

Supporting Information

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SI Materials and Methods

Plant Material. The Bay-0 × Shahdara RIL set [http://dbsgap.versailles.inra.fr/vnat/; (1)] was initially phenotyped in 4 different environments to map QTL affecting hypocotyl elongation. We used the core-pop164, a subset of 164 lines of the whole population designed to optimize QTL mapping while limiting the phenotyping effort. HIF350 was developed from an F7 line (RIL350) that still segregated for a single and limited genomic region around MSAT5.9, following a strategy described previously (2), which allowed for comparison of plants with alternative genotypes at *LIGHT5* in a common (although heterogeneous) background. Plants still heterozygous for the QTL region (thus segregating for the phenotype) were screened with adequate markers to isolate recombinants (rHIF) used in the fine-mapping process (see data below).

Distinct Bay-0 lines from the stock center were used to find variants at *LIGHT5*, especially the single seed descent (SSD) lines issued from the initial plant used to generate the RIL set (CS57923) called “Bay-0” throughout this paper and an SSD (41AV) from the Versailles resource center (http://dbsgap.versailles.inra.fr/vnat/) called “Bay-0[41AV]”, progeny of CS954.

Hypocotyl Measurements. Seeds were sterilized in 70% EtOH with 0.1% Triton X-100 for 10 min, then rinsed in 95% EtOH for 10 min. They were then resuspended in 0.1% agar and stratified in darkness at 4 °C for 3 days. Sixteen different genotypes (RILs or parental line, 16 seeds each) were spotted onto a 90-mm square Petri plate containing ½ MS salts and 0.7% phytagar without sugar. Plates were then exposed to white light for 6 days under the following continuous light treatments: 17 μmol·m⁻²·s⁻¹ (L1) or 10 μmol·m⁻²·s⁻¹ (L2) at either 22 or 26 °C. Each RIL was present once (16 seedlings) under L1 and once under L2 in each of the 2 distinct experiments performed at each temperature. Light treatments were always conducted side-by-side in a single chamber set to 1 temperature (2 × 11 plates). Plates were rotated every day within each treatment. White light was provided by three 20-W Cool White fluorescent and two 25-W incandescent bulbs in Percival E30B chambers; the R/FR ratio (650–680 nm/710–740 nm) was 1.06. At harvest, hypocotyls were transferred to acetate sheets and scanned on a flatbed scanner. Length was measured with Scion Image for Windows. Complete phenotypic data from RILs in four environments is included in Table 14 from our website’s supplementary information at www.inra.fr/vast/Files/Loudet_PNAS_SITables.xls.

Statistical Analysis and QTL Mapping. The complete set of data obtained by using the RILs was included in different analyses of variance (ANOVA) models to determine the specific effects of the “genotype”, “light”, “temperature,” and “residual” factors, and the interaction terms. Performed environment by environment, a similar analysis of variance with the “genotype” factor enabled us to quantify the broad-sense heritability (genetic variance/total phenotypic variance). Subsequent analyses used hypocotyl length mean values of an average of 16 seedlings (from 2 distinct experiments) per genotype per environment. Analysis of variance estimations were obtained by using the aov() function of the S-PLUS version 3.4 statistical package (Statistical Sciences).

The original set of 38 microsatellite markers and the genetic map obtained with MAPMAKER 3.0, (1); http://dbsgap.versailles.inra.fr/vnat/) were used to link phenotypic to genotypic variation. QTL analyses were performed by using the Unix version of QTL

CARTOGRAPHER 1.14 (3). Standard methods for interval mapping (IM) and composite interval mapping (CIM) were used as previously (1). Firstly, interval mapping (4) was carried out to determine putative QTL(s) involved in the variation of the trait. CIM model 6 of QTL CARTOGRAPHER was then performed on the same data: The closest marker to each local LOD score peak (putative QTL) was used as a cofactor to control the genetic background while testing at another genomic position. When a cofactor was also a flanking marker of the tested region, it was excluded from the model. The number of cofactors involved in our model reached a maximum of 4. The walking speed chosen for QTL analyses was 0.1 centiMorgans. The LOD significance threshold (2.3 LOD) was estimated from several permutation test analyses, as suggested by (5). Additive effects of detected QTL were estimated from CIM results, as representing the mean effect of the replacement of the Shahdara alleles by Bay-0 alleles at the studied locus. The contribution of each identified QTL to the total variance (R²) was estimated by variance component analysis, by using phenotypic values for each RIL. The model used the genotype at the closest marker to the corresponding detected QTL as random factors in ANOVA. Only homozygous genotypes were included in the analysis of variance. QTL × QTL interactions were searched for in the ANOVA analysis, as well as by using the “Pair-Scan” function of the WebQTL tool (http://www.genenetwork.org/).

