Induction of 9-cis-epoxycarotenoid dioxygenase in Arabidopsis thaliana seeds enhances seed dormancy

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Contributed by Roger N. Beachy, July 25, 2011 (sent for review April 11, 2011)

Full understanding of mechanisms that control seed dormancy and germination remains elusive. Whereas it has been proposed that translational control plays a predominant role in germination, other studies suggest the importance of specific gene expression patterns in imbibed seeds. Transgenic plants were developed to permit conditional expression of a gene encoding 9-cis-epoxycarotenoid dioxygenase 6 (NCED6), a rate-limiting enzyme in abscisic acid (ABA) biosynthesis, using the edcsyne receptor-based plant gene switch system and the ligand methoxyfenozide. Induction of NCED6 during imbibition increased ABA levels more than 20-fold and was sufficient to prevent seed germination. Germination suppression was prevented by fluridone, an inhibitor of ABA biosynthesis. In another study, induction of the NCED6 gene in transgenic seeds of nondormant mutants tt3 and tt4 reestablished seed dormancy. Furthermore, inducing expression of NCED6 during seed development suppressed vivipary, precocious germination of developing seeds. These results indicate that expression of a hormone metabolism gene in seeds can be a sole determinant of dormancy. This study opens the possibility of developing a robust technology to suppress or promote seed germination through engineering pathways of hormone metabolism.

Seed germination is completed by the emergence of the embryonic tissue from the seed coat (1), and plants have evolved a number of strategies to regulate germination. Seeds of many species go through dormancy, a period during which germination is suppressed under conditions that are normally favorable for germination (2). Dormancy allows seeds to germinate in appropriate seasons or at locations suitable for seedling growth and further development.

Seed dormancy and germination are controlled primarily by the balance of abscisic acid (ABA) and gibberellin (GA) (3). ABA is involved in the induction and maintenance of seed dormancy, whereas GA releases dormancy and induces germination. ABA and GA levels are determined by the relative rates of biosynthesis and deactivation of their chemical precursors and conjugates, the levels of which are mainly controlled by gene expression (4, 5). In contrast, hormone signal transduction is considered to be posttranslationally regulated. RGA-LIKE2 (RGL2), a repressor of seed germination, is subjected to the ubiquitin-proteasome pathway after perception of GA by GIBBERELLIN INSENSITIVE 1 (GID1), a GA receptor (6, 7). ABA INSENSITIVE 5 (ABI5), a germination repressor, is in the ABA signaling cascades that is affected by RGL2 (8), is also subjected to the ubiquitin-proteasome pathway (9). Interestingly, the direct target of RGA is XERICO, an H2-type RING protein that positively affects ABA accumulation (10). Thus, there is a feedback loop to the hormone metabolism pathways that balances the biosynthesis of ABA with its degradation, the outcome of which is an important determinant of dormancy and germination of seeds.

An objective of this study was to evaluate the role of expression of genes that control ABA production in germination of seeds. Earlier work supported the conclusion that germination is largely, or solely, based on translation of stored mRNAs and on functions of preexisting proteins in a study that used inhibitors of transcription and translation (11). On the other hand, data from studies that used transcriptome analyses support the suggestion that changes in gene expression are essential for release from dormancy and induction of germination-specific processes (12, 13).

We hypothesized that changing the expression of a key gene involved in synthesis of ABA during imbibition would be sufficient to modify hormone levels and germination of seeds. We tested this hypothesis by focusing on a key regulatory step of ABA biosynthesis, namely the cleavage of 9-cis-epoxycarotenoids to produce xanthoxin, catalyzed by 9-cis-epoxycarotenoid dioxygenases (NCEDs) (14, 15). NCED genes have been isolated from many agricultural species including maize (Zea mays) (16), tomato (Solanum lycopersicum) (17), potato (S. tuberosum) (18), avocado (Persea americana) (19), and orange (Citrus sinensis) (20). In Arabidopsis thaliana, NCED6 and NCED9 are the major genes involved in ABA biosynthesis in seeds. NCED6 is expressed in the endosperm and NCED9 in the endosperm and embryo of developing seeds (14).

