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Low temperature stimulates spatial molecular reprogramming of the *Arabidopsis* seed germination programme

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Research Paper

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Abstract

The timing of the germination of seeds is highly responsive to inputs from the environment. Temperature plays a key role in the control of germination, with low temperatures acting to stimulate this developmental transition in many species. In *Arabidopsis*, extensive gene expression changes have been reported at the whole seed level in response to cold, while much less is known about their spatial distribution across the diverse cell types of the embryo. In this study we examined the spatiotemporal patterns of promoter activity and protein abundance for key gibberellic acid (GA) and abscisic acid (ABA) factors which regulate the decision to germinate both during a time course of germination and in response to cold. Low temperature stimulated the spatial relocalization of these factors to the vasculature. The response of these seeds to dormancy-breaking stratification treatments therefore stimulates the distribution of both positive (GA) and negatively acting (ABA) components to this same cell type. This altered spatial pattern persisted following the transfer of seeds to 22°C, as well as after their rehydration, indicating that this alteration is persistent. These observations suggest that the vasculature plays a role in the low temperature-mediated stimulation of germination in this species, while novel cell types are recruited to promote germination in response to stratification.

Introduction

Seeds enable plants to move through both time and space, determining where and when plants are established (Koornneef *et al.*, 2002). The seed-to-seedling transition is therefore one of the major shifts in the plant life cycle (Finch-Savage and Leubner-Metzger, 2006; Springthorpe and Penfield, 2015).

Central to the control of the timing of the induction of germination is the role of the antagonistically acting hormones abscisic acid (ABA) and gibberellins (GA), which inhibit and promote this transition, respectively (Finkelstein *et al.*, 2008; Holdsworth *et al.*, 2008). The hormone balance theory which describes this relationship provides a molecular thresholding mechanism by which the development state of seeds is defined (Karssen and Lacka, 1986). The most abundant hormone is thought to define whether or not a seed transitions to the germination programme (Bradford and Trewavas, 1994). Biosynthetic (Olszewski *et al.*, 2002; Seo and Koshiba, 2002) and signalling pathways for ABA (Park *et al.*, 2009) and GA (Lee *et al.*, 2002; Murase *et al.*, 2008) have been identified, enabling the mechanistic regulation of the molecular agents which control dormancy and germination to be investigated.

Following the decision to germinate, the embryo within a seed commences growth (Koornneef *et al.*, 2002). This transition into a seedling is principally driven by cell expansion, rather than cell division (Bassel *et al.*, 2014; Sliwinska *et al.*, 2009). This discrete induction of growth following the initiation of the germination programme is promoted by GA (Groot and Karssen, 1987; Koornneef and Van der Veen, 1980) and its induction of gene expression associated with cell wall remodelling proteins which facilitate cell growth (Nakabayashi *et al.*, 2005; Dekkers *et al.*, 2013; Narsai *et al.*, 2017). These cell expansion-associated genes may be considered the downstream targets of the germination process in light of the central role they play in the regulation of embryo growth (Bassel, 2016).

Spatially distinct domains of gene expression programmes have been identified within the germinating *Arabidopsis* embryo using both gene expression analysis (Dekkers *et al.*, 2013) and the microscopic analysis of specific reporter constructs (Bassel *et al.*, 2014). The cellular sites of ABA and GA response and metabolism (Topham *et al.*, 2017), and growth-promoting cell wall-associated gene expression (Bassel *et al.*, 2014) have also been defined at single-cell resolution. In non-germinating *Arabidopsis* embryos, the radicle was found to be enriched for both ABA- and GA-associated synthesis and response components, leading to the proposal that this subdomain of the embryo acts as a decision-making centre in the control of seed

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dormancy (Topham *et al.*, 2017). Examination of gene expression associated with cell wall-associated gene expression revealed this to be first induced within the embryo radicle (Bassel *et al.*, 2014). These results collectively suggest that the radicle is where germination is initiated, a spatial site that overlaps with the decision-making centre.

The germination of seeds from an individual mother plant is typically non-uniform, with bet-hedging strategies being implemented (Bradford, 2002; Rowse and Finch-Savage, 2003; Springthorpe and Penfield, 2015; Mitchell *et al.*, 2016). This strategy is believed to improve plant fitness, while mechanisms underpinning this bet-hedging behaviour have been proposed (Johnston and Bassel, 2018).

In the context of food production systems, this bet-hedging trait is not favourable. Uniformity is a key objective in field-based agriculture at all stages of these systems. In order to achieve this, the germination of seeds must be synchronous once they have been planted. This co-ordinated crop establishment leads both to the suppression of weeds, and uniformity of the final product at harvest (Finch-Savage and Bassel, 2015). In light of this important role for uniformity in seed behaviour, procedures have been developed by commercial seed vendors which increase this population trait (Taylor *et al.*, 1998; Paparella *et al.*, 2015). In a process termed 'seed priming', seeds are held in suboptimal conditions for extended periods to repress germination (Finch-Savage *et al.*, 2004). Following the release of the seeds from this inhibitory treatment, the resulting germination profile of the seeds is more uniform. The mechanisms by which priming acts remain poorly understood, with protocols largely focusing on the efficacy of treatments rather than the mechanisms underpinning them. This limits the potential to enhance seed quality using these approaches.

Residual dormancy represents an obstacle to germination uniformity, and priming treatments can relieve seeds of this intrinsic block to germination. One method to eliminate dormancy in temperate seeds, including *Arabidopsis*, is to apply a low temperature treatment, termed 'stratification' (Yamauchi *et al.*, 2004). Widespread gene expression changes are associated with this low temperature response, but it remains unclear where these are located within the context of the multi-cellular embryo.

In this study we examined the spatiotemporal gene expression events underpinning the seed-to-seedling transition in *Arabidopsis*. We then compared these dynamic changes with those that occur in a seed that has been subjected to dormancy-relieving low temperature, in an effort to provide insight into the mechanism by which this germination-enhancing treatment is acting within seeds.

Materials and methods

Plant growth conditions

All plants in this study are in the Columbia background of *Arabidopsis*, and were grown in environmentally controlled cabinets, using 16 h light (23°C) and 8 h dark at 22°C. Seeds were collected when plants had stopped flowering, and placed in glassine bags for 4 weeks to reduce primary dormancy. Seeds were then cleaned by passing dried plant material through a fine mesh, and seeds were collected for use in subsequent experiments.

Germination assays

Germination of *Arabidopsis thaliana* seeds was conducted by plating 30 seeds in triplicate. Seeds were scored for radicle emergence (germination) every 4 h until 100% germination was reached. All seeds were on sterilized with 10% bleach and germinated on ½ MS 0.8% (w/v) agar plates in a growth room conditions with a 16 h light/8 h dark photoperiod at 22°C.

Cold treatment protocol

Arabidopsis thaliana seeds were cold treated as described previously (Sano *et al.*, 2017). Following 3 days of incubation at 4°C in the dark, seeds were transferred to 22°C in the light for 12 h before being dried. Seeds were dried by placing seeds between two layers or filter paper for 24 h. Reimbibition of seeds was done by plating them on ½ MS 0.8% (w/v) agar plates.

Generation of reporter constructs

Reporter constructs for *EXPANSIN* genes were generated using 2 kb of sequence upstream of the ATG start codon for each gene as previously described (Bassel *et al.*, 2014). Other reporter constructs come from previous publications, including *XTH18* and *XTH19* (Vissenberg *et al.*, 2005), *GID1A::GID1A-GUS* and *GID1C::GID1C-GUS* translational fusions (Suzuki *et al.*, 2009), *GA3ox1::GUS* and *GA3ox2::GUS* reporters (Hu *et al.*, 2008), *SCL3::GUS* (Zhang *et al.*, 2011), *AAO3::AAO3-GUS* and *ABA2::ABA2-GUS* (Seo *et al.*, 2006), and *RAB18::GUS* (Ghassemian *et al.*, 2000).

