Genetic Architecture of Natural Variation in Thermal Responses of Arabidopsis[1][OPEN]

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Wild strains of Arabidopsis (*Arabidopsis thaliana*) exhibit extensive natural variation in a wide variety of traits, including response to environmental changes. Ambient temperature is one of the major external factors that modulates plant growth and development. Here, we analyze the genetic architecture of natural variation in thermal responses of Arabidopsis. Exploiting wild accessions and recombinant inbred lines, we reveal extensive phenotypic variation in response to ambient temperature in distinct developmental traits such as hypocotyl elongation, root elongation, and flowering time. We show that variation in thermal response differs between traits, suggesting that the individual phenotypes do not capture all the variation associated with thermal response. Genome-wide association studies and quantitative trait locus analyses reveal that multiple rare alleles contribute to the genetic architecture of variation in thermal response. We identify at least 20 genomic regions that are associated with variation in thermal response. Further characterizations of temperature sensitivity quantitative trait loci that are shared between traits reveal a role for the blue-light receptor *CRYPTOCHROME2* (*CRY2*) in thermosensory growth responses. We show the accession Cape Verde Islands is less sensitive to changes in ambient temperature, and through transgenic analysis, we demonstrate that allelic variation at *CRY2* underlies this temperature insensitivity across several traits. Transgenic analyses suggest that the allelic effects of *CRY2* on thermal response are dependent on genetic background suggestive of the presence of modifiers. In addition, our results indicate that complex light and temperature interactions, in a background-dependent manner, govern growth responses in Arabidopsis.

Temperature is a critical environmental factor that has major effects on the growth, development, and distribution of plants across the globe (Fitter and Fitter, 2002; Samach and Wigge, 2005, 2013; Kotak et al., 2007; Penfield, 2008). With the predicted increase in global temperatures, and their potential impact on agricultural productivity, there are efforts to understand the genetic basis of temperature responses in plants. Traditionally, temperature effects have been studied in the context of extreme stress responses such as heat shock or cold shock (Kotak et al., 2007; Barrero-Gil and Salinas, 2013; Song et al., 2013; Storey and Storey, 2013). In recent times, there has been an interest in analyzing the response of plants to changes in their growth temperature within the nonstress range of l6°C to 27°C, as even small changes in temperature can have major impacts on plant growth and development (Wigge, 2013; Franklin et al., 2014). In this study, we refer to various phenotypic responses in plants that are attributable to small changes in ambient temperature as temperature/thermal response.

A few specific phenotypic responses are often used to uncover the genetic and molecular basis of thermal response in plants. These include temperature-induced changes in hypocotyl elongation, flowering time, and circadian clock as well as molecular phenotypes such as the expression levels of *At3g12580*, one of the 14 genes encoding HEAT SHOCK PROTEIN70 (HSP70; Gray et al., 1998; Blázquez et al., 2003; Halliday et al., 2003; Michael et al., 2003; Lempe et al., 2005; Balasubramanian et al., 2006b; Edwards et al., 2006; Koini et al., 2009; Kumar and Wigge, 2010; Kumar et al., 2012). In addition, cryptic phenotypes, which are often revealed in higher temperatures, are also used to uncover genes associated with variation in thermal response in plants (Queitsch et al., 2002, 2012; Sureshkumar et al., 2009).

Higher temperatures result in elongated hypocotyls, early flowering, and an increased *HSP70* expression (Gray et al., 1998; Blázquez et al., 2003; Balasubramanian et al., 2006b). Studies in the last decade have demonstrated

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that there is a genetic basis for most of the phenotypic responses observed upon temperature changes (Blázquez et al., 2003; Halliday et al., 2003; Balasubramanian et al., 2006b; Kumar and Wigge, 2010; Wigge, 2013). The temperature-induced hypocotyl elongation has been shown to be mediated by an increase in auxin levels, resulting from an increased expression of the *YUCCA* genes (e.g. *YUCCA8*), which encode enzymes involved in their biosynthesis (Gray et al., 1998; Koini et al., 2009; Franklin et al., 2011). The expression of the transcription factor *PHYTOCHROME INTERACTING FACTOR4* (*PIF4*) is induced at higher temperatures, which in turn leads to the increase in the expression of the *YUCCA* genes (Franklin et al., 2011). Recently, a role has been demonstrated for *DE-ETIOLATED1 (DET1)* and *ELONGATED HYPOCOTYL5 (HY5)* in controlling the hypocotyl elongation response by modulating *PIF4* expression according to the ambient temperature (Delker et al., 2014). *PIF4* has also been suggested to underlie temperature-induced early flowering in short days (Kumar et al., 2012).

Mutations in the *ACTIN-RELATED PROTEIN6* (*ARP6*) gene result in hyperactive thermal response even at lower temperatures (Kumar and Wigge, 2010). ARP6 is part of the SWR1 complex that plays a role in the incorporation/eviction of different histone variants (Deal et al., 2007). Based on this, it has been suggested that the incorporation/eviction dynamics of the histone variant H2A.Z on to the nucleosomes provide a direct mechanism for the perception of temperature (Kumar and Wigge, 2010). However, it has also been demonstrated that the presence of H2A.Z correlates with responsive genes and is not necessarily specific to temperature (Coleman-Derr and Zilberman, 2012).

On the other hand, the analysis of natural variation in flowering responses identified *FLOWERING LOCUS M* (*FLM*), which encodes a MADS domain containing floral repressor, to confer variation in thermosensory flowering response (Balasubramanian et al., 2006b). The function of *FLM* appears to be compromised at higher temperatures by changes in splicing patterns, resulting in derepression of *FLOWERING LOCUS T* (*FT*), and therefore the induction of flowering (Balasubramanian et al., 2006b; Balasubramanian and Weigel, 2006; Posé et al., 2013). Another MADS domain transcription factor SHORT VEGETATIVE PHASE (SVP), which interacts with FLM has also been shown to play a role in temperature-induced flowering, with the SVP protein being degraded at higher temperatures contributing to the derepression of *FT* (Lee et al., 2013). While there is agreement as to the thermal response in hypocotyl elongation, the genetic interaction between *FLM* and *PIF4* is yet to be analyzed (Verhage et al., 2014).

Here, we decipher and reveal extensive natural variation in thermal responses of Arabidopsis (*Arabidopsis thaliana*) by analyzing hundreds of accessions and recombinant inbred lines (RILs) across several traits. We show that the thermosensitivity measured in one trait is only weakly correlated with thermosensitivity in another trait. We use genome-wide association studies (GWASs) and quantitative trait locus (QTL) analysis to map loci for thermal response and show that the genetic architecture of thermal response comprises multiple genomic regions consisting of potentially rare alleles. We show allelic variation at *CRYPTOCHROME2* (*CRY2*) is associated with variation in thermal response. We identify Cvi-0 to be less responsive to temperature and demonstrate that the *CRY2*-Cvi-0 allele underlies this response. In addition to highlighting a role for CRY2 in thermal response, our results reveal complex genetic as well as genotype × environment interactions that link light and temperature response in plants.