Genetic analyses of loss- or gain-of-function mutants in ABA biosynthesis suggested that both NCED6 and NCED9 are important for induction and maintenance of dormancy. Arabidopsis nced6 nced9 double mutants showed a decrease in seed ABA content and reduced dormancy (14). However, mutations in these genes affect seed development, and it is not clear whether dormancy/germination phenotypes are the consequence of gene expression during seed development or only during imbibition. Although expression during both stages is probably important for control of germination, it is important to separate the events of seed development and germination.

To cause expression of NCED6 in a narrow stage of development, we adopted the plant gene switch system (PGSS), a chemically induced gene expression system. PGSS is based on the edcsyne receptor (EcR) and methoxyfenozide (MOF) and is free of drawbacks of other systems (21–23). The PGSS was used to induce transcription of NCED6 or NCED9 in imbibed seeds and in developing seeds under conditions that promote vivipary, precocious germination in developing seeds. Expression in imbibed seeds increased ABA production and restricted seed germination; fluridone, an inhibitor of ABA synthesis, inhibited this process. NCED9 was less effective than NCED6 in reducing germination. Induction of NCED6 during seed development increased seed dormancy and reduced or eliminated precocious germination.

Results

**Induction of NCED6 in Imbibed Seeds.** To test the hypothesis that induction of NCED6, a gene encoding the rate-limiting ABA


The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1112151108/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1112151108

PNAS October 11, 2011 vol. 108 no. 41 17225–17229
biosynthesis enzyme 9-cis-epoxycarotenoid dioxygenase (Fig. S1), suppresses seed germination, the coding region of NCED6 was cloned to the PGSS vector containing both the gene switch construct encoding the chimeric receptor protein AGE and the inducible promoter that is responsive to AGE upon addition of MOF (Fig. 1). Whereas the original VGE effector contains the VP16 activation domain from herpes simplex virus (V) (24), AGE (25) replaces the V domain with the activation domain from the rice bZIP protein RF2a (A) (26) (Fig. 1).

Wild-type A. thaliana Columbia-0 plants were transformed with Agrobacterium harboring AGE:NCED6 under the control of the cassava vein mosaic virus promoter (Fig. 1), a strong constitutive promoter. Twenty AGE:NCED6 transgenic lines were recovered, none of which exhibited phenotypes distinguishable from wild-type plants. Homozygous lines were isolated from five independent transgenic lines based on segregation of the antibiotic resistance trait and were used for further studies.

Induction of NCED6 in transgenic plants was effected by drenching the soil one time with watering solution containing Intrepid2F to ~60 μM active ingredient MOF (Materials and Methods). Plants were generally in the 10-rosette leaf stage. Ligand application caused accumulation of transcripts of the NCED6 gene only in transgenic plants (Fig. 2A); transcripts were not detected in the absence of ligand, or in wild-type plants either in the presence or absence of ligand. These results demonstrated that the inducible AGE:NCED6 constructs functioned in the transgenic plants.

Wild-type and homozygous seeds from plant line 5-176 (Fig. S2) were imbibed for 29 h in the presence or absence of MOF, and total RNA was extracted and used for Northern blot analysis. NCED6 was observed in the AGE:NCED6 seeds treated with ligand (Fig. 2B), but not in noninduced seeds or in seeds from nontransgenic plants.

Modified Germination of Seeds That Produce NCED6. To establish the impact of ABA on germination, seeds were induced to produce NCED6 by bathing them in water containing MOF (Fig. 3A). As controls, seeds of nontransgenic plants were treated in a similar manner, and transgenic seeds were germinated in water. Germination was suppressed in transgenic seeds treated with MOF but not in AGE:NCED6 seeds in the absence of ligand or in nontransgenic seeds treated with ligand.

