Developmental and phenotypic plasticity of *Arabidopsis thaliana* accessions across an ambient temperature range

Running title: Arabidopsis thermomorphogenesis

Carla Ibañez[1,2†], Yvonne Poeschl[3,4†], Tom Peterson[2], Julia Bellstädt[1,2], Kathrin Denk[1,2], Andreas Gogol-Döring[3,4,5], Marcel Quint[1,2] and Carolin Delker[1,2*]

[1]Institute of Agricultural and Nutritional Sciences, Martin Luther University Halle-Wittenberg, Betty-Heimann-Str. 5 , 06120 Halle (Saale), Germany

[2]Department of Molecular Signal Processing, Leibniz Institute of Plant Biochemistry, Weinberg 3, 06120 Halle (Saale), Germany

[3]Institute of Computer Science, Martin Luther University Halle-Wittenberg, Von-Seckendorff-Platz 1, 06099 Halle (Saale), Germany

[4]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

[5]Technische Hochschule Mittelhessen, Wiesenstr. 14, 35390 Gießen, Germany

cibanez@ipb-halle.de
yvonne.poeschl@informatik.uni-halle.de
peterson.tom@gmx.de
julia.bellstaedt@ipb-halle.de
kathrin.denk@landw.uni-halle.de
gogol-doering@idiv.de
marcel.quint@landw.uni-halle.de

† Co-first authors
* Author for correspondence:
carolin.delker@landw.uni-halle.de
Tel.: +49 345 5522 629

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Highlight

Comprehensive profiling of temperature responses in Arabidopsis reveals differential genotype and temperture effects on morphometric phenotypes and on vegetative and reproductive development.

Abstract

Global increase in ambient temperatures constitute a significant challenge to wild and cultivated plant species. Forward genetic analyses of isolated model temperature traits have resulted in the identification of several signaling and response components. However, a comprehensive knowledge about temperature sensitivity of different developmental stages and the contribution of natural variation therein is still scarce and fragmented at best. Here, we systematically analyze thermomorphogenesis throughout a complete life cycle in ten natural *Arabidopsis thaliana* accessions grown in four different temperatures ranging from 16 to 28 °C. We used Q_{10}, GxE, phenotypic divergence and correlation analyses to assess temperature sensitivity and genotype effects of more than 30 morphometric and developmental traits representing five phenotype classes. We found that developmental timing throughout the vegetative phase was primarily sensitive to temperature with only limited genotype effects indicating primarily thermodynamic effects and/or conserved regulation. Phenotypes associated with reproduction and various quantitative growth traits, however, were often sensitive to both genotype and temperature effects. Genotype-specific temperature responses may be attractive targets for future forward genetic approaches and accession-specific thermomorphogenesis maps may aid the assessment of functional relevance of known and novel regulatory components.

Introduction

Recurrent changes in ambient temperature provide plants with essential information about time of day and seasons. Yet, even small changes in mean ambient temperatures can profoundly affect plant growth and development which collectively can be summarized as thermomorphogenesis (Quint et al., 2016). In crops like rice, a season-specific increase in the mean minimum temperature of 1 °C results in a

~10 % reduction in grain yield (Peng et al., 2004) Likewise, up to 10 % of the yield stagnation of wheat and barley in Europe over the past two decades can be attributed to climate change (Moore and Lobell, 2015). Current projections indicate that mean global air temperatures will increase up to 4.8 °C by the end of the century (IPCC; Lobell and Gourdji, 2012). Global climate change will thus have significant implications on biodiversity and future food security.

Naturally, elevated ambient temperatures also affect wild species in their natural habitats. Long-term phenology studies of diverse plant populations have revealed an advance in first and peak flowering and alterations in the total length of flowering times (Fitter and Fitter, 2002; CaraDonna et al., 2014). Furthermore, estimates project that temperature effects alone will account for the extinction of up to one-third of all European plant species (Thuiller et al., 2005). As the impact of changes in ambient temperature on crop plants and natural habitats emerge, a comprehensive understanding of thermomorphogenesis throughout development becomes paramount.

Our present knowledge on molecular responses to ambient temperature signaling has significantly progressed by studies in *Arabidopsis thaliana.* Model thermomorphogenesis phenotypes such as hypocotyl elongation (Gray et al., 1998), hyponastic leaf movement (Zanten et al., 2009), and alterations in flowering time have served in various genetic approaches to identify relevant molecular components (reviewed in Quint et al., 2016). Extensive natural variation in these model traits has served as a valuable tool in the identification of regulatory components (Balasubramanian et al., 2006; Box et al., 2015; Raschke et al., 2015; Zhu et al., 2015; Sanchez-Bermejo et al., 2015; Lutz et al., 2015; Sanchez-Bermejo and Balasubramanian, 2016). So far, the main molecular players identified seem to function in response to both temperature and light stimuli and form a highly interconnected network of signaling elements. Prominent members of this network are photoreceptors such as CRYPTOCHROME 1 (CRY1, (Ma et al., 2016), PHYTOCHROME INTERACTING FACTOR 4 (PIF4, (Koini et al., 2009; Franklin et al., 2011; Proveniers and van Zanten, 2013), the DE-ETIOLATED 1 - CONSTITUTIVELY PHOTOMORPHOGENIC 1 – ELONGATED-HYPOCOTYL 5 (DET1-COP1-HY5) cascade (Toledo-Ortiz et al., 2014; Delker et al., 2014) and

EARLY FLOWERING 3 (ELF3) as a component of the circadian clock (Box et al., 2015; Raschke et al., 2015).

The investigation of signaling pathways that translate temperature stimuli into qualitative and quantitative developmental responses has so far largely been limited to either seedling development or flowering time. However, it seems likely that temperature responses in different phases of development require variations of a canonical signaling pathway. To enable the dissection of thermomorphogenic signaling at different developmental stages, it is vital to gather a comprehensive understanding of the diversity of temperature reactions throughout plant development.

According to basic principles of thermodynamics, temperature-induced changes in free energy will affect the rates of biochemical reactions. As these effects should occur generally, albeit to different extents, non-selective phenotypic responses can be expected to occur robustly and rather independently of genetic variation. Such traits may therefore be indicative of passive, thermodynamic effects on a multitude of processes. Alternatively, robust temperature responses may be due to thermodynamic effects on highly conserved signaling elements. These may be attractive targets for classic mutagenesis screens to identify the relevant regulatory components. In contrast, natural variation in thermomorphogenesis traits is likely the consequence of variability in one or several specific signaling or response components. It may be addressed by quantitative genetic approaches to identify regulators that contribute to variable temperature responses. Such genes would represent attractive candidates for targeted breeding approaches.

In this study we aim to (i) provide a map of developmental phenotypes that are sensitive to ambient temperature effects throughout a life cycle in the model organism *A. thaliana*, (ii) identify traits that are robustly affected by temperature with little variation among different accessions, and ask (iii) which traits are affected differentially by different genotypes and thus show natural variation in temperature responses.

To realize this, we performed a profiling of numerous developmental and morphological traits which can be sorted into five main categories: juvenile vegetative stage, adult vegetative stage, reproductive stage, morphometric parameters and

yield-associated traits. Phenotypes were analyzed in a subset of ten *A. thaliana* accessions which were grown at 16, 20, 24, and 28 °C in climate-controlled environments. Knowing that even a small randomly selected set of *A. thaliana* accessions covers a wide spectrum of genetic diversity (McKhann et al., 2004), we chose to analyze commonly used lab accessions such as Col-0, Ler-1 and Ws-2, accessions known to react hypersensitively to elevated temperature (e.g., Rrs-7, Delker et al., 2010, 2014), and parental lines of available mapping populations such as Bay-0, Sha, and Cvi-0.