RESULTS

Wild Strains of Arabidopsis Display Extensive Variation in Temperature Responses

Previous analyses indicated that there is substantial genetically controlled variation in thermal response to flowering time (Lempe et al., 2005; Balasubramanian et al., 2006b). To assess whether there is variation in thermal response in other phenotypes, we analyzed hypocotyl elongation in more than 5,000 plants representing 139 accessions at two different temperatures (23°C and 27°C in short days; Supplemental Table S1). There was substantial variation in hypocotyl lengths, with most accessions displaying longer hypocotyls at higher temperatures (Fig. 1A). ANOVA with the accessions as a factor of random effect and hypocotyl length as response revealed high heritability in hypocotyl elongation, with the genotypes accounting for 84% and 78% of the variation at 23°C and 27°C, respectively ($P < 0.0001$; Supplemental Table S2). This suggests that much of the observed variation is genetically controlled. When we combined the 23°C and 27°C data sets and analyzed the effects of temperature and genotype as well as genotype × temperature interactions through ANOVA with hypocotyl length as response, we found that 44% of variation could be attributed to the genotype ($P < 0.0001$). Consistent with the observed reduction in heritability (44% as opposed to 83% or 78% when data sets from different temperature were analyzed separately) with the combined data set, temperature accounted for 32% of the variation in hypocotyl lengths ($P < 0.0001$). Analysis of the reaction norms of accessions revealed that the response of the accessions to changes in temperature differed, suggestive of genotype × environment interactions (Fig. 1B; Supplemental Fig. S1). Consistent with this, through ANOVA, we quantified the effect of genotype × temperature interaction, which accounted for approximately 13% of the phenotypic variation ($P < 0.0001$). These findings suggested that there is extensive natural variation in thermal response in hypocotyl elongation.

To assess whether common factors modulate temperature sensitivity, we calculated the temperature sensitivity as the slope of the reaction norms for each of the accessions and compared the temperature sensitivity in

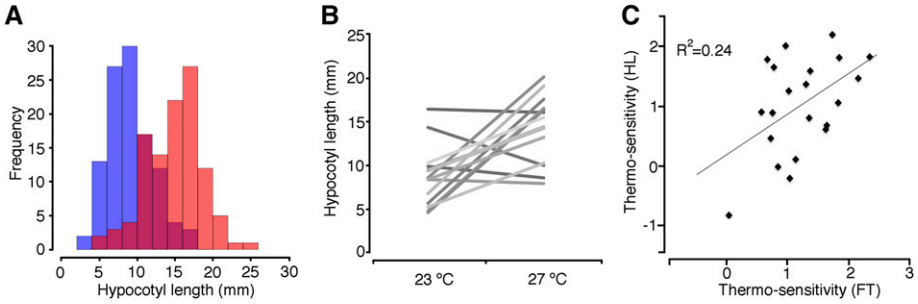

Figure 1. Natural variation in hypocotyl elongation response to temperature. A, Distribution of average hypocotyl lengths for accessions grown at 23°C (blue) or 27°C (red). The overlap is shown in a different color. B, Reaction norms of Arabidopsis accessions displaying differential thermal response in hypocotyl elongation. Only a small subset of accessions is shown. Crossing reaction norms indicate genotype × environment interactions. The reaction norms for all accessions are provided in Supplemental Figure S1. C, Correlation between temperature sensitivities in flowering time (FT) or hypocotyl elongation (HL). Temperature sensitivities were calculated from the slopes of the reaction norms of flowering time and hypocotyl elongation at 23°C and 27°C.

hypocotyl elongation with that of flowering time. We used the data from our current study and/or from our previous analysis (Supplemental Table S1; Balasubramanian et al., 2006b). There is a significant yet relatively small (R^2 = 0.24, $P < 0.0001$) correlation between thermosensitivities in flowering time and hypocotyl elongation (Fig. 1C). This suggests, while there are some common factors, phenotype-specific processes contribute to differential responses to temperature for different traits.

GWAS for Temperature-Induced Hypocotyl Elongation

To decipher whether there are common allelic variants that could explain the variation in thermal response in hypocotyl elongation, we took advantage of the genotypic information available through the 1,001 genomes project (http://www.1001genomes.org) and carried out association analysis for temperature sensitivity. The GWAS identified two regions that appear to be associated with temperature sensitivity (Fig. 2A; Supplemental Fig. S2). In chromosome 5, the Single Nucleotide Polymorphism (SNP) with the strongest association and the SNPs that are in linkage disequilibrium fell on the *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE2* (*SPL2*) gene (Fig. 2A). *SPL2* belongs to a family of transcription factors, which are targets for the temperature-responsive microRNA, *miR156* (Lee et al., 2010). Recently, it has also been shown that mutations in *SPL2* can modulate sensitivity to extreme temperature conditions, thus making *SPL2* a potential candidate gene for thermal response (Stief et al., 2014). Sequence analysis of *SPL2* using the 1,001 genomes project revealed several deletions in both the *SPL2* coding region as well as in the promoter, although further experiments are required to analyze the potential causal polymorphisms.

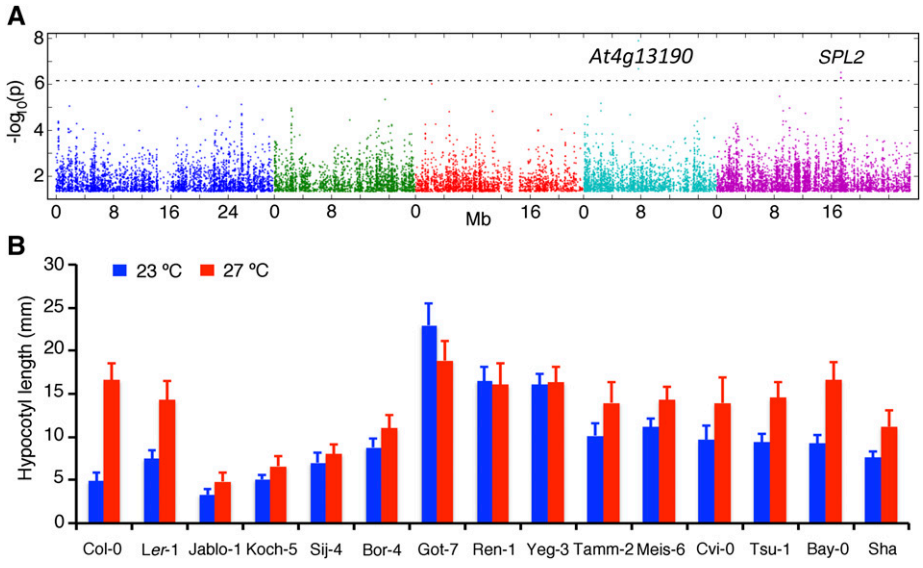

Figure 2. Genome-wide association analysis and the identification of strains with reduced thermal response in hypocotyl elongation. A, Manhattan plots for genetic association for temperature sensitivity in hypocotyl elongation generated through the GWAS Web Application. The plot generated through linear regression is shown here. For other models, refer to Supplemental Figure S1. The position of the candidate gene *SPL2* is shown. B, Hypocotyl elongation in accessions identified to be less responsive to ambient temperature changes including the parents of the RILs analyzed in this study.

The SNPs in the second region with significant association fell on a gene encoding a protein kinase (*At4g13190*), which is yet to be characterized (Fig. 2A). Association analysis using residuals of the regression of hypocotyl elongation at 23°C on to 27°C as a measure of temperature sensitivity provided similar results. In addition, the use of different statistical methods (Kruskal-Wallis analysis or approximate mixed model [AMM]) did not alter the Manhattan plots, although the significance of the association decreased in the AMM analysis (Supplemental Fig. S2). We did not find any additional SNPs with significant association. Thus, in contrast to flowering time where multiple common alleles have been found through GWAS (Atwell et al., 2010), there are fewer common allelic variants that appear to be associated with variation in temperature-induced hypocotyl elongation.

