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Gibberellins and Light-Stimulated Seed Germination

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Abstract

Bioactive gibberellins (GAs) promote seed germination in a number of plant species. In dicots, such as tomato and *Arabidopsis, de novo* GA biosynthesis after seed imbibition is essential for germination. Light is a crucial environmental cue determining seed germination in some species. The red (R) and far-red light photoreceptor phytochrome regulates GA biosynthesis in germinating lettuce and *Arabidopsis* seeds. This effect of light is, at least in part, targeted to mRNA abundance of GA 3-oxidase, which catalyzes the final biosynthetic step to produce bioactive GAs. The R-inducible GA 3-oxidase genes are predominantly expressed in the hypocotyl of *Arabidopsis* embryos. This predicted location of GA biosynthesis appears to correlate with the photosensitive site determined by using R micro-beam in lettuce seeds. The GA-deficient non-germinating mutants have been useful for studying how GA stimulates seed germination. In tomato, GA promotes the growth potential of the embryo and weakens the structures surrounding the embryo. Endo-β-mannanase, which is produced specifically in the micropylar endosperm in a GA-dependent manner, may be responsible for breaking down the endosperm cell walls to assist germination. Recently, a role for GA in overcoming the resistance imposed by the seed coat was also suggested in *Arabidopsis* from work with a range of seed coat mutants. Towards understanding the GA signaling pathway, GA response mutants have been isolated and characterized, some of which are affected in GA-stimulated seed germination.

Key words: Biosynthesis; Cellular localization; Germination; Gibberellin; Phytochrome; Seed coat

Introduction

Gibberellins (GAs) control many aspects of plant growth and development. For example, in *Arabidopsis thaliana,* bioactive GAs play an essential role in seed germination, leaf expansion, stem elongation, flowering, and flower development. GAs are diterpenes that are commonly biosynthesized from geranylgeranyl diphosphate (GGDP). The biosynthesis pathway converting GGDP to biologically active GAs, such as GA_1, and GA_4, has been well established (Hedden and Kamiya 1997). Genes encoding most of the GA biosynthesis enzymes have been isolated from *Arabidopsis* (Hedden and Phillips 2000; Helliwell and others 2001), allowing the regulation of GA biosynthesis to be analyzed in greater detail (Hedden and Phillips 2000; Yamaguchi and Kamiya 2000). By contrast, much less is known about GA perception and the subsequent signal transduction pathway. However, recent molecular genetic approaches in *Arabidopsis* and molecular and pharmacological studies in cereal aleurone have started to identify key components in the GA signaling pathway (Lovegrove and Hooley 2000; Sun 2000).

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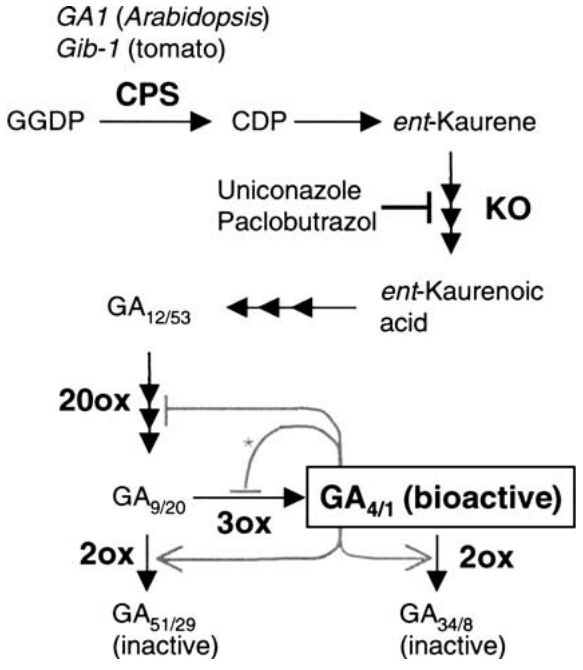

Figure 1. Major GA biosynthesis pathway. Bioactive GAs, GA_4, and GA_1, are indicated in the box. The inhibitory effect of uniconazole and paclobutrazol on KO is shown by the black T-bar. Gray arrows depict feedfoward regulation. Feedback inhibition is indicated by the gray T-bar. (*) In germinating *Arabidopsis* seeds, the *AtGA3ox2* gene, encoding GA 3-oxidase, is not subject to feedback regulation. GGDP, geranylgeranyl diphosphate; CDP, *ent*-copalyl diphosphate; CPS, *ent*-copalyl diphosphate synthase; KO, *ent*-kaurene oxidase; 20ox, GA 20-oxidase; 3ox, GA3-oxidase; 2ox, GA2-oxidase.

