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RESEARCH PAPER

Phytohormone signalling pathways interact with sugars during seed germination and seedling development

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Abstract

Exogenous glucose delays seed germination in *Arabidopsis thaliana* not only in wild type (WT), but also in a number of mutants in hormone signalling pathways. This study demonstrates that the *ABA Insensitive 3* (*ABI3*) gene in the ABA signalling pathway and the *RGA-like 2* (*RGL2*) and *SPINDLY* (*SPY*) genes in the GA signalling pathways all play important roles in the glucose-induced delay of seed germination. Transcription of the *ABI3* and *RGL2* genes is up-regulated by glucose. This study also supports the idea that different sugars such as the hexose stereoisomers, glucose, and mannose, delay or inhibit seed germination via different branches of the hormone signalling pathways. Analysis of post-germination seedling development of wild-type plants indicates that exogenous glucose supplied after germination may have a concentration-dependent stimulatory effect on root and shoot growth. Comparison of WT and *spy* seedling growth on different glucose concentrations suggests that the stimulatory effect of glucose is partially exerted via the GA or cytokinin signalling pathways. The effects of glucose on plant growth and development may be stimulatory or inhibitory depending on the developmental stage. The inhibitory effect on seed germination seems to be accomplished via the activation of the ABA signalling pathway, through ABI3, and inactivation of the GA signalling pathway through RGL2 and SPY. On the other hand, the stimulatory effect of glucose on seedling growth may involve the GA and/or cytokinin signalling pathways.

Key words: Abscisic acid, *Arabidopsis*, germination, gibberellic acid, glucose signalling.

Introduction

Seed germination is a critical step in the plant life cycle, and is regulated by many biotic and abiotic factors. In *Arabidopsis thaliana* seeds, the transition from dormancy to germination is controlled by external environmental factors such as light quality, moisture, and transient exposure to cold as well as several internal growth regulators (reviewed by Koornneef *et al*., 2002). Among the photohormones that play a role in *Arabidopsis* seed germination, gibberellin (GA) and abscisic acid (ABA) have the most pronounced effects (Koornneef *et al*., 2002). ABA establishes and maintains dormancy of seeds, whereas GA has the opposite effect, breaking dormancy and inducing seed germination (Steber *et al*., 1998). It appears that there are multiple ABA detection and signalling mechanisms (Himmelbach *et al*., 2003). Seed germination-based genetic screens have identified mutants affected in ABA biosynthesis or sensitivity. The latter include ABA-insensitive mutants and ABA-hypersensitive mutants (reviewed by Finkelstein *et al*., 2002). Many genes encoding enzymes involved in GA biosynthesis and catabolism have also been identified (Olszewski *et al*., 2002). Several genes encoding GA-signalling components involved in seed germination are also known (Swain *et al*., 2002; Tyler *et al*., 2004). RGL2 negatively regulates GA responses that primarily control seed germination (Lee *et al*., 2002). The *Arabidopsis* gene *SPY* is also a negative regulator of GA signalling. However, unlike RGL2 it also regulates all other developmental processes involving GA (Izhaki *et al*., 2001). In addition, SPY is an activator of cytokinin signalling pathway (Greenboim-Wainberg *et al*., 2005).

Sugars also act as regulatory molecules and play a pivotal role in the plant life cycle. Exogenous glucose application has been shown to delay seed germination and inhibit seedling development (reviewed by Gibson, 2005). Different levels of sugar supply have different effects on various

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stages of plant development, which may be related to endogenous ABA levels (Gibson, 2005). A delay in *Arabidopsis* seed germination was observed at a glucose concentration as low as 0.5% with the delay increasing with increasing glucose concentrations (Price *et al*., 2003; Dekkers *et al*., 2004). While intermediate glucose concentrations (1.5–3%) dramatically delay WT seed germination, similar concentrations of sorbitol or mannitol have very little effect, indicating that the effect of glucose is not simply osmotic (Price *et al*., 2003; Dekkers *et al*., 2004). Because ABA and GA are key internal regulators of *Arabidopsis* seed germination it seems likely that glucose and other sugars exert their inhibitory effects via the biosynthesis, degradation or signalling pathways for these hormones. Based on experiments with the exogenous application of hormones, hormone precursors, and hormone synthesis inhibitors, Dekkers *et al*. (2004) concluded that glucose is not acting via the biosynthesis of ABA, GA or ethylene. Price *et al*. (2003) suggested that glucose at intermediate and high concentrations may delay germination by slowing the decline of endogenous ABA concentration. Although these results suggest that glucose at higher concentrations may, at least partially, act via ABA, the components of the ABA response pathway involved in seed germination delay have not been identified (Price *et al*., 2003). It has been suggested that the glucose-induced delay of seed germination involves neither increasing ABA biosynthesis nor activation of ABA signal transduction via ABI1, ABI2, ABI4, or ABI5 gene products (Dekkers *et al*., 2004). Dekkers *et al*. (2004) also reported that *spy-5* is sensitive to glucose for the timing of germination and suggested that glucose does not act via GA signalling.

