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Light Regulation of Gibberellin Biosynthesis and Mode of Action

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Abstract

Some phenotypic effects produced in plants by light are very similar to those induced by hormones. In this review, the light-gibberellin (GA) interaction in germination, de-etiolation, stem growth, and tuber formation (processes regulated by GAs) are discussed. Germination of lettuce and *Arabidopsis* seeds depends on red irradiation (R), which enhances the expression of GA 3-oxidase genes (*GA3ox*) and leads to an increase in active GA content. De-etiolation of pea seedling alters the expression of *GA20ox* and *GA3ox* genes and induces a rapid decrease of GA_1 content. Stem growth of green plants is also affected by diverse light irradiation characteristics. Low light intensity increases stem elongation and active GA content in pea and *Brassica*. Photoperiod controls active GA levels in long-day rosette (spinach and *Silene*) and in woody plants (*Salix* and hybrid aspen) by regulating different steps of GA biosynthesis, mainly through transcript levels of *GA20ox* and *GA3ox* genes. Light modulation of stem elongation in light-grown plants is controlled by phytochrome, which modifies GA biosynthesis and catabolism (tobacco, potato, cowpea, *Arabidopsis*) and GA-response (pea, cucumber, *Arabidopsis*). In *Arabidopsis* and tobacco, *ATH1* (a gene encoding an homeotic transcription factor) is a positive mediator of a phyB-specific signal transduction cascade controlling GA levels by regulating the expression of *GA20ox* and *GA3ox*. Tuber formation in potato is controlled by photoperiod (through phyB) and GAs. Inductive short-day conditions alter the diurnal rhythm of *GA20ox* transcript abundance, and increases the expression of a new protein (PHOR1) that plays a role in the photoperiod-GA interaction.

Key words: De-etiolation; Germination; Gibberellins; Light; Photomorphogenesis; Photoperiod; Phytochrome; Stem elongation; Tuberization

Introduction

Many aspects of plant development are affected by light, and plants sense the different light characteristics (intensity, quality, direction, and duration) to adapt themselves to their environment and to optimize their growth and development (Chory 1997; McNellis and Deng 1995; Quail 1994; Smith 1994). Light is perceived by different photoreceptors [the phytochromes, the blue/UV-A (cryptochromes and phototropins), and the UV-B photoreceptors] (Batschauer 1998; Briggs and Olney 2001) that transduce the light signal to regulate gene expression through a series of signaling intermediates. The mechanism of light signal transmission and the characterization of some intermediates of the signal transduction pathway have been the subject of recent reviews (Batschauer 1998; Fankhauser and Chory 1997, 1999; Holm and Deng 1999; Nagy and Schäfer 2000; Quail and others 1995; Wei and Deng 1996).

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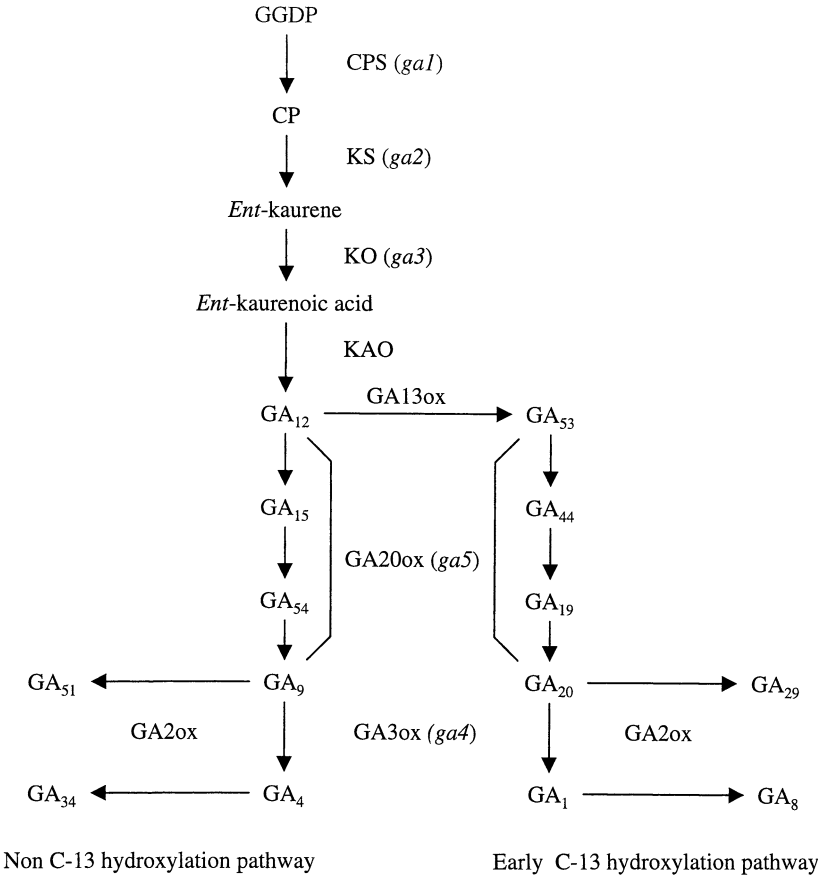