Temperature Sensitivity in Hypocotyl Elongation Segregates as a Multigenic Complex Trait

Because GWAS analysis identified only a couple of candidate regions, we looked for strains with reduced thermal response. To identify such accessions, we calculated the percentage of variance in hypocotyl elongation that can be attributed to temperature for each of the strains (Supplemental Table S1). For the majority (75%) of the strains, temperature accounted for more than 50% of the variation, with the median variance explained by temperature being 70.87%. We concentrated on the bottom quartile of the strains, with reduced temperature sensitivity, which included two different sets of behaviors (Fig. 2B). First, there were strains with impaired temperature-induced hypocotyl elongation in which the hypocotyl length was relatively short even at higher temperatures (e.g. Koch-5, Sij-4, Jablo-1, and Bor-4). Second, there were strains in which the hypocotyl length was much higher than the average at 23°C, suggesting that they exhibit a high temperature response even at lower temperatures (e.g. Got-7, Ren-1, Yeg-3, Meise-6, Tamm-2, and Cape Verde Islands [Cvi-0]). Among these strains, Sij-4 also displays a strong temperature-dependent growth defect, in addition to the absence of temperature-induced hypocotyl elongation. Further analysis of Sij-4 revealed that allelic variation at the *ICARUS1* locus, encoding a $tRNA^{His}$ guanylyl transferase, underlies this phenotype, indicating a role for fundamental processes in conferring variation in thermal response (Zhu et al., 2015). To decipher the genetic architecture of these accession-specific responses, we analyzed three F2 populations derived from the relatively temperature-insensitive strains (Tsushima [Tsu-0], Meise-6, and Yeg-3) with the reference strain Columbia (Col-0), which displays relatively higher sensitivity (Supplemental Fig. S3). All three analyzed F2 populations displayed a continuous distribution and lacked any bimodal distributions at either temperature, suggesting that multiple loci contribute to quantitative thermal responses in these strains.

Genetic Architecture of Temperature Response in RILs

Because our analysis suggested that thermal response is likely to be multigenic and quantitative, we chose to carry out QTL analysis using the RILs. We selected the Tsu-0 × Col-0, Cvi-0 × Col-0, and Bayreuth (Bay-0) × Shahdara (Sha) RILs in which the parental lines differed in their thermal response (Fig. 2B). To maximize capturing the variation associated with thermal response, we phenotyped all three RILs for several temperature sensitive traits (i.e. hypocotyl elongation, root elongation, and flowering time) across two different temperatures (23°C and 27°C). We observed high broad-sense heritabilities for all traits (Supplemental Table S2). Consistent with earlier observations, we observed longer roots, longer hypocotyls, and early flowering at higher temperatures across all RILs (Supplemental Tables S3–S5**)**. The genetic correlations between the different phenotypes were weak, suggesting that the underlying genetic factors are only partially shared between the phenotypes (Supplemental Table S6).

To identify the loci associated with thermal response, we applied two approaches. First, we identified QTLs that are detected only in one temperature, which indicates its temperature dependence. Second, we calculated temperature sensitivity for each of the three traits in the different RIL populations, used the same as phenotype, and carried out QTL analysis. We compiled the QTLs detected through these methods (Fig. 3; Table I; Supplemental Fig. S4; Supplemental Table S7). Among a total of 53 QTLs detected for a variety of traits, we observed 19 genomic regions that contribute to temperature response (Fig. 3; Table I). While some of the regions were detected as QTLs for multiple traits across populations, a majority of the QTLs were specific for individual traits, confirming that the analysis of multiple traits is required to capture variation in temperature response (Supplemental Table S7). Most QTLs (approximately 66%) for all the traits showed large effect explaining more than 10% of the phenotypic variance, while the remaining loci showed a moderate effect (5%–10%). Our analysis lacked the power to capture QTLs that accounted for less than 5% of phenotypic variation.

Allelic Variation at *CRY2* Modulates Temperature Sensitivity

Among the parental strains used in our analysis, Cvi-0 plants appeared very similar at 23°C and 27°C in several ways, suggesting that they had a reduced response to this change in temperature (Fig. 4, A–C). In contrast to Col-0, Cvi-0 displays high temperature (27°C)-associated phenotypes such as longer hypocotyls, elongated petioles, early flowering, and altered plant architecture at lower temperatures (23°C) as well (Fig. 4, A–C). QTL analysis for temperature sensitivity in both flowering time and hypocotyl elongation in the Col-0 × Cvi-0 RILs mapped to the top of chromosome 1

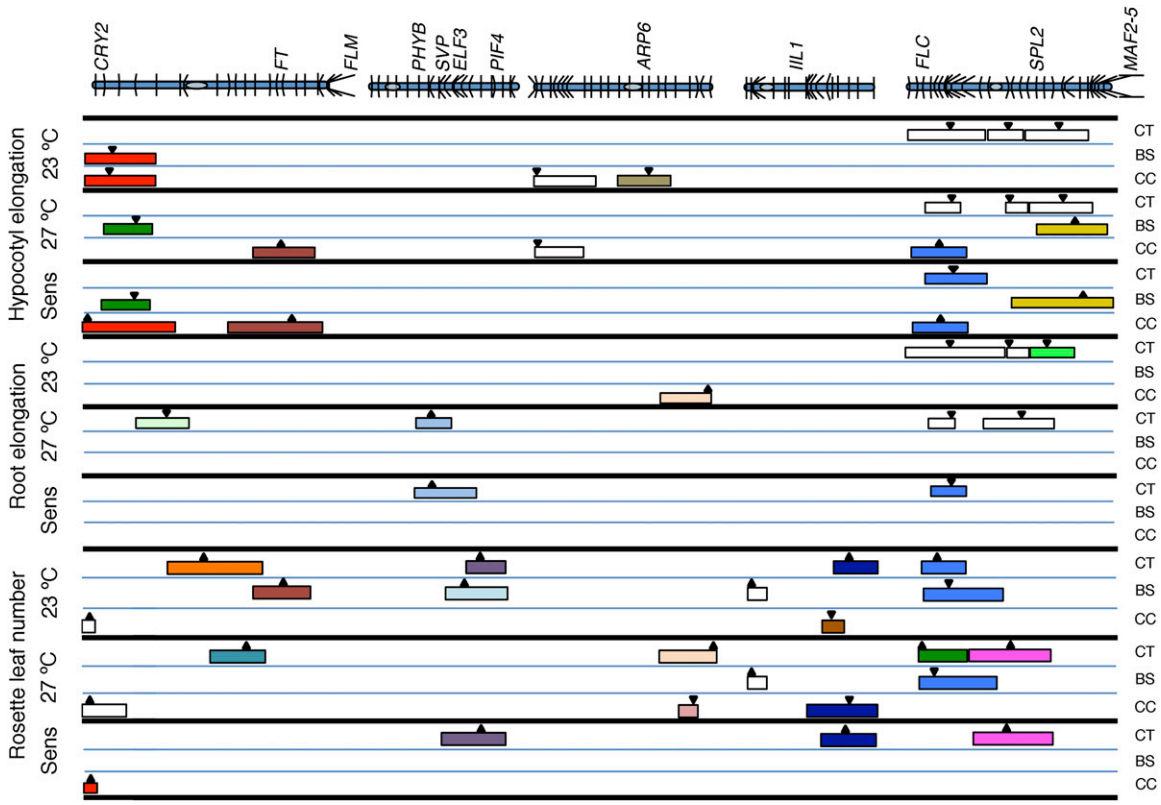

Figure 3. Comparative map positions of the QTLs. QTLs detected for hypocotyl elongation, root elongation, and rosette leaf number at two different temperatures (23°C and 27°C) as well as temperature sensitivity (Sens) for all three traits in three different RILs is depicted. The QTLs representing potentially the same loci are shaded in the same color, with the colored box representing two LOD confidence intervals. The arrow represents the position with the highest LOD threshold. The upward or downward arrows indicate that the Col-0 and Sha alleles increase and decrease the trait value, respectively. The positions of genes known to be associated with ambient temperature response are shown above. CT, Col-0 × Tsu-0; BS, Bay-0 × Sha; CC, Col-0 × Cvi-0; *MAF2-5, MADS AFFECTING FLOWERING2-5*.