High concentrations of glucose increased the accumulation of ABA in seedlings (Arenas-Huertero *et al*., 2000) and many of the sugar-insensitive mutants for post-germination seedling developmental abnormalities have been found to be allelic to genes involved in ABA biosynthesis or ABA signalling pathways (Gibson, 2005). For example, the *Arabidopsis* sucrose uncoupled-6 gene was found to be identical to one of the ABA-insensitive genes, *ABI4* (Huijser *et al*., 2000). In addition, mannose-induced inhibition of germination has also been demonstrated to involve the *ABI4* gene which has no function in the glucose pathway in seeds (Pego *et al*., 1999; Dekkers *et al*., 2004).

In this study, the possible mechanism of glucose-induced delay of seed germination via the stimulation of ABA signal transduction and/or inhibition of the GA signalling pathway is addressed. Seed germination and seedling development of several mutants of the ABA and GA signalling pathways were analysed during glucose treatment. The results indicate that ABA signal transduction is involved in the glucose-induced delay of seed germination via the *ABI3* gene. The GA signalling pathway is also involved in glucose effects on seed germination via *RGL2* and *SPY* genes. These data also suggest that there may be crosstalk between the ABA, GA, and glucose-signalling pathways that involves ABI3 and RGL2 during seed germination.

Materials and methods

Plant materials

Two ectotypes of *Arabidopsis thaliana* were used in this study: Columbia (Col) and Landsberg *erecta* (L*er*). Various *Arabidopsis* mutants with altered ABA and GA signalling were obtained from *Arabidopsis* stock centres. *spy5*, *abi2-1*, *abi3-1*, *abi4-1*, and *abi5-1* came from the Arabidopsis Biological Resource Center at Ohio State University (http://www.arabidopsis.org/abrc/), and *rgl2-1* (SGT625, Lee *et al*., 2002) came from the Nottingham Arabidopsis Stock Centre (http://arabidopsis.info/).

Germination, plant growth assays and RNA isolation

Before plating, seeds were sterilized in 6.25% sodium hypochlorite for 3 min and rinsed three times with sterile distilled water. Seeds were planted on 0.5× Murashige and Skoog medium (0.5MS, Caisson Laboratories) solidified with 0.8% agar (Fisher Scientific) and containing varying concentrations of glucose, mannose or hormones. Filter-sterilized hormone stock solutions were added after autoclaving to avoid the inactivation of hormones. α-D-Glucose and mannose were obtained from Acros Organics. Paclobutrazol (PAC), gibberellin (GA), and abscisic acid (ABA) were obtained from Sigma-Aldrich. *Arabidopsis* seeds were stratified for 3 d at 4 °C in the dark, then placed in a growth chamber with 70% humidity and under 18 h light and 6 h dark at 23 °C to facilitate germination. Germination (based on radicle emergence from the seed coat) was scored every 6–12 h or daily depending on the experiment. Each plate contained 90–110 seeds. Every experiment was repeated two or three times. In each experiment different batches of seeds were used. However, in each experiment, the WT and mutant seeds used were collected at the same time from the same age plants grown under the same conditions. Although the time-course of germination differed in each experiment, the trends in germination were the same for different batches of seeds. The data shown here is from one of these experiments. Plant growth was estimated by root length, number of leaves, and shoots weight at day 18. Seed germination and plant growth data were analysed using Microsoft Excel 2000. For germination tests, seeds collected at the same or similar times were used and stored at 4 °C before being used.

For mRNA expression analyses seeds were imbibed in distilled water for 7 d at 4 °C in the dark or imbibed in glucose or water for 72 h in the dark at 4 °C. Total RNA was extracted from these treated seeds for use in QRT-PCR. RNA extraction was performed as described previously (Vicient and Delseny, 1999).

Real-time PCR

Total RNA (2 μg) was treated with a DNA-*free* kit (Ambion INC.) and tested with real-time PCR for DNA contamination. The purified total RNA was used as a template to synthesize first-strand cDNA using a TaqMan Reverse Transcriptase kit (Roche) with oligo(dT) primers as per the manufacturer's instructions. Quantitative real-time PCR using first-strand cDNA as a template was carried out using an ABI Prism 7000 Sequence Detector with TaqMan Universal PCR Master Mix (Roche). PCR reactions were carried out in a final volume of 50 μl using gene-specific primers and probes in concentrations determined individually for each set of primers. Probes were modified with 6-FAM reporter dye at the 5′-end and TAMRA quencher at the 3′-end. All oligo synthesis and modifications were

done by Sigma-Genosys. Gene-specific primers and probes used were as follows: APT1 forward 5′-TGTTCCTTGCAACCGTC TTCT-3′, reverse 5′-TGGTTGAACGGTGGTTTGAG-3′, probe 5′-CCACCACCGTGCTCCTCCTTCG-3′; SPY forward 5′-GAGCT TGCTTTCCACTTTAATCCA-3′, reverse 5′-ATCAAGGTTGTCA CGGTCTTTGTA-3′, probe 5′-TGCTGAGGCTTGCAACAATTTG GGAGTAC-3′; RGL2 forward 5′-GGCTGCACAGTGGAGGAT TC-3′, reverse 5′-CGCGCTAGATCCGAGATGA-3′, probe 5′-TG AAATCCGCTGGGTTTGACCCG-3′; ABI3 forward 5′-CCATG-GAAGACATCGGAACCT-3′, reverse 5′-GGAGATACATCCTGC TTTTGTTGTT-3′, probe 5′-TCGTGTTTGGAACATGCGCTACA GGT-3′; ABI4 forward 5′-TTCCGGTAACTAATTCGACTTCGT-3′, reverse 5′-TTACACCCACTTCCTCCTTGTTC-3′, probe 5′-TCATCATGAGGTGGCGTTAGGGCA-3′.