GA promotes seed germination in many plant species. The importance of GA in stimulating seed germination is evident in *Arabidopsis* and tomato, where severe GA-deficient mutants require exogenous GAs to germinate (Koornneef and Van der Veen 1980; Koornneef and others 1990). In *Arabidopsis* and tomato, seeds imbibed in the presence of GA biosynthesis inhibitors (paclobutrazol and uniconazole) (Figure 1) fail to germinate, indicating that *de novo* GA biosynthesis upon imbibition is essential for seed germination to occur (Hilhorst and Karssen 1988; Nambara and others 1991). This suggests that the regulation of endogenous GA levels after seed imbibition is a crucial factor determining seed germination.

The GA-deficient non-germinating mutants of *Arabidopsis* and tomato (for example, *ga1* of *Arabidopsis* and *gib-1* of tomato) (Figure 1) have been useful systems for studying how GA stimulates seed germination. At least two roles for endogenous GAs in this process have been proposed (Hooley 1994): (1) to stimulate the growth potential of the embryo, as is indicated by the reduced growth rate of embryos of GA-deficient mutants and (2) the induction of hydrolases to weaken the structures surrounding the embryo. Thus, GA action is probably targeted to two separate tissues to promote seed germination in these species.

In this review, we discuss the regulation of GA biosynthesis and GA action during seed germination in selected plant species. First we review the regulation of GA biosynthesis during germination of lettuce and *Arabidopsis* seeds, where phytochrome-dependent germination and GA synthesis has been established. We also discuss how GA-deficient non-germinating seeds, produced by mutation or treatment with chemical inhibitors, have been used to study the mechanism for GA-promoted seed germination in tomato and to identify and characterize GA response mutants in *Arabidopsis*.

Regulation of GA Biosynthesis in Germinating Seeds

Regulation by Light

Seed germination, like other developmental processes, is controlled by multiple endogenous and environmental cues. Light is one of the major environmental factors that control seed germination. In many plant species, such as lettuce and *Arabidopsis*, appropriate light conditions are essential for seed germination to occur. Light-regulated seed germination was first recognized in lettuce seeds, in which red light (R) induces, but far-red light (FR) reversibly inhibits germination (Borthwick and others 1952). This finding led to the discovery of the R and FR photoreceptor phytochrome. Because GA can mimic the effect of R to stimulate germination of dark-imbibed seeds, it has been hypothesized that R promotes seed germination, at least in part, through increasing GA levels.

Phytochrome-regulation of GA biosynthesis has been studied in detail in germinating lettuce seeds. Toyomasu and others (1993) have shown that endogenous GA_1 (the major bioactive GA during lettuce seed germination), but not its immediate precursor GA_{20}, increases after R treatment. The effect of R on the GA_1 level is canceled when FR is irradiated after the R treatment. The reversible changes in the GA level in response to R and FR suggest the involvement of phytochrome. Because the precursor GA_{20} accumulates to a high level (about 100 times higher than GA_1) in dark-imbibed lettuce seeds, the conversion of GA_{20} to GA_1 is likely

to be the limiting step in the production of GA_1. Supporting this hypothesis, transcript levels for the *LsGA3ox1* (formerly *Ls3h)* gene, which encodes GA 3-oxidase, increase after R treatment (Toyomasu and others 1998). In contrast, mRNA levels of two GA 20-oxidase genes are induced by dark-imbibition alone, which is consistent with the accumulation of GA_{20} in the dark. These data indicate that phyotochrome controls the conversion of GA_{20} to GA_1, at least partially through altering *LsGA3ox1* mRNA abundance (Toyomasu and others 1998).