In addition to a meta-analysis of the phenotypic data, we provide accession-specific developmental reference maps of temperature responses that can serve as resources for future experimental approaches in the analysis of ambient temperature responses in *A. thaliana*.

Materials and methods

Plant material and growth conditions

Phenotypic parameters (Fig. 1, Supplementary Table S1) were assessed in *A. thaliana* accessions that were obtained from the Nottingham Arabidopsis Stock Centre (Scholl et al., 2000). Detailed information on stock numbers and geographic origin are listed in Supplementary Table S2. For seedling stage analyses, surface-sterilized seeds were stratified for 3 days in deionized water at 4 °C and subsequently placed on *A. thaliana* solution (ATS) nutrient medium (Lincoln et al., 1990) Seeds were germinated and cultivated in climate-controled growth cabinets (Percival, AR66-L2) at constant temperatures of 16, 20, 24 or 28 °C under long day photoperiods (16h light/8h dark) and a fluence rate of 90 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. We refrained from including a vernalization step because the primary focus of this study was to record morphology and development in response to different constant ambient temperature conditions.

Germination rates were assessed daily and hypocotyl, root length, and petiole angles were measured in 7 days old seedlings with ImageJ (http://imagej.nih.gov/ij/) and Root Detection (http://www.labutils.de/rd.html).

All other analyses were performed on soil-grown plants cultivated in growth cabinets (Percival) at a fluence rate of 140 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ and long day photoperiods (16h

light/8h dark). After imbibition for 3 days at 4 °C, seeds were grown in individual 5 x 5 cm pots, which were randomized twice a week to minimize position effects. Relative humidity of growth cabinets was maintained at 70 % and plants were watered by subirrigation. Plants were photographed daily for subsequent determination of phenotypic parameters using Image J (http://imagej.nih.gov/ij/). Determination of developmental progression largely followed the stages defined in Boyes et al. (2001). The vegetative growth period was divided in a juvenile phase (germination to initiation of the fifth rosette leave) and an adult vegetative stage (initiation of the sixth rosette leave to floral transition). At transition to the reproductive growth phase, the number of leaves was determined by manual counting in addition to recording the number of days after germination.

Spectrophotometric determination of chlorophyll content was performed as described in (Porra et al., 1989). Rates of germination and seedling establishment were determined from 100 individual seeds. Two different seed pools were generated by proportional merging of four different seed batches from individuals from one accession (1:1:1:1). Both sample pools were used in the actual experiments. Sterilized and stratified seeds were germinated on ATS medium without sucrose. Germination was determined in the first three days and seedling establishment data was recorded at day six. Morphological markers and time points of analysis are described in Supplementary Table S1. Data were recorded from three independent germination experiments of which one representative set is shown.

Data analysis

Data visualization and statistical analyses of the data were performed using the software R (Team R Core, 2012). For visualization of the data set, box plots were generated using the *boxplot* function contained in the graphics package. For visualization of the statistical measures, heat maps were generated using the *heatmap.2* function contained in the gplots package, which is available on http://cran.r-project.org.

ANOVA for single factors

ANOVAs for a single factor (either accession or temperature) were performed using the *anova* function contained in the R stats package. In case of temperature, the factor had four levels. In case of accession, the factor had ten levels. Tukey's 'Honest Significant Difference' test was used as post hoc test using the function *TukeyHSD* contained in the stats package.

GxE interaction

Variation in phenotype expression was analyzed by 2-way ANOVA according to Nicotra et al. (2010) and Whitman and Agrawal (2009) to test each phenotype for a significant effect of genotype (*G*, accession) or environment (*E*, temperature), and a significant genotype by environment interaction (GxE). Reaction norms for each analysis are shown in Supplemental Fig. S12.

Q_{10} temperature coefficient

The Q_{10} temperature coefficient was calculated according to Loveys et al. (2003) as

$$Q_{10} = \left(\frac{P_w}{P_c}\right)^{\frac{10}{T_w - T_c}}$$

where P_w and P_c are the trait values at the warmer and colder temperatures, respectively. T_w and T_c represent the corresponding temperatures in °C.

Index of phenotypic divergence (P_{st})

Calculation of the index of phenotypic divergence (P_{st}, Storz, 2002; Leinonen et al., 2006) as a measure to quantify variation in each phenotypic trait was calculated as previously described by Storz (2002) as

$$P_{st} = \frac{\sigma_b^2}{\sigma_b^2 + 2\sigma_w^2}$$

where σ_b^2 is the variance between populations, and σ_w^2 is the variance within populations. The ANOVA framework was used to partition the variances to get unbiased estimates for σ_b^2 and σ_w^2 .

Using the two factorial design, two types of indices of phenotypic variation of a trait/phenotype were considered separately. The index of phenotypic divergence for

genotypes (P_{st}^{gen}) at a defined temperature level can be computed to measure the effect/impact of the genotype on the variation whereas the index of phenotypic divergence for temperatures (P_{st}^{temp}) provides a measure for the effect of temperature on the observed variation for individual genotypes.

Results

To assess phenotypic plasticity in a range of ambient temperatures, *A. thaliana* plants were cultivated in parallel throughout an entire life cycle at four different temperatures (16, 20, 24 and 28 °C) under otherwise similar growth conditions (see Materials and methods for further details). More than 30 morphological and developmental traits were recorded representing the following five phenotype classes: juvenile vegetative, adult vegetative, and reproductive stages as well as morphometric and yield-associated phenotypes (Fig. 1 and Supplementary Table S1).

Temperature responses in the A. thaliana reference accession Col-0

In Col-0, almost all phenotypes analyzed in this study were affected by the cultivation in different ambient temperatures. Only seed weight and maximum height remained constant regardless of the growth temperature (Fig. 2A, Supplementary Fig. S1). Among the temperature-sensitive traits were several growth-associated phenotypes in the juvenile vegetative stage. Primary root length, hypocotyl and petiole elongation all increased with elevated temperatures which concurs with previously published data (Gray et al., 1998; Zanten et al., 2009). As another example, yield-related traits, such as the number of siliques per plant and the number of seeds per silique decreased with an increase in ambient temperature (Fig. 2A).

As reported previously, Col-0 plants showed a decrease in developmental time until flowering with increasing ambient temperatures (Balasubramanian et al., 2006). The transition from the vegetative to the reproductive phase at 28 °C occurred about 25 days earlier than at 16 °C (Fig. 2A). Similarly, the number of rosette leaves developed at time of bolting differed by 26 leaves between 28 °C and 16 °C (Fig. 2A).

The fact that only a very limited number of phenotypes was insensitive to cultivation in different temperatures clearly illustrates the fundamental impact of ambient temperature on plant growth and development.

Natural variation of temperature responses

To assess whether the observed temperature responses in Col-0 are robust among *A. thaliana* accessions or which of the responses may be affected by natural variation, phenotypic profiling was performed in nine additional *A. thaliana* accessions parallel to the analysis in Col-0 (Supplementary Table S2, Supplementary Fig. S1-S10). Naturally, a panel of ten accessions does not comprehensively represent the world-wide gene pool of *A. thaliana*. However, it can be expected that even 10 randomly chosen natural accessions represent ~70 % of the allelic diversity in the *A. thaliana* gene pool (McKhann et al., 2004). Hence, the general assessment of thermo-responsive development in *A. thaliana* as well as the identification and discrimination between traits that generally seem to exhibit natural variation and those that may be genetically fixed within the gene pool is a realistic aim even with a set of 10 accessions.

