

The receptor-like kinase *ERECTA* contributes to the shade-avoidance syndrome in a background-dependent manner

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- **Background and Aims** Plants growing at high densities perceive a decrease in the red to far-red (R/FR) ratio of incoming light. These changes in light quality trigger a suite of responses collectively known as the shade-avoidance syndrome (SAS) including hypocotyl and stem elongation, inhibition of branching and acceleration of flowering.
- **Methods** Quantitative trait loci (QTLs) were mapped for hypocotyl length to end-of-day far-red (EOD), a simulated shade-avoidance response, in recombinant inbred line (RIL) populations of *Arabidopsis thaliana* seedlings, derived from Landsberg *erecta* (Ler) and three accessions (Columbia, Col; Nossen, No-0; and Cape Verde Islands, Cvi-0).
- **Key Results** Five loci were identified as being responsible for the EOD response, with a positive contribution of Ler alleles on the phenotype independently of the RIL population. Quantitative complementation analysis and transgenic lines showed that *PHYB* is the candidate gene for *EODRATIO5* in the Ler × Cvi-0 RIL population, but not for two co-localized QTLs, *EODRATIO1* and *EODRATIO2* mapped in the Ler × No-0 and Ler × Col RIL populations, respectively. The *ERECTA* gene was also implicated in the SAS in a background-dependent manner. For hypocotyl length EOD response, a positive contribution of *erecta* alleles was found in Col and Van-0, but not in Ler, Cvi-0, Hir-1 or Ws. Furthermore, pleiotropic effects of *ERECTA* in the EOD response were also detected for petiole and lamina elongation, hyponastic growth, and flowering time.
- **Conclusions** The results show that the analysis of multiple mapping populations leads to a better understanding of the SAS genetic architecture. Moreover, the background- and trait-dependent contribution of *ERECTA* in the SAS suggest that its function in shaded natural environments may be relevant for some populations in different phases of plant development. It is proposed that *ERECTA* is involved in canalization processes buffering the genetic variation of the SAS against environmental light fluctuations.

Key words: *ERECTA*, shade-avoidance syndrome (SAS), natural genetic variation, phytochrome B (phyB), far-red end-of-day response, EOD response, quantitative trait loci, QTL.

INTRODUCTION

Individual plant species thrive in specific environments, some growing well across a wide area, others being restricted to survival in very narrow ecological niches. Within a niche, a species must compete for space, light and other resources with the same and other species. The shade-avoidance syndrome (SAS) is a strategy of major adaptive significance to plants (Schmitt, 1997). The SAS is highly widespread in most species growing in open habitats and depends on the ability of the plant to perceive the presence of neighbours to anticipate competition for scarce resources such as light in crowded stands (Schmitt, 1997). A plant canopy reduces the ratio of red to far-red light (R/FR) by the efficient absorption of red light by photosynthetic pigments and the relative increase of FR photons (Smith, 1982). Plants perceive this light quality change through the phytochrome system and respond very rapidly, enhancing SAS, including via vegetative structure elongation, hyponastic response and acceleration of flowering (Casal, 2012).

In terms of molecular mechanisms involved in the SAS, the reduction of R/FR in shaded environments leads to rapid

transcriptional changes principally mediated by phytochrome B (phyB). Shade light signals stabilize PIF4 and PIF5 proteins promoting elongation growth by the synthesis and redirection of auxin and favouring the degradation of DELLA proteins (Djakovic-Petrovic *et al.*, 2007; Lorrain *et al.*, 2008). PIF4 and PIF5 promote SAS by directly binding to G-boxes present in the promoter of early shade genes. To avoid an exaggerated SAS, some transcription factors such as HFR1, PAR1 and PAR2, expression of which is transcriptionally induced by shade, directly interact with PIF4 and PIF5 forming non-DNA-binding heterodimers that prevent their ability to induce the expression of elongation genes (Hornitschek *et al.*, 2009; Galstyan *et al.*, 2011). In addition, B-box-containing zinc finger transcription factors (BBX) can promote or inhibit hypocotyl growth in simulated and natural shade light (Crocco *et al.*, 2011). In low R/FR, *bbx21* and *bbx22* mutants show an exaggerated elongation response and the *bbx21bbx22* double mutant restores the SAS response in the *cop1* mutant background, indicating that these genes are also part of the feedback inhibition of shade-avoidance responses downstream of COP1 (Crocco *et al.*, 2010).

Phenotypes within most species are not fixed and show significant levels of natural genetic variation between individuals. In the model plant *Arabidopsis thaliana*, extensive natural variation in the SAS has been explored for seedling hypocotyl growth and flowering acceleration (Botto and Smith, 2002; Botto and Coluccio, 2007; Jiménez-Gómez et al., 2010; Coluccio et al., 2011). An SAS causal gene has recently been identified by analysing the genetic variation in the recombinant inbred line (RIL) population between Bay and Sha genotypes (Jiménez-Gómez et al., 2010; Coluccio et al., 2011). In both of these studies, the authors demonstrated that the *ELF3* gene is a component of SAS signalling involved in the elongation and flowering responses, the Sha-*ELF3* allele being less functional than the Bay-*ELF3* allele in low R/FR. The causal mechanism of *ELF3* genetic variation appears to be a single amino acid change in the Sha-*ELF3* allele (Coluccio et al., 2011). Besides, natural alleles of *PHYD* and *PIF4* contribute differentially to SAS (Aukerman et al., 1997; Brock et al., 2010). Using a genome-wide association analysis, it was suggested that polymorphisms with small effects could be responsible for the natural variation in hypocotyl growth under shade (Filiault and Maloof, 2012).

The leucine-rich repeat receptor-like Ser/Thr kinase gene *ERECTA* modulates plant developmental programmes as well environmental and biotic responses (Torii et al., 1996; van Zanten et al., 2009; Fu et al., 2009). Among the most characteristic phenotypes associated with the *erecta* mutation are the short stature and the reduced internode and pedicel lengths of inflorescences (Torii et al., 1996). The *ERECTA* locus has been implicated in the control of hypocotyl growth under continuous blue light, far-red and red pulses, and darkness (Borevitz et al., 2002; Botto et al., 2003). In *A. thaliana* plants, *ERECTA* is involved in the ethylene-induced hyponastic response, allowing an upward leaf movement response through differential petiole growth (van Zanten et al., 2010). Moreover, *ERECTA* has been implicated in the control of leaf, flower and fruit development, resistance to pathogens, and the regulation of transpiration (Douglas et al., 2002; Godiard et al., 2003; Llorente et al., 2005; Masle et al., 2005). In spite of the extensive characterization of *ERECTA* functions in plant development, its participation in the SAS is largely unknown.

