

Developmental and phenotypic plasticity of *Arabidopsis thaliana* accessions

2 across an ambient temperature range

4 Running title: *Arabidopsis* thermomorphogenesis

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Highlight

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

38 Abstract

Global increase in ambient temperatures constitute a significant challenge to wild and
40 cultivated plant species. Forward genetic analyses of isolated model temperature
traits have resulted in the identification of several signaling and response
42 components. However, a comprehensive knowledge about temperature sensitivity of
different developmental stages and the contribution of natural variation therein is still
44 scarce and fragmented at best. Here, we systematically analyze
thermomorphogenesis throughout a complete life cycle in ten natural *Arabidopsis*
46 *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
48 temperature sensitivity and genotype effects of more than 30 morphometric and
developmental traits representing five phenotype classes. We found that
50 developmental timing throughout the vegetative phase was primarily sensitive to
temperature with only limited genotype effects indicating primarily thermodynamic
52 effects and/or conserved regulation. Phenotypes associated with reproduction and
various quantitative growth traits, however, were often sensitive to both genotype and
54 temperature effects. Genotype-specific temperature responses may be attractive
targets for future forward genetic approaches and accession-specific
56 thermomorphogenesis maps may aid the assessment of functional relevance of
known and novel regulatory components.

58

Introduction

60 Recurrent changes in ambient temperature provide plants with essential information
about time of day and seasons. Yet, even small changes in mean ambient
62 temperatures can profoundly affect plant growth and development which collectively
can be summarized as thermomorphogenesis (Quint et al., 2016). In crops like rice, a
64 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
66 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
68 that mean global air temperatures will increase up to 4.8 °C by the end of the century
(IPCC; Lobell and Gourdj, 2012). Global climate change will thus have significant
70 implications on biodiversity and future food security.

Naturally, elevated ambient temperatures also affect wild species in their natural
72 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
74 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
76 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
78 understanding of thermomorphogenesis throughout development becomes
paramount.

80 Our present knowledge on molecular responses to ambient temperature signaling
has significantly progressed by studies in *Arabidopsis thaliana*. Model
82 thermomorphogenesis phenotypes such as hypocotyl elongation (Gray et al., 1998),
hyponastic leaf movement (Zanten et al., 2009), and alterations in flowering time
84 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
86 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.,
88 2015; Sanchez-Bermejo et al., 2015; Lutz et al., 2015; Sanchez-Bermejo and
Balasubramanian, 2016). So far, the main molecular players identified seem to
90 function in response to both temperature and light stimuli and form a highly
interconnected network of signaling elements. Prominent members of this network
92 are photoreceptors such as CRYPTOCHROME 1 (CRY1, (Ma et al., 2016),
PHYTOCHROME INTERACTING FACTOR 4 (PIF4, (Koini et al., 2009; Franklin et
94 al., 2011; Proveniers and van Zanten, 2013), the DE-ETIOLATED 1 -
CONSTITUTIVELY PHOTOMORPHOGENIC 1 – ELONGATED-HYPOCOTYL 5
96 (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.,
98 2015; Raschke et al., 2015).

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

According to basic principles of thermodynamics, temperature-induced changes in
108 free energy will affect the rates of biochemical reactions. As these effects should
occur generally, albeit to different extents, non-selective phenotypic responses can
110 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
112 processes. Alternatively, robust temperature responses may be due to
thermodynamic effects on highly conserved signaling elements. These may be
114 attractive targets for classic mutagenesis screens to identify the relevant regulatory
components. In contrast, natural variation in thermomorphogenesis traits is likely the
116 consequence of variability in one or several specific signaling or response
components. It may be addressed by quantitative genetic approaches to identify
118 regulators that contribute to variable temperature responses. Such genes would
represent attractive candidates for targeted breeding approaches.

120 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
122 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
124 differentially by different genotypes and thus show natural variation in temperature
responses.

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

yield-associated traits. Phenotypes were analyzed in a subset of ten *A. thaliana*
130 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*
132 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,
134 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
136 as Bay-0, Sha, and Cvi-0.

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

142 **Materials and methods**

Plant material and growth conditions

144 Phenotypic parameters (Fig. 1, Supplementary Table S1) were assessed in *A.*
thaliana accessions that were obtained from the Nottingham Arabidopsis Stock
146 Centre (Scholl et al., 2000). Detailed information on stock numbers and geographic
origin are listed in Supplementary Table S2. For seedling stage analyses, surface-
148 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.,
150 1990) Seeds were germinated and cultivated in climate-controlled growth cabinets
(Percival, AR66-L2) at constant temperatures of 16, 20, 24 or 28 °C under long day
152 photoperiods (16h light/8h dark) and a fluence rate of 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$. We
refrained from including a vernalization step because the primary focus of this study
154 was to record morphology and development in response to different constant ambient
temperature conditions.