Figure 1. Diagram of the two main gibberellin biosynthesis pathways in plants. GGDP, geranylgeranyl diphosphate; CP, *ent*-copalyl diphosphate; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase; ox, oxidase; *ga1*, *ga2*, *ga3*, *ga4*, and *ga5*, GA biosynthesis mutants of *Arabidopsis*.

The effect of light on plant growth and developmental processes can be substituted or mimicked in some cases (for example, germination, stem elongation, and flowering) by hormone application or by the reduction of their content, both with biosynthesis inhibitors or with mutations in biosynthetic genes (Chory and others 1996; Reid 1993). It is, therefore, not surprising that many studies have attempted to implicate alterations of hormone biosynthesis, catabolism, and/or response, particularly for the gibberellins (GAs) (Kamiya and García-Martínez 1999), in the control of these physiological processes by light.

Most of the genes encoding GA biosynthesis enzymes have been cloned (Hedden and Kamiya 1997; Helliwell and others 1998, 2001; Thomas and others 1999) (Figure 1) and this has allowed the study of light regulation of GA biosynthesis at the molecular level. By comparison, although biochemical and genetic approaches have identified several components of the GA signal transduction pathway (Bethke and Jones 1998; Sun 2000), little is known about the effect of light on the response of the tissues to GAs. Several recent reviews have dealt with the interaction between light and GA biosynthesis and metabolism (Chory and Li 1997; Hedden 1999; Hedden and Kamiya 1997; Hedden and Phillips 2000; Kamiya and Garcia-Martinez 1999; Kraepiel and Miginiac 1997). This review will try to cover the topic more comprehensively, particularly the latest developments. It will be restricted to the physiological processes of germination, de-etiolation, stem elongation, and tuber formation, where a clear light-GA interaction exists and where significant advances have been made recently. Flowering, another physiological process for which there is also evidence to suggest that GAs mediate the effect of light (Blázquez and others 1999; Levy and Dean 1998) will not be discussed in this review.

Seed Germination

Many seeds require light (white light, WL, or red irradiation, R) and GAs to germinate (Karssen 1995; Yamaguchi and Kamiya, this issue). The effect of light can be abolished by treating the seeds with inhibitors of GA biosynthesis (for instance, paclobutrazol), and the light requirement may be substituted by GA application in many species

