

ERECTA controls low light intensity-induced differential petiole growth independent of phytochrome B and cryptochrome 2 action in *Arabidopsis thaliana*

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Plants can respond quickly and profoundly to changes in their environment. Several species, including *Arabidopsis thaliana*, are capable of differential petiole growth driven upward leaf movement (hyponastic growth) to escape from detrimental environmental conditions. Recently, we demonstrated that the leucine-rich repeat receptor-like Ser/Thr kinase gene *ERECTA*, explains a major effect Quantitative Trait Locus (QTL) for ethylene-induced hyponastic growth in *Arabidopsis*. Here, we demonstrate that *ERECTA* controls the hyponastic growth response to low light intensity treatment in a genetic background dependent manner. Moreover, we show that *ERECTA* affects low light-induced hyponastic growth independent of Phytochrome B and Cryptochrome 2 signaling, despite that these photoreceptors are positive regulators of low light-induced hyponastic growth.

Plants must adjust growth and reproduction to adverse environmental conditions. Among the strategies that plants employ to escape from unfavorable conditions is differential petiole growth-driven upward leaf movement, called hyponastic growth. *Arabidopsis thaliana* is able to exhibit a marked hyponastic response upon flooding, which is triggered by endogenous accumulation of the gaseous phytohormone ethylene.¹ Moreover, a similar response is triggered upon low light intensity perception and in response to supra-optimal temperatures.²⁻⁵ By tilting

the leaves to a more vertical position during submergence and shading, the plants restore contact with the atmosphere and light, respectively. The kinetics of the hyponastic growth response induced by the various stimuli is remarkably similar. This led to the hypothesis that shared functional genetic components may be employed to control hyponastic growth. Yet, at least part of the signaling cascades is parallel, as the hormonal control of the response differs between the stimuli. Low light-induced hyponastic growth for example does not require ethylene action.² Whereas the response to heat is antagonized by this hormone.⁵ The abiotic stress hormone abscisic acid (ABA) antagonizes ethylene-induced hyponastic growth and stimulates heat-induced hyponastic growth.^{5,6} Moreover, ethylene-induced hyponasty does not involve auxin action⁷ whereas both heat- and low light-induced hyponasty require functional auxin signaling and transport components.^{2,5}

In our recent paper, published in *The Plant Journal*,⁸ we employed Quantitative Trait Locus (QTL) analysis to identify loci involved in the control of ethylene-induced hyponastic petiole growth. By analyzing induced mutants and by complementation analysis of naturally occurring mutant accessions, we found that the leucine-rich repeat receptor-like Ser/Thr kinase gene *ERECTA* (*ER*) is a positive regulator of ethylene-induced hyponastic growth and most likely is causal to one of the identified QTLs. In addition, we demonstrated that the ER dependency is

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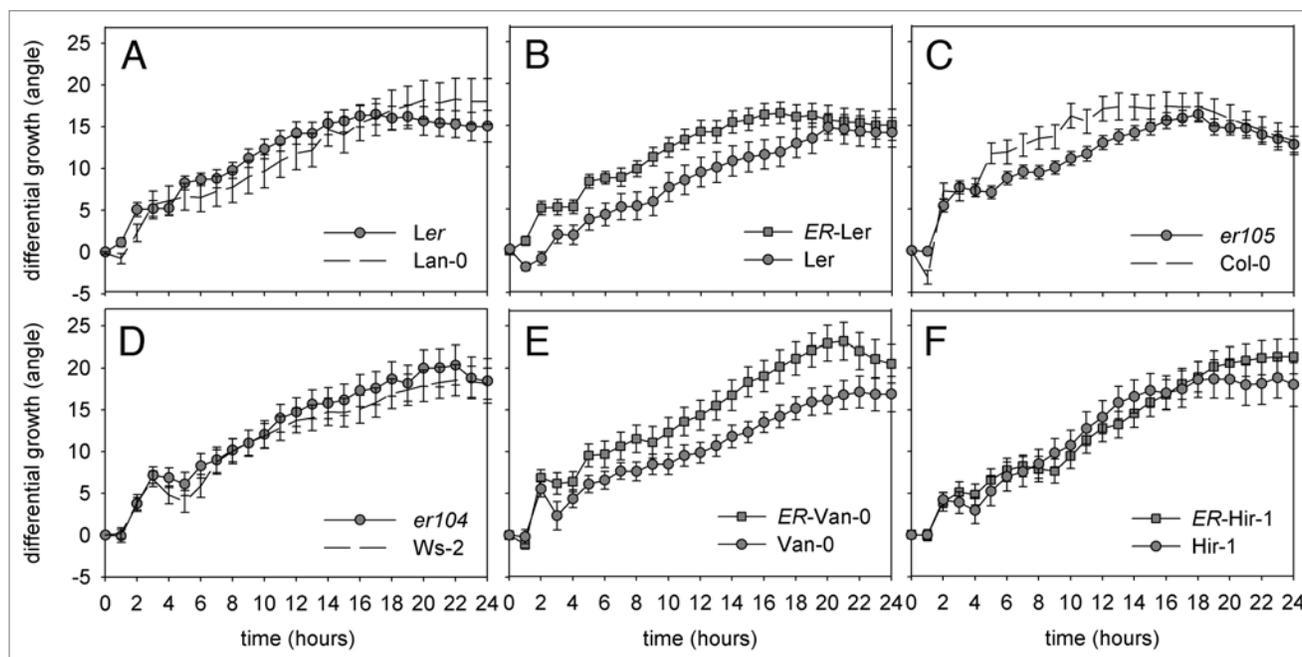