(Fig. 4D). RILs that carry the Cvi-0 allele at this QTL displayed reduced temperature sensitivity due to longer hypocotyls and early flowering at lower temperatures.

Within the confidence intervals for these QTLs, Cvi-0 is known to carry a hyperactive gain-of-function allele of *CRY2* that leads to early flowering, and this phenotype has been attributed to the function of *CRY2* as a blue-light receptor in light response (El-Din El-Assal et al., 2001). A hyperactive photoreceptor would increase sensitivity to light and thus lead to a shorter hypocotyl. However, Cvi-0 exhibits longer hypocotyls at both temperatures. To test whether the *CRY2*-Cvi-0 allele is the underlying locus for the longer hypocotyls and reduced temperature sensitivity of Cvi-0, we generated artificial microRNAs against *CRY2* in the Cvi-0 background (*35S*::*amiRCRY2*) in which the *CRY2* expression levels were severely reduced (Supplemental Fig. S5A). We assessed the transgenic plants for hypocotyl elongation and flowering time at varied temperatures (Fig. 5). *35S*::*amiRCRY2* lines displayed shorter hypocotyls compared with Cvi-0, suggesting that Cvi-*CRY2* contributes to the longer hypocotyls observed in Cvi-0 (Fig. 5A). However, this inhibition of hypocotyl elongation was observed only at 23°C, and the *35S*::*amiRCRY2* lines displayed temperature-induced hypocotyl elongation and restored temperature sensitivity. This suggests that the *CRY2*-Cvi-0 allele contributes to the longer hypocotyls observed at lower temperatures and *CRY2* is one of the genes underlying the compromised ambient thermal response observed in the Cvi-0 accession (Fig. 5A). Consistent with this, the *35S*::*amiRCRY2*-Cvi-0 lines also flowered later, again, only at lower temperatures, thus restoring temperature sensitivity in flowering time as well (Fig. 5B). In addition, *35S*::*amiRCRY2*-Cvi-0 plants also displayed short petioles and altered plant architecture and appeared similar to Col-0 plants grown at lower temperatures, suggesting that *CRY2*-Cvi-0 allele underlies the high-temperature phenotype exhibited by Cvi-0 even under lower thermal regimes (Supplemental Fig. S5, B and C).

To test whether allelic variation at *CRY2* modulates temperature sensitivity, we generated transgenic plants expressing *CRY2* alleles from Col-0 ($_{p}CRY2_{Col}$::$CRY2_{Col\text{-}0}$) or Cvi-0 ($_{p}CRY2_{Cvi}$::$CRY2_{Cvi\text{-}0}$) in the *cry2-1* mutant background (Guo et al., 1998) and compared the

Table I. *Summary of temperature-sensitive QTLs detected in this study*

ColTsu, Col-0 × Tsu-0 RILs; ColCvi, Col-0 × Cvi-0 RILs; BaySha, Bay-0 × Sha RILs; Chr, chromosome; Position, genomic position of the marker with highest LOD threshold; Interval, 2× LOD confidence interval for the QTL; RLN, rosette leaf number.

RIL	Chr	Position	Interval	Trait	Category	QTL
CviCol	1	0.59	0.59–1.62	RLN	Sensitivity	1
CviCol	1	0.59	0–5.0	Hypocotyl length	Sensitivity	1
CviCol	1	2.99	0–9.19	Hypocotyl length	23Specific	1
BaySha	1	3.21	0–8.6	Hypocotyl length	23Specific	1
BaySha	1	4.99	2.00–8.19	Hypocotyl length	Sensitivity	2
BaySha	1	6.37	2–8.19	Hypocotyl length	27Specific	2
ColTsu	1	10.57	6.53–13.27	Root length	27Specific	3
ColTsu	1	15.04	10.57–22.79	RLN	23Specific	4
ColTsu	1	19.79	15.4–22.79	RLN	27Specific	5
CviCol	1	25.1	17.8–29.3	Hypocotyl length	Sensitivity	6
CviCol	1	25.1	21.59–29.3	Hypocotyl length	27Specific	6
BaySha	1	25.82	20.63–29.01	RLN	23Specific	6
ColTsu	2	9.65	7.6–11.84	Root length	27Specific	7
ColTsu	2	9.65	6.93–14.66	Root length	Sensitivity	7
BaySha	2	12.98	10–19.42	RLN	23Specific	8
ColTsu	2	14.66	9.65–18.0	RLN	Sensitivity	9
ColTsu	2	14.66	11.84–18	RLN	23Specific	9
CviCol	3	14.92	11–17.99	Hypocotyl length	23Specific	10
CviCol	3	20.19	18.19–20.72	RLN	27Specific	11
CviCol	3	22.14	16.67–22.14	Root length	23Specific	12
ColTsu	3	23.04	16–23	RLN	27Specific	12
CviCol	4	11.87	10.6–13.17	RLN	23Specific	13
ColTsu	4	14.17	10.0–17.0	RLN	Sensitivity	14
ColTsu	4	14.17	12.53–17.04	RLN	23Specific	14
CviCol	4	14.8	8.91–17.67	RLN	27Specific	14
ColTsu	5	1.01	0–7.99	RLN	27Specific	15
BaySha	5	2.77	1.5–12.1	RLN	27Specific	16
ColTsu	5	3.44	1.01–7.99	RLN	23Specific	16
BaySha	5	4.64	1.47–14	RLN	23Specific	16
CviCol	5	4.74	0–9.85	Hypocotyl length	Sensitivity	16
CviCol	5	4.74	0–7.99	Hypocotyl length	27Specific	16
ColTsu	5	5.31	2.33–7.99	Root length	Sensitivity	16
ColTsu	5	5.31	2.33–10	Hypocotyl length	Sensitivity	16
ColTsu	5	13.04	5–18.74	RLN	Sensitivity	17
ColTsu	5	13.04	7.99–18.74	RLN	27Specific	17
ColTsu	5	18.74	15.79–21.84	Root length	23Specific	18
BaySha	5	23.87	17.25–25.92	Hypocotyl length	23Specific	19
BaySha	5	23.87	13.96–25.92	Hypocotyl length	Sensitivity	19

transgenic lines for their temperature sensitivity. While the $_{p}CRY2_{Col}$::$CRY2_{Col\text{-}0}$ plants displayed temperature sensitivity in hypocotyl elongation, $_{p}CRY2_{Cvi}$::$CRY2_{Cvi\text{-}0}$ plants were insensitive to temperature (Fig. 5C). In addition, the temperature insensitivity was also observed in flowering time, petiole elongation, and plant architecture, confirming that allelic differences at *CRY2* underlie variation in temperature sensitivity between the Col-0 and Cvi-0 accessions of Arabidopsis (Fig. 5C; Supplemental Fig. S6, A–C).