GUS histochemical staining

Arabidopsis thaliana embryos were dissected from seeds using a scalpel and forceps using a Leica SD6 binocular microscope. Embryos were stained in 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid, cyclohexylammonium salt (X-Gluc) solution with 0.1 M sodium phosphate buffer (pH 7.0), 0.1% Triton X-100 and 2 mM X-Gluc (Sigma). Embryos were stained at 37°C until the blue substrate became visible, or for 24 h. Samples were fixed in a 3:1 ethanol/acetic acid, 500:1 DMSO, 1% Tween 20 fixative solution for 24 h and cleared in a chloral hydrate solution until embryos were clear for imaging. Embryos were imaged using a Leica DM500 light microscope.

Results

Cold treatment of Arabidopsis seeds

The impact of cold treatment on the speed of germination in *Arabidopsis* seeds was investigated by imbibing seeds in the dark at 4°C for 3 days. Seeds were then transferred to 22°C in the light for 12 h before being dried, then reimbibed. This protocol was selected based on its inclusion of a low temperature treatment and increase in the speed at which *Arabidopsis* seeds germinate (Sano *et al.*, 2017). Using both the Col and Ler ecotypes, we confirmed that the speed at which germination is completed is reduced following this process (Fig. 1).

Visualizing molecular dynamics within germinating Arabidopsis embryos

Underpinning the transition from seed to seedling is a sequence of dynamic molecular events that unfold with the germinating embryo. These dynamic gene expression and epigenetic changes have been characterized previously on a genome-wide scale

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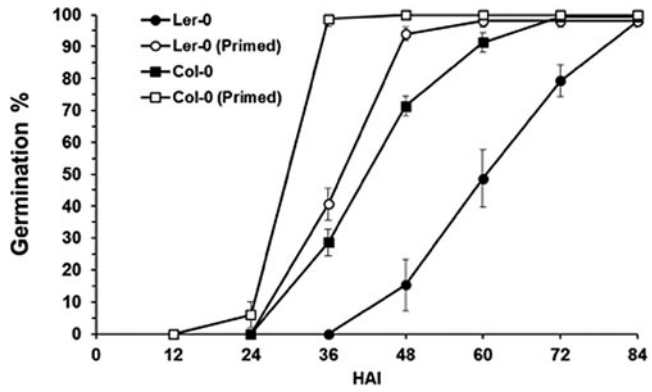


Fig. 1. Germination percentage of primed and unprimed Col-0 and Ler seeds at 22°C. Values are mean germination (%), and error bars are SE ($n = 3$). HAI, Hours after imbibition.

(Nakabayashi *et al*., 2005; Dekkers *et al*., 2013; Narsai *et al*., 2017). We sought to build upon this work by investigating the spatio-temporal dynamics of candidate genes using reporter constructs and light microscopy. The visualization of key reporters across the developmental sequence from seed to seedling enables the dynamic processes underpinning this transition to be understood. Comparing the expression pattern of these reporters in seeds across the cold treatment process may therefore provide insight into the stage at which the treatment is arresting this dynamic developmental programme, or associated changes in the molecular programme.

Three major classes of reporter were selected. The first represent genes that encode proteins targeted to the cell wall, and promote cell expansion (Cosgrove, 2005). The other two classes represent genes and proteins associated with ABA and GA synthesis, perception and response. The optical heterogeneity

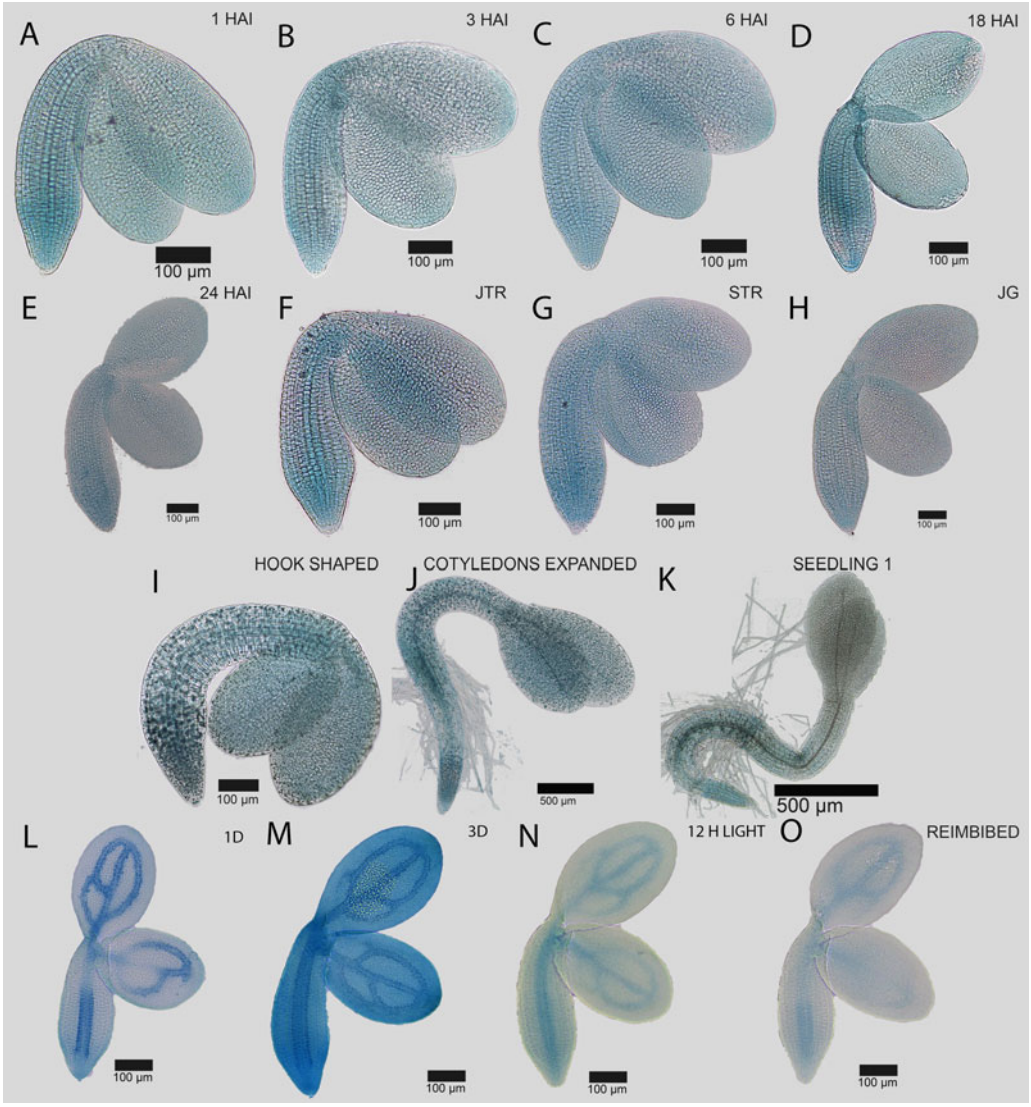


Fig. 2. Spatial and temporal dynamics of the *GID1A::GID1A-GUS* reporter during the seed-to-seedling transition in *Arabidopsis.* GID1A protein abundance in the germinating embryo at (A) 1 HAI, (B) 3 HAI, (C) 6 HAI, (D) 18 HAI, (E) 24 HAI, (F) just testa ruptured (JTR), (G) late testa rupture (STR), and germinated seedlings just after the completion of germination (H), (I) hook stage seedling, (J) recently expanded cotyledons and (K) a fully established seedling. Pattern of *GID1A::GID1A-GUS* activity in an embryo following (L) 1 day and (M) 3 days of priming treatment at 4°C in the dark, (N) 12 h at 22°C in the light, and (O) following reimbibition. Black bars indicate the scale in each image.

