

RESEARCH PAPER



GA signaling is essential for the embryo-to-seedling transition during Arabidopsis seed germination, a ghost story

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ABSTRACT

The plant hormone gibberellin (GA) stimulates developmental transitions including seed germination, flowering, and the transition from juvenile to adult growth stage. This study provided evidence that GA and the GA receptor *GID1* (*GA-INSENSITIVE DWARF1*) are also needed for the embryo-to-seedling transition in Arabidopsis. The *ga1-3* GA biosynthesis mutant fails to germinate unless GA is applied, whereas the *gid1abc* triple mutant fails to germinate because it cannot perceive endogenous or applied GA. Overexpression of the *GID1a*, *GID1b*, and *GID1c* GA receptors rescued the germination of a small percentage of *ga1-3* seeds without GA application, and this rescue was improved by dormancy-breaking treatments, after-ripening and cold stratification. While *GID1* overexpression stimulated *ga1-3* seed germination, this germination was aberrant suggesting incomplete rescue of the germination process. Cotyledons emerged before the radicle, and the resulting “ghost” seedlings failed to develop a primary root, lost green coloration, and eventually died. The development of *ga1-3* seedlings overexpressing *GID1* was rescued by pre-germinative but not post-germinative GA application. Since the *gid1abc* mutant also exhibited a ghost phenotype after germination was rescued by cutting the seed coat, we concluded that both GA and *GID1* are needed for the embryo-to-seedling transition prior to emergence from the seed coat.

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Introduction

Plant embryos survive for long periods of time in a quiescent and developmentally arrested state in dry seeds (reviewed by Bewley et al.¹). This allows their prolonged storage for use in propagating crop plants. This state of suspended animation must be reversed during germination. The term germination in the strict sense refers to the event when the embryonic root or radicle emerges from the seed coat. However, the germination process begins with water uptake, and involves many steps needed to reactivate metabolism and commence seedling development. These processes include cellular and DNA repair, the initiation of respiration, the initiation of RNA transcription and protein translation, the induction of hydrolytic enzymes for the mobilization of stored reserves, loosening of barrier tissues, and cell expansion culminating in radicle emergence. Radical emergence results from enzymatic weakening of the seed coat coupled with the internal force generated by the elongation of the embryonic axis. Given the complexity of the process, many different defects could potentially prevent germination, especially in aged seeds. Once germinated, a seedling must proceed through all critical developmental stages associated with growth, flowering, and fertility to complete its life cycle and ensure the next generation of viable seeds.

Seed dormancy can prevent the germination of viable seeds under favorable environmental conditions (reviewed in Finkelstein et al.²). Dormancy prevents germination out of season and ensures species survival of natural catastrophes as seeds

in the soil. The seeds of many temperate species are dormant at maturity, then acquire the ability to germinate through dormancy-breaking processes including after-ripening (a period of dry storage) and cold imbibition of water (also called cold stratification or moist chilling). The length of time required for dormancy loss through dry after-ripening is genetically determined. This together with transcriptomic studies suggests that measurable differences in regulatory factors control the change in germination potential with after-ripening of dry seeds.^{3,4} The plant hormone abscisic acid (ABA) induces dormancy during seed maturation and maintains dormancy in mature seeds (reviewed in Finkelstein et al.²), whereas the plant hormone gibberellin A (GA) stimulates germination and has been implicated in dormancy loss through after-ripening and cold stratification.^{5–7} Seeds of GA biosynthetic mutants fail to germinate unless GA is applied in Arabidopsis and tomato.^{8,9}

The Arabidopsis *GAI* gene encodes copalyl synthase, an early enzyme needed for GA biosynthesis.^{9,10} Mutations in this and other biosynthesis genes cause lack of *de novo* GA biosynthesis and GA-rescued phenotypes, including failure to germinate, dwarfism, delay in or failure to flower, and infertility.^{9,11} The *ga1-3* allele is a 5-kb deletion, resulting in a null allele with trace levels of GA hormone.^{12,13} These phenotypes reveal that GA stimulates germination, cell division and elongation, and flower development. GA also stimulates many developmental events in the plant life cycle including embryo development, the transition from embryo

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to seedling development, the development of leaf primordia in the shoot apical meristem, and the juvenile to adult phase transition.^{14–18}

GA stimulates plant growth and development by targeting negative regulators of GA responses, called DELLA (Asp-Glu-Leu-Leu-Ala) proteins, for destruction by the ubiquitin-proteasome pathway.^{19–21} GA signaling in Arabidopsis requires GA biosynthesis, the three GA receptors (GID1a, GID1b, and GID1c), and the F-box protein SLY1 (SLEEPY1) for proteolytic DELLA destruction (reviewed in Hauvermale et al.²²). GA binding to the GID1 receptor causes a conformational change in GID1 allowing the receptor to bind DELLA protein. The SLY1 F-box protein recognizes and binds DELLA when in complex with GID1. Thus, GA and GID1 stimulate DELLA polyubiquitination by the SCF^{SLY1} E3 ubiquitin ligase, leading to DELLA destruction by the 26S proteasome pathway.^{19,23,24} This lifts DELLA repression of GA responses including seed germination. Loss of GA perception in the *gid1abc* triple mutant and loss of GA signaling in the *sly1* mutant lead to similar phenotypes as those seen in the *gal-3* mutant, except that these phenotypes cannot be rescued by GA application.^{25,26,27} The failure of germination in *gid1abc* and *sly1* mutants can only be rescued by artificially removing the seed coat.