The aim of this work was to improve our understanding of the SAS genetic architecture of hypocotyl elongation to an end-of-day FR signal (EOD), a light treatment that simulates shade conditions in nature. First, we describe the natural genetic variation in EOD response among 108 accessions. Secondly, we conducted a quantitative trait loci (QTLs) analysis using three sets of *A. thaliana* RIL populations, having Landsberg *erecta* (Ler) as a common parent. Thirdly, we demonstrate that *PHYB* is the candidate gene for *EODRATIO5*, but not for two co-localized QTLs, suggesting *PHYB* background-dependent effects on shade. Finally, we focused on *ERECTA* as a candidate gene for *EOD6*. We found that the *ERECTA* gene is implicated in the SAS in a background-dependent manner. Furthermore, *ERECTA* polymorphic effects in EOD responses were detected for other SAS traits, suggesting that its role in shaded environments is relevant for some populations in different phases of plant development.

MATERIALS AND METHODS

Plant material

Arabidopsis thaliana accessions were obtained from the Arabidopsis Biological Resource Center (ABRC, Ohio State University, USA). RIL populations derived from crosses between Ler and No-0 (Magliano et al., 2005), Ler and Col (Lister and Dean, 1993), and Ler and Cvi-0 populations (Alonso Blanco et al., 1998) were also obtained from the ABRC. We also used near isogenic lines (NILs) polymorphic for *ERECTA* between Ler and Cvi-0 genotypes (Keurentjes et al., 2007); *PHYB* transgenic lines carrying 35S::*PHYB*-Cvi and 35S::*PHYB*-Ler alleles in *phyB-9* mutant Col background (Filiault et al., 2008); *ERECTA* transgenic lines in Landsberg *erecta* (Ler), Vancouver-0 (Van-0) and Hiroshima (Hir-1) backgrounds carrying *ERECTA*-Col (van Zanten et al., 2009), and *er-104* (Torii et al., 1996) and *er-2* (CS3401), *erecta* mutants in Ws and Col backgrounds, respectively.

Experimental conditions

We cultivated plants at 23 °C under 16/8-h light/dark long-day conditions. Seeds were harvested and kept dry in darkness. For the experiments, 15 seeds of each genotype were sown on 0.8 % agar contained in clear plastic boxes and incubated in darkness at 5 °C for 4 d. Thereafter, the boxes with seeds were transferred to 22 °C and irradiated with a red light (R) for 3 h to promote homogeneous germination. After 24 h incubation in darkness, seedlings were exposed to WL and EOD treatments. The WL treatment consisted of growing the seedlings in a white light chamber with 8/16-h light/dark short-day photoperiod at 22 °C (WL, 85 $\mu\text{mol m}^{-2} \text{s}^{-1}$, otherwise as indicated in the text). The EOD treatment consisted of adding 30 min of far-red light at the end of WL (FR, 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The FR light was provided by incandescent lamps in combination with an RG9 filter (Schott, Mainz, Germany). On day 4, the ten tallest of 15 seedlings of each box for each genotype were measured in at least three replicates.

For vegetative and flowering traits, we followed the experimental protocols indicated above. Five days after germination, seedlings were transplanted on a mixture of peat moss/vermiculite/perlite (1 : 3 : 3 ratio) contained in plastic pots. The pots were transferred and randomly distributed into a chamber under short day conditions (8/16-h light/dark) at 22 °C. The light treatment was done with mercury lamps (60 $\mu\text{mol m}^{-2} \text{s}^{-1}$). At the end of the photoperiod, half of the pots received a pulse of 30 min of FR light (45 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Over 6 weeks, we measured petiole and lamina length, and leaf angle in the youngest leaf totally expanded. Leaf angle, defined as the angle between the petiole and a line perpendicular to the soil surface, was measured at the middle of the photoperiod with a protractor. Flowering time, determined as leaf number at bolting, was measured by counting only primary rosette leaves when the flowering stem reached 2 cm in length. All light measurements were made with a spectrophotometer (SpectroSense2/2 + ; Skye Instrument Ltd, Llandrindod Wells, UK).

Data collection and statistical analysis

We measured hypocotyl length in WL and EOD. We calculated an index of shade response as the ratio of EOD/WL

following similar protocols used previously (Botto and Smith, 2002). Briefly, we estimated EOD response as the ratio of EOD/WL hypocotyl length. A similar criterion was used to calculate petiole and lamina length, and leaf angle. Because flowering time is accelerated by the EOD treatment, we estimated the EOD response for flowering as WL/EOD to maintain the index of shade response between a range of values similar to the other traits (Botto and Smith, 2002). Mean differences among treatments were tested by Student's *t*-test. Analyses were carried out with GraphPad Prism, Version 5.01 (GraphPad Software, Inc., La Jolla, CA, USA).

QTL mapping analysis

Forty-six, 66 and 99 markers obtained from Ler × No-0 (Magliano *et al.*, 2005), Ler × Col (Lister and Dean, 1993) and Ler × Cvi (Alonso-Blanco *et al.*, 1998) RIL populations were used for QTL analysis, respectively. MAPMAKER/EXP 3.0 was used to construct the linkage map (Lander *et al.*, 1987). Linkage groups were verified with a minimum LOD = 2.5 and a maximum distance of 50 cM (Kosambi function). Both the linkage map data and the RIL phenotypic data were then imported to QTL Cartographer version 2.0 obtained from <http://statgen.ncsu.edu/qtlcart/WQTLCart.htm> (Wang *et al.*, 2004). For the QTL analysis, we included 94 lines for the Ler × No-0 RIL population, 85 lines for the Ler × Col RIL population and 127 lines for the Ler × Cvi RIL population. We ran the data as absolute values in the WL and EOD responses. The likelihood, location, additive effect and percentage of variance explained by each QTL were calculated using model 6 based on the composite interval mapping (CIM) method (Zeng, 1994). QTL cofactors were initially selected by using forward-backward stepwise multiple regression. Mapping was conducted with a walking speed of 0.5 cM and a window size of 3 cM. For precise determination of significant QTLs, the LOD thresholds for each linkage group were calculated by a permutation test method (Doerge and Churchill, 1996) with 1000 permutations at the permutation significance level ($P < 0.05$). The support interval of each QTL was constructed using the 2-LOD rule with the confidence intervals being defined by all those values falling within 2-LOD score of the maximum value (Lynch and Walsh, 1998).