Germination of *Arabidopsis* seeds is also dependent on phytochrome. In this species, the phytochrome gene family consisting of five members (*PhyA* to *PhyE*) has been characterized (Sharrock and Quail 1989). Using the *phyA* and *phyB* null mutants, the roles of PhyA and PhyB in light-induced seed germination have been studied (Shinomura 1997). PhyB is the major family member that is stored in dormant seeds and is responsible for the typical photoreversible response shortly after the start of imbibition. After a long period of imbibition in the dark, PhyA plays a role in the irreversible response to irradiation with extremely low levels of light over a wide range of wavelengths (very low fluence response) (Shinomura and others 1996). Although PhyD- and PhyE-deficient mutants have been isolated in *Arabidopsis* (Aukerman and others 1997; Devlin and others 1998), their role in photo-induced seed germination has not been fully characterized.

Derkx and others (1994) reported that light-imbibed wild-type seeds contain higher levels of bioactive GAs than dark-imbibed ones, indicating that light influences GA biosynthesis. More recently, the effect of R on transcript levels of the *Arabidopsis AtGA3ox1* and *AtGA3ox2* genes (formerly *GA4* and *GA4H*, respectively), both of which encode GA 3-oxidases, has been analyzed (Yamaguchi and others 1998). Both *AtGA3ox1* and *AtGA3ox2* mRNA accumulation is rapidly elevated by a brief R pulse, which is reversed by FR. In the *phyB* mutant, *AtGA3ox2* expression is not increased by R, which indicates that PhyB primarily mediates the induction of *AtGA3ox2* by R. The phytochrome species that control R-induced *AtGA3ox1* expression have yet to be investigated. *AtGA3ox1* and *AtGA3ox2* transcript levels are still increased by R in the non-germinating *ga1-3* mutant seeds. This supports the idea that the R-induced transcript accumulation is not a consequence of seed germination, but presumably a direct effect of light. The *phyB* mutant seeds are able to germinate through the action of other phytochromes including PhyA (Shinomura and others 1996). It would be interesting to examine whether the *AtGA3ox* genes are up-regulated by light in the PhyA-mediated very low fluence response.

Regulation by GA Activity

Accumulating evidence indicates that levels of bioactive GAs are regulated by GA activity (Hedden and Phillips 2000; Yamaguchi and Kamiya 2000). This homeostatic control is achieved by feedback inhibition of the synthesis of bioactive GAs and feedforward regulation of GA deactivation. The feedback inhibition mechanism decreases transcript accumulation of GA 20-oxidase and GA 3-oxidase genes, while the feedforward regulation increases mRNA levels of GA 2-oxidase genes (Figure 1).

In germinating *Arabidopsis* seeds, bioactive GA_4 levels are markedly elevated in the GA-insensitive *gai-1* mutant seeds relative to wild-type seeds (Derkx and others 1994), which suggests that the homeostatic regulation of GA_4 levels by GA activity operates at this developmental stage. Of the two GA 3-oxidase genes expressed during seed germination, transcript accumulation of the *AtGA3ox1* gene is up-regulated in the *ga1-3* mutant seeds and down-regulated by application of exogenous GA_4. In contrast, *AtGA3ox2* mRNA levels are not affected by GA_4 levels (Yamaguchi and others 1998). These results indicate that only *AtGA3ox1,* but not *AtGA3ox2,* is under negative feedback regulation. *In situ* hybridization experiments have shown that *AtGA3ox1* and *AtGA3ox2* transcripts are co-localized in the same cell types (Yamaguchi and others 2001). Because the *AtGA3ox1* gene is subject to feedback regulation, this gene would contribute to the homeostasis of the hormone concentrations. On the other hand, *AtGA3ox2,* which is not down-regulated by GA activity, may function to increase GA levels over the homeostatic range. Thus, *AtGA3ox2* would play a crucial role in providing bioactive GAs sufficient for germination. This hypothesis needs to be examined by analyzing endogenous GA contents and seed germination in loss-of-function *AtGA3ox2* mutants. The differential regulation of *AtGA3ox1* and *AtGA3ox2* genes may represent part of the mechanism to control levels of bioactive GAs to alter plant development.

