# Phenotypic plasticity and ecotypic variations in growth and flowering time of *Arabidopsis thaliana* (L.) under different light and temperature conditions

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Four ecotypes of *A. thaliana* (L.) (Ct-1, Pf-0, Old-1 and Per-1) from low to high latitudes were grown under different light (300 mol photon m$^{-2}$s$^{-1}$ and 150 mol photon m$^{-2}$s$^{-1}$) and temperature (22 and 14 ºC) conditions to investigate their effects on phenotypic plasticity and ecotypic variations in plant growth and first flowering time. The results suggest that in *A. thaliana* low temperature decreases both phenotypic plasticity and ecotypic variations in first flowering time and total dry matter at final harvest under different light intensities. Relative growth rate is the most stable parameter of *A. thaliana* that is hardly affected by ecotype (no effect), light (no effect) or temperature (small effect) and this may one of the reason why *A. thaliana* is widely distributed on earth as a result of adaptations to different environments.

**Keywords**: *Arabidopsis thaliana*, Flowering, Latitude, Phenotypic plasticity, Temperature

*Arabidopsis thaliana*, (L.), a herbaceous monocarpic annual plant of Brassicaceae, is widely distributed on earth from about N 10º to about N 60º[1,2]. Therefore, *A. thaliana* is an interesting model species for understanding the mechanisms of plant adaptations to a wide range of environments along latitude. Latitude is an important determinant of local environmental conditions that affect plant growth and development. The annual mean values of both daily radiation integral and average daily air temperature decrease approximately linearly with increasing latitude even though the annual mean value of day length remains constant across latitude[3]. Low-latitude areas tend to have higher temperatures, which accelerate metabolic activities, cell growth and photosynthesis and thus the growth of a whole plant[1]. In contrast, at high latitudes, temperature is low and limits photosynthesis and thus plant growth[1,4,5,6]. Plants from lower latitudes generally tend to have shorter vegetative periods and earlier flowering times than plants from higher latitudes. These changes are genetically regulated within a species[4,7,8]. For example, Li *et al.*[1] found that leaf area ratio (i.e. ratio of total leaf area to total mass of a plant; LAR) increased and unit leaf rate (i.e. increase in plant mass per unit time interval per unit leaf area of a plant; ULR) decreased with increasing latitude in 40 ecotypes of *A. thaliana*, resulting in an almost constant relative growth rate (i.e. relative increase in plant mass per unit time interval with respect to the initial plant mass; RGR) across latitude.

*Arabidopsis thaliana* has a wide distribution from low to high latitudes, and its ecological variations and adaptations have drawn much attention also from a view point of plant physiology[2]. Ecotypic variations in morphology[7,9,10], phenology[7,9,11], growth rate[1] and life history[10,12] of *A. thaliana* with latitude have been well documented. However, these have been studied almost independently and interrelationships between these variations have rarely been studied. Since plant life history and geographic distribution are greatly affected by environments, it is important to study the adaptive relationships between environmental stresses and genetic characteristics of ecotypes regarding both RGR (vegetative growth) and flowering time (reproduction) under different light and temperature

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conditions. However, the previous study of Li *et al.*[1] surveyed only the relationship between plant size and RGR of 40 ecotypes of *A. thaliana* from various latitudes and Shibaike *et al.*[13] surveyed only flowering time in eight natural populations of *A. thaliana* in Japan.

In nature, both light intensity and temperature change simultaneously. However, interactive effects of light and temperature on plant growth and reproduction have not been studied so far; the effects of either light or temperature have been studied almost independently[14,16]. In this communication, the relationships between first flowering time and growth rate under controlled conditions of light and temperature are reported because light intensity and temperature appear to have different effects on the physiological characteristics of different *A. thaliana* ecotypes from different latitudes at the leaf level[17]. Here, it is also expected that the phenotypic plasticity of growth and reproduction within each ecotype in response to changing environments is important for the ecological adaptations of *A. thaliana*[18,19]. Therefore, not only the interactive effects of light intensity and temperature on different *A. thaliana* ecotypes (i.e. ecotypic variations) have been investigated but also plasticity within each ecotype in growth rate and first flowering time in response to different light and temperature conditions; with a view to answer how do variations in light intensity and temperature interactively affect the various aspects of *A. thaliana* ecotypes?