QTL confirmation assays

To reduce the QTL interval of *EODRATIO1* in the Ler × No-0 RIL population, we generated heterogeneous inbred lines (HIF) using the advantage that some RILs are still individually segregating for one limited genomic region (Tuinstra *et al.*, 1997). Using PCR, we screened F_7 individuals of RIL152 that were still heterozygous for marker PLS5 to select the HIF152 marker polymorphic at PLS5 and with homogenous alleles in the rest of the genome. We then selected recombinant HIF (rHIF) by successive PCRs using seven polymorphic indel markers in the interval mapping between CIW3 and NGA1126 (Salathia *et al.*, 2007). We also included two polymorphic markers at two positions of *PHYB* (sites 3 and 12, Filiault *et al.*, 2008). For *PHYB* quantitative complementation analysis, we crossed No-0, Col, Cvi-0 and Ler parental lines with a *PHYB* + or *phyB* mutant in Ler (*phyB-1*) or Col

(SALK_069700) following the protocols suggested by Mackay (2001). To evaluate the quantitative complementation with the *phyB* mutation, the phenotype of F_1 individuals was analysed by a two-way ANOVA with tester genotypes as the 'genotype' factor and *phyB* mutation as the 'phyB' factor. Quantitative complementation of the *phyB* mutant alleles was detected using the non-significant 'genotype' by 'phyB' factor interaction (Mackay, 2001). Functional variation of *PHYB* alleles from Ler and Cvi-0 was confirmed by evaluating hypocotyl growth in at least three independent transgenic lines carrying *35S::PHYB-Cvi* and *35S::PHYB-Ler* alleles in a *phyB-9* mutant Col background (Filiault *et al.*, 2008).

RESULTS

Natural genetic variation for an EOD response among 108 *Arabidopsis* accessions

We analysed the phenotypic correlation for hypocotyl length in *Arabidopsis* seedlings grown under WL and EOD among 108 accessions distributed between 15 and 63 °N. Hypocotyl length varied between 1.55 and 5.86 mm in WL, and between 3.66 and 8.13 mm in EOD (Supplementary Data Fig. S1A, B). To identify the capacity of the accessions to respond to shade, we calculated different indexes for shade-avoidance elongation response. We obtained similar results using log-transformed and untransformed data, and for different ratio indexes of shade response (data not shown). Therefore, from here on we detail only the results from the absolute untransformed data and the ratio of hypocotyl length in EOD/WL (hereafter EOD response), a standardized shade response index used previously (Botto and Smith, 2002). The EOD response was between 1.30 and 2.73. Accessions with low EOD response included Got-7, Tsu-1, No-0 and Cvi-0, whereas Got-22 and Eden-1 appeared as the most responsive genotypes to the EOD response (Fig. 1). We found a slight positive tendency for the EOD response with an increase in latitude (Fig. 1, $r = 0.33$, $P = 0.0004$), which was negatively correlated with the pattern of response in WL ($r = -0.31$, $P = 0.0011$, Supplementary Data Fig. S1A). To

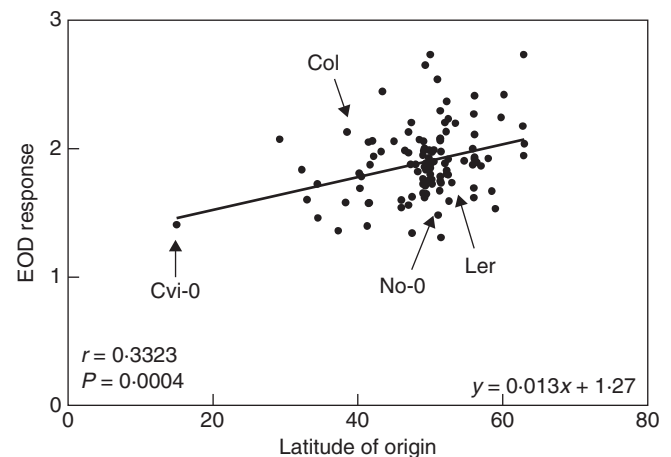


FIG. 1. Natural variation in the EOD response for hypocotyl length in 108 accessions. The linear regression equation and the Pearson correlation index (r) with P value are also shown.

confirm that the EOD treatment used in this work simulated natural shade conditions mediated principally by phyB, we studied the effects of EOD in *phyB* and *phyAphyB* double mutants in Ler and Col backgrounds (Supplementary Data Fig. S2A). As expected, the *phyB* mutants had a null EOD response, indicating that phyB is the phytochrome promoting the shade response (Supplementary Data Fig. S2B).

QTL mapping for EOD response in three RIL populations sharing Ler as the common parental genotype

We conducted a QTL quantitative approach using three sets of *A. thaliana* RIL populations, having Ler as a common parent. We used Ler × No-0, Ler × Col and Ler × Cvi-0 RIL populations (Lister and Dean, 1993; Alonso-Blanco et al., 1998; Magliano et al., 2005). We analysed the phenotypic variation of the three RIL populations in WL and EOD (Supplementary Data Fig. S3A, C, E, and Table S1). The four parental lines showed a range of variation for the EOD response between 1.13 for No-0 and 2.47 for Col (Supplementary Data Fig. S3B, D, F, and Table S1). The contribution of each parent to the EOD response was Col > Ler > Cvi-0 > No-0 (Supplementary Data Table S1). The average EOD response was 1.20 ± 0.02 for Ler × No-0, 1.75 ± 0.11 for Ler × Cvi-0 and 2.13 ± 0.12 for Ler × Col RIL populations (Supplementary Data Table S1). The genetic component of the phenotypic variation (heritability, H^2) ranged between 0.54 and 0.84 for WL, 0.59 and 0.84 for EOD, and 0.30 and 0.63 for the EOD response (Supplementary Data Table S1).