Thermocycler conditions were 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The mRNA level for each gene was determined using the standard curve method according to the manufacturer's instructions (ABI Prism 7000 Sequence Detection System User Guide). APT1 (adenosine phosphoribosyl transferase) transcript level in each sample was used as an internal control (Arroyo *et al*., 2003). The mean value from triplicate samples was used to calculate the transcript level. Results were analysed using Microsoft Excel.

Results

Components of both ABA and GA signalling pathways are involved in seed germination delay caused by exogenous glucose

The seed germination kinetics of several mutants involved in ABA and GA signalling were investigated on plates containing intermediate concentrations of glucose. High glucose concentrations were not used here because, under those conditions, the delay of germination is partially caused by osmotic effects (Price *et al*., 2003). Mutants in the ABA and GA signalling pathways, *abi3-1* and *rgl2-1*, that are known to be involved in germination but have not been included in previous studies, were tested. *spy5*, that was previously found to be glucose sensitive, or even hypersensitive for the germination delay was also retested and used as a control (Dekkers *et al*., 2004).

Germination of L*er* (wild-type control), *abi3*, *rgl2*, and *spy* seeds in the sugar-free control plates approached 90–100% after 42 h. Moderate concentrations (1.5% and 2.5%) of glucose delayed seed germination at 42 h in all the lines tested as shown in Fig. 1. However, the mutants were affected less severely than the wild type (WT). The mutant seeds of *rgl2*, *spy*, and *abi3*, all have a significantly higher germination rate than WT for both glucose concentrations used here (Fig. 1). The dramatic increase of germination frequency of *abi3* begins at 36 h and approaches 100% at 72 h on 1.5% glucose plates. The germination frequency of WT on 1.5% glucose begins to increase at 48 h and only approaches 40% at 72 h. The germination frequency of *abi3* begins to increase dramatically at 42 h and approaches 100% at 96 h on 2.5% glucose. The germination of WT increases very slowly on 2.5% glucose and reaches only 31% at 120 h.

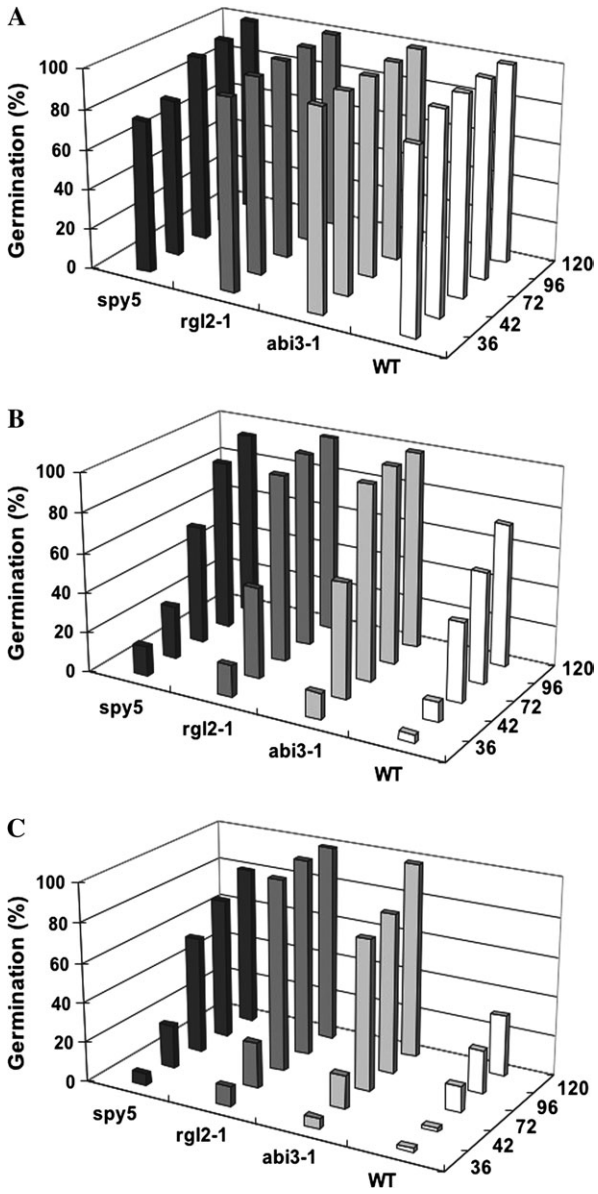

Fig. 1. (A–C) Effects of glucose on seed germination. Time-course of *abi3-1*, *rgl2-1*, *spy5*, and WT (L*er*) seed germination on 0.5MS (A), 0.5MS containing 1.5% glucose (B), and 0.5MS containing 2.5% glucose (C). Points on the *z*-axis are in hours after transfer of plates to the growth chamber.

The germination kinetics of *rgl2* mutant seeds is similar to *abi3* seeds (Fig. 1). Dekkers *et al.* (2004) also reported that the *spy* mutant was as sensitive or even more sensitive than WT to glucose. However, these results indicate that *spy* is partially resistant to glucose during germination. The germination of *spy* seeds was above 90% at 120 h on 1.5% glucose plates and about 80% at 120 h with 2.5% glucose treatment (Fig. 1B, C). The data suggest that both the ABA signalling pathway via ABI3 and the GA signalling pathway via RGL2 and SPY are involved in the glucose delay of seed germination.

