



Let the sunshine in: Absciscic acid modulates shade avoidance by inducing hyponasty movement in *Arabidopsis*

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Shade avoidance is a remarkable example of the plasticity exhibited by plants in response to environmental signals. Shade-avoiding plants need to perform an array of morphogenic adjustments upon the sensing of changes in light quality. Such changes in light are perceived as a decrease in the ratio of red to far-red (R/FR) wavelengths caused, for example, by neighboring plants competing for light (Ballaré and Pierik, 2017).

One of the signature responses to low R/FR (LRFR) involves the upward repositioning of leaves to maximize light capture, known as hyponasty. Although the pivotal function of auxin in regulating LRFR-induced hyponasty has been well-studied (Michaud et al., 2017; Pantazopoulou et al., 2017), knowledge on the role of other phytohormones in this phenomenon is scarce. In this issue of *Plant Physiology*, Michaud et al. (2022) report that abscisic acid (ABA) plays a crucial role in mediating LRFR-induced hyponasty. They demonstrate that exposure to LRFR rapidly increases the biosynthesis of ABA, which is necessary for the upward movement of leaves in the proximity of competing neighbors.

By mining available genome-wide expression data in the petioles of *Arabidopsis* (*Arabidopsis thaliana*) plants growing under shade-simulating LRFR conditions, the authors found the rapid upregulation of genes encoding NINE-CIS-EPOXYCAROTENOID DIOXYGENASE (NCED), which catalyzes the rate-limiting step in ABA biosynthesis. Reverse transcription–quantitative PCR (qPCR) validated that expression of *NCED3* was upregulated in leaf blades, and expression of both *NCED3* and *NCED5* was upregulated in plant petioles after LRFR exposure. Consistent with this

observation, they further found that LRFR exposure enhances ABA accumulation in the plant.

PHYTOCHROME-INTERACTING FACTORS (PIFs), particularly PIF4, PIF5, and PIF7, are transcription factors that act as master regulators of the transcriptional landscape in response to shade conditions (Fernández-Milmanda and Ballaré, 2021). Michaud et al. found that LRFR exposure failed to induce ABA biosynthesis in a *pif4pif5pif7* triple mutant. Concurrently, the rapid induction of *NCED3* expression under LRFR was absent in *pif4pif5pif7*, indicating the potential role of PIFs in *NCED3* transcription. Using chromatin immunoprecipitation–qPCR, the authors showed that PIF4 binds directly to the promoter sequence of *NCED3* upon LRFR exposure, indicating a regulatory role of PIF4 in ABA biosynthesis in LRFR.

To establish the importance of ABA accumulation in the LRFR-induced hyponasty response, Michaud et al. tested leaf movement after LRFR exposure in plants with compromised ABA biosynthesis, like the *nced3nced5* double mutant and the *aba-deficient2* (*aba2*) mutant. The mutants showed limited ability to mount the hyponastic response upon LRFR, measured as small leaf movement after exposure to shade-mimicking conditions, whereas their leaf movements under white light (WL) (High R/FR) were similar to wild-type (WT) (Figure 1B).

To evaluate the role of ABA signaling in the hyponastic response, the authors evaluated a sextuple mutant of PYRABACTIN RESISTANCE1-LIKE family receptors (*112458*) and a quadruple mutant (*Qabi2*) for the protein phosphatases of the ABA co-receptor PHOSPHATASE 2CA, HYPERSENSITIVE TO ABA1, ABA INSENSITIVE 1 (ABI1) and