We mapped seven QTLs for WL, six QTLs for EOD and five QTLs for the EOD response (Supplementary Data Table S2). Most were specific to each trait, suggesting that the genetic architecture of the SAS elongation response depends on specific loci of small effects: *WL2*, *WL4* and *WL6* to WL; *EOD2–EOD5* to EOD and *EODRATIO2–EODRATIO5* to the EOD response (Supplementary Data Table S2; Fig. 2). On the other hand, *WL1*, *WL3*, *WL5*, *WL7*, *EOD1* and *EOD6* were common to WL and EOD conditions, but not significant for the EOD response, suggesting that these loci are involved in general elongation without effects in the EOD response (Supplementary Data Table S2; Fig. 2).

The five *EODRATIO* QTLs were mapped on chromosomes 1, 2 and 5 and explained between 7 and 33 % of the total phenotypic variation (Fig. 2; Supplementary Data Table S2). The Ler alleles of the five *EODRATIO* QTLs contributed positively to the EOD response, having the smallest effects in *EODRATIO5* and the largest effects in *EODRATIO3* (0.07 and 0.27, respectively; Supplementary Data Table 2). Interestingly, the *EODRATIO1*, 2 and 5 QTLs identified in the three RIL populations co-localized on chromosome 2, intervals of which contained *PHYB* but not *ERECTA* (Supplementary Data Table 2; Fig. S2). Finally, *EODRATIO3* mapped to the middle of chromosome 5 in the Ler × Col RIL population and *EODRATIO4* mapped to the top of chromosome 1 in the Ler × Cvi-0 RIL population (Supplementary Data Table S2; Fig. 2B, C).

Confirmation of EODRATIO1 into an interval of 1.2 Mb

In the Ler × No-0 RIL population, *EODRATIO1* was mapped for EOD response and WL, suggesting that this

QTL might underlie variation in the shade-avoidance response primarily by controlling elongation in sun conditions with high R/FR. For *EODRATIO1* confirmation, we selected a heterogeneous inbred family (HIF152) polymorphic for the PSL5 marker on chromosome 2 (Fig. 3). The No-0 alleles at PSL5 in the HIF152 reduced the EOD response significantly. This accounted for a higher hypocotyl length in WL and also in EOD compared with Ler alleles (Supplementary Data Fig. S4A, B). By successive PCRs with polymorphic markers in the interval between CIW3 and NGA1126 of *EODRATIO1*, we selected rHIF. While rHIF13 and rHIF88 did not show EOD response differences, rHIF44 and rHIF16 showed the EOD response, confirming *EODRATIO1* to an interval of 1.2 Mb, between 8148 and 9354 kb, a region that includes the *PHYB* gene (Fig. 3C). To evaluate whether *PHYB* is the candidate gene for *EODRATIO1*, we selected the rHIF36 with polymorphic alleles for the *PHYB* gene but with homozygous alleles in the confirmed interval of this QTL. Polymorphic alleles at rHIF36 did not display a significant EOD response, suggesting that *PHYB* is not responsible for *EODRATIO1* (Fig. 3A).

PHYB is the candidate gene for EODRATIO5 but not for EODRATIO1 or EODRATIO2

By quantitative complementation analysis, we confirmed that *PHYB* gene variation did not account for *EODRATIO1* (Supplementary Data Fig. S5A). F_1 individuals of *PHYB* + and *phyB* showed that No-0 and Ler alleles were able to complement the dysfunction of the *PHYB* mutant allele (P genotype × *phyB* = 0.11). Because *PHYB* was also mapped to the intervals of *EODRATIO2* and *EODRATIO5*, we investigated whether *PHYB* is the candidate gene for these QTLs. By quantitative complementation analysis we confirmed that *PHYB* did not account for *EODRATIO2* (P genotype × *phyB* = 0.43; Supplementary Data Fig. S5B). However, two pieces of evidence suggest that *PHYB* is the candidate gene for *EODRATIO5*. First, the *PHYB* + Ler allele failed to complement *phyB* mutation in F_1 individuals (P genotype × *phyB* = 0.0006, Fig. 4A). Secondly, transgenic lines carrying *PHYB* alleles of Ler or Cvi-0 in the *phyB-9* mutant background showed that the *PHYB*-Ler allele has a higher contribution to the EOD response than the *PHYB*-Cvi allele (Fig. 4B).

ERECTA is the candidate gene for EOD6

While No-0, Col and Cvi-0 carry wild-type *ERECTA* alleles, Ler contains an induced mutation in the leucine-rich repeat receptor-like Ser/Thr kinase *ERECTA* gene (Torii et al., 1996). In our analysis, three QTLs were mapped at the *ERECTA* marker: *EOD2* and *EOD4* for EOD in Ler × No-0 and Ler × Col, respectively; and *EOD6* for WL and EOD in Ler × Cvi-0 RIL populations (Supplementary Data Table S2). As expected for the *ERECTA* function in plant development, the additive effects of Ler carrying the *erecta* mutation at *EOD2* and *EOD6* were lower than No-0 and Cvi-0, respectively. However, *ERECTA* appears not to be responsible for *EOD4* because opposite allelic effects were found (Supplementary Data Table S2). Using polymorphic NILs at the *ERECTA* region, we confirmed the positive contribution