## Materials and Methods

*Growth conditions*—Seeds of four different ecotypes of *A. thaliana* (L.), Ct-1 (original habitat: N 37.5°, E15°), Pf-0 (N 48.5°, E9°), Old-1 (N 53°, E8°) and Per-1 (N58°,E 56°) from low to high latitudes were obtained from the SENDAI *Arabidopsis* Seed Stock Centre (SASSC), Japan. About 90% of the 40 *A. thaliana* ecotypes investigated by Li *et al.*[1] are distributed from N 35° to N 60°. Therefore, in this experiment four ecotypes from nearly equidistant habitats along the latitudinal gradient between N 35° and N 60° were selected.

The seeds were sown in 5 cm (width), 5 cm (height) and 7 cm (depth) square plastic pots containing a mixture of 1:1 vermiculite and perlite. The sowing density was four seeds per pot. Each pot contained one ecotype and there were six pots for each ecotype per tray. In total, 24 pots of four ecotypes were arranged by using split-plot design in one tray. There were four trays for each controlled growth condition. Cold dark pre-treatment at 4 °C for four days was given in growth chambers (Nippon Medical and Chemical Instruments Co Ltd, Japan).

All controlled growth chambers were adjusted to the following different growth conditions: 16 h light and 8 h dark cycle; 300 mol photon m$^{-2}$s$^{-1}$ or 150 mol photon m$^{-2}$s$^{-1}$; 22 °C or 14 °C. Two replications of chambers for each growth condition were used. Ecotypes were exposed to 16 h light because *Arabidopsis* is a long day plant. The flowering time of *A. thaliana* is strongly influenced by photoperiod length and exposure of plants to a long photoperiod of 16 h light promotes flowering, while flowering is delayed under a short photoperiod of 10 h[20]. The employed 300 mol photon m$^{-2}$s$^{-1}$ and 150 mol photon m$^{-2}$s$^{-1}$ light intensities refer to the actual conditions in the field experienced by this species[21]. *A. thaliana* coexists with many taller plants in natural vegetation; therefore, *A. thaliana* may receive a light intensity of around 150-300 mol photon m$^{-2}$s$^{-1}$ in the field, even though this species often occurs in open habitats[21]. The employed temperature of 22 °C approximately simulates that of original habitats of Ct-1 and Pf-0, while the temperature of 14 °C that of Old-1 and Per-1[1]. On the sowing day, each tray was sufficiently supplied with 2,500-fold HYPONeX nutrient solution (HYPONeX Osaka, Japan). The nutrient solution (500 mL) was supplied to each tray once a week until the end of the experiment. Water (500 mL) was given twice a week for each tray.

Only seedlings that emerged from the soil surface on the same day were retained and others were thinned to a density of one seedling per pot to avoid the effect of time of seedling emergence.

*Measurement*—For all four ecotypes, three harvests were made on day 17, 24 and 31 after seedling emergence. Plants were separated into root, stem and leaf, dried and weighed for each organ. RGR was estimated by regression analysis in which the dependent variable *y* was the natural log-transformed dry weight of an individual plant and the independent variable *x* was the time of harvest (days after emergence in this study)[22,1]; RGR was given as the slope of the regression line. LAR (cm$^2$mg$^{-1}$), SLA (specific leaf area, i.e. ratio of leaf area to leaf mass, cm$^2$mg$^{-1}$) and ULR (mg cm$^{-2}$day$^{-1}$) were calculated according to Hunt[22] and Li *et al.*[1]. The day when flower buds were visually seen was recorded as first flowering time (FT) for each ecotype. Root:shoot

ratio (RSR) was calculated at the final harvest. Experiments were repeated twice to check reproducibility. The data of these two experiments were pooled for statistical analyses.