Germination was suppressed in many individual seed lots from the five homozygous independent transgenic lines that were imbibed in water containing MOF, with near-complete suppression of radicle emergence and absence of seedling establishment (Fig. S2). The majority (77–89%) of the induced AGE:NCED6 seeds that failed to complete germination were arrested after testa rupture (Fig. 3B, Left). The remainder of arrested seeds (11–25% of seeds) exhibited endosperm rupture, although radicles did not continue to grow (Fig. 3B, Right). These events mimic germination suppression that occurs when exogenous ABA is added during germination (27).

Fig. 1. Schematic representation of the plant gene switch system with the AGE vector used in this study. The Ecr-based PGSS is an efficient, inducible system with a unique ligand that essentially meets many of the criteria for field application; furthermore, few significant drawbacks (e.g., toxicity of the ligand) of this system have been observed. The PGSS consists of three basic components. First is a receptor protein that responds to a suitable ligand to activate gene expression. The chimeric receptor protein AGE comprises an activation domain (A) from rice bZIP protein RF2a (26) (highlighted in yellow), the DNA-binding domain of yeast GAL4 protein (G; amino acids 1–147) (41) (highlighted in green), and the ecysodyne-binding region from the Ecr from the spruce budworm C. fumiferana (E; amino acids 206–539) (42) (highlighted in pink). Second is an inducible promoter that when bound with the receptor activates expression of a hormone metabolism gene (e.g., NCED6) linked with the promoter (5XUAS). Third is a ligand that acts as an inducer, MOF (22, 46) (highlighted in orange). MOF is the active compound of Intrepid2F, which has been approved by the US Environmental Protection Agency and was used in this study. CsVM, cassava vein mosaic virus promoter.

Fig. 2. Induction of NCED6 in AGE:NCED6 transgenic lines. Expression of NCED6 was examined in WT and AGE:NCED6 transgenic rosettes after 48 h of soil drenching (A) and in seeds 29 h after imbibition (B) in the absence (−) or presence (+) of ligand. Equal loading images of ribosomal RNA (rRNA) are shown.

Fig. 3. Induced suppression of germination of AGE:NCED6 seeds. (A) Photographs showing an example of germination suppression observed in AGE:NCED6 seeds. −, uninduced; +, induced by ligand. The ligand did not affect WT seed germination. (B) Photographs of induced AGE:NCED6 seeds that were arrested after testa rupture (Left) and endosperm rupture (Right) (see text for details).
It is known that *Arabidopsis* testa is impermeable to some small molecules (11, 28); thus, it is possible that MOF does not enter seeds in advance of rupture of the testa. In contrast, *Arabidopsis* endosperm seems to be permeable to the ligand, because many seeds were arrested immediately after testa rupture (Fig. 3B); furthermore, induction of *NCED6* expression was detected at 29 h imbibition when most seeds had completed testa rupture but not radicle protrusion (Fig. 2B).

**Altered ABA Levels in Seeds.** The suppression of germination that followed expression of the *NCED6* gene was presumably caused by changes in ABA biosynthesis. This was confirmed by quantifying ABA levels in seeds using mass spectrometry. In the absence of MOF, ABA levels in both wild-type and *AGE:NCED6* seeds were relatively low and similar to each other. In contrast, following application of the ligand, there was a marked increase in ABA in *AGE:NCED6* seeds (Fig. 4). Indeed, the ABA levels in induced *AGE:NCED6* seeds (Fig. 4) were equivalent to those reported in *cyp707a2*, a mutation that results in hyperdormant seeds (29). *CYP707A2* encodes ABA 8'-hydroxylase (29), an enzyme that degrades ABA, and hyperdormancy in this line is due to an increase in the amount of ABA due to reduced amounts of the enzyme.

The degree of induction of expression of the *NCED6* gene was not established in this study, although increases in amounts of gene transcript were significant (Fig. 2). It is clear that induction of *NCED6* by the gene switch was sufficient to cause large increases in ABA levels.