156 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
158 Root Detection (<http://www.labutils.de/rd.html>).

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

light/8h dark). After imbibition for 3 days at 4 °C, seeds were grown in individual 5 x 5
162 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
164 subirrigation. Plants were photographed daily for subsequent determination of
phenotypic parameters using Image J (<http://imagej.nih.gov/ij/>). Determination of
166 developmental progression largely followed the stages defined in Boyes et al. (2001).
The vegetative growth period was divided in a juvenile phase (germination to
168 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
170 number of leaves was determined by manual counting in addition to recording the
number of days after germination.
172 Spectrophotometric determination of chlorophyll content was performed as described
in (Porra et al., 1989). Rates of germination and seedling establishment were
174 determined from 100 individual seeds. Two different seed pools were generated by
proportional merging of four different seed batches from individuals from one
176 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.
178 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
180 described in Supplementary Table S1. Data were recorded from three independent
germination experiments of which one representative set is shown.

182

Data analysis

184 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
186 generated using the *boxplot* function contained in the graphics package. For
visualization of the statistical measures, heat maps were generated using the
188 *heatmap.2* function contained in the gplots package, which is available on
<http://cran.r-project.org>.

190

192

ANOVA for single factors

194 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
196 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*
198 contained in the stats package.

200 GxE interaction

Variation in phenotype expression was analyzed by 2-way ANOVA according to
202 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
204 significant genotype by environment interaction (*GxE*). Reaction norms for each
analysis are shown in Supplemental Fig. S12.

206

*Q*₁₀ temperature coefficient

208 The *Q*₁₀ temperature coefficient was calculated according to Loveys et al. (2003) as

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

210 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.

212

Index of phenotypic divergence (*P*_{st})

214 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
216 previously described by Storz (2002) as

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

218 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
220 unbiased estimates for σ_b^2 and σ_w^2 .

Using the two factorial design, two types of indices of phenotypic variation of a
222 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
224 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
226 temperature on the observed variation for individual genotypes.

228 **Results**

To assess phenotypic plasticity in a range of ambient temperatures, *A. thaliana* plants
230 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
232 methods for further details). More than 30 morphological and developmental traits
were recorded representing the following five phenotype classes: juvenile vegetative,
234 adult vegetative, and reproductive stages as well as morphometric and yield-
associated phenotypes (Fig. 1 and Supplementary Table S1).

236

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

238 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
240 constant regardless of the growth temperature (Fig. 2A, Supplementary Fig. S1).
Among the temperature-sensitive traits were several growth-associated phenotypes
242 in the juvenile vegetative stage. Primary root length, hypocotyl and petiole elongation
all increased with elevated temperatures which concurs with previously published
244 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
246 decreased with an increase in ambient temperature (Fig. 2A).

As reported previously, Col-0 plants showed a decrease in developmental time until
248 flowering with increasing ambient temperatures (Balasubramanian et al., 2006). The
transition from the vegetative to the reproductive phase at 28 °C occurred about 25
250 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).

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

Natural variation of temperature responses

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

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

294 Similarly to the response observed in Col-0 (Fig. 2), all analyzed genotypes showed
296 a temperature-induced acceleration of vegetative development as indicated by
298 negative $\log_2 Q_{10}$ values with low variability among accessions (Fig 3A + B,
300 Supplementary Fig. S1-S10). Considerably higher variation was observed in $\log_2 Q_{10}$
302 values of traits related to reproductive stages. As all accessions investigated were
304 principally able to flower despite the lack of an extended cold period, none of them
306 strictly required a vernalization treatment to transition to the reproductive phase. In
308 contrast to the other accessions, Got-7 and Rrs-7, however, showed a significant
310 delay in flowering time with increasing temperature (Fig. 3B). Got-7, for example, did
312 not flower within the first 90 days of cultivation when grown in 24 or 28 °C. Thus,
314 initiated leaf senescence at bolting stage prevented accurate determination of leaf
316 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 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.

318

Genotype, Environment, and GxE interaction analysis

320 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
322 plots for each phenotype are shown in Supplementary Fig. S12. Each of the
analyzed traits showed significant effects of genotype, environment (temperature)
324 and GxE interaction (Supplementary Table S3). Surprisingly, this included all juvenile
and adult vegetative stages despite their seemingly uniform impression of
326 temperature responses given by the Q_{10} values (Fig. 3A + B).