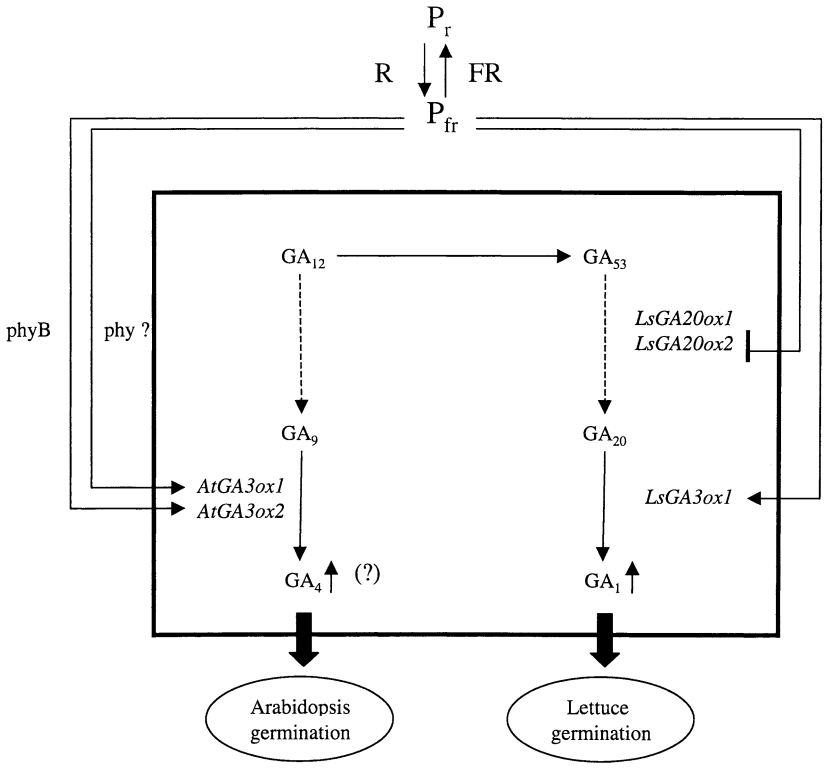

Figure 2. Regulation of GA metabolism by R during the germination of *Arabidopsis* and lettuce seeds. (?), not confirmed.

(Plummer and others 1997). Early experiments on the effect of R and far-red (FR) light on lettuce (*Lactuca sativa* L., cv Grand Rapids) seed germination led to the identification of the phytochrome photoreceptor (Borthwick and others 1952). This was followed by the suggestion, from application experiments, that phytochrome affects germination through the action of GAs (Ikuma and Thimann 1960). It has been proposed that phytochrome might act by (1) stimulating the synthesis of GAs, (2) increasing the response of seeds to endogenous GAs, or (3) decreasing the level of an inhibitor of germination. It is only recently, mainly through work with lettuce and *Arabidopsis thaliana*, that the relationship between light and GA on germination has begun to be clarified.

Lettuce

Lettuce seeds (cv Grand Rapids) do not germinate in the dark, and the application of GA_1, but not that of GA_{20}, its immediate metabolic precursor, induces germination. This indicates that GA_1 is the main biologically active GA in lettuce seeds (Toyomasu and others 1993). The content of endogenous GA_1 (the active GA in lettuce) starts increasing within 3 h after a 10-min pulse of R treatment, well in advance of germination induced by R (detected from about 10 h after R treatment) (Toyomasu and others 1993). The contents of GA_{19} and GA_{20}, the two immediate GA_1 precursors, were relatively high in the seed compared with GA_1 (about 4 and 100 times more, respectively) and were not affected by R. GA_1 is synthesized from GA_{53} by two different 2-oxoglutarate dependent enzymes, GA 20-oxidase and GA 3-oxidase, acting in sequence (Figure 1), and cDNA clones of two GA 20-oxidase genes (*LsGA20ox1* and *LsGA20ox2*) and of one GA 3-oxidase gene (*LsGA3ox1*) have been isolated from lettuce (Toyomasu and others 1998). The expression of *LsGA20oxl* and *LsGA20ox2* is induced by imbibition alone in the absence of light irradiation. The level of *LsGA20ox2* mRNA decreases with R treatment, whereas that of *LsGA20ox1* is unaffected by light. In contrast, the expression of *LsGA3ox1* is induced significantly by R within 2 h of treatment and this effect is canceled by FR (Toyomasu and others 1998) (Figure 2). These results suggest that R promotes GA_1 synthesis by inducing *LsGA3ox1* expression via phytochrome action.

Transcript levels of *GA20ox* and *GA3ox* are subjected to feed-back regulation, in many cases, by the action of active GAs (Cowling and others 1998; Hedden and Kamiya 1997). Therefore, the decrease of *LsGA20ox2* transcripts in R-treated seeds could be a consequence of feed-back regulation as a result of the increased GA_1 content in germinating seeds. Interestingly, the expression of *LsGA20ox1* does not

seem to be subject to feed-back regulation by the endogenous GA_1, nor that of *LsGA3ox1* (which increased).