**Recovery of Germination After Treatment with Fluridine.** To confirm that inhibition of germination of induced *AGE:NCED6* seeds was due to production of ABA, seeds were germinated in the presence of fluridone, a carotenoid biosynthesis inhibitor (30). Fluridone inhibits phytoene desaturase, a key enzyme in the carotenoid biosynthetic pathway (31), upstream of the role of *NCED6* in ABA biosynthesis (Fig. S1), and treating seeds with fluridone can reduce ABA synthesis (32). Seeds of plants containing the *AGE:NCED6* gene were germinated with coapplication of MOF and fluridone to determine the effect on germination. As described above, the suppression of germination of induced *AGE:NCED6* seeds was fully rescued by fluridone (Fig. 5) and seeds that were induced germinated and developed to seedlings, although they were etiolated due to the herbicidal effects of the chemical. These results support the conclusion that specific suppression of germination in seeds of *AGE:NCED6* plants induced with MOF was dependent on ABA biosynthesis.

**Suppression of Germination in Nondormant Mutant Seeds.** Transgenic lines were also developed in a nondormant line of *Arabi-

![Fig. 4.](Image) Increased ABA levels in induced *AGE:NCED6* seeds. ABA levels in WT and *AGE:NCED6* seeds incubated for 29 h in the absence (−) or presence (+) of ligand are shown. Data indicate average ± SD (*n* = 3). DW, dry weight.

**Fig. 5.** Suppression of germination in *AGE:NCED6* seeds induced by MOF and recovery of germination by 10 μM fluridone, an inhibitor of ABA biosynthesis. Percentage germination of seeds of WT and three *AGE:NCED6* lines (5-176, 8-181, and 15-195) in the absence (−) or presence (+) of ligand. Data from three experiments of 50–100 seeds per sample; average ± SD (*n* = 3).

**Suppression of Precocious Germination in Developing Seeds.** The induction experiments clearly indicate that an increased accumulation of *NCED6* transcripts and concomitant increase in ABA levels can be a determinant of germination. We also induced *NCED6* in developing seeds, focusing on its function to prevent vivipary. Preliminary experiments had indicated that the peak expression of *NCED6* was during the long-green silique stage (Fig. S5) in *Arabidopsis*. Developing siliques of the *AGE:NCED6* lines were treated or not treated with ligand under conditions that favor precocious germination. Germination from the developing seeds contained in green siliques of the *AGE:NCED6* lines was effectively suppressed by treatment with MOF, whereas those incubated held under similar conditions but without MOF underwent precocious germination (Fig. 6). Suppression of vivipary was also observed in yellow-stage siliques, that is, those that are more mature (Fig. S6), when siliques were treated with MOF. These results indicated that increasing...
NCED6 gene expression significantly alters the dormancy of developing seeds.

Discussion

The role of ABA in inducing and maintaining seed dormancy is well-known; it is also well-known that NCED proteins are rate-limiting in ABA synthesis. However, direct evidence that NCED is involved in seed dormancy remained to be established. Mutants in which ABA degradation is prevented exhibit reduced seed germination (29), indicating that a specific level of ABA is necessary to maintain seed dormancy. Constitutive overexpression of NCED1 in tomato seeds suppressed seed germination; however, constitutive overexpression increased ABA levels in many plant tissues and caused undesirable phenotypes in plant development (37, 38). To alter ABA levels to specific levels for seed dormancy requires a tighter control of expression of an NCED gene(s). In this study, conditional expression was used to control the production of NCED.

Genes encoding *A. thaliana* NCED6 and NCED9 were expressed in *A. thaliana* using the Ecr-based system and MOF was used to activate gene expression. The studies provided conclusive evidence that induction of NCED6 gene expression, and to a lesser degree NCED9, during imbibition led to high levels of ABA production, which in turn restricted seed germination. When the ABA synthesis inhibitor fluridone was administered concurrently with induction of gene expression, production of ABA was reduced and the amount of seed germination was increased. Similarly, inducing expression of NCED6 in detached siliques during seed development repressed vivipary, presumably by increasing production of ABA.

Other workers reported that inducing expression of a bean (*Phaseolus vulgaris*) NCED gene in tobacco (*Nicotiana tabacum*) seeds using the dexamethasone-inducible system resulted in incomplete suppression of germination (39). Incomplete suppression might result from expression of a heterologous NCED gene or incomplete penetration of the gene switch system that was used.