Previous work showed that overexpression of the three GA receptors in the GA biosynthesis mutant *gal-3* increased sensitivity to GA stimulation of seed germination.⁵ This effect was stronger when seeds first experienced the dormancy-breaking treatments of cold stratification and dry after-ripening. Subsequently, we made the anecdotal observation that *gal-3* seeds in which HA-tagged *GID1a*, *GID1b*, or *GID1c* were overexpressed on the 35 promoter (*HA:GID1-OE*) were able to germinate at a very low efficiency. This phenomenon of germination in a GA-deficient mutant was not noticed initially because the resulting seedlings were small and failed to grow. This study characterized *GID1* rescue of *gal-3* germination, and the post-germination developmental arrest we have termed the *gal-3* “ghost” phenotype.

Materials and methods

Germplasm

Genotypes included in this study were wild-type (Landsberg *erecta* or *Ler*), *gal-3*, and *gal-3 HA:GID1a-OE*, *gal-3 HA:GID1b-OE*, and *gal-3 HA:GID1c-OE*, as previously described.^{9,28,29,5} The *GID1-OE* overexpression lines carry the *HA:GID1(a-c)* translational fusion constructs overexpressed on the Cauliflower Mosaic Virus 35S promoter. The *gid1abc* triple mutant in the Columbia (Col) ecotype is fully infertile. It is maintained as a *gid1a* heterozygote with the genotype *gid1b-1/gid1b-1 gid1c-2/gid1c-2 gid1a-1/+*.²⁵

Growth conditions

Seeds were plated on MS-agar (0.5x Murishige and Skoog salts, 0.8% agar) plates buffered with MES pH 5.5 (2-(N-morpholino) ethanesulfonic acid, Sigma-Aldrich), subjected to 3 days of cold stratification at 4°C to obtain uniform germination. After cold stratification, plates were imbibed in the light at 22°C until they reached the 4 leaf stage, at which time they were transferred to soil

for propagation. To promote germination, *gal-3* seeds were plated in the presence of 10 μM GA₃. Plants were grown in potting soil in Conviron™ growth chambers as in¹⁹. Both *gal-3* and *gal-3 HA:GID1* overexpression lines were sprayed once a week with 10 μM GA₃ to rescue fertility until they reached the green silique stage. Mature seeds were stored in open tubes for 2 weeks at room temperature, then stored in closed tubes either at room temperature for after-ripening or at –20°C to preserve dormancy.

Germination assays

For germination experiments evaluating the effects of GA and dormancy breaking treatments on seed germination in *gal-3* and *gal-3 HA:GID1-OE* lines, three biological replicates of at least 100 seeds each were surface sterilized with 10% bleach/0.01% SDS for 10 minutes, washed with sterile water, and then plated on MS-agar plates supplemented with the indicated concentration of GA₄. Stocks of 10 mM GA₄ (Sigma) were solubilized in 70% ethanol and stored at –20°C until use. Plates were either cold stratified (imbibed at 4°C for 3 days) and then imbibed in the light at 22°C or placed directly into the light without cold stratification. Seed germination was scored daily over 9 days. The mean percent germination was calculated using three biological replicates.

For characterization of the ghost-like phenotype in *gid1abc* triple mutant seedlings, three biological replicates of at least 100 seeds each were surface sterilized with 10% bleach/0.01% SDS for 10 minutes and plated on MS-agar plates. Plates were cold stratified at 4°C for 3 days and then imbibed in the light at 22°C. Seed germination was monitored over the course of 9 days and then seedling development was tracked for an additional 12 days.

Table 1. The effects of *HA:GID1-OE* on *gal-3* germination with cold and after-ripening.

Genotype	¹ Treatment	² Percent Germination with after-ripening			
		2 wk	8 wk	24 wk	52 wk
<i>Ler</i>	NC	100	100	100	100
	C	100	100	100	100
<i>gal-3</i>	NC	0.0	0.3	0.0	0.0
	C	0.0	0.5	1.3	2.0
<i>gal-3</i> + 1 μM GA ₄	NC	98.0	98.0	99.0	100
	C	99.0	98.5	99.0	100
<i>HA:GID1a-OE</i>	NC	0.0	1.5	2.9	4.0
	C	1.3	3.8	3.8	4.0
<i>HA:GID1b-OE</i>	NC	0.0	0.7	6.7	6.0
	C	3.8	9.4	7.0	6.0
<i>HA:GID1c-OE</i>	NC	0.0	1.3	5.1	6.0
	C	0.6	2.8	5.2	6.0

¹C = with cold stratification, NC = without cold stratification.