Arabidopsis

Germination of *Arabidopsis* seeds in the dark is very poor compared with germination in WL, and the application of GA_{4+7} reverses the effect of darkness (Derkx and Karssen 1993). The effect of light is mediated by phytochrome because R stimulates seed germination and the effect of R is reversed by FR (Yang and others 1995). In *Arabidopsis* there are at least five genes encoding phytochromes *(AtPhyA-E)* (Quail and others 1995). PhyB is the main phytochrome involved in the germination of *Arabidopsis* seeds, although phyA also plays a role, depending on the kind of irradiation (Shinomura and others 1994, 1996). Genes encoding different enzymes of the GA-biosynthetic pathway have been cloned from *Arabidopsis: AtCPS* (locus *GA1*, which encodes *ent*-copalyldiphosphate synthase, CPS), AtKS (locus *GA2*, which encodes *ent*-kaurene synthase, KS), *AtKO* (locus *GA3*, which encodes *ent*-kaurene oxidase, KO), *AtKAO* (which encodes *ent*-kaurenoic acid oxidase), *AtGA3ox1* and 2 (which encode GA 3-oxidases), and *AtGA20ox1*, 2, and *3* (which encode GA 20-oxidases) (Figure 1). Severe alleles of gene mutants that block the synthesis of enzymes early in the GA biosynthesis pathway, *ga1 (AtCPS), ga2 (AtKS),* and *ga3 (AtKO)*, do not germinate without exogenous application of GAs, showing that GAs are necessary for germination of *Arabidopsis* seeds. In contrast, even the putative null allele *ga4-2 (AtGA3ox1)* can germinate without GAs, suggesting the presence of another GA 3-oxidase in germinating seeds (Hedden and Proebsting 1999; Ross and others 1997). A second GA 3-oxidase gene (*AtGA3ox2*, previously *GA4H*), which is predominantly expressed during seed germination, has been isolated (Yamaguchi and others 1998). The transcript levels of both *AtGA3ox1* and *AtGA3ox2* genes increase in imbibed seeds after R treatment (Yamaguchi and others 1998) (Figure 2) and, at least in the case of *AtGA3ox2*, this is due probably to activation of transcription (Yamaguchi and others 2001). Results from *in situ* RNA hybridization and GUS reporter expression have shown that both genes are expressed predominantly in the hypocotyl (Yamaguchi and others 2001), and this observation is of interest because hypocotyl is the most sensitive tissue to R induction of seed germination, at least in lettuce (Inoue and Nagashima 1991). Although the endogenous GAs have not been analyzed in *Arabidopsis* seeds, R treatment is expected to increase the level of GA_4 (the major biologically active GA in *Arabidopsis*) (Figure 2). In the phyB-deficient *phyB-1* mutant, *AtGA3ox1* expression, but not that of *AtGA3ox2*, is induced by R (Yamaguchi and others 1998). This indicates that while the R-induced expression of *AtGA3ox2* is mediated by phyB, the R-induced expression of *AtGA3ox1* is medidated by a different phytochrome (Figure 2). Moreover, in contrast to the *AtGA3ox1* gene (Cowling and others 1998), the application of GA_4 shows that the levels of *AtGA3ox2* transcripts are not regulated by a feedback inhibition mechanism in germinating seeds (Yamaguchi and others 1998). The two GA 3-oxidases of *Arabidopsis*, therefore, seem to play different physiological roles during light induction of seed germination.

Seedling De-Etiolation

Seedlings grown in the dark show an etiolated phenotype as a result of the skotomorphogenesis (dark development) developmental program, which leads to extremely elongated internodes and the presence of an apical hook, with an absence of leaf development (Fosket 1994). Exposure of etiolated seedlings to light produces seedling de-etiolation, the first step of photomorphogenesis (light development), characterized morphologically by inhibition of stem elongation, opening of the apical hook, and leaf and chloroplast development (Wei and Deng 1996).

Photomorphogenesis is a complex process in which different hormones, mainly GAs, brassinosteroids, and cytokinins seem to play a role (Chory and others 1997; Kraepiel and Miginiac 1997). It was observed long ago that etiolated pea plants are much taller than plants grown continuously under WL, and that GA_3 application enhances stem elongation of light-grown pea plants to values similar to that of etiolated plants (Lockhart 1956). This suggested that GAs are involved in the regulation of stem elongation during de-etiolation, and for many years made pea an ideal plant model system for studying the light-GA interaction. Inhibition of stem elongation by R (Kende and Lang 1964) and its reversal by FR (Behringer and others 1990; Campell and Bonner 1986) indicate that the photoreceptor is a phytochrome. The involvement of GAs in the de-etiolation process is also supported by a decrease of stem elongation of dark-grown pea seedlings treated with inhibitors of GA biosynthesis, and the reversal of this inhibition by active GAs (Campell and Bonner 1986; Kende and Lang 1964; Reid 1983; Sponsel and Reid 1992). However, there has been a long-standing

controversy over the relationship between light and GAs in photomorphogenesis (de-etiolation), particularly over whether the light inhibition of stem elongation mediated by phytochrome is the result of (1) a decrease in the content of active GAs (Campell and Bonner 1986; Sponsel 1986, in pea; Toyomasu and others 1992, in lettuce), or (2) a reduction of GA sensitivity of the stem (Reid 1988; Weller and others 1994, in pea; Ross and others 1992, in sweetpea; Toyomasu and others 1994, in rice).