To approximate and to compare temperature sensitivity of traits among different accessions, we calculated Q_{10} values for each individual trait and phenotype class for each analyzed genotype (Loveys et al., 2003). The Q_{10} quotient represents the factor by which a trait value changes if the ambient temperature increases by 10 °C. We calculated geometric means of all possible pairwise combinations of temperatures to minimize effects potentially caused by different response curves and used the $\log_2 Q_{10}$ for visualization as to retain high resolution in the presentation of the data.

Similarly to the response observed in Col-0 (Fig. 2), all analyzed genotypes showed a temperature-induced acceleration of vegetative development as indicated by negative $\log_2 Q_{10}$ values with low variability among accessions (Fig 3A + B, Supplementary Fig. S1-S10). Considerably higher variation was observed in $\log_2 Q_{10}$ values of traits related to reproductive stages. As all accessions investigated were principally able to flower despite the lack of an extended cold period, none of them strictly required a vernalization treatment to transition to the reproductive phase. In contrast to the other accessions, Got-7 and Rrs-7, however, showed a significant delay in flowering time with increasing temperature (Fig. 3B). Got-7, for example, did not flower within the first 90 days of cultivation when grown in 24 or 28 °C. Thus, initiated leaf senescence at bolting stage prevented accurate determination of leaf number at the onset of flowering.

A direct comparison of leaf number and time of development further corroborates a sudden increase in variation at the transition to flowering (Supplementary Fig. S11). However, at 16 °C and 20 °C several accessions contribute to the overall variability in the graph, whereas at 24 °C and 28 °C, C24 and Rrs-7 are the main determinants of variation due to their massive number of leaves corresponding to an extension of the vegetative growth phase (Supplementary Fig. S11). Got-7 likely would increase this variation at 24 and 28 °C, but is missing in these plots due to the lack of flowering transition within 90 days. Here, the lack of vernalization may at least partially be a significant factor. However, since all accessions were able to flower at temperatures of 16 and 20 °C an essential requirement for vernalization can be excluded.

Taken together, juvenile and adult vegetative development remained highly conserved, whereas the reproductive stage and yield-associated traits showed higher between-accessions and within-accession variability, as indicated by the ranges/dimensions of the box plots in Fig. 3A. Here, high variation within a phenotype class indicates that temperature effects on individual traits within that class are highly variable. The strongest within-accession variation was observed for morphometric phenotypes such as hypocotyl and petiole elongation. In contrast, a high between-accessions variability is indicative for differential responses of different genotypes which was most prominent in reproductive stage traits.

The differential variances of $\log_2 Q_{10}$ values among the two vegetative and the other phenotype classes indicated that genotype and environment effects may contribute differentially to phenotypic plasticity of different traits. We therefore next quantified the contribution of genotype and and the contribution of temperature effects on the phenotypic plasticity. We first used a 2-factorial ANOVA to assess which phenotypes show significant changes that can be attributed to genotype (G, accession), environment (E, temperature), and/or GxE interaction. Subsequently, we used the variance partitioning approach (Storz, 2002; Leinonen et al., 2006; Gay et al., 2008; Whitlock, 2008) to dissect and quantify the extent of the individual genotype and temperature effects on the phenotypic variation in more detail.

Genotype, Environment, and GxE interaction analysis

Each phenotypic trait was subjected to a 2-factorial ANOVA to address which of the analyzed factors (G, E, GxE) had significant effects on the phenotype. Reaction norm plots for each phenotype are shown in Supplementary Fig. S12. Each of the analyzed traits showed significant effects of genotype, environment (temperature) and GxE interaction (Supplementary Table S3). Surprisingly, this included all juvenile and adult vegetative stages despite their seemingly uniform impression of temperature responses given by the Q_{10} values (Fig. 3A + B).

Therefore, we made use of a previously described variance partitioning approach (Storz, 2002; Leinonen et al., 2006; Gay et al., 2008; Whitlock, 2008) to further dissect the individual extent of temperature and genotype effects on the observed variation. Specifically, we calculated the index of phenotypic divergence (P_{st}, Storz, 2002) at each analyzed temperature as a measure of genotype effects P_{st}^{gen} on the trait of interest. To complement this analysis, we also estimated the variation occurring across temperatures for each of the analyzed accessions P_{st}^{temp} (Supplemental Fig. S13), which enabled us to assess the temperature effect for the trait of interest for specific genotypes.

Genotype effects

The 2-factorial ANOVA design of the GxE interaction analysis has shown that the genotype significantly affects the variation of the phenotypic traits. The variance partitioning index for genotype effects (P_{st}^{gen}) provides a quantitative assessment of the extent of genotype contribution to variation at individual temperatures.

P_{st}^{gen} values showed highly variable patterns among the different traits and phenotype classes. Regardless of the individual temperature, genotype effects on developmental timing throughout the vegetative phase was generally very low (Fig. 4A). This finding corroborates the impression gained from the analysis of Q_{10} values (Fig. 3). However, genotype effects on later stages of adult vegetative development seem to increase with higher temperatures which may be the significant effect observed in the ANOVA-based GxE interaction assessment.

Similarly, increasing genotype effects at higher temperatures were also observed for

reproductive traits. For those traits, P_{st}^{gen} values at 16°C were already considerably stronger than for vegetative growth stages and increased further with elevated temperatures. A contrasting pattern of decreasing genotype effects with an increase in temperatures was observed for total plant height indicating that here, natural variation in growth is higher at lower temperatures (Fig. 4A). Yield-associated phenotypes in general showed only low genotype effects on variation, indicating that under our experimental conditions variation in trait expression in this category is primarily affected by temperature.

Other phenotypes display rather differential or less gradual genotype effects among different temperatures. For example, the genotype impact on variation in hypocotyl and petiole length sharply increases from 24 to 28°C, indicating a certain buffering capacity or a threshold for natural variation (Fig. 4A).

In some cases, such as flowering time, a strong genotype effect seems to correlate also with a strong general temperature sensitivity as indicated by the high between-accessions variability in Q_{10} values (Fig. 4A and Fig. 3B). However, this does not seem to be a general principle. In case of root length, for example, low genotype effects were observed (Fig. 4C), even though the phenotype was highly sensitive to a change in ambient temperature (Fig. 3B).

Temperature effects

We also used the variance partitioning approach to analyze the extent of the significant impact of temperature on phenotypic variation that was detected in the GxE interaction analysis (Supplementary Table S3). Therefore, we calculated the index for temperature effects (P_{st}^{temp}) on the variation of phenotypic plasticity across all four temperatures within each of the ten accessions (Fig. 4B). While the P_{st}^{gen} provided information on the genotype effect and thus, the overall natural variation of trait expression at different temperatures, the P_{st}^{temp} provides information primarily on the temperature-induced variability for each accession individually.

The heatmap representation of temperature effects (Fig. 4B) partially complements the genotype effect results. For example, variation in vegetative development showed

strong temperature effects (high P_{st}^{temp}), whereas P_{st}^{gen} values were generally low (Fig. 4B). Interestingly, temperature effects in juvenile vegetative stages seemed to be lower (for seedling establishment and 2 rosette leave stage) than in later vegetative stages with the exception of germination which showed strong temperature effects in most accessions.