Therefore, we made use of a previously described variance partitioning approach
328 (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
330 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
332 trait of interest. To complement this analysis, we also estimated the variation
occurring across temperatures for each of the analyzed accessions P_{st}^{temp}
334 (Supplemental Fig. S13), which enabled us to assess the temperature effect for the
trait of interest for specific genotypes.

336

Genotype effects

338 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
340 partitioning index for genotype effects (P_{st}^{gen}) provides a quantitative assessment of
the extent of genotype contribution to variation at individual temperatures.

342 P_{st}^{gen} values showed highly variable patterns among the different traits and
phenotype classes. Regardless of the individual temperature, genotype effects on
344 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
346 (Fig. 3). However, genotype effects on later stages of adult vegetative development
seem to increase with higher temperatures which may be the significant effect
348 observed in the ANOVA-based GxE interaction assessment.

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

350 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
352 temperatures. A contrasting pattern of decreasing genotype effects with an increase
in temperatures was observed for total plant height indicating that here, natural
354 variation in growth is higher at lower temperatures (Fig. 4A). Yield-associated
phenotypes in general showed only low genotype effects on variation, indicating that
356 under our experimental conditions variation in trait expression in this category is
primarily affected by temperature.

358

Other phenotypes display rather differential or less gradual genotype effects among
360 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
362 capacity or a threshold for natural variation (Fig. 4A).

In some cases, such as flowering time, a strong genotype effect seems to correlate
364 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
366 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
368 change in ambient temperature (Fig. 3B).

370 *Temperature effects*

We also used the variance partitioning approach to analyze the extent of the
372 significant impact of temperature on phenotypic variation that was detected in the
GxE interaction analysis (Supplementary Table S3). Therefore, we calculated the
374 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}
376 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
378 the temperature-induced variability for each accession individually.

The heatmap representation of temperature effects (Fig. 4B) partially complements
380 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
382 (Fig. 4B). Interestingly, temperature effects in juvenile vegetative stages seemed to
be lower (for seedling establishment and 2 rosette leave stage) than in later
384 vegetative stages with the exception of germination which showed strong
temperature effects in most accessions.

386 Many traits exhibit highly differential temperature effects among accessions in the
sense of one accession demonstrating a particularly strong temperature effect on a
388 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
390 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,
392 whereas considerably higher P_{st}^{temp} values were determined for the other
accessions. Accessions which exhibit strong temperature effects on phenotypic
394 variation may be interesting candidates for forward genetic approaches to identify the
contributing molecular regulatory components.

396

Comparison of temperature and genotype effects

398 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
400 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
402 accessions.

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

412

Temperature effects on yield and propagation

414 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
416 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
418 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
420 opposite trend even though the overall yield was severely reduced at both
temperatures (Supplemental Fig. S14). This illustrates that the extension of the
422 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
424 with common logic as a longer vegetative phase means also more biomass and
assimilates that can be translocated into the seeds.

426 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
428 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
430 same or the respective other temperature. Seedling establishment (= fully opened
cotyledons) after 6 days showed reproducible differences among the different
432 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,
434 seedling establishment rates were above 97 %. However, seeds collected from
plants grown at 28 °C seem to show higher seedling establishment rates when grown
436 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
438 development at higher temperatures, putatively involving epigenetic processes. While
these effects were repeatedly observed for individual seed pools, extensive analysis
440 of seeds collected from independently cultivated parental lines need to be analyzed
to substantiate these observations.

442

444

Correlation of phenotypic temperature responses

446 Finally, we analyzed putative correlations in temperature responses (28 vs. 16 °C)
among different phenotypes to assess potential links among traits and evaluate
448 whether individual phenotype responses are indicative of temperature responses in
general. We used Pearson correlation coefficients for pairwise comparisons of trait
450 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
452 phenotypes covered a wide range (Supplementary Fig. S15). Particularly high
correlation values were observed among flowering time, hypocotyl length and seed
454 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
456 successfully used in classic forward genetic approaches (such as hypocotyl
elongation) are also at least partially indicative for plant temperature responses in
458 later stages of development.

460 **Discussion**

Increased ambient temperatures have been shown to affect thermomorphogenesis
462 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
464 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.*
466 *thaliana* grown in four different ambient temperatures. Furthermore, including several
distinct *A. thaliana* accessions reduced potential genotype-specific biases in the data
468 and allowed the analysis of temperature and genotype effects on the variation
observed in different phenotypic traits.

