NON-STRESSFUL TEMPERATURE CHANGES AFFECT TRANSGENERATIONAL PHENOTYPIC PLASTICITY ACROSS THE LIFE CYCLE OF *ARABIDOPSIS THALIANA* PLANTS

Ailén Authier[1,2,4], Pablo Cerdán[1,3], Gabriela Auge[1,2,4]*

[1] Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET). Argentina; [2]Instituto de Biociencias, Biotecnología y Biología Traslacional (iB3), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Buenos Aires, Argentina; [3] Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas Buenos Aires – Consejo Nacional de Investigaciones Científicas y Tecnológicas (IIBBA – CONICET). Buenos Aires, Argentina.

**For correspondence: auge.gabriela@inta.gob.ar*

ABSTRACT

Background and Aims. Plants respond plastically to seasonal changes, often resulting in an adaptation to environmental variation. Even though much is known about how seasonality regulates developmental transitions within generations, transgenerational effects of non-stressful environmental changes are just beginning to be unveiled. This study aimed to evaluate the effects of ambient temperature changes on the expression of transgenerational plasticity in key developmental traits of *Arabidopsis thaliana* plants.

Methods. We grew Columbia-0 plants in two contrasting temperature environments (18 and 24°C) during their whole life cycles, or the combination of those before and after bolting (18-24°C and 24-18°C) across two generations. We recorded seed germination, flowering time and reproductive biomass production for the second generation, and seed size of the third generation.

Key Results. The environment during the whole life cycle of the first-generation of plants, even that experienced before flowering, influenced the germination response and flowering time of the second generation. These effects showed opposing directions in a pattern dependent on the life stage experiencing the cue in the first-generation. On the contrary, reproductive biomass production depended on the immediate environment of the progeny generation. Finally, the seed area of the third generation was influenced positively by correlated environments across generations.

Conclusions. Our results suggest that non-stressful environmental changes affect the expression of key developmental traits across generations, although those changes can have contrasting effects depending on the parental and grandparental life stage that perceives the cue. Thus,

transgenerational effects in response to non-stressful cues may influence the expression of life history traits and potential adaptation of future generations.

KEYWORDS: plasticity, germination, flowering, *Arabidopsis thaliana*, reproductive biomass, seeds

INTRODUCTION

Plants have evolved in seasonal environments, adjusting their phenology to optimal environmental conditions across the year. Plants achieve this by integrating environmental information through the perception of cue changes, initiating a signaling cascade and adjusting development programs, altogether resulting in a concerted response to the environment. This response is maintained until conditions are changed and this cycle starts all over again (Anderson *et al.* 2011). Phenotypic plasticity, in which a certain genotype displays a range of phenotypic responses to face daily and seasonal changes, is essential for the survival, growth and reproduction of individuals (Liancourt *et al.* 2013). The informational value of environmental changes that can induce phenotypic plasticity depends on the selective pressure exerted by that environmental cue (Karban *et al.* 1999). If the phenotypic plasticity displayed by plants is consistent with the environment and not detrimental to fitness, then it acquires adaptive value (Dudley and Schmitt 1996).

Plants can respond to changes in the environment that occurred during their life cycle and those of previous generations (Gutterman 1982; Sultan 1996, 2000; Donohue and Schmitt 1998; Donohue 2009; Willis *et al.* 2014). Much of the work done on effects across generations has been focused on the study of establishment or maintenance of responses to stressful conditions, such as presence of pathogens, extreme temperatures (high or low), drought, etc. (reviewed in Bonduriansky 2021; Auge et al. 2023). However, the effects of environmental factors associated with non-stressful seasonal changes and their correspondence with plant responses across generations remain to be revealed.

Adequate developmental transition control requires the perception of seasonal cues in order for them to be properly coordinated throughout the life cycle (Auge, *et al.* 2017). For annual plants—which germinate, flower and reproduce over the course of a year—there are two key developmental transitions: the transition from vegetative to reproductive stages (flowering) and the seed to seedling stages (germination) (Donohue *et al.* 2010; Ehrlén 2015; Auge, *et al.* 2017). The timing of these transitions is under strong natural selection and is finely regulated by environmental conditions, both responding to temperature, light quality, and photoperiod, among other cues (Simpson and Dean 2002; Amasino 2004; Michaels *et al.* 2005; Holdsworth *et al.* 2008; Donohue *et al.* 2010; Munguía-Rosas *et al.* 2011; Ehrlén 2015). Plants integrate this information in such a way that the environmental conditions experienced by mother plants influence the behavior of the progeny (J. Marshall and Uller 2007; English *et al.* 2015). The correct integration of environmental information within and across generations would allow future generations to respond adaptively to their own environments. *Arabidopsis thaliana* plants show transgenerational plasticity in multiple traits in response to changes of the parental environment (Groot *et al.* 2017; Lampei 2019; Alvarez *et al.* 2020). *A. thaliana* mostly displays an annual winter life history in its native range, experiencing fluctuating temperatures during its life cycle: seeds germinate in autumn when temperature drops and humidity increases, and plants overwinter as a rosette. The spring with longer days and warmer temperatures induces flowering, and the seeds are produced and dispersed at the end of this season finishing the cycle. The hot and dry summer induces seed dormancy, but the arrival of the autumn with cooler and more humid days breaks this dormancy and induces germination, starting the cycle all over again (Casas et al. 2012). A disturbance in the environment in any of the seasons could induce a change in the adjustment of the life cycle. For example, if the autumn is too dry, seeds may not

germinate until the arrival of spring, thus shifting the entire life cycle into a completely different season and part of it probably happening during the summer, which can markedly reduce survival (Zacchello et al. 2020; Postma and Ågren 2022). However, some *A. thaliana* populations have also evolved a spring/summer annual phenology, which allows them to complete a whole life cycle after dispersion of seeds in the spring when environmental conditions are met (a compromise between environments before and after seed dispersal) (Penfield and Springthorpe, 2012; Huang *et al.* 2014). Nonetheless, as different life stages have different tolerances to environmental conditions, plants must adjust their developmental transitions so that each stage matches the optimal environment that allows for their development and survival. Even mild changes can affect the timing of those transitions, shifting development to suboptimal growing conditions (Gray and Brady 2016).

Changing temperatures, in particular when experienced by the *A. thaliana* parental generation, result in phenotypic plasticity in the progeny (Whittle *et al.* 2009; Suter and Widmer 2013a, b; He *et al.* 2014; Burghardt *et al.* 2016; Groot *et al.* 2017; Auge, Blair, *et al.* 2017; Alvarez *et al.* 2020). In addition, hot temperatures have negative effects in all stages of the life cycle of this species (Zinn *et al.* 2010; Groot *et al.* 2017), creating a potential selective agent in their natural habitat (Wolfe and Tonsor 2014). The parental environment also influences quantity, viability and vigor of progeny seeds (Valencia-Díaz and Montaña 2005; Wang *et al.* 2015; Li *et al.* 2017; Huang *et al.* 2018; Awan *et al.* 2018). Altogether, these traits, which are under selection, could affect fitness of the next generation (Larios *et al.* 2014; Lu *et al.* 2016).