²For each condition, 3 biological replicates and n = 100. After-ripening time in weeks (wk).

Table 2. An ANOVA summary from a Tukey's pairwise comparison determining significant interactions among and between genotypes, treatments (cold or no cold), and after-ripening.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Genotype (Geno)	3	31.18774071	10.39591357	110.61	<0.0001
Treatment (Treat)	1	10.24538460	10.24538460	109.01	<0.0001
After-ripening (AR)	3	31.72070122	10.57356707	112.50	<0.0001
Rep	2	0.29601524	0.14800762	1.57	0.2152
Geno*Treat	3	2.28134864	0.76044955	2.88	0.0406
Geno*AR	9	4.79955619	0.53328402	2.12	0.0376
Geno*Treat*AR	24	18.34487438	0.76436977	8.13	<0.0001

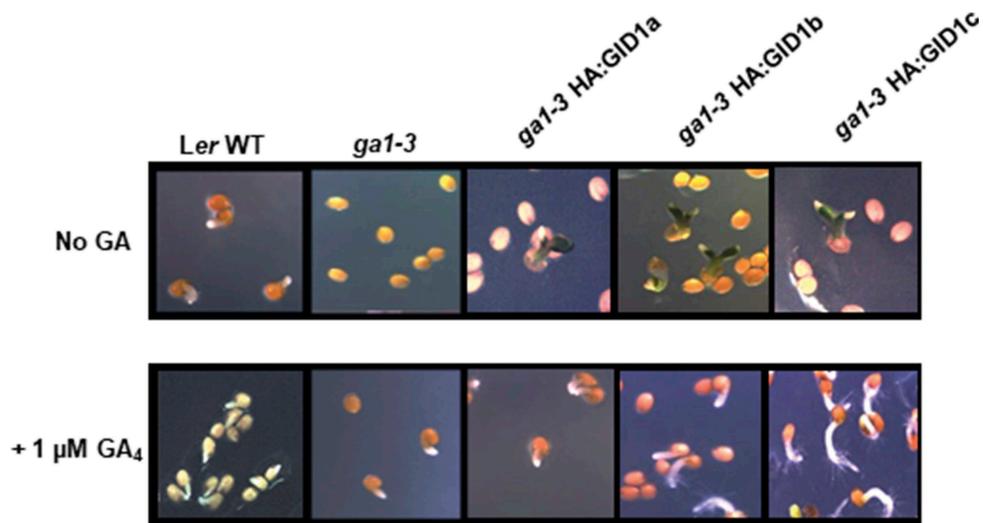


Figure 1. *ga1-3 HA:GID1-OE* germination resulted in abnormal seedlings. Normal germination of *Ler* and of *ga1-3* in the presence of 1 μM GA_4 (radicle emergence first), is contrasted with abnormal germination of *ga1-3 GID1-OE* seeds in the absence of GA. *ga1-3 GID1-OE* seeds germinated cotyledons first, and cotyledons had white tips. This “ghost” phenotype was rescued in *ga1-3 GID1-OE* lines when seeds were imbibed in the presence of 1 μM GA_4 .

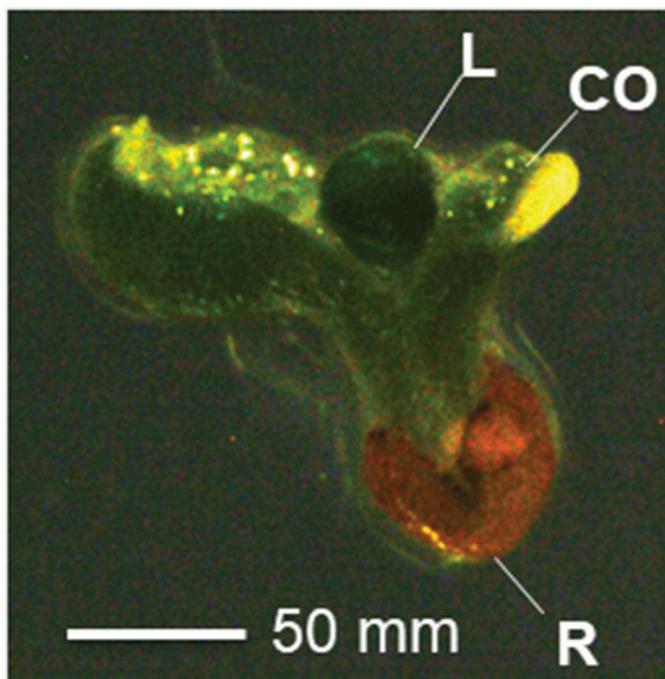


Figure 2. An abnormal “ghost” *ga1-3 HA:GID1b-OE* seedling. In the absence of GA, *ga1-3 HA:GID1-OE* seeds germinated with cotyledons (CO) emerging before the radicle/root (r). The resulting seedlings showed failure in hypocotyl elongation and cotyledon expansion, although first leaves (l) did emerge. These seedlings eventually died. Scale bar = 50 mm.