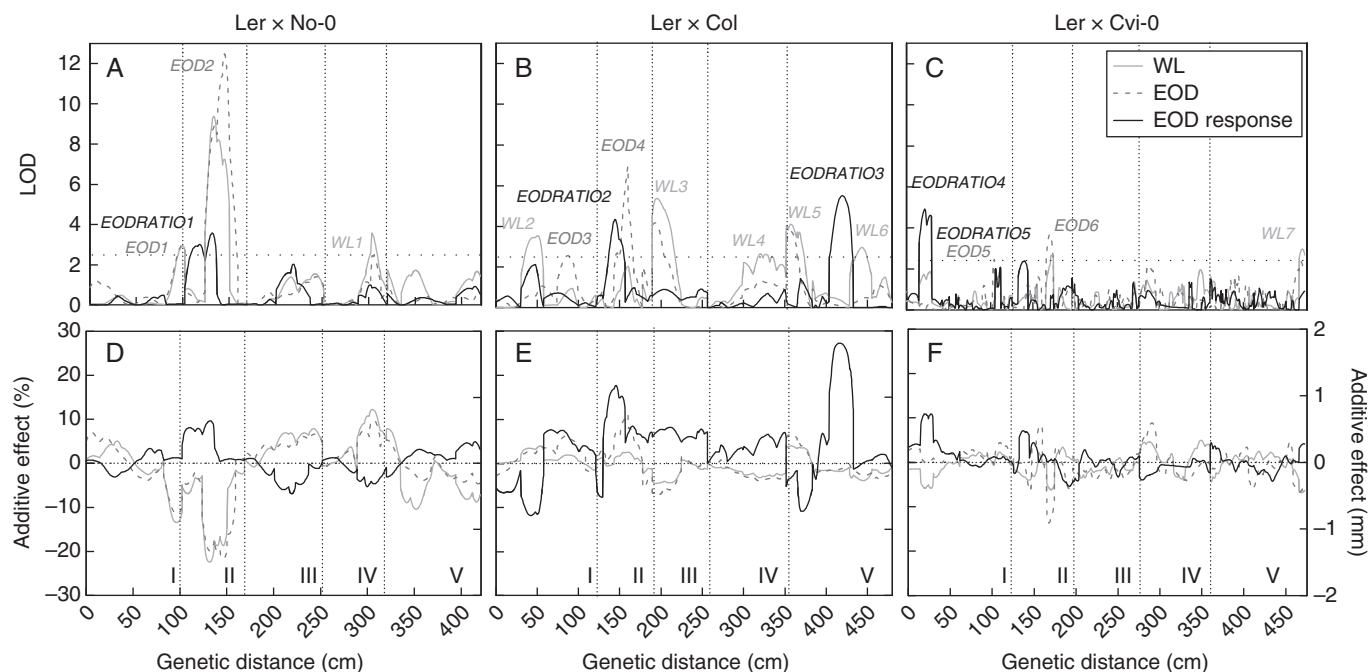


FIG. 2. QTL mapping for the WL, EOD and EOD responses in three mapping populations sharing the Ler parent. LOD and additive effects are shown along accumulative distance (cM) of the five chromosomes of *Arabidopsis* (A, D for Ler \times No-0; B, E for Ler \times Col; and C, F for Ler \times Cvi-0 RIL populations). WL, EOD and EODRATIO numbers of QTLs are arbitrary. Horizontal dotted lines represent significant thresholds for LOD scores. In the additive effect graphs, positive values indicate a positive contribution of Ler alleles and negative values indicate a positive contribution response of the alternative parent for each trait. Roman numerals at the bottom of the graphs indicate the chromosome number.

of Cvi-*ERECTA* alleles at *EOD6* for the hypocotyl elongation in WL and EOD (Fig. 5). Furthermore, the EOD response was not significantly affected by *ERECTA* polymorphisms between Ler and Cvi-0, confirming the output of the QTL analysis (Fig. 5; Supplementary Data Table S2).

The effects of *ERECTA* depends on the genetic background

To have a better understanding of the *ERECTA* function in shade, we analysed the EOD response in polymorphic lines at the *ERECTA* gene in five genetic backgrounds. Using point mutations at the *ERECTA* gene in Col and Ws, and *ERECTA* transgenic lines in Ler, Van-0, Ws and Hir-1 accessions, we demonstrated that *ERECTA* effects in the EOD response are dependent on the genetic background (Fig. 6C). The *erecta* allele in Col and Van-0 showed a significantly higher EOD response compared with those genotypes carrying the *ERECTA* allele. Although the *ERECTA* allele did not have effects on the EOD response in Ler, Ws and Hir-1, the seedlings of these genotypes showed a higher elongation in WL and EOD as expected for the *ERECTA* function in cell expansion (Fig. 6A, B).

ERECTA is pleiotropic to other SAS traits

The SAS is present along the life cycle of the plants. To understand the genetic-dependent function of *ERECTA* in the SAS, we analysed the effects of *ERECTA* in the shade response of vegetative and flowering traits. The *erecta* allele increased the EOD response in some traits depending on the genetic background, increasing leaf angle in Ws, petiole and lamina length in Van-0, and flowering shade response in Ws

(Fig. 7). However, the *ERECTA* allele in the Ler background increased the EOD response for petiole and lamina length (Fig. 7B, C). The *ERECTA* allele in Ws also increased the EOD response for petiole elongation (Fig. 7B). Depending on the genetic background, the *ERECTA* allele increased petiole and lamina elongation significantly, and petiole angle in WL and EOD conditions, while the *erecta* allele in the Ws background promoted the number of leaves at flowering in WL (Supplementary Data Fig. S6).

DISCUSSION

In the present study, we exploited natural variation for shade-avoidance responses mapping QTLs in three RIL populations sharing Ler as a common parental genotype. SAS genetic variation was explained by several QTLs of small effect that fell into two groups. Six QTLs appeared in both WL and EOD light conditions. These loci could be responsible for the variation in general elongation without causing variation in the SAS. The second group of QTLs explained a more specific SAS genetic variation. In this group, three and two QTLs mapped specifically for WL and EOD, respectively. Furthermore, to identify specific QTLs associated with the SAS, we estimated the EOD response trait as the ratio of hypocotyl length in EOD/WL, a standardized shade response index used previously (Botto and Smith, 2002). We mapped five *EODRATIO* QTLs. *EODRATIO1* was also found in WL, but the other four *EODRATIO* QTLs mapped in different loci to those identified in WL and EOD conditions (Fig. 2 and Supplementary Data Table S2). We suggest that candidate genes for *EODRATIO1* might underlie variation in the