Genotype × Environment Interactions Modify the Effects of *CRY2*-Cvi-0

The effects of the *CRY2*-Cvi-0 allele on hypocotyl elongation differed between the Cvi-0 and Col-0 backgrounds. While displaying temperature insensitivity in both backgrounds, the *CRY2*-Cvi-0 allele conferred longer hypocotyls in the Cvi-0 background and shorter hypocotyls in the Col-0 background (Fig. 5, A and C), suggesting the presence of background-specific modifiers of *CRY2* function in this response. In addition, F1 plants derived from a Col-0 × Cvi-0 cross exhibited temperature-induced hypocotyl elongation, indicating that the temperature sensitivity conferred by the *CRY2*-Cvi-0 is recessive in nature (Supplemental Fig. S7A). This is in contrast to flowering time, where the *CRY2*-Cvi-0 allele acts as a dominant allele, which is attributed to the increased stability of the CRY2-Cvi-0 protein (El-Din El-Assal et al., 2001). However, temperature does not seem to affect the stability of the CRY2 protein, as previous studies found no obvious differences between different temperatures (Gould et al., 2013). Therefore, we wondered whether differential light × temperature interactions might modulate

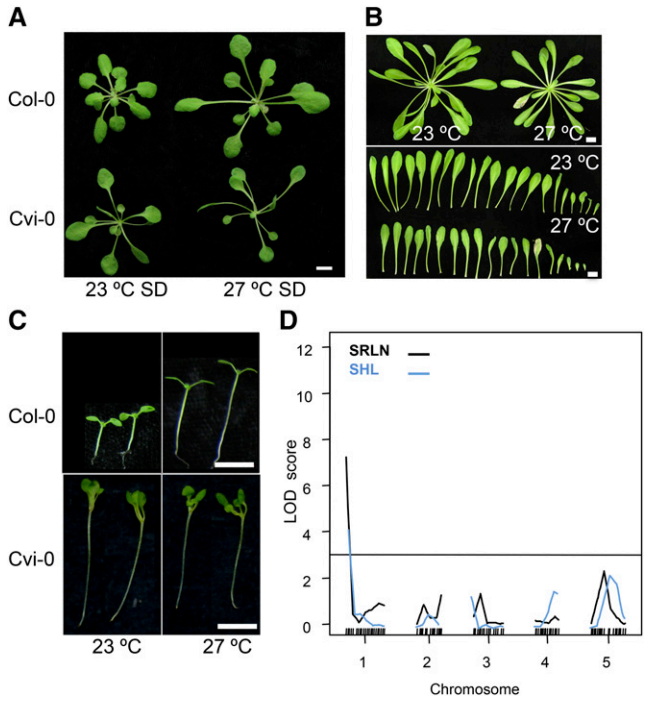

Figure 4. Reduced sensitivity to temperature in Cvi-0 maps to the top of chromosome 1. A, Architecture of Col-0 or Cvi-0 plants grown at 23°C or 27°C. B, Petiole elongation in Cvi-0 at 23°C compared with 27°C. C, Temperature-induced hypocotyl elongation in Col-0 compared with Cvi-0. D, QTL analysis of temperature sensitivity in hypocotyl elongation (light blue) or flowering time (black) in the Col-0 × Cvi-0 RILs. Bars = 6 mm (A, C, and D) and 10 mm (B). SRLN and SHL represent temperature sensitivity in rosette leaf number (SRLN) and hypocotyl length (SHL).

the discrepancy in hypocotyl elongation observed in both backgrounds.

The *CRY2*-Cvi-0 has been previously shown to confer enhanced cotyledon unfolding during photomorphogenesis (Botto et al., 2003). Therefore, we hypothesized that a short hypocotyl may result from enhanced cotyledon opening, which may inhibit hypocotyl elongation. To test this, we compared the effects of *CRY2*-Cvi-0 and *CRY2*-Col-0 alleles on cotyledon opening in the *cry2-1* background. The cotyledon unfolding was significantly higher in the plants carrying the *CRY2*-Cvi-0 allele compared with the plants harboring the *CRY2*-Col-0 allele in the *cry2-1* mutant background across all the conditions tested (Fig. 5D; Supplemental Fig. S6E). In addition, the $_{p}CRY2_{Cvi}$::$CRY2_{Cvi\text{-}0}$ plants in the *cry2-1* mutant background displayed cotyledon opening very early compared with $_{p}CRY2_{Col}$::$CRY2_{Col\text{-}0}$ plants at both temperatures (Fig. 5D). This is consistent with the hypothesis that the hypocotyl elongation stops after cotyledon opening, resulting in the shorter hypocotyls. This effect was seen in both Col-0 (carrying the endogenous *CRY2*-Col-0 allele) as well as the *cry2-1* mutants, suggesting that this photomorphogenetic phenotype conferred by the *CRY2*-Cvi-0 is dominant in the Col-0 background (Fig. 5D; Supplemental Fig. S6D). However, this phenotype was not that obvious in the Cvi-0 accession, which harbors the *CRY2*-Cvi-0 allele (Fig. 5D). While the Cvi-0 plants did display mild cotyledon opening, it was quite weak compared with the $_{p}CRY2_{Cvi}$::$CRY2_{Cvi\text{-}0}$ plants in the Col-0 background (Fig. 5D; Supplemental Fig. S6D). Therefore, it appears that the early cotyledon opening in the Col-0 background results in short hypocotyls at both temperatures, contrasting its effect of longer hypocotyls in the Cvi-0 background at both temperatures. Thus, genetic background modifies the effect of the *CRY2*-Cvi-0 allele on light response, which in turn alters the temperature-induced hypocotyl elongation response.

To assess the underlying mechanism for this background-dependent effect, we analyzed the expression levels of *CRY2* in Col-0, Cvi-0, and the different transgenic lines (Supplemental Fig. S8). We found the $_{p}CRY2_{Col}$::$CRY2_{Col\text{-}0}$ plants to be expressing *CRY2* similar to Col-0 or Cvi-0, while there was a major increase in the expression levels of the *CRY2*-Cvi-0 allele in the Col-0 background (Supplemental Fig. S8). This suggests that the observed phenotypic differences could be attributed at least partially to the differential activity of the *CRY2*-Cvi-0 promoter in the Col-0 and Cvi-0 backgrounds.

Because the original *CRY2* allelic variation was defined through the analysis of Landsberg *erecta* (L*er*) and Cvi-0 accessions, we analyzed the F1 plants derived from the L*er* × Cvi-0 cross, which revealed that the temperature insensitivity segregated as a recessive trait in this cross as well (Supplemental Fig. S7A). In addition, the near-isogenic line *EDI*-NIL (El-Din El-Assal et al., 2001), which harbors the genomic region encompassing the *CRY2* locus from Cvi-0 in the otherwise L*er* background, also displayed temperature sensitivity, suggesting the presence of modifiers in the L*er* background that suppress the effects of the *CRY2*-Cvi-0 allele. Consistently, transgenic plants in the L*er* background carrying the L*er* or Cvi-0 alleles for *CRY2* also displayed temperature-induced hypocotyl elongation (Supplemental Fig. S7B). Thus, genetic modifiers in different backgrounds modulate the light × temperature interactions conferring the differential responses seen in Arabidopsis accessions.