The objective of this study was to investigate whether non-stressful ambient temperature changes would influence plasticity across generations in *A. thaliana* plant traits (germination, flowering time, reproductive biomass production and seed size), also evaluating the impact of timing of

those environmental effects and their persistence in the life cycle. Since environmental changes do not necessarily have to be stressful in nature to promote plasticity, we hypothesize that transgenerational plasticity can also occur in response to non-stressful changes, leading to adaptive transgenerational effects throughout the life cycle of plants. The environmental conditions experienced during the entire life cycle of the parental generation, even before flowering, will influence the germination response, flowering time, seed yield and size of subsequent generations. These findings would imply that non-stressful stimuli might influence the expression of life traits and potentially contribute to the adaptive ability of future plant generations.

MATERIALS AND METHODS

Plant material and temperature treatments

In this study, we used the *Arabidopsis thaliana* Columbia-0 (Col-0) ecotype. This accession is a highly inbred line typically used in plant biology labs, and displays early flowering and intermediate dormancy, which allow us to properly assess the phenotypes of interest of this study (Somssich 2019).

A first generation of plants (G0) were initially grown to homogenize the environment in which seeds of the first generation (G1) were produced with the goal of removing any undesired transgenerational effects. G0 seeds were imbibed in a plate with agar-agar 0.6% w/v (Plant Agar, Duchefa Biochemie) for 4 days at 4°C in darkness. Seeds were then transferred using a micropipette to soaked 180 ml pots with a mix of 2:1 soil:perlite (Grow Mix Multipro, Agroquímica Larrocca SRL.). Pots were immediately placed in a growth chamber (white light

sources were Neutral LED tubes 4100K, PAR~130 µmol m^{-2} s^{-1}) where seeds were allowed to germinate at 24°C in a long day photoperiod (LD, 16h light / 8h darkness). One week after germination, excess seedlings were thinned leaving one focal plant per pot. Plants were grown at 24°C and LD until harvest. Plants were watered regularly (2-3 times a week) and fertilized once with Hakaphos 0.1% w/v (Compo Agricultura) immediately after bolting. Seeds were harvested when 2/3 of the fruits were dry and placed in a hermetic container with silica gel for 3 days. Seeds from 3 different individual plants were pooled and stored in 1.5ml tubes to generate the G1 seed stock, and then kept in a fridge until used for experiments.

To explore the temperature effects on the expression of plasticity across generations, we designed a multigenerational experiment in which we grew plants at two temperatures that are known to have contrasting effects on *A. thaliana* growth: 18 and 24°C (Sánchez-Lamas *et al.* 2016). We either grew the plants during their whole life cycles at those temperatures (18-18 and 24-24), or they were moved to the contrasting temperature after bolting (18-24 and 24-18), obtaining a combination of four possible thermal environments (Table 1).

Treatment 3 would emulate what Columbia-0 plants (a spring/summer annual ecotype) encounters in natural conditions: progression of cooler to warmer temperatures during the life cycle would indicate the passage through spring into the summer. Comparing responses between treatments 1 vs. 3 and 2 vs. 4 would provide information about the effect of the environment after flowering (reproductive growth) on the development of the next generation, while comparing responses between treatments 1 vs. 2 and 3 vs 4 would inform us on the effects of the environment during early development of the previous generation (vegetative growth, before flowering).

G1 plants growth

G1 seeds were incubated in the conditions described above, then transferred to pots with a mix of soil and perlite (2:1) and randomly placed in LD at 24°C for pots of treatments 1 and 3, or at 18°C for pots of treatments 2 and 4 (see Table 1 and previous section for growth condition specifications). Seeds were allowed to germinate and after one-week, excess seedlings were thinned to leave a focal plant per pot with a total of 12 pots (plants) per treatment. Plants growing at both temperatures were watered regularly (3 times a week for the ones growing at 24°C, and 1-2 times a week for those growing at 18°C) and fertilized once with Hakaphos 0.1% w/v (Compo Agricultura, Argentina) after bolting. Plants of treatments 1 and 4 were kept in the same conditions during their whole life cycle; those of treatments 2 and 3 were moved to the contrasting temperature (moved between chambers) after bolting was observed. All plants were randomized weekly within the chambers and containing trays to avoid positional effects. For this, plants were checked every other day for floral bud emergence. As growth at both temperatures may result in differences in flowering time and plant development, planting was staggered to synchronize seed harvest across all treatments. Seeds from 12 individual plants per treatment (independent biological replicates) were harvested on the same date and kept in separate tubes in dry storage until used for germination testing.

G2 seeds germination assay

To evaluate the maternal effects on germination, and their interaction with the seed's environment, freshly harvested seeds (3 days after harvest) were imbibed in agar-agar plates (0.6% w/v, Plant Agar, Duchefa Biochemie) and immediately incubated in LD at 18 or 24°C.

Twenty seeds were sowed per plate, one plate per replicate (12 maternal plants), G1 treatment (4 treatments), and seed incubation condition (2 temperatures), giving a total of 96 plates. Plates were considered as our unit of analysis. Germination was counted daily during the first week of incubation and then every other day until seeds reached a germination *plateau* (no new germinants in two consecutive counting days). A seed was considered germinated when radicle protrusion was observed. Final germination proportion was calculated as the number of germinated seeds divided by the total number of viable seeds per plate. A seed was considered viable if it kept firmness to touch at the end of the assay.

G2 plants flowering time, reproductive biomass production and seed area

To explore the effects of the first-generation environments on the second generation's flowering time and reproductive biomass production, and the interaction between environments of both generations, G2 plants were grown in the same treatments as G1 plants using a fully factorial experimental design (Figure 1). For this we used a *single seed descent* procedure: G2 seeds descendant from each G1 biological replicate plants (n = 12) grown in different conditions were sown in individual agar-agar plates, stratified at 4°C in darkness for 4 days, transferred to pots and grown in the temperature treatment combinations as described above (Treatments 1-4, Table 1). Plants were moved randomly within the chambers and containing trays on a weekly basis to avoid positional effects. In this way, we grew plants in one of each 16 possible combinations of first- and second-generation environments (192 plants total, Figure 1).

Flowering time: Flowering time was recorded as the number of rosette leaves developed from germination to bolting (floral bud emergence).

Reproductive biomass production: G2 plants were kept until they reproduced. G3 seeds were harvested after plants senesced and stored in separate 1.5ml tubes by individual (12 biological replicates per treatment). Reproductive biomass production was recorded by weighting total seed yield per tube using a precision scale.

Seed size: The seed size measurement was carried out by taking pictures with a NIKON D3300 camera (ISO 1600, 1/160 seconds, f/8, 55 mm) of 20-40 seeds from each biological replicate with a millimeter ruler as a reference to the scale (and an identifier). Photographs were processed with the ImageJ software (Schneider *et al.* 2012). Firstly, the image scale was established in millimeters. Then, the seeds in the picture were selected and transformed to a binary image, selecting those objects inside the HSB coloration space with brightness between 0-203, saturation between 159-255 and hue between 0-57. The selected values that did not correspond to seeds were eliminated manually and a pixel line was added between overlapped seeds to consider unique values and reduce overestimation of area values. Final seed area measurements and total number of seeds were obtained using the tool "Analyze Particles".