Mannose affects germination via ABI3 but not via RGL2 or SPY

Arabidopsis seed germination is inhibited by mannose in a concentration-dependent manner starting at concentrations much lower than other sugars (Pego *et al*., 1999). It was tested whether mutants in the GA and ABA signalling pathways, *abi3-1*, *rgl2-1*, and *spy*, that were shown here to be resistant to glucose, are also resistant to mannose inhibition of seed germination. The effect of mannose on *spy* and *rgl2* germination had not been reported and the reported results for *abi3-1* have been conflicting. Laby *et al*. (2000) found that *abi3-1* is as sensitive as WT to 1.7 mM mannose while Huijser *et al*. (2000) reported that *abi3-1* shows partial resistance to 5 mM mannose. WT and mutant seeds were tested on three concentrations of mannose. The germination of mutant seeds was assayed at the 8th and 10th days after transfer to the growth chamber. A comparison of germination on mannose-free plates and three concentrations (5 mM, 7.5 mM and 10 mM) of mannose is shown in Fig. 2. The germination frequency of *abi3* seeds on 5 mM mannose plates approached 40% while those of WT, *rgl2*, and *spy* were all below 10% on day 10 (Fig. 2). The germination frequency of *abi3* seeds on higher concentrations of mannose, 7.5 mM and 10 mM, was only about 10%, but no seeds of WT, *rgl2*, and *spy* germinated under these conditions (Fig. 2). These results indicate that mannose repression of *Arabidopsis* seed germination does not involve GA signalling pathways through RGL2 or SPY gene products, but that ABA signalling pathway is involved in this process via ABI3 in addition to the previously reported effect of ABI4 (Pego *et al*., 1999).

Effects of ABA and GA on rgl2, abi3, *and* spy *seed germination*

To determine if there might be an interaction between the ABA and GA signalling pathways during glucose-induced delay of germination, glucose-insensitive alleles used in this study were tested for resistance to PAC, an inhibitor of GA biosynthesis, and to ABA during seed germination. *Spy-3* has been shown to be partially ABA insensitive and *abi3-1* is known to germinate in the presence of GA biosynthesis inhibitors (Nambara *et al*., 1991; Steber *et al*., 1998). It was found that *spy-5* shows WT sensitivity to ABA. While the germination of *spy-5* and WT seeds is inhibited by ABA application (Fig. 3A, B) the germination of *rgl2-1* seeds is greater than that of WT with both 1 μM and 3 μM ABA treatment (Fig. 3A, B). These data demonstrate that the *rgl2-1* mutant is at least partially resistant to ABA in a concentration-dependent manner. As shown in Fig. 3C and D, *abi3-1* seed germination reached a high percentage (>90%) on day 6 on both 10^{-5} M and 10^{-4} M PAC plates. The germination of *abi3* seeds is similar to that of *rgl2* and *spy* but the germination of *abi3* seeds is slightly delayed at the higher concentration of PAC (Fig. 3C, D). All three mutants, *abi3*, *rgl2*, and *spy* were highly resistant to PAC inhibition of seed germination. These results suggest that ABI3 may inhibit seed germination via negative regulation of the GA signalling pathway. ABI3 is a transcription factor and could therefore exert its influence on the GA signalling pathway via transcriptional activation of negative regulators such as RGL2 or SPY.

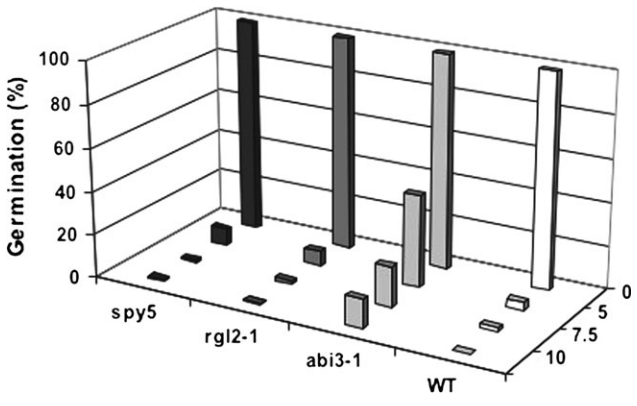

Fig. 2. Effects of mannose on seed germination. Percentage germination of WT and mutants, 10 d after transfer to a growth chamber, on 0.5MS containing different concentrations of mannose. Concentration of mannose (mM) is indicated on the *z*-axis.

Transcription of genes involved in glucose-induced delay of seed germination

The RGL2 transcription levels were analysed in *abi3* and WT seeds imbibed in H_2O at 4 °C in the dark for 7 d. The relative RGL2 mRNA levels in *abi3* mutant seeds decreased 3-fold relative to WT seeds (Table 1). This result suggests that ABI3 positively controls the *RGL2* gene expression. Since the *rgl2* mutant is slightly resistant to ABA inhibition of seed germination and RGL2 belongs to the GRAS family of putative transcriptional regulators (Pysh *et al*., 1999), the ABI3 transcription levels in *rgl2* and WT seeds were also compared. ABI3 mRNA levels in *rgl2* mutant seeds decreased by 7-fold compared with those in WT seeds (Table 1). This suggests that RGL2 may be involved in the activation of *ABI3* expression. There was no significant difference in *SPY* expression in either *rgl2* or *abi3* backgrounds (Table 1). These data suggest that both ABI3 and RGL2 may be involved in the crosstalk between the ABA and GA signalling pathways and that it may be accomplished by reciprocal transcriptional activation.