DISCUSSION

Multiple Rare Alleles Rather Than Common Alleles Underlie Most of the Variation in Thermal Responses in Arabidopsis

In this study, we have uncovered the genetic architecture of temperature-dependent growth responses in Arabidopsis. Thermal response can be measured using a variety of phenotypes. While we observed correlation between temperature sensitivities for flowering time and hypocotyl elongation among accessions, there was almost no correlation between thermal responses measured through different traits in the RILs (Supplemental Table S6), suggesting that the underlying molecular mechanisms modulating thermosensitivity differ

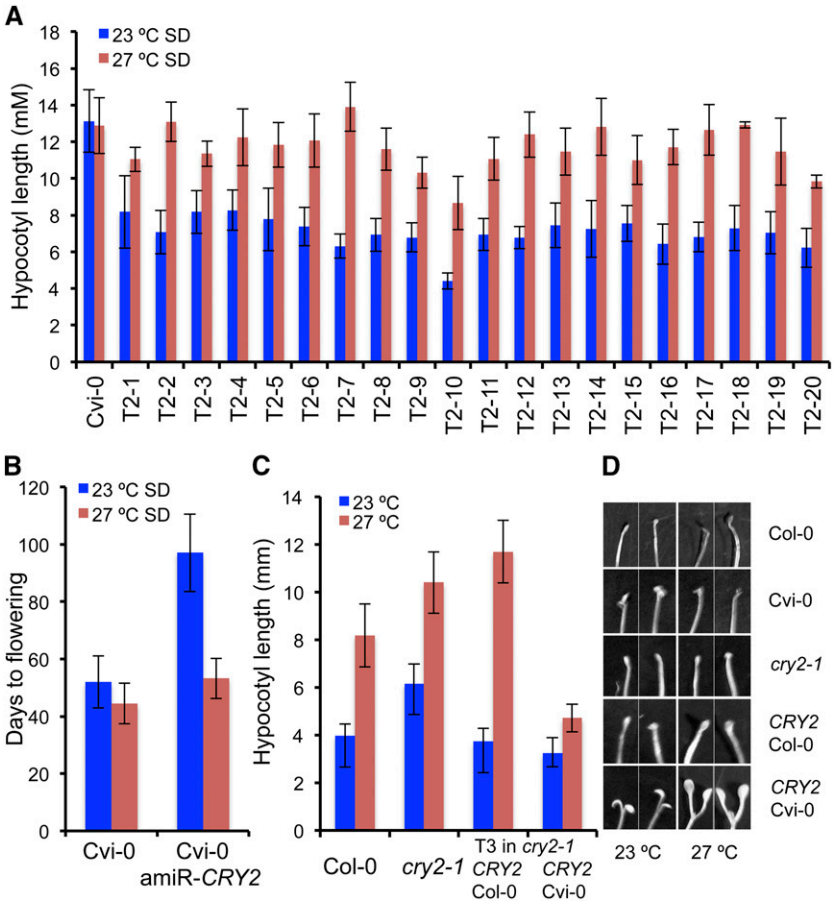

Figure 5. Allelic variation at *CRY2* modulates thermal responses in Arabidopsis. A, Restoration of temperature sensitivity in hypocotyl elongation in plants in which *CRY2* function is compromised via artificial microRNA against *CRY2*. Hypocotyl elongation at two different temperatures in 20 independent T2 families of *35S*::*amiRCRY2* lines in the Cvi-0 background compared with Cvi-0. B, Restoration of temperature sensitivity in flowering time in *35S*::*amiRCRY2* in the Cvi-0 background, measured as days to flowering. C, Hypocotyl elongation at different temperatures in T3 transgenic plants carrying either the Col-0 or the Cvi-0 allele in the *cry2-1* background compared with Col-0 and *cry2-1*. D, Cotyledon opening response in Col-0, Cvi-0, *cry2-1*, and transgenic plants carrying either the Col-0 (*CRY2pro*::*CRY2*-Col-0) or the Cvi-0 allele (*CRY2pro*::*CRY2*-Cvi-0) in the Col-0 background in dark. SD, Short-day condition.

between distinct traits. Consistent with this, out of the 12 QTLs detected for temperature sensitivity, only two QTLs were shared between traits and RILs. Nevertheless, we have identified several genomic regions that modulate temperature responses in specific traits. Therefore, our results show that analysis of temperature response using individual traits fails to capture the majority of variation in thermal response.

We have carried out GWAS for hypocotyl elongation and temperature sensitivity in this response. Unlike flowering time, for which several strong associations have been reported (Atwell et al., 2010), we found no associations with hypocotyl length and detected only two regions associated with temperature sensitivity in hypocotyl elongation. This suggests that the genetic architecture of thermal response in hypocotyl elongation is quite different from the genetic architecture of flowering time (Salomé et al., 2011). However, there is extensive genetically controlled phenotypic variation, as the heritabilities for hypocotyl elongation at both temperatures were quite high. Therefore, it appears that multiple loci contribute to thermal responses in hypocotyl elongation. Consistent with this, QTL analysis uncovered multiple genomic regions that display temperature-dependent effects. This suggests that most of the variation in thermal response is attributable to alleles that occur at relatively low frequency in populations, which are not easily detectable through association analysis. This is consistent with the findings that often genes with demonstrated allelic effects in hypocotyl elongation have been identified only in a subset of strains, with common allelic variation having been reported only for the *PHYTOCHROME B* (*PHY B*) and *PHYC*, although rare alleles of *PHYA*, *PHYD*, and *CRY2* have been described (Aukerman et al., 1997; El-Din El-Assal et al., 2001; Maloof et al., 2001; Balasubramanian et al., 2006a; Filiault et al., 2008). Thermal response, a complex trait, modulated both by genetic and genotype × environment interactions, may benefit from multiple loci/alleles, which may allow incremental changes in a smaller scale that could suit adaptations to multiple environments. Overall, our findings suggest that most of the variation in thermal response may be conferred through rare alleles rather than common alleles in natural populations.

GWAS and QTL Analysis Identifies Loci Associated with Thermal Response

Several earlier studies have shown that the expression level of *miR156* changes in response to growth

temperature (Lee et al., 2010; An et al., 2011; May et al., 2013; Stief et al., 2014). *miR156* levels regulate developmental transitions in plants, including the floral transition (Wang, 2014). Most of the *miR156*-related responses have been explained through its role in down-regulating the *SPL* family of transcription factors (Wang, 2014). Among the *SPL* family of transcription factors, *SPL2* is relatively less explored, and recently, it has been linked with heat shock memory and thermo tolerance (Stief et al., 2014). Our studies have identified a strong association between allelic variations at *SPL2* and temperature sensitivity in hypocotyl elongation, making *SPL2* an obvious candidate gene for ambient temperature response. The SNPs with strongest associations were within the promoter of the *SPL2* gene. However, the promoter region of *SPL2* appears to harbor several deletions, and thus further analysis is required to confirm a role for *SPL2* and to work out the potential underlying mechanisms for this response.

The QTL mapping revealed several regions that appear to modulate sensitivity to temperature, which include known as well as additional candidate genes for thermal response. Consistent with earlier findings, a QTL for flowering time in the Bay-0/Sha population based around the *FLM* gene was detected only at 23°C but not at 27°C (Supplemental Table S7), suggesting that real differences are captured in our approach. One of the QTL for thermosensitive growth responses across multiple traits/RILs mapped to the top of chromosome 5, which includes the candidate gene *FLOWERING LOCUS C* (*FLC*). *FLC*, in addition to playing a key role in vernalization response (Michaels and Amasino, 1999; Sheldon et al., 1999), has also been linked to pleiotropic effects, including temperature compensation of the circadian clock, thermosensitivity in flowering time, and plant architecture (Michaels and Amasino, 1999; Sheldon et al., 1999; Poduska et al., 2003; Edwards et al., 2005, 2006; Balasubramanian et al., 2006b; Sibout et al., 2008). Therefore, it is conceivable that *FLC* could be the underlying locus for this QTL for thermal response.

