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Maternal temperature effects on dormancy influence germination responses to water availability in *Arabidopsis thaliana*

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ABSTRACT

With climate change, germination cuing to water availability is expected to be especially important for seedling survival. Here, we examined germination responses to low water potential and tested whether dormancy status mediates these responses. We considered both genetically based dormancy (genotypes with allelic variation in dormancy genes) as well as dormancy imposed by the environment (low seed-maturation temperature or short duration of dry afterripening). We examined (a) germination capacity at low water potential, (b) germination acceleration in response to pre-incubation at low water potential, and (c) secondary dormancy induction by low water potential. We found that both environmentally imposed dormancy and genetically based dormancy influenced germination responses to low water potential. Specifically, dormancy established via introgression of a strong dormancy allele and dormancy induced by low seed-maturation temperatures both reduced the ability to germinate at low water potential. Pre-incubation at low water potential accelerated germination, but the rate differed between both dormancy-inducing environments and among dormancy genotypes. Prolonged incubation at low water potential induced secondary dormancy, and this effect was greater in fresher (more dormant) seeds and in seeds that were matured at low temperature (a dormancy-inducing treatment). Although genotypes also varied in secondary dormancy induction, their level of primary dormancy did not predict their induction into secondary dormancy. Environmentally induced dormancy also influenced the expression of genetic differences in germination responses to low water potential. Thus environmentally determined dormancy influences not only germination responses to low water potential but also their evolutionary potential.

1. Introduction

Plant performance often depends on the accurate use of environmental cues to control phenology, or the seasonal timing of biological events. Phenology is considered to be one of the primary factors to influence the performance of organisms in novel climates that result from climate change or dispersal (Bradshaw and Holzapfel, 2008; Chuine and Beaubien, 2001; Menzel et al., 2006; Parmesan, 2006; Walther et al., 2002; Willis et al., 2008). The phenology of germination is particularly consequential, not only because the seedling stage is vulnerable to many environmental factors, but also because the seasonal timing of seed germination can influence the environmental conditions experienced by all subsequent life stages (Baskin and Baskin, 1998; Donohue et al., 2005; Eriksson, 2002; reviewed in Gutterman, 1994; Weinig, 2000). As a consequence, the seasonal timing of germination can be under extremely strong natural selection (Donohue et al., 2005; Huang et al., 2010), is likely to be a strong selective sieve for populations colonizing novel environments (Kronholm et al., 2012; Montesinos-Navarro et al., 2012), and it can have ramifying effects on whole life cycles (Burghardt et al., 2015b; Chiang et al., 2013). Identifying the major environmental factors that contribute to variation in germination behavior is therefore necessary to predict plant performance under diverse environmental conditions that accompany climate change or range expansion.

Seed dormancy prevents germination under environmental conditions that would normally permit germination in non-dormant seeds (Baskin and Baskin, 1998; Bewley, 1997; Simpson, 1990). Physiological dormancy is the most prevalent form of seed dormancy (Baskin and Baskin, 1983), and it allows seeds to postpone germination until specific environmental conditions are

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encountered that release the constraint on germination. High dormancy is associated with low germination proportions and a reduced ability to germinate over a wide range of conditions, whereas low dormancy is associated with higher germination proportions at a wider range of conditions. Dormancy is a dynamic state that can be distinguished as two types: primary dormancy, which is established during seed maturation and can vary with seed-maturation conditions such as temperature, and secondary dormancy, which is induced by environmental conditions experienced after dispersal and subsequent to the loss of primary dormancy (Baskin and Baskin, 1998; Bewley et al., 2013; Cadman et al., 2006). Primary dormancy is gradually alleviated through a process called afterripening, and when seeds become imbibed, secondary dormancy can be induced if seeds are exposed to unfavorable conditions. It is the interaction between factors that break primary dormancy, elicit germination, and induce secondary dormancy that ultimately determine germination timing (Baskin and Baskin, 1998; Forcella et al., 2000).

Once dormancy is broken, water availability strongly regulates the timing and probability of germination (Baskin and Baskin, 1998; Bewley et al., 2013). Rainfall can be highly variable within and between years (Clauss and Venable, 2000) and is not always indicative of suitable growing conditions if moisture is ephemeral. In some cases, seeds that germinate quickly after the onset of rain may gain a head start over others, but rapid germination could also result in mortality if drought occurs soon after. Seeds must therefore respond appropriately to such ephemeral cues. In annual plant species, one strategy is to capitalize on moisture resources as soon as they become available in order to increase the chances of establishment. Alternatively, the risk of germinating into an unfavorable environment can be spread across the seed cohort, such that only a fraction of seeds are competent to germinate when moisture conditions are permissive (Gremer and Venable, 2014; Venable and Lawlor, 1980). A third option is to not germinate until moisture conditions are optimal and persistent.

Germination timing in response to dynamically fluctuating temperature and water availability has been accurately predicted in agronomic applications using hydrothermal time models (Alvarado and Bradford, 2002; Bradford, 2002, 2005; Hardegree et al., 2003). Empirically estimated parameters that describe germination responses to temperature and water availability (i.e. water potential, or Ψ) are used to predict the rate of progress towards germination. The key parameters used to describe germination responses to water availability include base water potential, Ψ_b, or the lowest water potential at which germination can be completed, and minimum water potential, Ψ_{min}, or the lowest water potential necessary for metabolic advancement to occur while still preventing radicle protrusion. Germination speed is proportional to the difference between ambient Ψ and Ψ_b, with larger differences resulting in faster germination. Seeds with a high (less negative) Ψ_b therefore have a narrower range of moisture conditions that permit germination, and they exhibit slower germination compared to seeds with a low Ψ_b. Under field conditions, seeds with a higher Ψ_b could prevent precocious germination when water is available but growing conditions are otherwise unfavorable for growth. In a recent long-term field study of a community of desert annuals, low Ψ_b was shown to be significantly associated with higher germination proportions within a year, later germination during the season, and higher demographic variance across years (Huang et al., 2015), indicating that germination responses to water potential can have important phenological and demographic consequences in natural systems.

Lack of emergence does not necessarily mean that germination-related processes are static, as processes related to germination and dormancy can still proceed even at water potentials that do not permit germination. For instance, seeds may still accumulate progress towards germination at water potentials below Ψ_b if the ambient water potential is above Ψ_{min}. Under low-moisture conditions, seeds may become partially imbibed and achieve a head start on germination, as evidenced by faster germination upon subsequent exposure to permissive hydric conditions. This enhancement effect on germination is often utilized in agriculture to improve crop performance – in practice, it is referred to as seed priming – and studies have identified multiple cellular processes that occur during seed priming, including protein synthesis, nucleic acid synthesis, and DNA repair mechanisms (Chen and Arora, 2013; Paparella et al., 2015). In nature, seeds on the soil surface experience fluctuating cycles of wetting and drying throughout the year, and a number of studies have reported improved predictions of field emergence by accounting for priming dynamics (Allen et al., 2000; Cheng and Bradford, 1999; Rowse and Finch-Savage, 2003). In contrast to priming, prolonged exposure to non-permissive water potentials may actually induce secondary dormancy (Auge et al., 2015). Advancement towards germination at non-permissive water potentials is not always a desirable response, if the onset of rain, for example, coincides with other environmental conditions that are unfavorable. In this instance, the ability of seeds to re-enter dormancy can prevent germination under unfavorable conditions and may be crucial for seedling survival.

The dormancy status of a seed influences the range of environmental conditions that are permissive for germination; as dormancy is lost, the permissive range broadens (Forcella et al., 2000). Hydrothermal models of germination have incorporated dormancy dynamics as changes in Ψ_b, and thereby the range of Ψ over which germination can occur, as dormancy is alleviated (Bair et al., 2006; Batlla and Benech-Arnold, 2004; Bauer et al., 1998; Christensen et al., 1996; Hardegree et al., 2013; Meyer et al., 2000). The empirical accuracy of such models suggests that changes in dormancy may directly influence germination responses to water potential. Both genetic and environmental mechanisms contribute to dormancy levels, but it has yet to be determined if dormancy induced by these different mechanisms leads to similar germination responses to moisture. To understand how dormancy contributes causally to germination responses to Ψ requires direct manipulation of genetic and environmental factors that control dormancy. Exploring how environmentally and genetically based variation in dormancy influences sensitivity to Ψ is necessary to understand how germination phenology may vary across environments with different water availability, including environments of the future.