Cellular Localization

To predict how GA promotes seed germination, it is helpful to determine cellular localization of GA biosynthesis. Comparison of the sites of GA synthesis and response would be necessary to in-

vestigate whether GA acts as an intercellular signal. Because GAs are present in plant tissues at very low levels, direct examination of cellular distribution of GAs with anti-GA antibodies has not been practical. The recent isolation of genes encoding GA biosynthesis enzymes is allowing us to determine the distribution of their transcripts and, thus, to predict the sites of GA production in plants.

Using *in situ* hybridization and promoter-GUS gene fusion constructs, cell-specific expression patterns of GA biosynthesis genes have been analyzed in germinating *Arabidopsis* seeds (Yamaguchi and others 2001). *AtGA3ox1* and *AtGA3ox2* transcripts accumulate predominantly in the cortex of embryo axis, suggesting that this is the major site of synthesis of bioactive GAs. The cortical cells are enlarged and appear more vacuolated during the initial growth of the embryonic axis, perhaps as a result of the response to GAs (Yamaguchi and others 2001). Thus, GA synthesis and response may be spatially correlated in the embryo. Inoue and Nagashima (1991) have utilized micro-beam irradiation of R to determine photo-perceptive sites in germinating lettuce seeds. Irradiation of the hypocotyl with R results in efficient induction of seed germination, while irradiation of the cotyledons and radicle tip does not. The effect of R on the hypocotyl of lettuce embryos is interesting because the R-inducible *AtGA3ox1* and *AtGA3ox2* genes are predominantly expressed in the hypocotyl of *Arabidopsis* embryos. The spatial correlation between the perception of R and GA synthesis supports the idea that the R-stimulated GA synthesis, at least in part, mediates R-promoted seed germination (Figure 2). Bioactive GAs produced in the cortex of the embryonic axis may cause cell enlargement *in situ*, and perhaps act in other tissues as well, including the envelope surrounding the embryo, as discussed below (Figure 2).

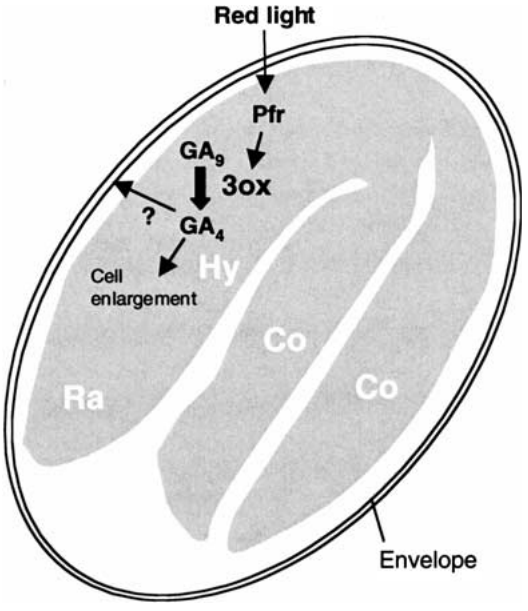

Figure 2. Model for R-induced germination of *Arabidopsis* seeds. Pfr, formed by red light irradiation, increases mRNA levels of GA 3-oxidase, which catalyzes the production of bioactive GAs. Cell-specific expression patterns of GA 3-oxidase genes predict that bioactive GAs are mainly synthesized in the cortex of the hypocotyl. Bioactive GAs produced in the hypocotyl may stimulate cell enlargement *in situ*. The arrow with a question mark indicates the hypothetical role of GA in weakening the envelope tissue in germinating *Arabidopsis* seeds. 3ox, GA 3-oxidase; Co, cotyledon; Hy, hypocotyl; Ra, radicle.

GA Signaling and Response During Seed Germination

Although the requirement of endogenous GA for seed germination is well documented in tomato and *Arabidopsis*, the exact role of GA in stimulating seed germination needs to be further investigated. Recent studies in tomato have identified genes that are expressed prior to radicle emergence in a GA-dependent manner. Molecular genetic approaches have been used to identify components in the GA signal transduction pathway. Some of these GA response mutants have been shown to affect GA-dependent seed germination in *Arabidopsis* and tomato.