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

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

480 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 482 significant, were limited and mostly showed similar accelerations by increasing temperatures in all analyzed genotypes. This observation may be indicative for 484 extensive thermodynamic effects on (conserved) regulatory mechanisms involved in this process. Indeed, thermomorphogenic responses are often speculated to be 486 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 488 coefficient (Q_{10}) for plant development was demonstrated for germination rates and plant respiration (Hegarty, 1973; Atkin and Tjoelker, 2003). The strong temperature 490 effect on the acceleration of developmental timing throughout the vegetative phase, which was only weakly affected by genotypes supports this theory (Fig. 4B). When 492 adopting the terms of “passive” and “active” temperature effects as proposed by (Penfield and MacGregor, 2014), timing of vegetative development would represent a 494 passive temperature response that might be caused by thermodynamic effects on metabolic rates and enzyme activities or on highly conserved signaling/response 496 components.

On the other hand, phenotypes that show a high degree of genotype and 498 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 500 would represent “active” temperature effects (Penfield and MacGregor, 2014) Natural variation in thermomorphogenic responses could be caused by different 502 polymorphisms of signaling or response genes ranging from alteration in gene sequence to expression level polymorphism (Delker and Quint, 2011). As they 504 provide keys to altered temperature responses that could be utilized in specific breeding approaches, these genes would thus be of high interest.

506 Several phenotypes analyzed here have the potential to contribute to adaptation to environmental conditions. Particularly hypocotyl and petiole elongation as well as 508 hyponastic leaf movement (increased petiole angles) have previously been shown to

improve leaf cooling by increased transpiration rates (Crawford et al., 2012; Bridge et
510 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
512 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
514 significantly affects the timing of further development. Alternatively, these processes
might involve similar signaling elements. In fact, PIF4 and ELF3 are central
516 regulators integrating multiple environmental stimuli and have been shown to be
involved in both, temperature-induced hypocotyl elongation and the induction of
518 flowering (Koini et al., 2009; Kumar et al., 2012; Box et al., 2015; Raschke et al.,
2015).

520 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
522 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
524 components could be important targets of adaptation to growth in higher ambient
temperatures.

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

530 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
532 breeding. The work presented here may inspire new approaches for temperature
research in non-reference accessions as some temperature responses were much
534 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,
536 initial evidence for trans-generational effects require further analysis to account for
potential epigenetic transduction of temperature cues on growth and development.

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

542 accessions. While robust temperature-sensitive phenotypes might indeed be caused
544 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.

546 **Supplementary data**

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

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

Table S3: GxE interaction

550 Fig. S1: Summary of Col-0 thermomorphogenesis

Fig. S2: Summary of Bay-0 thermomorphogenesis

552 Fig. S3: Summary of C24 thermomorphogenesis

Fig. S4: Summary of Cvi-0 thermomorphogenesis

554 Fig. S5: Summary of Got-7 thermomorphogenesis

Fig. S6: Summary of Ler-1 thermomorphogenesis

556 Fig. S7: Summary of No-0 thermomorphogenesis

Fig. S8: Summary of Rrs-7 thermomorphogenesis

558 Fig. S9: Summary of Sha thermomorphogenesis

Fig. S10: Summary of Ws-2 thermomorphogenesis

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

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

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

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

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

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570

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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.

