Hormone- and Light-Mediated Regulation of Heat-Induced Differential Petiole Growth in Arabidopsis[\textsuperscript{W}][\textsuperscript{OA}]

Martijn van Zanten, Laurentius A.C.J. Voesenek, Anton J.M. Peeters*, and Frank F. Millenaar\textsuperscript{1}

Plant Ecophysiology, Institute of Environmental Biology, Utrecht University, 3584 CH Utrecht, The Netherlands

Plants react quickly and profoundly to changes in their environment. A sudden increase in temperature, for example, induces differential petiole growth-driven upward leaf movement (hyponastic growth) in Arabidopsis (Arabidopsis thaliana). We show that acclimations that face the strongest fluctuations in diurnal temperature in their natural habitat are least sensitive for heat-induced hyponastic growth. This indicates that heat-induced hyponastic growth is a trait subject to natural selection. The response is induced with kinetics remarkably similar to ethylene- and low light-induced hyponasty in several accessions. Using pharmacological assays, transcript analysis, and mutant analyses, we demonstrate that ethylene and the photoreceptor protein phytochrome B are negative regulators of heat-induced hyponastic growth and that low light, phytochrome A, auxin, polar auxin transport, and abscisic acid are positive regulators of heat-induced hyponastic growth. Furthermore, auxin, auxin polar transport, phytochrome A, phytochrome B, and cryptochromes are required for a fast induction of heat-induced hyponastic growth.

Temperature is an important environmental factor that varies over seasons but also pronouncedly during the day. Supraoptimal temperatures are among the most damaging abiotic factors in crop plants (Mittler, 2006; Barnabás et al., 2008), and minor changes in temperature can already have dramatic effects on plants, for example, on fine-tuning of several key processes in plant development such as germination (Toh et al., 2008), floral transition (Halliday et al., 2003; Balasubramanian et al., 2006), and circadian clock entrainment and compensation (Samach and Wigge, 2005; Gould et al., 2006; Penfield, 2008).

A study on the natural variation of leaf angles in Arabidopsis (Arabidopsis thaliana) accessions originating from different geographic origins revealed that leaves of accessions found at lower latitudes are more erect than those from northern accessions (Hopkins et al., 2008). A recent report by Koini and colleagues (2009) showed that a sudden increase in temperature induces an increase in leaf inclination (hyponastic growth), which is controlled by PHYTOCHROME-INTERACTING FACTOR4 (PIF4).

Hyponastic growth is also associated with shade and submergence avoidance and brings leaves toward light and air, respectively (Ballaré, 1999; Cox et al., 2003; Pierik et al., 2004; Millenaar et al., 2005, 2009). Accumulation of the gaseous hormone ethylene is the key trigger for the induction of hyponastic growth in submerged plants, including Arabidopsis (Millenaar et al., 2005). Interestingly, reduced light intensity (low light) triggers a hyponastic growth response with similar kinetics as ethylene (Millenaar et al., 2005). Both genetic and pharmacological data indicated that auxin and polar auxin transport are involved in low light-induced, but not in ethylene-induced, hyponastic growth, whereas the response to low light is ethylene independent (Millenaar et al., 2009). Based on these data, we hypothesized that ethylene and low light induce hyponastic growth via largely independent routes but probably share functional components downstream, which explains the phenotypical resemblance.

In this report, we demonstrate that a rapid temperature shift (from 20°C to 38°C) induces hyponastic growth with highly similar kinetics to ethylene- and low light-induced hyponasty. Leaf angles of naturally occurring accessions that face the strongest fluctuations in diurnal temperature in their natural habitat are least sensitive to heat, suggesting that this trait is subject to natural selection. The regulation of this response was studied by a combination of pharmacological experiments, gene expression studies, and mutant analyses. We present evidence that ethylene and phytochrome B are negative regulators and that low light, phytochrome A, auxin, and abscisic acid (ABA) are positive regulators of heat-induced hyponastic growth.

\textsuperscript{1} Present address: De Ruiter Seeds, Leeuwenhoekweg 52, 2660 BB Bergschenhoek, The Netherlands.

* Corresponding author; e-mail a.j.m.peeters@uu.nl.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Anton J.M. Peeters (a.j.m.peeters@uu.nl).

\textsuperscript{W} The online version of this article contains Web-only data.

\textsuperscript{OA} Open Access articles can be viewed online without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.109.144386
RESULTS

Heat-Induced Leaf Inclination in Arabidopsis

To characterize the response of Arabidopsis petioles to elevated temperatures in detail, petiole angles were measured after 7 h of exposure to different temperatures, using the standard accessions Columbia-0 (Col-0), Landsberg erecta (Ler), and Wassilewskija-2 (Ws-2). A positive correlation for all accessions was found in the temperature range from approximately 16°C to approximately 38°C, demonstrating that Arabidopsis petioles actively react with a differential growth-driven upward leaf movement (heat-induced hyponastic growth) to changes in environmental temperature (Fig. 1A). Below 16°C, none of the tested accessions changed petiole angles, and above approximately 30°C, the response of Col-0 and Ler reached a plateau whereas Ws-2 leaf angles continued to increase.

From this experiment, it is not conclusive if petiole angles adjust to the absolute temperature, to the relative change in temperature, or to both. Therefore, petiole angles of Col-0 plants that were pregrown for 3 weeks at 10°C were measured. In agreement with the results of Hopkins et al. (2008), this cold (vernalization) pretreatment induced a significant increase (P < 0.001) in initial petiole angle (35.9° ± 0.6°) relative to plants grown at 20°C (25.8° ± 0.4°). The dose-response curve was similar regardless of the pregrowth temperature, but cold-pregrown plants lost the ability of heat-induced hyponasty at temperatures exceeding approximately 32°C (Fig. 1A). Moreover, as cold-pregrown plants showed higher initial angles, the absolute angles of cold-pregrown plants were significantly higher (P < 0.001) than in Col-0 grown at 20°C (Fig. 1B). These results seem to suggest that the degree of heat-induced hyponastic growth is determined by absolute temperature. Regarding the responses of all tested accessions, a shift from 20°C to 38°C induced a strong hyponastic growth response, and this is used throughout the experiments.

A time-lapse camera setup (Cox et al., 2003; Millenaar et al., 2005) was employed to monitor the kinetics of heat-induced hyponastic growth. In Col-0, the response starts within 1 h, the angle change per unit of time is maximal after approximately 3 h, and the maximal angle is reached around 7 h after start of the treatment (Fig. 1C). Heat-induced hyponastic growth is irreversible once induced, since placing the plants back to the control temperature after 3 or 6 h did not alter the maximum response angle (amplitude) at 7 h. However, plants placed back at the control temperature were less able to maintain high leaf angles after the response maximum throughout the experimental period, which resulted in a faster decline of leaf angles after reaching the maximum response.