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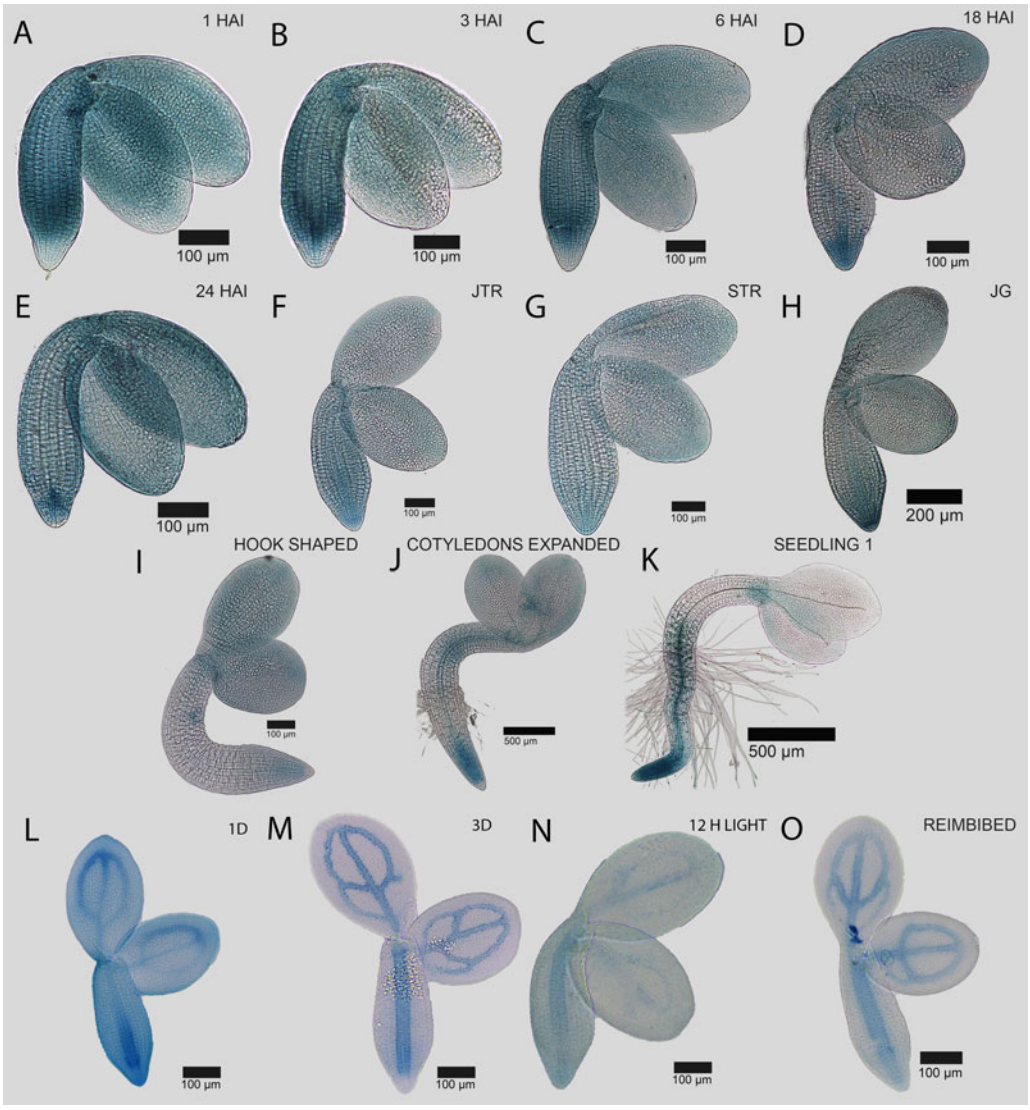


Fig. 3. Spatial and temporal dynamics of the *GID1C::GID1C-GUS* reporter during the seed-to-seedling transition in *Arabidopsis.* GID1C protein abundance in the germinating embryo at (A) 1 HAI, (B) 3 HAI, (C) 6 HAI, (D) 18 HAI, (E) 24 HAI, (F) early testa rupture, (G) late testa rupture, and germinated seedlings just after the completion of germination (H), (I) hook stage seedling, (J) recently expanded cotyledons and (K) a fully established seedling. Pattern of *GID1C::GID1C-GUS* activity in an embryo following (L) 1 day and (M) 3 days of priming treatment at 4°C in the dark, (N) 12 h at 22°C in the light, and (O) following reimbibition. Black bars indicate the scale in each image.

of mature *Arabidopsis* embryos makes it not possible to visualize fluorescent proteins deep within samples (Moreno, 2006). In order to achieve this, samples can be clarified and the β-glucuronidase (GUS) reporter (Jefferson, 1989) observed throughout all cells of the tissue (Truernit *et al.*, 2008). We made use of this system to examine the spatial and temporal changes that occur in germinating *Arabidopsis* seeds.

GA synthesis and signalling during the seed-to-seedling transition

GA is required for the induction of germination of intact *Arabidopsis* seeds (Koornneef and Van der Veen, 1980). The patterns of gene expression associated with this induction of GA synthesis show cell type-specific profiles (Yamaguchi *et al.*, 2001; Ogawa *et al.*, 2003), which are modulated by cold temperatures (Yamauchi *et al.*, 2004) and light (Yamaguchi *et al.*, 2001). This in turn leads to GA responses that act to promote downstream cell wall-associated gene expression in seeds (Cao *et al.*, 2006). Central to this induction are the *GIBBERELLIN INSENSITIVE DWARF* (*GID*) receptors (Ueguchi-Tanaka *et al.*, 2005), DELLA proteins (Lee *et al.*, 2002) and the *SCARECROW-LIKE3* (*SCL3*) transcription factor which controls germination responses (Zhang *et al.*, 2011). GUS reporters for GA-synthesis, -signalling and -response components were examined to understand the spatiotemporal events underpinning this hormone response that stimulates the germination process.

The hormone GA is perceived by the *GID1* receptors (Ueguchi-Tanaka *et al.*, 2005). The presence of GID1A or GID1C proteins is a primary requirement for a cell to be able to respond to this hormone. We examined the distribution of these receptors using GUS translational fusions (Gallego-Giraldo *et al.*, 2014). During the germination process, both of these proteins were broadly distributed across the embryo, with a slight bias

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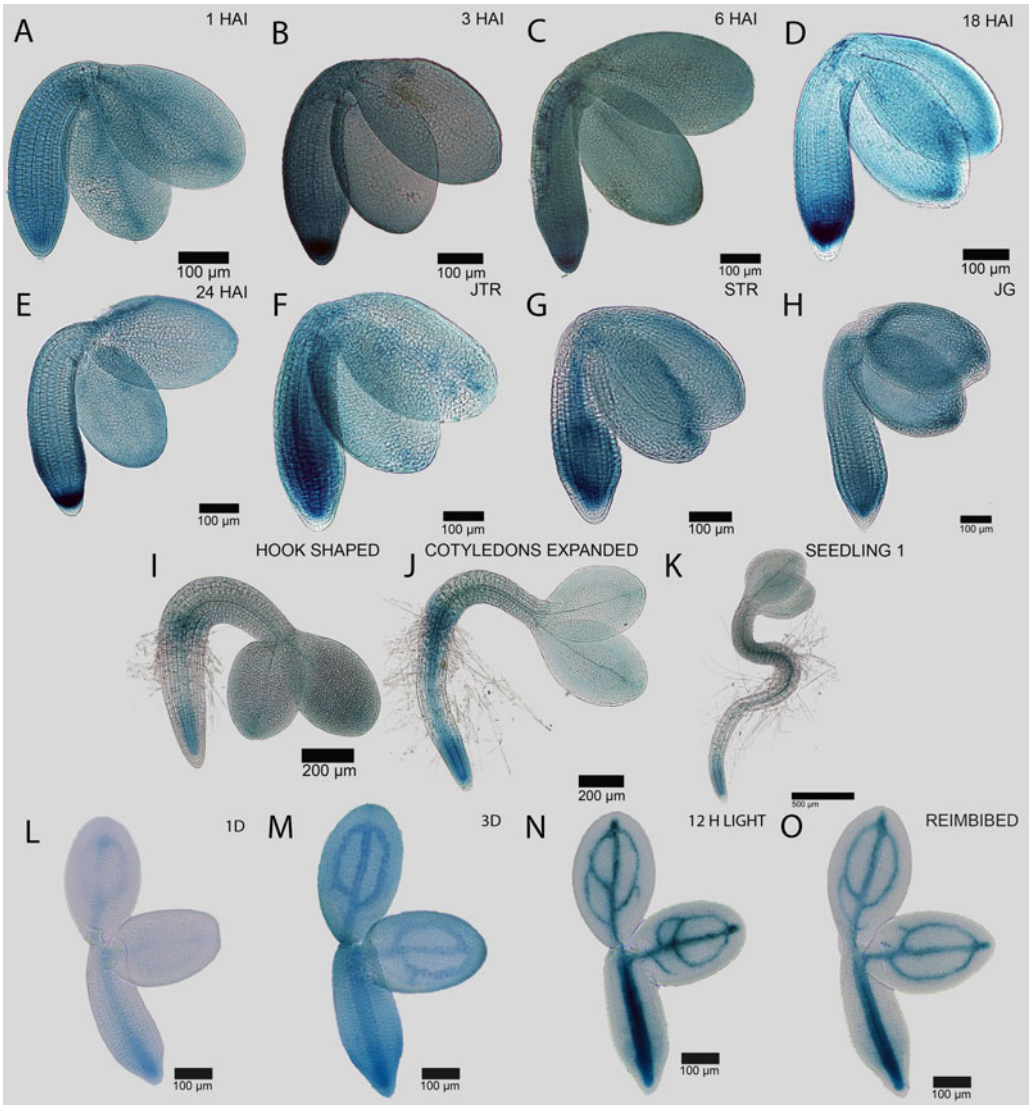