Statistical analysis

All statistical analyses were conducted using R v.4.2.2 (R Core Team 2022). To test for effects of the different temperature environments on G2 seed germination with plates as unit of analysis (independent biological replicates), we fit generalized linear models using 'glm' and then performed type-III likelihood ratio tests (χ^2 test for goodness of fit) using the 'Anova' function in the 'car' package (Fox and Weisberg 2019). As germination is a binomial trait expressed in form of proportions, we used a logit link function to run the tests. We first analyzed the effects of

the whole life cycle environment of the G1 plants ('G1 Env' with four levels: treatments 1-4 and 18-18 as reference), the seed environment ('Incubation' with two levels: 18 and 24°C), and their interaction ('G1 Env' × 'Incubation'). Then, we analyzed the environmental effects before, 'BF', and after flowering, 'AF', and their interaction, 'BF × AF' (two levels, 18 and 24°C), to evaluate whether the timing of those changes had an influence on the germination response. All the independent variables were considered as fixed.

To test for effects of G1 and G2 environments (independent variables 'G1 Env' and 'G2 Env' with four levels each: treatments 1-4) and their interactions on the response variables flowering time, reproductive biomass production and seed size, which are normally distributed, we used the 'aov' function in the 'stats' package (R Core Team 2022) to perform a factorial ANOVA. To interpret the interactions, we next analyzed the effect of each generation's environment on the response variables: we tested the effects of the G2 environment for the group of descendants within each G1 treatment, and the effects of the G1 environment within each G2 treatment.

To estimate the magnitude and direction of the transgenerational effects on the response variables germination and flowering time, we fit generalized linear models with the 'brglm' (biased reduced generalized linear models, Kosmidis and Firth 2021) and 'glm' packages (Fox and Weisberg 2010) to obtain the 'Estimate' representing the change in log odds with associated 95% confidence intervals: positive values indicate that 24°C induced germination (increased germination proportion) or delayed flowering (higher number of leaves) when compared with 18°C; and confidence intervals not overlapping zero indicate the effects are significant.

For all instances, when testing for multiple comparisons, a correction was conducted using the Holm method of 'p.adjust' in the 'stats' package (R Core Team 2022).

RESULTS

Germination responses of a second generation are influenced by the first-generation's environment before and after flowering

To explore the temperature effects across the whole life cycle on early life responses of the next generation, we analyzed the germination response of the G2 seeds from G1 plants grown in different thermal environmental combinations (Table 1, Figure 1). The first generation and seed incubation environments had a significant effect on germination proportion separately (G1 Env $\chi^2 = 422.7$, df = 3, $p < 2.2\text{x}10^{-16}$; Incubation $\chi^2 = 54.5$, df = 1, $p = 1.6\text{x}10^{-13}$; Figure 2a), which follows previous reports on progeny germination and confirm that our treatments are properly establishing maternal temperature effects. However, the incubation temperature did not modify the expression of responses to the environment of the first generation (non-significant G1 Env × Incubation interaction, $\chi^2 = 5.6$, df = 3, $p = 0.13$).

To explore the influence of timing of those environmental changes, we analyzed the effect of temperatures before (BF) and after flowering (AF) separately on the final germination proportion (for this analysis the non-significant three-way interaction, AF × BF × Incubation, was dropped as it did not improve the model). Temperature before and after flowering significantly interacted to regulate seed germination proportion (significant BF × AF, Table 2a). As expected, temperature after flowering strongly affected germination: for both incubation conditions, seeds developed and matured at warmer temperatures germinated more than those seeds produced at cooler temperatures (comparisons 18-18 vs 18-24, and 24-18 vs 24-24; Table 2; Figure 2a and Supplementary Figure 1). In addition, the environment experienced by the G1 plants before flowering significantly affected seed germination at both incubation temperatures: at equal environments after flowering, warmer temperatures before flowering reduced progeny

germination (comparisons 18-18 vs 24-18, and 18-24 vs 24-24; Table 2; Figures 2 and Supplementary Figure 1).

In addition, germination proportion was highly correlated with germination speed (number of seeds germinated per day), *i.e.* the environments across generations that promoted germination also induced faster germination and *vice versa* (Pearson's correlation = 0.967, $p < 0.001$; Figure 2b).

Persistence of transgenerational effects in flowering time is dependent on the progeny's environment

When evaluating the persistence of the G1 environment effects further down in the life cycle of the progeny, our results showed a significant interaction between the environments of both generations on flowering time (significant G1 Env × G2 Env interaction; Table 3; Supplementary Figure 2, Supplementary Figure 3).

When G2 plants were grown at 18°C, we observed a significant effect of the environment of G1 plants before and after flowering (Figure 3). We observed contrasting effects on flowering time of G2 plants that were dependent on the life stage experiencing the environmental cue in the previous generation: warm temperatures before flowering accelerated bolting, while when experienced after flowering, flowering was delayed (Supplementary Figure 3), strikingly similar to what was observed for the germination response (Supplementary Figure 1).

Warmer temperatures experienced by the second generation accelerated flowering time, regardless of the environment of G1 plants (Table 3, Figures 3 and Supplementary Figure 2). However, this effect was larger when G1 plants were grown in the combination 18-24°C

(Supplementary Figure 2). This might be due to the warmer temperature being a strongly inductive flowering environment, which combined with a long day photoperiod, masks the expression of the transgenerational effects.

Effects of environments of consecutive generations on reproductive biomass production

To analyze whether the transgenerational effects on traits related to fitness further down in the second-generation life cycle are persistent, we quantified the environmental effects of both generations on reproductive biomass production of G2 plants. We observed that in our experimental conditions, the environment of the first generation did not affect seed production of G2 plants (Figure 4). Reproductive biomass production strongly depended on the immediate environment: G2 plants grown at 18-18 (dark blue) and 24-18 (light blue) produced the maximum and minimum total seed weight, respectively, regardless of the treatment of the first generation (Figure 4). These results are reflected in the non-significant interaction of the statistical analysis (non-significant G1 Env × G2 Env).

Seed size is influenced by the interaction of environments across generations

To further the analysis of the persistence of transgenerational effects, we measured the area of G2 and G3 seeds, a trait strongly related to fitness. G2 seed size was not affected by the environment of the previous generation (Supplementary Figure 4). However, the results showed that, under our experimental conditions, the environment of both generations interacted to influence G3 seed size (Figure 5). In addition, the most favorable environment of G2 plants (the one that produces seeds with larger size) was 18-18 (dark blue) for all G1 treatments, indicating

that the most immediate generation is still a large determinant of fitness, as observed for seed weight. Interestingly, we observed that there is an effect on seed size when environments are matched across generations: the seeds from G2 plants that grew under the same environmental conditions as those of the previous generation showed higher size compared to those where there is no correlation of environments.

DISCUSSION

The environment that the first generation of plants encountered throughout their entire life cycle had a strong influence on the expression of many developmental traits in the subsequent generation. We found that the first generation's environment exerts short-term effects on the next generation's germination response and long-term effects on plant phenotypes during another critical developmental transition, flowering. These temperature effects can be contrasting (change direction) and are dependent on the life stage of the previous generation that experienced those changing temperatures. However, these effects are weakened and disappear later in the life cycle since we did not find a significant contribution on seed production across generations, implying that within-generation effects are favored. But in the third generation, we observed that seed size, a proxy for propagule investment, was positively influenced when environments across generations matched.