The outcomes of this study provide a foundation for developing technologies related to seed germination, for example, to reduce vivipary in agricultural species that produce partially dormant seeds. For example, preharvest sprouting can occur during wheat production if moist conditions are encountered late in the growing season (40). It is critical to understand the mechanisms of both suppression and promotion of seed germination and to develop technologies to control seed development, dormancy, and germination to reduce unwanted vivipary. Gene-switching technologies can be used to address this and similar problems in seed development.

There are multiple inducible gene expression systems other than the PGSS used in the current study. Some have been used successfully in experiments to modify seed germination (8). However, many systems use ligands that may not be readily adapted to applications in agricultural practices, such as steroid hormones or antibiotics. Although a moderate concentration of methoxyfenozide was used in these studies (a dilution of Intrepid2F to ~62 μM MOF active ingredient), other experiments conducted using *AGE:NCED6* seeds have indicated that the ligand can be diluted to 0.45 μM to suppress germination (Fig. S7). Intrepid2F has been approved by the US Environmental Protection Agency (EPA Regulation 62719-442) for field use as a receptor-specific insecticide, making use of this gene switch potentially relevant to use in agriculture. Furthermore, in addition to inducible expression, NCED6 expression during seed development can potentially be enhanced, for example by using other promoters, to cause spontaneous hyperdormancy and prevent preharvest sprouting.

Materials and Methods

Vector Construction and Plant Transformation. The coding regions of NCEDs were ligated to the restriction sites in the *AGE* gene switch vector (25). Details of NCED amplification and ligation are described in SI Materials and Methods. The AGEC vector contains the activation domain from rice bZIP protein RF2a (26), the DNA-binding domain of yeast GAL4 protein (G; amino acids 1–147) (41), and the ecdysone-binding region from the EcR from the spruce budworm *Choristoneura fumiferana* (E; amino acids 206–539) (42) (Fig. 1). The sequences in the transformation vectors were verified again (termed *AGE:NCED6* and *AGE:NCED9*). The transformation vectors were introduced into *Agrobacterium tumefaciens* strain GV3101 by electroporation, and the resulting strains were used to transform *Arabidopsis thaliana* Columbia-0 by the floral dip method (43).

Induction Experiments. For the induction of *AGE:NCED6* plants at ~10-rosette stages, diluted (10,000×) Intrepid2F (Dow AgroSciences) solution that contained 62 μM MOF as an active ingredient was applied directly to the soil in pots containing *Arabidopsis* seedlings by drenching. Seedlings were harvested 2 d after induction and frozen at ~80 °C before RNA extraction. For the induction experiments in imibed seeds, seeds were placed in 3-cm plastic Petri dishes on two layers of filter paper (no. 2; Whatman) moistened with 3.5 mL water or Intrepid2F (10,000×) and incubated at 4 °C for 3 d and at 22 °C for 29 h (gene expression analysis) or 5 d (germination tests). For germination recovery, 10 μM fluridone was included with Intrepid2F.

Fig. 6. Suppression of precocious germination in *AGE:NCED6* siliques. (A) Immature green siliques incubated for 12 d on agar media. (Upper) WT and *AGE:NCED6* lines (5-176, 8-181, and 15-133) incubated in the absence (−) or presence (+) of ligand. Note that the induced *AGE:NCED6* siliques exhibit little germination. (Lower) Representative images of precocious germination in the absence of ligand (−IP) and suppression of precocious germination in the presence of ligand (+IP) in *AGE:NCED6* siliques. (B) Results of precocious germination tests of WT and *AGE:NCED6* siliques (three independent lines, two individual plants for each line), in the absence (−) or presence (+) of ligand. Data indicate average ± SD (n = 3).
Gene Expression Analyses. Methods for gene expression analysis are described in SI Materials and Methods.

ABA Quantification. ABA was quantified at the Donald Danforth Plant Science Center Proteomics and Mass Spectrometry Facility. The method has been published previously (44), but was modified to ABA. Details are described in SI Materials and Methods.