Figure 1. Characterization of the hyponastic growth response to different temperatures. A, Dose-response relation of temperature and petiole angles after 7 h of treatment with different temperatures relative to the initial angle. Symbols are as follows: 20°C-grown (solid lines) Ws-2 (circles), Col-0 (diamonds), and Ler (triangles) and 10°C-pregrown Col-0 (squares, dashed line). Angles were pairwise subtracted, which gives the difference between the angles of treated and control plants at each time point (Benschop et al., 2007). B, Absolute (initial) petiole angles of Col-0 pregrown at 20°C (gray bars) or at 10°C (black bars) after 7 h of treatment at the indicated temperatures. C, Col-0 petiole angle kinetics upon exposure to heat (38°C) for 24 h (triangles) and of reversal from 38°C to 20°C after 3 h (circles) and 6 h (squares). Depicted angles are pairwise subtracted. Error bars represent SE; n > 10. *** P < 0.001.
The Amplitude of Heat-Induced Hypostatic Growth Correlates with the Natural Diurnal Temperature Range of Arabidopsis Accessions

To compare heat-induced hypostatic growth with ethylene- and low light-induced hypostatic growth (Millenaar et al., 2005, 2009), we exposed five frequently used accessions to these three signals (Fig. 2). Strong similarities in responses were found in Col-0, Ler, and C24 (Fig. 2, A–C). Elevated temperature induced a much stronger response than exposure to ethylene or low light in Ws-2 (Fig. 2D). Finally, Cape Verde Islands-0 (Cvi-0) lacked a marked response to heat, ethylene, and low light relative to the air-control treatment (Fig. 2E).

To directly compare heat-induced hypostatic growth among the five accessions, a pairwise subtraction was performed to correct for diurnal and/or circadian petiole movements (Benschop et al., 2007; Fig. 2F). Cvi-0, which originates from near the equator, is a weak responder, whereas C24, originating from the Portuguese accession Coimbra (Schmid et al., 2006),

Figure 2. Natural variation in heat-induced hypostatic growth. A to E, Effects of exposure to air control (20°C; gray circles), heat (20°C to 38°C; black triangles), ethylene (5 μL L⁻¹; solid lines), and low light (from 200 to 20 μmol m⁻² s⁻¹; dashed-dotted lines) on absolute petiole angles (in degrees) of Col-0 (A), Ler (B), C24 (C), Ws-2 (D), and Cvi-0 (E). Note that the angle at time 0 reflects the initial petiole angle of the accessions prior to treatment. F, Pairwise subtracted angles of heat-induced hypostatic growth of the five accessions from A to E. G, Response amplitude, which is the difference in absolute angles at time 0 (initial petiole angle) and the absolute angle after 7 h of heat treatment of 21 Arabidopsis accessions. H, Pearson correlation ($r^2$) between response angle change values from G and diurnal temperature range at the geographical origin of the accessions. The connecting line results from linear regression. Error bars represent se; $n = 10$. 

van Zanten et al. 2009 Plant Physiol. 151: 1448-1457

Copyright © 2009 American Society of Plant Biologists. All rights reserved.
has an intermediate response. On the contrary, central European accessions (Ler, Col-0, and Ws-2) showed strong responses. These data suggest a correlation between geographic origin and amplitude of heat-induced hyponastic growth. To test this, we measured the absolute leaf angle increase to 7 h of heat treatment in 21 selected accessions, together roughly covering the biogeographical latitudinal distribution range of Arabidopsis (Fig. 2G; Supplemental Table S1). Considerable variation was observed in the amplitude of the response after 7 h of treatment (less than 5° for Pak-1 to approximately 30° in Be-0). No significant correlations between the amplitude of heat-induced hyponastic growth with latitude, longitude, or altitude were found (Supplemental Fig. S1), although average temperature itself was strongly negatively correlated to latitude ($r^2 = -0.76$).

Subsequently, we analyzed if local environmental conditions at the collection sites correlated with the amplitude of heat-induced hyponastic growth (Supplemental Table S2). For this aim, mean annual climate data acquired over a 30-year period were examined (New et al., 1999; Supplemental Table S2). A small but significant negative correlation ($P = 0.032$, $r^2 = -0.22$) was observed between maximum response angles at 7 h after induction and diurnal temperature range (i.e. the differences between maximum and minimum temperature during the day; Fig. 2H). This might indicate that plants from a location where daily temperatures are highly fluctuating respond less to heat than plants from relatively temperature stable environments.

**Ethylene Is a Negative Regulator of Heat-Induced Hyponastic Growth**

Because ethylene-induced hyponastic growth phenocopies heat-induced hyponastic growth (Fig. 2, A–C), we hypothesized that ethylene may be a downstream component of heat-induced hyponasty. To test this, we first measured ethylene release upon heat treatment in Col-0. In the first hours after treatment, ethylene release tended to decrease relative to the ethylene release during control temperatures, and this became significant ($P < 0.05$) 3 to 6 h after the start of the heat treatment (Fig. 3A).

Transcription of the ethylene biosynthesis gene 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID OXIDASE4 (ACO4) and the ethylene receptor ETHYLENE RESPONSE SENSOR2 (ERS2) increases in the presence of ethylene (Wilkinson et al., 1995; Hua and Meyerowitz, 1998; Vrijezen et al., 1999). We tested the expression of these ethylene marker genes in plants subjected to heat. Quantitative reverse transcription (RT)-PCR analysis (Fig. 3, B and C) did not reveal increases in transcript levels of these ethylene marker genes, whereas the heat-inducible positive control marker (Busch et al., 2005) HEAT SHOCK TRANSCRIPTION FACTOR A2 (HSFA2) was strongly up-regulated (Fig. 3D).

As a third approach to test ethylene involvement in heat-induced hyponasty, ethylene-related mutants were assayed. The ethylene-insensitive lines ethylene insensitive4 (ein4) and ethylene response1 (etr1) showed enhanced heat-induced hyponastic growth (Fig. 3, E and F). Enhancement was not observed in ein2 (Fig. 3G). A mutant with constitutive ethylene response, constitutive triple response1 (ctr1), the triple loss-of-function mutant with a strong constitutive ethylene response phenotype, etr1 ein4 etr2, and ethylene over-producer1 (eto1; 2.6× more ethylene release than in the wild type) all had marked decreases in amplitude of the response to heat treatment (Fig. 3, H–J). Consistent with a role for ethylene as negative modulator in heat-induced hyponastic growth, pretreatment with the ethylene receptor antagonist 1-methylcyclopropene (1-MCP) resulted in an enhanced hyponastic growth response to heat (Fig. 3K). Finally, application of ethylene and heat simultaneously resulted in a reduced hyponastic growth response compared with heat treatment alone. Particularly, these plants were less able to maintain high leaf angles after the response maximum (Fig. 3K).