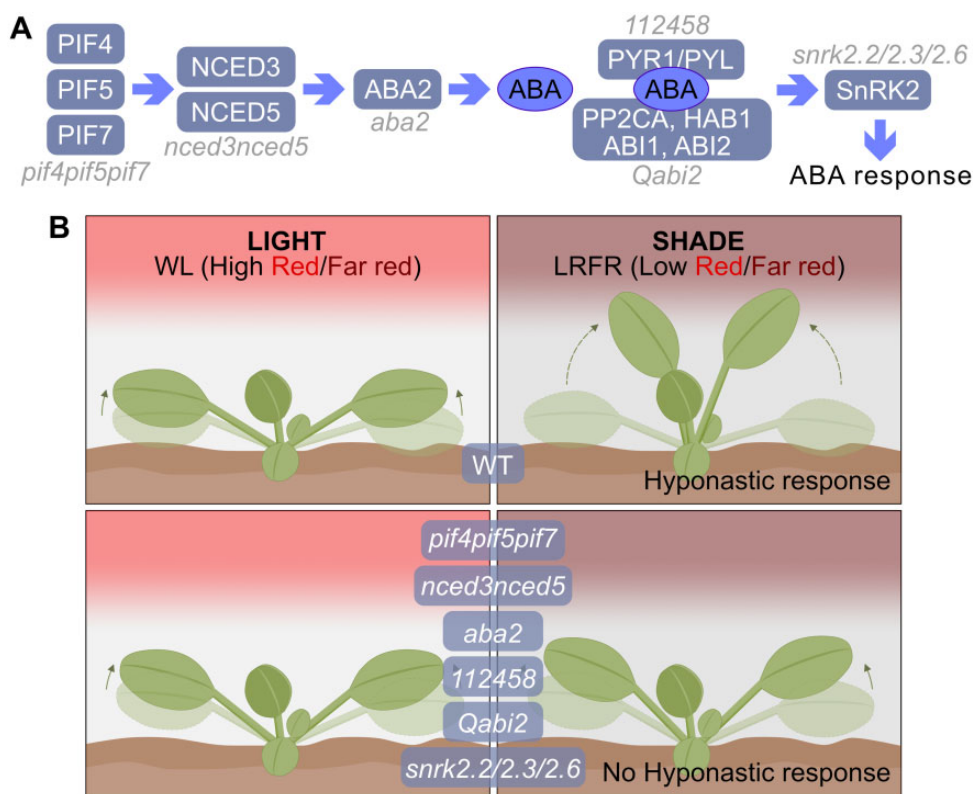


Figure 1 ABA biosynthesis and signaling are necessary for establishing the hyponastic response upon LRFR light conditions. A, PIF4/5/7 are transcription factors controlling ABA biosynthesis. In particular PIF4 binds directly to the promoter of the gene encoding NCED3, which, together with ABA DEFICIENT 2 (ABA2), is part of the ABA biosynthetic pathway. The core ABA signaling components include PYRABACTIN RESISTANCE 1-LIKE receptors of ABA, PHOSPHATASE 2CA, HYPERSENSITIVE TO ABA1, ABA INSENSITIVE 1 and 2, which are protein phosphatases of the ABA co-receptor family, and SNF1-RELATED PROTEIN KINASE kinases that activate transcription factors inducing ABA responses. Mutant lines are indicated accordingly under or over each component. B, WT plants exposed to shade (LRFR) generate a hyponastic response that move the leaves upward in search for light, a response that is highly limited in the indicated mutants for ABA biosynthesis and signaling. Figure created in Affinity Designer, version 1.10.5.

2 (ABI2) (Figure 1). Furthermore, they evaluated a triple mutant of the SNF1-RELATED PROTEIN KINASE kinases, which are targets of ABA-dependent phosphorylation (Figure 1A). Without exception, all the multiple mutants tested exhibited a limited hyponasty response phenotype after LRFR exposure, establishing a pivotal role of ABA signaling in the leaf shade-avoidance response (Figure 1B).

LRFR induces auxin production in the leaf blade, which ultimately mediates the hyponastic leaf movement (Michaud et al., 2017). Considering this, Michaud et al. (2022) compared leaf movements of WT and *aba2* after exogenous application of auxin (Indole-3-acetic acid, IAA) to their leaf tips. The *aba2* mutant exhibited a reduced hyponastic response compared to WT upon IAA application, indicating that auxin-induced leaf movements require ABA biosynthesis.

Since ABA is a key regulator of stomatal movements, the authors tested whether the increased levels of ABA under LRFR affect leaf transpiration rate. By comparing the transpiration rates and stomatal apertures under WL and LRFR conditions in WT plants, the authors found that LRFR-induced ABA accumulation does not interfere with the transpiration rate. Alternatively, the hyponastic movement was unaffected

in mutants that have altered transpiration rate. These results confirmed that ABA-dependent hyponasty under LRFR is not linked to stomatal transpiration. Through cell-type-specific disruption of ABA signaling, the authors found that a functional ABA pathway must operate in multiple cell types in the leaves for the hyponastic movement.

Historically, with few exceptions, elongation of hypocotyls and petioles have been used as the primary readout for studying the mechanisms of shade avoidance (Ballaré and Pierik, 2017). The findings from Michaud et al. (2022) substantially add to our understanding of the mechanism of altered leaf movements, which is an equally important attribute under foliar shade. Accumulating evidence emphasizes the importance of the interactions between light and ABA signaling pathways in multiple aspects of plant development (Yadukrishnan and Datta, 2021). While Michaud et al. (2022) unveil an important molecular link between LRFR signaling and ABA biosynthesis, it will be interesting to look for other possible points of cross-talk with key ABA signaling components. The existence of effective feedback mechanisms might be necessary to regulate the ABA pathway, considering the highly fluctuating nature of LRFR signals in natural environments. Another question is how the

ability of ABA to promote LRFR-induced hyponasty is influenced by abiotic stress conditions, during which ABA performs additional functions. This is particularly intriguing because ABA imparts an antagonistic effect on shade-induced hypocotyl and petiole elongation under salinity stress (Hayes et al., 2019).

To further dissect the mechanism by which ABA induces shade avoidance, it will be necessary to understand the dynamics of ABA accumulation upon LRFR treatments. Tissue grinding and solvent extractions for ABA quantification are reliable methods yet lack the resolution to visualize ABA accumulation in different cell types. Approaches using genetically encoded sensors such as ABAleon, ABACUS, or the more recent 2-in-1 GEFs (genetically encoded fluorescent indicators) (Jones et al., 2014; Waadt et al., 2014, 2020) may allow the evaluation of both the spatial and temporal dynamics of ABA accumulation on intact cells of leaf blades and petioles, for example.

Conflict of interest statement. The authors declare that there is no conflict of interest.

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