Fine-Mapping. Phenotyping during the fine-mapping process was performed in the same framework as described in “Hypocotyl Measurements” under 22 °C / L1 conditions. Two sets of recombinants were isolated within the candidate region segregating in HIF350: The first one was screened from the initial HIF350 over the 16.173–18.050-Mb interval and the second one was screened from one of the positive recombined HIF (rHIF) obtained in the first round over the 17.405–17.692 Mb interval. Screens for recombinants involved respectively 600 and 4,000 individuals. Recombinant genotypes were determined by using microsatellite or indel markers, then CAPS when previous types of markers were exhausted, and finally direct sequencing of the SNPs to precisely localize recombination breakpoints. Once recombinants had been isolated they were tested for the segregation of the hypocotyl phenotype by progeny testing (96 seedlings individually phenotyped and genotyped per rHIF).

Following a strategy described in (6), advanced rHIF crosses were generated from 2 different rHIFs recombined immediately to the north or immediately to the south of the *LIGHT5* interval (and with adequate genotype elsewhere) that do segregate for the QTL phenotype, giving rise to lines arHIF47.

Transgenic Complementation. Genomic fragments spanning the predicted ORFs of the three genes in the *LIGHT5* interval were amplified from rHIF138-13 and subcloned in a topoTA vector. The resulting insert was sequenced, and transferred by using the GATEWAY system to a plant transformation vector containing the 35S promoter and a 3′ YFP tag. rHIF138-8 plants were transformed to complement the short hypocotyl phenotype. T2 plants (*TZP-OX*) were used for all of the described experiments.

Microarray Analysis. Microarray experiments were carried out per Affymetrix protocols and as described (7). Briefly, 7 day-old tissue was harvested under either continuous blue at subjective dawn, or every 4 hours (starting at dawn) under 12 hours light (white, 30 μM)/12 hours dark cycles (22 °C) over 1 day (6 time points). A total of 64 samples representing all genotypes under

both conditions in quadruplicate were collected. Tissue was collected in 2-ml microcentrifuge tubes with three ball bearings, frozen immediately in liquid nitrogen and stored at -80°C . Tissue was disrupted by using a Retsch shaker and extracted by using RNAeasy (QIAGEN) with on column RNase-free DNase treatment. Resulting RNA was checked for quality and then labeled probe was made with $5\ \mu\text{g}$ of RNA by using the Affymetrix kit (Affymetrix). A total of 33 samples were used for microarray analysis: Blue, 5 genotypes (*TZP-OX*, rHIF138-8, rHIF138-13, arHIF47-2, and arHIF47-5), three replicates; 12 hours light (white, $30\ \mu\text{M}$)/12 hours dark cycles, 3 genotypes (*TZP-OX*, rHIF138-8, and rHIF138-13), 6 time points (0, 4, 8, 12,

16, and 20 hours after lights on). Probe was hybridized to Arabidopsis ATH1 GeneChip arrays overnight, washed and scanned by using the standard Affymetrix protocol.

Hybridization intensities from all microarrays were normalized together by using gcRMA implemented in the R statistical package. The blue dataset was then separated and differentially expressed genes were identified by using linear modeling with the limma bioconductor package in R (8). Time course data were analyzed for differentially expressed genes by using the time points as replicates. Cycling genes were identified as described (7).

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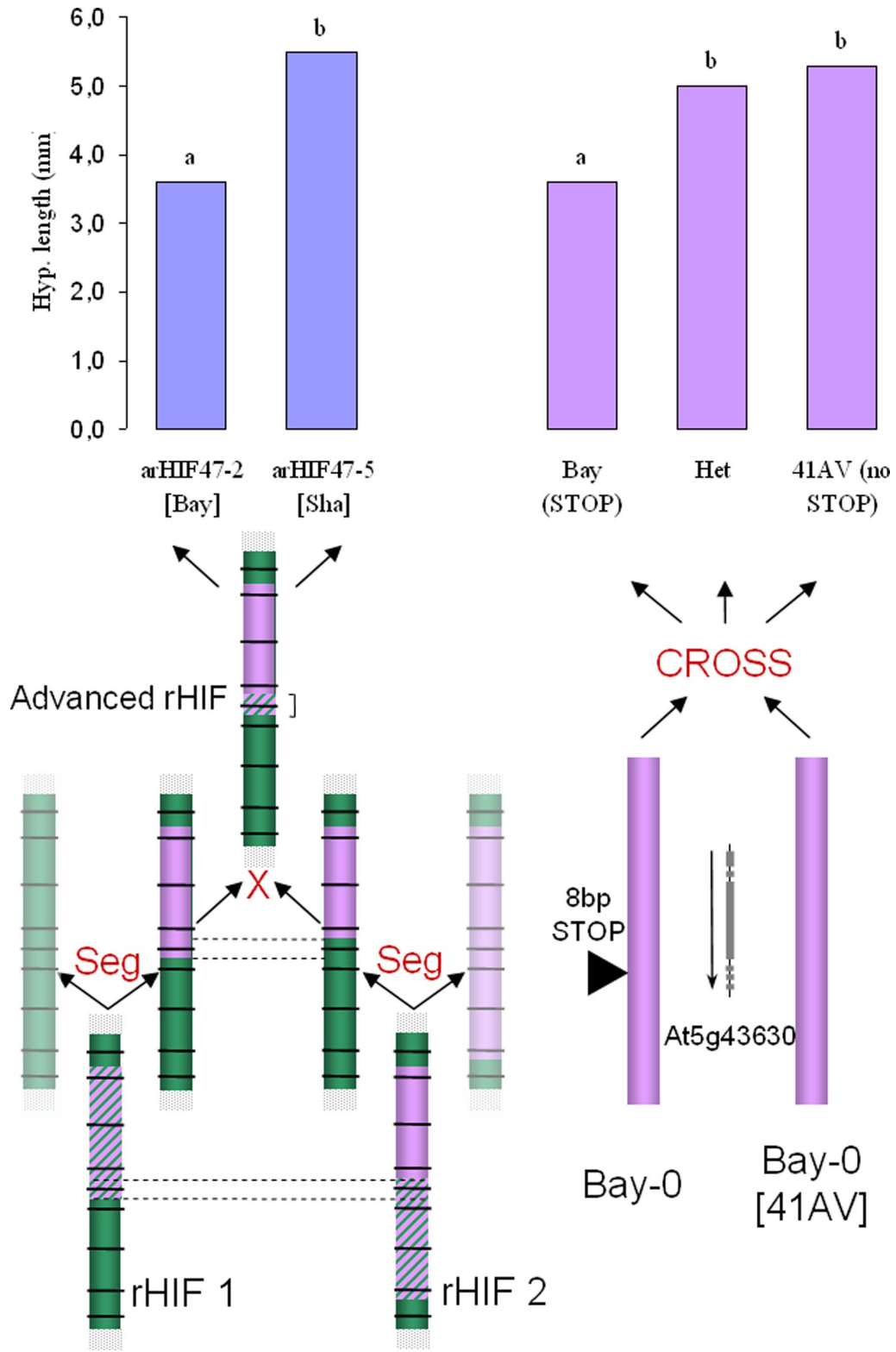