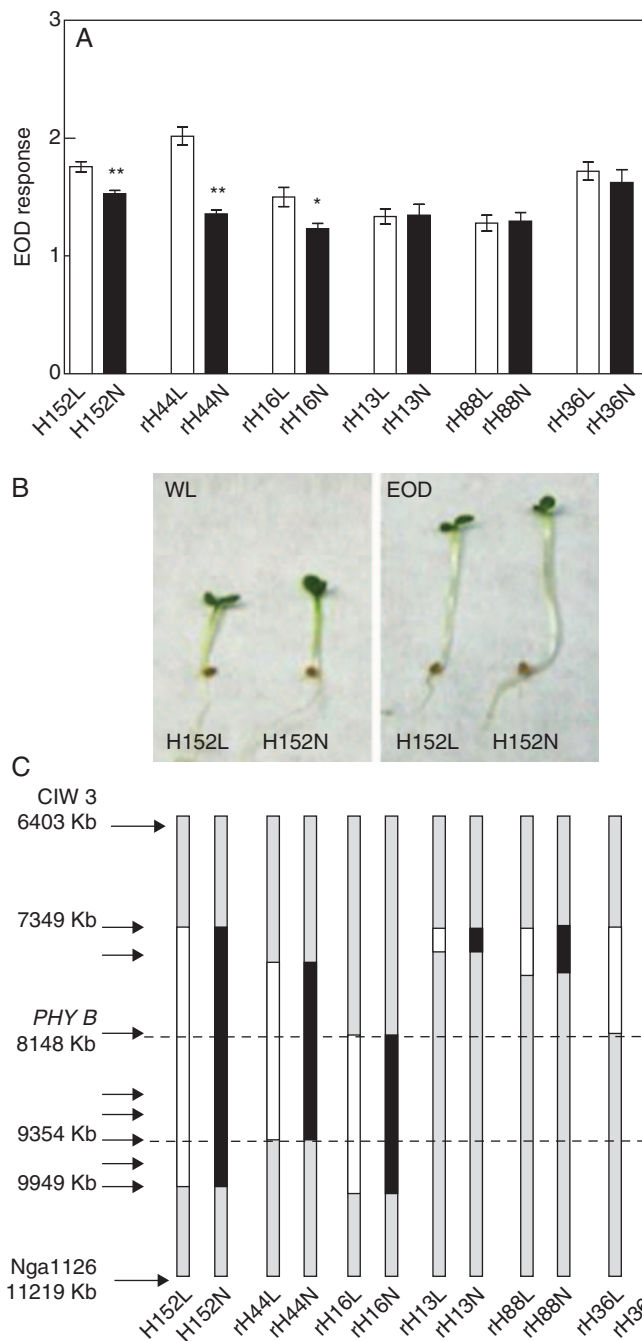


FIG. 3. *EODRATIO1* is confirmed to an interval of 1.2 Mb on chromosome 2. (A) Hypocotyl length for HIF and rHIF obtained from RIL152 polymorphic at the PSL5 marker. Each bar represents mean \pm s.e.m. Significant differences between means were identified by Student's *t*-test and are indicated: * $P < 0.05$ and ** $P < 0.001$. (B) Photographs of HIF152 grown under WL or EOD. (C) Diagram of the segregation area for HIF152 and rHIF152 lines on chromosome 2. The segregation area for each line is shown as heterozygous (Ler = white and No-0 = black) or homozygous (grey). Marker positions are indicated by arrows.

shade-avoidance response primarily by controlling hypocotyl elongation in open areas, where the density of plants is low. Interestingly, using the same Ler \times No-0 RIL population, a flowering time QTL co-localized with *EODRATIO1* when *Arabidopsis* plants were grown in isolation at high R/FR ratios but not at high densities (Botto and Coluccio, 2007).

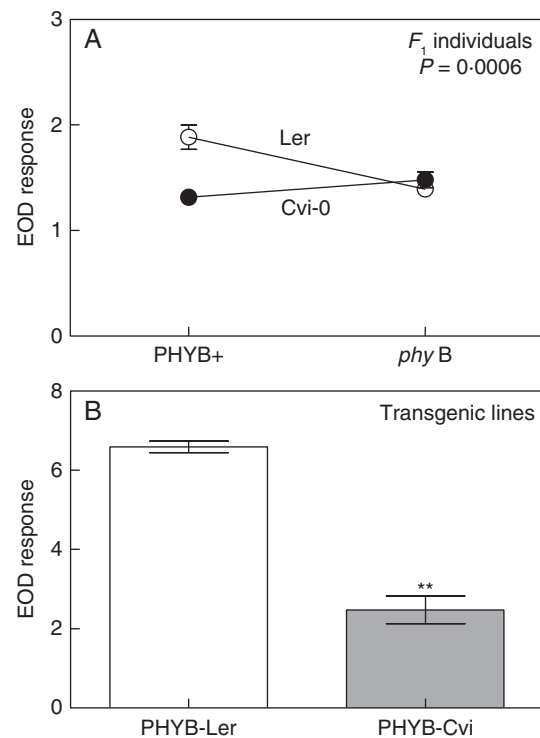


FIG. 4. *PHYB* is the candidate gene for *EODRATIO5*. (A) Quantitative complementation test for the EOD response. The P value of the interaction between tester genotype ('genotype' factor) and *phyB* mutation in Ler background ('phyB' factor) is given. Each point represents the mean \pm s.e.m. (B) EOD response in transgenic lines carrying *35S::PHYB-Cvi* and *35S::PHYB-Ler* alleles. Each bar represents mean \pm s.e.m. Significant differences between means were identified by Student's *t*-test and are indicated: ** $P < 0.01$.

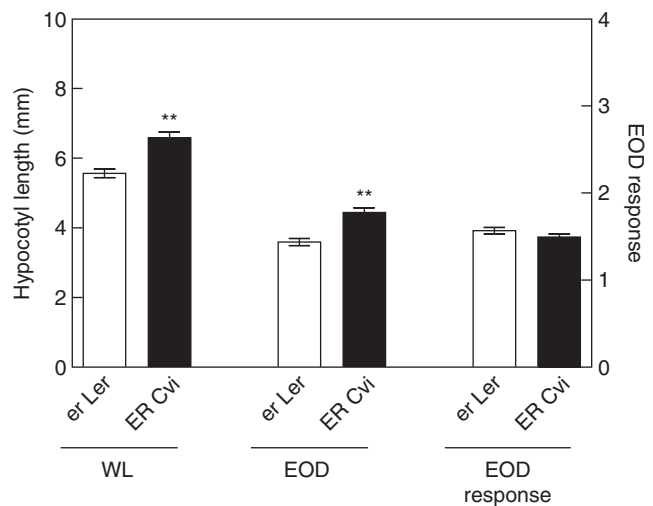


FIG. 5. *ERECTA* is the candidate gene for *EOD6*. Hypocotyl length in WL and EOD, and EOD response of NIL carrying polymorphic *ERECTA* allele originating from the Ler \times Cvi-0 RIL population. Each bar represents mean \pm s.e.m. Significant differences between means were identified by Student's *t*-test and are indicated: ** $P < 0.001$.

