoding several light and oxidative stress response factors (*32*). This phenotype may explain why the light stress–induced changes in H_2O_2 levels in the local and systemic leaves of the *abi1-1* mutant are attenuated (Fig. 5B). The results presented in Figs. 3 to 5 support a model in which ABA is required to trigger the rapid light stress–induced local and systemic stomatal closure responses of *Arabidopsis*, but that this response may involve the function of other plant hormones such as SA and JA (*29*–*31*). Nevertheless, the results shown in Fig. 5 represent a single time point (10 min) following light stress application and may not reveal transient changes in ABA, SA, and/or JA during earlier time points.

To further investigate the role of ABA, JA, and SA in mediating local and systemic stomatal responses to light stress in *Arabidopsis*, we measured the concentrations of ABA, JA, the JA precursor 12-oxophytodienoic acid (OPDA), and SA in local and systemic leaves of wild-type (Col) and *rbohD* plants subjected to local light stress treatment for various time periods. In response to a local application of light stress in wild-type plants, ABA transiently accumulated in local leaves at 1 min and in systemic leaves at 4 min (Fig. 6A). This increase was attenuated in the *rbohD* mutant (Fig. 6A), suggesting that ABA synthesis and/or release from its conjugated forms (*12*) is required for ROS production by RBOHD. OPDA transiently increased in local and systemic leaves of wild-type plants between 1 and 8 min after local light

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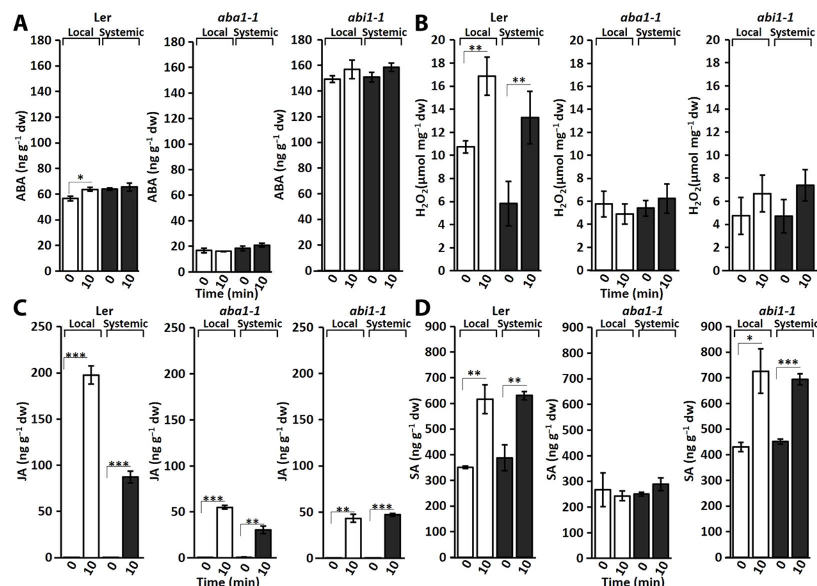

Fig. 5. ABA is required for H_2O_2 and SA accumulation in local and systemic leaves of *Arabidopsis* plants subjected to local application of light stress. (**A** to **D**) Accumulation of ABA (A, n = 30 plants for each group), H_2O_2 (B, n = 30 plants for each group), jasmonic acid (JA) (C, n = 30 plants for each group), and salicylic acid (SA) (D, n = 30 plants for each group) in local and systemic leaves of wild-type Ler, *aba1-1*, and *abi1-1* plants in response to local application of light stress. Statistical significance was determined by a one- or two-tailed Student's t test as described in (*25*). Results are presented as the means ± SE, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

stress application (Fig. 6B), an increase that was also attenuated in the *rbohD* mutant (Fig. 6B). In contrast, in wild-type plants, the JA accumulation peaked in the local leaves at 8 min and in the systemic leaves at 4 min (Fig. 6C), and the systemic, but not the local, accumulation of JA was attenuated in the *rbohD* mutant (Fig. 6C). Similar to OPDA, JA, and ABA, SA transiently accumulated in systemic leaves at 4 min (fig. S5), and this increase was attenuated in the *rbohD* mutant (fig. S5). However, in contrast to JA and ABA, SA steadily accumulated in local leaves with a transient peak at 1 min that was not attenuated in *rbohD* plants (fig. S5). The findings shown in Fig. 6 (A to C) and fig. S5 support the possibility that an interplay between ABA, JA, and ROS regulates stomatal responses in local and systemic leaves of *Arabidopsis* in response to light stress.

SLAC1, GHR1, and LOX1 are involved in the local and/or systemic stomatal responses of *Arabidopsis* to light stress

To further study the role of ABA, JA, and ROS in mediating rapid stomatal regulation during light stress, we studied the local and systemic stomatal responses of *slac1*, *ghr1*, and *lox1* mutants to local application of light stress in *Arabidopsis*. SLAC1 and GHR1 function in ABA-dependent and ABA-independent stomatal closure pathways (*36*–*39*), whereas LOX1 (lipoxygenase 1) is involved