Arabidopsis thaliana offers unique potential for investigating the genetic basis of germination responses to seasonal environmental factors, including Ψ. It is broadly distributed across diverse seasonal environments and exhibits a range of life-histories caused by variation in flowering and germination timing (Ratcliffe 1965; Donohue, 2009; Thompson, 1994). Environmental factors associated with flowering time, especially temperature during seed maturation, have strong effects on dormancy and germination in this species, such that seed maturation under cool conditions induces strong dormancy (Chiang et al., 2011; Donohue et al., 2007; Kendall and Penfield, 2012; Springthorpe and Penfield, 2015). Natural allelic variants of loci involved in dormancy have been identified (Alonso-Blanco et al., 2003; Bentsink et al., 2010; Huang et al., 2010; Laserna et al., 2008) and introgressed onto a common genetic background, allowing experimental studies of the combined effects of genetically and environmentally based differences in dormancy on germination responses to specific environmental factors, such as water availability.

Here, we examined germination responses to water potential and tested whether genetically and environmentally determined dormancy status mediates these responses. To manipulate

dormancy, we used genotypes with allelic variation at dormancy loci, and we also matured seeds at two temperatures known to induce different dormancy levels. Specifically, we focused on three germination responses to low water potential (Fig. 1), by addressing the following questions: (1) Does dormancy status alter the ability of seeds to complete germination over a range of water potentials? (2) Does dormancy status influence the ability of seeds to accrue progress towards germination at water potentials below those that permit the completion of germination? (3) Does dormancy status – determined by genetic factors, environmental factors, and afterripening – alter secondary dormancy induction in response to low water potential?

2. Materials and methods

2.1. Genetic material and seed production

We used genotypes of *A. thaliana* that contain contrasting natural alleles at quantitative trait loci (QTL) associated with primary dormancy and germination. We used two common accessions, Landsberg *erecta* (*Ler*) and Columbia (Col), to compare variation between the two most commonly used laboratory lines. To isolate allelic effects, we used three near isogenic lines (NILs) with alleles from the Cape Verde Island (Cvi) accession introgressed into the *Ler* background (Alonso-Blanco et al., 2003; Bentsink et al., 2006): *Ler-DOG1*$_{Cvi}$ contains the strongly dormant Cvi allele of *Delay Of Germination-1* (*DOG1*). *Ler-DOG6*$_{Cvi}$ contains the dormant allele of *Delay Of Germination-6* (*DOG6*), *Ler-FLC*$_{Cvi}$ contains the active Cvi allele of *Flowering Locus C* (*FLC*), which is associated with higher germination (Chiang et al., 2009). Thus, *Ler-DOG1*$_{Cvi}$, *Ler-DOG6*$_{Cvi}$ have higher dormancy (reduced germination) compared to *Ler*, while *Ler-FLC*$_{Cvi}$ has lower dormancy (increased germination) compared to *Ler*.

To induce different levels of primary seed dormancy, seeds were matured at two temperatures: 14 °C promotes strong primary dormancy, and 25 °C induces less dormancy (Donohue et al., 2007; Kendall et al., 2011; Kendall and Penfield, 2012). These temperatures are within the range experienced during seed-maturation in *A. thaliana*. To synchronize the harvest of seeds across seed-maturation treatments, seed sowing was staggered across treatments. After seven days of dark stratification at 4 °C, replicates of all genotypes were sown into pots filled with Metromix 360 (Scotts

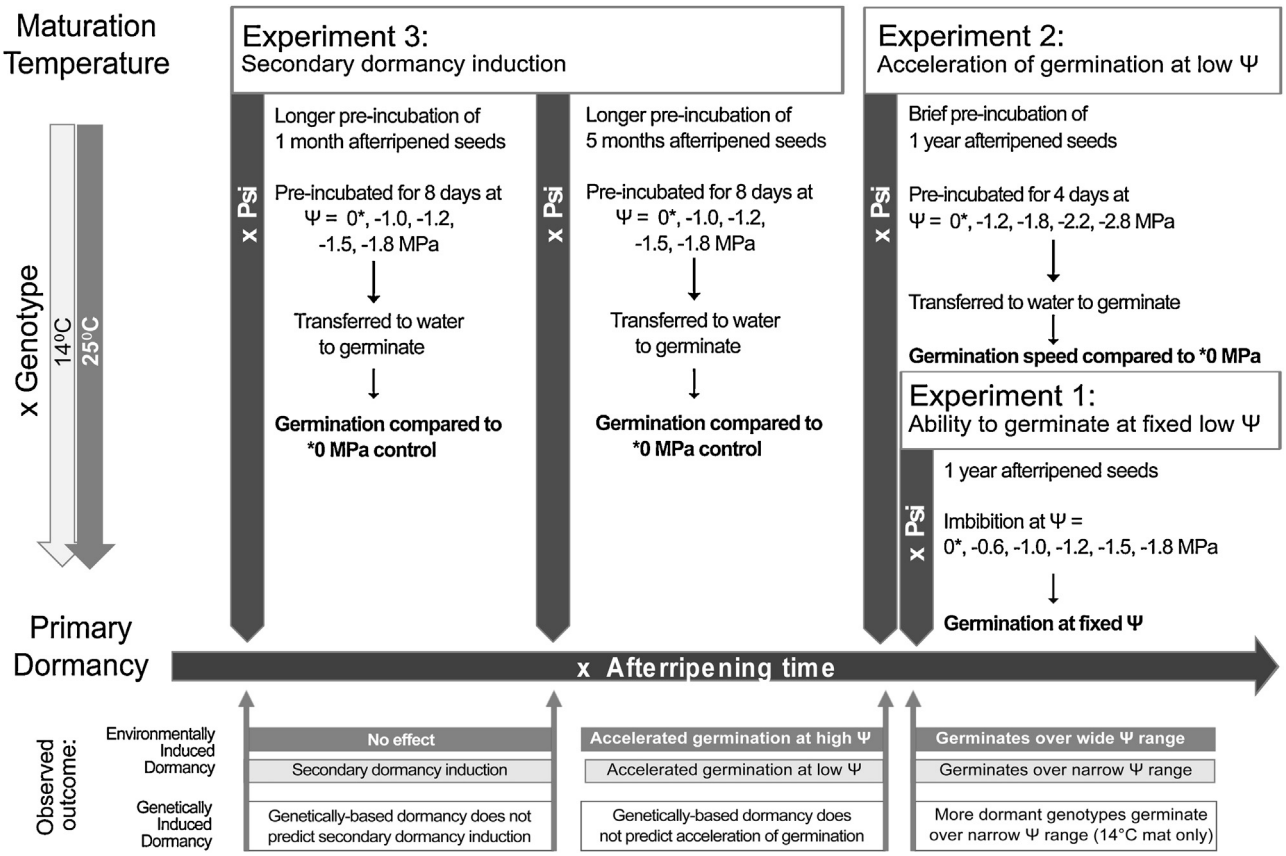

Fig. 1. Schematic summarizing the experiments employed in this study (upper figure) and their observed outcomes (bottom panel). Seeds of different "Genotypes" were matured under two "Maturation temperatures" (far left); both genotype and maturation temperature determine the initial level of "Primary Dormancy" (far left). Seed-maturation treatments are shaded according to temperature: 14 °C (light gray/black text) and 25 °C (dark gray/white text). Seeds lose dormancy during "Afterripening" (axis on bottom; left to right, with left being time of harvest). The experiments were conducted after different durations of afterripening, indicated by the intersection of the arrow from each experiment onto the axis of "Afterripening". Beneath each experiment, which is labeled according to the germination behavior that was assessed, is a summary of the experimental procedures, presented as a sequence of treatments conducted for each experiment, indicated by arrows from top to bottom. Each experiment included a manipulation of water potential ("Psi"), and their specific values are indicated as "**Ψ**" in each experiment. "*"denotes the control treatment. The relevant germination metric assessed for each experiment is indicated in boldface. The bottom panel summarizes the observed responses for each experiment; up-arrows connect the observed outcome to the experiment it pertains to. "Environmentally Induced Dormancy" indicates the observed germination behavior under each seed-maturation temperature (as above, 14 °C is indicated by light gray/black text; 25 °C is indicated by dark gray/white text). "Genetically Induced Dormancy" indicates how germination behavior depended on dormancy genotype. Interactions between environmentally and genetically induced dormancy (Genotype × Maturation interactions) are not indicated in this figure, but they are discussed in the text.