We found several genes for which natural variation has been reported to colocalize with some of the QTLs that we detected for thermal response. The region around the *FT* gene, which has been previously implicated in thermal response, was identified as a region for temperature sensitivity in hypocotyl elongation (Balasubramanian et al., 2006b; Schwartz et al., 2009; Liu et al., 2014). We found QTLs encompassing the *EARLY FLOWERING3* (*ELF3*) gene as a QTL for temperature sensitivity in flowering time in the Col-0/Tsu-0 and Bay/Sha RILs (Thines and Harmon, 2010; Undurraga et al., 2012; Mizuno et al., 2014; Box et al., 2015). Similarly, a QTL around *PIF4* was found as a QTL for temperature sensitivity in flowering time in the Col-0/Tsu-0 population (Brock et al., 2010; Kumar et al., 2012; Delker et al., 2014). The MADS domain transcription factor *SVP* plays a role in thermal response, it has also been identified as a candidate conferring natural variation through both association mapping and QTL analysis, and its effect is also dependent on the genetic background (Lee et al., 2007, 2013; Atwell et al., 2010; Méndez-Vigo et al., 2013). We found a QTL for thermosensitivity in root length mapping to this region. Further analysis is required to verify any of these genes are the causal genes for the identified QTLs. There were some regions that appeared in more than one RIL/trait, for which we did not find any obvious candidate genes. These include the bottom of chromosome 4, which was detected as a QTL for temperature sensitivity in flowering in the Col-0/Tsu-0 population and as a QTL for flowering time only at 27°C in the Col-0/Cvi-0 population.

Cvi-0 Is Less Sensitive to Small Changes in Ambient Temperature

Several studies have revealed that the accession Cvi-0 is unusual in both phenotypic as well as genotypic characteristics (Alonso-Blanco et al., 1998; Rao and Davis, 1999; Borsani et al., 2001; El-Din El-Assal et al., 2001; Nordborg et al., 2005; Tessadori et al., 2009; Vasseur et al., 2011, 2014). Our findings show that within the tested ambient temperature range (23°C–27°C), Cvi-0 exhibits high temperature-associated phenotypes such as early flowering, longer hypocotyls, and elongated petioles even at lower temperatures. Cvi-0 was collected from the Cape Verde islands, where the fluctuations in average temperatures throughout the year is fairly minimal (23°C–27°C for most months) when compared with other natural habitats for Arabidopsis. Therefore, our findings might reflect a relaxed sensitization to temperature within this range, which may suit local environmental conditions. Our observations are also consistent with previous studies, which have shown that Cvi-0 is less sensitive to stomatal opening in response to environmental changes (Monda et al., 2011; Vasseur et al., 2014). Leaf temperature measurements in response to CO_2 revealed that, unlike Col-0, Cvi-0 displayed minimal changes in leaf temperature (Monda et al., 2011). Furthermore, it has been shown that the heat-induced leaf hyponasty response is reduced in the Cvi-0 accession (van Zanten et al., 2009), consistent with our findings. A recent study by Suter and Widmer (2013) also found the Cvi-0 accession to be less responsive to a 3-d heat shock treatment. However, our studies do not rule out differential thermal responses in Cvi-0 in other temperature ranges.

We have shown that the *CRY2*-Cvi-0 allele is at least partially responsible for this reduced temperature sensitivity. Knocking down *CRY2* in the Cvi-0 background increased sensitivity to temperature across a variety of traits supporting this hypothesis. The restoration of temperature sensitivity in the *amiRCRY2*-Cvi-0 lines is mostly due to a suppression of hypocotyl elongation at lower temperature conditions, suggesting that *CRY2*-Cvi-0 confers the high-temperature response at lower temperatures. Previous studies have shown that the *CRY2*-Cvi-0 allele inhibits hypocotyl elongation in response to an increase in blue light (El-Din El-Assal et al.,

2001). Therefore, this role of *CRY2*-Cvi-0 in inducing hypocotyl elongation is likely to be independent of its role in light signaling. However, the exact mechanism through which *CRY2*-Cvi-0 confers temperature insensitivity remains unknown. Previous studies have shown that the stability of the *CRY2* protein does not change in response to a change in temperature (Gould et al., 2013). While *CRY2*-Cvi-0 is more stable than *CRY2*-L*er*, as shown earlier (El-Din El-Assal et al., 2001), it is still unclear how a stable *CRY2*-Cvi-0 modulates temperature perception and response. It is interesting to note that although *CRY2* is known very well for its role in blue-light perception, it has also been implicated in sensing redox status and magnetism as well as entrainment of the circadian clock by temperature (Kaushik et al., 2007; Gegear et al., 2008; Yoshii et al., 2010; Fogle et al., 2011). Therefore, it is conceivable that CRY2-Cvi-0, due to its increased stability, may also modulate thermal response, although further studies are required to explore the underlying mechanism.

Thermal Responses Are Modified through Light × Temperature Interactions in a Background-Dependent Manner

Plant response to environmental changes is often a combinatorial response to multiple cues. Our study provides a nice example for how light and temperature interact to elicit a phenotypic response. We have shown that the *CRY2*-Cvi-0 allele leads to cotyledon opening even in darkness. This is consistent with earlier findings (Botto et al., 2003; Vasseur et al., 2014), where it has been demonstrated that the cotyledon unfolding to hourly pulses of far-red light is stronger in the Cvi-0 accession compared with Col-0 and CRY2-Cvi-0 may be contributing to altered thermal responses. However, in contrast to earlier studies, we have analyzed the effects of the alleles in a common loss-of-function background (*cry2-1* in the Col-0 background), which allows us to decipher some of these interactions precisely. The *CRY2*-Cvi-0 allele is a gain-of-function allele with respect to its blue-light response and acts in a dominant manner. The dominant gain-of-function in blue-light signaling leads to an early cotyledon opening even in dark. Once cotyledons are open, the hypocotyl elongation stops. Thus, in the Col-0 background, we observed abolition of the temperature-induced hypocotyl elongation, because the cotyledons are opened due to the hyperactive light signaling conferred by *CRY2*-Cvi-0. The increased expression levels of *CRY2*-Cvi-0 specifically in the Col-0 background could possibly be the underlying mechanism for the same. While analysis of an individual phenotype would suggest that *CRY2*-Cvi-0 in the Col-0 background is insensitive in temperature-induced hypocotyl elongation, the underlying mechanism appears to the hyperactive light signaling conferred by the *CRY2*-Cvi-0, which leads to early cotyledon opening. However, it remains unclear as to how the *CRY2*-Cvi-0 confers differential response in petiole elongation in the Cvi-0 and Col-0 backgrounds. Nevertheless, the thermal response phenotype is an indirect effect of light × temperature interactions. On the other hand, we observed that these effects of this allele are highly attenuated in the Cvi-0 background, demonstrating how light and temperature interactions in a background-dependent manner modulate phenotypic responses in Arabidopsis.

Our findings support that unlike flowering time, the genetic architecture of the natural variation in thermal response likely comprises multiple small-effect alleles that occur at low frequency across global populations. We have uncovered several potential genomic regions, which can now be explored further to identify the causal genes that modulate natural variation in thermal response. We have characterized one of the underlying QTLs, which reveal a role for allelic variation at *CRY2* in mediating natural variation in thermal response. Our findings with *CRY2* demonstrate the complexity of light and temperature interactions and the effect of various genetic backgrounds on this interaction. Future studies would uncover the modifiers that regulate the light × temperature interactions, which lead to differential growth responses in plants.