Many traits exhibit highly differential temperature effects among accessions in the sense of one accession demonstrating a particularly strong temperature effect on a specific trait, while another accession may show low to no temperature effects (e.g. chlorophyll content in Ler-1 *vs*. Bay-0). This is particularly obvious for yield-related traits such as total number of seeds per plant and silique as well as silique length. Here, temperature effects on phenotype variation were low for Col-0, C24 and Bay-0, whereas considerably higher P_{st}^{temp} values were determined for the other accessions. Accessions which exhibit strong temperature effects on phenotypic variation may be interesting candidates for forward genetic approaches to identify the contributing molecular regulatory components.

Comparison of temperature and genotype effects

As each phenotypic trait has been assigned a value for genotype and temperature effects, they can now be compared directly to assess which of the two has a stronger influence on the phenotypic plasticity. To allow a direct comparison of effects, we first computed mean values for P_{st}^{gen} across all temperatures and P_{st}^{temp} across all accessions.

A scatterplot of mean P_{st}^{gen} and P_{st}^{temp} values for each trait clearly visualizes the predominant temperature effect on changes in the timing of vegetative growth stages (Fig. 4C, Supplementary Fig. S13). In contrast, morphometric phenotypes displayed considerably higher degrees of genotype effects with similarly high temperature effects. This combination of factorial effects is most prominent for phenotypes associated with the transition to reproductive development. Phenotypes associated with late developmental stages were generally less affected by both factors with a general tendency of slightly higher genotype than temperature effects (Fig. 4C, Supplementary Fig. S13).

Temperature effects on yield and propagation

Several yield-associated phenotypes such as total number of seeds, seed size and seed weight showed varying degrees of temperature sensitivity, likely caused by the partially distinct temperature effects on individual accessions (Fig.2B, Fig. 4B). A comparison of total seed numbers harvested from plants grown at 28 °C or 16 °C clearly illustrates that for most accessions higher temperatures cause a strong decrease in total yield (Fig. 5A, Supplementary Fig. S14). However, Got-7 showed an opposite trend even though the overall yield was severely reduced at both temperatures (Supplemental Fig. S14). This illustrates that the extension of the vegetative growth phase positively affects yield (it has to be noted that in the case of Got-7 this observation might be affected by the lack of vernalization). This is in line with common logic as a longer vegetative phase means also more biomass and assimilates that can be translocated into the seeds.

The observed differences in yield and some of the seed size parameters prompted us to inspect potential trans-generational effects of ambient growth temperatures on the following generation. We therefore tested the rates of seedling establishment of seeds collected from plants grown at 16 °C and 28 °C when cultivated again at the same or the respective other temperature. Seedling establishment (= fully opened cotyledons) after 6 days showed reproducible differences among the different samples. Seeds collected from plants grown at 16 °C showed almost no differences in seedling establishment when germinated at 16 or 28 °C (Fig. 5B). In both cases, seedling establishment rates were above 97 %. However, seeds collected from plants grown at 28 °C seem to show higher seedling establishment rates when grown under the same temperature (28 °C) compared to seeds germinated at 16 °C (Fig. 5B). This selective response may indicate trans-generational priming of seeds for development at higher temperatures, putatively involving epigenetic processes. While these effects were repeatedly observed for individual seed pools, extensive analysis of seeds collected from independently cultivated parental lines need to be analyzed to substantiate these observations.

Correlation of phenotypic temperature responses

Finally, we analyzed putative correlations in temperature responses (28 vs. 16 °C) among different phenotypes to assess potential links among traits and evaluate whether individual phenotype responses are indicative of temperature responses in general. We used Pearson correlation coefficients for pairwise comparisons of trait ratios (28 vs. 16 °C) among all accessions. As to be expected from the varying degrees of genotype and temperature effects on different traits, correlations among phenotypes covered a wide range (Supplementary Fig. S15). Particularly high correlation values were observed among flowering time, hypocotyl length and seed production (Fig. 5C), indicating that traits with strong adaptive potential seem to be affected similarly. Moreover, these data reveal that model phenotypes that have been successfully used in classic forward genetic approaches (such as hypocotyl elongation) are also at least partially indicative for plant temperature responses in later stages of development.

Discussion

Increased ambient temperatures have been shown to affect thermomorphogenesis for selected phenotypes. A systematic assessment of developmental and phenotypic plasticity across a complete life cycle has, to the best of our knowledge, been lacking so far. This study aims to provide such a solid base of temperature effects on plants by consecutive profiling of plant growth and development throughout a life cycle of *A. thaliana* grown in four different ambient temperatures. Furthermore, including several distinct *A. thaliana* accessions reduced potential genotype-specific biases in the data and allowed the analysis of temperature and genotype effects on the variation observed in different phenotypic traits.

All of the 34 analyzed phenotypes were significantly affected by different growth temperatures, natural variation, and GxE interactions illustrating the fundamental impact of ambient temperature on plant development (Supplementary Table 3, Supplementary Fig. S1-S10). The analysis of phenotypic divergence allowed the further dissection of phenotypes based on the extent of temperature and genotype effects. First, we identified phenotypes that were primarily affected by temperature and showed small genotype-induced variation. Second, we identified phenotypes that

additionally or even predominantly showed genotype effects on the observed phenotypic variation.

Developmental timing of juvenile and adult vegetative growth was significantly affected by genotype and temperature (Supplementary Table S3). Yet, temperature was the dominant factor in the observed variation (Fig. 4). Genotype effects, albeit significant, were limited and mostly showed similar accelerations by increasing temperatures in all analyzed genotypes. This observation may be indicative for extensive thermodynamic effects on (conserved) regulatory mechanisms involved in this process. Indeed, thermomorphogenic responses are often speculated to be primarily caused by broad or general effects of free energy changes on biochemical reactions (e.g. enzyme activities). The validity of the early proposed temperature coefficient (Q_{10}) for plant development was demonstrated for germination rates and plant respiration (Hegarty, 1973; Atkin and Tjoelker, 2003). The strong temperature effect on the acceleration of developmental timing throughout the vegetative phase, which was only weakly affected by genotypes supports this theory (Fig. 4B). When adopting the terms of “passive” and “active” temperature effects as proposed by (Penfield and MacGregor, 2014), timing of vegetative development would represent a passive temperature response that might be caused by thermodynamic effects on metabolic rates and enzyme activities or on highly conserved signaling/response components.

On the other hand, phenotypes that show a high degree of genotype and temperature effects might rather be influenced by one or more specific genes that contribute to trait expression in a quantitative manner. As such, these phenotypes would represent “active” temperature effects (Penfield and MacGregor, 2014) Natural variation in thermomorphogenic responses could be caused by different polymorphisms of signaling or response genes ranging from alteration in gene sequence to expression level polymorphism (Delker and Quint, 2011). As they provide keys to altered temperature responses that could be utilized in specific breeding approaches, these genes would thus be of high interest.