Fig. 4. Spatial and temporal dynamics of the *SCL3::GUS* reporter during the seed to seedling transition in *Arabidopsis.* Promoter activity in the germinating embryo at (A) 1 HAI, (B) 3 HAI, (C) 6 HAI, (D) 18 HAI, (E) 24 HAI, (F) early testa rupture, (G) late testa rupture, and germinated seedlings just after the completion of germination (H), (I) hook stage seedling, (J) recently expanded cotyledons and (K) a fully established seedling. Pattern of *SCL3::GUS* promoter activity in an embryo following (L) 1 day and (M) 3 days of priming treatment at 4°C in the dark, (N) 12 h at 22°C in the light, and (O) following reimbibition. Black bars indicate the scale in each image.

towards the axis (Figs 2 and 3). Following 1 day of cold treatment in the dark at 4°C, both of these proteins became enriched to the vascular cells of the embryo (Figs 2L and 3L). The localization persisted until 3 days in the cold at 4°C and in the light at 22°C for a further 12 h (Figs 2M–N and 3M–N). Dehydration and rehydration of seeds did not alter the localization of GA receptors to the vasculature (Figs 2O and 3O). Cold treatment, therefore, altered the cellular site of perception of the germination-stimulating hormone GA from across the embryo to principally within the vasculature.

The *SCL3::GUS* reporter acts as a useful proxy to understand the cellular sites where GA responses are occurring (Zhang *et al.*, 2011). Activity of this reporter is enriched in the radicle during the early stages of seed germination (Fig. 4A–E) and progressively moves along the hypocotyl and into the cotyledons until the completion of germination (Fig. 4F–H). Following germination, *SCL3::GUS* activity is enriched in the root (Fig. 4I–K), and presumably in the endodermis where it has been reported previously to be present (Zhang *et al.*, 2011). *SCL3::GUS* activity was enriched within the vasculature from the earliest stage of cold treatment sampled until the rehydration of seeds (Fig. 5L–O). GA response as indicated by this reporter therefore closely followed the pattern of GA perception indicated by the GID1A and GID1C reporters.

The final step and rate-limiting of GA synthesis is catalysed by the enzyme *GIBBERELLIN 3 β-HYDROXYLASE* (*GA3ox*) (Olszewski *et al.*, 2002). Examination of the *GA3ox1::GUS* promoter reporter (Mitchum *et al.*, 2006) indicated a broad initial induction across the embryo radicle, followed by movement into the hypocotyl as germination progresses (Supplementary Fig. S1A–G). In response to cold, a broad domain of activity of this promoter was observed across the embryo (Supplementary Fig. S1L–N). This GA-synthesis promoter therefore does not become enriched within the vasculature as do the perception and response components.

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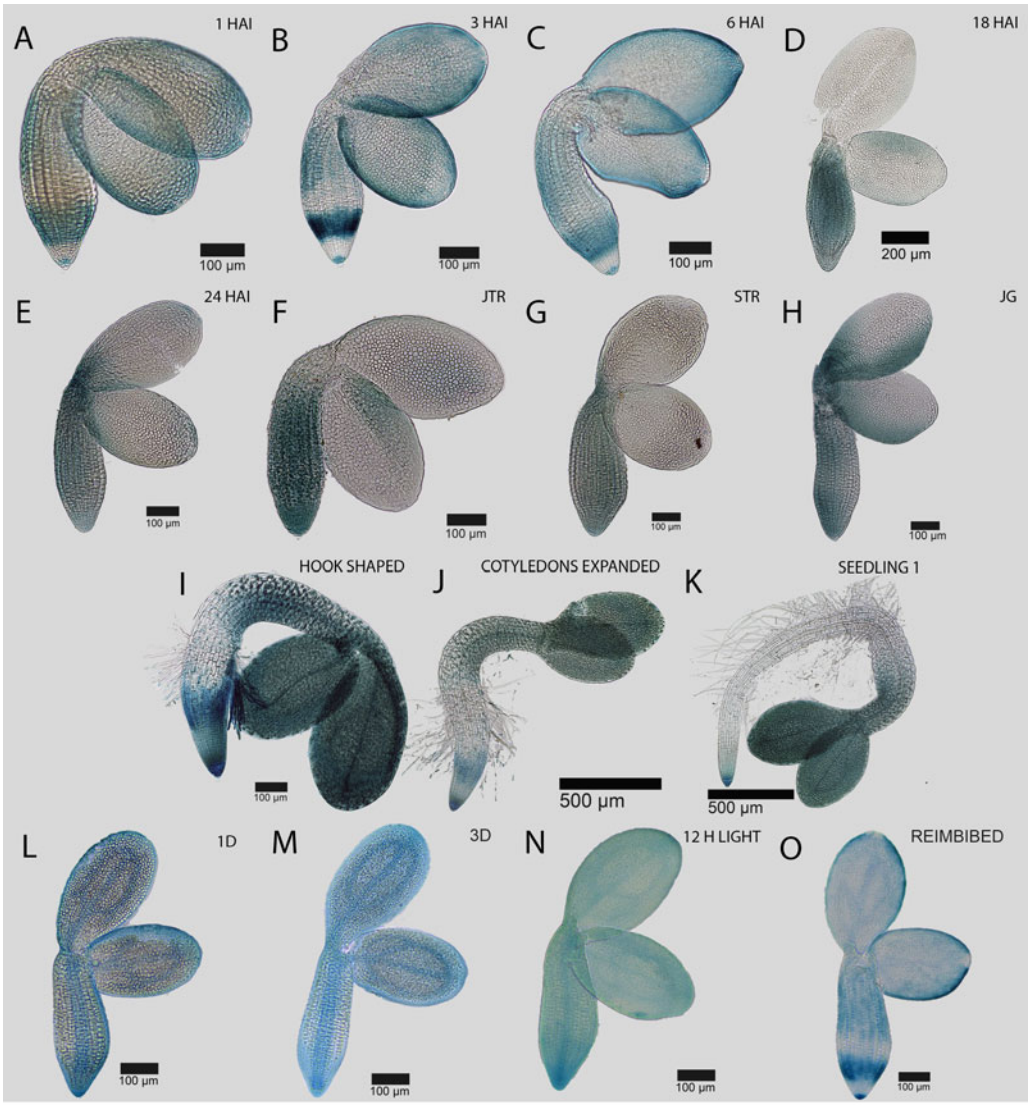


Fig. 5. Spatial and temporal dynamics of the *EXPA1::GUS* reporter during the seed-to-seedling transition in *Arabidopsis.* Promoter activity in the germinating embryo at (A) 1 HAI, (B) 3 HAI, (C) 6 HAI, (D) 18 HAI, (E) 24 HAI, (F) early testa rupture, (G) late testa rupture, and germinated seedlings just after the completion of germination (H), (I) hook stage seedling, (J) recently expanded cotyledons and (K) a fully established seedling. Pattern of *EXPA1::GUS* promoter activity in an embryo following (L) 1 day and (M) 3 days of priming treatment at 4°C in the dark, (N) 12 h at 22°C in the light, and (O) following reimbibition. Black bars indicate the scale in each image.