Precocious Germination Experiments. Developing siliques at the long-green stage (Fig. 35) were collected, slightly opened at the replum-valve margin using a surgical blade, sterilized with 70% (vol/vol) ethanol for 1 min and 25% bleach for 10 min, and then plated on 0.7% (wt/vol) agar containing 1% (wt/vol) sucrose and Murashige and Skoog salt (45), with or without Intrepid2F (10,000 μM). For three independent homzygous age:NCED6 lines and wild type, 10 siliques from each of three individual plants were divided into two groups of five, which were plated in the presence or absence of ligand, respectively. Germination was examined after 12 d of incubation.

ACKNOWLEDGMENTS. We are grateful to Isabelle Debeaujon, Institut National de la Recherche Agronomique, France, for her advice on tt mutants, and Natalya Goloviznina and Theresa Nguyen, Oregon State University, for their assistance in microscopy and precocious germination experiments. This work was supported by a Fundación Séneca Fellowship (to C.-M.-A.) and National Science Foundation Grant IBN-0237562 (to H.N.).
Supporting Information

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SI Materials and Methods

Vector Construction and Plant Transformation. NCED6 and NCED9 DNA was amplified from Arabidopsis thaliana Columbia-0 genomic DNA (no introns in either gene) using gene-specific primers (NCED6 forward 5'-CATAGTGTGTCACAAGTCA-3' and reverse 5'-ACGAAGAGATTTGAGCTGT-3'; NCED9 forward 5'-CGAATGTCTACATCGGT-3' and reverse 5'-AGGTCTGCAAGGAGATG-3') and high-fidelity DNA polymerase PrimeSTAR (Takara). Using amplified fragments as templates, the coding regions were amplified with restriction enzyme sites (BstBI/ApaI for NCED6; Xhol/XmaI for NCED9). The conditions for PCR were: one cycle at 94 °C (4 min), one cycle at 90 °C (2 min), touchdown cycles (94 °C for 15 s, 72 °C → 66 °C for 15 s, and 72 °C for 30 s) (one cycle for each temperature), and 30 cycles at 94 °C (15 s), 65 °C (15 s), and 72 °C (30 s), followed by extension at 72 °C (7 min). The NCED6 and NCED9 coding regions with restriction sites were cloned to Zero Blunt TOPO vectors (Invitrogen) and verified by sequencing.

Gene Expression Analyses. For RNA gel-blot analysis, total RNA was extracted from Arabidopsis seedlings, siliques, or seeds using standard phenol-SDS extraction. Equal amounts (2 μg) of total RNA were separated on a 1.3% (wt/vol) agarose gel containing 7% (vol/vol) formaldehyde, transferred to a positively charged nylon membrane (Hybond-N+; Amersham Biosciences), and UV-cross-linked. To make the RNA probes, NCED6 cDNA in TOPO pCR 4.0 vector (Invitrogen) was transcribed using a digoxigenin-labeled NTP mixture (Roche Applied Science) and T7 RNA polymerase (Ambion). Overnight hybridization was done at 60 °C in hybridization buffer containing 50% (vol/vol) deionized formamide, 4% (wt/vol) SDS, 5× SSC, and ~100 ng/mL RNA probe followed by 15 min of prehybridization at the same temperature. The membranes were washed once for 25 min with 2× SSC and 0.1% (wt/vol) SDS at 60 °C and twice for 25 min with 0.2× SSC and 0.1% (wt/vol) SDS at 60 °C. They were then blocked for 30 min with 5% (wt/vol) nonfat milk in 0.1 M maleic acid buffer (pH 7.5) containing 0.15 M NaCl and 0.3% (vol/vol) Tween 20 (buffer A) and were incubated with alkaline phosphatase-conjugated anti-digoxigenin antibody (1:15,000 dilution) for 1 h at 25 °C. After washing with buffer A, the membranes were subjected to chemiluminescence detection. The signal was detected on X-ray film.