Several phenotypes analyzed here have the potential to contribute to adaptation to environmental conditions. Particularly hypocotyl and petiole elongation as well as hyponastic leaf movement (increased petiole angles) have previously been shown to

improve leaf cooling by increased transpiration rates (Crawford et al., 2012; Bridge et al., 2013). As such, variation in any of these traits could significantly impact on photosynthesis rates and affect further growth and development. In fact, the ratio of hypocotyl elongation showed a high correlation with the ratio of flowering induction and yield (28 vs. 16 °C, Fig. 5C). This could indicate that early seedling development significantly affects the timing of further development. Alternatively, these processes might involve similar signaling elements. In fact, PIF4 and ELF3 are central regulators integrating multiple environmental stimuli and have been shown to be involved in both, temperature-induced hypocotyl elongation and the induction of flowering (Koini et al., 2009; Kumar et al., 2012; Box et al., 2015; Raschke et al., 2015).

In addition, natural allelic variation in the circadian clock components *ELF3* and in the regulation of *GIGANTEA* have recently been shown to directly affect PIF4-mediated hypocotyl elongation in response to elevated temperatures (de Montaigu et al., 2015; Box et al., 2015; Raschke et al., 2015). Therefore, PIF4 and PIF4-regulating components could be important targets of adaptation to growth in higher ambient temperatures.

The increasing number of identified genes and allelic variations that contribute to specific phenotypic changes in response to elevated ambient temperatures argue against a general explanation of morphological and developmental changes due to passive thermodynamic effects.

Exploiting natural genetic variation to identify genes that are involved in the regulation of temperature effects on specific traits can provide new leads for plant breeding. The work presented here may inspire new approaches for temperature research in non-reference accessions as some temperature responses were much more pronounced in accessions other than Col-0 (Fig. 3 + 4). Specific approaches will depend on the focus on either yield- or biomass-associated traits. In addition, initial evidence for trans-generational effects require further analysis to account for potential epigenetic transduction of temperature cues on growth and development.

In conclusion, our work provides a map that allows the dissection of thermomorphogenesis in phenotypic traits that are either robustly affected by temperature or traits that are differentially affected by temperature among different

accessions. While robust temperature-sensitive phenotypes might indeed be caused by thermodynamic acceleration of metabolism, natural genetic variation of temperature responses implicate the relevance of specific regulatory cascades that can be instrumental to future breeding approaches.

Supplementary data

Table S1: List of recorded phenotypes and association to phenotype classes

Table S2: Identity and geographic origin of analyzed *A. thaliana* accessions

Table S3: GxE interaction

Fig. S1: Summary of Col-0 thermomorphogenesis

Fig. S2: Summary of Bay-0 thermomorphogenesis

Fig. S3: Summary of C24 thermomorphogenesis

Fig. S4: Summary of Cvi-0 thermomorphogenesis

Fig. S5: Summary of Got-7 thermomorphogenesis

Fig. S6: Summary of Ler-1 thermomorphogenesis

Fig. S7: Summary of No-0 thermomorphogenesis

Fig. S8: Summary of Rrs-7 thermomorphogenesis

Fig. S9: Summary of Sha thermomorphogenesis

Fig. S10: Summary of Ws-2 thermomorphogenesis

Fig. S11: Natural variation in developmental timing (leaves vs. days)

Fig. S12: Reaction norm plots of each phenotype for each of the analyzed genotypes

Fig. S13: Mean and standard deviation of P_{st} values.

Fig. S14: Temperature effect on yield (absolute values)

Fig. S15: Correlations among temperature response ratios (28 vs. 16 °C)

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Figure legends

Fig. 1: Phenotypic profiling approach

Schematic representation of the accessions, cultivation temperatures (°C) and phenotype classes used in the phenotypic profiling approach. Numbers indicate individual traits listed in Supplementary Table S1 and are color-coded according to the corresponding phenotype class. Blue squares indicate phenotypes sorted into the '*morphometric phenotypes*' class. Their position is indicative for the developmental stage at time of assessment.

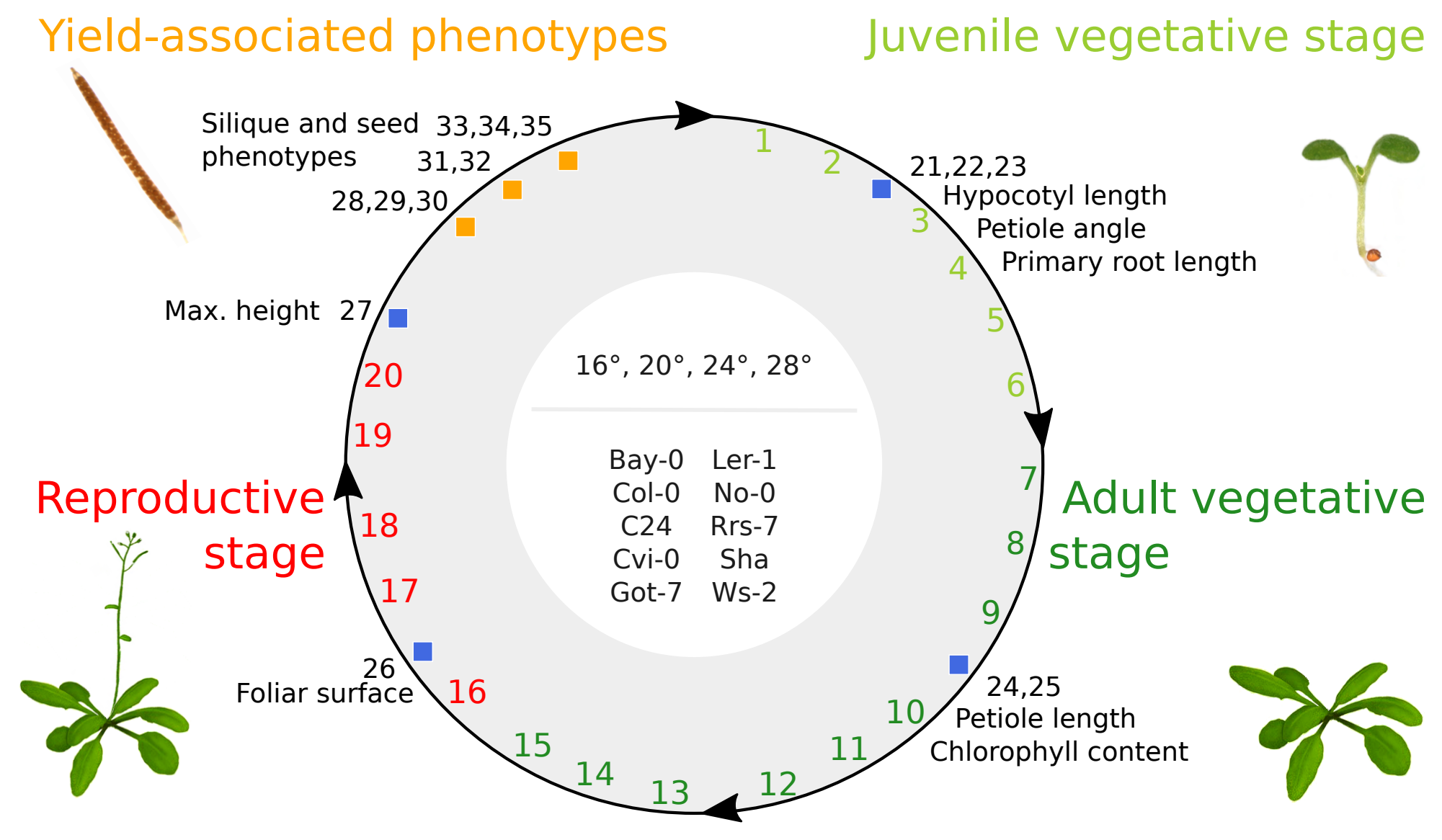
Fig. 2: Col-0 growth and development in response to different ambient temperatures
(A) Quantification of phenotypic traits recorded at different growth temperatures. Box plots show median and interquartile ranges (IQR), outliers (> 1.5 times IQR) are shown as circles. Units for each trait are specified in Supplementary Table S1. Different letters denote statistical differences (P > 0.05) among samples as assessed by one-factorial ANOVA and Tukey HSD. (B) Summary of temperature effects on developmental timing. Circles denote medians, bars denote IQRs (n > 15). Times of phenotypic assessment for selected traits in (A) are indicated by asterisks.