We found that seeds developed and matured at warmer temperatures germinated more than those of mother plants experiencing cooler temperatures. This behavior was expected and consistent with multiple reports showing that increasing temperatures after flowering reduce dormancy levels (Penfield and Springthorpe 2012; Footitt *et al.* 2013; Burghardt *et al.* 2016). Maternal

effects can contribute to a bet-hedging strategy to spread germination across the year and help seeds face unpredictable environmental conditions. Warming temperatures during seed maturation may accelerate germination to fit a whole new generation before summer strikes (Burghardt *et al.* 2016), or it may reduce the ability of seeds to cycle back into a deep secondary dormant state (Auge *et al.* 2015). In a natural environment, dispersal closer to summer would indicate that the dormant period should be shorter for seeds to ensure germination in the fall. In turn, the environment experienced by the mother plant before flowering significantly affected progeny germination but in an opposite manner to the effect of the same environment experienced after flowering: at equal environments after flowering, warmer temperatures experienced early in the life cycle influenced the behavior of the next generation of seeds, even before the plants committed the developmental program from vegetative to reproductive growth. This could have the same effect as cooler temperatures during seed maturation on the response of progeny germination, allowing seeds to explore a wider range of environments to ensure germination occurs in optimal conditions. Early life temperature changes have been shown to have a strong effect on progeny germination (Chen et al. 2014; Penfield and MacGregor 2017; Auge et al. 2017). Progeny seeds of the Landsberg *erecta* ecotype had a high level of primary dormancy when mother plants were grown at 16°C before flowering, showing a similar effect of that environment when experienced after flowering (Chen *et al.* 2014). Our results showed the opposite trend (cooler temperatures before flowering promoted germination), and this might be due to different environmental sensitivities across populations of the same species and local adaptation (Burghardt *et al.* 2016; Colicchio 2017). However, it strongly reinforces the fact that the environment during the whole life cycle of the mother plant influences early trait responses in the progeny generation.

The response to environments that have been experienced by previous generations could restrict the expression of adaptive phenotypes and affect fitness. Nevertheless, this response could also add information that helps synchronizing life cycles in correlated environments among generations (Auge, *et al.* 2017). We observed that the environment of the first generation affected the flowering time of the second generation—*i.e.*, the flowering time of the progeny was influenced by the maternal environment. We also observed that the flowering response to the environment of the previous generation depended on the environment of the present generation. In this case, warmer temperatures (24°C) masked the expression of the maternal effects on flowering time. Plants would be expected to favor within-generational over transgenerational plasticity if they are challenged by increasingly stressful environments, in this way allowing them to adjust their phenotypes to match the current conditions more optimally (Lampei 2019; Dury and Wade 2020). It would be interesting then to find if shortening days would permit effects due to the interaction of the environments of both generations at the warmer temperature tested in this study. However, this approach would need to account for potential transgenerational effects of the photoperiod, as it has been shown previously (Imaizumi *et al.* 2017). On the other hand, when the progeny environment allowed for maternal effects to be expressed (18°C), the environment of the mother plant 'before flowering' regulated the response of the second generation to its own environment, regardless of the maternal environment 'after flowering'. Cooler temperatures experienced within-generation delay flowering in *Arabidopsis* plants (Blázquez *et al.* 2003; Lorenzo *et al.* 2016) and this effect is also true across-generations: we found that offspring of mothers grown in cooler temperatures flowered later than those from mothers grown in warmer temperatures. Maternal environments have been shown to affect progeny flowering responses in different conditions: salinity and heat stress experienced by

mother plants could advance flowering (Suter and Widmer 2013a; Moriuchi *et al.* 2016). Our results are consistent with those reported by Groot *et al.* (2017) in which the third generation of plants grown in warmer temperatures showed earlier flowering in cases where their lineage was grown under the same conditions. The phenotypic plasticity of the progeny associated with the response to warming experienced by several generations could be understood as an adaptive strategy to the seasonal variation of temperature through generations, since high temperatures have negative effects on the life cycle of *A. thaliana* (Zinn *et al.* 2010), becoming a potential selection agent in its natural habitat (Wolfe and Tonsor 2014). Thus, the parental effects we observed are likely adaptive as the maternal environment is more accurately predicting the progeny's due to matching environmental changes (Leverett *et al.* 2016; Auge *et al.* 2017; Alvarez *et al.* 2021).

After flowering, the accumulation of reproductive biomass and seed size are measurements related to the resources invested in producing the next generation and are a strong indicator of fitness. These can be measured as total seed weight or seed area for a fitness proxy. We have not observed a maternal or two-generation effect (interaction of first- and second-generation environments) on the seed yield, but a strong within-generation effect instead. In line with this, the largest seeds were produced when G2 plants were grown at the cooler environment during their whole life cycles (regardless of the G1 plant treatments) indicating that the environment of the present generation is strongly determining fitness, matching the observed effect on seed weight. In addition, the observed detrimental effect of the warmer temperature suggests that for the summer annual Col-0, and probably for other *A. thaliana* ecotypes as well, the coolest environment is the most favorable. Given that the daily temperature range for Col-0 in its defined natural range is between 15-16/21-22°C (TAIR), it is highly likely that 24°C has a mild-stress

effect on the production of reproductive biomass and allocation of nutrient supply (Groot *et al.* 2017). Additionally, seed size showed a strong response to the interaction between first and second generation environments, indicating that transgenerational effects are present in, at least, one trait related to propagule investment (Wang *et al.* 2015). Further, we observed that a correlation between environments of both generations (*i.e.*, when maternal and progeny environments matched) increased seed size, indicating that fitness enhances when previous environments are repeated (Marshall and Uller 2007; Auge *et al*. 2023) . In consequence, when comparing total seed weight and individual seed size, there is a trade-off between traits (number of seeds determined by weight and size). These results suggest that the adaptive strategy would be to produce seeds with more resources (larger seeds) to the detriment of seed number. This strategy has been theorized to favor the evolution of parental effects when selection on viability (*i.e.*, survival) is acting (Kuijper and Johnstone 2021). Altogether, the results suggest that when experiencing environments that either favor or impair reproduction, within-generation effects are likely to constrain the expression of plasticity and the evolution of parental effects.

Our study demonstrates that adaptive transgenerational plasticity arises when environmental changes across generations are minimized and temporally correlated, favoring phenotypes related to fitness advantages (Hoyle and Ezard 2012; Ezard *et al.* 2014). We did find that maternal effects could be concealed and that within-generation plasticity was favored when maintaining transgenerational effects could lead to less desirable phenotypes, such as delaying flowering at higher temperatures. To determine how the balance between within- and across-generations effects would contribute to plasticity, providing adaptive local variation and the best expression of life cycles, we would need to explore these response patterns and fitness costs/benefits in additional ecotypes. Even though environmental effects are strong across

generations, as demonstrated in this and other studies (Groot *et al.* 2017; Auge *et al.* 2017; Latzel *et al.* 2023), their direction and strength show significant genetic variation (Groot *et al.* 2017; Auge *et al.* 2017; Matesanz *et al.* 2022; Latzel *et al.* 2023), and our findings should be confirmed in other *A. thaliana* populations with different life histories. Nevertheless, our study shows that even non-stressful environmental changes, like ambient temperatures that fall within the range of what *A. thaliana* plants typically experience in nature, can have significant consequences for plant development and fitness over generations and may enable the process of adaptation to broader environmental changes.

