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Effects of the Gibberellin Biosynthetic Inhibitor Uniconazol on Mutants of *Arabidopsis*[1]

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ABSTRACT

Using the gibberellin (GA) biosynthetic inhibitor Uniconazol, we determined that *det1*, a mutant that no longer requires light to be germinated, still requires GA synthesis for germination. This result suggests that dark inhibition of germination in *Arabidopsis* may be due to inhibition of GA synthesis by the *DET1* gene product in mature wild-type seeds. Similar experiments with mutants that lack seed dormancy due to a reduced sensitivity to abscisic acid (*abi*) have shown that *abi1* and *abi3* no longer require GA for germination. Furthermore, by shifting wild-type seeds to inhibitor at 6-hour intervals during imbibition, we determined that GA synthesis is only required during the first 24 hours of the imbibition process to reverse abscisic acid-induced dormancy in *Arabidopsis*.

The role of phytohormones in regulating seed germination is often regarded as a balance between the germination-promoting hormone GA[2] and the inhibiting hormone ABA (7, 9). However, genetic studies in which a collection of *Arabidopsis* mutants with defects in hormone metabolism was used suggested that GA and ABA are temporally separated and probably respond to different environmental cues (reviewed in ref. 8).

Mutants defective in ABA synthesis (*aba*) or that cannot respond to ABA (*abi*) lack dormancy at all stages of development even though maximal ABA synthesis in wild-type *Arabidopsis* peaks relatively early in seed development (8, 10, 11). Furthermore, the reversal of ABA-induced dormancy in mature seeds normally requires a light-mediated induction of GA synthesis during imbibition (3, 13). Although little is known about how light promotes GA synthesis, the isolation of a collection of mutants (*det*) that no longer requires light for germination has permitted the identification of at least three genes involved in this process (1, 2).

In an attempt to determine whether GA synthesis is required in mutants with aberrant germination, we analyzed the germination response of *aba-1*, *abi1*, *abi2*, *abi3*, and *det1* by imbibing seeds on the GA biosynthetic inhibitor Uniconazol (6). Alterations in GA synthesis are easily scored in *Arabidopsis* because loss of GA synthesis results in loss of germination (12).

We also used Uniconazol to inhibit GA synthesis at various times during seed imbibition to determine how long GA synthesis is required to break dormancy of wild-type seeds.

MATERIALS AND METHODS

Plant Material

The *Arabidopsis thaliana* (L.) Heynh lines Landsberg *erecta* and Columbia were used in this investigation. These ecotypes were found to respond to GA_3 and the GA biosynthesis inhibitor Uniconazol in a similar way. The *aba-1* (isolation No. A26), *abi1* (AII), *abi2* (EII), and *abi3* (CIV) mutant lines which were obtained from M. Koornneef were all descended from Landsberg *erecta* (10–12). The *det1* mutant, derived from the Columbia ecotype, was kindly provided by J. Chory (2). The GA biosynthesis inhibitor Uniconazol was a gift from the Pesticides Research Laboratories, Takarazuka Research Center, Sumitomo Chemical Co. Ltd., Japan.

Growth Conditions

Growth conditions for both sterile and nonsterile experiments have been previously described (4). GA_3 and/or Uniconazol were dissolved in ethanol and added to the media after sterilization. All seeds were imbibed at 2°C for 5 d and then transferred to room temperature. Petri plates were maintained at 22 to 24°C under 24 h of light for 7 d, after which the seeds were scored for germination.

For dark germination experiments, seeds were sterilized and plated as described above under green safelight conditions. Plates were wrapped in tinfoil three times and stored at 22 to 24°C for 7 d, after which germination was scored.

The percentage of germination among different batches of seeds varied according to the age of the seeds. Seeds that were harvested and stored 6 months or more before being tested were more resistant to Uniconazol at the level of germination than seeds that had been stored for shorter periods (2–7 weeks). Because younger seeds produced a more clear-cut response to the inhibitor, seeds of this age were used for all experiments.

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[2] Abbreviations: GA, gibberellin; Uniconazol, (E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (experimental code No. S-3307).