By using a candidate gene approach, we confirmed *ERECTA* as the gene responsible for *EOD6*. The leucine-rich repeat receptor-like Ser/Thr kinase gene *ERECTA* is involved in the plant developmental programme as well as in environmental

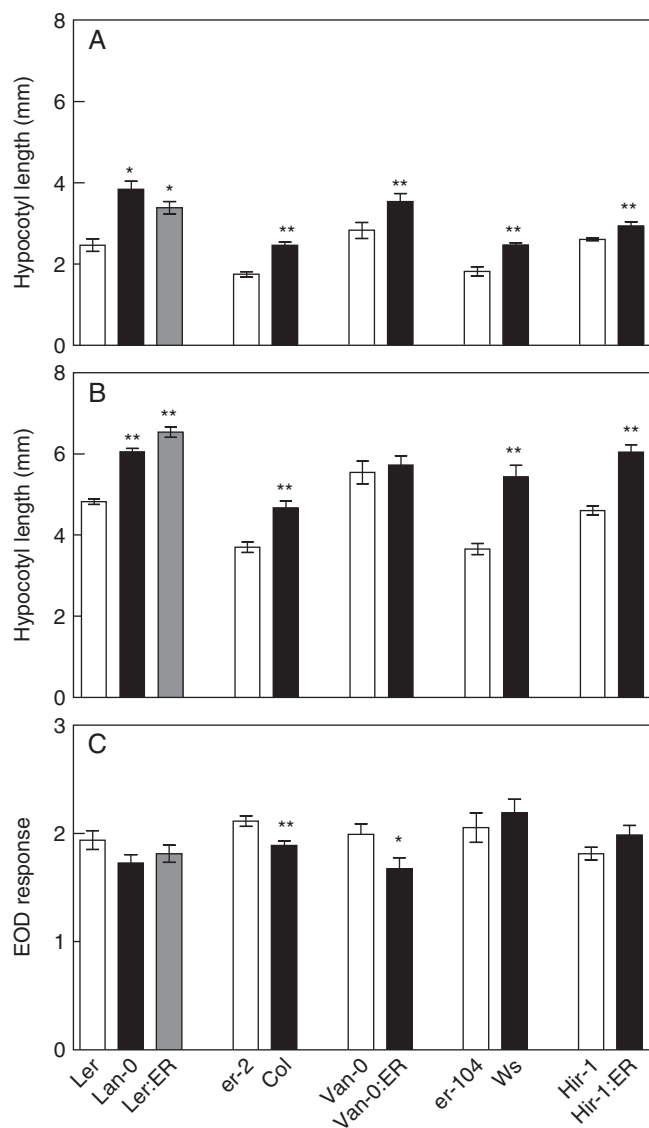


FIG. 6. *ERECTA* effects on shade-avoidance responses depend on the genetic background. Hypocotyl length in WL (A), EOD (B) and EOD response (C). Each bar represents mean \pm s.e.m. Significant differences between means were identified by Student's *t*-test and are indicated: * $P < 0.05$ and ** $P < 0.001$.

and biotic responses controlling cell elongation (Torii *et al.*, 1996; van Zanten *et al.*, 2009; Fu *et al.*, 2009). It has been described that *ERECTA* controls spatial and temporal patterns of epidermal cell number and size because the *erecta* allele leads to a low cell expansion rate (Tisné *et al.*, 2011). It is therefore not surprising that *ERECTA* increased the plant growth elongation in WL and EOD light conditions. The novelty of our study is the demonstration that *ERECTA* is implicated in the SAS in a background-dependent manner and its effects are pleiotropic to other shade-avoidance responses. The *erecta* allele promoted the EOD response of hypocotyl growth in Col and Van-0, but not in Ler, Cvi-0, Hir-1 or Ws. Furthermore, the contribution of *ERECTA* in the EOD response was also found for leaf hyponastic growth, lamina and petiole elongation, and flowering time, suggesting that its role in shaded environments is relevant

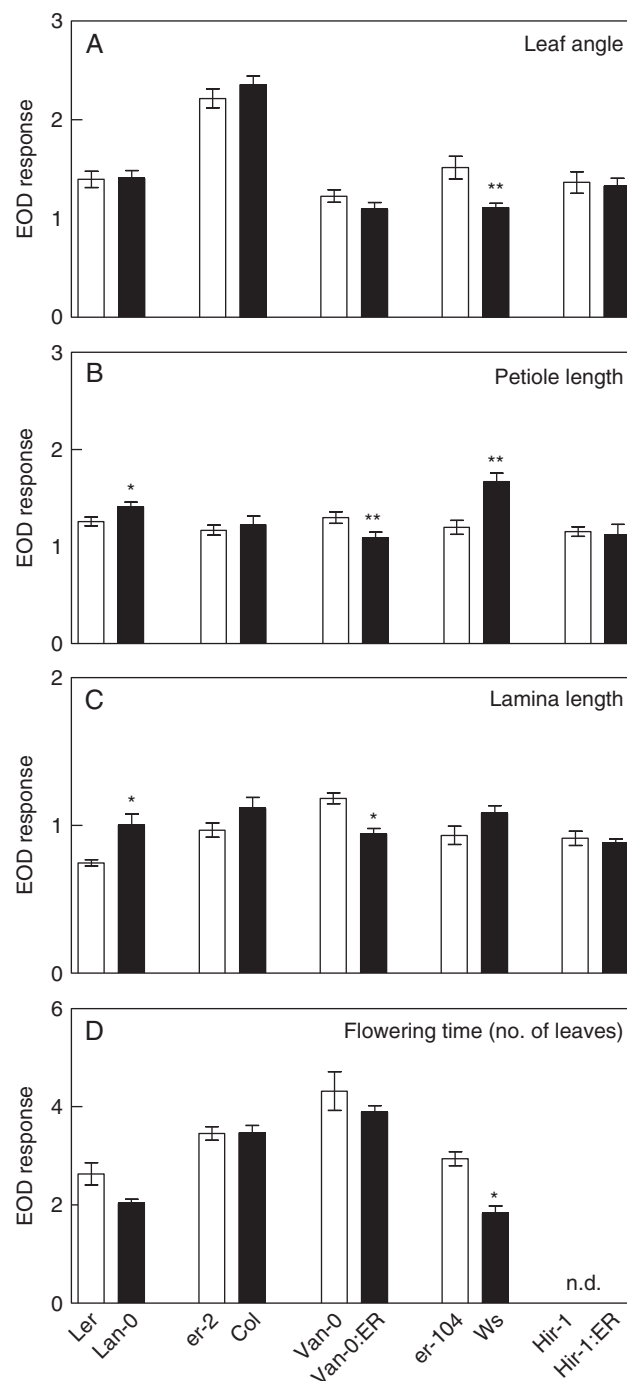


FIG. 7. *ERECTA* function in the shade-avoidance syndrome is pleiotropic. EOD response for (A) leaf angle, (B) petiole length, (C) lamina length, and (D) number of leaves at flowering. Each bar represents mean \pm s.e.m. Significant differences between means were identified by Student's *t*-test and are indicated: * $P < 0.05$ and ** $P < 0.001$. nd, not determined.