*Statistical analyses*—To investigate the effects of ecotype (E), light intensity (L) and temperature (T) on FT, total dry weight at final harvest (DW) and RSR at final harvest, three-way ANOVA (analysis of variance) was first performed, in which FT, DW or RSR was a dependent variable, and E, L and T were treated as factors. There were significant interaction effects among E, L and T. Therefore, one-way ANOVA was performed for 16 levels (groups) of $\{E_k, L_i, T_j\}$, where, k = 1: Ct-1, 2: Pf-0, 3: Old-1, 4: Per-1; i = 1: 300 mol photon $m^{-2}s^{-1}$, 2:150 mol photon $m^{-2}s^{-1}$; j = 1: 22 ℃, 2:14 ℃. Each level represents the data (FT, DW or RSR) obtained for each ecotype under each light intensity at each temperature. In order to investigate the effects of different light and temperature conditions on each ecotype and differences among the four ecotypes, a Bonferroni multiple comparison was performed. Log transformation of the variables was made when necessary. The differences in RGR between ecotypes (E) or growth conditions (L, T) were assessed by multiple comparison of the slopes of regression lines among 16 levels of {E, L, T} in which log (total dry weight of an individual plant) was treated as the dependent variable and time after emergence as the independent variable.

To check correlations between FT, RGR, LAR, SLA, ULR, DW or RSR, correlation analysis was performed.

## Results

Flowering occurred earlier at 22 ℃ (within 31 days) than at 14 ℃ (within 57 days), namely, plasticity of FT in response to temperature was found under each light intensity in each ecotype (Fig. 1A; Table 1). Plasticity of FT in response to light was found at 22 ℃ (except for Ct-1) but not at all at 14℃ (Fig. 2A, Table 1).

Plasticity of RGR in response to temperature was found under 150 mol photon $m^{-2}s^{-1}$ (except for Per-1) but not at all under 300 mol photon $m^{-2}s^{-1}$ (Fig. 1B; Table 1). Plasticity of RGR in response to light was not found at all at each temperature (Fig. 2B; Table 1).

Plasticity of DW in response to temperature was found under each light intensity (except for Per-1) (Fig. 1C; Table 2). Plasticity of DW in response
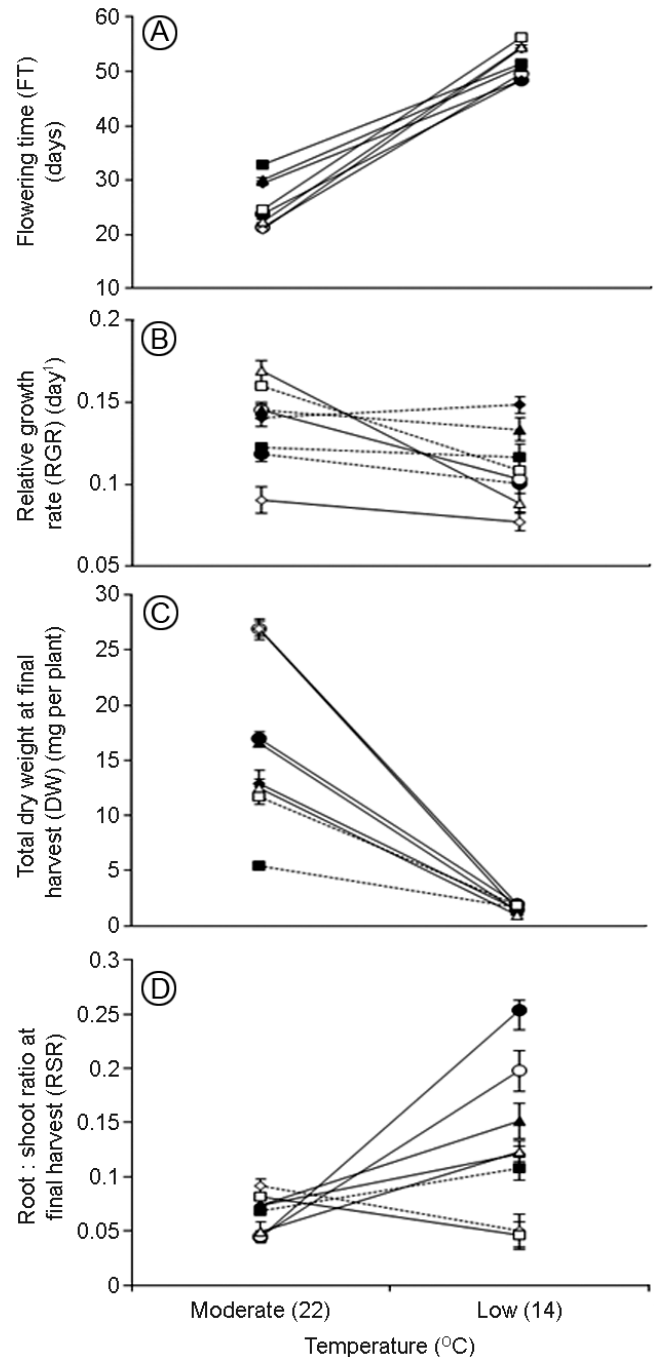