Sierra, Marysville, OH, USA) and then moved to full spectrum light at 20 °C in a 12-hour light cycle to allow germination. After 10 days, seedlings were vernalized (4 °C, 10-hour light cycle) for 28 days to promote flowering before being placed into either 14 °C or 25 °C. Plants were grown under the two constant temperature regimes in a 12-hour light cycle in EGC Model GC8-2 Plant Growth Chambers (Chagrin Falls, OH). Twelve maternal plants were grown at each temperature. Replicate plants were randomly distributed over three chambers containing four maternal replicate plants each, and pot positions were rotated on a weekly basis within each chamber. Plants were fertilized twice before bolting with a 300 ppm solution of Peter's Professional 20–20–20 fertilizer (The Scotts Company, Marysville, OH, USA). Watering was withheld for two weeks when siliques approached maturity, and seed harvest occurred on the same day for both temperature treatments. Seeds were stored at ambient room temperature (~21–23 °C at 22% RH) in a humidity-controlled dessicator (Secador® 4.0 Auto-Dessicator Cabinets, Bel-Art Products, Pequannock, NJ, USA) until used for germination assays.

For practical purposes, seeds used in Experiment 3 were harvested from different maternal plants than seeds used in Experiments 1 and 2, although growing conditions were identical across all experiments.

2.1.1. Germination assays

All germination assays were conducted at a constant temperature of 16 °C with a 12-hour photoperiod in Percival Model GR41LX incubation chambers (Percival Scientific Inc., Perry, IA). This temperature has been shown to be near the optimum temperature of germination for these genotypes (Burghardt et al., 2015a). For all assays, twelve seeds of each genotype were sown onto 60 mm petri plates containing Whatman P5 filter paper saturated with one of four PEG-8000 solutions or pure water as a control. PEG, or polyethylene glycol, is a high molecular weight polymer that can be dissolved in aqueous solution at different concentrations to create solutions with various osmotic pressures. Solutions with greater amounts of PEG solute result in a more negative water potential.

Seeds of each genotype, seed-maturation temperature (14 °C or 25 °C), and afterripening duration were either germinated or pre-incubated for a specified length of time (as indicated in each subsection) at a range of low water potentials in the light. For Experiments 2 and 3, pre-incubated seeds were rinsed and transferred to new 35 mm plates containing filter paper and fresh water (0 MPa). At the time of transfer to fresh water, control plates were prepared by placing seeds with no pre-incubation into plates containing fresh water. Germination was scored at regular intervals (also indicated below) until germination plateaued (the time to plateau is indicated for each experiment below). Seed viability was determined at the end of the experiment by assessing firmness to touch. The final number of germinants and the total number of viable seeds were recorded, to give the final germination proportion for each plate.

2.2. Experiment 1: germination at fixed water potentials

2.2.1. Experimental treatments

The first goal of this study was to measure the ability of seeds to germinate at low water potential, and specifically to test how dormancy, genotype, and seed-maturation temperature altered that ability. We used seeds afterripened for 59 weeks ("one year" hereafter), because the commonly used parameter of base water potential, Ψ_b, is a measure of the lowest water potential at which radicle emergence can occur in non-dormant (afterripened) seeds. However, dormancy persisted even after one year of afterripening in some treatments, so this experiment measured the persistence of the influence of these dormancy manipulations on the range of water potentials at which germination could be completed.

Preliminary experiments indicated that seeds matured at 14 °C did not germinate at water potentials below −1.2 MPa, whereas seeds matured at 25 °C did germinate. Thus, seeds matured at 14 °C were incubated at a higher range of water potentials (0, −0.6, −1.2, −1.8, −2.2 MPa), and seeds matured at 25 °C were incubated at a lower, extended range of water potentials (0, −1.2, −1.8, −2.2, −2.8 MPa). Nine replicate plates per genotype and seed-maturation treatment were used at each water potential. Germination was scored every other day for four weeks, until germination reached a clear plateau even at the lowest water potentials. Final germination proportion was used as the dependent variable in all analyses, with petri plate as the independent unit of analysis.

2.2.2. Statistical analysis

To test whether seeds with different dormancy levels, imposed by different alleles at dormancy loci (Geno) or seed-maturation temperature (Mat) differed in germination ability under different constant water potentials (Psi), we analyzed the final proportion of seeds that germinated with logistic regression (Proc LOGISTIC in SAS 9.4; SAS Institute, Cary NC) using Fisher's scoring optimization (ML) algorithm and Firth's penalized likelihood to accommodate issues of quasi-separation caused by extreme germination proportions (0% or 100%) in some treatments. Quasi-separation is common in logistic/probit regression when certain combinations of predictor variables lead to all or nothing outcomes. Firth's bias-reduced penalized likelihood accommodates separation issues and was therefore employed in our regression model. The lowest water potential treatments (−2.2 and −2.8 MPa) resulted in no germination, and were therefore excluded from analyses to minimize problems with severe data separation. First, we fit a full model that included all interactions and that used "final number of germinants/total viable seeds" per petri plate as the dependent variable, and Geno, Mat, and Psi as fixed factors. The Psi × Mat interaction was highly significant as well as all fixed factors (Supplemental Table S1), so we next analyzed each maturation temperature separately.

We estimated the lowest water potential at which germination could be completed. This measure is not identical to Ψ_b of a standard hydrotime model, which requires nearly 100% germination at 0 MPa and 50% in at least one other water potential treatment to obtain a good fit; some of our treatments did not fully lose primary dormancy, especially cold-matured seeds, so we instead estimated the lowest water potential at which germination could proceed to completion in order to gain insight on the limits on germination in the presence of water stress. We performed linear regressions of final germination proportion of individual petri plates (our independent unit of analysis) as a function of water potential for each genotype in each maturation treatment separately. The lowest water potential treatments resulted in no germination for any genotype or maturation treatment (−2.2 and −2.8 MPa) and were omitted from the regression. We then used the model coefficients to determine the water potential at which $y = 0$; that is, the water potential at which we would expect no germination to occur, hereafter referred to as the "baseline Ψ".

2.3. Experiment 2: enhancement of germination by pre-incubation at low water potential

2.3.1. Experimental treatments

The second goal of this study was to test whether dormancy manipulations can alter the degree to which seeds progress towards germination at low water potential, even if they cannot

complete germination at such low water potentials. To this end, we pre-incubated one-year afterripened seeds at a range of low water potentials (−1.2, −1.8, −2.2, −2.8 MPa) for four days in the light – allowing progress towards germination, if they were capable of it – before transferring them to water to germinate. If seeds germinated more efficiently when pre-incubated, we inferred that progress towards germination had been made during the pre-incubation at low water potential. Plates were surveyed daily for germinants over a period of 12 days. To ensure germination had reached a clear plateau in all treatments, the final number of germinants and the total number of viable seeds were recorded after 16 days, to give the final germination proportion for each plate.

2.3.2. Statistical analysis

To test for significant effects of the pre-incubation treatment on germination, and to test whether its effect differed among genotypes and seed-maturation temperatures, we examined whether pre-incubation at low water potential altered germination compared to untreated seeds, with respect to both germination proportion and germination speed (mean time to germination, or "MTG"). The magnitude, or "degree of response" to the pre-incubation treatment was calculated as the difference in final germination proportion between treated and untreated control seeds of the same maternal plant. For germination speed, the raw time to germination (day) was determined for each seed and then used to calculate the mean time to germination (MTG) for each petri plate, and differences were calculated with respect to an un-treated control of the same maternal plant, and those differences (+6 to accommodate negative values) were log-transformed (for each Geno × Mat combination: 12 replicate plates per water potential treatment, 12 un-treated control plates). Un-germinated seeds and seeds that germinated during pre-incubation were omitted from this calculation. Unlike raw germination proportions and germination speeds, the difference in germination proportion and germination speed between treated and control seeds were normally distributed (proportions) or could be transformed to normality (speed) and so were analyzed with analysis of variance using the GENMOD procedure in SAS 9.4 (SAS Institute, Cary NC).

In the full model, which used the degree of response as the dependent variable, all interactions were included, and Geno, Mat, and Psi were treated as fixed factors. Because the Geno × Mat interaction was highly significant, we next analyzed each seed-maturation temperature separately, with Geno, Psi, and Geno × Psi as predictors. When we detected significant effects of genotype or interactions with genotype in the above models, we performed separate analyses for each genotype compared to L*er*, to determine which genotypes differed significantly from the L*er* control genotype. Bonferroni corrections were conducted to adjust for multiple comparisons to L*er* (four comparisons). Analyses of absolute germination proportions and speed (as opposed to analysis of differences in germination proportion and germination speed compared to untreated control seeds) are available in Supplemental materials (Table S3).