GA treatment of *ga1-3 HA:GID1-OE* seedlings

ga1-3 HA:GID1-OE seeds that germinated in the absence of GA were transferred to MS-agar plates containing 1 μM GA_4 where indicated. Alternatively, 1 μM GA_4 was added to MS-agar plates containing ungerminated *ga1-3* and *ga1-3 HA:GID1-OE* seeds. All plates were placed in the light at 22°C for an additional 12 days. Seed germination and seedling growth were monitored daily.

GA dose-response curves of *ga1-3 HA:GID1-OE* lines

To evaluate the minimum amount of GA_4 needed to rescue the ghost phenotype, *ga1-3 HA:GID1-OE* seeds were after-ripened for 2 months, then plated in the presence of 0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, or 1 μM GA_4 . The 0 μM GA_4 MS-agar plates contained 70% ethanol at the maximum concentration used in the dose-response curve to serve as a “mock” control for the effect of the solvent. Up to 5000 seeds from each *ga1-3 HA:GID1-OE* were screened to ensure a total of 100 seedlings for each condition. Seeds were surface sterilized with 10% bleach/0.01% SDS for 10 minutes and plated on MS agar plates with or without GA_4 . Plates were cold stratified for 3 days at 4°C and then placed in the light at 22°C. Seed germination and seedling growth were monitored daily for a total of 12 days.

Micrographs

All seedling photographs were taken with a Leica MZ6 light microscope with 8X magnification using the Infinity1 camera and Infinity Capture software (Luminera).

Statistical analysis

For all germination experiments, data was transformed using a square root transformation. For both germination and GA dose-response experiments, statistical significance was assessed using an analysis of variance (ANOVA) with a Tukey’s all pairwise comparison (SAS v9.4).

Results

GID1 overexpression, after-ripening, and cold stratification stimulate *ga1-3* seed germination without GA

To characterize the *HA:GID1-OE* rescue of *ga1-3* germination, the effect of overexpression without and with the dormancy-breaking treatments of dry after-ripening and cold-stratification was