Fig. 1—Within-ecotype plasticity in flowering time (A), relative growth rate (B), total dry weight at final harvest (C) and root: shoot ratio at final harvest (D) of four *A. thaliana* ecotypes grown at moderate (22 ℃) and low (14 ℃) temperature under 300 mol photon $m^{-2}s^{-1}$ (closed symbols) and 150 mol photon $m^{-2}s^{-1}$ (open symbols). Ct-1, ● and ○; Pf-0, ◆ and ◇; Old-1, ▲ and △; Per-1, ■ and □ under 300 mol photon $m^{-2}s^{-1}$ and 150 mol photon $m^{-2}s^{-1}$, respectively. Solid lines represent significant difference ($P$ <0.05), while dashed lines represent non-significant difference ($P$ >0.05). Mean ± SE for each ecotype and each growth condition ($n$= 5).

Table 1—The results of Bonferoni multiple comparison for RGR (upper right) and (FT) (lower left) of four ecotypes of *Arabidopsis thaliana* in the one-way ANOVA of 16 levels (groups).

| | E1 L1 T1 | E1 L2 T1 | E1 L1 T2 | E1 L2 T2 | E2 L1 T1 | E2 L2 T1 | E2 L1 T2 | E2 L2 T2 | E3 L1 T1 | E3 L2 T1 | E3 L1 T2 | E3 L2 T2 | E4 L1 T1 | E4 L2 T1 | E4 L1 T2 | E4 L2 T2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E1 L1 T1 | | | | | | | | | | | | | | | | |
| E1 L2 T1 | | | | | * | | | | | | | | | | | |
| E1 L1 T2 | * | * | | | | | | | | | | | | | | |
| E1 L2 T2 | * | * | | | | | | | | | | | | | | |
| E2 L1 T1 | | * | * | * | | | | | | | | | | | | |
| E2 L2 T1 | | * | * | * | | | | * | | | | | | | | |
| E2 L1 T2 | * | * | | | * | * | | | | * | | RGR | | * | | |
| E2 L2 T2 | * | * | | | * | * | | | | | | | | | | |
| E3 L1 T1 | | * | * | * | | * | * | * | | | | | | | | |
| E3 L2 T1 | | * | * | | | * | * | * | | | * | * | | | | |
| E3 L1 T2 | * | * | | | * | * | | | * | * | | | | | * | |
| E3 L2 T2 | * | * | | | * | * | | | * | * | | | | | | |
| E4 L1 T1 | * | * | | | * | * | | | * | * | | | | | | |
| E4 L2 T1 | | * | * | | | | * | * | | | * | * | * | | | |
| E4 L1 T2 | * | * | | | * | * | | | * | * | | | | | * | |
| E4 L2 T2 | * | * | * | | * | * | * | | * | * | * | FT | | * | | |

RGR= relative growth rate; FT= flowering time.