Because the *ABI3* and *ABI4* genes both encode transcription factors and seem to be involved in the mannose-induced inhibition of seed germination, it was necessary to determine whether these genes act in the same pathway by controlling each other's expression. The ABI3 transcript levels were compared in *abi4* and WT (Col) as well as the ABI4 transcript accumulation in *abi3* and WT (L*er*) seeds. The expression of these genes is the same in WT and

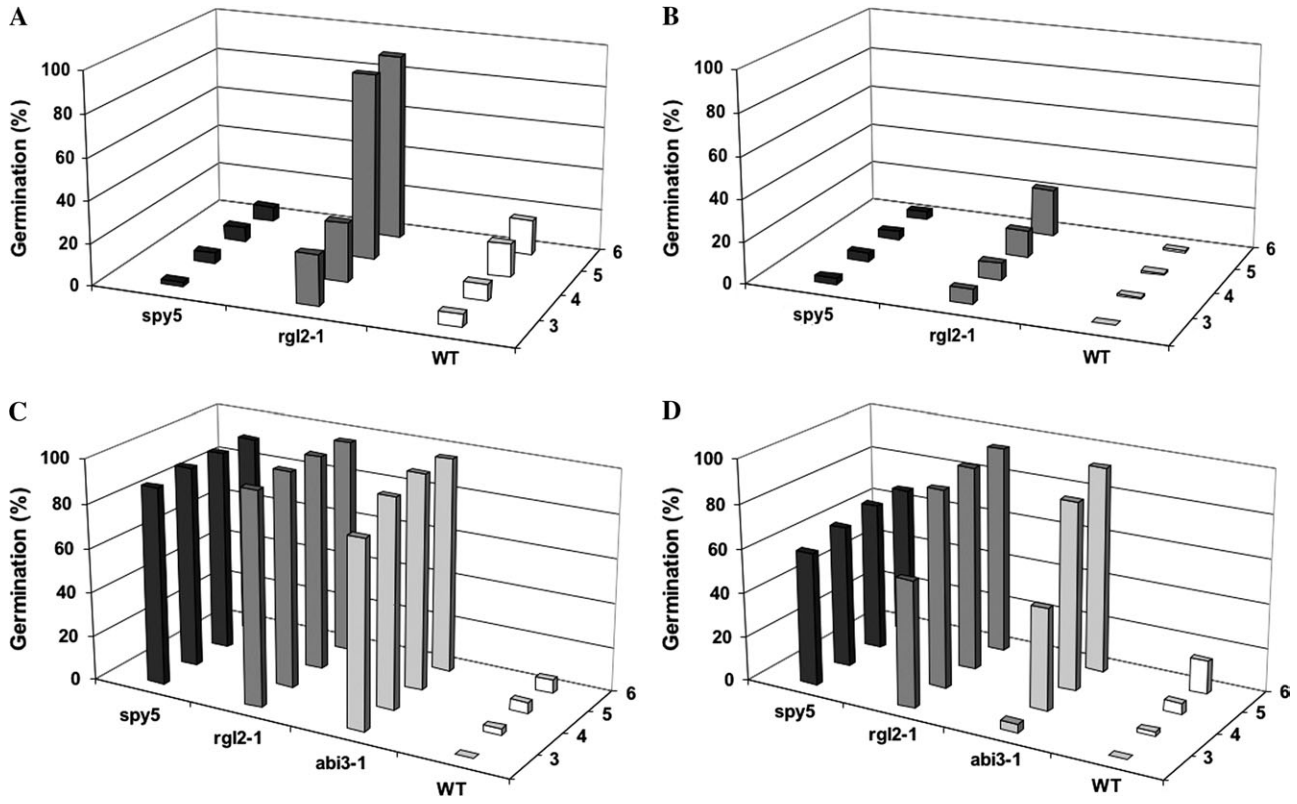

Fig. 3. (A–D) Effects of ABA and PAC on seed germination. Percentage germination of WT and mutants, on 0.5MS containing: 1 μM ABA (A), 3 μM ABA (B), 10^{-5} M PAC (C), 10^{-4} M PAC (D). Time (d) after transfer to the growth chamber is indicated on the *z*-axis.

Table 1. *Relative mRNA levels*

Genotypes	Genes		
	ABI3	*RGL2*	*SPY*
abi3-1[a]		0.31±0.12	1.21±0.44
rgl2-1[a]	0.14±0.04		1.80±0.11
WT[b]	2601.4±43.6	46.6±3.7	
WT[c]		2.5±0.1	

[a] mRNA levels in mutant seeds/mRNA levels in L*er* seeds imbibed in H_2O at 4 °C for 7 d.
[b] mRNA levels in WT (L*er*) seeds imbibed in glucose solution/mRNA levels in WT seeds imbibed in H_2O at 4 °C for 3 d.
[c] mRNA levels in WT (L*er*) seeds imbibed in ABA solution/mRNA levels in WT seeds imbibed in H_2O at 4 °C for 3 d.

mutant backgrounds (data not shown) suggesting that ABI3 and ABI4 do not control each other's transcription.