The physiological parameter Ψ_{min} is defined as the lowest water potential required for metabolic advancement to occur (Tarquis et al., 1992). Thus, when ambient water potential is below Ψ_b for a given seed, radicle protrusion is prevented but germination processes can still proceed if the water potential is above Ψ_{min}. Based on this definition, Ψ_{min} was estimated as the water potential at which no advancement of germination was observed. To estimate the value of Ψ_{min}, we fit a linear regression model using the mean time to germination compared to untreated control as the dependent variable (MTG of treated seeds/MTG of untreated control seeds of the same maternal plant), and Psi treatment as a continuous response variable. We fit a model for every combination of Geno × Mat separately and used the linear regression equation to determine the *x*-intercept when $y = 1$. An intercept of $y = 1$ (as opposed to 0) indicates the water potential at which the germination speed of the treated seeds was the same as the untreated control.

2.4. Experiment 3: secondary dormancy induction by longer pre-incubation at low water potential

2.4.1. Experimental treatments

It has been shown that prolonged incubation at low water potential can induce secondary dormancy in *A. thaliana* (Auge et al., 2015). The third goal of this study, therefore, was to test whether the ability of incubation at low water potential to induce secondary dormancy depended on dormancy that was imposed by different genotypes, seed-maturation temperatures, or durations of afterripening. To this end, we pre-incubated seeds of each genotype, seed-maturation temperature (14 °C or 25 °C), and afterripening duration (one or five months) at a range of low water potentials (−1.0, −1.2, −1.5, and −1.8 MPa) in the light for 8 days before transferring them to water to germinate. The pre-incubation period in this experiment was twice as long as that used in Experiment 2. We assayed eleven replicate plates per genotype, seed-maturation temperature, and afterripening duration at each water potential. Plates were surveyed for germinants over a period of 20 days, to allow fresh seeds sufficient time to reach maximal germination capacity. For some treatment combinations, particularly seeds with low dormancy and high (near 0 MPa) water potentials during pre-incubation, the extended period of pre-incubation led to some germination occurring before the transfer to water; these germinants were not included in the sample used to calculate germination proportions and germination speed of seeds after transfer to pure water.

2.4.2. Statistical analysis

To test for significant effects of the pre-incubation treatment, and to test whether that response depended on genotype, seed-maturation temperature, or afterripening, we analyzed the magnitude of response to the pre-incubation treatment using the GENMOD procedure in SAS 9.4 (SAS Institute, Cary, NC). The degree of secondary dormancy induction by pre-incubation was calculated as the difference in final germination proportion between treated and untreated control seeds of the same maternal plant, for each afterripening duration. In cases in which we could not use same the maternal plant, a plant of the same genotype, maturation temperature, and growth chamber was used for the calculation. The degree of secondary dormancy induction was used as the dependent variable, and genotype (Geno), seed-maturation temperature (Mat), duration of afterripening (Afterripe), and water potential (Psi) were treated as fixed factors.

First we used a full model, which included all interactions. The Geno × Afterripe × Mat interaction was significant, so we next analyzed each Afterripe level separately, with Geno, Mat, Psi, and their two- and three-way interactions as predictors. We also analyzed each seed-maturation temperature separately, with Geno, Afterripe, Psi, and their two- and three-way interactions as predictors. Because some interactions with Afterripe and Mat treatment were significant in these submodels, we then analyzed each combination of Afterripe and Mat separately with Geno, Psi, and Geno × Psi as predictors. When we detected significant effects of genotype or interactions with genotype in the above models, we performed separate analyses for each genotype compared to L*er*, to determine which genotypes differed significantly from the L*er* control genotype. To adjust for multiple comparisons to the L*er* genotype (four comparisons), we conducted Bonferroni corrections.

We also estimated and analyzed the difference in germination speed (mean time to germination) between treated and untreated control seeds in a similar manner as germination proportion. Because the focus of this study was on secondary dormancy induction, we present the analysis of germination speed in Supplemental materials (Table S4 and S5; Text S2).

3. Results

3.1. Experiment 1: dormancy increases the water potential required for germination

Cold seed-maturation temperature induced strong dormancy that persisted even after one year of afterripening (Fig. 2, Supplemental Table S1). Genotypes also differed in dormancy after one year of afterripening, with L*er*-*DOG1*$_{Cvi}$ and Col being the most dormant, and L*er*, L*er*-*DOG6*$_{Cvi}$, and L*er*-*FLC*$_{Cvi}$ having comparably high levels of germination. The effect of water potential (Psi) interacted significantly with seed-maturation temperature ($X^2 = 18.46$, df = 2, $P < 0.0001$; Supplemental Table S1), so the two seed-maturation treatments were analyzed separately.

Water potential significantly influenced germination of all genotypes and seed-maturation treatments (Table 1,Fig. 2), such that germination proportions decreased with decreasing water potential (increased water stress). When matured at 25° C, seeds of all genotypes were non-dormant, as shown by nearly 100% germination at the 0 MPa water potential. Germination proportions sharply declined at −1.2 MPa, and no germination was observed beyond −1.8 MPa for any genotype. When matured at 14° C, germination proportions were also high for seeds incubated at 0 MPa, with the exception of Col and L*er*-*DOG1*$_{Cvi}$, indicating that cold temperatures during seed maturation induced stronger dormancy for these genotypes. These genotypes did not germinate at any other water potential except −0.6 MPa, and proportions were extremely low in this treatment (<10%) and limited to a single seed of one replicate plant. Baseline water potentials were also lower for seeds matured at 25° C (ranging from −1.62 to −2.12 MPa) compared to those matured at 14 °C (ranging from −1.43 to −1.54 MPa; Fig. 3A). Therefore, cold seed-maturation temperature impeded the ability of seeds to germinate at low water potential.

The strength (slope) of the response to water potential did not differ among genotypes in either maturation temperature, as indicated by non-significant interactions between genotype and Psi (Table 1). However, because some genotypes were more dormant than others (and therefore started at lower germination proportions even at 0 MPa), genotypes did differ in the lowest water potential at which germination could occur (Fig. 3A). Specifically, the baseline water potential was lowest for L*er*-*FLC*$_{Cvi}$, a genotype with shallow dormancy, especially at the hot seed-maturation temperature, whereas it was consistently highest for Col, a genotype with high dormancy.

In sum, dormancy, whether induced environmentally via manipulation of seed-maturation temperature or genetically via allelic changes, impeded the ability to germinate at low water potentials. Despite genetic differences in baseline water potential, imposed by persistent differences in dormancy among genotypes, the slope of the response to water potential did not significantly vary with allelic variation in dormancy. Therefore, genetic differences in the ability to germinate under water stress appear to be because of intrinsic dormancy differences rather than differences in responses to water potential per se.

3.2. Experiment 2: germination progresses even at low water potentials that prevent the completion of germination

The pre-incubation treatment did not consistently influence germination proportions (Supplemental Tables S2 and S3A; see Supplemental Text S1 for discussion); however, it did influence the speed of germination. Control seeds exhibited faster germination when matured at 25 °C compared to 14 °C (Fig. 4), and the effect of the pre-incubation treatment on absolute germination speed varied among genotypes and seed-maturation temperatures (Geno × Mat × Psi effect: $X^2 = 22.04$, df = 12, $P = 0.037$; Supplemental Table S3B). Germination was generally accelerated by pre-incubation, and the magnitude of acceleration was greatest among seeds matured at 14 °C, although the effect was stronger in seeds matured at 25 °C than those matured at 14 °C (Psi × Mat effect: $X^2 = 24.20$, df = 3, $P < 0.0001$). When seeds were matured at 25 °C, germination was accelerated by pre-incubation at high but not low water potentials, as indicated by a significant effect of Psi on the difference in germination speed from the control (Table 2). Seeds that were matured at 14 °C also accelerated germination in response to pre-incubation, but the magnitude of response was similar across water potential treatments.