In one-way ANOVA of 16 levels (groups) of ecotype ($E_k$), light ($L_i$), temperature ($T_j$) } where, k = 1: Ct-1, 2: Pf-0, 3: Old-1, 4: Per-1; i = 1: 300  mol photon m$^{-2}$s$^{-1}$, 2: 150  mol photon m$^{-2}$s$^{-1}$; j = 1: 22 ℃, 2: 14 ℃. Each level (group) represents data of RGR or FT obtained for each ecotype under each light intensity at each temperature.

*, significant difference at $P < 0.05$. Blank blocks represent not significantly different cases ($P > 0.05$).

to light was found at 22 ℃ (except for Old-1) but not at 14 °C (except for Per-1) (Fig. 2C, Table 2).

Plasticity of RSR in response to temperature was found under each light intensity (except for Per-1under 300  mol photon m$^{-2}$s$^{-1}$and Pf-0 under 150  mol photon m$^{-2}$s$^{-1}$) (Fig. 1D; Table 2). Plasticity of RSR in response to light was not found at each temperature (except for Ct-1 at 14 °C) (Fig. 2D; Table 2).

At 22 ℃, ecotypic variations were found in FT under 300  mol photon m$^{-2}$s$^{-1}$ and DW under each light intensity, whereas RGR or RSR showed no ecotypic variations under each light intensity (Table 1 and 2; also see Fig. 2). At 14 °C, however, there were no ecotypic variations in FT, RGR or DW under each light intensity; ecotypic variation was found only in RSR under each light intensity (Table 1 and 2).

For the correlation analysis, we pooled all four ecotypes together. All four ecotypes together showed no significant correlations between FT and RGR, LAR or SLA under different light and temperature conditions (Table 3). However, they showed a negative correlation between FT and ULR, DW or RSR under different light and temperature conditions (Table 3) ($P < 0.01$, $n = 16$). All four ecotypes together showed no correlations between RGR and FT, LAR, SLA, ULR, DW or RSR but there was a negative correlation between LAR and ULR under different light and temperature conditions (Table 3) ($P < 0.01$, $n = 16$). Under different light and temperature conditions, all four ecotypes together showed positive correlations between DW and RSR (Table 3) ($P < 0.01$, $n = 16$).

## Discussion

The objective of this study was to determine how variations in light intensity and temperature interactively affect within-ecotype plasticity and ecotypic variations in the growth rate and first flowering time of *A. thaliana* ecotypes.