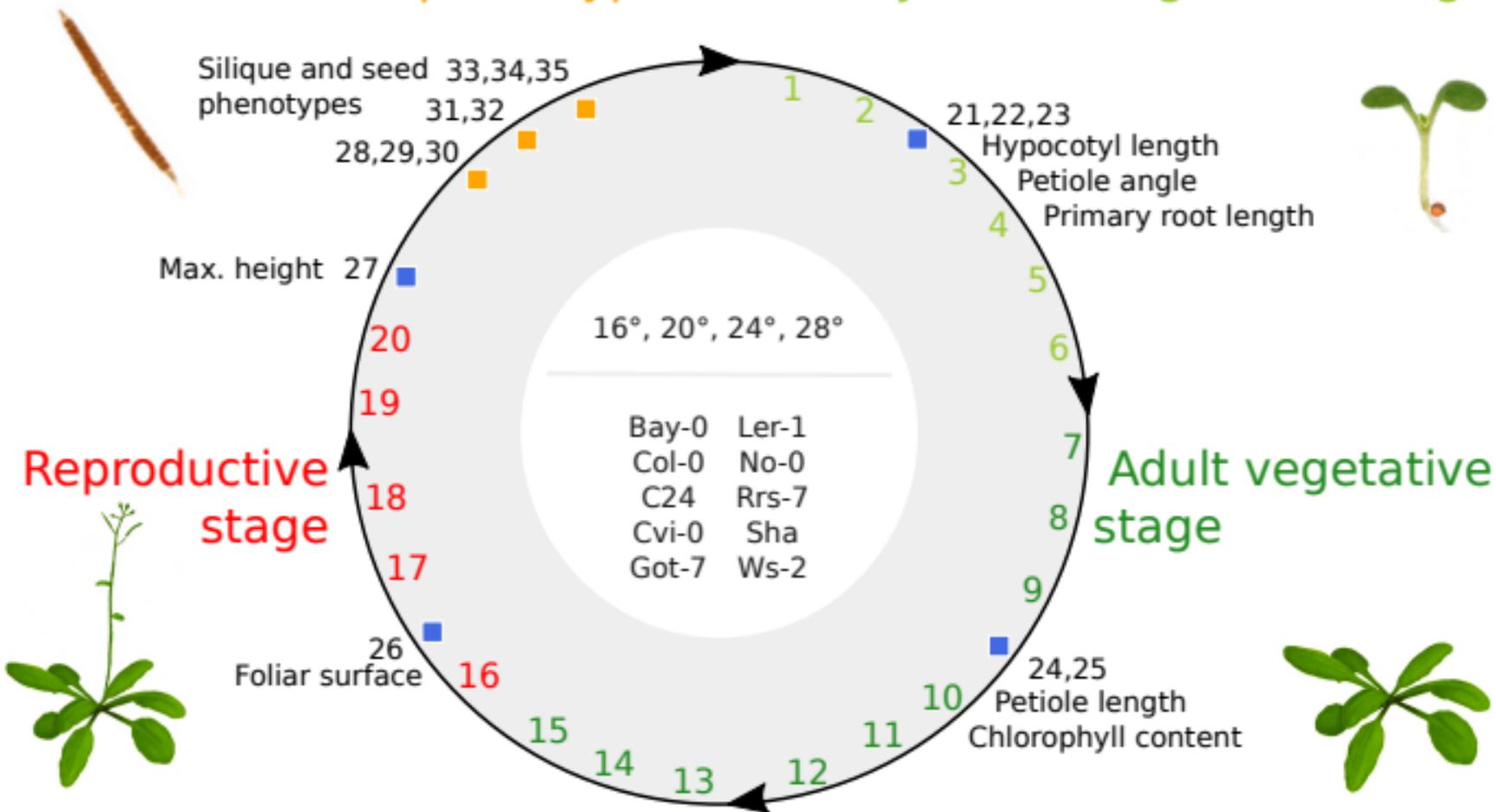
Fig. 5 Yield, transgenerational effects and phenotypic correlations