for certain populations in different phases of development. Other quantitative mapping studies also found a QTL mapped at the *ERECTA* marker for hypocotyl elongation in de-etiolated seedlings of a Ler \times Cvi-0 RIL population grown in darkness, continuous blue light, or red and far-red pulses of light (Borevitz *et al.*, 2002; Botto *et al.*, 2003).

Here we propose that *ERECTA* is involved in canalization processes buffering the genetic variation of the SAS. Canalization is the ability of organisms to buffer their developmental processes against environmental fluctuations (Waddington, 1942; Meiklejohn and Hartl, 2002). Several kinases, such as *ERECTA*, are client proteins of Hsp90 that can act as a persuasive buffer against genetic variation (Jarosz and Linquist, 2010). In fact, Hsp90 protein modulated the expression of genetic variation and developmental stability in hypocotyl growth and flowering in *A. thaliana* (Sangster et al., 2008a, b). In one of these studies, an HSP90-responsive QTL affecting hypocotyl length was epistatic to the *erecta* mutation but not to wild-type *ERECTA*, suggesting that the pleiotropic *erecta* mutation may substitute Hsp90 inhibition to reveal a polymorphism at a second locus (Sangster et al., 2008a). In an independent study, Hall et al. (2007) found a QTL at *ERECTA* contributing to microenvironmental canalization for leaf number under long-day but not short-day photoperiods, the *ERECTA* effects being allele-specific and also dependent on the genetic background. The patterns of canalization could differ between pleiotropic traits because microenvironmental variation affects the degree of developmental buffering. In some circumstances, selection would favour reduced canalization and increased sensitivity to microenvironmental variations so that individuals maintain sufficient plasticity to respond to this variation. In summary, we suggest that the canalization penetrance of *ERECTA* in the SAS could be the result of the evolutionary trajectory of each genotype in different light environments.

Five *EODRATIO* QTLs were identified in this work. Interestingly, in all cases, the Ler alleles controlled hypocotyl elongation growth positively. This suggests a stronger function of Ler alleles than Col, Cvi-0 and No-0 alleles in the EOD response. In addition to the three *EODRATIO* QTLs co-localized on chromosome 2, we mapped *EODRATIO3* to the middle of chromosome 5 in the Ler × Col RIL population (Supplementary Data Table S2). Interestingly, Coluccio et al. (2011) also detected a QTL co-localized with *EODRATIO3* in the Bay × Sha RIL population, the Bay alleles having a positive contribution to hypocotyl elongation under shade. The candidate gene falling into the *EODRATIO3* interval is *TZP/PLUS3*, a gene controlling cell-wall synthesis involved in hypocotyl length variation between Bay and Sha (Loudet et al., 2008). We also mapped *EODRATIO4* to the top of chromosome 1 in the Ler × Cvi-0 RIL population. Several genes involved in the phyA responses, such as *PHYA*, *PIF3* (Leivar and Quail, 2011) and *CRY2* (Botto et al., 2003), mapped to the *EODRATIO4* interval. Recently, it had been shown that genetic variants of phyA signalling loci are significantly associated with simulated shade in a genome-wide association study with 200 *Arabidopsis* accessions (Filiault and Maloof, 2012).

We have shown here that *PHYB* is the candidate gene for *EODRATIO5* mapped in the Ler × Cvi-0 RIL population, but not for two co-localized *EODRATIO* QTLs detected in Ler × Col and Ler × No-0 RIL populations. We also found a lower sensitivity response of Cvi-*PHYB* than the Ler-*PHYB* allele under WL and EOD responses. Seedlings of both genotypes exposed to continuous light also showed a low red sensitivity response for the Cvi-*PHYB* allele (Filiault et al., 2008). Interestingly, in a sequence diversity analysis

of *PHYB*, Ler, Col and No-0 *PHYB* variants segregated together in a Central European clade and the Cvi-0 variant grouped with four Spanish accessions in an isolated clade (Filiault et al., 2008). Furthermore, we showed a mild positive tendency to display a higher EOD response for hypocotyl elongation in a north–south latitudinal cline, probably as a consequence of a lower light sensitivity in WL of the south-located genotypes (Supplementary Data Fig. S1). However, in certain cases, the evolution of shade response would be the consequence of local adaptations to light variations. For example, we found two accessions from the same geographical location in Germany, Got-7 and Got-22, with contrasting EOD response (1.35 vs. 2.74, respectively, Fig. 1). In fact, the distribution of each population could be the result of individual adaptation to a particular habitat integrating complex networks of light signals and other environmental signals to increase its fitness.

While this manuscript was under final revision, Patel et al. (2013) reported similar findings in the *Plant Journal*. These authors found that *ERECTA* function in shade environments is dependent on growth temperature and genetic background.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Figure S1: natural variation for hypocotyl length in WL and EOD for 108 accessions of *Arabidopsis thaliana*. Figure S2: shade-avoidance response for hypocotyl growth in wild-type, *phyA* and *phyB*. Figure S3: frequency distribution in the WL, EOD and EOD response for Ler × Cvi-0, Ler × Col and Ler × No-0 RIL populations. Figure S4: *EODRATIO1* in WL and EOD is confirmed by HIF and rHIF polymorphic markers between CIW3 and NGA1126 at chromosome 2. Figure S5: interaction between tester genotype ('genotype' factor) and *phyB* mutation ('phyB' factor). Figure S6: leaf angle, petiole length, lamina length and number of leaves at flowering, measured in plants cultivated in WL and EOD. Table S1: summary statistics for hypocotyl length under WL, EOD and EOD response. Table S2: QTL mapping for hypocotyl length in WL and EOD and EOD response in three RIL populations sharing Ler as a common parental background.

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