Overall, these data consistently indicate that ethylene is a negative regulator of heat-induced hyponastic growth.

**Low Light Enhances Heat-Induced Hyponastic Growth**

Plant responses to high temperature and low red to far-red (R:FR) ratio, mimicking natural canopy signals, are highly similar (Gray et al., 1998; Halliday et al., 2003; Halliday and Whitelam, 2003; Penfield, 2008). This is perhaps best supported by Koini et al. (2009), who showed that responses to high temperature are mediated by PIF4. Complementing low R:FR/spectral shade, we tested here for possible interactions between spectral neutral low light and heat signals in hyponastic growth. We applied low light and heat simultaneously to Col-0 and Ler. In both accessions, the combined treatment (heat + low light) resulted in a strong enhancement of hyponastic growth amplitude as compared with plants subjected to either treatment alone (Fig. 4, A and B).

The roles of various photoreceptors in heat-induced hyponastic growth were studied with photoreceptor mutants in the Ler genetic background. The phytochrome A (phyA) mutant showed delayed induction of the response but exhibited a wild-type amplitude after 6 h (Fig. 4C). Interestingly, the response of phyB was delayed, but we observed enhanced petiole angles compared with the wild type for the remainder of the experimental period (up to 24 h; Fig. 4D). The phyA phyB double mutant was initially delayed but was indistinguishable from the wild type after 24 h (Fig. 4E). Thus, phyA appears to antagonize the enhanced response of phyB in heat-induced hyponastic growth. To further test if PhyB is a negative regulator of heat-induced hyponastic growth, we assayed phyB9 in Col-0. Interestingly, this mutant had an enhanced response to
heat throughout the 24-h experimental period (Fig. 4F). The response of the leaky chromophore-deficient mutant long hypocotyl2/genome uncoupled3, which has reduced levels of all five phytochromes (phyA to phyE), was similar to that of the phyA phyB double mutant (Fig. 4G). This suggests that phyC and phyD do not play prominent roles in the response, although their involvement cannot be completely ruled out.

Heat-induced hyponastic growth was slightly delayed in the cryptochrome1 cryptochrome2 (cry1 cry2) double mutant (Fig. 4H) compared with the wild-type (wt) response. K. Effect of exposure to heat on Col-0 petiole angles in the presence of ethylene (5 μL L⁻¹; black circles) or 1-MCP (gray triangles) compared with plants in heat (solid line) or ethylene (dashed-dotted line). Angles resulted from pairwise subtraction. Error bars represent s; n > 10.

In summary, our results demonstrate that PhyA, PhyB, and Cry photoreceptor proteins are required for proper induction of heat-induced hyponastic growth in Ler. PhyB act as a negative regulator of the response amplitude in both the Col-0 and Ler backgrounds, and loss-of-function phyA rescues this effect, at least in Ler. Notably, the heat-induced hyponastic growth response of phyB in both Col-0 and Ler mimicked the response of the combination treatment of heat and low light in the respective wild types.

The contrasting effects of ethylene and low light on heat-induced hyponastic growth allowed studying prioritization of the signals. The enhanced hyponastic growth response as the result of simultaneous application of heat and low light could be repressed by additional ethylene in Col-0 and Ler (Fig. 5, A and B). In agreement, the combination of heat plus ethylene repressed the enhanced amplitude of heat-treated phyB in both Col-0 and Ler to a level similar to a single ethylene treatment (Fig. 5, C and D). Similarly, the ethylene overproducer eto1-1 (Fig. 5F) and the consti-
tutive ethylene signaling mutant ctr1 (Fig. 5G) lacked any additive effect of simultaneous application of low light and heat. Together, these data suggest that ethylene is a dominant negative signal with respect to heat-induced hyponastic growth.

Auxin Signaling and Polar Auxin Transport Are Required for Heat-Induced Hyponastic Growth

Auxin and polar auxin transport (PAT) are required for low light-induced hyponastic growth in Arabidopsis (Vandenbussche et al., 2003; Millenaar et al., 2009), and auxin plays a role in submergence-induced hyponasty in Rumex palustris (Cox et al., 2004). Moreover, auxin has been associated with responses to high temperature (Gray et al., 1998; Koini et al., 2009).

To test the involvement of auxin in heat-induced hyponasty, we pharmacologically inhibited the auxin efflux carriers with 2,3,5-triiodobenzoic acid (TIBA) and naphthylphthalamic acid (NPA). These treatments reduced and abolished heat-induced hyponasty, respectively (Fig. 6A). This indicates that auxin and PAT are required for heat-induced hyponastic growth. We examined the expression of the auxin activity marker IAA19/MSG2 (Tatematsu et al., 2004) by quantitative RT-PCR. IAA19/MSG2 mRNA levels did not change dramatically (less than two times for each time point), although expression was enhanced 3 to 6 h after initiation of heat treatment compared with controls (Fig. 6B), suggesting that our heat treatment might enhance auxin activity.

Auxin is perceived by the F-box protein TRANS-PORT INHIBITOR RESPONSE1 (TIR1) and AUXIN SIGNALING F-BOX (AFB) proteins (Dharmasiri et al., 2005a, 2005b; Kepinski and Leyser 2005). The tir1 mutant showed a delayed response and a slightly reduced amplitude (Fig. 6C), whereas the quadruple auxin receptor mutant tir1 afb1 afb2 afb3 exhibited a similar delay but also a strongly reduced hyponastic growth response amplitude (Fig. 6D). This suggests that redundancy exists among the TIR/AFB proteins in heat signaling with respect to hyponastic growth. In agreement with the pharmacological data, auxin efflux carrier mutants pin-formed7 (pin7) and pin3 showed delayed and abolished responses, respectively (Fig. 6, E and F).

Together, these data suggest that auxin signaling and PAT are required for a fast induction and maximum amplitude of heat-induced hyponastic growth.

ABA Is a Positive Regulator of Heat-Induced Hyponastic Growth

ABA is a negative regulator of ethylene-induced hyponastic growth in R. palustris and Arabidopsis (Cox et al., 2004; Benschop et al., 2007). Application of exogenous ABA did not change the hyponastic growth response of the petiole to heat (Fig. 7A). Application of
the ABA biosynthesis inhibitor fluoridon repressed the amplitude of the response. This suggests that ABA is a positive regulator of heat-induced hyponastic growth and that endogenous ABA levels may saturate the response.