Direct evidence that de-etiolation involves an alteration of GA metabolism in pea has been obtained by two independent laboratories (Ait-Ali and others 1999; Gil and García-Martínez 2000). It was found that GA_1 (the active GA in pea) content in the apical shoots of etiolated pea decreases rapidly upon transfer to WL, to about 25% within 2 h of WL treatment, and to trace levels after 4 h. The effect of light on GA_1 content was reversed when the plants were transferred again to darkness after 6 h of WL. However, contrary to expectations (Sponsel and Reid 1992), light increases the transcript levels for GA 20-oxidase and GA 3-oxidase in the apical shoot, indicating that the decrease in GA_1 content induced by light may not be explained by a reduction in the amount of these GA-biosynthetic enzymes. The increase of transcript accumulation, at least that of GA 20-oxidase, seems to be regulated by both phyA and phyB (Ait-Ali and others 1999). This agrees with results showing that pea de-etiolation by R is controlled by both phyA and phyB (Weller and others 2001). The *PsGA20ox1* transcript accumulation is probably the result of feedback inhibition due to the reduction of the GA_1 level, because it does not occur when the seedlings are treated with GA_1 before irradiation. The concentration of GA_8, the inactive product of GA_1 metabolism, increases in irradiated seedlings (Gil and García-Martínez 2000), suggesting that GA 2β-hydroxylation activity may increase during de-etiolation and thus regulate the level of GA_1. Clones from two genes coding GA 2-oxidases of pea (*PsGA2ox1* and 2) have been isolated (Lester and others 1999; Martin and others 1999) and should help to clarify this issue. Recent work claims that the drop in GA_1 concentration after exposure to light is actually controlled by phyA (not phyB) and a blue-light receptor, which down-regulate the expression of *PsGA3ox1* and up-regulate that of *PsGA2ox2*, while phyB regulates the response to GA_1 (J.B. Reid and others 2001, Abstract presented at the 17th IPGSA Congress, Brno, unpublished) (Figure 3). It is of interest, however, that the decrease of GA_1 content is transient and limited to about the first 24 h after transfer to WL; the GA_1 content in de-etiolated plants increases again up to a level similar to that found in plants grown continuously in WL (O'Neill and others 2000), probably as a result of chloroplast and leaf development. This may explain why Weller and others (1994) could not find differences of GA_1 content between shoots of etiolated and light-grown seedlings. It has been suggested that the lower rate of growth of light-grown plants compared with etiolated ones with similar levels of GA_1, would be due to a reduced sensitivity of light-grown plants to GAs (O'Neill and others 2000).

A gene encoding a putative serine carboxypeptidase, isolated from pea by differential display (*PsCP*, accession number AJ251969), is expressed in the shoot, but not in the cotyledons, of etiolated seedlings. Transcripts of the gene cannot be detected in paclobutrazol-treated seedlings, and their abundance decreases upon transfer of the seedlings to WL, parallel to the decrease of GA_1 content described before. The decrease of *PsCP* transcript levels in WL is negated by GA_3 application (M. Cercós, C. Urbez, J. Carbonell, personal communication). These results suggest that *PsCP* may play a role in the action of GA_1 during de-etiolation.

Stem Elongation

In light-grown plants, stem elongation is modulated by different light characteristics, mainly its intensity, quality (kind of radiation), and duration (photoperiod) (Smith 1994). The mediation of GAs in the plant response to these aspects of light irradiation has been investigated in different species (Figure 3).

Light Intensity

Decrease of light intensity reduces photosynthesis and leads to a reduction in dry-matter production, but also to an increase in stem elongation, which is inversely proportional to the WL irradiance intensity (Potter and others 1999). In pea, *Lotus tenuis* and *Brassica napus* it has been found that low irradiance increases the active GA levels. In the case of pea (Gawronska and others 1995), the increase in GA_1 content (up to three times) was proportional to the reduction of light intensity [down to 10% of control; control (100%) = 386 μmol m^{-2} s^{-1}] and showed a good positive correlation with stem elongation. The contents of other GAs, mainly GA_{20}, the immediate precursor of GA_1, and GA_8 and GA_{29}, the products of inactivating enzymes, also increased in pea under low irradiance conditions. This indicates that light intensity affects the flux of