Fig. S2. *LIGHT5* is At5g43630 and the causal polymorphism is the 8-bp insertion in Bay-0. Two rHIFs segregating for *LIGHT5* and defining the limits of the candidate interval were crossed to generate an advanced rHIF (arHIF47) segregating solely for the 7-kb candidate region. Phenotypes are shown for the arHIF47 progeny fixed for either the Bay or Sha 7-kb region. Bay-0[41AV] lacks the 8-bp insertion causing the early stop in At5g43630 with the rest of the genome being the same as in Bay-0. Phenotypes are shown from F2 plants between the two isogenic parents. Different letters on bars indicate significantly different means ($P < 0.01$; least significant difference test).

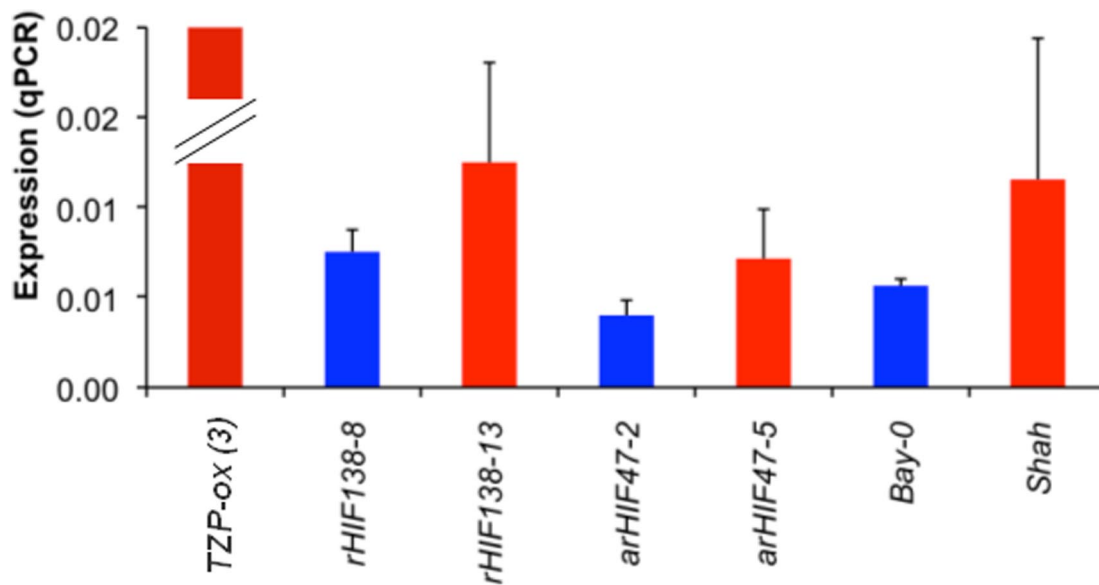


Fig. S4. TZP expression is correlated with increased hypocotyl growth. Plants were grown under continuous blue light for 5 days and tissue was collected at dawn. Expression was measured by qPCR. Expression of TZP was higher in lines with longer hypocotyls, including *TZP-OX* (3), *rHIF138-13*, *arHIF47-5*, and Shahdara. The bar for *TZP-OX* (3) was truncated because of the expression being on a different scale than the rest of the lines.