Cell growth-associated gene expression during the seed-to-seedling transition

Gene expression for enzymes that promote cell wall weakening act as a proxy to understand which cells are having their growth promoted (Cosgrove, 2005). These downstream targets of the germination process have been demonstrated to play a regulatory role in the control of this transition (Lü *et al.*, 2013). Proteins encoded by *EXPANSIN* (*EXPA*) and *XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE* (*XTH*) genes promote cell expansion in *Arabidopsis* cells (Vissenberg *et al.*, 2005). We examined the activity of the promoters encoding members of the *EXPA* and *XTH* multigene families that are conditionally unregulated in response to the induction of the germination program (Nakabayashi *et al.*, 2005; Bassel *et al.*, 2008; Dekkers *et al.*, 2013).

The spatiotemporal patterns of gene expression associated with growth-promoting cell wall modifying gene expression may serve as a proxy to understand spatial control of cell expansion (Cosgrove, 2005). A series of expansin genes are induced in germinating *Arabidopsis* embryos (Nakabayashi *et al.*, 2005) and promoter-GUS reporters to these genes have been generated previously (Bassel *et al.*, 2014; Stamm *et al.*, 2017). These lines were examined using light microscopy over the time course of seed germination and early seedling establishment to explore the genetic control of cell expansion. *EXPA1::GUS* is first induced in cells of the embryonic radicle, and spreads progressively up the axis and into the cotyledons during germination (Fig. 5A–F). Following germination, promoter activity is increasingly focused to the root tip and cotyledons, being excluded from the majority of the root and hypocotyl (Fig. 5G–K).

During cold treatment, the *EXPA1::GUS* promoter becomes enriched within the vascular cells of the embryo. This follows the pattern of upstream GA perception and response (Figs 2–4). Following the cold exposure, the spatiotemporal pattern observed

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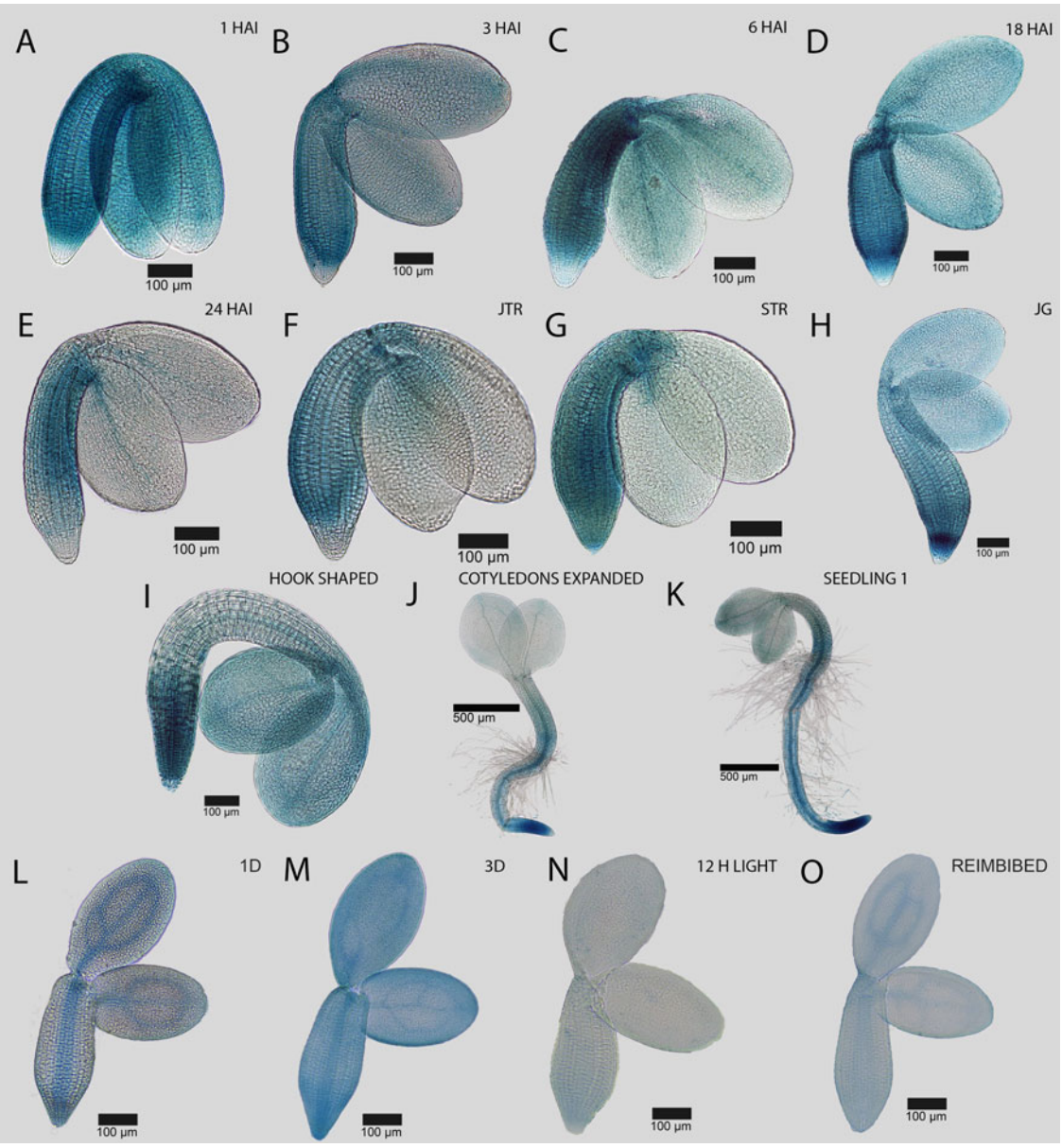


Fig. 6. Spatial and temporal dynamics of the *AAO3::AAO3-GUS* reporter during the seed to seedling transition in *Arabidopsis*. AAO3 protein abundance in the germinating embryo at (A) 1 HAI, (B) 3 HAI, (C) 6 HAI, (D) 18 HAI, (E) 24 HAI, (F) early testa rupture, (G) late testa rupture, and germinated seedlings just after the completion of germination (H), (I) hook stage seedling, (J) recently expanded cotyledons and (K) a fully established seedling. Pattern of *AAO3::AAO3-GUS* activity in an embryo following (L) 1 day and (M) 3 days of priming treatment at 4°C in the dark, (N) 12 h at 22°C in the light, and (O) following reimbibition. Black bars indicate the scale in each image.

between 3–6 hours after imbibition (HAI) of regular germination (Fig. 5B,C). The GA-responsive promoter *EXPA1::GUS* therefore follows the vasculature relocalization of gene expression associated with stratification. This is, however, not strictly true for all cell wall remodelling enzyme promoters, and was not observed for either *EXPA15::GUS* (Supplementary Fig. S2) or *XTH19::GUS* (Supplementary Fig. S3).

ABA synthesis and signalling during the seed-to-seedling transition

ABA plays a central role in the control of seed dormancy, and in mediating stress responses, limiting the germination of non-dormant seeds (Kushiro *et al*., 2004). We examined the expression of reporters associated with ABA synthesis and response over the time course of germination and compared this with the pattern in cold-treated seeds to determine at which stage low temperature arrests the ABA programme.