Figure 1. ERECTA involvement in low light-induced hyponasty. Effect of exposure to low light (spectral neutral reduction in light intensity from 200 to 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$) on the kinetics of hyponastic petiole growth in *Arabidopsis thaliana*. (A) mutant (circles) *Ler* and wild type (dashed line) *Lan-0*, (B) *Ler* and *Ler* complemented (*ER*-; squares) with the *Col-0* *ERECTA* allele (*ER-Ler*), (C) *er105* and *Col-0* wild type, (D) *er104* and *Ws-2* wild type, (E) natural mutant *Van-0* and *Van-0* complemented with the *Col-0* *ER* allele (*ER-Van-0*), (F) natural mutant *Hir-1* and *Hir-1* complemented with the *Col-0* *ER* allele (*ER-Hir-1*). Petiole angles were measured using time-lapse photography and subsequent image analysis. Data is pairwise subtracted, which corrects for diurnal petiole movement in control conditions. For details on this procedure, growth conditions and materials, transformation protocol, treatments, data acquisition and all analyses see.^{1,8} Error bars represent standard errors; $n \geq 12$.

not via ER mediated control of ethylene production or sensitivity.

Since low light-induced hyponasty does not require ethylene action,² ER may be part of the proposed shared signaling cascade leading to hyponastic growth where ethylene and low light signals meet. Therefore, we studied low light intensity-induced hyponasty in various *erecta* mutants. Moreover, natural occurring *er* mutant accessions complemented with a functional, *Col-0* derived, *ER* allele were tested. The response of *Lan-0* (*Lan-0*; with functional *ER*) to low light was indistinguishable from the response of Landsberg *erecta* (*Ler*) (Fig. 1A). However, complemented *Ler* (*ER-Ler*) showed an enhanced response compared to *Ler* (Fig. 1B). The response of mutant *er105* was slightly attenuated compared to the wild type Columbia-0 (Fig. 1C). Mutant *er104*, however, showed an indistinguishable hyponastic growth phenotype to low light compared to the wild type Wassilewskija-2 (*Ws-2*) (Fig. 1D). Complementation of the natural occurring *erecta* mutant accession Vancouver-0 (*Van-0*) resulted in an

enhanced hyponastic growth response to low light (Fig. 1E), whereas this was not the case for Hiroshima-1 (*Hir-1*) (Fig. 1F). Together, these data suggest that ER acts as positive regulator of low light-induced hyponastic growth and therefore may be part of the shared signaling cascade towards differential petiole growth. Yet, the effect is strongly dependent on the genetic background since the effects were not observed in every accession tested.

Phytochrome B (PhyB) and Cryptochrome 2 (Cry2) photoreceptor proteins are required for a full induction of low light-induced hyponastic growth.² We transformed the *phyb5 cry2* mutant⁹ (*Ler* genetic background) with *Col-0* derived *ER*. This complementation did not restore the ability of *phyb5 cry2* to induce hyponastic growth to neither ethylene (data not shown) nor low light conditions (Fig. 2A). Mutant *phyb5 cry2* plants have a typical constitutive shade avoidance phenotype, reflected by severely elongated organs. This includes enhanced inflorescence and silique length

and thin inflorescences (Fig. 2B–D). Complementation with *ER* resulted in a significant additional effect on these parameters (Fig. 2B–D). Together, this suggests that ER is not an integral part of PhyB nor Cry2 signaling with respect to (hyponastic) growth. Moreover, PhyB and Cry2 control of plant architecture does not require ER action. Rather, ER seems to mediate growth via genetic interaction with light-reliant growth mechanisms, instead of being downstream of photoreceptor action. Studies on the effects of ER on shade avoidance responses and various hormone responses, including cytokinin and auxin, led to the similar conclusion, suggesting a possible role for ER as a molecular hub coordinating light- and hormone-mediated plant growth.^{10,11} One could speculate that ER fine-tunes other (than light) environmental clues with light signaling components. A comparable conclusion was drawn previously for gibberellin (GA) reliant growth mechanisms, as *er* enhanced the negative effect on plant size of the *short internode* (*shi*) mutation¹² and *er* represses the positive