To investigate further the effect of ABI3 and RGL2 on seed germination delay by glucose, ABI3 and RGL2 mRNA levels were compared in WT seeds imbibed in 2.5% glucose with seeds imbibed in H_2O alone. ABI3 mRNA level in seeds imbibed in glucose is more than 2500 times higher than that of H_2O-treated seeds (Table 1). This indicates that glucose dramatically induces ABI3 gene expression on a transcriptional level. The RGL2 mRNA level in seeds treated with glucose is more than 42 times higher than that of H_2O-treated seeds (Table 1). These data suggest that glucose delays seed germination via transcriptional induction of both RGL2 and ABI3 genes.

Glucose has a stimulatory effect on seedling growth in a concentration-dependent manner

High glucose concentrations, 6% and above, have been shown to have an inhibitory effect on seedling growth and development (Gibson, 2005). This inhibitory effect is restricted to a very narrow time window of about 48 h from the start of seed imbibition (Gibson *et al*., 2001). All the mutants used in this study show WT sensitivity to this inhibitory effect of glucose (not shown). The effects of different concentrations of glucose on the later stages of seedling development were investigated. To avoid the effects of glucose on the timing of germination and on early seedling development, all seeds were first germinated on sugar-free plates and then similar-sized seedlings were transferred on the 4th day to plates containing different concentrations of glucose (1.5%, 2.5%, 5%, and 7% glucose). WT seedlings on all glucose concentrations proceeded to develop true leaves (Fig. 4A). The effects of different concentrations of exogenous glucose application on seedling growth are evident several days after transfer from glucose-free media (not shown). Figure 4A shows representative WT shoots, on day 18, from a glucose-free plate and four different glucose concentrations. Seedling