Delayed first flowering of *A. thaliana* ecotypes at 14 °C than at 22 °C under each light intensity indicates that low temperature lowers whole plant growth and development (Fig. 1A). Moreover, low temperature, 14 ℃, reduced within-ecotype plasticity and the effect of light intensity on ecotypic variation in FT (Figs 1A, 2A), while at 22 °C there
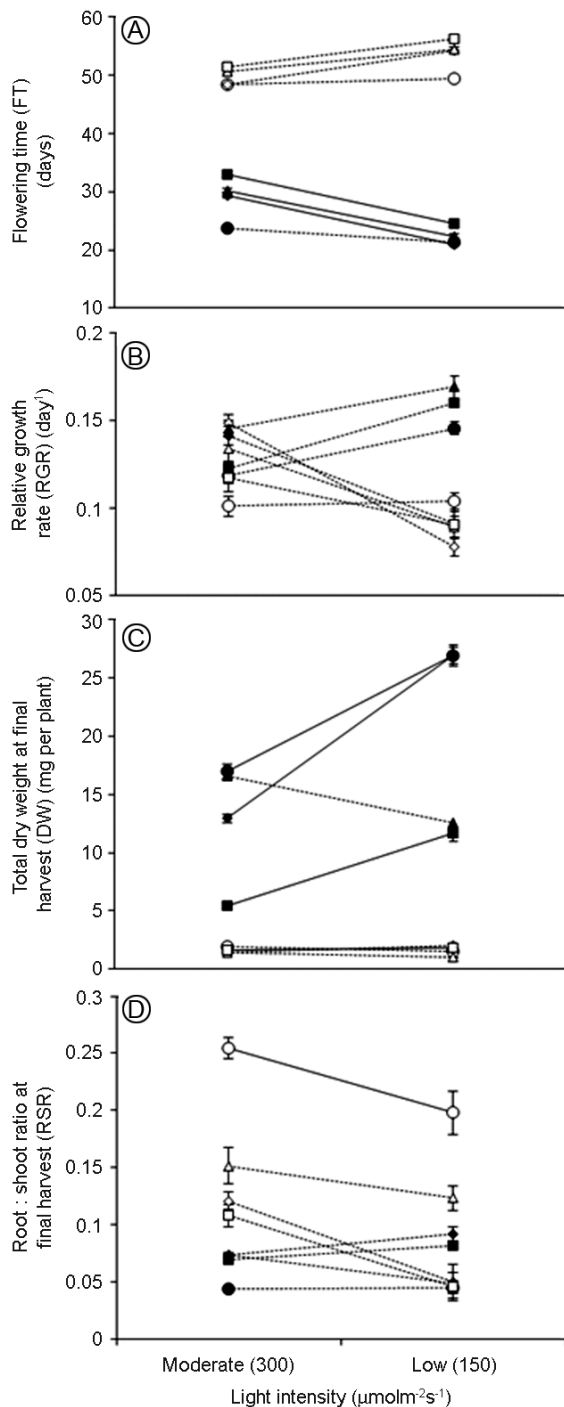
Fig. 2—Within-ecotype plasticity in flowering time (A), relative growth rate (B), total dry weight at final harvest (C) and root: shoot ratio at final harvest (D) of four *Arabidopsis thaliana* ecotypes grown under moderate (300 mol photon $m^{-2}s^{-1}$) and low light intensity (150 mol photon $m^{-2}s^{-1}$) at 22 ℃ (closed symbols) and 14 ℃ (open symbols). Ct-1, ● and○; Pf-0, ◆ and ◇; Old-1, ▲ and △; Per-1, ■ and □ at 22 ℃ and 14 ℃, respectively. Solid lines represent significant difference (*P* <0.05), while dashed lines represent non-significant difference (*P*>0.05). Mean ± SE for each ecotype and each growth condition (*n*= 5).

was great within-ecotype plasticity of FT between two light intensities. At 22 °C, earlier flowering and greater DW in all four ecotypes under 150 mol photon $m^{-2}s^{-1}$ than under 300 mol photon $m^{-2}s^{-1}$ (Figs 2A and C), indicating that low light conditions are advantageous for the growth of *A. thaliana*. In support of present results, Callahan & Pigliucci[21] reported that in the field, flowering occurred earlier at shadier sites compared to less shady sites in the wild populations of *A. thaliana*.

