Supporting Information

Footitt et al. 10.1073/pnas.1116325108

SI Materials and Methods

Seed Production. Seeds were produced (February–May 2007) in a temperature-controlled greenhouse. Heating and venting were set to provide a temperature of 16–18 °C during the 16-h day and 10–15 °C at night. Supplementary lighting (400 W high-pressure sodium lamps) (Osram) was supplied when light intensity fell below 300 W m^2 during the 16-h day. Nondormant seeds of the *Arabidopsis thaliana* ecotype Cape Verdi Isle (Cvi) were sown into compost (Levingtons F2/sand/vermiculite at a ratio of 6/1/1) in multicell trays held in capillary matting-lined seed trays. Mature seeds were harvested by hand threshing and equilibrated at 15% relative humidity/5 °C for 7 d to produce an equilibrium moisture content of 5–7% on a dry-weight basis. Seeds were stored at −80 °C in sealed tubes.

Seed Burial. To reduce seed mortality, seeds were dressed with Metalaxyl (Hockley International) at 1 g active fungicide/kg seeds (1). Seeds were then dispersed in Ballotini balls (100- to 250-μm diameter) (Potters Ballotini) of the same particle size as the sand in the sandy loam soil in the field trial area at a density of 40 seeds/g of Ballotini balls in125-μm mesh nylon bags (Clarcor-UK) and sealed with a WeLoc Weland M. bag clip (size PA110). For each time point, one bag for physiological analysis and four bags for molecular analysis were buried at a depth of 5 cm in a random plot design. SM200 soil moisture sensors (Delta-T Devices) and Thermistore temperature probes (Betatherm) linked to a data logger (Delta-T Devices) recorded soil moisture and temperature at seed depth in dummy bags (2).

Seed Recovery. Samples for molecular analysis were recovered from the field in the dark. A light-proof box with sealed arm holes in the top was placed over the burial site, and the base was sealed with soil to exclude light. The samples were then exhumed and placed in a laminated foil bag (Moore and Buckle) sealed with a WeLoc PA150 clip. In the laboratory under a green safe light, seeds were immediately separated from the Ballotini balls in cold water to remove soil. Ballotini balls and seeds were transferred to a 50-mL centrifuge tube, allowed to settle, and then washed three times. Tubes were gently shaken, causing seeds to migrate over the Ballotini balls to the tube walls where they were removed with a pastette (3 mL) (Jencons) to 2-mL Eppendorf tubes and immediately frozen in liquid nitrogen and stored at −80 °C. Seeds for physiological analysis were separated from Ballotini balls in the light in a similar fashion as above and immediately used for dormancy testing.

Dormancy Testing. Seeds recovered for physiological analysis were surface-sterilized in a 0.125% sodium hypochlorite solution [household bleach (5% sodium hypochlorite) diluted to 2.5%] for 5 min and then washed three times in water. Although household bleach is an effective seed disinfectant, excessive exposure and high concentrations of it can break dormancy (3, 4). Using Ultipette BARKY CP-100 tips (Barkey Instruments International), 50 seeds were plated out in triplicate plates (124 × 88 × 22 mm) (Stewart Plastics). Each plate contained two sheets of Whatman 3MM chromatography paper and 8 mL of the appropriate solution. Plates were placed in sealable freezer bags and incubated at 20 °C under continuous light, and germination was scored at 2- to 3-d intervals for up to 28 d. Germination was recorded as the emergence of the radical through the seed coat. Nitrate sensitivity was tested by incubating seeds on 10 mM KNO_3 as above. Gibberellic acid (GA) sensitivity was tested by exposing seeds to 5–250 μM GA in 1.7 mM citric acid/3.3 mM K_2HPO_4 buffer at pH 5.0 as above.