The final step of ABA synthesis is catalysed by the enzyme *ALDEHYDE OXIDASE 3* (*AAO3*) (Seo *et al*., 2006). The distribution of this protein is principally localized to the embryo axis during germination (Fig. 6A–G). During the early stages of cold treatment, the protein becomes enriched in the vasculature (Fig. 6L), where it becomes progressively less abundant. A similar pattern of ABA synthesis protein redistribution is with the ABA-synthesis protein *ABA DEFICIENT 2* (*ABA2*), which catalyses the penultimate step in hormone synthesis. During the early stages of germination, the protein is most abundant in the

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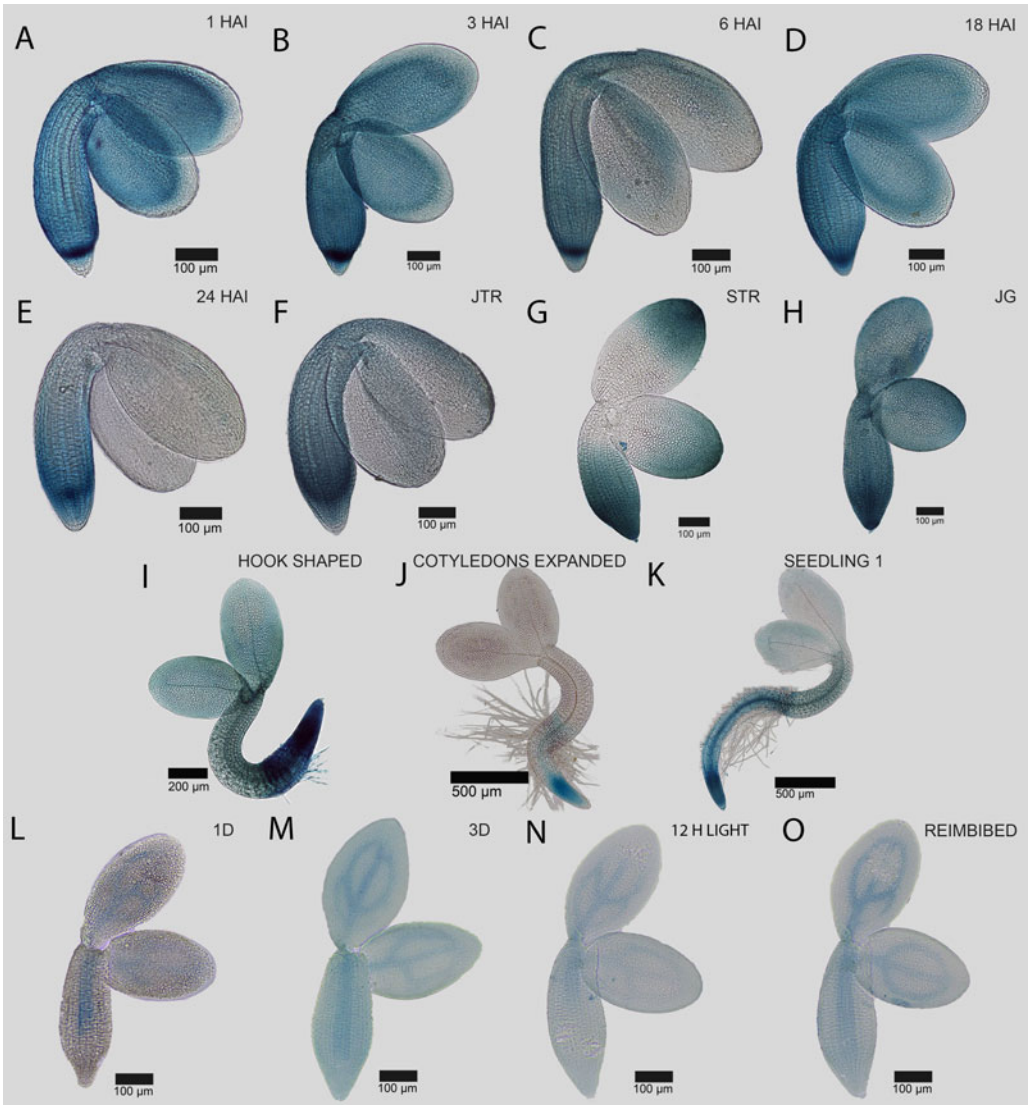


Fig. 7. Spatial and temporal dynamics of the *ABA2::ABA2-GUS* reporter during the seed to seedling transition in *Arabidopsis*. ABA2 protein abundance in the germinating embryo at (A) 1 HAI, (B) 3 HAI, (C) 6 HAI, (D) 18 HAI, (E) 24 HAI, (F) early testa rupture, (G) late testa rupture, and germinated seedlings just after the completion of germination (H), (I) hook stage seedling, (J) recently expanded cotyledons and (K) a fully established seedling. Pattern of *ABA2::ABA2-GUS* activity in an embryo following (L) 1 day and (M) 3 days of priming treatment at 4°C in the dark, (N) 12 h at 22°C in the light, and (O) following reimbibition. Black bars indicate the scale in each image.

radicle (Fig. 7A–G), yet during low temperature exposure becomes enriched within the vasculature (Fig. 7L–O). The sites of ABA synthesis overlap with those of GA perception and response during and following *Arabidopsis* seed stratification.

The *RESPONSE TO ABA18* (*RAB18*) promoter has been characterized previously as responsive to ABA, and acts as a useful proxy to determine where ABA-mediated transcriptional responses are occurring (Lång and Palva, 1992). Over the early stages of germination, this reporter is principally concentrated in the radicle, with some activity present on the outer margins of the cotyledons (Fig. 8A–D). This pattern continues until the seed has completed germination (Fig. 8E–H), where it then becomes focused first in the root of the early seedling (Fig. 8J) then the lower hypocotyl at a later stage of seedling development (Fig. 8K). Embryos from seeds that are being stratified at 4°C in the dark show *RAB18::GUS* in the vasculature, but this then changes to a spotty pattern in the axis and cotyledons once seeds are moved to 22°C in the light (Fig. 8L–O). ABA response therefore follows a vascular localization, but only at low temperatures.

Discussion

This study sought to investigate how low germination-stimulating temperatures impact the spatiotemporal dynamics of GA and ABA. By characterizing the sequential steps underlying seed germination for gene expression associated with cell growth, and hormone synthesis and response, a molecular developmental chronology was established. This was compared with seeds during the low temperature treatment to determine whether spatial changes were invoked by this treatment.

Generally speaking, molecular components and promoter activities were redistributed to the vascular cells of the embryo after low temperature treatment. This was observed for

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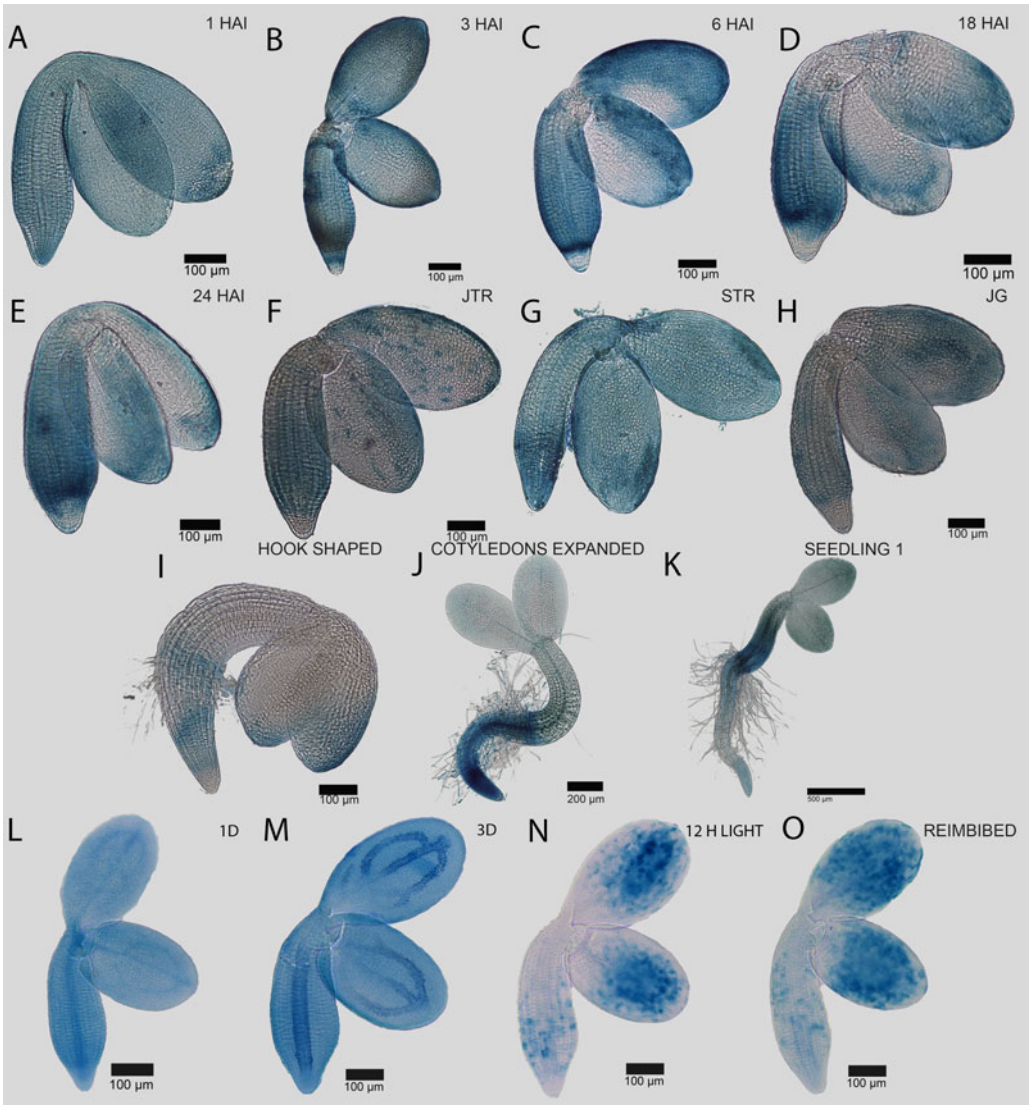