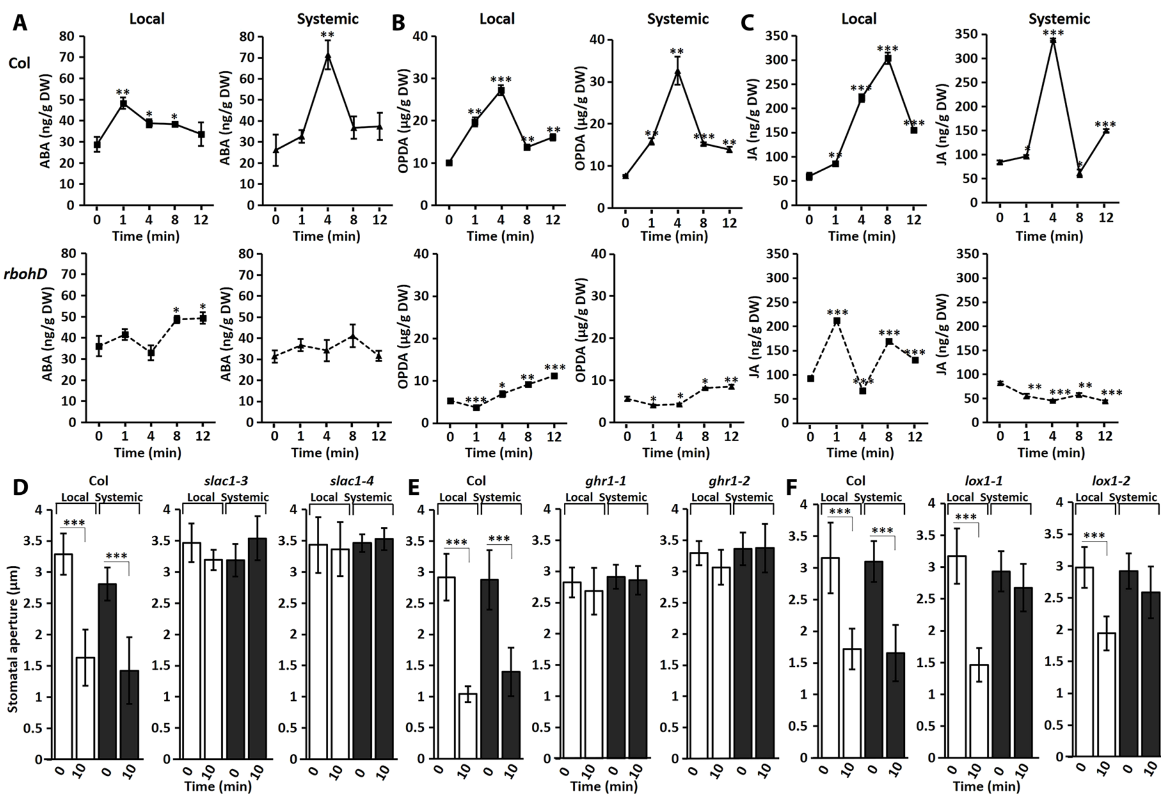

Fig. 6. Transient accumulation of ABA, OPDA, and JA in local and systemic leaves of *Arabidopsis* plants subjected to local application of light stress, and a role for SLAC1, LOX1, and GHR1 in the local and/or systemic stomatal closure response of *Arabidopsis* to light stress. (**A**) Changes in the level of ABA in local (left) and systemic (right) leaves of wild-type (Col; top) and *rbohD* mutants (*rbohD*; bottom). n = 20 plants for each group (**B**) and (**C**), similar to (A), however, showing changes in 12-oxophytodienoic acid (OPDA) (B) and JA (C). n = 20 plants for each group (**D**) and (**E**). Impaired local and systemic stomatal closure response of *Arabidopsis slac1* (**D**) and *ghr1* (**E**) mutants (Col background) to local application of light stress. n = 500 stomata from 30 different plants in each group. (**F**) Impaired systemic, but not local, stomatal closure response of *Arabidopsis lox1* mutants to local application of light stress. n = 500 stomata from 30 different plants for each group. Statistical significance was determined by a one- or two-tailed Student's t test as described in (*25*). Results are presented as the means ± SE, ***$P < 0.005$, **$P < 0.01$, and *$P < 0.05$.

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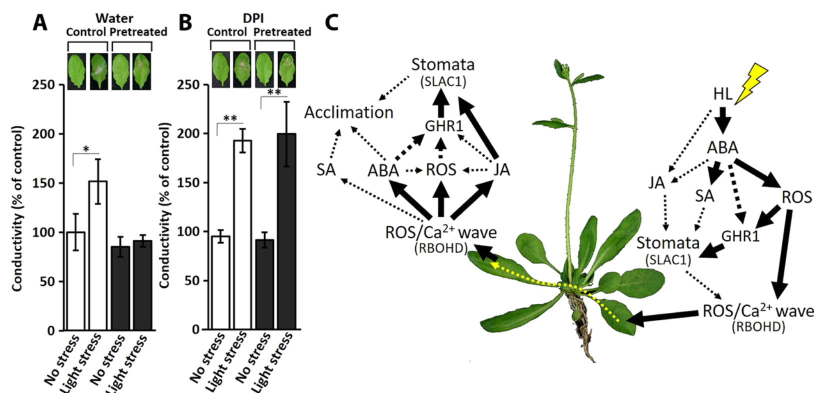

Fig. 7. Enhanced acclimation of systemic leaves is dependent on leaf-to-leaf rapid signaling, and a model for the interplay between ABA, ROS, and JA during rapid systemic stomatal responses of *Arabidopsis* to light stress. (**A** and **B**) Enhanced acclimation of systemic leaves to light stress. Plants were pretreated for 10 min with light stress on a local leaf (pretreated). Control plants were untreated (control). After 15 min, the systemic leaves of the treated or untreated plants were subjected to light stress (light stress) for 60 min and sampled for ion leakage measurements and imaging. Control plants were not given the 60-min light stress (no stress). Water (A) or DPI (50 μM) (B) was applied in agar at the midpoint between the local and systemic leaves (as in Fig.2A). Statistical significance was determined by a one- or two-tailed Student's *t* test as described in (*25*). Results are presented as the means ± SE (for each time point *n* = 30 different plants in each group), $^{**}P < 0.01$, $^{*}P < 0.05$. (**C**) A proposed model for the rapid systemic stomatal closure response of *Arabidopsis* to local application of light stress. The triggering of stomatal responses, accumulation of SA and H_2O_2, and the initiation of the ROS/Ca^{2+} wave in local leaves requires ABA. The closure of stomata in systemic leaves requires the ROS/Ca^{2+} wave and an interplay between JA, ABA, and ROS. The stomatal responses of both systemic and local leaves require GHR1 and SLAC1. Solid arrows represent tested interactions, and dashed arrows represent hypothetical interactions that require further studies.

in ABA-independent stomatal regulation pathways (*37*). *slac1* (Fig. 6D) and *ghr1* (Fig. 6E) mutants were impaired in their local and systemic stomatal closure response to light stress. In contrast, *lox-1* (Fig. 6F) mutants were impaired in their systemic, but not local, stomatal closure responses. These findings demonstrate a key role for GHR1 and SLAC1 in regulating rapid local and systemic stomatal responses and highlight a possible involvement of JA in regulating systemic stomatal responses to light stress.

The rapid leaf-to-leaf signaling pathway promotes systemic acclimation to light stress

To determine the physiological importance of the systemic leaf-to-leaf signal for plant acclimation to light stress, we tested light stress–induced injury in systemic leaves 10 min after the activation of the systemic signal by a local application of light stress. This assay was performed with plants that had water or DPI applied to the midpoint between the local and systemic leaves (similar to Fig. 2A). Ion leakage, which is a measure of leaf injury (*32*), was significantly higher in the systemic leaves of plants exposed to light stress than in those of plants in which the local leaves were not pretreated with light (Fig. 7A; compare the white bars). In contrast, no injury was detected in light-stressed systemic leaves of plants in which the local leaf was pretreated with light stress for 10 min (Fig. 7A; compare the black bars). The application of the ROS/Ca^{2+} wave inhibitor DPI at the midpoint between the local and systemic leaves (similar to Fig. 2A) suppressed this acclimation process, providing further evidence that it required a ROS wave–dependent systemic signal (Fig. 7B).