Inhibitor Shift Experiments

Wild-type Columbia seeds were sterilized and plated on 0.8% agar containing no nutrients as described above. After the seeds were plated, 20 of them were transferred every 6 h, for 48 h after imbibition, to one of three plates containing: basal media (control), basal media plus 10^{-4} M Uniconazol, basal media plus 10^{-4} M Uniconazol/10^{-5} M GA_3. Seven days after plating, the seeds were scored for germination.

RESULTS

Effect of Uniconazol on Germination of Wild-Type and Mutant Seeds

At a concentration of 10^{-4} M, Uniconazol was found to inhibit germination and was completely reversed by the addition of GA_3 (Table I). At concentrations in which germination was not severely inhibited (10^{-7} M), plants were still dwarfed in stature compared with control plants (data not shown). This is in agreement with genetic studies in which leaky GA biosynthetic mutations were used and that showed that the amount of GA required for germination is lower than that required to sustain cell elongation (12).

When *aba-1*, *abi1*, or *abi3* were imbibed on a concentration of 10^{-4} M Uniconazol, germination was similar to that without inhibitor (Table I). Therefore, it appears that the two ABA-insensitive mutants are similar to the biosynthetic mutant in that they are GA independent for germination. However, the response of *abi2* is more similar to wild type in that its germination is inhibited by Uniconazol (Table I). This result is surprising because this mutant apparently lacks dormancy in the absence of the inhibitor and is resistant to exogenous ABA (Table I, column 3).

To determine whether *det1* mutants have altered synthesis or response to GA, *det1* mutant seeds were scored for dark germination in the presence of Uniconazol. We found that *det1* is unable to germinate in the presence of Uniconazol unless exogenous GA_3 is provided (Table II). Thus, it appears that the *det1* mutant still requires GA for germination and, therefore, must synthesize GA in the absence of light.

Table I. *Germination Response in Wild-Type (WT) and Mutant Seeds with Altered ABA Metabolism on Uniconazol or ABA*

The concentration of Uniconazol and ABA were 10^{-4} and 10^{-6} M, respectively. Controls contained no inhibitors. WT + GA, GA (10^{-5} M) included in the medium. Results are the means of three independent experiments.

Genotype	Germination		
	Control	Uniconazol	ABA
Wild type			
WT	100	0	0
WT + GA	100	100	
ABA biosynthetic mutant			
aba 1	71	60	
ABA-insensitive mutants			
abi1	95	95	100
abi2	100	18	100
abi3	100	97	79

Table II. *Germination Response to Uniconazol in Wild-Type (WT) and det1 Arabidopsis Seeds in the Dark*

The concentration of Uniconazol and GA_3 were 10^{-4} and 10^{-5} M, respectively. Controls contained no GA or Uniconazol.

Genotype	Germination			
	Control	GA_3	Uniconazol	
			−GA	+GA
	%			
WT	23	100	0	69
det1	100	100	0	100

Inhibitor Shift Experiments

Seeds can be exposed to Uniconazol at any time during seed imbibition, it is, therefore, possible to inhibit GA synthesis at discrete points in the germination process and to determine the time during germination at which GA must be synthesized to break seed dormancy. Seeds shifted to the inhibitor in the first 6 h of imbibition did not germinate. A slight increase in germination was detected by 12 h with complete reversal seen by 24 h (Fig. 1). This result implies that the GA that is synthesized during the first 24 h of imbibition is sufficient to reverse the effects of ABA-induced dormancy and to allow seeds to germinate.