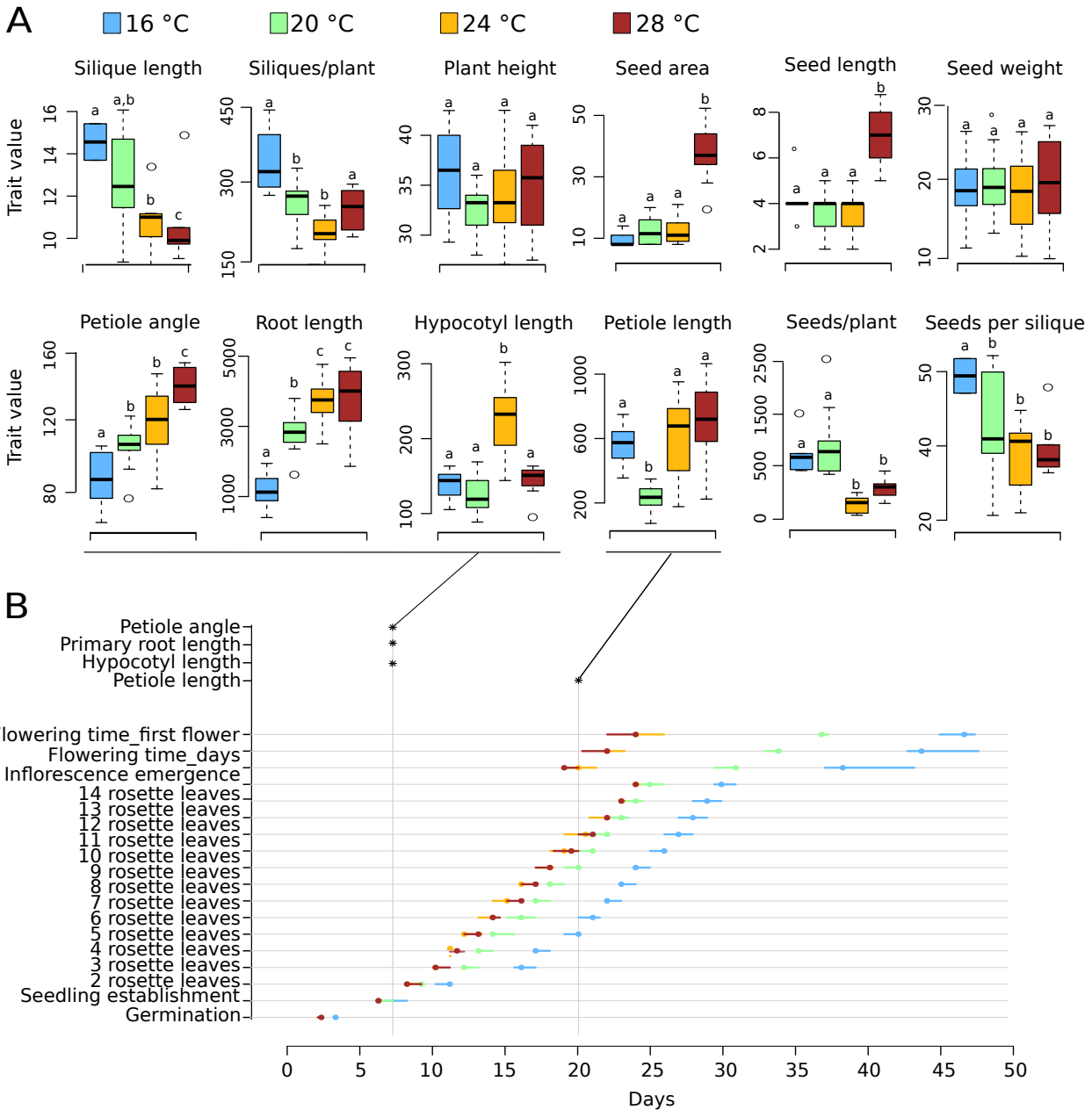
Fig. 3 Natural variation in temperature sensitivity of phenotypic traits (Q_{10})
Mean $\log_2 Q_{10}$ values for each accession (A) summarized in box plots for each phenotype class and (B) presented as a heatmap for all individual phenotypes. (A) Box plots show median and interquartile ranges (IQR), whiskers range from min. to max. values. (B) positive (increasing) and negative (decreasing) $\log_2 Q_{10}$ values are shown in yellow and blue, respectively with a $\log_2 Q_{10}$ cut-off value of 2 for better resolution. Missing data are denoted in light gray.

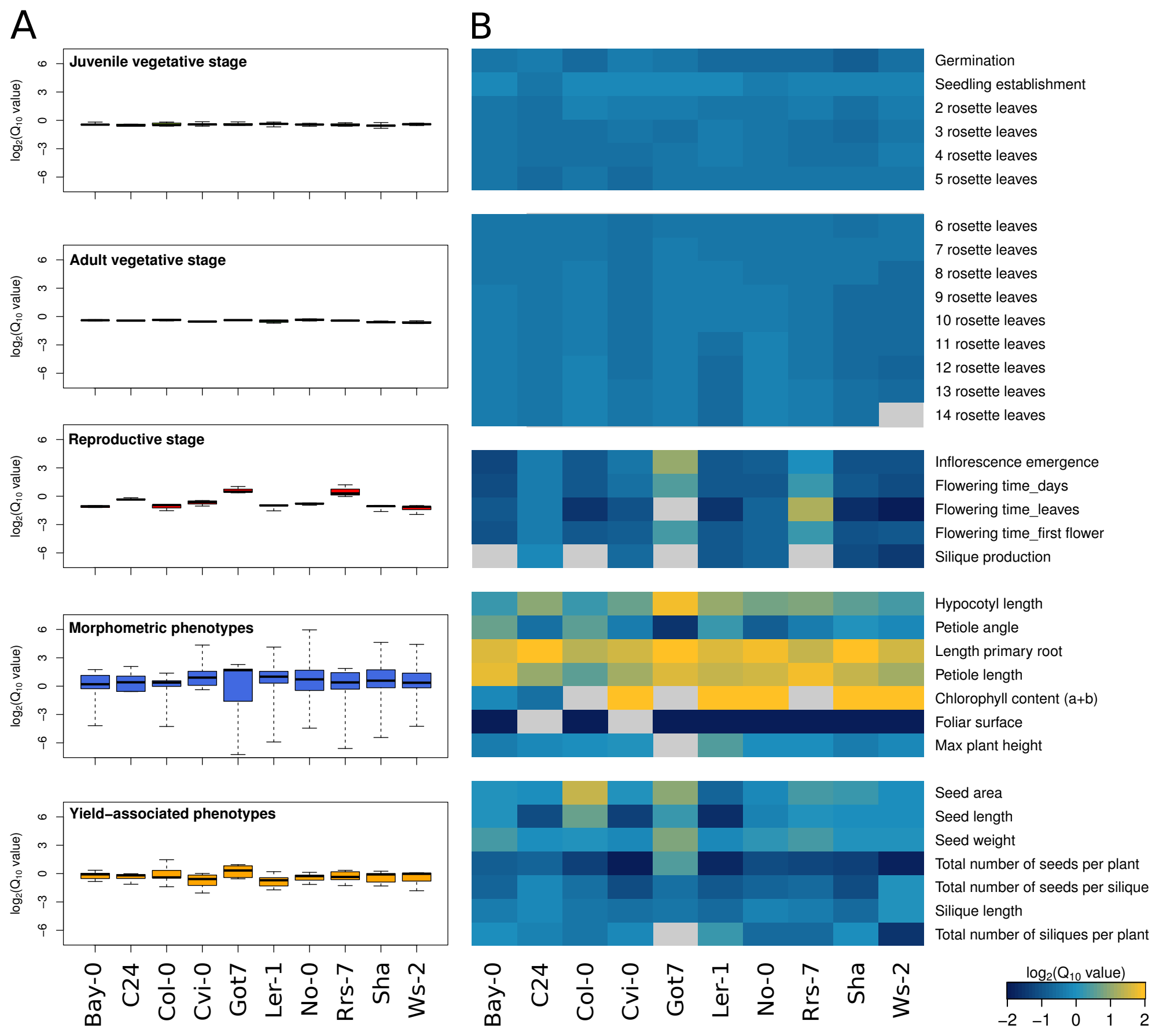
Fig. 4 Genotype and temperature effects on phenotypic variation
Heat map representations of (A) genotype effects P_{st}^{gen} and (B) temperature effects P_{st}^{temp} on all recorded phenotypes. Missing data is shown in light gray. (C) Scatter plot of mean P_{st}^{gen} and P_{st}^{temp} values over all temperatures and accessions, respectively. Phenotypes are color-coded according to the phenotype class shown in Fig. 1 and described in Supplementary Table S1. A scatter plot including standard deviations is shown in Supplementary Fig. S13.