ABA-INSENSITIVE1 (ABI1) is negative regulator of ABA signaling (Leung et al., 1997; Hoth et al., 2002). We found that ABI expression was modestly enhanced (approximately two times at 3 and 6 h) under heat treatment (Fig. 7B), suggesting that heat may desensitize ABA signaling by enhanced ABI1 expression. In agreement, the constitutive ABA-hypersensitive era1-2 mutant showed enhanced heat-induced hyponastic growth response (Fig. 7C), and several ABA biosynthesis and ABA-insensitive mutants (Fig. 7, D and G–I) showed repressed and delayed heat-induced hyponasty. In contrast, aba2 (Fig. 7E) exhibited a wild-type response, and the amplitude of aba3 (Fig. 7F) was slightly enhanced. This apparent contradiction may be explained by the observation that specifically these two ABA-related mutants have increased levels of ethylene release (LeNoble et al., 2004), which may interfere with heat-induced hyponastic growth (Fig. 3). In accordance, we found a strongly significant (P < 0.001) increase in ethylene release in aba2-1 and aba3-1 (0.63 ± 0.7 and 0.62 ± 0.05 nL g⁻¹ fresh mass [FM] h⁻¹, respectively, compared with 0.36 ± 0.02 nL g⁻¹ FM h⁻¹ in wild-type Col-0) in the adult vegetative tissues that were used in this study, whereas all other tested ABA-related mutants did not have significant differences in ethylene release (data not shown). Overall, our results indicate that ABA and ABA signaling are positive regulators of heat-induced hyponastic growth.

**DISCUSSION**

Heat-Induced Hyponastic Growth as an Adaptive Response

Petiole hyponasty is a resource-directed reorientation of plant organs to escape from diminished growth conditions. The response is associated with escape from complete submergence and reduced light intensities as well as canopy signals (Cox et al., 2003; Millenaar et al., 2005; Mullen et al., 2006). Here, we studied the kinetics of elevated temperature-induced hyponastic growth in Arabidopsis and showed that petiole angles reversibly adjust, in a dose-dependent manner, to changes in their growth temperature in a range of physiologically relevant temperatures. This is in agreement with Millenaar et al. (2005), who found a modest increase in leaf angle when plants were transferred from 20°C to 30°C, and with the observations of Koini et al. (2009). Notably, the strongest response was observed at approximately 38°C, after which the responses attenuated. This is in accordance with Millenaar et al. (2005), who found a modest increase in leaf angle when plants were transferred from 20°C to 30°C, and with the observations of Koini et al. (2009). Notably, the strongest response was observed at approximately 38°C, after which the responses attenuated. This is in accordance with results of McCabe and Leaver (2000), who showed that a temperature of 38°C is just sublethal for Arabidopsis plants in which no acquired thermotolerance was induced before.

Strong natural variation was observed for the amplitude of the heat-induced hyponastic growth response among natural accessions (Fig. 2). A latitudinal cline in initial petiole angles was described previously (Hopkins et al., 2008). Yet, the observed high-temperature response amplitude was not correlated with latitude. However, heat response amplitude negatively correlated to diurnal temperature range at the accession...
collection sites. Thus, accessions that on average face large daily temperature fluctuations in their natural habitat showed the weakest hyponastic growth response to heat. Tempering hyponastic growth may be adaptive to save resources in a strong fluctuating daily environment, while responding to temperature changes in an otherwise stable temperature environment may potentially bring fitness benefits. Leaf (re)positioning can be explained by functional arguments that steep leaf angles have efficient solar light capture in the morning and winter, whereas it may optimize photosynthesis by avoiding overirradiation, excess heat flux, and extensive water loss at midday and during summer (King, 1997; Falster and Westoby, 2003). In agreement, Fu and Ehleringer (1989) showed that heliotropic leaf movements in common bean (*Phaseolus vulgaris*) are controlled by air temperature and that leaves are positioned such that photosynthesis is close to optimal. Moreover, a positive correlation between leaf angles and air temperature has been observed in *P. vulgaris* and *Phaseolus acutifolius* (Yu and Berg, 1994). Analogously, Gray et al. (1998) suggested that in Arabidopsis, heat-induced growth is employed to reposition the photosynthesizing tissues away from heated soil and consequently allow better access to evaporative cooling by moving air. Thus, heat-induced hyponastic growth in Arabidopsis may also be employed for optimization of photosynthesis.

**Light Signaling Interacts with Heat in Hyponastic Growth**

Light and temperature signaling are tightly connected (Mazzella et al., 2000; Blázquez et al., 2003; Penfield, 2008; Koini et al., 2009). For example, early flowering of *phyB* mutants is repressed at low growth temperatures (16°C). In contrast, *PhyA*, *PhyD*, and *PhyE*, acquire a more prominent functional role at 16°C (Halliday et al., 2003; Halliday and Whitelam, 2003). This cross talk is response specific, as the typical, elongated *phyB* phenotype is temperature independent (Halliday et al., 2003). Moreover, phototropic curvature of Arabidopsis seedlings was delayed by increased temperatures (Orbović and Poff, 2007).

Accordingly, our study established extensive cross talk between spectral neutral low light and heat signaling with respect to hyponastic growth. Low light intensity enhanced the amplitude of heat-induced hyponastic growth (Fig. 4), and this response was mimicked in *phyB* mutants. This is in agreement with the work of Larkindale and Knight (2002), who showed that heat-induced oxidative damage in Arabidopsis was prevented in the dark. This indicates that oxidative damage to membranes resulted from photoinhibition of the electron transport chain. Strikingly, a similar inhibition of the photosynthesis-related electron transport chain using 3-(3,4-dichlorophenyl)-1,1-dimethylurea induced hyponastic growth in normal...
Figure 7. Role of ABA in heat-induced hyponastic growth. A, Effects of heat treatment on petiole angles of Col-0 treated with 20 μM ABA (gray triangles) or soil drained with 100 μM fluoridom (gray diamonds) compared with their respective mock treatments (ABA, black circles; fluoridom, black squares). B, Quantitative RT-PCR analysis of ABI1 in control plants in air (gray circles) and heat treatment (black squares). Expression values are normalized to 1 at time 0 (n > 3). C to I, Effects of heat on petiole angles of ABA-related mutants (black circles) compared with the wild-type (wt) response (solid lines). Angles resulted from pairwise subtraction. Error bars represent ±s; n > 10.

Hormonal Control of Heat-Induced Hyponastic Growth

Ethylene action protects against, and facilitates in the repair of, heat-induced oxidative damage and predominantly functions in basal thermotolerance in Arabidopsis. In this respect, ethylene-insensitive mutants were more susceptible to heat-induced oxidative damage (Larkindale and Knight, 2002; Larkindale et al., 2005). In agreement with this, our data on ethylene production and insensitive and hypersensitive/overproducing mutants, together with pharmacological data (Fig. 3), strongly suggest that ethylene is a negative regulator of heat-induced hyponastic growth. In contrast to other ethylene-insensitive mutants, ein2 did not show an enhanced hyponastic phenotype (Fig. 3). Perhaps altered ABA levels, as observed in ein2 (Ghassemian et al., 2000), interfere in an unknown manner with the response. Yet, we found that ABA is a positive modulator of heat-induced hyponastic growth (Fig. 7).