Thermodormancy was tested on water as above at 5, 10, 15, 20, and 25 °C. Seed viability was also tested at 20 °C using 100 μM GA/50 μM fluridone (Apollo Scientific) in citrate/phosphate buffer (pH 5.0). Depth of dormancy was determined by dry afterripening seeds at 20 °C in the dark at an equilibrium moisture content of 55% relative humidity. Loss of dormancy was evaluated by periodic germination testing on water at 20 °C in the light. From the resulting afterripening curves, the depth of dormancy, or AR50, was determined as the time required in dry storage to remove dormancy in 50% of the population.

Thermal Germination Window. Using the thermodormancy data described above, the thermal germination window was determined for germination ≥10%. The mean weekly air temperature was used as an indicator of soil surface temperature to establish a thermal baseline for germination as set by the environment. Germination ≥10% at a temperature greater than or equal to the soil surface temperature indicates when the permissive temperature for germination equals or exceeds the thermal baseline. In this situation, germination is possible in the field on exposure to light.

Seedling Emergence. To evaluate seedling emergence in the field, four replicate 17.5-cm^2 rigid black pots (BHGS Horticultural) were buried leaving a 2-cm lip above the ground. The pots were filled with sterilized soil to 3 cm from the top. The soil surface was covered in 125-μm nylon mesh to allow soil disturbance without incorporating seeds into deeper layers. A total of 500 seeds were then sown over the surface and covered in 1 cm of sterile soil. At monthly intervals, the soil was disturbed to expose seeds to light. Subsequent seedling emergence in each plot was recorded and seedlings were removed.

Abscisic Acid Analysis. The abscisic acid (ABA) content of three independent biological samples was determined at six time points covering the annual cycle. Hormone analysis used deuterated standards and analysis was by ultra performance liquid chromatography/electro spray ionization tandem mass spectroscopy (5–7) performed by the National Research Council Plant Biotechnology Institute, Saskatoon, Canada.

Quantitative PCR. RNA was extracted from seeds using the RNAqueous kit (Ambion) in conjunction with a plant RNA isolation aid (Ambion). RNA quality was determined using a spectrophotometer (Nanodrop) and electrophoretically with an Agilent Bioanalyzer. RNA was treated with RNase-free DNase I (Roche Diagnostics) to remove contaminating genomic DNA. Synthesis of cDNA was performed on 2 μg total RNA using SuperScript II Reverse Transcriptase (Invitrogen) and random pentadecamer primers (Invitrogen) following the manufacturer's instructions. Quantitative RT-PCR was performed in 384-well plates with a LightCycler 480 Real-Time PCR instrument (Roche Diagnostics) using the LightCycler 480 SYBR Green I Master kit (Roche Diagnostics). The gene specific-primer pairs used are listed in Table S1. For *DOG1*, a universal primer pair was used to cover all splice variants (8). Of the genes evaluated for use as reference genes (9) that appear in our seed dormancy microarray data (10, 11), the housekeeping gene At4g34270 (Tip 4-like) had the lowest fold change in expression and coefficient of variation across 13 different dormancy states and was selected as the reference gene for normalization purposes. The data at each time point are derived from analysis in triplicate of three independent biological samples. Data were

normalized against an internal calibration curve (0–1/1,000 dilutions) of the gene of interest, and the reference gene at the time point was ascertained by PCR and array data to have the highest expression of the gene of interest. Reactions contained 5 μL of SYBR Green I Master, 2 μL PCR-grade water, 1 μL of 10 mM forward and reverse primers, and 1 μL cDNA (diluted 1:10) in a final volume of 10 μL. PCR was carried out under the following conditions: one cycle at 95 °C for 10 min followed by 50 cycles at 95 °C for 30 s, 65 °C for 30 s, and 72 °C for 30 s. Data were analyzed using LightCycler 480 software, version 1.5 (Roche Diagnostics).