DISCUSSION

Our study uncovered a rapid signaling pathway that coordinated the response of different leaves within the same plant to light stress. Thus, each leaf within the plant could act as an autonomous unit that sends and/or receives a light stress–induced signal that affects its acclimation, as well as that of other nonstressed leaves. A sudden increase in light intensity perceived by a particular leaf could therefore mediate a canopy-wide stomatal response that will prepare distant leaves for the possibility of being subjected to an intense light stress event (Fig. 1). Our study further demonstrated that the rapid systemic signal that mediates this leaf-to-leaf communication response depended on RBOHD and the ROS/Ca^{2+} wave (Fig. 2), and on the presence of the plant hormone ABA in the local leaf for its initiation (Figs. 3 to 6). Although the role of ABA in triggering this response was not entirely clear, ABA appeared to be required for H_2O_2 accumulation and the initiation of the ROS/Ca^{2+} wave in the local leaf (Figs. 3 to 6). This role of ABA is in agreement with previous studies that showed enhanced ABA accumulation during light stress in *Arabidopsis* (*3*) and a requirement for ABA in triggering the accumulation of different light stress response transcripts (*4*, *19*).

Although ABA appears to play a key role in triggering the light stress–induced rapid systemic stomatal closure response (Figs. 3 to 6), it may not be directly involved in signaling for stomatal closure in systemic leaves (Figs. 4F, 5, and 6F, and fig. S4). Thus, additional hormones such as JA and/or H_2O_2 may be involved in mediating systemic stomatal responses (Figs. 5 and 6). Some stomatal closure pathways are activated in response to biotic stimuli to prevent bacterial pathogens from entering the leaf (*36*, *37*). Many of these pathways depend on H_2O_2 and converge with the canonical ABA stomatal closure pathway upstream of SLAC1 (*36*–*39*). The rapid local and systemic stomatal responses of *Arabidopsis* to light stress described in this work (Fig. 1) depended on SLAC1 (Fig. 6D) and GHR1 (Fig. 6E). The stomatal closure responses in the local or systemic leaves of *Arabidopsis* differed in their dependency on LOX1, which is involved in JA signaling (Fig. 6F) (*37*). This finding could indicate that two different pathways are involved in the stomatal closure responses described in this work: one in local leaves, which is JA-independent, and the other in systemic leaves, which is JA-dependent (Fig. 6F). This finding highlights the complex spatial and temporal hormonal signaling networks that are likely to mediate local and systemic stomatal responses in plants.

We propose that ROS accumulation and the activation of the RBOHD-dependent ROS/Ca^{2+} wave in local leaves in response to light stress are ABA-dependent (Fig. 7C). The accumulation of ROS in local leaves could subsequently trigger a SLAC1-dependent stomatal closure through GHR1. Alternatively, the accumulation of ABA in the local leaf could trigger stomatal closure through ABI2 and GHR1 (*38*). We further propose that once the RBOHD-dependent ROS/Ca^{2+} wave reaches the systemic leaf, it triggers ABA and JA accumulation and SLAC1-dependent stomatal closure through the function of GHR1 and, potentially, JA (Fig. 7C). Further studies are needed to examine this model and the role of ABI2, SA, and other plant hormones such as nitric oxide and auxin in these pathways. In addition, because alterations in ABA biosynthesis are also linked to nonphotochemical quenching and antioxidant processes (*40*), the light stress systemic

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signal may also be initiated in response to changes in nonphotochemical quenching. Because both biotic (flagellin 22–induced) and abiotic (light stress–induced) systemic signaling pathways require the ROS/Ca^{2+} wave (Fig. 2) (*12*, *22*, *26*) and involve JA and SA signaling (Figs. 5 and 6 and fig. S5) (*36*–*39*), it is possible that the ROS/Ca^{2+} wave is a functional linkage used by the plant in different biotic-abiotic interactions or overlapping signal transduction pathways.

The rate of the stomata and acclimation responses to light stress described in this work (Figs. 1 and 7A) is much faster than many previously reported systemic responses to pathogens or abiotic stresses (*6*–*10*, *41*–*43*). Our results showing that plants can rapidly coordinate the stomatal responses of different leaves within their canopy and acclimate to light stress (Figs. 1 and 7A) therefore shed new light on plants as being highly coordinated and dynamic multicellular organisms.

MATERIALS AND METHODS

Plant material and growth conditions

A. thaliana Col-0 (cv. Columbia-0), *rbohD*, *aba2-1*, *aba3-1*, *slac1* (*slac1-3*, *SALK_099139*; *slac1-4*, *SALK_137265*), *ghr1* (*ghr1-1*, *SALK_031493C*; *ghr1-2*, *SALK_033702C*), and *lox1* (*lox1-1*, *SALK_059431C*; *lox1-2*, *SALK_000058C*) mutants (*12*, *21*, *22*, *36*, *38*), and Ler-0 (cv. Landsberg erecta), *aba1-1*, and *abi1-1* mutants (*35*) were grown in peat pellets (Jiffy-7, Jiffy; http://jiffygroup.com/en/) at 23°C under short day growth conditions (8-hour light/16-hour dark; 50 μmol m^{-2} s^{-1}). Col plants expressing the *Zat12*:: or *WRKY40::Luciferase* reporter construct were grown and imaged with a NightOWL LB983 NC100 (Berthold, https://berthold.com/) imager as described in (*22*, *32*). All plants were grown under highly controlled conditions without the application of any chemical or treatment that could induce a stress or pathogen response before conducting the experiments. All experiments were conducted with a minimum of 6 to 10 different plants per technical repeat, time point, treatment, or genetic background. Because of the use of half a leaf for metabolic analyses, many more plants were used per technical repeat in experiments involving measurements of hormones or H_2O_2. All experiments were conducted in three biological repeats, each with three technical repeats (for a minimal total of 24 to 30 different plants per time point). All plants used for this study were grown in a random mixed-plot design to prevent position effects and other growth and sample size effects. Data acquisition and data analysis were separated and conducted by different individuals to prevent bias.

Light stress and ABA application

Plants were grown in cookies for 30 to 45 days under short day conditions (50 μmol m^{-2} s^{-1}). Local leaves were exposed to light stress (2000 μmol m^{-2} s^{-1}) at 21°C for periods of 0, 1, 5, and 10 min using a fiber-optic light source (ACE I; Schott) as described in (*12*) or sprayed with ABA (50 μM) as described in (*35*). Light stress was applied to a circular area (about 0.75 cm in diameter) located at the tip of a mature fully expanded rosette leaf. Leaves were immediately sampled and divided along their mid vain. Half of each leaf was used for measuring stomatal aperture as described below (*35*, *44*), and the other half was flash-frozen in liquid nitrogen and used for H_2O_2, quantitative real-time polymerase chain reaction (qRT-PCR), ABA, SA, and JA analysis as described in (*12*, *22*, *32*, *35*). The entire sampling process (from leaf detachment to initial stomata imprint and frozen material) was conducted within 10 to 15 s. For humidity experiments, plants were placed in a closed chamber (51 cm × 25 cm × 11 cm; length × width × height, respectively) connected to a cool mist humidifier (GUL540D1V1) for 30 min. This treatment kept the plants at a relative humidity of 80 to 85% for the entire incubation time.