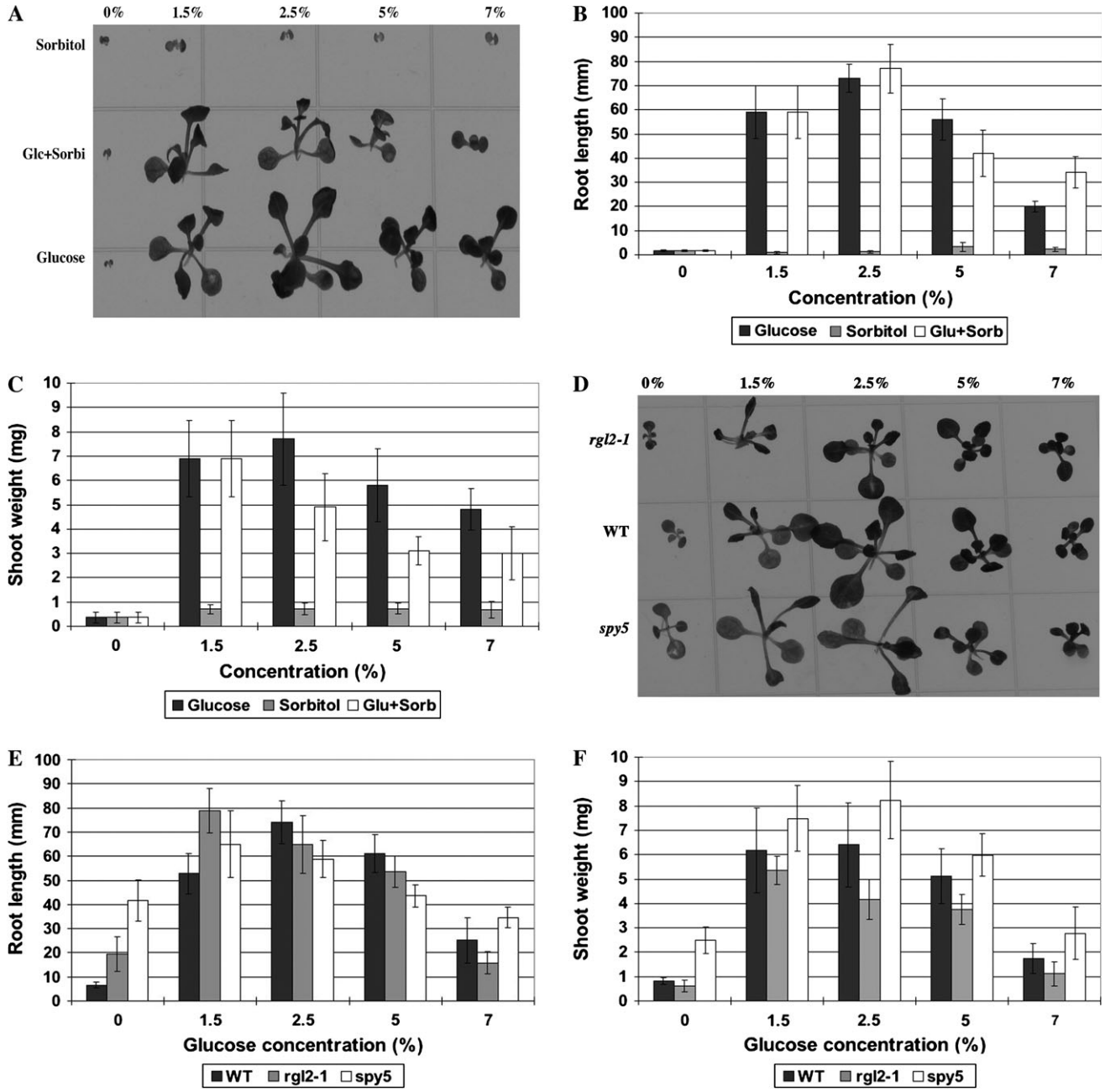

Fig. 4. (A–F) Comparison of glucose and sorbitol effect on WT seedling development (A–C) and effects of glucose on GA signalling mutant seedling growth (D–F) analysed on day 18 after transfer to the growth chamber. In the 'glucose plus sorbitol' groups, the 1.5% treatment is glucose alone, 2.5% treatment is 1.5% glucose plus 1% sorbitol, 5% treatment is 1.5% glucose plus 3.5% sorbitol, and the 7% treatment is 1.5% glucose plus 5.5% sorbitol. Representative WT rosettes grown on various concentrations of glucose, sorbitol, and glucose plus sorbitol total concentrations are indicated above rosettes (A). Root length (B) and shoot weight (C) of WT grown on various concentrations of glucose, sorbitol and glucose plus sorbitol. WT and GA signalling mutant rosettes (D), root length (E), and shoot weight (F) grown on various concentrations of glucose. Glucose concentrations (%) are indicated above rosettes in (D). Error bars represent standard deviations.

growth on sugar-free plates is stunted (Fig. 4). Primary roots are very short, and true leaves are not visible on day 10 (not shown). Seedlings from plates containing increasing concentrations of sorbitol alone are indistinguishable from seedlings from sugar-free plates on day 18 (Fig. 4A–C). By contrast, seedlings from plates containing glucose have much longer roots and larger shoots (Fig. 4A–C). Seedlings from intermediate glucose concentrations, 1.5% and 2.5%, have the longest roots and the largest leaves (Fig. 4A–C). Root length and shoot weight increased with intermediate levels of glucose and decreased at high glucose concentrations. Rosette development of WT on 7% glucose plates is better than that on 1.5% glucose plus 5.5% sorbitol plates (Fig. 4A). The above results indicate

that glucose has a stimulatory effect on WT seedling development and that the inhibitory effect of very high levels of glucose is osmotic. The application of 1.5–5% glucose resulted in a significant growth stimulation. WT roots were on average at least nine times longer (Fig. 4B) and rosettes attained at least five times larger mass (Fig. 4C). There were no significant differences between root and shoot growth of the *rgl2* mutant and WT at day 18 (Fig. 4D–F). By contrast, *spy* grows considerably better than WT without glucose and similarly to WT seedlings with glucose (Fig. 4D–F). The roots of the *spy* mutant are much longer, at least five times than those of WT on glucose-free plates (Fig. 4E). The rosettes of *spy,* on glucose-free plates, appear at least twice the size of WT on day 18 (Fig. 4D) and attain three times higher mass (Fig. 4F). These results indicate that glucose has a stimulatory effect on both root and shoot growth in WT seedlings and that *spy* is less dependent on glucose supply for seedling growth.

Discussion

Glucose levels as low as 0.5% considerably delay *Arabidopsis* seed germination (Price *et al*., 2003). This delay is not due to osmotic stress because concentrations below 6% of sorbitol have no effect on the timing of seed germination. Glucose at intermediate and high concentrations may affect germination by slowing the decline of endogenous ABA concentration (Price *et al*., 2003). However, the components of the ABA signalling pathway such as ABI1, ABI2, ABI4, and ABI5 are not involved in this process (Price *et al*., 2003; Dekkers *et al*., 2004).

There have been at least three different glucose-signalling pathways proposed, two of which appear to be hexokinase-dependent (Moore *et al*., 2003). Because the glucose analogue, 3-*O*-methylglucose has a very similar effect on the timing of germination as glucose and because it is not a good substrate for hexokinase (HXK) it was suggested that glucose delays seed germination via an HXK-independent pathway (Dekkers *et al*., 2004). Because mannose, a stereoisomer of glucose, is a good substrate for HXK and it inhibits *Arabidopsis* seed germination at very low concentrations, concentrations at which other sugars have no effect, it was suggested that it acts via an HXK-dependent pathway (Pego *et al*., 1999). The idea that mannose and glucose inhibit germination via different pathways is further supported by the findings that ABI4 is involved in mannose, but not glucose, effects on germination (Pego *et al*., 1999; Dekkers *et al*., 2004). The data presented here show that *rgl2* and *spy* mutants in the GA signalling pathway are resistant to glucose-induced delay but not to mannose inhibition of germination. These data are consistent with the idea that mannose and glucose inhibit germination through different pathways, and further demonstrate that mannose inhibition of seed germination is not via the GA signalling pathway through RGL2 or SPY.

One of the ABA-insensitive genes may be involved in both the glucose-induced delay of germination and the mannose inhibition of germination. The *abi3-1* mutant is resistant to glucose and partially resistant to mannose inhibition of seed germination, which suggests that ABI3 may be a common factor in both mannose and glucose pathways to inhibit seed germination. Therefore, mannose inhibits seed germination not only via ABI4 in the ABA signalling pathway but also via ABI3. It remains to be determined whether ABI3 and ABI4 exert their effects through the same branch of the ABA signalling pathway or possibly act in parallel.