RGR of *A. thaliana* showed no plasticity between two temperatures under 300 mol photon $m^{-2}s^{-1}$, but under 150 mol photon $m^{-2}s^{-1}$ RGR was statistically somewhat lower at 14 °C than at 22 °C (Fig. 1B), indicating that plant growth rate was affected by the interactive effect of light and temperature conditions. However, at each of 22 ℃ and 14 ℃, there was no ecotypic variation in RGR under each light intensity or within-ecotype plasticity between two light intensities (Figs 1B and 2B; Table 1). Li *et al.*[1] found a negative correlation between RGR and the latitude of the original habitats of 40 ecotypes of *A. thaliana* grown in a green house under natural conditions; however, the correlation was very weak (*r* = 0.45, *P*<0.01) and the variation with latitude was very small (minimum: 0.21 $day^{-1}$ – maximum: 0.25 $day^{-1}$ from the regression line). In the present study, RGR of *A. thaliana* was also fairly stable, hardly affected by ecotype (no effect), light (no effect) or temperature (small effect); however, FT was fairly variable as affected by ecotype, light and temperature, and negatively dependent on ULR and DW (Table 3). Under different light and temperature conditions, there was no correlation between RGR and FT in all four ecotypes, although this relation might change with another set of ecotypes. Kobayashi *et al.*[23] found that an ecotype of *Plantago asiatica* (a perennial forb) with large RGR flowered earlier than an ecotype with small RGR in the vegetative period. There was a negative correlation between FT and ULR under different light and temperature conditions in all four ecotypes, suggesting that the increased photosynthetic productivity promoted flowering (Table 3).

At 22 ℃ under 300 mol photon $m^{-2}s^{-1}$, DW showed a negative significant correlation with FT in all four ecotypes, suggesting that Ct-1 is a fast growing south ecotype with earlier flowering time and greater dry weight than a north ecotype Per-1 (Figs 2A and 2C). In parallel to this, it is well known that plants from low-latitude habitats tend to have shorter

Table 2—The results of Bonferoni multiple comparison for DW (upper right) and RSR (lower left) of four ecotypes of *Arabidopsis thaliana* in the one-way ANOVA of 16 levels (groups).

| | E1 L1 T1 | E1 L2 T1 | E1 L1 T2 | E1 L2 T2 | E2 L1 T1 | E2 L2 T1 | E2 L1 T2 | E2 L2 T2 | E3 L1 T1 | E3 L2 T1 | E3 L1 T2 | E3 L2 T2 | E4 L1 T1 | E4 L2 T1 | E4 L1 T2 | E4 L2 T2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E1 L1 T1 | | * | * | * | | * | * | * | | | * | * | * | * | * | * |
| E1 L2 T1 | | | * | * | * | | * | * | * | * | * | * | * | * | * | * |
| E1 L1 T2 | * | * | | | * | * | | | * | * | | | | * | | |
| E1 L2 T2 | * | * | * | | * | * | | | * | * | | | | | | |
| E2 L1 T1 | | | * | * | | * | * | * | | | * | * | * | | * | * |
| E2 L2 T1 | | | * | * | | | * | * | * | * | * | * | * | * | * | * |
| E2 L1 T2 | * | * | * | | * | | | | * | * | | | | * | | * |
| E2 L2 T2 | | | * | * | * | | | | * | * | DW | | * | | | |
| E3 L1 T1 | | | * | * | | | | | | | * | * | * | * | * | * |
| E3 L2 T1 | | | * | * | | | | | | | * | * | * | | | |
| E3 L1 T2 | * | * | | * | * | | * | * | * | | | | | | | |
| E3 L2 T2 | * | * | * | | | | * | * | * | | | | | | | |
| E4 L1 T1 | | | * | * | | | | | | | * | * | | * | | * |
| E4 L2 T1 | | | * | * | | | | | | | * | RSR | | | * | |
| E4 L1 T2 | * | * | * | | | | | | | * | | | | | | * |
| E4 L2 T2 | | | * | * | | * | * | | | | * | * | | * | | |

DW= total dry weight; RSR= root shoot ratio.

In one-way ANOVA of 16 levels (groups) of ecotype ($E_k$), light ($L_i$), temperature ($T_j$) } where, k = 1: Ct-1, 2: Pf-0, 3: Old-1, 4: Per-1; i = 1: 300 mol photon $m^{-2}s^{-1}$, 2: 150 mol photon $m^{-2}s^{-1}$; j = 1: 22 ºC, 2: 14 ºC. Each level (group) represents data of DW or RSR obtained for each ecotype under each light intensity at each temperature.