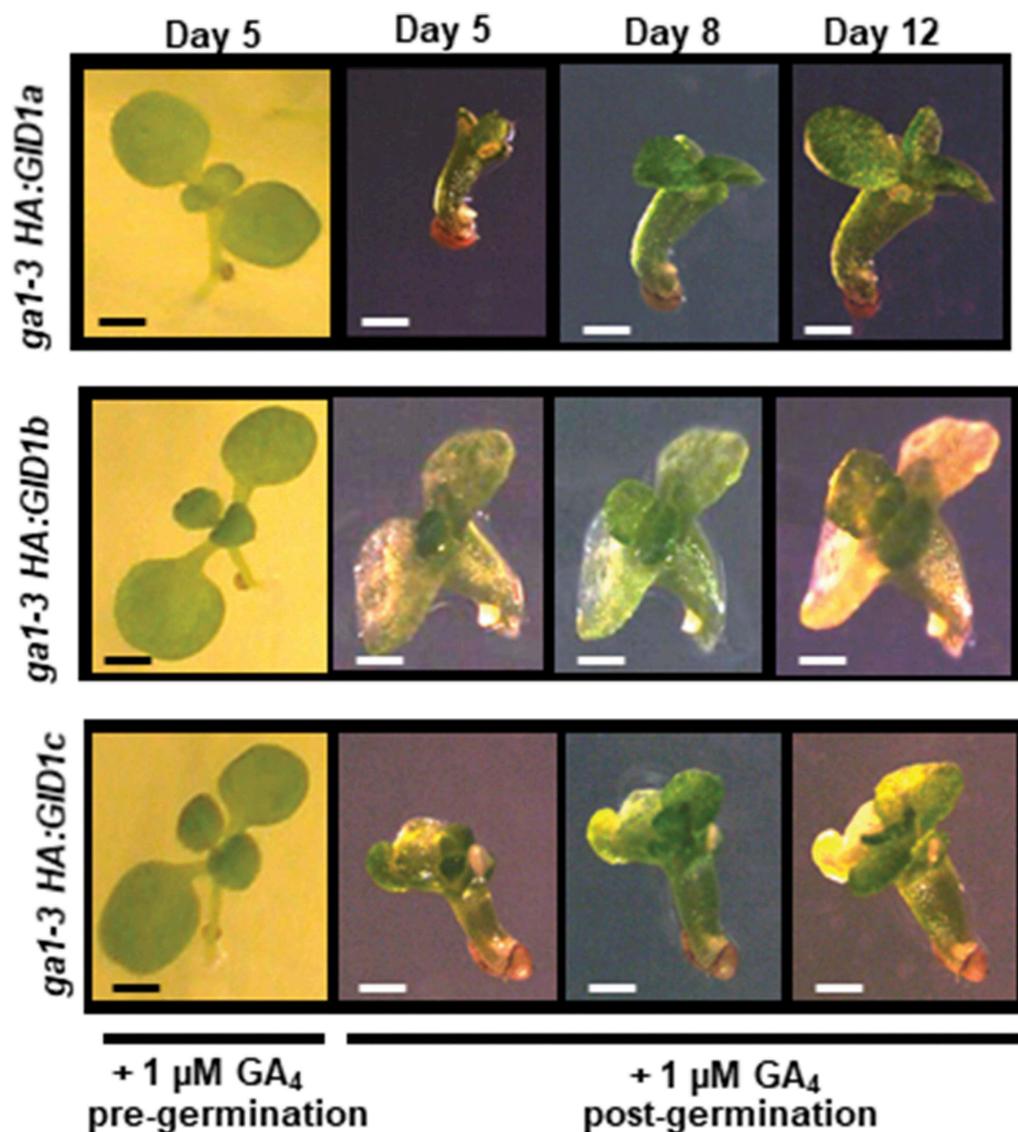


Figure 3. Post-germinative GA application failed to rescue *ga1-3 HA:GID1-OE* seedling development. When germinated in the presence of 1 μM GA_4 *ga1-3 HA:GID1-OE* lines developed a normal seedling phenotype. When GA_4 was added after germination seedling growth and development was abnormal, and seedlings gradually lost green coloration and died. Black or white scale bars = 50 μm .

examined. An after-ripening time course was conducted for seeds of wild-type (WT) Ler, *ga1-3*, *ga1-3 HA:GID1a-OE*, *ga1-3 HA:GID1b-OE*, and *ga1-3 HA:GID1b-OE* followed by germination at 22°C for 9 days without GA hormone. Germination was examined with and without cold stratification for 3 days at 4°C before incubation at 22°C. Germination of *ga1-3 HA:GID1-OE* seeds occurred within 5–9 days of imbibition in the light at 22°C while Ler WT germinated within 24 hrs (Figure S1). The combination of long after-ripening and cold stratification resulted in no more than 2% germination in untransformed *ga1-3*, whereas *GID1* gene overexpression resulted in 1.3 to 9.4% germination ($n = 100$; Table 1).

Based on an ANOVA with Tukey's pairwise comparison, each *GID1* overexpression construct significantly stimulated *ga1-3* germination, and this effect was enhanced by both after-ripening and cold stratification (Table 1; Table 2). At 2 and 8 weeks (wk) of after-ripening, *GID1* gene overexpression only stimulated *ga1-3* germination when the seeds were cold

stratified. *GID1* overexpression significantly stimulated *ga1-3* germination both with and without cold stratification when seeds had been after-ripened for 24 and 52 wk ($p < .0001$; Table 1; Figure S2). The strongest stimulation of germination was seen with *GID1b* overexpression when seed dormancy was broken through 8 wk of after-ripening in concert with cold stratification. Interestingly, the *ga1-3 HA:GID1b-OE* germination increased with 8 wk after-ripening, then decreased with longer 24 and 52 wk of after-ripening (Figure S2).

Germination of *ga1-3* without GA results in aberrant seedling development that cannot be rescued by post-germination GA application

While *GID1* overexpression did allow *ga1-3* seeds to germinate at a low frequency in the absence of GA, the resulting seedlings failed to develop normally. During wild-type Ler germination, seedlings emerged radicle first, then the cotyledons expanded, the radicle