FUNDING INFORMATION

This work was supported by Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación (Agencia I+D+i), Argentina [grants no. PICT2016-0386 and PICT2020-A-00072 awarded to GA, and PICT2016-0944 awarded to PC].

ACKNOWLEDGEMENTS

We thank Dr Mariana Barber, Tomas Deguer, Maria Clara Fontana and Virginia Ibarra for their technical help, and Dr Nora Madanes for valuable comments on the manuscript.

LITERATURE CITED

Alvarez M, Bleich A, Donohue K. **2020**. Genotypic variation in the persistence of transgenerational responses to seasonal cues*. *Evolution* **74**: 2265–2280.

Alvarez M, Bleich A, Donohue K. **2021**. Genetic differences in the temporal and environmental stability of transgenerational environmental effects. *Evolution* **75**: 2773–2790.

Amasino R. **2004**. Vernalization, Competence, and the Epigenetic Memory of Winter. *The Plant Cell* **16**: 2553–2559.

Anderson JT, Willis JH, Mitchell-Olds T. **2011**. Evolutionary genetics of plant adaptation. *Trends in Genetics* **27**: 258–266.

Auge GA, Blair LK, Burghardt LT, *et al.*2015. Secondary dormancy dynamics depends on primary dormancy status in *Arabidopsis thaliana*. *Seed Science Research* **25**: 230–246.

Auge GA, Blair LK, Neville H, Donohue K. **2017**. Maternal vernalization and vernalization-pathway genes influence progeny seed germination. *New Phytologist* **216**: 388–400.

Auge GA, Leverett LD, Edwards BR, Donohue K. **2017**. Adjusting phenotypes via within- and across-generational plasticity. *New Phytologist*: 343–349.

Auge G, Hankofer V, Groth M, Antoniou-Kourounioti R, Ratikainen I, Lampei C. **2023**. Plant environmental memory: implications, mechanisms and opportunities for plant scientists and beyond. *AoB PLANTS* **15**: plad032.

Awan S, Footitt S, Finch-Savage WE. **2018**. Interaction of maternal environment and allelic differences in seed vigour genes determines seed performance in *Brassica oleracea*. *The Plant Journal* **94**: 1098–1108.

Blázquez MA, Ahn JH, Weigel D. **2003**. A thermosensory pathway controlling flowering time in Arabidopsis thaliana. *Nature Genetics* **33**: 168–171.

Bonduriansky R. 2021. Plasticity across Generations In: Phenotypic Plasticity & Evolution. CRC Press.

Burghardt LT, Edwards BR, Donohue K. **2016**. Multiple paths to similar germination behavior in *Arabidopsis thaliana*. *New Phytologist* **209**: 1301–1312.

Casas RR de, Kovach K, Dittmar E, Barua D, Barco B, Donohue K. **2012**. Seed after-ripening and dormancy determine adult life history independently of germination timing. *New Phytologist* **194**: 868–879.

Chen M, MacGregor DR, Dave A, *et al.*2014. Maternal temperature history activates Flowering Locus T in fruits to control progeny dormancy according to time of year. *Proceedings of the National Academy of Sciences* **111**: 18787–18792.

Colicchio J. **2017**. Data from: Transgenerational effects alter plant defense and resistance in nature. : 99836 bytes.

Donohue K. **2009**. Completing the cycle: maternal effects as the missing link in plant life histories. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**: 1059–1074.

Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG. **2010**. Germination, Postgermination Adaptation, and Species Ecological Ranges. *Annual Review of Ecology, Evolution, and Systematics* **41**: 293–319.

Donohue K, Schmitt J. **1998**. Maternal environmental effects in plants: adaptive plasticity. In: New York, NY, USA: Oxford University Press, 137–158.

Dudley SA, Schmitt J. **1996**. Testing the Adaptive Plasticity Hypothesis: Density-Dependent Selection on Manipulated Stem Length in *Impatiens capensis*. *The American Naturalist* **147**: 445–465.

Dury GJ, Wade MJ. **2020**. When mother knows best: A population genetic model of transgenerational versus intragenerational plasticity. *Journal of Evolutionary Biology* **33**: 127–137.

Ehrlén J. **2015**. Selection on flowering time in a life-cycle context. *Oikos* **124**: 92–101.

English S, Pen I, Shea N, Uller T. **2015**. The Information Value of Non-Genetic Inheritance in Plants and Animals. *PLOS ONE* **10**: e0116996.

Ezard THG, Prizak R, Hoyle RB. **2014**. The fitness costs of adaptation via phenotypic plasticity and maternal effects (D Marshall, Ed.). *Functional Ecology* **28**: 693–701.

Footitt S, Huang Z, Clay HA, Mead A, Finch- Savage WE. **2013**. Temperature, light and nitrate sensing coordinate Arabidopsis seed dormancy cycling, resulting in winter and summer annual phenotypes. *The Plant Journal* **74**: 1003–1015.

Fox J, Weisberg S. **2019**. *An R Companion to Applied Regression*. Thousand Oaks, CA: Sage.

Gray SB, Brady SM. **2016**. Plant developmental responses to climate change. *Developmental Biology* **419**: 64–77.

Groot MP, Kubisch A, Ouborg NJ, *et al.*2017. Transgenerational effects of mild heat in *Arabidopsis thaliana* show strong genotype specificity that is explained by climate at origin. *New Phytologist* **215**: 1221–1234.

Gutterman Y. **1982**. Phenotypic maternal effect of photoperiod on seed germination. The physiology and biochemistry of seed development, dormancy and germination, 67(79), 27-59.

He H, de Souza Vidigal D, Snoek LB, *et al.*2014. Interaction between parental environment and genotype affects plant and seed performance in Arabidopsis. *Journal of Experimental Botany* **65**: 6603–6615.

Holdsworth MJ, Bentsink L, Soppe WJJ. **2008**. Molecular networks regulating Arabidopsis seed maturation, after- ripening, dormancy and germination. *New Phytologist* **179**: 33–54.

Hoyle RB, Ezard THG. **2012**. The benefits of maternal effects in novel and in stable environments. *Journal of The Royal Society Interface* **9**: 2403–2413.

Huang Z, Footitt S, Tang A, Finch- Savage WE. **2018**. Predicted global warming scenarios impact on the mother plant to alter seed dormancy and germination behaviour in Arabidopsis. *Plant, Cell & Environment* **41**: 187–197.

Imaizumi T, Auge G, Donohue K. **2017**. Photoperiod throughout the maternal life cycle, not photoperiod during seed imbibition, influences germination in *Arabidopsis thaliana*. *American Journal of Botany* **104**: 516–526.

Karban R, Agrawal AA, Thaler JS, Adler LS. **1999**. Induced plant responses and information content about risk of herbivory. *Trends in Ecology & Evolution* **14**: 443–447.

Kosmidis I, Firth D. **2021**. Jeffreys-prior penalty, finiteness and shrinkage in binomial-response generalized linear models. *Biometrika* **108**: 71–82.

Kuijper B, Johnstone RA. **2021**. Evolution of epigenetic transmission when selection acts on fecundity versus viability. *Philosophical Transactions of the Royal Society B: Biological Sciences* **376**: 20200128.