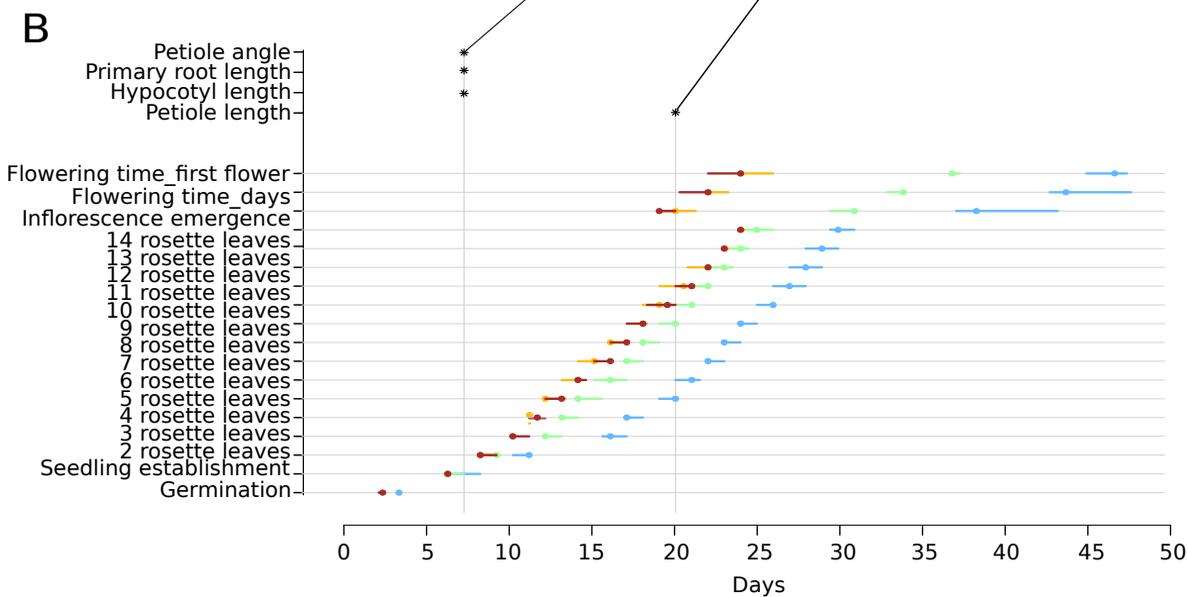
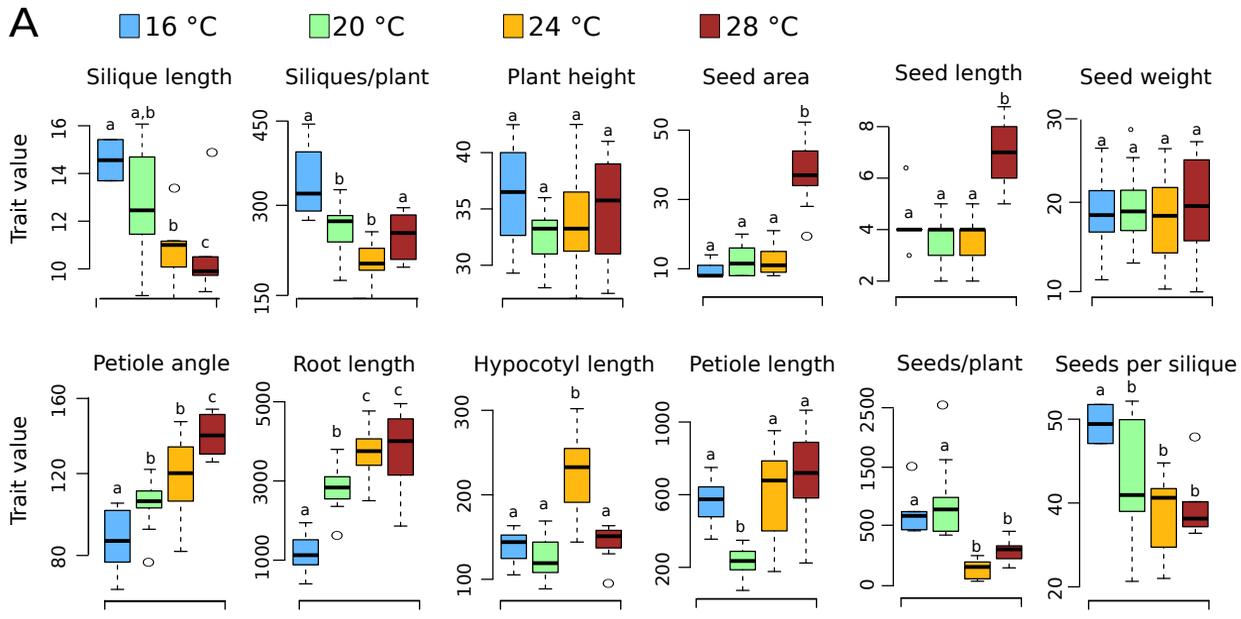
(A) Comparison of yield among 28 and 16 °C. Box plots show relative seed numbers and SEM of 28 °C (vs. 16 °C mean). Values < 1 indicate a reduction of seed numbers compared to the 16 °C mean. Different letters denote significant differences ($P < 0.05$) as assessed by two-factorial ANOVA of absolute 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.