Stomatal aperture

Stomatal aperture was measured as described in (*35*, *44*). Briefly, local and systemic leaves were cut, and their lower surface was immediately stuck to a microspore slide with a medical adhesive (Hollister). After 1 to 2 min, the leaf was peeled away under distilled water. The lower epidermis imprint stuck to the glass was then visualized under the microscope, and stomatal images were recorded. Measurements of stomatal aperture were performed using the imaging software ImageJ, version 6. At least 500 different stomata were measured from 10 different plants for each time point, treatment, or genetic background.

Leaf thermal imaging

Thermal imaging of plants was performed as described in (*21*), with a few modifications. In brief, 4- to 5-week-old plants growing under short day conditions in low light (50 μmol m^{-2} s^{-1}) were placed under the thermal imaging camera at a distance of about 30 cm. After an equilibration period of 20 to 30 min, light stress was applied to a local leaf as described above, and thermal images were captured using a FLIR A655SC infrared camera (FLIR Systems), from local and systemic leaves with a defined region of interest (that did not overlap with the lighted area on the treated leaf), at various time points. Images were saved on a computer and were analyzed using ResearchIR software. One image was captured every 10 s, and a mean of six images from six different leaves was calculated for each experimental time point per technical repeat.

Inhibitor studies

To inhibit the propagation of the ROS/Ca^{2+} wave from the local to the systemic leaf, a drop of 0.3% agarose-containing water, 50 μM DPI, or 2 mM lanthanum chloride ($LaCl_3$) was placed at the midpoint between the local and systemic leaf for 15 min. Local tissue was then subjected to light stress or ABA application, and stomatal responses and H_2O_2 accumulation were measured in the local and systemic leaves.

Electrolyte leakage, qRT-PCR, SA, ABA, JA, and H_2O_2 measurements

Electrolyte leakage was measured as described in (*32*). qRT-PCR was performed and quantified as described in (*12*) using a StepOnePlus Real-Time PCR System (Applied Biosystems, http://appliedbiosystems.com/). The following primers were used: *Zat12*, TGGGAAGAGAGTG-GCTTGTTT (forward) and TAAACTGTTCTTCCAAGCTCCA (reverse); and *EF1-α*, GAGCCCAAGTTTTTGAAGA (forward) and CTAA-CAGCGAAACGTCCCA (reverse). Concentrations of ABA, SA, and JA in local and systemic leaves were measured using ultraperformance liquid chromatography as described in (*35*). The accumulation of H_2O_2 in local and systemic tissues was measured using Amplex Red (Molecular Probes, Invitrogen, http://invitrogen.com/) as described in (*32*).

Statistical analysis

Statistical significance was determined by a one- or two-tailed Student's *t* test as described in (*25*). Results are presented as the means ± SE (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$).

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SUPPLEMENTARY MATERIALS

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Fig. S1. The light stress–induced stomatal closure response of *Arabidopsis*.
Fig. S2. An increase in leaf temperature accompanies the stomatal closure response of local and systemic leaves to light stress applied to the local leaf.
Fig. S3. ABA application to a local leaf triggers the ROS wave in local and systemic leaves.
Fig. S4. Leaf temperature measurements of wild-type Ler and *aba1-1* and *abi1-1* mutants subjected to light stress applied to the local leaf.
Fig. S5. Transient accumulation of SA in local and systemic leaves of *Arabidopsis* plants subjected to local application of light stress.