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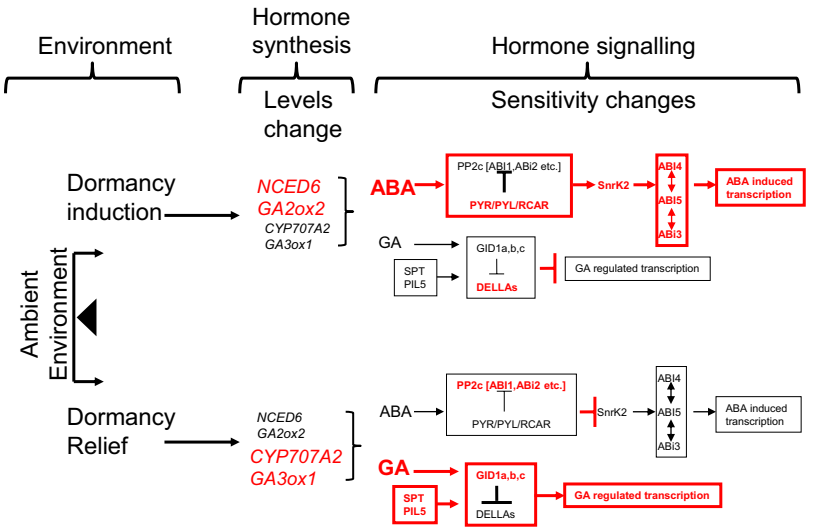

Fig. S1. A schematic summary of ABA and GA metabolism and signaling genes implicated in the regulation of seed dormancy where environmental signals influence the relative balance of dormancy induction and relief. When induction or relief are induced, the pathways indicated in red dominate. Active GA levels increase just before radical emergence, suggesting that it plays a key role in the regulation of germination; the key stages of GA metabolism during this process have been described (1). GA3-oxidase is responsible for the final step in GA biosynthesis to produce active GAs. Subsequent degradation is via GA2-oxidase. Key genes responsible for ABA biosynthesis, degradation, and conjugation during *Arabidopsis* seed germination have been identified and investigated (e.g., 2–6). NCEDs are the primary regulatory step in ABA synthesis; subsequent inactivation is by hydroxylation (CYP707) or conjugation with sugars (7). The balance of these processes regulates ABA content. Hormone signaling, especially that resulting from the dynamic balance of the hormones abscisic acid (ABA) and gibberellic acid (GA), is a key component of the interacting networks regulating germination (4, 8). Understanding of ABA signal transduction is developing rapidly, and a model has recently emerged in which PYR/PYL/RCAR receptors bind to ABA to remove the repression by PP2cs of downstream signaling via SnRK2s to ABRE-binding transcription factors (ABI3, ABI4, ABI5) (9). On the other side of this balance, DELLA proteins repress GA responses and therefore germination potential (10). DELLAs are degraded to remove this repression on forming a complex with GA and GID1 receptors (11). These signaling pathways are influenced by a diverse range of environmental signals as germination potential increases, principally temperature and light. Key components of the interaction between these environmental signals and GA are the two phytochrome-interacting bHLH transcription factors, PIL5 and SPT. Both factors repress germination potential in the dark and at low temperature, respectively. PIL5 represses cell-wall–modifying genes similar to the DELLAs. It also represses *GA3ox1* and *CYP707A2* and enhances *GA2ox1, NCED6*, and DELLA expression, whereas *SPT* represses *GA3ox1* expression (12, 13). PIL5 and SPT in turn are inactivated by DELLAs (RGL2 and RGA2) (13). PIF proteins are released when the GID protein–GA complex binds DELLA proteins to target their degradation by the proteosome (14).

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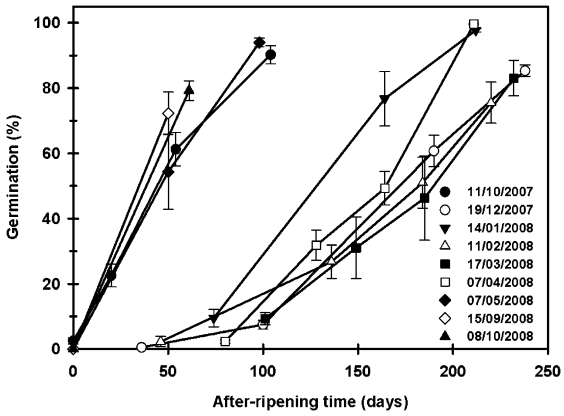

Fig. S2. Mean dry afterripening times of seeds from four independent biological replicates before burial (11/10/2007) and when recovered from the field at intervals where the time to afterripening in 50% of the population was greater than zero. Data represent the mean ± SEM (n = 4).