Lampei C. **2019**. Multiple simultaneous treatments change plant response from adaptive parental effects to within-generation plasticity, in *Arabidopsis thaliana*. *Oikos* **128**: 368–379.

Larios E, Búrquez A, Becerra JX, Lawrence Venable D. **2014**. Natural selection on seed size through the life cycle of a desert annual plant. *Ecology* **95**: 3213–3220.

Latzel V, Fischer M, Groot M, *et al.*2023. Parental environmental effects are common and strong, but unpredictable, in Arabidopsis thaliana. *New Phytologist* **237**: 1014–1023.

Leverett LD, Auge GA, Bali A, Donohue K. **2016**. Contrasting germination responses to vegetative canopies experienced in pre- vs. post-dispersal environments. *Annals of Botany* **118**: 1175–1186.

Li R, Chen L, Wu Y, *et al.*2017. Effects of Cultivar and Maternal Environment on Seed Quality in Vicia sativa. *Frontiers in Plant Science* **8**: 1411.

Liancourt P, Spence LA, Song DS, *et al.*2013. Plant response to climate change varies with topography, interactions with neighbors, and ecotype. *Ecology* **94**: 444–453.

Lorenzo CD, Sanchez- Lamas M, Antonietti MS, Cerdán PD. **2016**. Emerging Hubs in Plant Light and Temperature Signaling. *Photochemistry and Photobiology* **92**: 3–13.

Lu JJ, Tan DY, Baskin CC, Baskin JM. **2016**. Effects of germination season on life history traits and on transgenerational plasticity in seed dormancy in a cold desert annual. *Scientific Reports* **6**: 25076.

Marshall D, Uller T. **2007**. When is a maternal effect adaptive? *Oikos* **116**: 1957–1963.

Matesanz S, Ramos-Muñoz M, Rubio Teso ML, Iriondo JM. **2022**. Effects of parental drought on offspring fitness vary among populations of a crop wild relative. *Proceedings of the Royal Society B: Biological Sciences* **289**: 20220065.

Michaels SD, Himelblau E, Kim SY, Schomburg FM, Amasino RM. **2005**. Integration of Flowering Signals in Winter-Annual Arabidopsis. *Plant Physiology* **137**: 149–156.

Moriuchi KS, Friesen ML, Cordeiro MA, *et al.*2016. Salinity Adaptation and the Contribution of Parental Environmental Effects in Medicago truncatula (R Balestrini, Ed.). *PLOS ONE* **11**: e0150350.

Munguía-Rosas MA, Ollerton J, Parra-Tabla V, De-Nova JA. **2011**. Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured: Phenotypic selection on flowering phenology. *Ecology Letters* **14**: 511–521.

Penfield S, MacGregor DR. **2017**. Effects of environmental variation during seed production on seed dormancy and germination. *Journal of Experimental Botany* **68**: 819–825.

Penfield S, Springthorpe V. **2012**. Understanding chilling responses in *Arabidopsis* seeds and their contribution to life history. *Philosophical Transactions of the Royal Society B: Biological Sciences* **367**: 291–297.

Postma FM, Ågren J. **2022**. Effects of primary seed dormancy on lifetime fitness of *Arabidopsis thaliana* in the field. *Annals of Botany* **129**: 795–808.

R Core Team. **2022**. R: A language and environment for statistical computing. R Foundation for Statistical Computing,.

Sánchez-Lamas M, Lorenzo CD, Cerdán PD. **2016**. Bottom-up Assembly of the Phytochrome Network (C Fankhauser, Ed.). *PLOS Genetics* **12**: e1006413.

Schneider CA, Rasband WS, Eliceiri KW. **2012**. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**: 671–675.

Simpson GG, Dean C. **2002**. *Arabidopsis* , the Rosetta Stone of Flowering Time? *Science* **296**: 285–289.

Somssich M. **2019**. *A short history of Arabidopsis thaliana (L.) Heynh. Columbia-0*. PeerJ Inc.

Sultan SE. **1996**. Phenotypic Plasticity for Offspring Traits in Polygonum Persicaria. *Ecology* **77**: 1791–1807.

Sultan SE. **2000**. Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science* **5**: 537–542.

Suter L, Widmer A. **2013a**. Environmental Heat and Salt Stress Induce Transgenerational Phenotypic Changes in Arabidopsis thaliana. *PLoS ONE* **8**.

Suter L, Widmer A. **2013b**. Phenotypic Effects of Salt and Heat Stress over Three Generations

in Arabidopsis thaliana. *PLoS ONE* **8**.

TAIR. *The Arabidopsis Information Resource*. https://www.arabidopsis.org/servlets/TairObject?type=species_variant&id=90. 24 Jan. 2023.

Valencia-Díaz S, Montaña C. **2005**. Temporal variability in the maternal environment and its effect on seed size and seed quality in Flourensia cernua DC. (Asteraceae). *Journal of Arid Environments* **63**: 686–695.

Wang L, Wang HL, Zhang K, Tian CY. **2015**. Effects of maternal nutrient and water availability on seed production, size and germination of heterocarpic *Atriplex aucheri*. *Seed Science and Technology* **43**: 71-79.

Whittle CA, Otto SP, Johnston MO, Krochko JE. **2009**. Adaptive epigenetic memory of ancestral temperature regime in Arabidopsis thaliana. This paper is one of a selection of papers published in a Special Issue from the National Research Council of Canada – Plant Biotechnology Institute. *Botany* **87**: 650–657.

Willis CG, Baskin CC, Baskin JM, *et al.*2014. The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. *New Phytologist* **203**: 300–309.

Wolfe MD, Tonsor SJ. **2014**. Adaptation to spring heat and drought in northeastern Spanish *Arabidopsis thaliana*. *New Phytologist* **201**: 323–334.

Zacchello G, Vinyeta M, Ågren J. **2020**. Strong stabilizing selection on timing of germination in a Mediterranean population of *Arabidopsis thaliana*. *American Journal of Botany* **107**: 1518–1526.

Zinn KE, Tunc-Ozdemir M, Harper JF. **2010**. Temperature stress and plant sexual reproduction: uncovering the weakest links. *Journal of Experimental Botany* **61**: 1959–1968.

FIGURE LEGENDS

Figure 1. Experimental design of the multigenerational experiment to test for ambient temperature effects on the expression of transgenerational plasticity. A single seed descent method (represented as black lines) was used to run a fully factorial experiment in which we evaluated the interaction between parental and progeny environments on progeny developmental traits (germination, flowering time, reproductive biomass production and seed area, indicated to the right). Grey outlined boxes represent generations of plants (G0-G3). Black circles represent life cycle endpoints of plants grown in standard conditions (G0). Colored circles represent life cycle endpoints of plants (G2 and G3) grown in the different treatments (dark blue: treatment 1, 18-18; light blue: treatment 2, 24-18; light orange: treatment 3, 18-24; and dark orange: treatment 1, 24-24). Full combination of treatments after growing two generations is indicated in the boxes at the bottom of the figure (treatment of first generation – treatment of second generation; color boxes follow the treatment of the first generation).