REFERENCES AND NOTES

1. J. Kromdijk, K. Glowacka, L. Leonelli, S. T. Gabilly, M. Iwai, K. K. Niyogi, S. P. Long, Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science* **354**, 857–861 (2016).
2. Z. Li, S. Wakao, B. B. Fischer, K. K. Niyogi, Sensing and responding to excess light. *Annu. Rev. Plant Biol.* **60**, 239–260 (2009).
3. G. Galvez-Valdivieso, M. J. Fryer, T. Lawson, K. Slattery, W. Truman, N. Smirnoff, T. Asami, W. J. Davies, A. M. Jones, N. R. Baker, P. M. Mullineaux, The high light response in *Arabidopsis* involves ABA signaling between vascular and bundle sheath cells. *Plant Cell* **21**, 2143–2162 (2009).
4. M. Gorecka, R. Alvarez-Fernandez, K. Slattery, L. McAusland, P. A. Davey, S. Karpinski, T. Lawson, P. M. Mullineaux, Abscisic acid signalling determines susceptibility of bundle sheath cells to photoinhibition in high light-exposed *Arabidopsis* leaves. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **369**, 20130234 (2014).
5. K.-J. Dietz, Efficient high light acclimation involves rapid processes at multiple mechanistic levels. *J. Exp. Bot.* **66**, 2401–2414 (2015).
6. S. Karpinski, H. Reynolds, B. Karpinska, G. Wingsle, G. Creissen, P. Mullineaux, Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. *Science* **284**, 654–657 (1999).
7. Z. Guo, F. Wang, X. Xiang, G. J. Ahammed, M. Wang, E. Onac, J. Zhou, X. Xia, K. Shi, X. Yin, K. Chen, J. Yu, C. H. Foyer, Y. Zhou, Systemic induction of photosynthesis via illumination of the shoot apex is mediated sequentially by phytochrome B, auxin and hydrogen peroxide in tomato. *Plant Physiol.* **172**, 1259–1272 (2016).
8. J. B. Rossel, P. B. Wilson, D. Hussain, N. S. Woo, M. J. Gordon, O. P. Mewett, K. A. Howell, J. Whelan, K. Kazan, B. J. Pogson, Systemic and intracellular responses to photooxidative stress in *Arabidopsis*. *Plant Cell* **19**, 4091–4110 (2007).
9. M. J. Gordon, M. Carmody, V. Albrecht, B. Pogson, Systemic and local responses to repeated HL stress-induced retrograde signaling in *Arabidopsis*. *Front. Plant Sci.* **3**, 303 (2012).
10. M. Carmody, P. A. Crisp, S. d'Alessandro, D. Ganguly, M. Gordon, M. Havaux, V. Albrecht-Borth, B. J. Pogson, Uncoupling high light responses from singlet oxygen retrograde signaling and spatial-temporal systemic acquired acclimation. *Plant Physiol.* **171**, 1734–1749 (2016).
11. R. Mittler, E. Blumwald, The roles of ROS and ABA in systemic acquired acclimation. *Plant Cell* **27**, 64–70 (2015).
12. N. Suzuki, G. Miller, C. Salazar, H. A. Mondal, E. Shulaev, D. F. Cortes, J. L. Shuman, X. Luo, J. Shah, K. Schlauch, V. Shulaev, R. Mittler, Temporal-spatial interaction between reactive oxygen species and abscisic acid regulates rapid systemic acclimation in plants. *Plant Cell* **25**, 3553–3569 (2013).
13. J. A. Raven, Speedy small stomata? *J. Exp. Bot.* **65**, 1415–1424 (2014).
14. E. Merilo, P. Jalakas, H. Kollist, M. Brosché, The role of ABA recycling and transporter proteins in rapid stomatal responses to reduced air humidity, elevated CO_2, and exogenous ABA. *Mol. Plant* **8**, 657–659 (2015).
15. C. Pastenes, P. Pimentel, J. Lillo, Leaf movements and photoinhibition in relation to water stress in field-grown beans. *J. Exp. Bot.* **56**, 425–433 (2005).
16. Ü. Niinemets, M. Reichstein, M. Staudt, G. Seufert, J. D. Tenhunen, Stomatal constraints may affect emission of oxygenated monoterpenoids from the foliage of *Pinus pinea*. *Plant Physiol.* **130**, 1371–1385 (2002).
17. R. Zweifel, J. P. Bohm, R. Hasler, Midday stomatal closure in Norway spruce–reactions in the upper and lower crown. *Tree Physiol.* **22**, 1125–1136 (2002).
18. C. Barta, F. Loreto, The relationship between the methyl-erythritol phosphate pathway leading to emission of volatile isoprenoids and abscisic acid content in leaves. *Plant Physiol.* **141**, 1676–1683 (2006).
19. M. J. Fryer, L. Ball, K. Oxborough, S. Karpinski, P. M. Mullineaux, N. R. Baker, Control of *Ascorbate Peroxidase* 2 expression by hydrogen peroxide and leaf water status during excess light stress reveals a functional organisation of *Arabidopsis* leaves. *Plant J.* **33**, 691–705 (2003).
20. L. Rizhsky, H. Liang, J. Shuman, V. Shulaev, S. Davletova, R. Mittler, When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. *Plant Physiol.* **134**, 1683–1696 (2004).
21. S. Merlot, A.-C. Mustilli, B. Genty, H. North, V. Lefebvre, B. Sotta, A. Vavasseur, J. Giraudat, Use of infrared thermal imaging to isolate Arabidopsis mutants defective in stomatal regulation. *Plant J.* **30**, 601–609 (2002).
22. G. Miller, K. Schlauch, R. Tam, D. Cortes, M. A. Torres, V. Shulaev, J. L. Dangl, R. Mittler, The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. *Sci. Signal.* **2**, ra45 (2009).
23. R. Mittler, S. Vanderauwera, N. Suzuki, G. Miller, V. B. Tognetti, K. Vandepoele, M. Gollery, V. Shulaev, F. Van Breusegem, ROS signaling: The new wave? *Trends Plant Sci.* **16**, 300–309 (2011).
24. S. Gilroy, N. Suzuki, G. Miller, W. G. Choi, M. Toyota, A. R. Devireddy, R. Mittler, A tidal wave of signals: Calcium and ROS at the forefront of rapid systemic signaling. *Trends Plant Sci.* **19**, 623–630 (2014).
25. M. J. Evans, W.-G. Choi, S. Gilroy, R. J. Morris, A ROS-assisted calcium wave dependent on the AtRBOHD NADPH oxidase and TPC1 cation channel propagates the systemic response to salt stress. *Plant Physiol.* **171**, 1771–1784 (2016).
26. U. Dubiella, H. Seybold, G. Durian, E. Komander, R. Lassig, C.-P. Witte, W. X. Schulze, T. Romeis, Calcium-dependent protein kinase/NADPH oxidase activation circuit is required for rapid defense signal propagation. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 8744–8749 (2013).
27. W.-G. Choi, M. Toyota, S.-H. Kim, R. Hilleary, S. Gilroy, Salt stress-induced Ca^{2+} waves are associated with rapid, long-distance root-to-shoot signaling in plants. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 6497–6502 (2014).
28. M. M. Drerup, K. Schlucking, K. Hashimoto, P. Manishankar, L. Steinhorst, K. Kuchitsu, J. Kudla, The Calcineurin B-like calcium sensors CBL1 and CBL9 together with their interacting protein kinase CIPK26 regulate the Arabidopsis NADPH oxidase RBOHF. *Mol. Plant* **6**, 559–569 (2013).
29. S. Munemasa, F. Hauser, J. Park, R. Waadt, B. Brandt, J. I. Schroeder, Mechanisms of abscisic acid-mediated control of stomatal aperture. *Curr. Opin. Plant Biol.* **28**, 154–162 (2015).
30. S.-Y. Park, P. Fung, N. Nishimura, D. R. Jensen, H. Fujii, Y. Zhao, S. Lumba, J. Santiago, A. Rodrigues, T.-f. F. Chow, S. E. Alfred, D. Bonetta, R. Finkelstein, N. J. Provart, D. Desveaux, P. L. Rodriguez, P. McCourt, J.-K. Zhu, J. I. Schroeder, B. F. Volkman, S. R. Cutler, Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* **324**, 1068–1071 (2009).
31. B. R. Acharya, S. M. Assmann, Hormone interactions in stomatal function. *Plant Mol. Biol.* **69**, 451–462 (2009).
32. N. Suzuki, A. R. Devireddy, M. A. Inupakutika, A. Baxter, G. Miller, L. Song, E. Shulaev, R. K. Azad, V. Shulaev, R. Mittler, Ultra-fast alterations in mRNA levels uncover multiple players in light stress acclimation in plants. *Plant J.* **84**, 760–772 (2015).
33. M. Okamoto, Y. Tanaka, S. R. Abrams, Y. Kamiya, M. Seki, E. Nambara, High humidity induces abscisic acid 8′-hydroxylase in stomata and vasculature to regulate local and systemic abscisic acid responses in Arabidopsis. *Plant Physiol.* **149**, 825–834 (2009).
34. F. Armstrong, J. Leung, A. Grabov, J. Brearley, J. Giraudat, M. R. Blatt, Sensitivity to abscisic acid of guard-cell K+ channels is suppressed by abi1-1, a mutant Arabidopsis gene encoding a putative protein phosphatase. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9520–9524 (1995).
35. S. I. Zandalinas, D. Balfagón, V. Arbona, A. Gómez-Cadenas, M. A. Inupakutika, R. Mittler, ABA is required for the accumulation of APX1 and MBF1c during a combination of water deficit and heat stress. *J. Exp. Bot.* **67**, 5381–5390 (2016).
36. A. Guzel Deger, S. Scherzer, M. Nuhkat, J. Kedzierska, H. Kollist, M. Brosché, S. Unyayar, M. Boudsocq, R. Hedrich, M. R. G. Roelfsema, Guard cell SLAC1-type anion channels mediate flagellin-induced stomatal closure. *New Phytol.* **208**, 162–173 (2015).
37. J.-L. Montillet, N. Leonhardt, S. Mondy, S. Tranchimand, D. Rumeau, M. Boudsocq, A. V. Garcia, T. Douki, J. Bigeard, C. Laurière, A. Chevalier, C. Castresana, H. Hirt, An abscisic acid-independent oxylipin pathway controls stomatal closure and immune defense in *Arabidopsis*. *PLOS Biol.* **11**, e1001513 (2013).
38. D. Hua, C. Wang, J. He, H. Liao, Y. Duan, Z. Zhu, Y. Guo, Z. Chen, Z. Gong, A plasma membrane receptor kinase, GHR1, mediates abscisic acid- and hydrogen peroxide-regulated stomatal movement in *Arabidopsis*. *Plant Cell* **24**, 2546–2561 (2012).
39. H. Hõrak, M. Sierla, K. Tõldsepp, C. Wang, Y.-S. Wang, M. Nuhkat, E. Valk, P. Pechter, E. Merilo, J. Salojärvi, K. Overmyer, M. Loog, M. Brosché, J. I. Schroeder, J. Kangasjärvi, H. Kollist, A dominant mutation in the HT1 kinase uncovers roles of MAP kinases and GHR1 in CO2-induced stomatal closure. *Plant Cell* **28**, 2493–2509 (2016).
40. M. Szechyńska-Hebda, M. Lewandowska, S. Karpiński, Electrical signaling, photosynthesis and systemic acquired acclimation. *Front. Physiol.* **8**, 684 (2017).
41. S. A. Mousavi, A. Chauvin, F. Pascaud, S. Kellenberger, E. E. Farmer, *GLUTAMATE RECEPTOR-LIKE* genes mediate leaf-to-leaf wound signalling. *Nature* **500**, 422–426 (2013).
42. M. Szechynska-Hebda, J. Kruk, M. Górecka, B. Karpińska, S. Karpiński, Evidence for light wavelength-specific photoelectrophysiological signaling and memory of excess light episodes in *Arabidopsis*. *Plant Cell* **22**, 2201–2218 (2010).