Fig. 8. Spatial and temporal dynamics of the *RAB18::GUS* reporter during the seed-to-seedling transition in *Arabidopsis.* Promoter activity in the germinating embryo at (A) 1 HAI, (B) 3 HAI, (C) 6 HAI, (D) 18 HAI, (E) 24 HAI, (F) early testa rupture, (G) late testa rupture, and germinated seedlings just after the completion of germination (H), (I) hook stage seedling, (J) recently expanded cotyledons and (K) a fully established seedling. Pattern of *RAB18::GUS* promoter activity in an embryo following (L) 1 day and (M) 3 days of priming treatment at 4°C in the dark, (N) 12 h at 22°C in the light, and (O) following reimbibition. Black bars indicate the scale in each image.

GA-perception, GA-response, cell wall modifying gene expression, ABA-synthesis and ABA-response-associated genes and proteins. The reason why this redistribution occurs remains unclear; however, the ubiquity with which it happens across a broad range of reporters suggests that placing all components into a single cell type is consistent.

In primary dormant *Arabidopsis* seeds, the cells that respond to each ABA and GA are enriched within the radicle, and separated in the vasculature and root cap, respectively (Topham *et al.*, 2017). This spatial separation endowed the system with the ability to process alternating temperatures. This ability to process changing temperature was lost in models where hormone responses are contained within the same cell. The spatial relocalization of both ABA- and GA-signalling components to the vasculature following cold treatment suggests that this complex temperature processing capacity is absent. This may be a consequence to residual dormancy being alleviated and this no longer being needed. This work sheds light on the molecular and cellular basis of cold-stimulated seed germination, and may provide cellular targets for genetic manipulation to enhance seed quality.

Supplementary Material. To view Supplementary Material for this article, please visit: https://doi.org/10.1017/S0960258519000266.

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References

Bassel, G.W. (2016) To grow or not to grow? *Trends in Plant Science* **21**, 498–505.

Bassel, G.W., Fung, P., Chow, T.F., Foong, J.A., Provart, N.J. and Cutler, S.R. (2008) Elucidating the germination transcriptional program using small molecules. *Plant Physiology* **147**, 143–155.

Bassel, G.W., Stamm, P., Mosca, G., de Reuille, P.B., Gibbs, D.J., Winter, R., Janka, A., Holdsworth, M.J. and Smith, R.S. (2014) Mechanical constraints imposed by 3D cellular geometry and arrangement modulate growth patterns in the Arabidopsis embryo. *Proceedings of the National Academy of Sciences of the USA* **111**, 8685–8690.

Bradford, K.J. (2002) Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Science* **50**, 248–260.

Bradford, K.J. and Trewavas, A.J. (1994) Sensitivity thresholds and variable time scales in plant hormone action. *Plant Physiology* **105**, 1029–1036.

Cao, D., Cheng, H., Wu, W., Soo, H.M. and Peng, J. (2006) Gibberellin mobilizes distinct DELLA-dependent transcriptomes to regulate seed germination and floral development in Arabidopsis. *Plant Physiology* **142**, 509–525.

Cosgrove, D.J. (2005) Growth of the plant cell wall. *Nature Reviews Molecular Cell Biology* **6**, 850–861.

Dekkers, B.J.W., Pearce, S., van Bolderen-Veldkamp, R.P., Marshall, A., Widera, P., Gilbert, J., Drost, H.G., Bassel, G.W., Muller, K., King, J.R. *et al.* (2013) Transcriptional dynamics of two seed compartments with opposing roles in *Arabidopsis* seed germination. *Plant Physiology* **163**, 205–215.

Finch-Savage, W., Dent, K. and Clark, L. (2004) Soak conditions and temperature following sowing influence the response of maize (*Zea mays* L.) seeds to on-farm priming (pre-sowing seed soak). *Field Crops Research* **90**, 361–374.

Finch-Savage, W.E. and Bassel, G.W. (2015) Seed vigour and crop establishment: extending performance beyond adaptation. *Journal of Experimental Botany* **67**, 567–591.

Finch-Savage, W.E. and Leubner-Metzger, G. (2006) Seed dormancy and the control of germination. *New Phytologist* **171**, 501–523.

Finkelstein, R., Reeves, W., Ariizumi, T. and Steber, C. (2008) Molecular aspects of seed dormancy. *Annual Review of Plant Biology* **59**, 387–415.

Gallego-Giraldo, C., Hu, J., Urbez, C., Gomez, M.D., Sun, T.P. and Perez-Amador, M.A. (2014) Role of the gibberellin receptors GID 1 during fruit-set in *Arabidopsis*. *Plant Journal* **79**, 1020–1032.

Ghassemian, M., Nambara, E., Cutler, S., Kawaide, H., Kamiya, Y. and McCourt, P. (2000) Regulation of abscisic acid signaling by the ethylene response pathway in *Arabidopsis*. *The Plant Cell* **12**, 1117–1126.

Groot, S.P.C. and Karssen, C.M. (1987) Gibberellins regulate seed-germination in tomato by endosperm weakening – a study with gibberellin-deficient mutants. *Planta* **171**, 525–531.

Holdsworth, M.J., Bentsink, L. and Soppe, W.J. (2008) Molecular networks regulating *Arabidopsis* seed maturation, after-ripening, dormancy and germination. *New Phytologist* **179**, 33–54.

Hu, J., Mitchum, M.G., Barnaby, N., Ayele, B.T., Ogawa, M., Nam, E., Lai, W.-C., Hanada, A., Alonso, J.M. and Ecker, J.R. (2008) Potential sites of bioactive gibberellin production during reproductive growth in *Arabidopsis*. *The Plant Cell* **20**, 320–336.

Jefferson, R. (1989) The GUS reporter gene system. *Nature* **342**, 837–838.

Johnston, I.G. and Bassel, G.W. (2018) Identification of a bet-hedging network motif generating noise in hormone concentrations and germination propensity in *Arabidopsis*. *Journal of The Royal Society Interface* **15**, 20180042.

Karssen, C. and Lacka, E. (1986) A revision of the hormone balance theory of seed dormancy: studies on gibberellin and/or abscisic acid-deficient mutants of *Arabidopsis thaliana*, pp. 315–323 in M. Bopp (ed.), *Plant Growth Substances*, 1985. Berlin-Heidelberg: Springer.

Koornneef, M., Bentsink, L. and Hilhorst, H. (2002) Seed dormancy and germination. *Current Opinion in Plant Biology* **5**, 33–36.