MATERIALS AND METHODS

Plant Material and Growth Conditions

All the accessions used in this study are listed with additional information in Supplemental Table S1. Seeds for accessions and the mutants were obtained from the Arabidopsis Biological Resource Center, the Institut National de la Research Agronomique, or RIKEN. The *EDI*-NIL and the *CRY2* transgenic lines in the L*er* background were gifted by Dr. Carlos Alonso-Blanco. The *cry2* mutants in the L*er* background (*fha-1–fha-3*) and the Col-0 background (*cry2-1*, CS3732) have been previously described (Koornneef et al., 1991; Guo et al., 1998). The transgenic lines harboring the *CRY2*-Col-0 and *CRY2*-Cvi-0 alleles were generated in this study (see below). The plants were grown in controlled growth chambers (Percival Scientific) or growth rooms at 23°C or 27°C under short-day (16-h-dark/8-h-light) conditions. Plants for transformation were grown in long day (16-h-light/8-h-dark) conditions. Relative humidity was maintained at 65% in all conditions. The plants were watered periodically.

Scoring of Flowering Time, Hypocotyl Elongation, and Primary Root Length

Flowering time was measured using either days to flowering or rosette leaf number, as described previously (Lempe et al., 2005). Hypocotyl elongation was measured either in plates or in soil. For the plate measurements, seeds were sterilized either by washing in 70% (v/v) ethanol with 0.1% (v/v) Triton X-100 for 3 to 5 min followed by 95% (v/v) ethanol for 1 to 2 min or by washing in bleach for 20 min followed by a water wash. Twenty seeds for each genotype (accession or RIL) were spotted onto 0.5× or 1× Murashige and Skoog media (pH 5.7) with or without Suc. Plates were placed in light- and humidity-controlled Percival chambers. For measurements in soil, seeds were stratified for 4 d in 0.1% (w/v) agarose and sown in soil, and the plants were cultivated in growth rooms at 23°C or 27°C in short days. Ten-day-old seedlings from soil were transferred to acetate sheets and scanned on a flatbed scanner. Plates were photographed along with a scale. Hypocotyl length was measured with ImageJ64 for Mac (National Institutes of Health). We found the heritability for this trait to be substantially higher in soil when compared with the plates across multiple experiments. For root length, plates were photographed on day 5, and the root length was measured through ImageJ64 as described above. To analyze photomorphogenesis/skotomorphogenesis, different genotypes were sown on

Murashige and Skoog plates, covered with aluminum foil, and then kept vertically in Percival growth chambers for 10 d at the appropriate temperatures and light conditions. Plates were scanned after 10 d, and the plants were analyzed for their growth response, including cotyledon opening. Cotyledon opening was analyzed by measuring the angle between the embryonic leaves through ImageJ. To analyze allelic effects of *CRY2*, plants representing three independently derived stable transgenic lines at T3 harboring the $pCRY2_{Cvi}$::$CRY2_{Cvi\text{-}0}$ and $pCRY2_{Col}$::$CRY2_{Col\text{-}0}$ transgenes were analyzed twice, but, for comparison, data from a single transgenic line used for the phenotypic analysis is shown.

Statistical Analyses

Statistical analyses were done using JMP or SPSS (SAS Institute), Microsoft Excel, and R. The Col-0/Tsu-0, Col-0/Cvi-0, and Bay-0/Sha RILs have been previously described (Loudet et al., 2002; Simon et al., 2008). For hypocotyl elongation, at least 20 plants per RIL per temperature (23°C and 27°C short day) were measured. For flowering time analysis, six plants per RIL per temperature were grown in growth rooms in a completely randomized design. Broad-sense heritability was calculated as between-line variance divided by total variance. The total variance was partitioned into between-line variance and the residuals in a one-way ANOVA model using the genotype as a single factor of random effect and the phenotype as the response. Genetic correlations between the traits were calculated as described previously (Lempe et al., 2005). Temperature sensitivity was measured as described previously using the slope of the reaction norms (Lempe et al., 2005). To assess the effects of genotype, environment, and genotype × environment interactions, the entire data set (i.e. hypocotyl length for all plants across both temperature conditions) was analyzed using ANOVA, and the variance was partitioned. All the phenotypic data used for the analyses of various statistics are provided in Supplemental Table S2.

GWAS and QTL Analyses

The GWAS analyses were conducted using the GWAS Web Application online tool that harbors the genotypic information from the 1,001 genomes project (http://www.1001genomes.org; Seren et al., 2012). GWAS was conducted using the linear regression model/Kruskal-Wallis or AMM approach. Significant associations were found for temperature sensitivity in hypocotyl elongation. Temperature sensitivity measured either through the slope of the reaction norm or the residuals of the regression of hypocotyl length at 23°C on to 27°C gave similar results. The phenotypic data used for GWAS are provided in Supplemental Table S1. After initial analysis with AMM, we used the SNP with the highest association in the *SPL2* gene as a cofactor and reran the association analyses. We analyzed the sampling bias on GWAS by systematically removing sets of eight accessions multiple times and found that it did not make any difference to the detected associations. QTL mapping was performed using the Multiple QTL modeling in R/qtl (http://www.biostat.jhsph.edu/kbroman/qtl/). Empirical log of the odds (LOD) threshold was determined through analysis of 5,000 permutations, which typically resulted in LOD scores around 2.5 for most of the phenotypes. As a conservative measure, we considered QTLs whose LOD score was above 3.

Generation of Transgenic Plants

A *CRY2* genomic DNA fragment containing 2 kb of promoter sequence was PCR amplified from Col-0 and Cvi-0 using Phusion DNA polymerase with primers described in the Supplemental Table S8. The fragment was then cloned into a Gateway vector pDONR207 and then moved to destination vector pFK202 and used for Col-0 and *cry2-1* mutant plant transformation. An artificial microRNA against *CRY2* was constructed using the primers described in Supplemental Table S8, cloned under the *Cauliflower mosaic virus* 35S promoter (*35S::amiRCRY2*), and transformed into the Cvi-0 plants. Due to the requirement of long dormancy for germination of the Cvi-0 strain, transgenic seeds were sown after 3 to 4 months of storage. The presence of the *CRY2* transgene was confirmed by genotyping using primers described in Supplemental Table S8 (El-Din El-Assal et al., 2001). Multiple independent T1 lines were analyzed for each of the transgenic plants and for specific analysis; independent T2 or T3 lines were used as specified. The specific mutations representing different *CRY2* alleles were confirmed in transgenic plants by sequencing. The expression of *CRY2* in transgenic plants was quantified by quantitative real-time PCR with SYBR Green on a LightCycler system (LightCycler 480, Roche) using primers described in Supplemental Table S8.

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Reaction norms that represent thermal response across all the analyzed strains.

Supplemental Figure S2. Genome-wide association analysis of temperature sensitivity in hypocotyl elongation.

Supplemental Figure S3. Frequency distributions of hypocotyl lengths for F2 populations.

Supplemental Figure S4. QTL maps for multiple traits in different RIL populations.

Supplemental Figure S5. Cvi-0 plants in which *CRY2* function is compromised display temperature sensitivity in petiole elongation.

Supplemental Figure S6. Allelic variation at *CRY2* confers variation in temperature-associated architectural phenotypes.

Supplemental Figure S7. The effect of *CRY2*-Cvi-0 allele is dependent on the genetic background.

Supplemental Figure S8. Comparison of expression levels of *CRY2* in transgenic lines.

Supplemental Table S1. Descriptions and the phenotypes of accessions used in the GWAS analysis.

Supplemental Table S2. Summary statistics for accessions and the recombinant inbred lines.

Supplemental Table S3. Phenotypic data from the Col-0 × Tsu-0 RIL population.

Supplemental Table S4. Phenotypic data from the Col-0 × Cvi-0 RIL population.

Supplemental Table S5. Phenotypic data from the Bay-0 × Sha RIL population.

Supplemental Table S6. Genetic correlations between various traits.

Supplemental Table S7. QTL identified in this work using different RILs.

Supplemental Table S8. Oligos used in this study.

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