Role of GA in Seed Germination

The non-germinating *gib-1* mutant of tomato has proved a useful system for analyzing the physiological and molecular basis of GA-stimulated seed germination. Although *gib-1* mutant seeds require exogenous GA for germination, removal of the endosperm and testa layers around the radicle tip causes germination (Groot and Karssen 1987). Similarly, when the envelope covering the *Arabidopsis* embryo is mechanically removed, the *ga1* embryos can grow into dwarf plants (Silverstone and others 1997; Telfer and others 1997). These observations suggest that one of the roles of GA in stimulating seed germination is to overcome the mechanical resistance conferred by the envelope surrounding the embryo.

Groot and Karssen (1987) have analyzed weakening of the endosperm and testa by measuring the puncture force needed to break through these layers

in tomato seeds. In wild-type seeds, the puncture force decreases before radicle emergence, but this does not occur in the *gib-1* seeds until GA is added exogenously. However, when de-embryonated seed-halves (isolated endosperm and testa) of the *gib-1* mutant are incubated with isolated wild-type embryos, the puncture force decreases significantly. These results indicate that the breakage of the endosperm and testa is dependent on GA, and suggest that endogenous GAs produced in the embryos are responsible for weakening these tissues to stimulate germination. This hypothesis is consistent with the expression of GA 3-oxidase genes in the embryos, but not in the envelope in germinating *Arabidopsis* seeds (Yamaguchi and others 2001). Because the *gib-1* embryos have a reduced growth rate in comparison with wild-type embryos, GA also plays a role in facilitating the growth of embryos during germination (Groot and Karssen 1987).

In tomato, GA-dependent breakdown of the endosperm cell wall results in the release of sugars, of which the major component is mannose (Groot and others 1988). Nonogaki and Morohashi (1996) have identified endo-β-mannanase activity exclusively in the micropylar region (where the radicle protrudes) of the endosperm prior to germination. Tissue print *in situ* hybridization confirmed that *LeMAN2* mRNA (encoding an endo-β-mannanase) accumulated specifically in the micropylar endosperm cap before radicle emergence (Nonogaki and others 2000). These results suggest that GA may promote the production of endo-β-mannanase to facilitate the weakening of the endosperm cell walls. The galactomannan-hydrolyzing activity in the micropylar region is photoreversibly modulated by R and FR treatments, which also control tomato seed germination (Nomaguchi and others 1995). If GA biosynthesis in germinating tomato seeds is regulated by phytochrome, as demonstrated in lettuce and *Arabidopsis* seeds (Toyomasu and others 1998; Yamaguchi and others 1998), the light-dependent galactomannan-hydrolyzing activity may be a consequence of light-modulated endogenous GA levels. However, the growth potential of the embryo, which could also be GA-dependent, is not inhibited by FR (Nomaguchi and others 1995). It would be interesting to examine whether phytochrome regulates GA levels during tomato seed germination.

Expansins are known to be involved in cell wall extension, possibly by disrupting hydrogen bonding between the cell wall components (Cosgrove 1998). In addition, expansins present in non-growing cells may contribute to tissue softening. Genes encoding expansins have been characterized in germinating tomato seeds (Chen and Bradford 2000). One of the expansin genes, *LeEXP4,* is GA-inducible and is specifically expressed in the micropylar endosperm cap prior to radicle protrusion. Also, *LeEXP4* expression is inhibited by FR treatment, which also inhibit seed germination. Differential cDNA display analysis was carried out to identify GA-responsive genes in radicle tips and endosperm caps of imbibed *gib-1* mutant seeds. The results have shown that genes coding for subunits of vacuolar H^+-ATPase are induced by GA treatment, and that their transcripts are particularly abundant in the micropylar region before germination (Cooley and others 1999). In addition, genes encoding class I β-1,3-glucanase and chitinase are up-regulated by GA in the micropylar endosperm cap (Wu and others 2001). Tissue-specific expression of these GA-dependent genes in the micropylar endosperm caps supports the role of GA in facilitating the breakdown of the mechanical barrier. Whether the products of these genes are directly involved in the tissue weakening remains to be demonstrated.