Figure 2. Effect of the first generation's environment on germination proportion of *Arabidopsis thaliana* Columbia-0 seeds. Graphs show final germination proportion (a) or germination speed (b) of seeds from mother plants grown in a combination of non-stressful ambient temperature environments (18 and 24°C) before and after flowering (BF-AF, x-axis): 1) 18-18 (dark blue), 2) 24-18 (light blue), 3) 18-24 (light orange) and 4) 24-24 (dark orange). Response of seeds incubated at 18°C (left panel) and 24°C (right panel) are shown as boxplots.

Figure 3. Effects of environment changes experienced by the first-generation on flowering time of *Arabidopsis thaliana* Columbia-0 progeny plants. Graph shows the number of leaves at bolting (y-axis) for each progeny treatment, 18°C (left) and 24°C (right; x-axis). Colors indicate

the environment of G1 plants: 1) 18-18 (dark blue), 2) 24-18 (light blue), 3) 18-24 (light orange), and 4) 24-24 (dark orange).

Figure 4. Effects of environments across generations on reproductive biomass production of *Arabidopsis thaliana* Columbia-0 G2 plants. Graph shows total seed weight (mg) from G2 plants (colors) grown in a combination of G1 environments before and after flowering (BF-AF, x-axis): 1) 18-18 (dark blue), 2) 24-18 (light blue), 3) 18-24 (light orange), and 4) 24-24 (dark orange). Top right: results of the analysis of variance testing (ANOVA) for environmental effects and their interactions (G1 Env and G2 Env).

Figure 5. Environmental effects of progeny and maternal environments on the area of *Arabidopsis thaliana* Columbia-0 G3 seeds. Graph shows seed area (mm^2) for seeds from G2 plants (colors) descendent from G1 plants grown in a combination of environments before and after flowering (BF-AF, x-axis): 1) 18-18 (dark blue), 2) 24-18 (light blue), 3) 18-24 (light orange), and 4) 24-24 (dark orange). Bottom right: results of the analysis of variance (ANOVA) testing for environmental effects and their interactions.

TABLES

Table 1. Experimental design of the multigenerational experiment.

Treatment	*Temperature before flowering (BF)*	*Temperature after flowering (AF)*
1	18	18
2	24	18
3	18	24
4	24	24

Table 2. Effects of temperature before (BF) and after flowering (AF), and during seed incubation (IT) on progeny germination proportion. The table shows effects of temperature BF, AF and IT and their two-way interactions (a, Full model); and of temperature BF and AF, and their two-way interaction by incubation temperature (b, IT = 18°C and c, IT = 24°C). Tests based on type III analyses of likelihood ratios from a generalized linear model with a logit link function (for the full model the three-way interaction was dropped as preliminary analyses showed it did not improve the model). χ^2 values, degrees of freedom (df) and *p* values are shown in the table.

Variable	a) Full model			b) IT = 18°C			c) IT = 24°C		
	χ^2	df	*p*	χ^2	df	*p*	χ^2	df	*p*
BF	24.6	1	1.69×10^{-6}	22.9	1	1.69×10^{-6}	33.5	1	6.98×10^{-9}
AF	163.8	1	$< 2.2 \times 10^{-16}$	139.2	1	$< 2.2 \times 10^{-16}$	227.1	1	$< 2.2 \times 10^{-16}$
IT	58.1	1	2.54×10^{-14}	--			--		
BF × AF	20.5	1	6.07×10^{-6}	3.6	1	0.057	16.9	1	0.057
BF × IT	4.9	1	0.027	--			--		
AF × IT	0.3	1	0.551	--			--		

Table 3. Effects of maternal environment on rosette leaf number of progeny plants. Table shows the results of an analysis of variance indicating *F*-values, degrees of freedom (df) and *p* values for the effects of the first (G1 Env) and second-generation environments (G2 Env), and their interactions (a, Full model); or the effects of the first-generation environment on plants grown at 18°C (b) or 24°C (c).

Variable	**a) Full model**			**b) G2 = 18°C**			**c) G2 = 24°C**		
	F	df	*p*	*F*	df	*p*	*F*	df	*p*
G1 Env	14.23	2	2.55×10^{-6}	19.27	2	2.56×10^{-7}	0.87	2	0.417
G2 Env	87.66	1	2.9×10^{-16}	--			--		
G1 Env × G2 Env	18.63	2	7.57×10^{-8}	--			--		