Ethylene is the pivotal trigger of submergence-induced hyponastic petiole growth in Arabidopsis and other species (Cox et al., 2003; Millenaar et al., 2005; Benschop et al., 2007), but it was not required for low light-induced hyponastic growth (Millenaar et al., 2009). The combined (triple) ethylene + low light + heat treatment repressed the cumulative effect of heat and low light on hyponastic growth (Fig. 5). The combination treatment of low light and ethylene did
not result in an altered hyponastic growth response (Millenaar et al., 2005). Therefore, we conclude that the inhibitory effect of ethylene in the triple combination treatment (heat + low light + ethylene; Fig. 5) is due to repression of specifically heat signaling components rather than low light-specific components.

Altogether, the data suggest that ethylene is a general antagonist of heat effects in Arabidopsis. However, temperature increase (20°C–29°C) induced hypocotyl elongation independent of ethylene action (Gray et al., 1998). This might indicate that the specific regulation of heat-induced hyponastic growth in hypocotyls differs from the regulation in vegetative adult plants (this study) and seedlings (Larkindale and Knight, 2002; Larkindale et al., 2005) or, alternatively, that ethylene is not required per se in the response to relatively mild temperature increases used by Gray et al. (1998).

Remarkably few studies have described the role of auxin in temperature-related processes, whereas the roles of auxin and polar auxin transport are relatively well understood in differential growth responses, including hyponasty (Vandenbussche et al., 2003; Cox et al., 2004; Millenaar et al., 2009). Enhanced levels of free auxin have been observed in high temperature-treated Arabidopsis hypocotyls (Gray et al., 1998). Furthermore, auxin was required for high temperature-induced hypocotyl elongation. This is in agreement with our pharmacological and mutant analyses showing that auxin is required for heat-induced hyponastic growth. IAA19/MSG2 marker gene expression in petiole tissue tended to increase during 38°C heat treatment, although differences were small. This is consistent with the observations made by Gray et al. (1998) and Koini et al. (2009), who showed increased expression of the auxin-responsive genes IAA4 and IAA29 in hypocotyls upon heat treatment (29°C and 28°C, respectively) and suggested that heat may sensitize the petioles to auxin.

The auxin-mediated regulation of heat-induced hyponastic growth shows remarkable parallels with low light-induced hyponastic growth that was also attenuated by TIBA and abolished by NPA treatment (Millenaar et al., 2009). Moreover, similar to heat, a full induction of low light-induced hyponasty required AFB1, AFB2, AFB3, TIR1, PIN3, and PIN7 (Millenaar et al., 2009).

ABA is involved in the induction of acquired thermotolerance in bromegrass (Bromus inermis), maize (Zea mays), creeping bentgrass (Agrostis stolonifera), and Arabidopsis (Larkindale and Huang, 1994; Robertson et al., 1994; Gong et al., 1998; Larkindale and Knight, 2002). Moreover, heat stimulates ABA synthesis in Arabidopsis seeds, which prevents germination at nonoptimal temperatures (Toh et al., 2008). In contrast, ABA does not seem to be involved in high temperature-induced hypocotyl elongation (Gray et al., 1998).

ABA is a negative regulator of ethylene-induced hyponastic growth in Arabidopsis (Benschop et al., 2007) and submergence-induced hyponasty in R. palustris (Cox et al., 2004). In sharp contrast, our data showed that ABA is a positive regulator of heat-induced hyponastic growth (Fig. 7). However, not all tested ABA-related mutants exhibited reduced heat-induced hyponastic growth. aba2-1 and aba3-1 showed normal, or even slightly exaggerated, heat-induced responses, which might be due to their leaky nature (Léon-Kloosterziel et al., 1996). Nonetheless, these lines also showed enhanced ethylene release (Le Noble et al., 2004). Ethylene is a negative regulator of heat-induced hyponastic growth (Fig. 3), but may explain why aba2 and aba3 had a close to wild-type/exaggerated response, in an unknown manner. In agreement, aba2 and aba3 showed only moderate effects on acquired and basal thermotolerance relative to abi1-1 and abi2-1 (Larkindale et al., 2005). However, the same was true for aba1 and abi3 lines that did show a markedly reduced heat-induced hyponastic growth response. Additionally, aba2-1 and aba-1 were affected in ethylene-induced hyponastic growth (Benschop et al., 2007).

Vice versa, aba1-1 and abi2-1 did not show an altered response to ethylene but did show a clearly reduced amplitude upon heat treatment, and aba1-1, abi1-1, abi3-1, and abi2-1 exhibited decreased hyponastic responses to heat and were unaffected (aba1-1 and abi2-1) or enhanced (abi1-1 and abi2-1) in ethylene (Benschop et al., 2007). Overall, these data suggest that the net effects of ABA on ethylene- and heat-induced hyponastic growth are opposite and that the ABA-related genetic components employed in the regulation of the response differ between the treatments.

ABI1 is a negative regulator of ABA signaling and ethylene-induced hyponastic growth (Benschop et al., 2007). ABI expression was enhanced by ethylene (De Paepe et al., 2004; Benschop et al., 2007) and ABA (Leung et al., 1997; Hoth et al., 2002) application. During heat treatment, ABI1 acts as a positive regulator of hyponastic growth. Yet, ABI1 transcription was enhanced during heat treatment (Fig. 7B). This suggests that heat modulates (desensitizes) petioles for hyponastic growth partly via transcriptional regulation of ABI1.

CONCLUSION

Regulation of hyponastic growth is complex, and different environmental stimuli are integrated in the control of this differential petiole growth response in Arabidopsis. We demonstrated that ethylene act as dominant negative regulator, and auxin and ABA as positive regulators, of heat-induced hyponastic growth. Heat and low light act additively on hyponastic growth. Ethylene-induced hyponastic growth is independent from auxin signaling (Millenaar et al., 2009), and ABA is a negative regulator of ethylene-induced hyponasty (Benschop et al., 2007). In contrast, low light-induced hyponasty depends on auxin and is independent of ethylene signaling (Millenaar et al., 2009).
2009). Thus, factors required for heat-induced hyponastic growth are both overlapping and different from low light signaling toward hyponastic growth, and the same might be true for factors controlling ethylene-induced hyponastic growth.