Furthermore, genotypes differed in the degree to which pre-incubation at low water potential accelerated germination (significant Geno effect in Table 2). Specifically, pre-incubation significantly delayed germination of Col and accelerated germination of L*er*-*DOG6*$_{Cvi}$ seeds matured at 14 °C, but not at 25 °C (significant Geno effect; Table 2). Although 25 °C-matured seeds of most genotypes exhibited accelerated germination after pre-incubation compared to the untreated control, Col seeds were only slightly accelerated after pre-incubation at −1.2 MPa, and at −2.8 MPa germination was slower than the untreated control (Fig. 4). Furthermore,

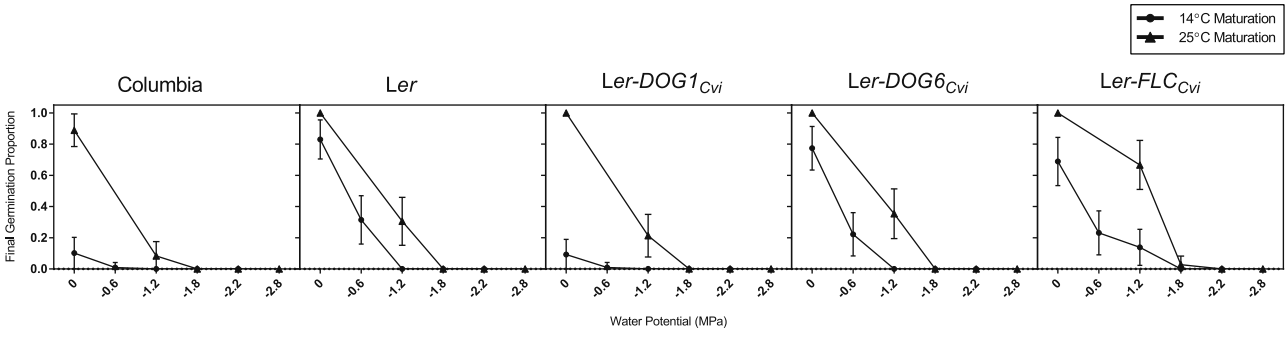

Fig. 2. Germination proportions at constant water potentials (Experiment 1). Final germination proportion of afterripened seeds matured at 14 °C (circles) and 25 °C (triangles) and imbibed at different water potentials. For seeds matured at 25 °C, germination was not assessed at −0.6 MPa, and the range of water potentials was extended to include −2.8 MPa. More negative values indicate lower water potential (higher water stress). Lines connect genotypic means and bars indicate standard errors around those means.

Table 1
Results of logistic regression analysis of final germination proportions of seeds incubated at constant water potentials (Experiment 1). The model tests for effects of genotype (Geno) and water potential (Psi) on final germination proportion for each seed-maturation temperature (25 °C or 14 °C) separately. Results are presented for a model that included all genotypes (Main model), and also for each genotype compared to L*er* (Geno vs. L*er*). P-values for pairwise comparisons were Bonferroni corrected. X^2 values are based on Type III analyses. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	Hot maturation (25 °C)			Cold maturation (14 °C)		
Final proportion	Geno	Psi	Geno x Psi	Geno	Psi	Geno x Psi
Geno vs. Ler	*df = 1*	*df = 3*	*df = 3*	*df = 4*	*df = 3*	*df = 12*
Col	2.31	69.56***	3.14	4.54	69.60***	6.83
L*er-FLC*$_{Cvi}$	2.63	58.47***	0.99	0.42	165.12***	7.6
L*er-DOG1*$_{Cvi}$	0.001	61.16***	0.007	4.68	67.08***	7.28
L*er-DOG6*$_{Cvi}$	0.03	60.25***	0.09	0.16	182.28***	0.17
Main model	*df = 4*	*df = 2*	*df = 8*	*df = 4*	*df = 3*	*df = 12*
All genos	13.91**	148.89***	7.69	14.87**	180.69***	18.90

pre-incubation at −1.8 and −2.2 MPa had less effect on germination speed for the Col genotype (25° C only) as compared to L*er* (significant Geno effect; Table 2). These differences are not obviously related to differences in dormancy, however, since Col was more dormant than L*er* before pre-incubation, but L*er-DOG6*$_{Cvi}$ had dormancy similar to L*er*. Unfortunately, we could not assess whether pre-incubation accelerated germination in the most dormant genotype matured at 14 °C – L*er-DOG1*$_{Cvi}$ – because of its very low germination proportions.

In seeds matured at 25 °C, the minimum water potential at which progress towards germination was made ("Ψ_{min}") was similar for genotypes on the L*er* background, but it was higher for Col (Fig. 3B). Interestingly, estimates of Ψ_{min} are lower than the estimates of baseline water potential (Experiment 1; Fig. 3A), indicating that germination can proceed at low water potentials even if germination cannot be completed at such low water potential. For seeds matured at 14 °C, pre-incubation enhanced the germination speed of L*er-DOG6*$_{Cvi}$ and L*er-FLC*$_{Cvi}$ genotypes even at the lowest water potential that we measured, so we were unable to estimate Ψ_{min} in these seeds. However, Ψ_{min} differed significantly between L*er* and Col genotypes matured at 14 °C (L*er* = −3.72 ± 0.24 MPa, Col = −2.05 ± 0.41 MPa), and this difference may be due to the strong temperature-induced dormancy observed in Col.

In sum, pre-incubation at low water potential accelerated germination in most genotypes, indicating that progress towards germination occurs at low water potential even when seeds cannot germinate at those low water potentials. This effect was more consistent in hot-matured seeds with less temperature-induced dormancy, but its effect varied among genotypes in a manner that cannot be attributed to genotypic differences in dormancy.

3.3. Experiment 3: longer pre-incubation at low water potential induces secondary dormancy in a manner that depends on dormancy

Control seeds without pre-incubation exhibited high germination proportions in all treatments, with slightly lower germination

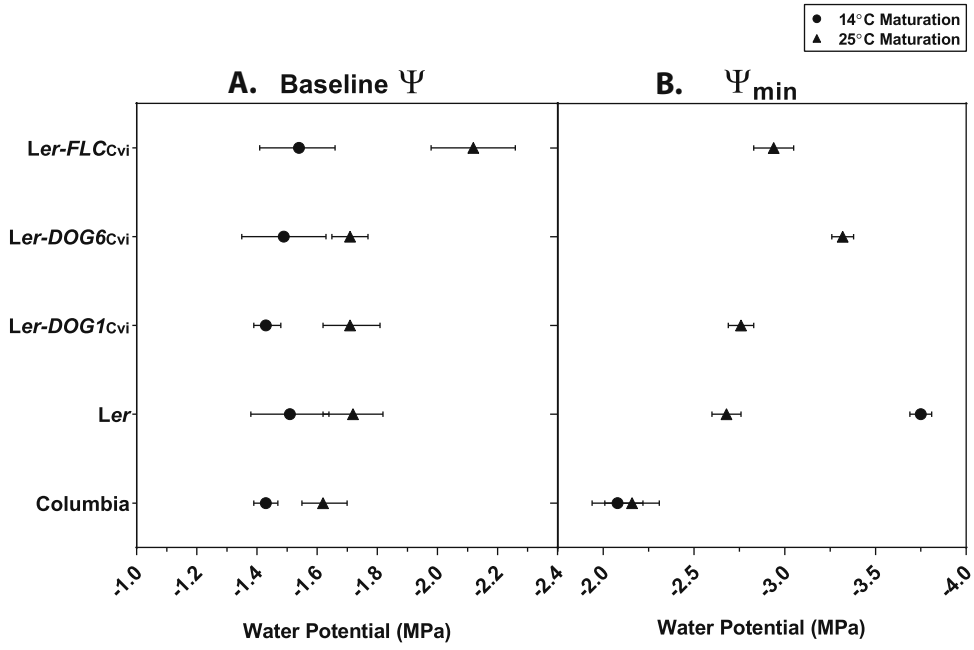

Fig. 3. (A) Baseline Ψ estimates and 95% CI for genotypes matured at 14° C and 25° C. Estimates were determined by performing linear regressions of final germination proportion (data from Experiment 1) as a function of water potential for each genotype in each maturation treatment separately. Baselines reflect the water potential at which we would expect no germination to occur. (B) Ψ_{min} estimates and 95% CI for genotypes matured at 14° C and 25° C. Estimates were determined by performing linear regressions of relative mean time to germination (data from Experiment 2) compared to untreated control as the dependent variable (MTG of treated seeds/MTG of untreated control seeds for each plant), and Psi treatment as a continuous response variable. We fit a model for every combination of Geno × Mat separately and used the linear regression equation to determine the water potential at which treated and untreated seeds do not differ in germination speed.