Abscisic Acid Quantification. Samples were ground in liquid nitrogen, and internal standards (10 mL of 2.5 mM) were added. Samples were extracted with 1.5 mL acetoniitrile/methanol (1:1 vol/vol). After lyophilization, samples were resolubilized in 200 mL of 50% MeOH. For LC separation, two monolithic C18 columns (Onyx, 4.6 mm × 100 mm; Phenomenex) with a guard cartridge were used at a flow rate of 1 mL/min. The gradient was from 40% solvent A [0.1% (vol/vol) acetic acid in MilliQ water], held for 2 min, to 100% solvent B [90% acetonitrile (vol/vol) with 0.1% acetic acid (vol/vol)] in 5 min. The LC was held at 100% B for 3 min and then ramped back to initial conditions and reequilibrated for an additional 2 min. To minimize variation from the autosampler, the sample loop was overfilled with 52 mL of sample and the sample storage temperature was set to 8 °C. The LC-MS/MS system was composed of a Shimadzu LC system with a LEAP CTC PAL autosampler coupled to an Applied Biosystems 4000 QTRAP mass spectrometer equipped with a Turbolon Spray (TIS) electrospray ion source. Source parameters were set to: CUR (curtain gas): 25; GS1 (ion source gas 1): 50; GS2: 50 (arbitrary unit); CAD (collision gas): high; IHE (interface heater): on; TEM (temperature): 550 °C; IS (ionspray voltage): ~4,500. Both quadrupoles (Q1 and Q3) were set to unit resolution. Analyst software (AB Sciex, version 1.4.2) was used to control sample acquisition and data analysis. To maximize sensitivity, abscisic acid (ABA) standard solutions were infused into the 4000 QTRAP with a syringe pump (no. 22; Harvard) at 10 mL/min to select multiple reaction-monitoring (MRM) transitions and optimize compound-dependent parameters for MRM detection. A standard curve was established for the method. For quantitation, a series of standards was prepared containing different concentrations of ABA mixed with deuterium-labeled ABA (250 pmol/sample). Correction factors were obtained by adjusting the ratio of standard peak areas to that of internal standards in all samples. The peak areas of endogenous ABA were normalized with the corresponding internal standard and then calculated according to the standard curve.
Fig. S1. Schematic representation of ABA biosynthesis and deactivation pathways. The figure is not comprehensive but highlights the key pathway related to the genes and active hormone focused on in this work (highlighted in black). A single arrow does not represent a single reaction. │ indicates the site of inhibition in the carotenoid biosynthesis by fluridone. The figure is modified from ref. 1. GGDP, geranylgeranyl diphosphate. See main text for gene names.


Fig. S2. Suppression of seed germination induced by ligand in multiple seed lots of AGE:NCED6 homozygous lines. −, uninduced; +, induced with ligand. WT, wild-type Col-0. Data indicate average ± SD (n = 3).

Fig. S3. Suppression of seed germination induced by ligand in AGE:NCED6 homozygous lines constructed in the transparent testa (tt) mutant. −, uninduced; +, induced with ligand. tt3 and tt4: control tt3 and tt4 mutant seeds without AGE:NCED6. Data indicate average ± SD (n = 3).
Fig. S4. Suppression of seed germination induced by ligand in multiple seed lots of *AGE:NCED9* homozygous lines. −, uninduced; +, induced with ligand. Data indicate average ± SD (*n* = 3).

Fig. S5. Stage specificity of *NCED6* expression during *Arabidopsis* seed development. RNA was extracted from siliques at stage I (green siliques < 0.5 cm), II (green siliques > 1.0 cm), III (yellow fresh siliques), and IV (brown dry siliques). *NCED6* peaked around stage II. Equal loading images of ribosomal RNA (rRNA) are shown.

Fig. S6. Suppression of precocious germination in *AGE:NCED6* yellow siliques. Results of precocious germination tests of WT and *AGE:NCED6* siliques (three independent lines, two individual plants for each line), in the absence (−) or presence (+) of ligand. Data indicate average ± SD (*n* = 3).
Fig. S7. Inhibition of germination of *AGE:NCED6* seeds in different concentrations of methoxyfenozide.