Nevertheless, the similarities in kinetics suggest that the signaling routes may converge and affect a similar set of functional components (Millenaar et al., 2009). Recently, Koini et al. (2009) demonstrated that heat-induced hyponasty was abolished in the pif4 mutant. PIF4 is required for induction of shade-avoidance phenotypes. Active PhyB physically interacts with PIF4 and targets the protein for degradation and subsequently inhibits cell elongation (Huq and Quail, 2002; Lorrain et al., 2008). Accordingly, phyB constitutive shade-avoidance phenotypes are attenuated in phyB pif4 double mutants. Heat induces a transient increase in PIF4 transcript levels (Koini et al., 2009). Together, these data facilitate a model in which PhyB-dependent low light signaling (Millenaar et al., 2009) and heat signaling toward hyponastic growth converge on PIF4. Perhaps also, cross talk of these signals with ethylene occurs via PIF proteins, as at least PIF5 affects ethylene levels (Khanna et al., 2007). Our results suggest that loss of phyA countered the enhanced hyponastic growth phenotype of phyB (Fig. 4). In agreement, phyA responses do not involve PIF4 action and PhyA may be dominant over PhyB in this response (Huq and Quail, 2002). Therefore, we cannot exclude that PIF-independent pathways (in addition) control heat-induced hyponastic growth.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis (Arabidopsis thaliana) seeds were from the Nottingham Arabidopsis Stock Centre (with accession numbers in parentheses) or were gifts of the authors who described the mutant. All accessions are described in Supplemental Table S1. Ethylene mutants are all in Col-0; etr1-1 (N8075; Kieber et al., 1993), ein2-1 (N3071; Guzman and Ecker, 1990), ein1-1 (N8053; Roman et al., 1995), eto1-1 (N3072; Guzman and Ecker, 1990), etr1-4 (Chang et al., 1993), etr1-6 ein4-2 etr3-3 (Hua and Meyerowitz, 1998). Photoreceptor mutants are in Ler, unless stated otherwise: cry1 cry2 (Hyf fla1; Yanovsky et al., 2000), bg2 (N68; Koornneef et al., 1980); Kohchi et al., 2001), phyA-201 (N6219; Nagatani et al., 1993), phyA-201 phyB-5 (N6224; Reed et al., 1994), phyB-9 in Col (Reed et al., 1993). Auxin mutants are in the Col-0 background unless stated otherwise: pin3-4 (N9063; Friml et al., 2002), pin7-1 (N9056; Friml et al., 2003), tir1-1 (N3579; Ruegger et al., 1997), tir1-1 afb1-1 afb2-1 afb3-1 in Col/Ws (Dharmasiri et al., 2005b). ABa-related mutants are in the Col or Ler background: aba2-1 (N156; Leon-Kloosterziel et al., 1996), aba1-3 (N157; Leon-Kloosterziel et al., 1996), egl-2 (Cutler et al., 1996), aba1-1 (N21; Koornneef et al., 1982), aba1-2 (N22; Koornneef et al., 1984; Leung et al., 1994), aba1-1 (N23; Koornneef et al., 1984; Leung et al., 1997), aba1-3 (N24; Koornneef et al., 1982).

Plants were grown on a fertilized mixture of potting soil and perlite (1:2, v/v) as described by Millenaar et al. (2005) at 20°C. Geographic parameters of the collection sites of individual accessions were extracted from the Climate Baseline Data of the Intergovernmental Panel on Climate Change (IPCC).-1 (photosynthetic active radiation) by covering the plants with specially neutral cloth, which did not influence light quality as checked with a LICOR-1800 spectroradiometer (LI-COR).

Ethylene was applied in continuous flow-through (75 L h

Pharmacological Treatments

Gaseous 1-MCP (1 μL L

Computerized Image Analysis of Angle Kinetics and Calculations

Hyponastic growth kinetics experiments were conducted using an automated time-lapse photography setup (Cox et al., 2003; Millenaar et al., 2005; Benschop et al., 2007). Plants were placed singly in glass cuvettes with petioles of study perpendicular to the camera. Leaves that were obscuring the petiole base were removed. Additionally, petioles were marked at the petiole/lamina junction with orange paint (Decofin). These preparations did not influence the responses (data not shown). All conditions were kept to the conditions in the growth chambers until the experiment started.

Photographs of two petioles per plant were taken every 10 min. To enable continuous photography, no dark period was included in the 24-h experimental period. Angles were measured between the orange point at the petiole/lamina junction and a fixed basal point of the petiole, compared with the horizontal, using the KS400 (version 3.0) software package (Carl Zeiss Vision) and a customized macro. Angles were pairwise subtracted, which gives the angle difference between treated and control plants at each time point (Benschop et al., 2007), to correct for diurnal/circadian leaf movements of control plants. New se values were calculated by taking the square root from the summation of the two squared se values. Plants used for single-time-point angle measurements were manually photographed. Angles were measured using ImageJ (http://rsb.info.nih.gov/ij). For all replicate plants, angles of two petioles were averaged prior to further analysis.

Geographic Climate Data

Geographic parameters of the collection sites of individual accessions were taken from the Natural Variation in Arabidopsis Web site (http://dbgap.versailles.inra.fr/vnat/) or from the authors describing the accessions (Supplemental Table S1). Environmental data of the collection sites (0.5° latitude × 0.5° longitude surface land area plots) of the used accessions were extracted from the Climate Baseline Data of the Intergovernmental Panel on Climate Change.
Regulation of Heat-Induced Hyponasty in Arabidopsis

Ethylene Release

Ethylene release from rosettes was measured as described by Millenaar et al. (2005). Whole rosettes of approximately 300 mg were placed in a syringe. Ethylene was allowed to accumulate in the syringe for 15 to 20 min and subsequently analyzed on a gas chromatograph (GC955; Synspec). This short time frame prevented wound-induced ethylene production, which commenced only after 25 min (data not shown).

Real-Time RT-PCR

Col-0 petioles were snap frozen in liquid nitrogen. RNA was isolated from the RNeasy extraction kit (Qiagen). Genomic DNA removal, cDNA synthesis, and real-time RT-PCR were performed as described by Millenaar et al. (2005, 2009). Real-time RT-PCR data were calculated with the comparative Ct method (Livak and Schmittgen, 2001) expressing mRNA values relative to β-Tubulin-6. Primers used are listed in Supplemental Table S3.

Supplemental Data

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

Supplemental Figure S1. Correlation between amplitude of heat-induced hyponastic growth and geographic parameters.

Supplemental Table S1. Natural variation in response to heat in Arabidopsis accessions.

Supplemental Table S2. Correlations between heat-induced hyponastic petiole growth and climate data at the collection sites of the used Arabidopsis accessions.

Supplemental Table S3. Primers for quantitative RT-PCR analysis used in this study.

Supplemental Literature S1.

ACKNOWLEDGMENTS

We thank Ronald Pierik for comments on the manuscript, Maarten Terlou for technical assistance, Ian Wright for software tools, and M. Estelle, M. Koomneef, C. Lin, G.E. Schaller, and G.C. Whitelam for sharing mutant lines.

Received July 7, 2009; accepted September 7, 2009; published September 9, 2009.