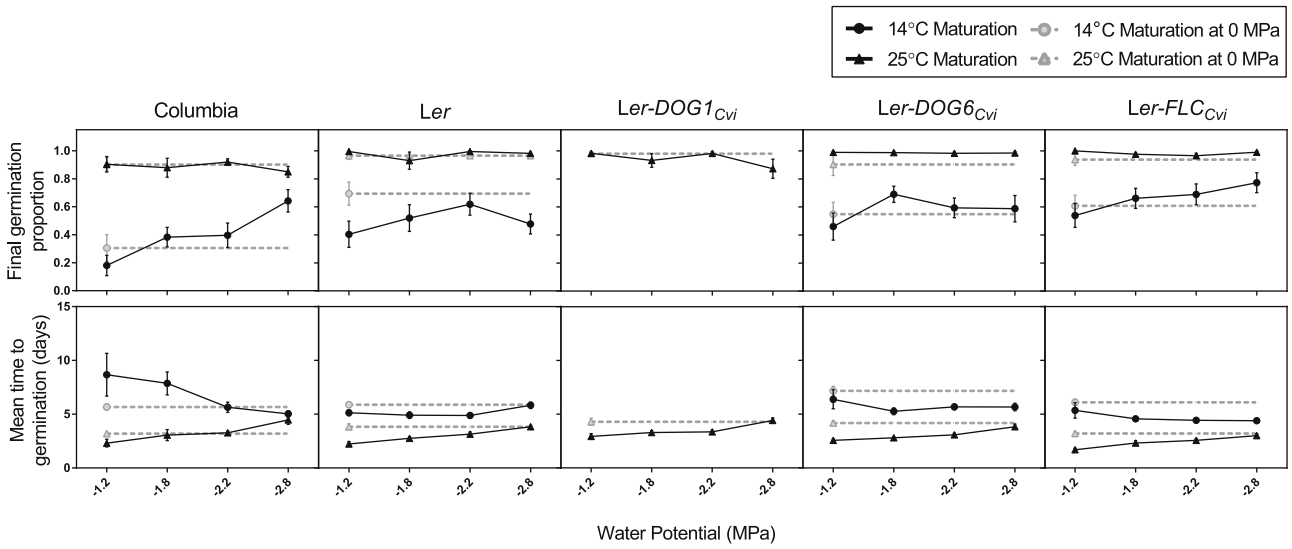

Fig. 4. Effect of brief pre-incubation at low water potential on germination (Experiment 2). Germination proportion (upper) and germination speed (lower) of afterripened seeds pre-incubated for four days at a range of low water potentials and subsequently transferred to water (0 MPa). Reaction norms for each genotype and seed-maturation temperature (triangles = 25 °C or circles = 14 °C) are presented as a function of pre-incubation water potential (Psi) for pre-incubated seeds (solid lines) of each maturation temperature. Lines connect genotypic means, and bars indicate standard errors around those means. Un-incubated seeds are plotted as dashed lines and grey symbols for comparison. Because *Ler-DOG1*$_{Cvi}$ seeds matured at 14 °C exhibited very low germination proportions, germination speed was unable to be calculated for that combination.

proportions in fresh seeds matured under cold temperatures (Fig. 5). Primary dormancy was low in all treatments and for all genotypes, with the exception of *Ler-DOG1*$_{Cvi}$ matured at 14 °C.

Overall, prolonged pre-incubation at low water potential reduced germination proportions, and such secondary dormancy induction was less effective at lower potentials (Fig. 5). Therefore, a minimum amount of water is necessary to induce secondary dormancy. The full model did not detect a significant Geno × Afterripe × Mat × Psi interaction. However, the interaction between Geno, Afterripe, and Mat was highly significant ($X^2 = 47.14$, df = 4, $P < 0.0001$), indicating that genotype effects on secondary dormancy induction are influenced by maturation temperature and afterripening. The Afterripe × Mat interaction was also highly significant in the full model ($X^2 = 28.10$, df = 1, $P < 0.0001$), indicating that the effect of afterripening on secondary dormancy induction differs depending on the seed-maturation temperature. We therefore conducted separate analyses for each afterripening and seed-maturation treatment.

Seeds matured at 14 °C were more readily induced into secondary dormancy by prolonged pre-incubation than seeds matured at 25 °C, but only when seeds were fresh, as indicated by a significant Psi × Mat interaction for fresh but not afterripened seeds (Table 3). Therefore, seeds with the weakest dormancy (hot-matured and afterripened) were not induced into secondary dormancy by prolonged pre-incubation at low water potential, but seeds with the strongest dormancy (cold-matured and fresh) were induced most into secondary dormancy.

Genotypes differed in the degree of dormancy induction by prolonged pre-incubation, but genotype effects were significantly stronger in seeds matured at 14 °C than at 25 °C when seeds were fresh (Tables 3 and 4; significant Geno × Mat for fresh seeds). No genotype was significantly induced into secondary dormancy when matured at 25° C when seeds were fresh (Table 4A). For seeds matured at 14 °C, fresh seeds of Col and *Ler-FLC*$_{Cvi}$ were less induced at more negative (lower) water potentials than *Ler* and *Ler-DOG6*$_{Cvi}$ genotypes, and *Ler-DOG1*$_{Cvi}$ did not exhibit altered germination because it had persistent primary dormancy (Fig. 5). As before, these genotypic differences in secondary dormancy induction are not convincingly associated with genetic differences in primary dormancy, since primary dormancy differed little among genotypes under these conditions (except for *Ler-DOG1*$_{Cvi}$, which had such deep primary dormancy that secondary dormancy could not be detected). In afterripened seeds, prolonged pre-incubation especially at the higher water potentials induced some secondary dormancy; all genotypes exhibited secondary dormancy induction when seeds were matured at 14 °C, but only Col exhibited secondary dormancy induction when seeds were matured at 25 °C. Therefore, cold temperature during seed-maturation enhanced the expression of genetic variation in the degree to which secondary dormancy was induced, as compared to hot maturation temperatures, but only significantly so when seeds were fresh (Table 4).

Prolonged pre-incubation appears to have similar effects on germination speed as did shorter periods of pre-incubation (Supplemental Fig. S1; Supplemental Table S6). In fresh seeds, prolonged pre-incubation accelerated germination of seeds matured at 25° C, but it had little effect on the germination speed

Table 2
Analysis to test for enhanced germination speed after brief pre-incubation at low water potential (Experiment 2) for each seed-maturation temperature separately. Results of Type III analysis of variance to test for effects of genotype (Geno) and water potential (Psi) on the degree of response (difference in germination speed between un-incubated and pre-incubated seeds) for each seed-maturation temperature (Mat) separately. Results are presented for a model that included all genotypes (Main model), and also for each genotype compared to *Ler* (Geno vs. *Ler*). Significance levels of pairwise tests were Bonferroni corrected. X^2 values are based on likelihood ratios. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Difference in MTG	Hot maturation (25 °C)			Cold maturation (14 °C)		
Geno vs. Ler	Geno *df = 1*	Psi *df = 3*	Geno × Psi *df = 3*	Geno *df = 1*	Psi *df = 3*	Geno × Psi *df = 3*
Col	13.69***	29.25***	0.17	7.78*	3.23	10.14
Ler-FLC$_{Cvi}$	0.38	31.86***	0.85	6.04	1.18	2.91
Ler-DOG1$_{Cvi}$	0.02	20.28***	1.20	–	–	–
Ler-DOG6$_{Cvi}$	1.84	30.67***	0.61	6.90*	0.67	1.84
Main model	*df = 4*	*df = 3*	*df = 12*	*df = 3*	*df = 3*	*df = 9*
All genos	32.19***	68.73**	4.20	29.81***	2.62	9.06