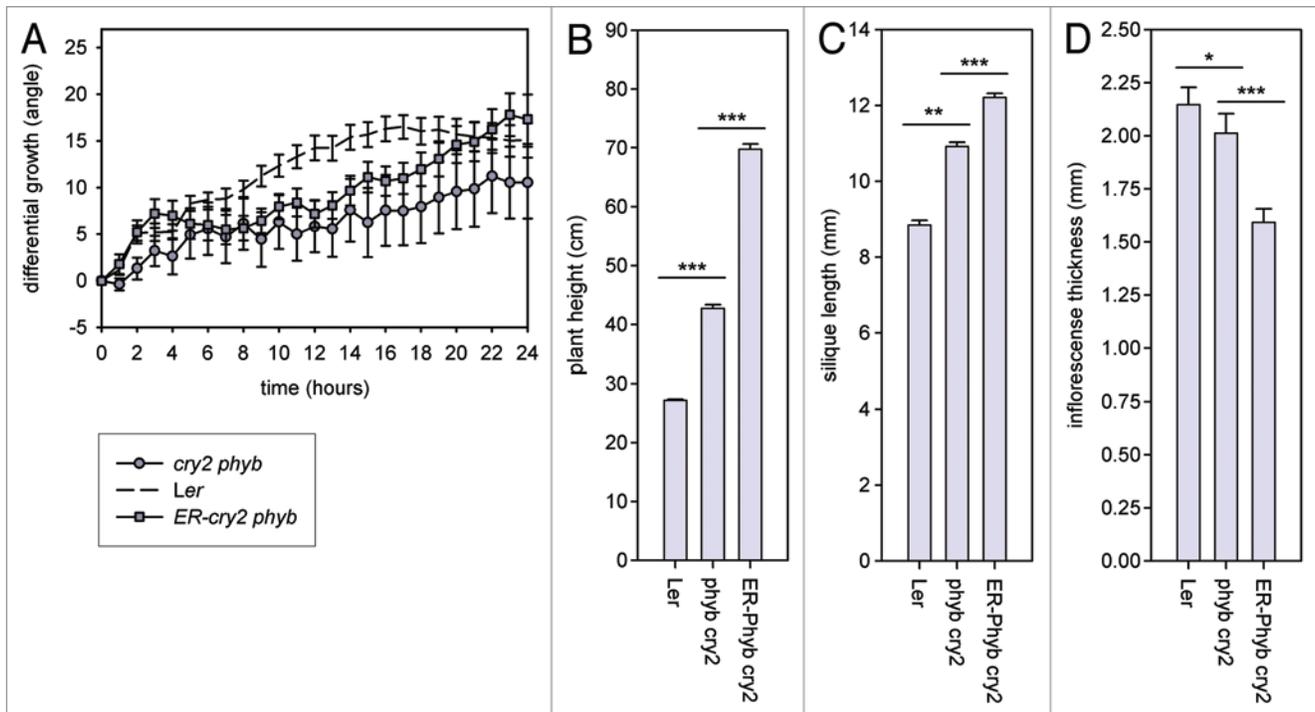


Figure 2. Effects of *ERECTA* on light signaling. (A) Effect of exposure to low light (spectral neutral reduction in light intensity from 200 to 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$) on the kinetics of hyponastic petiole growth of *Ler* (dashed lines), the photoreceptor double mutant *phyb5 cry2* (circles) and this mutant complemented with the Col-0 *ERECTA* (*ER-phyb cry2*; squares). For details see legend Figure 1. (B) Plant height, (C) silique length and (D) inflorescence stem thickness of the above mentioned lines. These parameters were measured when the last flower on the plant developed a silique. Plant height was measured from root/shoot junction to inflorescence top. Stem thickness was measured ~1 cm above the root/shoot junction with a caliper and silique lengths were measured from representative pedicels in the top ~10 cm of the main inflorescence stem. Error bars represent standard errors; $n \geq 12$. Significance levels; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = non significant, by Students t-test.

effect of the *spindly* mutation in a GA independent manner.¹³

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