DISCUSSION

We used the GA biosynthesis inhibitor Uniconazol to determine the role of GA in a number of different mutants with altered germination. It has been reported that this class of

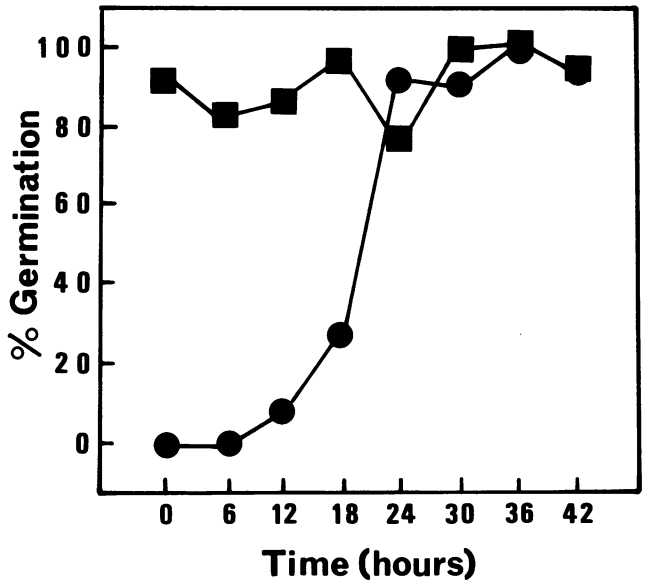

Figure 1. Germination of wild-type *Arabidopsis* imbibed for various times on Uniconazol (●, 10^{-4} M) or Uniconazol plus GA_3 (■, 10^{-5} M). Each point, time of shift. The percentage of germination was determined by dividing the number of germinated seeds on Uniconazol or Uniconazol plus GA by the number of germinating seed on the control plate. Similar results were seen in two independent experiments.

growth retardants also effects sterol and ABA biosynthesis (5, 14). However, the strong resistance to Uniconazol in the ABA mutants and the complete reversal of the inhibitor by GA in wild-type seeds suggest these side reactions do not substantially interfere with GA-induced germination in *Arabidopsis* at the concentrations used in this study.

On this basis, it appears that the DET1 gene product is one of the factors that determines when GA is synthesized during germination. Unlike wild-type seeds, the *det1* mutant does not require exogenous GA_3 for good germination in the absence of light (2). However, because *det1* is unable to germinate in the presence of Uniconazol, it appears that this mutant still requires GA for germination and, therefore, must synthesize GA independently of light (Table II).

Molecular studies of *det1* suggest that the DET1 gene may encode a transcriptional repressor (1). In this context our results suggest that GA biosynthetic genes may also be negatively controlled by the DET1 gene at the level of transcription. Moreover, the positive effect of light on wild-type germination implies that the function of photoactive phytochrome in germination may be to inhibit DET1 synthesis or action.

That an ABA biosynthetic mutant germinates on Uniconazol suggests that GA is not required to activate the biochemical machinery required for germination if ABA biosynthesis is reduced. Similar conclusions has been suggested by Koornneef and co-workers (10) using a double mutant defective in both GA and ABA biosynthesis. As with ABA biosynthetic mutants, the inability to respond to ABA due to an *abi1* or *abi3* mutation can also remove the requirement for GA synthesis and action during germination. Surprisingly, however, *abi2*, which does not appear to respond to ABA, still requires GA for germination. It is possible that the *abi2* allele used in this study was leaky, and therefore, the seeds are weakly dormant, making the interpretation of this result difficult. The fact that among the ABA-insensitive mutants *abi2* has the lowest resistance to exogenous ABA would support the above notion (data not shown).

Alternatively, it could be argued that ABA-insensitive mutants can be subclassified into two groups based on their GA requirement for germination. Although the biochemical basis of these two groups is not clear, the *abi2* response to Uniconazol suggests that it may be possible to genetically separate the establishment of dormancy via ABA from GA-induced germination.

Considered as a whole, the studies presented here suggest that after GA synthesis is released from DET1 repression the sole purpose of GA action during germination is to reverse ABA-induced dormancy. Furthermore, it appears that GA response genes need only be active for the first 24 h of imbibition to complete this process (Fig. 1). After this time other cues are used to activate genes involved in later stages of germination.

The response of mutants defective in ABA metabolism to Uniconazol suggests that it should be possible to isolate mutants that are resistant to this inhibitor using germination as an assay. Such a screening tool that may identify new genes involved in the establishment of dormancy, and the germination response in *Arabidopsis* is now in progress.

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