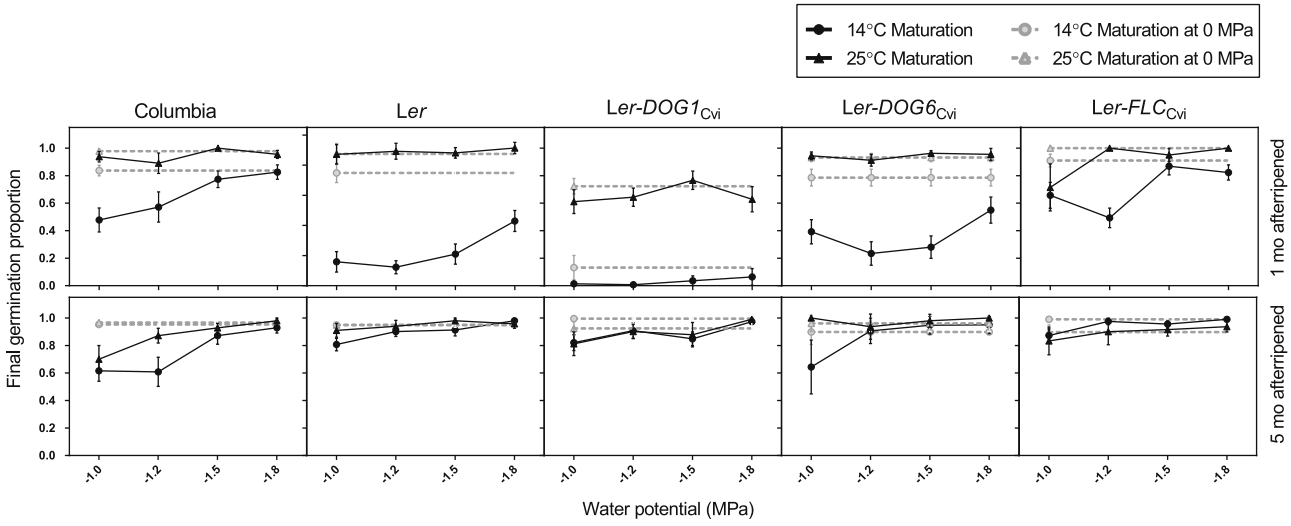

Fig. 5. Induction of secondary dormancy in response to prolonged pre-incubation at low water potential (Experiment 3). Germination proportion of seeds pre-incubated at a range of low water potentials for eight days and subsequently transferred to water (0 MPa). Seeds of each genotype and seed-maturation temperature (25 °C or 14 °C) were afterripened for 1 month (upper panel) and 5 months (lower panel). The mean final germination proportion in water for seeds at each pre-incubation (Psi) treatment are represented by solid lines and un-incubated seeds as dotted lines. Bars indicate standard errors around genotypic means.

(with the exception of Col) of seeds matured at 14° C (Supplemental Tables S4 and S5). Regardless of maturation temperature, pre-incubation effects in afterripened seeds were generally less apparent (Supplemental Tables S4 and S6). More detailed discussion of effects of prolonged pre-incubation on germination speed can be found in Supplemental materials (Text S2).

Taken together, these results demonstrate how factors that are typically associated with increased dormancy, such as low amounts of afterripening and cold seed-maturation temperatures, appear to enhance secondary dormancy induction by prolonged pre-incubation at low water potential. Genotypic differences in secondary dormancy induction, however, are not able to be explained by genetically based differences in primary dormancy.

4. Discussion

Environmentally imposed dormancy influenced germination responses to low water potential, but genetically based dormancy did not always influence those responses in a comparable manner (Fig. 1). Dormancy imposed by both genetic and environmental factors reduced the ability of seeds to germinate at low water potential, but genetic variation in dormancy did not predict the degree to which pre-incubation at low water potential accelerated germination. Moreover, while environmentally determined primary dormancy reflected the degree of secondary dormancy induction by prolonged imbibition at low water potential, genetic variation in primary dormancy did not. Environmentally induced dormancy also influenced the expression of genetic differences in germination responses to low water potential. Thus environmentally determined dormancy influences not only germination responses to low water potential but also their evolutionary potential.

Cold seed-maturation temperature hindered the ability to complete germination at low water potentials. This result suggests that seeds matured at high temperatures may germinate more readily under low water availability compared to cold-matured seeds, since they have a lower baseline Ψ. This type of opportunistic germination strategy has been observed in seeds from arid habitats with unpredictable rain events and has been described as an adaptive strategy in habitats where moisture is the primary limiting factor for germination (Estrelles et al., 2015). The direction of this response also supports the hypothesis that Ψ_b declines as dormancy is lost (Batlla and Benech-Arnold, 2015; Bair et al., 2006). Some consistent differences were noted between genotypes, indicating that allelic

Table 3
Analysis of secondary dormancy induction by prolonged pre-incubation at low water potential (Experiment 3). Results of Type III analysis of variance to test for effect of water potential (Psi) on the difference in germination proportion between pre-incubated and untreated control seeds. Left-hand columns: Results of Type III analysis of variance for each seed-maturation temperature separately. Results are presented for a model with all genotypes (Main model) as well as for each genotype compared to *Ler* (Geno vs. *Ler*). Significance levels were corrected for multiple comparisons to *Ler* via Bonferroni corrections. Interactions with afterripening test whether effects of Geno and Psi differ between afterripening treatments. The three-way interaction between Geno × Afterripe × Psi was non-significant for both hot- and cold-matured seeds. Right-hand columns: Results of Type III analysis of variance for each afterripening treatment separately. Interactions with Mat test whether effects of Geno and Psi differ between seed-maturation temperatures. The three-way interaction between Geno × Mat × Psi was non-significant for both fresh and afterripened seeds. X^2 values are based on likelihood ratios. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Difference in final proportion	Hot maturation (25 °C)		Cold maturation (14 °C)		Fresh		Afterripened	
Geno vs. Ler	Geno × Afterripe *df = 1*	Psi × Afterripe *df = 3*	Geno × Afterripe *df = 1*	Psi × Afterripe *df = 3*	Geno × Mat *df = 1*	Psi × Mat *df = 3*	Geno × Mat *df = 1*	Psi × Mat *df = 3*
Col	0.15	3.64	51.54***	0.81	26.14***	8.20	3.30	2.45
Ler-FLC$_{Cvi}$	3.12	1.31	30.66***	12.05*	27.50***	8.41	0.34	2.78
Ler-DOG1$_{Cvi}$	0.72	0.69	44.89***	1.53	27.83***	2.50	0.80	1.33
Ler-DOG6$_{Cvi}$	0.11	0.31	1.07	11.28*	1.54	6.74	0.03	11.84*
Main model	*df = 4*	*df = 3*	*df = 4*	*df = 3*	*df = 4*	*df = 3*	*df = 4*	*df = 3*
All genos	3.58	1.20	76.57***	6.99	55.46***	9.76*	6.23	2.11

Table 4
Analysis of secondary dormancy induction by prolonged pre-incubation at low water potential (Experiment 3). Results of Type III analysis of variance of the difference in germination proportion between pre-incubated and un-incubated control seeds, to test for effects of pre-incubation at low water potential (Psi), Genotype (Geno), and to test whether effects of pre-incubation varied among genotypes (Geno × Psi) for seeds in each seed-maturation temperature and afterripening treatment separately. (A) Seeds afterripened for one month. (B) Seeds afterripened for 5 months. Results are presented for a model with all genotypes (Main model) as well as for each genotype compared to *Ler* (Geno vs. *Ler*). Significance levels were corrected for multiple comparisons to *Ler* via Bonferroni. The X^2 values presented here are based on likelihood ratios. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

A.	Difference in final proportion	Hot maturation (25 °C)			Cold maturation (14 °C)		
	Fresh seeds *Geno vs. Ler*	Geno *df = 1*	Psi *df = 3*	Geno × Psi *df = 3*	Geno *df = 1*	Psi *df = 3*	Geno × Psi *df = 3*
	Col	0.91	0.17	0.22	40.96***	7.08***	0.92
	Ler-FLC$_{Cvi}$	2.78	1.03	1.02	38.20***	7.09***	1.54
	Ler-DOG1$_{Cvi}$	1.42	0.19	0.35	42.55***	1.70	0.78
	Ler-DOG6$_{Cvi}$	0.02	0.05	0.05	2.27	4.40**	0.30
	Main model	*df = 4*	*df = 3*	*df = 12*	*df = 4*	*df = 3*	*df = 12*
	All genos	1.41	1.16	0.68	16.01***	7.82***	1.06
B.	Difference in final proportion	Hot maturation (25 °C)			Cold maturation (14 °C)		
	Afterripened seeds *Geno vs. Ler*	Geno *df = 1*	Psi *df = 3*	Geno × Psi *df = 3*	Geno *df = 1*	Psi *df = 3*	Geno × Psi *df = 3*
	Col	0.18	2.89*	1.87	12.25***	7.27***	1.97
	Ler-FLC$_{Cvi}$	0.37	0.48	0.22	0.02	5.99***	0.36
	Ler-DOG1$_{Cvi}$	0.10	0.72	0.70	3.69	4.66**	0.33
	Ler-DOG6$_{Cvi}$	0.24	0.89	0.59	0.36	9.21***	1.68
	Main model	*df = 4*	*df = 3*	*df = 12*	*df = 4*	*df = 3*	*df = 12*
	All genos	0.28	2.97*	0.99	6.79***	14.22***	1.93*

variation in dormancy may alter the ability to germinate at low water potential. However, these genotypic differences were more subtle than the environmental effects that produced highly contrasting dormancy phenotypes and that were associated with pronounced differences in baseline Ψ.