LITERATURE CITED

Balsubramanian S, Sureshkumar S, Lempe J, Weigel D (2006) Potent induction of Arabidopsis thaliana flowering by elevated growth temperature. PLoS Genet 2: e106

Ballare CL (1999) Keeping up with the neighbours: phytochrome sensing and other signalling mechanisms. Trends Plant Sci 4: 97–102

Barnabás B, Jäger K, Fehér A (2008) The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ 31: 11–38

Benschop JJ, Millenaar FF, Smeets ME, van Zanten M, Voesenek LACJ, Peeters AJM (2007) ABA antagonizes ethylene-induced hyponastic growth in Arabidopsis. Plant Physiol 143: 1013–1023

Blázquez MA, Ahn JH, Weigel DA (2003) Thermosensory pathway controlling flowering time in Arabidopsis thaliana. Nat Genet 33: 168–171

Boyes DC, Zayed AM, Ascenzi R, McCaskill AJ, Hoffman NE, Davis KR, Görlich J (2001) Growth stage-based phenotypic analysis of Arabidopsis: a model for high throughput functional genomics in plants. Plant Cell 13: 1499–1510

Busch W, Wunderlich M, Hoffich F (2005) Identification of novel heat shock factor-dependent genes and biochemical pathways in Arabidopsis thaliana. Plant J 41: 1–14

Chang C, Kwok SE, Bleeker AB, Meyerowitz EM (1993) Arabidopsis ethylene-response gene ETR1: similarity of product to two-component regulators. Science 262: 539–544

Cox MCH, Benschop JJ, Vreeburg RM, Wagemaker CAM, Moritz T, Peeters AJM, Voesenek LACJ (2004) The roles of ethylene, auxin, abscisic acid, and gibberellin in the hyponastic growth of submerged Rumex palustris petioles. Plant Physiol 136: 2948–2960

Cox MCH, Millenaar FF, de Jong van Berkel YM, Peeters AJM, Voesenek LACJ (2003) Plant movements: submergence-induced petiole elongation in Rumex palustris depends on hyponastic growth. Plant Physiol 132: 282–291

Cutler S, Ghassemian M, Bonetta D, Cooney S, McCourt PA (1996) Protein farnesyl transferase involved in abscisic acid signal transduction in Arabidopsis. Science 273: 1239–1241

De Paepe A, Vuysteke M, van Hummelen P, Zabeau M, Van der Straaten D (2004) Transcriptional profiling by cDNA-AFLP and microarray analysis reveals novel insights into the early response to ethylene in Arabidopsis. Plant J 39: 537–559

Dharmasiri N, Dharmasiri S, Estelle M (2005a) The F-box protein TIR1 is an auxin receptor. Nature 435: 441–445

Dharmasiri N, Dharmasiri S, Weijers D, Lechner E, Yamada M, Hobbie L, Ehrismann JS, Jürgens G, Estelle M (2005b) Plant development is regulated by a family of auxin receptor F box proteins. Dev Cell 9: 199–219

Falster DS, Westoby M (2003) Leaf size and angle vary widely across species: what consequences for light interception? New Phytol 158: 509–525

Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, Offringa R, Jürgens G (2003) Eflux-dependent auxin gradients establish the apical–basal axis of Arabidopsis. Nature 426: 147–153

Friml J, Wissniewska J, Benkova E, Mendgen K, Palme K (2002) Lateral relocation of auxin efflux regulator PIN3 mediates tropism in Arabidopsis. Nature 415: 806–809

Fu QA, Ehleringer JR (1989) Heliotropic leaf movements in common beans controlled by air temperature. Plant Physiol 91: 1162–1167

Ghassemian M, Nambara E, Cutler S, Kawaiwa H, Kamiya Y, McCourt P (2000) Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis. Plant Cell 12: 1117–1126

Gong M, Li YJ, Chen SZ (1998) Abscisic acid induced thermotolerance in maize seedlings is mediated by Ca2+ and associated with antioxidant systems. J Plant Physiol 153: 488–496

Gould PD, Locke JC, Larue C, Southern MM, Davis SJ, Hanano S, Moyle R, Milich R, Putterill J, Millar AJ, et al (2006) The molecular basis of temperature compensation in the Arabidopsis circadian clock. Plant Cell 18: 1177–1187

Gray WM, Ostin A, Sandberg G, Romano CP, Estelle M (1998) High temperature promotes auxin-mediated hypocotyl elongation in Arabidopsis. Proc Natl Acad Sci USA 95: 7197–7202

Guzman P, Ecker JR (1990) Exploiting the triple response of Arabidopsis to identify ethylene-related mutants. Plant Cell 2: 513–523

Halliday KJ, Salter MG, Thingnaes E, Whitelam GC (2003) Phytochrome control of flowering is temperature sensitive and correlates with expression of the floral integrator FT. Plant J 33: 875–885

Halliday KJ, Whitelam GC (2003) Changes in photoperiod or temperature alter the functional relationships between phytochromes and reveal roles for phyD and phyE. Plant Physiol 131: 1913–1920

Hoppkins R, Schmitt J, Stinchcombe JR (2008) A latitudinal cline and response to vernalization in leaf angle and morphology in Arabidopsis thaliana (Brassicaceae). New Phytol 179: 155–164

Hoth S, Morgante M, Sanchez JP, Hanafey MK, Tingey SV, Chua NH (2002) Genome-wide gene expression profiling in Arabidopsis thaliana reveals new targets of abscisic acid and largely impaired gene regulation in the abf1-1 mutant. J Cell Sci 115: 4891–4900

Hua J, Meyerowitz EM (1998) Ethylene responses are negatively regulated by a receptor gene family in Arabidopsis thaliana. Cell 94: 261–271

Huq E, Quail PH (2002) PIN4, a phytochrome-interacting bHLH factor, functions as a negative regulator of phytochrome B signaling in Arabidopsis. EMBO J 22: 2441–2450