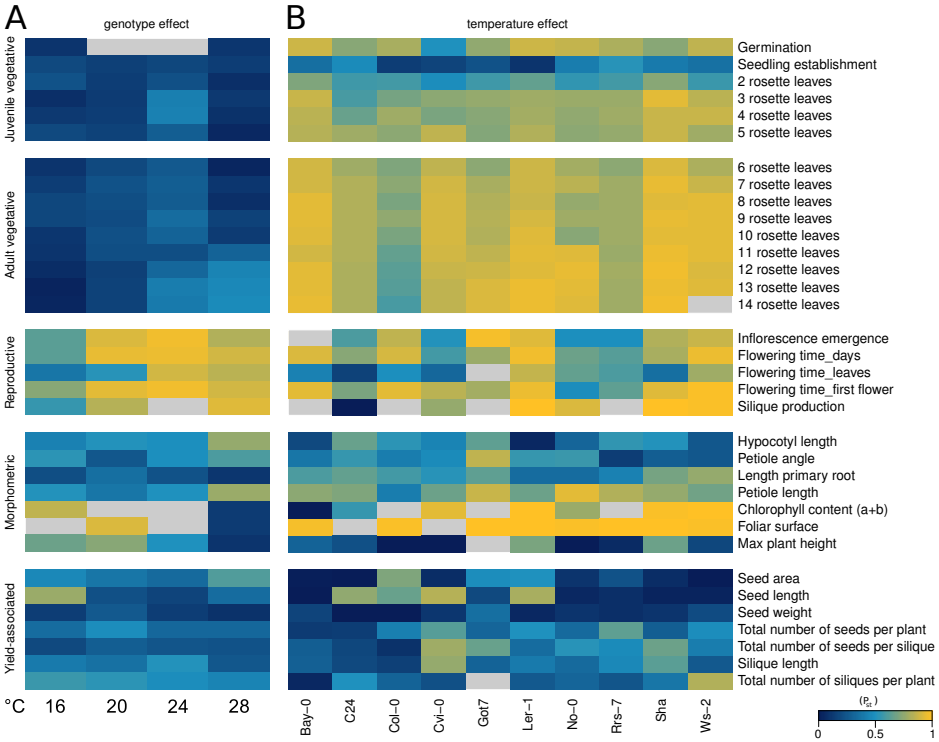
Fig. 5 Yield, transgenerational effects and phenotypic correlations
(A) Comparison of yield among 28 and 16 °C. Box plots show relative seed numbers and SEM of 28 °C (vs. 16 °C mean). Values < 1 indicate a reduction of seed numbers compared to the 16 °C mean. Different letters denote significant differences (P < 0.05) as assessed by two-factorial ANOVA of absolute data shown in Supplementary Fig S14. (B) Germination rate of seeds collected from plants grown at 16 or 28 °C for an entire life cycle were analyzed for subsequent germination at 16 °C and 28 °C.

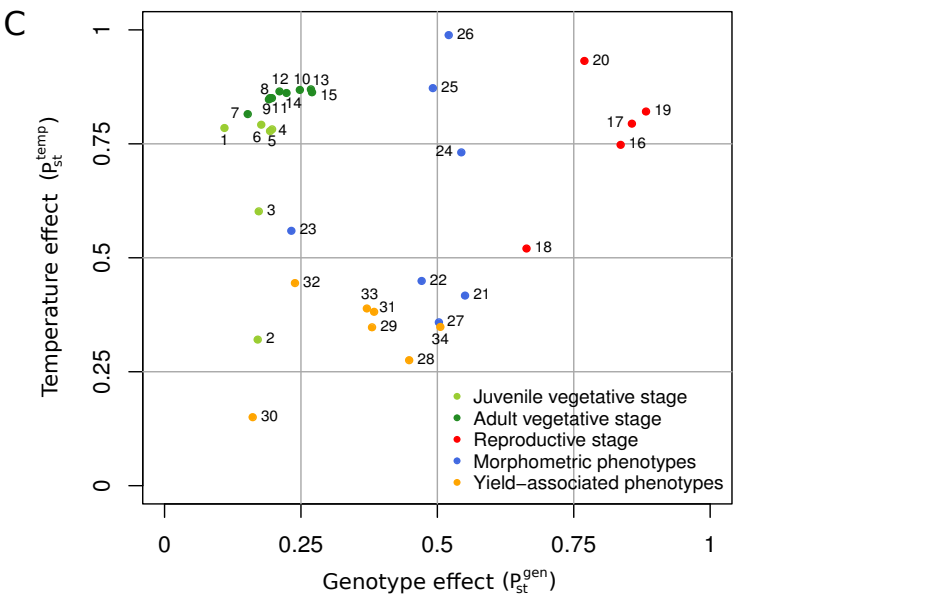
The experiment was performed three times with similar results of which one representative result is shown. (C) Scatter plot of selected phenotypes with strong temperature effects. Pearson correlation coefficients (r) of trait values are shown in the upper right corners. See Supplementary Fig. S15 for complete set of pair-wise comparisons among traits.