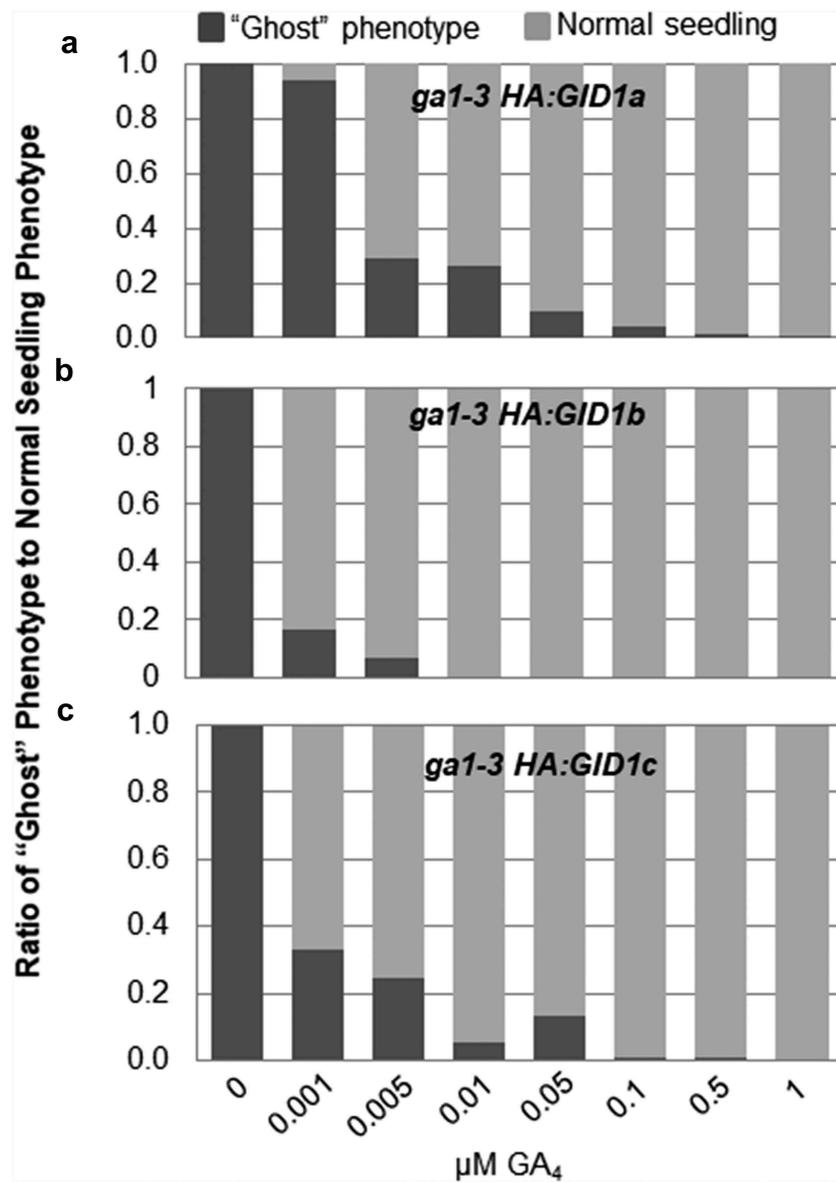


Figure 4. The ghost seedling phenotype is rescued by GA in a dose-dependent manner. Two month after-ripened (a) *ga1-3 HA:GID1a-OE*, (b) *ga1-3 HA:GID1b-OE*, and (c) *ga1-3 HA:GID1c-OE* seeds were plated without GA₄ or with the indicated GA₄ concentration. Plates were cold stratified for 3 days in the dark, and then moved to 22°C in the light. The ratio of “ghosts” (dark gray) to normal seedlings (light gray) is shown.

elongated into a root, and the first leaves developed (Figure 1). The *ga1-3* and *ga1-3 HA:GID1-OE* seedlings emerged cotyledons-first rather than radicle first. Thus, it appeared that cotyledon expansion rather than radicle elongation generated the force needed to emerge from the seed coat. After germination, the cotyledons did not expand normally, and the radicle and hypocotyl did not elongate (Figure 2). Many of these abnormal seedlings had white-tipped cotyledons that never became green. The radicles remained small, resembling those of an embryo (Figure 3). Eventually, these seedlings lost their green coloration, became translucent, and died. Thus, we refer to this as a “ghost” seedling phenotype. To summarize, *GID1* overexpression rescued *ga1-3* germination in the strict sense of emerging from the seed coat but was unable to fully rescue the germination process since seedlings were unable to complete the embryo-to-seedling transition.

To determine whether GA application could rescue the germination and abnormal growth of the *ga1-3 HA:GID1-OE*

overexpression lines, seeds and seedlings were transferred to plates containing 1 μM GA₄. GA treatment fully rescued germination and normal post-germinative growth of the seeds that failed to germinate after imbibing for 10 days at 22°C in the absence of GA (Figure 1; Figure 3). In contrast, ghost *ga1-3 HA:GID1-OE* seedlings that germinated in the absence of GA, failed to recover after 5–12 days of incubation in the light in the presence of GA (Figure 3). Although the true leaves of *ga1-3 HA:GID1-OE* ghost seedlings began to grow, there was no additional development of the primary root, and seedling death associated with cotyledon bleaching occurred within 10 to 14 days after germination. These results suggest that GA hormone is needed prior to germination to stimulate the transition from embryo to seedling development.

Next a GA dose-response experiment was used to determine the GA₄ concentration required during germination to rescue the post-germinative seedling development of *ga1-3 HA:GID1a-OE*,