Koornneef, M. and Van der Veen, J. (1980) Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. *Theoretical and Applied Genetics* **58**, 257–263.

Kushiro, T., Okamoto, M., Nakabayashi, K., Yamagishi, K., Kitamura, S., Asami, T., Hirai, N., Koshiba, T., Kamiya, Y. and Nambara, E. (2004) The *Arabidopsis* cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. *EMBO Journal* **23**, 1647–1656.

Lång, V. and Palva, E.T. (1992) The expression of a rab-related gene, rab18, is induced by abscisic acid during the cold acclimation process of *Arabidopsis thaliana* (L.) Heynh. *Plant Molecular Biology* **20**, 951–962.

Lee, S., Cheng, H., King, K.E., Wang, W., He, Y., Hussain, A., Lo, J., Harberd, N.P. and Peng, J. (2002) Gibberellin regulates *Arabidopsis* seed germination via RGL2, a GAI/RGA-like gene whose expression is up-regulated following imbibition. *Genes and Development* **16**, 646–658.

Lü, P., Kang, M., Jiang, X., Dai, F., Gao, J. and Zhang, C. (2013) RhEXPA4, a rose expansin gene, modulates leaf growth and confers drought and salt tolerance to Arabidopsis. *Planta* **237**, 1547–1559.

Mitchell, J., Johnston, I.G. and Bassel, G.W. (2016) Variability in seeds: biological, ecological, and agricultural implications. *Journal of Experimental Botany* **68**, 809–817.

Mitchum, M.G., Yamaguchi, S., Hanada, A., Kuwahara, A., Yoshioka, Y., Kato, T., Tabata, S., Kamiya, Y. and Sun, T.P. (2006) Distinct and overlapping roles of two gibberellin 3-oxidases in *Arabidopsis* development. *Plant Journal* **45**, 804–818.

Moreno, N., Bougourd, S., Haseloff, J. and Feijo, J. (2006) *Imaging Plant Cells*. New York: Springer.

Murase, K., Hirano, Y., Sun, T.-P. and Hakoshima, T. (2008) Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. *Nature* **456**, 459–463.

Nakabayashi, K., Okamoto, M., Koshiba, T., Kamiya, Y. and Nambara, E. (2005) Genome-wide profiling of stored mRNA in *Arabidopsis thaliana* seed germination: epigenetic and genetic regulation of transcription in seed. *Plant Journal* **41**, 697–709.

Narsai, R., Gouil, Q., Secco, D., Srivastava, A., Karpievitch, Y.V., Liew, L.C., Lister, R., Lewsey, M.G. and Whelan, J. (2017) Extensive transcriptomic and epigenomic remodelling occurs during *Arabidopsis thaliana* germination. *Genome Biology* **18**, 172.

Ogawa, M., Hanada, A., Yamauchi, Y., Kuwahara, A., Kamiya, Y. and Yamaguchi, S. (2003) Gibberellin biosynthesis and response during *Arabidopsis* seed germination. *Plant Cell* **15**, 1591–1604.

Olszewski, N., Sun, T.-P. and Gubler, F. (2002) Gibberellin signaling: biosynthesis, catabolism, and response pathways. *Plant Cell* **14**, S61–80.

Paparella, S., Araújo, S., Rossi, G., Wijayasinghe, M., Carbonera, D. and Balestrazzi, A. (2015) Seed priming: state of the art and new perspectives. *Plant Cell Reports* **34**, 1281–1293.

Park, S.Y., Fung, P., Nishimura, N., Jensen, D.R., Fujii, H., Zhao, Y., Lumba, S., Santiago, J., Rodrigues, A., Chow, T.F. *et al.* (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* **324**, 1068–1071.

Rowse, H. and Finch-Savage, W. (2003) Hydrothermal threshold models can describe the germination response of carrot (*Daucus carota*) and onion (*Allium cepa*) seed populations across both sub-and supra-optimal temperatures. *New Phytologist* **158**, 101–108.

Sano, N., Kim, J.-S., Onda, Y., Nomura, T., Mochida, K., Okamoto, M. and Seo, M. (2017) RNA-Seq using bulked recombinant inbred line populations uncovers the importance of brassinosteroid for seed longevity after priming treatments. *Scientific Reports* **7**, 8095.

Seo, M., Hanada, A., Kuwahara, A., Endo, A., Okamoto, M., Yamauchi, Y., North, H., Marion-Poll, A., Sun, T.P., Koshiba, T. *et al.* (2006) Regulation of hormone metabolism in *Arabidopsis* seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. *Plant Journal* **48**, 354–366.

Seo, M. and Koshiba, T. (2002) Complex regulation of ABA biosynthesis in plants. *Trends in Plant Science* **7**, 41–48.

Sliwinska, E., Bassel, G.W. and Bewley, J.D. (2009) Germination of *Arabidopsis thaliana* seeds is not completed as a result of elongation of the radicle but of the adjacent transition zone and lower hypocotyl. *Journal of Experimental Botany* **60**, 3587–3594.

Springthorpe, V. and Penfield, S. (2015) Flowering time and seed dormancy control use external coincidence to generate life history strategy. *eLife* **4**, e05557.

Stamm, P., Topham, A.T., Mukhtar, N.K., Jackson, M.D., Tomé, D.F., Beynon, J.L. and Bassel, G.W. (2017) The transcription factor ATHB5 affects GA-mediated plasticity in hypocotyl cell growth during seed germination. *Plant Physiology* **173**, 907–917.

https://doi.org/10.1017/S0960258519000266 Published online by Cambridge University Press

Suzuki, H., Park, S.H., Okubo, K., Kitamura, J., Ueguchi-Tanaka, M., Iuchi, S., Katoh, E., Kobayashi, M., Yamaguchi, I. and Matsuoka, M. (2009) Differential expression and affinities of *Arabidopsis* gibberellin receptors can explain variation in phenotypes of multiple knock-out mutants. *Plant Journal* **60**, 48–55.

Taylor, A., Allen, P., Bennett, M., Bradford, K., Burris, J. and Misra, M. (1998) Seed enhancements. *Seed Science Research* **8**, 245–256.

Topham, A.T., Taylor, R.E., Yan, D., Nambara, E., Johnston, I.G. and Bassel, G.W. (2017) Temperature variability is integrated by a spatially embedded decision-making center to break dormancy in *Arabidopsis* seeds. *Proceedings of the National Academy of Sciences of the USA* **114**, 6629–6634.

Truernit, E., Bauby, H., Dubreucq, B., Grandjean, O., Runions, J., Barthelemy, J. and Palauqui, J.C. (2008) High-resolution whole-mount imaging of three-dimensional tissue organization and gene expression enables the study of phloem development and structure in *Arabidopsis*. *Plant Cell* **20**, 1494–1503.

Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M., Chow, T.-Y., Yue-ie, C.H., Kitano, H. and Yamaguchi, I. (2005) GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. *Nature* **437**, 693–698.

Vissenberg, K., Oyama, M., Osato, Y., Yokoyama, R., Verbelen, J.P. and Nishitani, K. (2005) Differential expression of AtXTH17, AtXTH18, AtXTH19 and AtXTH20 genes in *Arabidopsis* roots. Physiological roles in specification in cell wall construction. *Plant & Cell Physiology* **46**, 192–200.

Yamaguchi, S., Kamiya, Y. and Sun, T. (2001) Distinct cell-specific expression patterns of early and late gibberellin biosynthetic genes during *Arabidopsis* seed germination. *Plant Journal* **28**, 443–453.

Yamauchi, Y., Ogawa, M., Kuwahara, A., Hanada, A., Kamiya, Y. and Yamaguchi, S. (2004) Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. *Plant Cell* **16**, 367–378.

Zhang, Z.L., Ogawa, M., Fleet, C.M., Zentella, R., Hu, J., Heo, J.O., Lim, J., Kamiya, Y., Yamaguchi, S. and Sun, T.P. (2011) Scarecrow-like 3 promotes gibberellin signaling by antagonizing master growth repressor DELLA in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the USA* **108**, 2160–2165.

https://doi.org/10.1017/S0960258519000266 Published online by Cambridge University Press