Seeds progressed towards germination at water potentials lower than the baseline Ψ (water potentials that are not permissive for germination), and the rate of progress depended on the seed-maturation temperature. Seeds matured at the warmer temperature were less dormant and accumulated more progress toward germination than cold-matured seeds when pre-incubated at sub-optimal water potential, as evidenced by the faster germination rates compared to un-incubated seeds. The rate of progress (or amount of acceleration) declined with increasing water potential as Ψ approached Ψ_{min}. Cold-matured seeds also accumulated progress towards germination during pre-incubation, but the advancement effect was similar across water potentials. This suggests that seeds with strong temperature-induced dormancy may have a lower Ψ_{min} which, coupled with a high baseline Ψ, could result in a greater range of water potentials for priming compared to non-dormant seeds matured at higher temperature. Such different priming responses of seeds matured at different temperatures may under some conditions result in more synchronized germination among seeds matured at different temperatures throughout the season. For instance, seeds matured and dispersed at cool temperatures in early spring, though not competent to germinate, may accrue progress towards germination at low water potentials between precipitation events. Eventually, when ambient conditions are above the baseline Ψ, cold-matured seeds could accelerate their germination and synchronize germination with seeds that were matured under warmer temperatures later in the season. However, whether the accumulated progress towards germination of cold-matured seeds exceeds that of hot-matured seeds would depend on how long water potentials remained below the Ψ_{min} of hot-matured seeds but above that for cold-matured seeds. By contrast, these differential responses could lead to disparate germination times between cohorts of seeds matured at different temperatures if ambient water potentials remain above the Ψ_{min} of hot-matured seeds. Such variation in germination timing could have fitness consequences depending on the optimal season of germination and the degree of unpredictability in environmental conditions. For example, plants of *Diptychocarpus strictus* that germinated in autumn exhibited a longer overall lifespan and had 3–10 times greater seed production compared to spring-germinated plants, suggesting that consistent germination in the autumn is adaptive (Lu et al., 2014). However, because of extreme environmental variation in that habitat, a bet-hedging strategy that produces multiple germination cohorts could reduce variation in fitness over time and contribute to the maintenance of those populations in temporally unpredictable environments.

The physiological adjustments that occur during seed priming have also been reported to impart stress tolerance under adverse conditions such as cold, drought, and salt stress and thereby influence seedling establishment and seed longevity (Chen and Arora, 2013; Elkoca et al., 2007). Seeds of *Wigandia urens* that experience natural priming under field conditions have been shown to increase germination synchrony and increase probability of establishment compared to non-primed seeds (González-Zertuche et al., 2001), and the advantages acquired through priming were still apparent after 2 years (Gamboa-deBuen et al., 2006). However, priming effects are not always beneficial and can influence seed longevity either positively or negatively depending on the duration of the priming treatment and the conditions experienced immediately after priming (reviewed in Paparella et al., 2015; Bewley et al., 2013). While some studies have proposed that wet–dry cycling resulting from rain events play a role in extending the longevity and persistence of aged seeds in the soil due to priming-related repair mechanisms (Long et al., 2011; Chen and Arora, 2013), others have found a negative correlation between priming and longevity (Tarquis et al., 1992). A recent study in *A. thaliana* found a negative correlation between dormancy and seed longevity that was proposed to be controlled by the *DOG1* locus. The authors hypothesized that dormant seeds occurring in humid

environments would have the ability to repair or prevent aging-related damage during dormancy cycling, whereas seeds in dry environments would require different mechanisms to maintain longevity (Nguyen et al., 2012, 2015). In the wild, it may be advantageous for seeds produced in cool environments to have a reduced ability to complete germination at the onset of rain while maintaining the ability to advance metabolically between precipitation events in order to reap the benefits of priming and maintain viability in the soil.

During incubation at low Ψ, the physiological parameters of Ψ_b and Ψ_{min} themselves can change (Cheng and Bradford, 1999), indicating that dormancy itself (in addition to priming) can change during fluctuating imbibition at water potentials below baseline Ψ. Wagmann et al. (2012) described geographical variation in drought-dependent dormancy release of *Beta vulgaris* ssp. *maritima* that was highly heritable. In their study, germination in the spring was positively correlated with dormancy alleviation by a single drought treatment at a Northern latitude, whereas at a southern latitude, several drought periods were required to release dormancy and resulted in the spreading of germination across multiple favorable seasons (spring and autumn). The authors proposed that such adjustments to local conditions were partially due to maternal effects. Our findings support this idea in that they explicitly show that non-genetic effects on dormancy characteristics can influence the ability of seeds to respond to Ψ. These combined findings suggest that the observed differential responses for hot- and cold-matured seeds may be important to optimize germination timing to occur at favorable times of year.

We also found that seeds matured at cool temperature were more easily induced into secondary dormancy by prolonged imbibition at low water potential than were seeds matured at warm temperature. Studies in several other species have reported similar dormancy-inducing effects of long exposure to water stress (Pekrun et al., 1997; Momoh et al., 2002). For instance, dormancy cycling in response to seasonal rainfall has been observed in tropical perennial species, such that seeds acquired secondary dormancy during the rainy season and lost dormancy during the subsequent dry season (Garcia et al., 2014), indicating that seasonal fluctuations in rain can modulate cycles of dormancy and patterns of germination. Auge et al. (2015) also found secondary dormancy induction through incubation at low water potential in *A. thaliana*, especially in cold-matured seeds. Interestingly, in our study, seeds matured under cool temperature had greater secondary dormancy induction even when they did not exhibit deeper primary dormancy, suggesting that seed-maturation temperature influences secondary dormancy induction somewhat independently of primary dormancy. Such induction of secondary dormancy is likely to delay germination under natural conditions. These results, combined with the potentially greater range of water potentials for seed priming, suggests that seeds matured at cooler temperatures may be particularly sensitive to fluctuating cycles in soil moisture and that dormancy regulation during imbibition may be operating at lower Ψ in cold-matured seeds than in hot-matured seeds. This would enable repeated transitions between dormant, non-dormant, and primed states under periods of low rainfall. Such cyclic fluctuations in soil water content have been shown to alter dormancy status of *Polygonum aviculare* seeds in a manner dependent on the seed hydration status (Batlla and Benech-Arnold, 2004). When considered within a seasonal context, the different priming and secondary-dormancy responses of hot-matured versus cold-matured seeds may work in concert under natural field conditions to either synchronize or spread the timing of germination among seeds matured at different temperatures throughout the season, depending on the precise water potential and how long it persists. This could make it difficult to predict germination time in environments with fluctuating water availability.

Hot seed-maturation masked germination differences between genotypes, whereas cold seed-maturation enhanced genotypic differences in germination responses to Ψ, despite genotypes having comparable levels of primary dormancy (with the exception of L*er*-*DOG1*$_{Cvi}$). In particular, cold-matured seeds of L*er* and *Ler*-*DOG6*$_{Cvi}$ were induced into secondary dormancy by all pre-incubation treatments when fresh, whereas Col and L*er*-*FLC*$_{Cvi}$ were induced in secondary dormancy at high but not low water potentials. Genetic differences in secondary dormancy induction were greatly reduced with afterripening for genotypes on the L*er* background, but Col continued to show secondary-dormancy induction at high water potentials. Thus seed-maturation and afterripening conditions can influence whether genetic differences in germination/dormancy responses to water potential are expressed. In particular, germination responses to water potential are expected to be able to respond to natural selection more when seeds are matured under cool temperature. By contrast, maturation under warmer temperatures is likely to reduce differences between genotypes and impede evolutionary responses (Sultan and Bazzaz, 1993; Ackerly et al., 2000).

The results presented here highlight the multifaceted influence of seed-maturation temperature in governing germination responses to water potential, and they demonstrate that its effects on germination and secondary dormancy remain relevant even when primary dormancy does not differ. These results also suggest that allelic differences in dormancy play an important role in determining germination responses to low water potential, especially in regions that experience low temperatures during reproduction and maturation. Furthermore, maturation temperature affects the extent to which genetic variation in dormancy traits are expressed and in turn, which genes are exposed to natural selection. Therefore maternal environmental effects can influence germination phenology considerably and as such should be incorporated into future efforts to predict germination timing under dynamically variable environments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.envexpbot.2016.02.011.

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