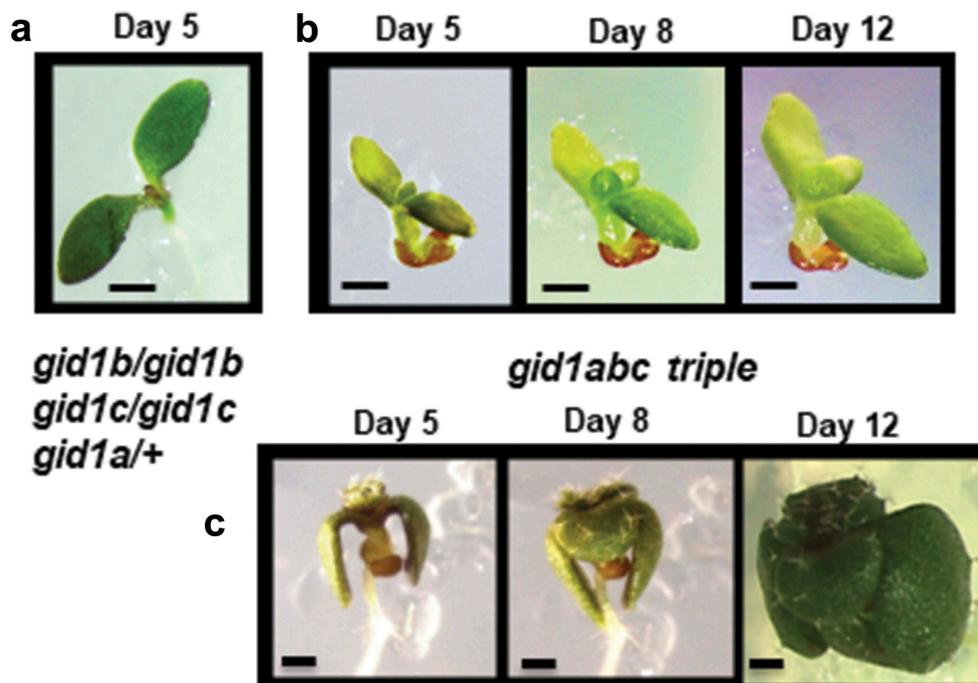


Figure 5. Ghost phenotype in a *gid1abc* triple mutant seedling. (a) A *gid1b/gid1b c/c a/+* heterozygous seedlings displayed a wild-type phenotype, whereas (b) the homozygous *gid1abc* triple mutant displayed a ghost-seedling phenotype similar to *gal-3 HA:GID1-OE* lines, including cotyledon-first germination, the failure of root elongation, and loss of green coloration in the cotyledons and first leaves. (c) A small percentage of *gid1abc* triple mutant completed the embryo-to-seedling transition, developing roots and shoots. Bar = 50 mm.

gal-3 HA:GID1b-OE, and *gal-3 HA:GID1c-OE*. These seeds were after-ripened for 2 months, then cold stratified before germinating on GA.⁵ Seeds were imbibed for 12 days on MS-agar containing increasing concentrations of GA₄ and 100 of the resulting seedlings were scored for normal versus ghost seedling phenotype. Three replicates of 100 germinated seeds each were examined for each GA concentration (Figure 4). Note that since *gal-3 HA:GID1c-OE* had 2% germination without GA, it was necessary to imbibe 5000 seeds to obtain 100 germinated seedlings. It appeared that the concentration of GA needed to exceed 50% germination under these conditions was also sufficient to rescue the ghost phenotype, 0.01 μM for *gal-3 HA:GID1b-OE* and 0.1 μM for *gal-3 HA:GID1a-OE* and *gal-3 HA:GID1c-OE*. The *gal-3 HA:GID1b-OE* line was most responsive and the *gal-3 HA:GID1a-OE* line least responsive to GA rescue of the ghost seedling phenotype. The minimum GA concentration at which ghosts were still observed was 0.005 μM for *gal-3 HA:GID1b-OE*, 0.05 μM for *gal-3 HA:GID1c-OE*, and 0.1 μM for *gal-3 HA:GID1a-OE*. When the ghost phenotype was observed over 12 days of imbibition at 0.005 μM GA₄, it was observed the *gal-3 HA:GID1b-OE* ghost seedlings were able to develop a short root before dying, suggesting that a partial rescue was possible (Figure S3).

Germination of the *gid1abc* triple mutant frequently results in a ghost-like seedling

If GA signaling is required for seedling growth and development, then we would expect a strong GA-insensitive mutant to exhibit a ghost phenotype like that observed in *gal-3*. The *gid1abc* triple mutant is infertile and cannot germinate unless the seed coat is cut. Thus, the *gid1abc* triple is maintained as

a *gid1a* heterozygote with the genotype, *gid1b/gid1b gid1c/gid1c gid1a/+* (Figure 5(a)).²⁵ The progeny of the heterozygote segregated 17% ungerminated seeds. It may be that this is slightly less than the expected 25% because *gid1a* and *gid1b* are about 22 Mb apart on Arabidopsis chromosome 3. In order to examine the post-germinative growth of the *gid1abc* homozygous triple mutant, seed coats of 63 ungerminated seeds were nicked with a sharp pair of dissection tweezers. All of the nicked seeds produced seedlings, of which only 4 produced “normal” seedlings exhibiting the GA dwarf phenotype, dark green cotyledons and true leaves, and elongated roots (Figure 5(c)). The remaining seedlings arrested in seedling development and resembled the *gal-3* ghost seedlings (Figure 5(b)). These seedlings failed to produce an elongated root, became glassy or translucent, lost their green coloration, and died without establishing seedling growth on MS-agar. Thus, 93.7% of *gid1abc* triple mutants exhibited a ghost seedling phenotype when seed germination was forced by cutting the seed coat. This indicates that GA perception by *GID1* is needed for efficient transition from embryo to seedling development during the germination process.