Figure 1

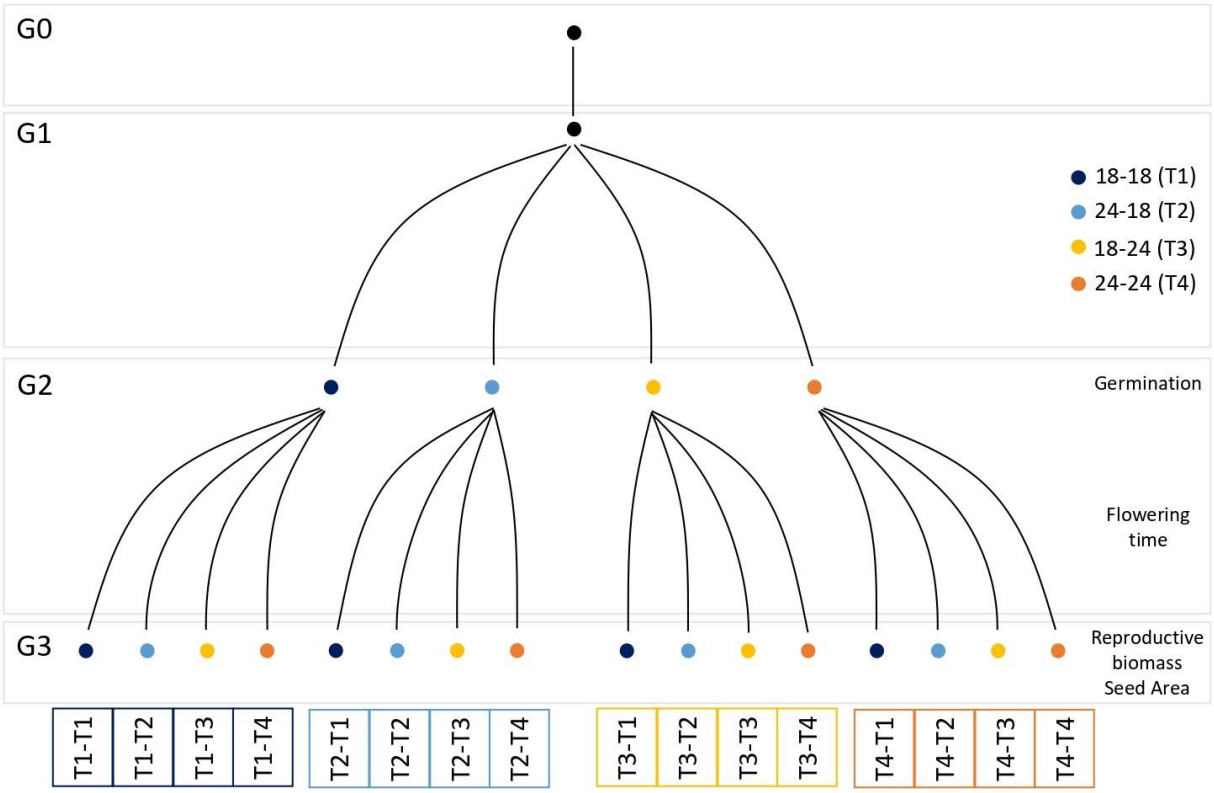

Figure 2

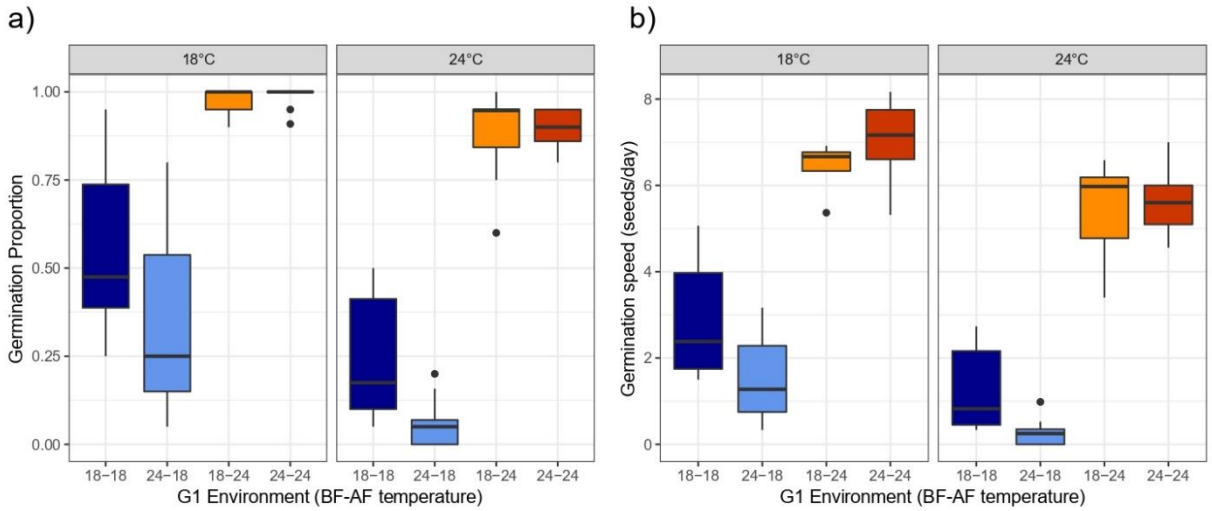

Figure 3

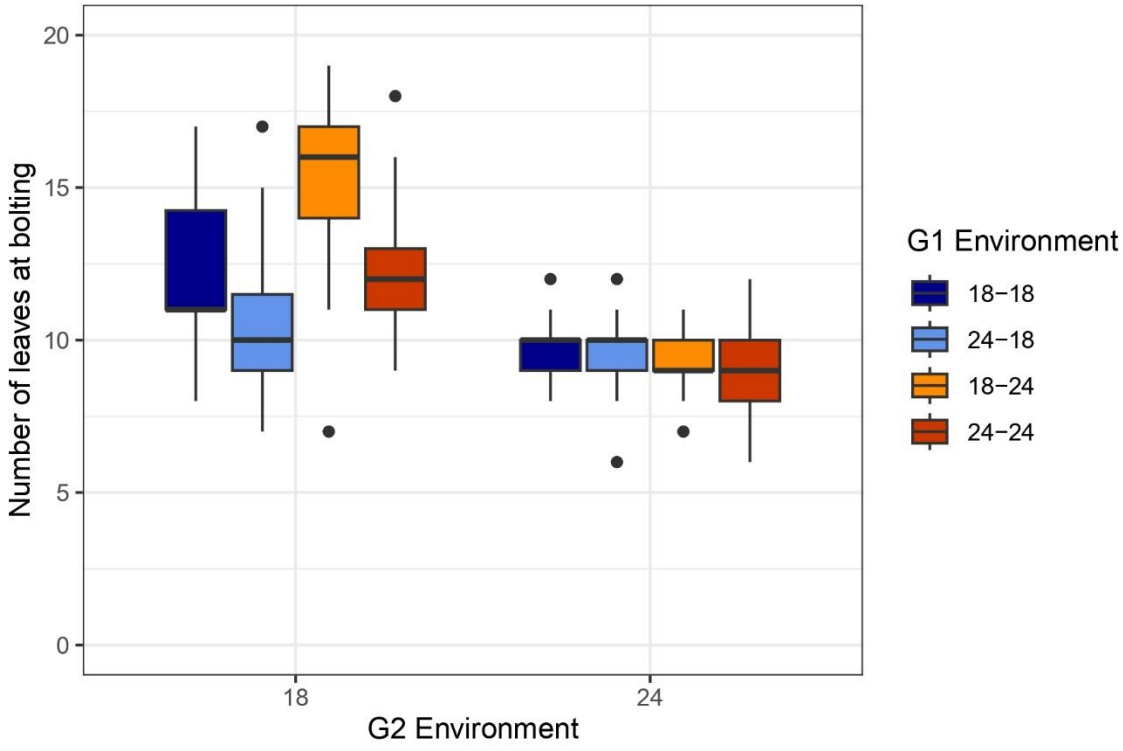

Figure 4

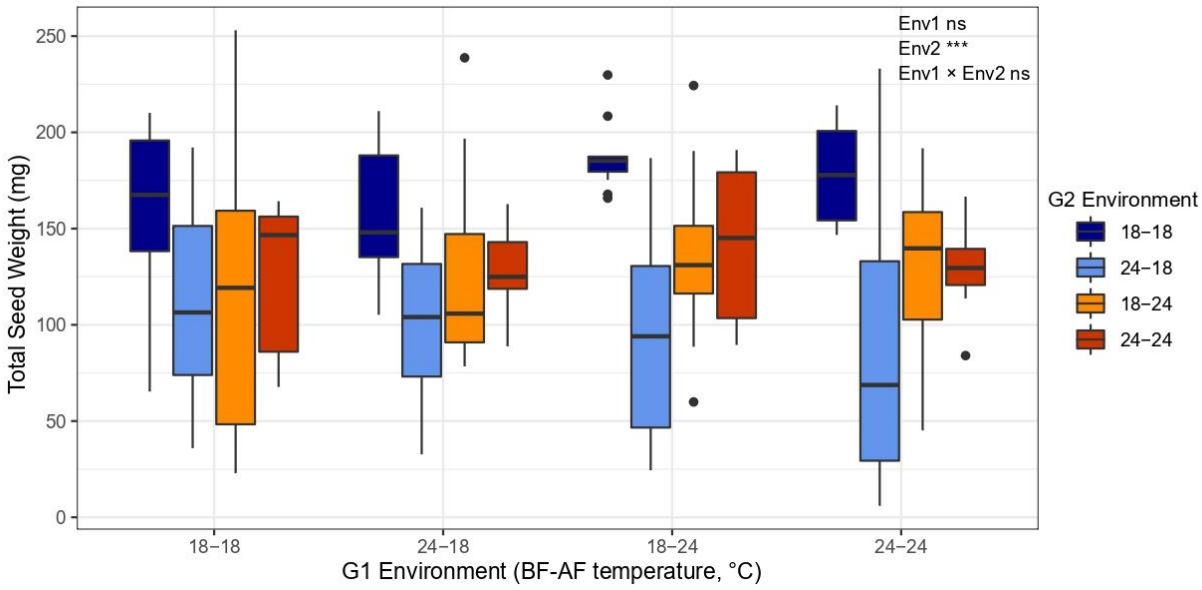

Figure 5