A variety of seed coat mutants of *Arabidopsis* have been used to analyze the importance of the seed envelope in preventing germination (Debeaujon and Koornneef 2000). *tt (transparent testa)* mutants lack the production of anthocyanin pigment in the testa. When *tt* mutations are introduced into the *ga1* background, they partially rescue germination of the *ga1* seeds without exogenous GAs. In addition, mutants with reduced mucilage on the testa surface *(ttg, gl2* and *ap2)* require less GA for germination. These data suggest that GA plays an essential role in overcoming the germination resistance imposed by the seed coat in *Arabidopsis.*

GA Response Mutants

Because GA plays an essential role in the germination of *Arabidopsis* and tomato seeds, GA response mutants should have altered ability to germinate. Genetic approaches have identified several GA signaling components, which include positive and negative regulators of the GA response. Recent studies on the GA signaling pathway are described in a comprehensive review article (Sun 2000). Here, we select some GA response mutants with emphasis on those related to seed germination.

Recessive *spy (spindly)* mutants have been identified because of their ability to germinate in the presence of a GA biosynthesis inhibitor, paclobutrazol (Jacobsen and Olszewski 1993). Because the *spy* mutations confer constitutive GA response during later vegetative and reproductive development as well, SPY is likely to be a negative regulator of GA signaling in most stages of *Arabidopsis* devel-

opment. *SPY* encodes a Ser/Thr *O*-linked N-acetylglucosamine transferase (OGTs), which is known to modify proteins by glycosylation of Ser/Thr residues in animals (Thornton and others 1999). Recently, Swain and others (2001) have reevaluated the *spy* mutant phenotype and suggested that SPY plays a role in plant development beyond its role in GA signaling. Whether SPY plays a specific role in GA signaling during seed germination still requires further studies.

Similar to the *spy* mutant, the *early flowering 1* (*EAF1*) mutant shows resistance to paclobutrazol during seed germination (Scott and others 1999). This recessive mutant has pale green leaves with elongated petioles, and displays an early flowering phenotype under both long- and short-day conditions. Based on the GA-related mutant phenotype, it is proposed that EAF1 may negatively control GA content or signaling. *procera* (*pro*) tomato is a recessive mutant that resembles GA-treated wild-type plants like the *Arabidopsis spy* mutants. The *pro* mutation can partially suppress the defects in the GA-deficient *gib-1* mutant, including the inability to germinate: the digenic *pro/gib-1* mutant requires less exogenous GAs for seed germination relative to the *gib-1* mutant (Van Tuinen and others 1999). Therefore, PRO may function as a negative regulator of GA-stimulated seed germination in tomato.

Because severe GA-deficient mutants require exogenous GA for germination in *Arabidopsis,* GA-insensitive mutants could display a non-germinating phenotype that is not rescued by exogenous GAs. However, in the *aba (ABA-deficient)* or *abi (ABA-insensitive)* mutant background, these GA-insensitive mutants may be able to germinate because some *aba* and *abi* mutations suppress the inability of GA-deficient seeds to germinate (Koornneef and others 1982; Nambara and others 1991). Steber and others (1998) have isolated suppressors of the *abi1-1* mutant, and one of the loci has been named *sleepy1 (sly1).* Germination of the *sly1/abi1-1* seeds is inhibited in the presence of 3 μM ABA, which does not inhibit germination of the parental line *abi1-1.* The *sly1* mutant is recessive and exhibits an infertile, dark green dwarf phenotype that is similar to GA-deficient mutants. In addition, the monogenic *sly1* mutant (without the *abi1-1* mutation) fails to germinate. These characteristics suggest that SLY1 is a positive regulator of GA response throughout plant development in *Arabidopsis.* Isolation of the *SLY1* gene may help to predict its role in the GA signaling pathway.

A mutation at the *COMATOSE* (*CTS*) locus in *Arabidopsis* results in a marked reduction in germination potential (Russell and others 2000). Application of bioactive GAs does not restore the impaired germination of the *cts* seeds, indicating that *cts* is a GA-insensitive non-germinating mutant. The germination of *cts* is partially rescued only when imbibed seeds are treated with prolonged chilling for stratification. Interestingly, the *cts* phenotype is not observed during other developmental stages. Because the *cts* mutation is recessive, CTS is proposed to be a positive regulator of seed germination and/or a negative regulator of embryo dormancy (Russell and others 2000).