Discussion

This study provided evidence that GA hormone functions prior to seedling emergence to stimulate the embryo-to-seedling developmental transition. Previous work suggested that GA is mainly needed to overcome the seed coat barrier during germination since *gal-3*, *sly1-2*, and *gid1abc* seed germination can be rescued by testa weakening or removal of the seed coat.^{23,25,30–32} However, absence of *GID1a*,

GID1b, and *GID1c* in *gid1abc* led to an inability to efficiently transition to seedling development after the seed coat was cut (Figure 5). Thus, GA signaling is required for this transition. Moreover, exogenous GA application could only rescue *gal-3 HA:GID1-OE* seedling growth and development if applied before seedling emergence from the seed coat. Thus, GA signaling stimulates the embryo-to-seedling transition prior to germination in the strict sense. This suggests that GA may play a critical role in reactivating metabolism and/or development during the germination process before germination in the strict sense. Future work will need to investigate which essential processes needed during germination fail during ghost seedling germination.

Overexpression of *GID1a*, *GID1b*, and *GID1c* was not expected to rescue *gal-3* seed germination because GA receptors were not expected to function in the absence of GA biosynthesis. Surprisingly, we found that *GID1* gene overexpression led to a low level of seed germination in *gal-3*, in the absence of GA biosynthesis and application. This suggests either that *GID1* proteins have some limited ability to function in germination without GA, or that the residual GA levels in *gal-3* seeds are sufficient to trigger some GA signaling and germination. It is known that *GID1* gene overexpression has no negative effect on the embryo-to-seedling transition since wild-type *Ler* transformed with *HA:GID1-OE* constructs complete normal plant development without arresting in the embryo-to-seedling transition.²⁹

While Arabidopsis *GID1a* and *GID1b* depend on GA-binding to interact with and target DELLA proteins for destruction, *GID1b* protein can bind DELLA protein with low affinity without GA.³³ GA enhances *GID1b* binding to DELLA. This GA-independent function may be why *GID1b* overexpression results in both the strongest increase in GA sensitivity during germination and in the strongest rescue of *gal-3* and *sly1-2* seed germination (Figure 3).⁵ However, we cannot rule out the possibility that *GID1* proteins have functions that occur in the absence of GA. That *GID1b* overexpression stimulates germination in a GA-deficient mutant is interesting given that *gid1* double mutant analysis revealed that *GID1b* can act as a negative regulator of seed germination, especially in the dark.³⁴ This suggests that the role of *GID1b* in regulating germination can depend on environmental context.

While the *gal-3 HA:GID1-OE* ghost seedlings were able to emerge from the seed coat, this germination was aberrant in that cotyledon expansion, not radicle elongation, provided the force needed to fracture the seed coat. Similar cases of cotyledon-first germination have been observed. The GA-insensitive *sly1* F-box mutants fail to germinate unless the seed coat is cut.²³ After nicking the *sly1* seed coat, it was observed that the seedlings germinated cotyledons first and that primary root elongation was either absent or delayed. The resulting *sly1* seedlings often have problems becoming established on soil, suggesting these seedlings may have difficulties with the embryo-to-seedling transition similar to, but milder than those of the *gid1abc* triple mutant. Cotyledon-first germination was observed when *gal-3* germination was partly rescued by mutations in DELLA genes, negative regulators of GA signaling. Penfield et al.³⁵ showed that higher dormancy was associated with smaller cotyledons in multiple dormant

Arabidopsis backgrounds, including *gal-3*, rescued to varying degrees by mutations in the five DELLA genes. This suggested that dormancy loss stimulates germination through increased cotyledon expansion. If DELLA-imposed dormancy blocks cotyledon expansion, then this suggests that *GID1* gene overexpression may partly lift DELLA-imposed dormancy allowing cotyledon expansion to break open the seed coat. Moreover, this might explain why dormancy-breaking treatments of cold stratification and after-ripening enhanced the cotyledon-first germination of *gal-3 HA:GID1-OE* lines. Previous work showed that increasing GA sensitivity with after-ripening is associated with increased accumulation of *GID1* proteins, suggesting that *GID1* plays a role in increasing germination potential.⁵

The ghost phenotype could be caused by many possible flaws in development, such as failure to initiate photosynthesis, cell division, or failure in shoot or root development. GA stimulates many aspects of plant development that might be needed for the embryo-to-seedling transition, including the transition to leaf development in the shoot apical meristem,³⁶ the mobilization of stored reserves during the embryo-to-seedling transition,³⁷ and stimulation of root meristem enlargement and root growth.³⁸ Aberrations in any of these functions may have caused the ghost phenotype. Future work will need to investigate the precise mechanisms causing the *gal-3* ghost phenotype.

Several lines of evidence suggest that GA signaling may be needed to trigger auxin-stimulated root growth and development during germination. Weaker alleles of the Arabidopsis auxin-insensitive mutant *monopteros* showed a failure in seedling root development similar to *gal-3* ghosts and reminiscent of the phenotypes observed when auxin transport is inhibited.³⁹ Interestingly, GA appears to stimulate root responsiveness to indole-3-acetic acid (IAA).^{40,41} Moreover, there appears to be a connection between GA and control of auxin transport.^{42,43} Previous work suggested that GA stimulates root gravitropism based on the fact that asymmetric GA localization in roots stimulated asymmetric localization of PIN2 auxin transporters by controlling PIN2 protein stability. Future work will need to examine if the ghost phenotype is associated with either defects in auxin transport or auxin-regulated vascular development.

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