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43. D. Wendehenne, Q. M. Gao, A. Kachroo, P. Kachroo, Free radical-mediated systemic immunity in plants. *Curr. Opin. Plant Biol.* **20**, 127–134 (2014).
44. R. Morillon, M. J. Chrispeels, The role of ABA and the transpiration stream in the regulation of the osmotic water permeability of leaf cells. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 14138–14143 (2001).

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Coordinating the overall stomatal response of plants: Rapid leaf-to-leaf communication during light stress

Amith R. Devireddy, Sara I. Zandalinas, Aurelio Gómez-Cadenas, Eduardo Blumwald and Ron Mittler

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Sending a canopy-wide message

A stress experienced by one part of a plant can be transmitted to other parts of the plant not directly exposed to the stress. Although not all leaves in the canopy of a plant may be exposed to light at the same time, it is beneficial to coordinate the closure of pores in leaves called stomata to prevent desiccation. Devireddy *et al.* showed that a wave of reactive oxygen species (ROS) and Ca^{2+} enabled leaves experiencing light stress in *Arabidopsis thaliana* plants to trigger stomatal closure in leaves not exposed to light. Stomatal closure required abscisic acid in light-stressed leaves and jasmonic acid in nonexposed leaves, hormones previously implicated in stomatal closure. This coordinated and dynamic response may enable plants to acclimate to light stress.

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Supplementary Materials for

Coordinating the overall stomatal response of plants: Rapid leaf-to-leaf communication during light stress

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This PDF file includes:

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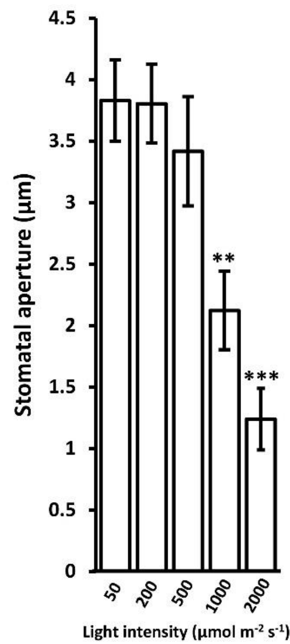


Fig. S1. The light stress–induced stomatal closure response of *Arabidopsis*. Stomatal aperture was measured in similar age and developmental stage *Arabidopsis* leaves grown under controlled conditions and subjected to different light intensities for 10 min. Plants were grown under short day conditions at 50 µmol m^{-2} sec^{-1}. Statistical significance was discriminated by a one- or two-tailed Student's t-test as described in (*25*). Results are presented as the mean ± SE (for each time point N=500 stomata from 30 different plants for each group). ***P < 0.005, **P < 0.01.