The semidominant *gai-1* mutant is a GA-insensitive semidwarf with reduced germination ability (Koornneef and others 1985; Derkx and Karssen 1993). Pre-chilling, but not exogenous GA, increases the germination of the *gai-1* seeds (Derkx and Karssen 1993). The loss-of-function alleles (for example, *gait-6)* are similar to wild-type plants in appearance (Peng and Harberd 1993), but they are more resistant to paclobutrazol for stem elongation and flower development than wild-type (Peng and others 1997). Thus, GAI is likely to be a negative regulator of the GA response. However, the *gai-t6* seeds do not germinate on 10^{-4} M paclobutrazol (where *spy* mutants can germinate). GAI is a putative transcriptional regulator that belongs to the GRAS family. The role of GAI and other members of the family (Sun 2000) during seed germination needs to be further investigated.

Light-Modulated GA Sensitivity

There is some evidence that environmental cues, such as light and temperature, can modulate sensitivity to exogenous GAs. Besides controlling the biosynthesis of GA, light has been shown to alter GA sensitivity during seed germination in *Arabidopsis.* Using the non-germinating *ga1* mutant seeds, Derkx and Karssen (1993) have compared the requirement of exogenous GA for germination in the dark versus continuous white light. The light-imbibed seeds were able to germinate at a lower dose of GA_{4+7} than the dark-imbibed seeds, indicating that white light increases the sensitivity to exogenous GA.

Yang and others (1995) have studied more specifically the roles of phytochrome in modulating GA sensitivity during *Arabidopsis* seed germination. They showed that R lowers, but FR reversibly elevates, exogenous GA levels required for germination of uniconazole-treated seeds. Because GA sensitivity is still altered by R and FR treatments in the digenic *phyA/phyB* null mutant, other phytochromes are likely to be responsible for sensing R

and FR. Supporting this hypothesis, Poppe and Schäfer (1997) reported that seed germination of the *phyA/phyB* double mutant is still under phytochrome control, based on its photoreversible response to R and FR. Identification of the phytochrome species modulating GA sensitivity would be informative for elucidating the mechanism for light-regulated seed germination. By contrast to the light-modulated GA sensitivity in *Arabidopsis,* GA sensitivity is not modified by R or FR in imbibed lettuce seeds, of which germination is similarly dependent on phytochrome (Toyomasu and others 1998). Therefore, the major effect of phytochrome on GA during lettuce seed germination is to control the levels of this hormone.

Conclusion and Perspectives

Phytochrome-regulation of GA biosynthesis has uncovered part of the mechanism for light-stimulated seed germination in lettuce and *Arabidopsis.* Pfr, formed after R treatment, increases transcript levels of GA 3-oxidase, which is directly responsible for the formation of bioactive GAs. Based on the cell-specific expression patterns of GA biosynthesis genes, the site of GA biosynthesis has been predicted in germinating *Arabidopsis* seeds. This should increase our knowledge on how GA stimulates seed germination, along with accumulating information on the GA response pathway. In tomato and *Arabidopsis,* physiological, genetic, and biochemical evidence suggests a role for GA in weakening the structures covering the embryo during germination.

To better understand light-induced seed germination, there are several questions to be addressed. One possible approach is to identify *cis-* and *trans*-acting factors participating in the regulation of GA biosynthesis genes by phytochrome. This would also contribute to understanding how multiple factors (for example, light and feedback inhibition) control GA biosynthesis genes. Besides studying the complex regulation of GA biosynthesis, identifying the molecular basis of light-modulated GA sensitivity is a key issue in fully understanding how light and GA signals are integrated. Obviously, isolation of more components in the GA signaling pathway is necessary for increasing our knowledge on how GA stimulates seed germination. Also, a comprehensive search for GA-responsive gene markers would be helpful to define more specifically the GA signaling pathway and to better characterize potential GA response mutants. These marker genes should also help to analyze cross-talk between GA signaling and other endogenous and environmental factors controlling seed germination.

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