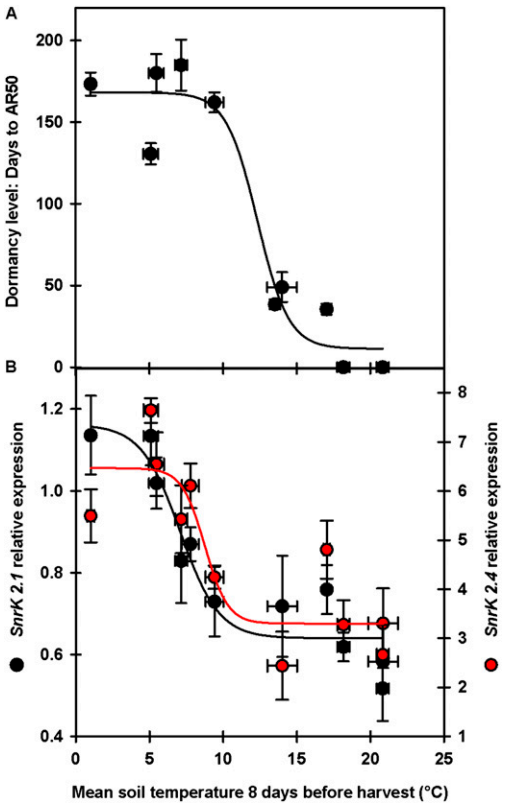

Fig. S3. The impact of soil temperature on dormancy level and the expression of *SnrK2.1* and *-2.4* in seeds recovered from the field. (*A*) Dormancy level in the field is strongly influenced by soil temperature (Fig. 1*A*). Here dormancy is seen to be strongly influenced by the cumulative effect of low and high temperature. As a result, dormancy increases as mean soil temperature decreases in the winter and decreases as mean soil temperature increases in late spring/early summer with a rapid transition zone between 9 and 14 °C. [AR50 regression curve is $y = 11.2840 + 156.8227/(1 + \exp(-(x - 12.3329)/-1.0577))$; $R^2 = 0.946$.] (*B*) A strong relationship was seen between mean soil temperature and the expression of *SnrK2.1* and *-2.4* (Figs. 1*A* and 2*B*) where the relationship is seen to be almost linear between 5 and 10 °C. [*SnrK2.1* regression curve is $y = 0.64 + 0.523/(1 + \exp(-(x - 0.708)/-1.32))$, $R^2 = 0.890$; *SnrK2.4* regression curve is $y = 3.297 + 3.171/(1 + \exp(-(x - 8.76)/-0.783))$; $R^2 = 0.776$.]

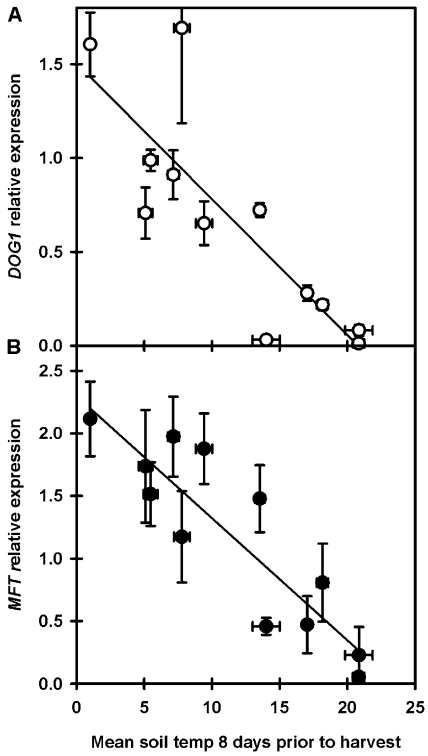

Fig. S4. The impact of soil temperature on the expression of *DOG1* and *MFT* in seeds recovered from the field. The expression of both genes followed changes in soil temperature in the field (Figs. 1*A* and 2*A*). Here (*A*) *DOG1* ($r = -0.836$) and (*B*) *MFT* ($r = -0.889$) are seen to be strongly influenced by the cumulative effect of temperature.