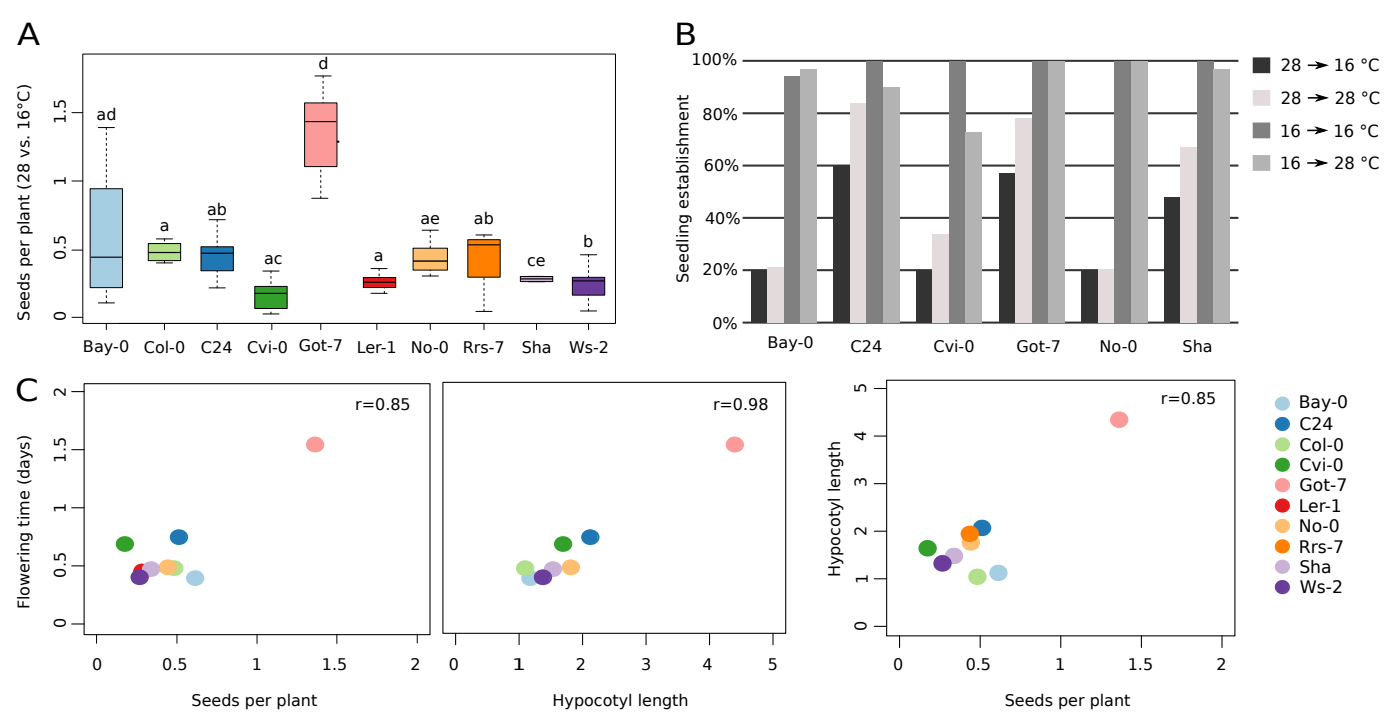

Yield-associated phenotypes
Juvenile vegetative stage
Silique and seed phenotypes
33,34,35
31,32
28,29,30
Max. height 27
21,22,23
Hypocotyl length
Petiole angle
Primary root length
16°, 20°, 24°, 28°
Bay-0 Ler-1
Col-0 No-0
C24 Rrs-7
Cvi-0 Sha
Got-7 Ws-2
Reproductive stage
Adult vegetative stage
26
Foliar surface
24,25
Petiole length
Chlorophyll content
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20


A
16 °C
20 °C
24 °C
28 °C
Silique length
Siliques/plant
Plant height
Seed area
Seed length
Seed weight
Petiole angle
Root length
Hypocotyl length
Petiole length
Seeds/plant
Seeds per silique
Trait value
B
Petiole angle
Primary root length
Hypocotyl length
Petiole length
Flowering time_first flower
Flowering time_days
Inflorescence emergence
14 rosette leaves
13 rosette leaves
12 rosette leaves
11 rosette leaves
10 rosette leaves
9 rosette leaves
8 rosette leaves
7 rosette leaves
6 rosette leaves
5 rosette leaves
4 rosette leaves
3 rosette leaves
2 rosette leaves
Seedling establishment
Germination
Days


A
B
Juvenile vegetative stage
Adult vegetative stage
Reproductive stage
Morphometric phenotypes
Yield−associated phenotypes
log2(Q10 value)
6
3
0
−3
−6
Germination
Seedling establishment
2 rosette leaves
3 rosette leaves
4 rosette leaves
5 rosette leaves
6 rosette leaves
7 rosette leaves
8 rosette leaves
9 rosette leaves
10 rosette leaves
11 rosette leaves
12 rosette leaves
13 rosette leaves
14 rosette leaves
Inflorescence emergence
Flowering time_days
Flowering time_leaves
Flowering time_first flower
Silique production
Hypocotyl length
Petiole angle
Length primary root
Petiole length
Chlorophyll content (a+b)
Foliar surface
Max plant height
Seed area
Seed length
Seed weight
Total number of seeds per plant
Total number of seeds per silique
Silique length
Total number of siliques per plant
Bay-0
C24
Col-0
Cvi-0
Got7
Ler-1
No-0
Rrs-7
Sha
Ws-2
log2(Q10 value)
−2
−1
0
1
2


A
genotype effect
B
temperature effect
Juvenile vegetative
Adult vegetative
Reproductive
Morphometric
Yield-associated
Germination
Seedling establishment
2 rosette leaves
3 rosette leaves
4 rosette leaves
5 rosette leaves
6 rosette leaves
7 rosette leaves
8 rosette leaves
9 rosette leaves
10 rosette leaves
11 rosette leaves
12 rosette leaves
13 rosette leaves
14 rosette leaves
Inflorescence emergence
Flowering time_days
Flowering time_leaves
Flowering time_first flower
Silique production
Hypocotyl length
Petiole angle
Length primary root
Petiole length
Chlorophyll content (a+b)
Foliar surface
Max plant height
Seed area
Seed length
Seed weight
Total number of seeds per plant
Total number of seeds per silique
Silique length
Total number of siliques per plant
°C 16 20 24 28
Bay–0 C24 Col–0 Cvi–0 Got7 Ler–1 No–0 Rrs–7 Sha Ws–2
(P_{st})
0 0.5 1


C
Temperature effect (P_{st}^{temp})
Genotype effect (P_{st}^{gen})
0 0.25 0.5 0.75 1
Juvenile vegetative stage
Adult vegetative stage
Reproductive stage
Morphometric phenotypes
Yield–associated phenotypes


A
Seeds per plant (28 vs. 16°C)
0
0.5
1
1.5
ad
a
ab
ac
d
a
ae
ab
ce
b
Bay-0
Col-0
C24
Cvi-0
Got-7
Ler-1
No-0
Rrs-7
Sha
Ws-2
B
Seedling establishment
100%
80%
60%
40%
20%
0%
28 → 16 °C
28 → 28 °C
16 → 16 °C
16 → 28 °C
Bay-0
C24
Cvi-0
Got-7
No-0
Sha
C
Flowering time (days)
r=0.85
r=0.98
r=0.85
Seeds per plant
Hypocotyl length
Hypocotyl length
Seeds per plant
Bay-0
C24
Col-0
Cvi-0
Got-7
Ler-1
No-0
Rrs-7
Sha
Ws-2