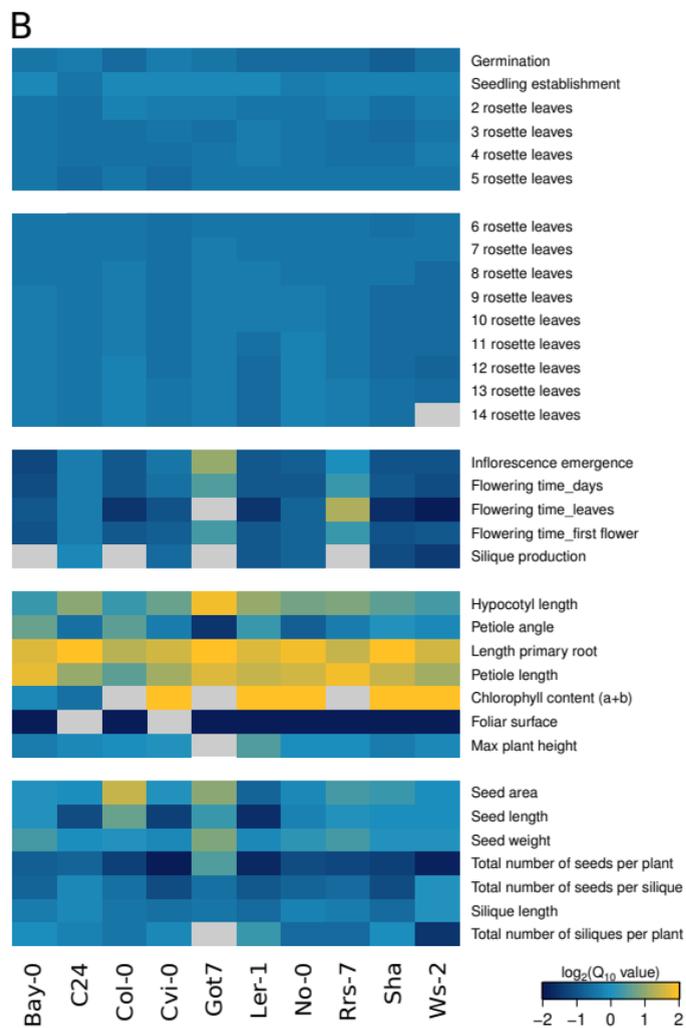
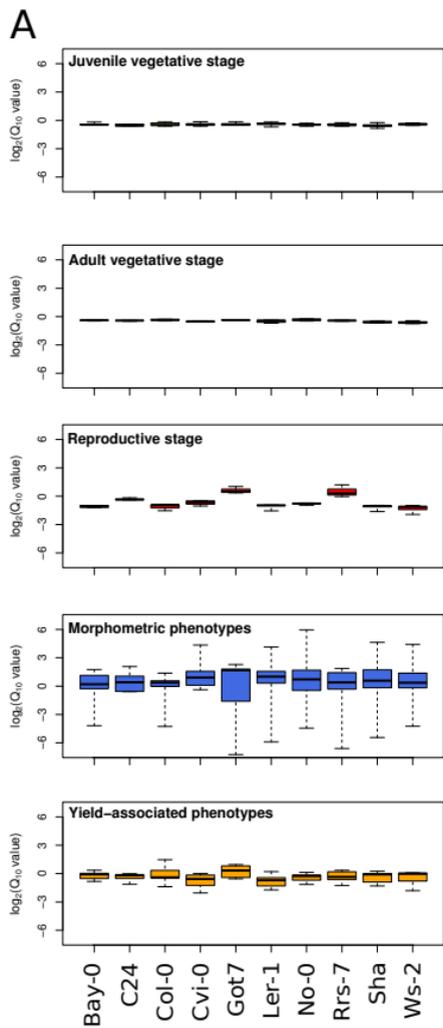
The experiment was performed three times with similar results of which one representative result is shown. (C) Scatter plot of selected phenotypes with strong temperature effects. Pearson correlation coefficients (r) of trait values are shown in the upper right corners. See Supplementary Fig. S15 for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

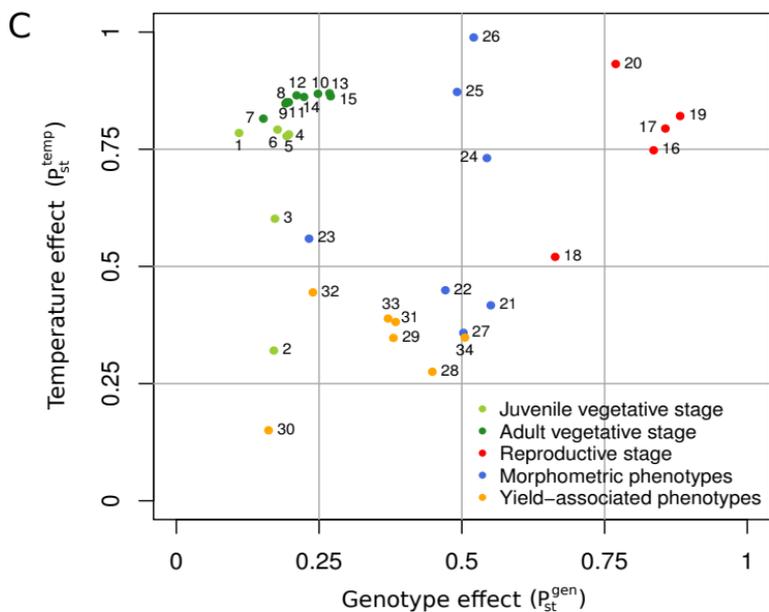
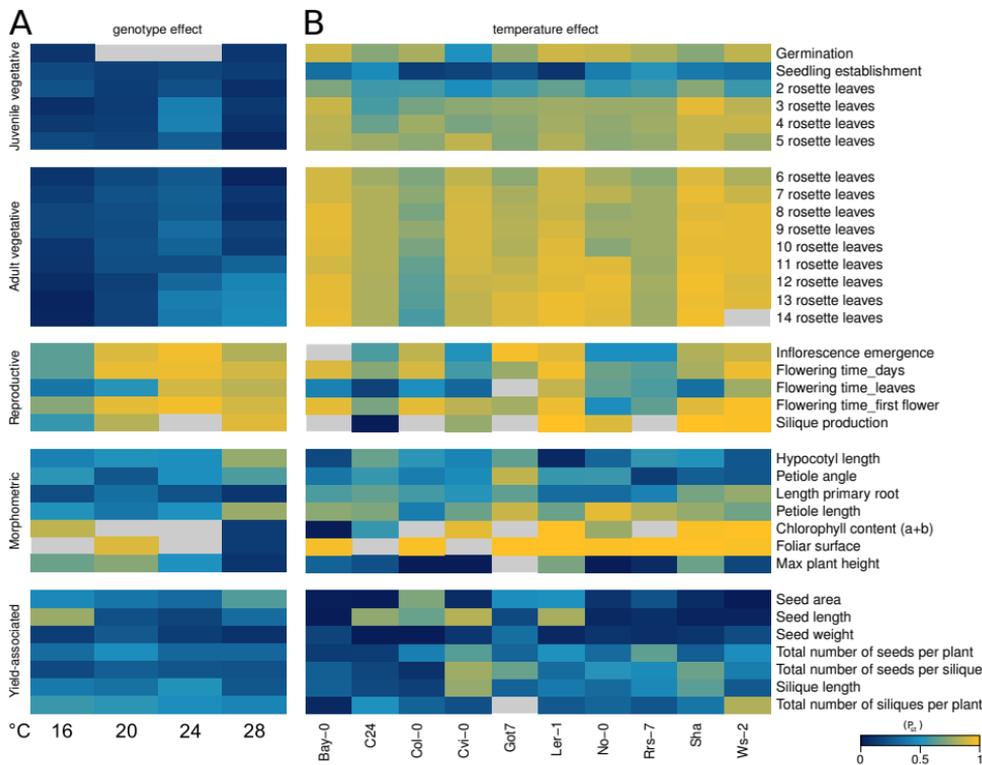
Yield-associated phenotypes