*, significant difference at $P < 0.05$. Blank blocks represent not significantly different cases ($P > 0.05$).

Table 3—The results of correlation analysis between different parameters

| | FT | RGR | LAR | SLA | ULR | DW | RSR |
|---|---|---|---|---|---|---|---|
| FT | | | | | | | |
| RGR | | | | | | | |
| LAR | | | | | | | |
| SLA | | | | | | | |
| ULR | — | | | — | | | |
| DW | — | | | | | | |
| RSR | — | | | | | + | |

(FT= flowering time; RGR= relative growth rate; LAR= leaf area ratio; SLA= specific leaf area; ULR= unit leaf rate, DW= total dry weight; RSR= root shoot ratio) of four ecotypes of *A. thaliana* under different light and temperature conditions. + or — sign indicates significant ($P < 0.05$) positive or negative correlation, respectively. Blank blocks represent not significantly different cases ($P > 0.05$).

vegetative periods than those from high-latitude habitats and this latitudinal change is genetically based[4,7,8]. In contrast, in *Plantago asiatica*, which is distributed at wide latitudes and altitudes, the plants from northern habitats showed greater leaf allocation and plant productivity than those from southern habitats[24]. However, such within-ecotype plasticity of DW and FT was not observed at 14 ºC between two light intensities (Figs 2A and 2C; Tables 1, 2). Additionally, very small DW at 14 ºC than at 22 ºC, suggesting that plants grown at low temperature exhibit reduced investment into the shoot and leaves which results in slow plant growth and late flowering (Figs 1A, 1C).

In contrast with the other parameters like FT and DW, RSR showed great within-ecotype plasticity at 14 ºC in response to light intensity, whereas it was not observed at 22 ºC (Fig. 1D). RSR was greater at 14 ºC than at 22 ºC in each ecotype except for Per-1 (Fig. 1D). This suggests that at 14 ºC roots grow bigger to get much water because low temperature stress reduces water absorption rate in relation to aquaporin content[25]. Actually, Oshima et al.[25] reported that in *Graptopetalum*, aquaporin content decreased with a decrease in temperature. In our study, biomass was allocated more to roots probably to compensate for low aquaporin content at a low temperature.

In many plant species, positive correlations between RGR and SLA, LAR [26,28] or ULR[29,30] have

been reported. Loveys *et al.*[16] reported that the correlations between RGR and its components (LAR and ULR) were greatly influenced by growth temperature in 16 fast- and slow-growing species. In our study, we did not find any relationships between RGR and its components under low to moderate light and temperature conditions, suggesting that low growth temperature might have reduced correlations between RGR and its components in *A. thaliana* (Table 3). However, the negative correlation between LAR and ULR (Table 3) indicates that under different light and temperature there was a trade-off between energy investment into leaf development and photosynthetic productivity.

## Conclusion

Low temperature lowers both within-ecotype plasticity and ecotypic variations in FT and DW of *A. thaliana* under different light intensities, which are great at a moderate temperature, and also reduces the correlations between these parameters. In our study, moderate light (300 mol photon m$^{-2}$s$^{-1}$) was stressful condition for plant growth, which lowered plasticity of growth (RGR). Low temperature (14 °C) was also stressful for FT, DW and growth (RGR) and lowered plasticity of them. The fairly stable RGR of *A. thaliana* under different light and temperature conditions (no ecotypic variations and very small within-ecotype plasticity of our study) as compared with many other species[23] may be part of the reason why this species is widely distributed on earth as a result of adaptations to different environments in terms of growth rate[2]. We presented the findings of a preliminary study with only four ecotypes but believe that our findings are general phenomena because our result of RGR with four ecotypes was the same as that of Li *et al.*[1] with 40 ecotypes regarding ecotypic variations. From our results, we hypothesize that stressful growth conditions for plants reduce plasticity of growth and flowering parameters. However, we need further investigation with more ecotypes to fully understand within-ecotype plasticity and ecotypic variations.

## Acknowledgement

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