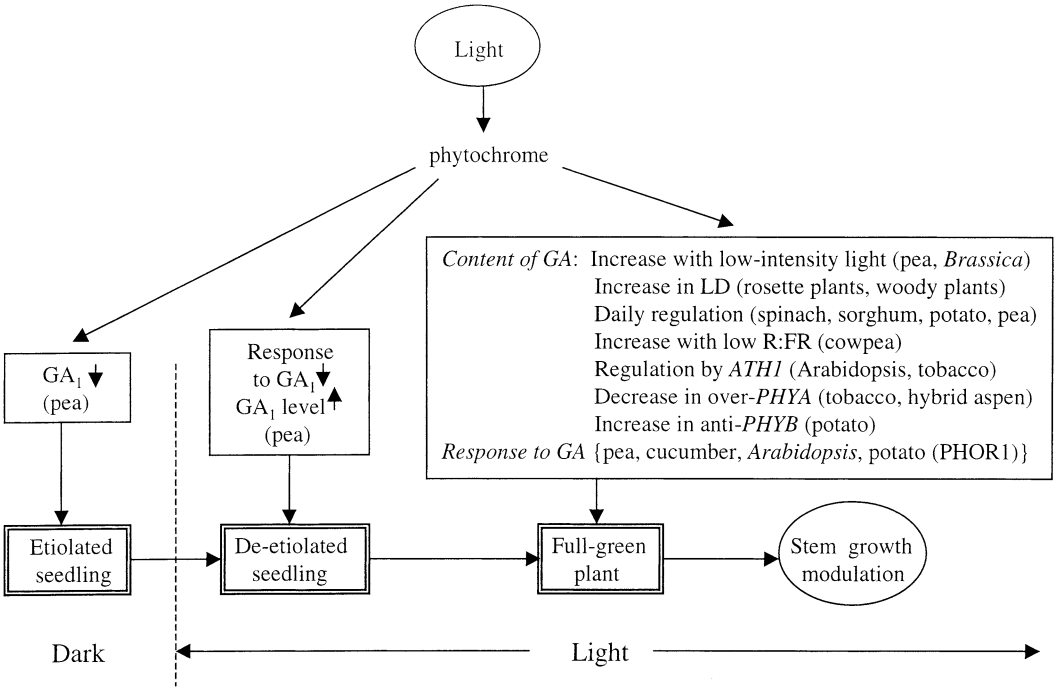

Figure 3. Effects of light on GA content and response during de-etiolation and stem growth modulation of green plants. *ATH1*, homeotic transcription factor gene of *Arabidopsis; PsCP,* serine carboxypeptidase gene of *Pisum sativum*.

at least the last part of the GA-biosynthetic pathway. In the case of *Lotus* (Clúa and others 1996) the contents of GA_1 and GA_3 were 3 to 5-fold higher in shoots of plants cultured under shade (400 µmol m^{-2} s^{-1}) compared with full sunlight (2400 µmol m^{-2} s^{-1}), while maintaining the same R:FR ratio (1.2). In *Brassica* (Potter and others 1999), GA_1 and GA_3 contents increased up to more than 20-fold, and those of GA_{20} and GA_8 more than 50 times, when irradiance was reduced from 500 to 25 µmol m^{-2} s^{-1}, while metabolic experiments indicate that GA_1 and GA_8 conjugation were reduced. Therefore, the effect of low irradiance on elongation seems to be due to an increase of GA_1 biosynthesis and to a decrease of GA_1 catabolism. In agreement with this conclusion, it has been found that irradiance intensity affects the transcript levels of *PsGA20ox1* in pea leaves, although the effect is opposite depending on the time of the day at which the material is collected: the amount of transcript was inversely proportional to light intensity at the end of the 16 h light period, while it correlated positively at the end of the 8 h dark period (Figure 4). It is necessary, therefore, to be cautious about drawing conclusions from gene expression analyses carried out at a single time of day.

Pea plants treated with paclobutrazol (to block endogenous GA biosynthesis) and GA_1 (2.5 µg per seed) elongate more when grown under 10% WL

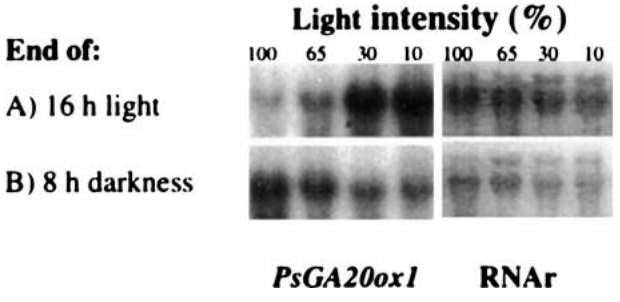

Figure 4. Effect of WL intensity on *PsGA20ox1* transcript levels in pea leaves. The RNA from the third leaves of 14 day-old plants, grown under 16 h light/8 h dark photoperiod, was analyzed (about 20 µg total RNA per lane) at the end of the 16 h light period (**A**) or at the end of the 8 h dark period (**B**). 100% = 200 µmol m^{-2} s^{-1}.