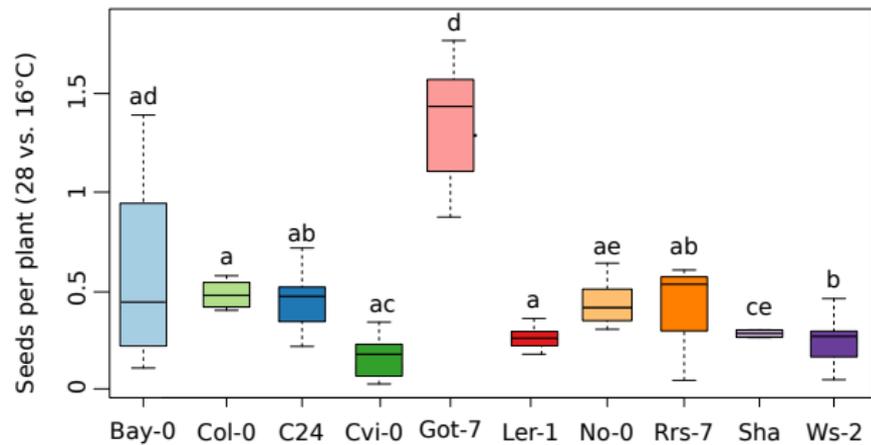
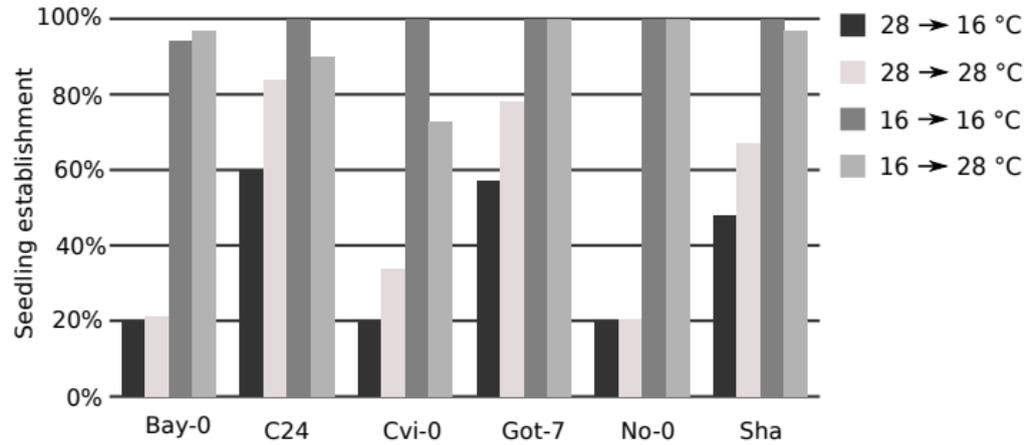
Juvenile vegetative stage









A**B****C**