Plant Physiol. Vol. 151, 2009
Kepinski S, Leyser O (2005) The Arabidopsis F-box protein TIR1 is an auxin receptor. Nature 435: 446–451
Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR (1993) CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the Raf family of protein kinases. Cell 72: 427–441
Khanna R, Shen Y, Marion CM, Tsuchisaka A, Theologis A, Schafer E, Quail PH (2007) The basic helix-loop-helix transcription factor FIF5 acts on ethylene biosynthesis and phytocrome signaling by distinct mechanisms. Plant Cell 19: 3915–3929
King DA (1997) The functional significance of leaf angle in Eucalyptus. Aust J Bot 45: 619–639
Kochi T, Mukougawa K, Frankenberk N, Masuda M, Yokota A, Lagarias JC (2001) The Arabidopsis HY2 gene encodes phytochromobilin synthase, a ferredoxin-dependent biliverdin reductase. Plant Cell 13: 425–436
Koini MA, Alvey L, Allen T, Tilley CA, Harberd NP, Whitelam GC, Franklin KA (2000) High temperature-mediated adaptations in plant architecture require the bHLH transcription factor PIF4. Curr Biol 10: 408–413
Koornneef M, Jorna ML, Brinkhorst-van der Swan DLC, Karssen CM (1997) The isolation of abscisic acid deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of Arabidopsis thaliana (L.). Heynh. Theor Appl Genet 91: 385–393
Koornneef M, Reutzel J, Karssen CM (1984) The isolation and characterization of abscisic acid-insensitive mutants of Arabidopsis thaliana. Physiol Plant 61: 377–383
Koornneef M, Rolf E, Spruit CJF (1980) Genetic control of light-induced hypocotyl elongation in Arabidopsis thaliana (L.) Heynh. Z Pflanzenphysiol 100: 147–160
Larkindale J, Hall JD, Knight MR, Vierling E (2005) Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiology 138: 882–897
Larkindale J, Huang BJ (1994) Thermotolerance and antioxidant systems in Agrostis stolonifera: involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide, and ethylene. J Plant Physiol 141: 405–413
Larkindale J, Knight MR (2002) Protection against heat stress-induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid. Plant Physiol 128: 682–695
Le Noble ME, Spollen WG, Sharp RE (2002) Protection against heat stress-induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid. Plant Physiol 128: 682–695
Lavenne J, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 25: 402–408
Lorraín S, Allen T, Duek PD, Whitelam GC, Fankhauser C (2008) Phytocrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. Plant J 53: 312–323
Mazzella MA, Bertero D, Casal JJ (2000) Temperature-dependent internode elongation in vegetative plants of Arabidopsis thaliana lacking phytocrome B and cryptochrome 1. Planta 210: 497–501
McCabe PF, Weaver C (2000) Programmed cell death in cell cultures. Plant Mol Biol 44: 359–368
Millenaar FF, Cox MCH, de Jong-van Berkel YEM, Wolschen RAM, Pierik R, Voesenek LACJ, Peeters AJM (2005) Ethylene-induced differential growth of petioles in Arabidopsis: analyzing natural variation, response kinetics, and regulation. Plant Physiol 137: 998–1008
Millenaar FF, van Zanten M, Cox MCH, Pierik R, Voesenek LACJ, Peeters AJM (2009) Ethylene and low light induce differential petiole growth in Arabidopsis through partly separate signaling pathways. New Phytol 184: 141–152
Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11: 15–19
Mullen JL, Weing C, Hangarter RP (2006) Shade avoidance and the regulation of leaf inclination in Arabidopsis. Plant Cell Environ 29: 1099–1106
Nagatani A, Reed JW, Chory J (1993) Isolation and initial characterization of Arabidopsis mutants that are deficient in phytocrome A. Plant Physiol 102: 269–277
New M, Huilme M, Jones P (1999) Representing twentieth-century space-time climate variability. I. Development of a 1961–90 mean monthly terrestrial climatology. J Clim 12: 829–856
Orbovic V, Poříl KL (2007) effect of temperature on growth and phototropism of Arabidopsis thaliana seedlings. J Plant Growth Regul 26: 222–228
Penfield S (2008) Temperature perception and signal transduction in plants. New Phytol 179: 615–628
Pierik R, Cuppens MLC, Voesenek LACJ, Visser EJW (2004) Interactions between ethylene and gibberellins in photytocrome-mediated shade avoidance responses in tobacco. Plant Physiol 136: 2928–2936
Reed JW, Nagatani A, Elich TD, Fagan M, Chory J (1994) Phytocrome A and phytocrome B have overlapping but distinct functions in Arabidopsis development. Plant Physiol 104: 1139–1149
Reed JW, Nagpal P, Poole DS, Furuya M, Chory J (1993) Mutations in the gene for the red/far-red light receptor phytocrome B alter cell elongation and physiological responses throughout Arabidopsis development. Plant Cell 5: 147–157
Reeved J, Ishikawa M, Gusta LV, MacKenzie SL (1994) Abscisic acid-induced heat tolerance in Brumus inermis Leyss cell-suspension cultures: Heat-stable, abscisic acid-responsive polypeptides in combination with sucrose confer enhanced thermostability. Plant Physiol 105: 181–190
Roman G, Lubarsky B, Kieber J, Rothenberg M, Ecker J (1995) Genetic analysis of ethylene signal transduction in Arabidopsis thaliana: five novel mutant loci integrated into a stress response pathway. Genetics 139: 1393–1409
Ruegger M, Dewey E, Hobbie L, Brown D, Bernasconi P, Turner J, Muday G, Estelle M (1997) Reduced naphthylphthalamic acid binding in the tir3 mutant of Arabidopsis is associated with a reduction in polar auxin transport and diverse morphological defects. Plant Cell 9: 745–757
Samach A, Wigge PA (2005) Ambient temperature perception in plants. Curr Opin Plant Biol 8: 483–486
Schmid KJ, Török O, Meyer R, Schmutz H, Hoffmann MH, Altmann T (2006) Evidence for a large-scale population structure of Arabidopsis thaliana from genome-wide single nucleotide polymorphism markers. Theor Appl Genet 112: 1104–1114
Tatematsu K, Kumagai S, Muto H, Sato A, Watahiki MK, Harper RM, Liscum E, Yamamoto KT (2004) MassHup encodes AUSA19, an auxin-regulated protein that functions together with the transcriptional activator NPR4/ARF7 to regulate differential growth responses of hypocotyl and formation of lateral roots in Arabidopsis thaliana. Plant Cell 16: 379–393
Tah S, Imamura A, Watanabe A, Nakabayashi K, Okamoto M, Jikumaru Y, Hanada A, Aso Y, Ishiyama K, Tamura N, et al. (2008) High temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in Arabidopsis seeds. Plant Physiol 146: 1368–1385
Vandenbussche F, Vriezen WH, Smalle J, Laarhoven LJ, Harren FJM, Van Der Straeten D (2003) Ethylene and auxin control the Arabidopsis response to decreased light intensity. Plant Physiology 133: 517–527
Vazquez WH, Hulzing K, Marinescu C, Voesenek LACJ (1999) 1-Aminocyclopropane-1-carboxylate oxidase activity limits ethylene biosynthesis in Rumex palustris during submergence. Plant Physiol 121: 189–195
Wilkinson JQ, Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ (1995) An ethylene-inducible component of signal transduction encoded by Never-riper. Science 270: 1807–1809
Yanovsky MJ, Mazzella MA, Casal JJ (2000) Quadruple photoreceptor mutant still keeps track of time. Curr Biol 10: 1013–1015
Yu F, Berg VS (1994) Control of paralleltropism in two Phaselos species. Plant Physiol 106: 1567–1573