It is clear from the results that glucose delays germination by activating the ABA signalling pathway via ABI3 and repressing the GA signalling pathway via SPY and RGL2. It has been shown that glucose application has a pronounced effect on ABI3 and RGL2, up-regulating both at the transcriptional level. The data also show that the *rgl2* mutant is partially resistant to ABA application and confirm that the *abi3* mutant is resistant to PAC, indicating that RGL2 may be involved in the ABA signalling pathway and that ABI3 is involved in the GA signalling pathway. These data on SPY, RGL2, and ABI3 transcription in *rgl2* and *abi3* mutant backgrounds indicate that RGL2 up-regulates ABI3 transcription and that ABI3 up-regulates RGL2 transcription. These data suggest that there is crosstalk between the ABA and GA signalling pathways and that RGL2 and ABI3 gene products are involved in this process.

These data also show that both *ABI3* and *RGL2* genes are induced by glucose, although whether glucose induces both genes 'directly' remains to be determined. Figure 5 shows our model of a possible mechanism of the glucose-induced delay of seed germination. Glucose leads to a dramatic increase in *ABI3* gene expression at the transcriptional level, which in turn activates the ABA signalling pathway resulting in the inhibition of seed germination.

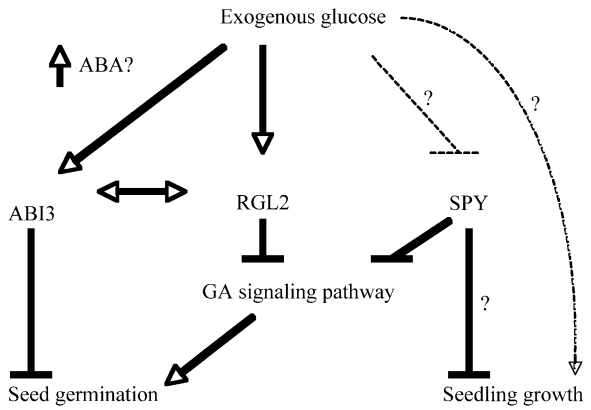

Fig. 5. Proposed model for the interactions between glucose and the ABA and GA signalling pathways during seed germination and the later stages of seedling development. See text for details.

The ABI3 gene product may also activate RGL2 transcription and thus lead to the inactivation of GA signalling pathway at the same time. Glucose also up-regulates RGL2 expression either directly or via ABI3 thus leading to the inhibition of seed germination by turning off the GA signalling pathway. The data also suggest that RGL2 may activate the ABA signalling pathway by up-regulating ABI3 transcription.

Glucose not only plays a role in germination but also in other aspects of the plant life cycle, such as seedling development. *Arabidopsis* seedlings germinated on 5% glucose plates fail to develop expanded cotyledons or true leaves (Laby *et al*., 2000). The data demonstrate that when young seedlings are transferred to glucose-containing media, following germination without glucose, that glucose has a stimulatory effect on both root and shoot growth and development. Moderate glucose levels (e.g. 1.5–5%) strongly stimulate root and true leaf development. Very high glucose levels also lead to better growth than the complete absence of glucose. Poorer growth on very high concentration compared with moderate concentrations of glucose may simply be associated with osmotic stress.

The interaction between sugar and plant hormone response pathways was indicated by studies that characterized sugar-hypersensitive and resistant mutants for seedling development. The mutants in the ABA signalling pathways such as *abi1*, *abi2*, and *abi3* are sensitive to the inhibitory effect of high glucose levels on early seedling development, but *abi4* and *abi5* are resistant (Finkelstein, 1994; Arenas-Huertero *et al*., 2000; Huijser *et al*., 2000; Laby *et al*., 2000). These experiments comparing glucose, sorbitol, and glucose plus sorbitol suggest that the effects of some of these mutations on the later stages of seedling development are related to osmotic stress resistance and are not specific to glucose.

The data also show that seedling growth of *spy-5* on glucose-free medium is much better than that of WT. It is possible that part of the stimulatory effect of glucose on seedling growth is via the activation of the GA signalling pathway since GA is necessary for root and leaf growth. In the *spy* mutant, the GA signalling pathway is on in the absence of the functional SPY gene product. Therefore in the *spy* mutant, glucose is not necessary to activate the GA signalling pathway. The reason why *spy* grows better with than without glucose may be that only a part of the stimulatory effect of glucose on seedling growth is via relieving the inhibitory effect of SPY. Since SPY also functions in cytokinin signalling, it cannot therefore be ruled out that glucose exerts it stimulatory effect via this pathway instead of or in addition to the GA signalling pathway (Greenboim-Wainberg *et al*., 2005).

In conclusion, glucose can delay seed germination through a different pathway from sugars such as mannose. The effect of glucose delay on seed germination is via genes in both the GA and ABA signalling pathways. Genes that were tested, *ABI3* and *RGL2*, may act via the same pathway in the glucose delay of seed germination. It has also been demonstrated that glucose, applied after germination, has a strong stimulatory effect on seedling growth and development. This effect is partially mediated via activation of GA and/or inactivation of cytokinin signalling pathways by relieving the inhibitory or the stimulatory effect of a key regulator, SPY. Therefore, exogenous glucose in moderate concentrations has opposite effects on plant growth and development depending on the developmental stage during which it is applied. The inhibitory and stimulatory effects of glucose are, at least in part, mediated via components of the ABA and GA signalling pathways.

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