intensity than under 50%, and more than the controls (100% = 386 µmol m^{-2} s^{-1}) (Gawronska and others 1995), suggesting that light intensity may also affect GA responsiveness. The results of GA_3 application to *Brassica* plants grown under low and high intensity light, however, did not seem to confirm this hypothesis (Potter and others 1999).

Light Duration: Photoperiod

The photoperiod (actually the length of the dark period) affects different developmental processes

such as stem elongation and bolting, bud dormancy, flowering, and tuber formation. The evidence is consistent with both phyA [in long day (LD) plants] and phyB [in short day (SD) plants], playing an important role in photoperiod perception (Jackson and Thomas 1997). Light perception occurs in the leaves but the response depends on the signal being transported to other parts of the plant. The evidence for a role for GAs in the response of stem elongation to photoperiod will be reviewed here. Although most of the work on the effect of photoperiod on GA metabolism has been carried out on the last steps of the GA biosynthesis pathway, catalyzed by dioxygenases, there is also some evidence for earlier steps being affected by photoperiod (Figure 1). For example, *ent*-kaurene accumulation in plants in which *ent*-kaurene oxidation was inhibited with tetcyclacis was three times higher in LD than in SD in spinach, and more than double in *Agrostemma githago* (Zeevaart and Gage 1993). Photoperiod may, therefore, affect the flux through the entire GA-biosynthetic pathway.

Spinach. The involvement of GAs in photoperiod-induced bolting of LD rosette plants is well documented, mainly as a result of work from Zeevaart's group. Stem growth of spinach (*Spinacia oleracea* L.) induced by 16 h of low intensity light from incandescent lamps following the 8 h of high intensity light (LD treatment) is prevented by the application of inhibitors of GA biosynthesis and reversed by GA_3 (Zeevaart and others 1993). Early results showed that the photoperiod regulates GA metabolism, controlling the conversion of GA_{19} to GA_{20} in spinach: [^{3}H]GA_{53} was metabolized completely to [^{3}H]GA_{20} in LD (which induces stem elongation), but only to [^{3}H]GA_{44} and [^{3}H]GA_{19}. in SD (Gianfagna and others 1983). It was found later, using cell-free extracts, that LD actually regulates the two steps GA_{53} to GA_{44} and GA_{19} to GA_{20}, but not GA_{44} (in the lactone form) to GA_{19} (Gilmour and others 1986), which may explain the lower content of GA_{19} and higher content of GA_{20} upon transfer from SD to LD conditions (Gilmour and others 1986; Metzger and Zeevaart 1980). This is probably the result of higher levels of transcripts of the *SoGA20ox1* gene (that encodes a GA 20-oxidase) in the shoot apex of spinach plants grown in LD compared with those grown in SD or in darkness (Wu and others 1996). Because the *in vitro* expression product of *SoGA20ox1* is capable of metabolizing GA_{53} to GA_{44}, and GA_{19} and GA_{20}, the *SoGA20ox1* gene may encode the enzyme purified partially by Gilmour and others (1986) that is regulated by photoperiod. It has been suggested that there is another GA 20-oxidase in spinach catalyzing specifically the conversion of GA_{44} to GA_{19} (Wu and others 1996), a step not regulated by LD (Gilmour and others 1986).

Although LD increases dramatically the content of GA_{20}, the active GA in the control of stem elongation in spinach is GA_1, and its content also increases progressively with the number of LD cycles (Zeevaart and others 1993). The effect of photoperiod on the expression of *GA3ox* genes in spinach, however, is still not known.

Silene. Work with *Silene armeria*, another LD rosette plant, has shown that stimulation of stem elongation by LD also depends on GAs because: (1) application of tetcyclacis prevents the effect of LD, and (2) the content of GA_{53} decreases with LD treatment, while the contents of GA_{19}, GA_{20}, and GA_1 increase (Talon and Zeevaart 1990). The increase of GA_1 is particularly high (up to 30 times) in the subapical tissues (0.5–3.5 mm) (Talon and others 1991a). It is of interest that the higher content of GA_1 in the apex of *Silene* depends on LD treatment to mature leaves, regardless of the kind of light treatement (SD, LD, or darkness) given to the apex (Talon and Zeevaart 1992), supporting the concept that a signal needs to be transported from the leaves for the modification of GA metabolism in the apex.