Table S1. qPCR primers

Gene ID	Annotation	Forward primer	Reverse primer
At5g57050	*ABI2*	TTCAGAATCTCTAAATGGCTGAAAC	AAAAGGACTAAAATCAGAGTTTCCTG
At3g24650	*ABI3*	CCTTGGAACAGGACGATGA	AAGAGAGGTTGGTGGTGGTG
At2g40220	*ABI4*	GAGATCCGAGAGCCACGTAA	GGAAGAGGAGGTGGAAGGAG
At2g36270	*ABI5*	TAATGGAGGAGGTGGTGGTG	CTGCTGCTTGTTGTTGATTGTT
At2g29090	*CYP707A2*	CAATTCCTTCTTCGCCACTC	ATGGTATGGACCTTGGTGGA
At5g45830	*DOG1*	GACGGCTACGAATCTTCAGG	AGCTAGCTGCTCCGCACTTA
At5g10140	*FLC*	GCTACTTGAACTTGTGGATAGCAA	GGAGAGGGCAGTCTCAAGGT
At1g30040	*Ga2ox2*	CTATTGGCTTCTTCTTCGGCTTG	AGTTCTTCGGCAACCATCTC
At1g15550	*Ga3ox1*	GCTACCTGTCCAACGCAAG	CGAGCCAGTGATGGTGAA
At3g05120	*GID1A*	GCTGCGAGCGATGAAGTTA	AACCCATGTATTGAGAGGAACC
At1g18100	*MFT*	GAGGCACAAATCCCTCAAGC	CCGAAAAGTACCAATATGTAACG
At3g24220	*NCED6*	TTCAAGATACCGACACTTCCTG	GGCGATTCTGCTCCATAGG
At1g77760	*NR1*	ATTCAGGCTTATACCACCAAGC	ACAATCGAGTCCTTCCTTCTCTC
At1g12110	*NRT1.1*	ACTGCGAACAAGCAAGTGG	ACCCGAAACCCGAGACAC
At2g20180	*PIL5*	CGTCACGGAGAGGATAATGAAG	AAAACCAGAGACTTCACACCAAG
At4g01026	*PYL7*	GTAAATGTCAAATCTGGTCTTCCA	TGCACAGTCAAGATCGAAGAGT
At4g17870	*PYR1*	GACGATTCGACAAACCACAA	GCGCATCTCGAAGTTTTGTT
At1g14920	*RGA2*	CGATTCGGCTTCTTCGTCTA	AGCATTTCAACCGCTTGTTT
At3g03450	*RGL2*	CTGCGTTTCCAAAGGAAGAG	GTCGGATCCTCTTGCTGCTA
At5g08590	*Snrk2.1*	TCATCCGGTTCAAGGAGGT	CGGCAGTACAGATTCGATCA
At1g10940	*Snrk2.4*	ATCTGTGATTTTGGTTATTCCAAGT	AGAAAGGACCTCAGGTGCAA
At4g36930	*SPT*	GATCTTTTAGGTCAGGTTGTCCATC	GTCATTCCAACGCAAACATAAC
At4g34270	*TIP41-like*	GTGAAAACTGTTGGAGAGAAGCAA	TCAACTGGATACCCTTTCGCA

Primer sets for the genes used to evaluate the dormancy-cycling behavior of the *Arabidopsis* ecotype Cvi in the field. See ref. 1 for *DOG1* universal primers for splice variants.

1. Bentsink L, Jowett J, Hanhart CJ, Koornneef M (2006) Cloning of DOG1, a quantitative trait locus controlling seed dormancy in Arabidopsis. *Proc Natl Acad Sci USA* 103:17042–17047.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)