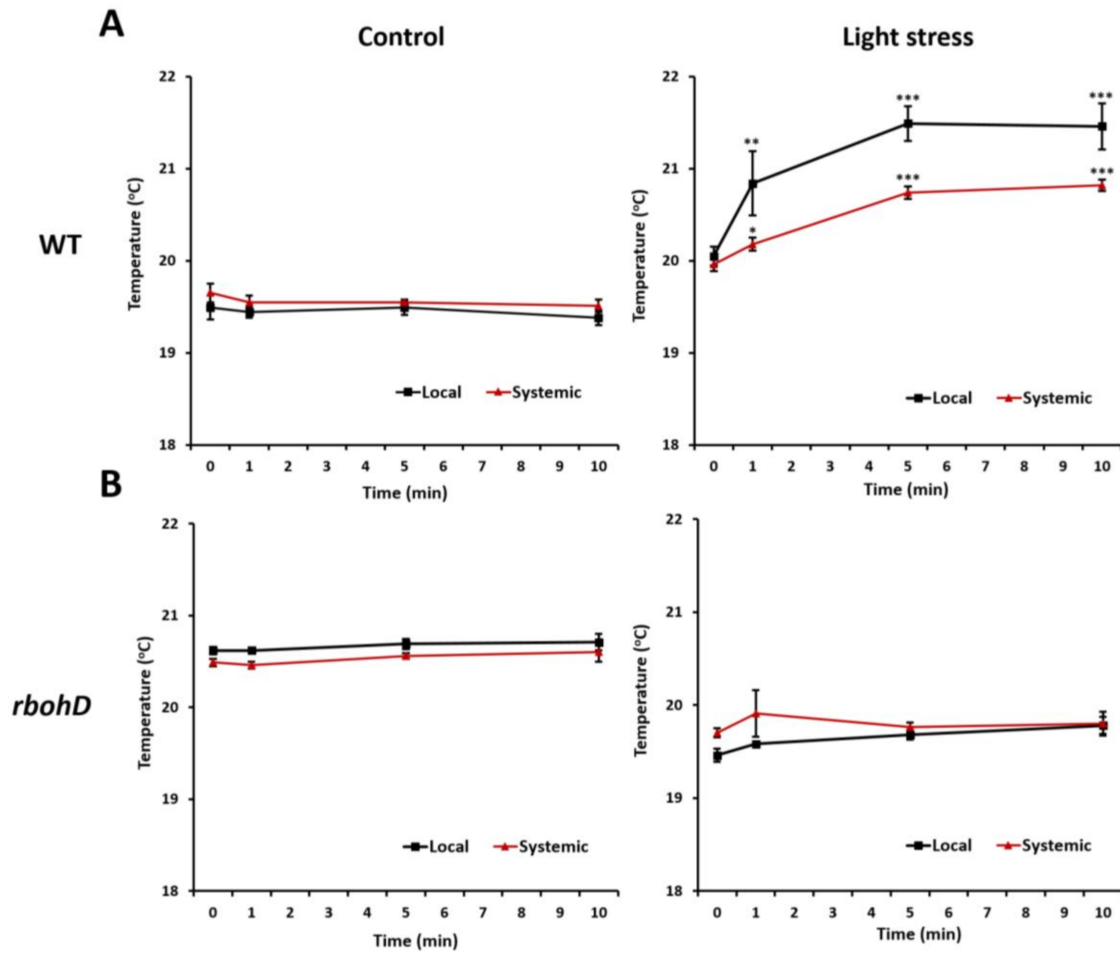
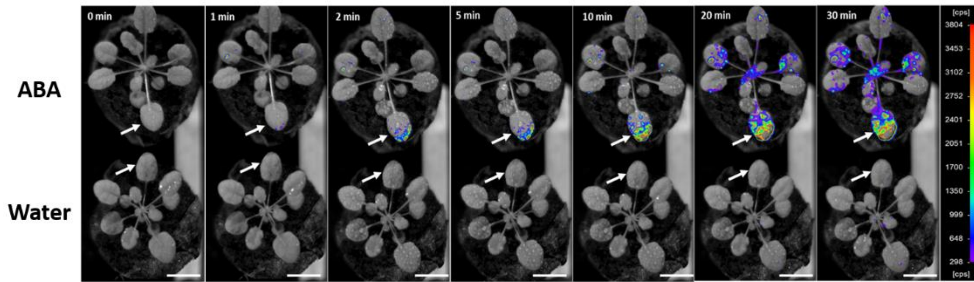


Fig. S2. An increase in leaf temperature accompanies the stomatal closure response of local and systemic leaves to light stress applied to the local leaf. (**A**) Leaf temperature of local and systemic leaves of wild type Arabidopsis plants (Col) subjected to a local light stress treatment. Left panel shows control plants and right panel shown plants subjected to a local light stress. N=18 different plants. (**B**) Leaf temperature of local and systemic leaves of Arabidopsis mutants lacking the respiratory burst oxidase homolog D enzyme (*rbohD*) subjected to a local light stress treatment. Left panel shows control plants and right panel shows plants subjected to a local light stress. N=18 different plants for each group. Statistical significance was determined by a one- or two-tailed Student's t-test as described in (*25*). Results are presented as the mean ± SE, ***P < 0.005, **P < 0.01, *P < 0.05.

A



B

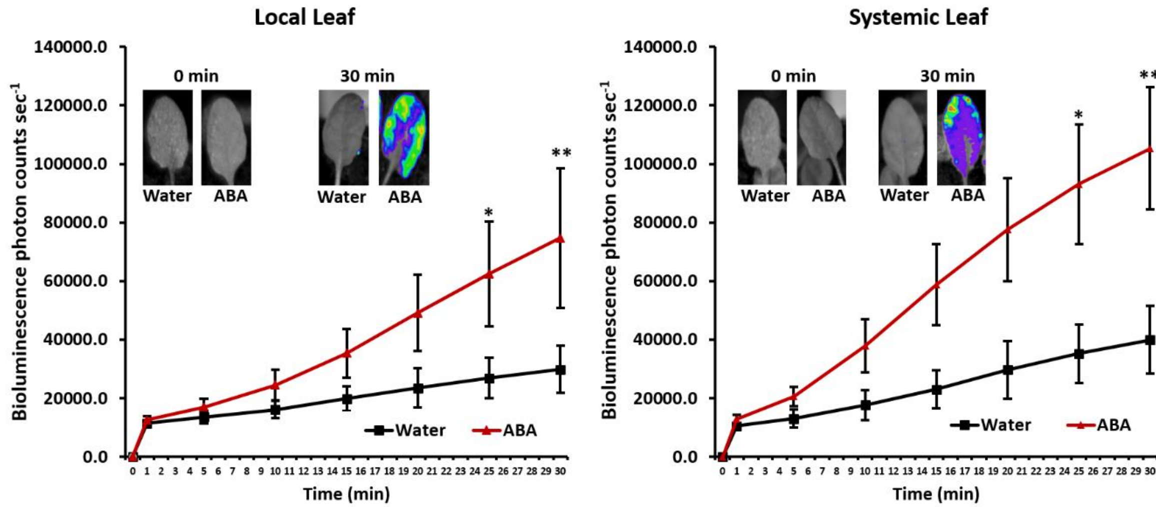


Fig. S3. ABA application to a local leaf triggers the ROS wave in local and systemic leaves. (**A**) Time-laps imaging of the *Zat12::Luciferase* reporter response in Arabidopsis plants following ABA (50 μM) application to a local leaf. (**B**) The response of *WRKY40::Luciferase* reporter plants to local application of ABA. Statistical significance was discriminated by a one- or two-tailed Student's t-test as described in (*25*). Results are presented as the mean ± SE (for each time point N=30 plants). **P < 0.01, *P < 0.05.

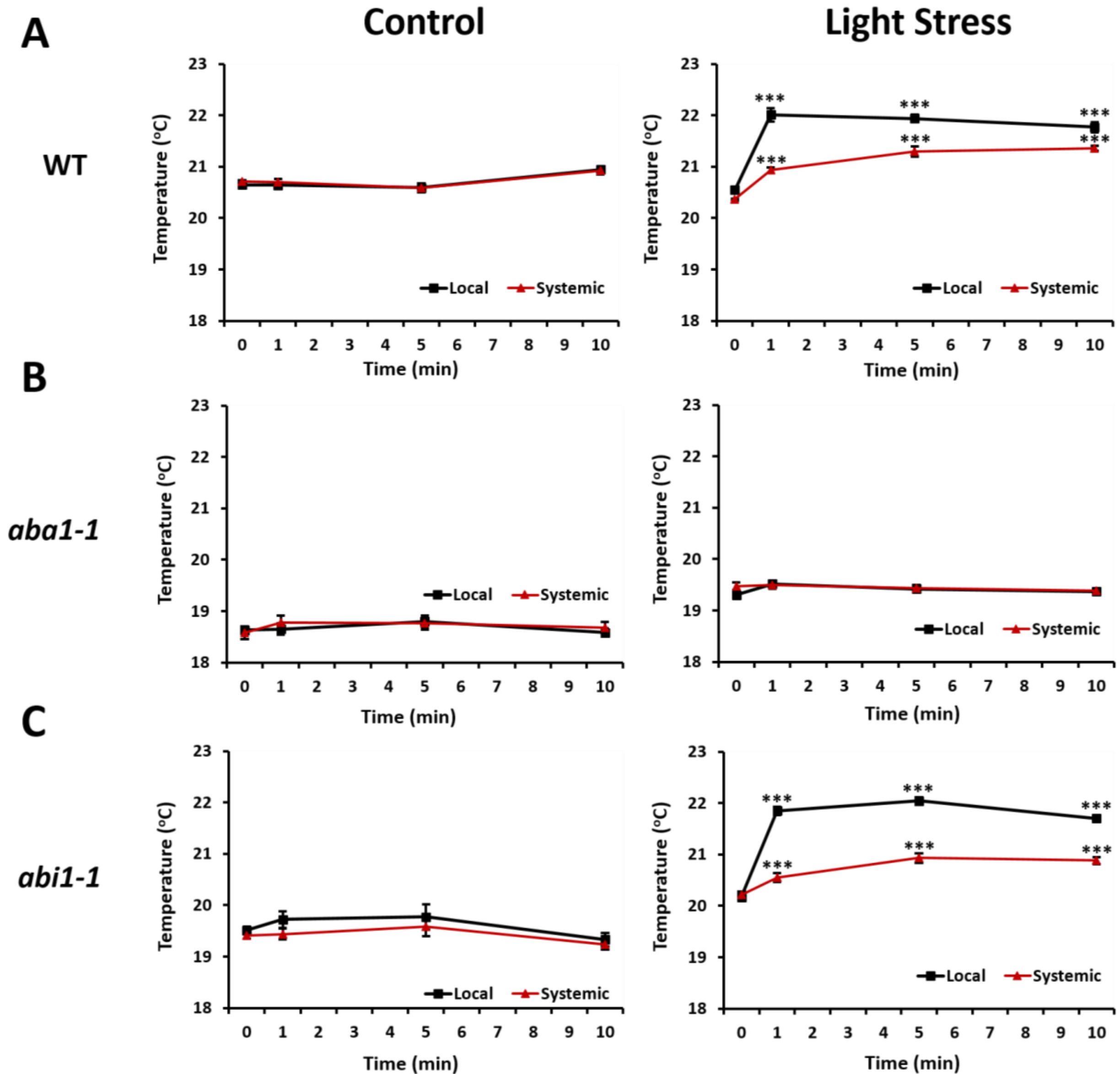


Fig. S4. Leaf temperature measurements of wild-type Ler and *aba1-1* and *abi1-1* mutants subjected to light stress applied to the local leaf. Leaf temperature of local and systemic leaves of wild type Arabidopsis (Ler) plants (**A,** N=18 different plants for each group), *aba1-1* (**B,** N=18 different plants for each group) and *abi1-1* (**C,** N=18 different plants for each group) mutants subjected to a local light stress treatment. Left panel shows control plants and right panel shows plants subjected to light stress. Statistical significance was determined by a one- or two-tailed Student's t-test as described in (*25*). Results are presented as the mean ± SE. ***P < 0.005.

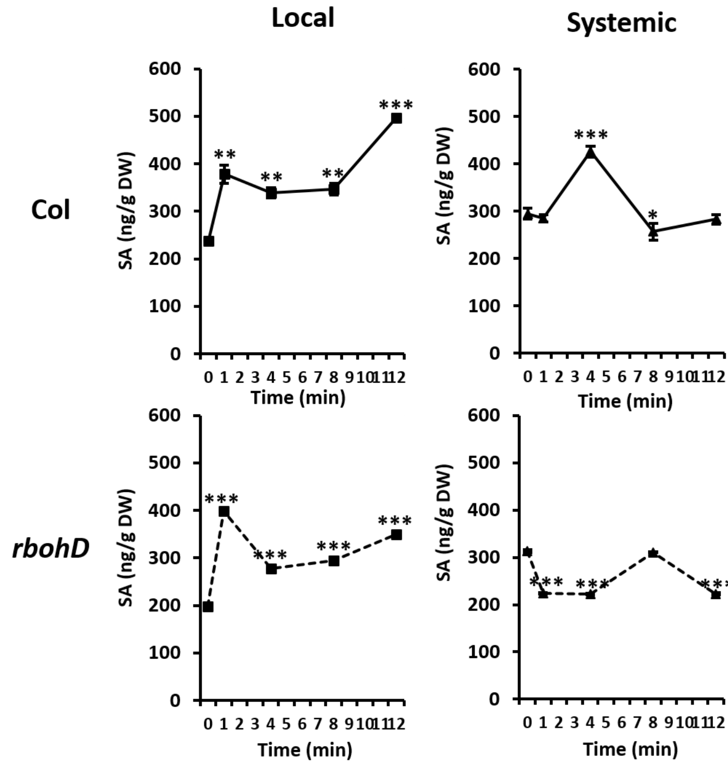


Fig. S5. Transient accumulation of SA in local and systemic leaves of *Arabidopsis* plants subjected to local application of light stress. Changes in the level of SA in local (left panels) and systemic (right panels) leaves of wild type (Col; top panels) and *rbohD* mutants (*rbohD*; bottom panels). Statistical significance was determined by a one- or two-tailed Student's t-test as described in (*25*). Results are presented as the mean ± SD (for each time point N=20 different plants), ***P < 0.005, **P < 0.01, *P < 0.05.