Arabidopsis. In *Arabidopsis*, the transcript levels of *AtGA20ox1* (previously *GA5*) also increase in plants transferred from SD to LD conditions (Xu and others 1997). The increase, however, was only about 40%, which may not be unexpected considering that *Arabidopsis* is a facultative LD plant.

The content of two active GAs, GA_1 and GA_4, increases in the rosette leaves of *Arabidopsis* when the plants are transferred from SD to LD, but the amount of transcript of *AtGA3ox1* is not affected by the photoperiod (Xu and others 1997). Therefore, the enhancement of stem elongation by LD in *Arabidopsis* may be controlled only by *GA20ox* genes. This is apparently in contrast with observations in *Pharbitis nil*, a SD plant, where the metabolism of [^{3}H]GA_{20} to putative [^{3}H]GA_1, as well as the epicotyl response to applied GA_{20}, were larger in LD (continuous light) than in SD (Yang and others 1996).

Woody Plants. In many woody plants, SD induce cessation of stem growth and formation of terminal buds, while LD sustain stem elongation. In *Salix pentandra*, the application of GA_{20} and GA_1, but not that of GA_{53}, GA_{44}, and GA_{19}, stimulates stem elongation under SD (Junttila and Jensen 1988). The contents of GA_{20} and GA_1 in *Salix* increase when the plants are transferred to LD (Olsen and others 1995a): the increase of GA_1 is transient and localized

to the 5–10 mm subapical zone (Olsen and others 1995b, 1997a), a result similar to that found previously in *Silene* (Talon and others 1991a). Although this suggests that the photoperiod controls the conversion of GA_{19} to GA_{20} in *Salix*, no effect of photoperiod was observed, however, on the conversion of [^{3}H]GA_{19} to [^{3}H]GA_{20} (Olsen and others 1995c). In the Norway spruce (*Picea abies* [L.] Karst.), GA_1, and GA_4, but not GA_{20}, induced shoot elongation in SD; this observation, together with the lower content of GA_4 and GA_1 in SD than under continuous light, indicates that LD may control GA biosynthesis at the level of GA 3-oxidation (Moritz 1995). The cloning of genes coding *GA20ox* and *GA30x* in *Salix* and *Picea* is necessary to clarify this issue.

In the hybrid aspen (*Populus tremula* L. × P. *tremuloides* Michs.), the sustained stem growth under LD conditions is associated with higher GA_{19}, GA_{20}, and GA_1 contents in the shoot compared with SD conditions (Olsen and others 1997b). Transfer from LD to SD reduces the level of GA_1 (more in the young leaves than in the internodes) and produces the accumulation of GA_{19} and GA_{20} in early elongating internodes, and of GA_{19} in leaf blades, indicating that SD reduce GA 20-oxidase activity (Olsen and others 1997b). This hypothesis is supported by the expression of a *GA20ox* gene from hybrid aspen (*PttGA20ox1*), which is negatively regulated (transcript levels) by SD in expanding leaf tissue (Eriksson and Moritz 2001). Moreover, when [^{14}C]GA_{12} was applied to shoots of plants grown under SD there was an accumulation of [^{14}C]GA_{53}, probably the main *in vivo* substrate of *PttGA20ox1* (Eriksson and Moritz 2001).

The over-expression of oat *PHYA* in hybrid aspen reduces stem growth, as a result of shorter internodes, and overcomes the increase of GA content induced by LD in non-transformed plants (Olsen and others 1997b). The transcript levels of *PttGA20ox1* were also reduced in transgenic aspen over-expressing *PHYA* in LD (Eriksson and Moritz 2001).

Daily Changes of Gibberellin Content and Metabolism

In spinach, a LD plant, the levels of all GAs analyzed from the early C-13 hydroxylation pathway are higher at the end of the light than at the end of the dark period, suggesting an enhanced flux through the GA-biosynthetic pathway in light (Talon and others 1991b). The content of some GAs has been shown to change diurnally in *Sorghum bicolor*, a SD plant (for example, GA_{20} and GA_1 levels increase at lights-on, peak in the afternoon, and decrease to a minimum in darkness) (Foster and Morgan 1995). However, no significant differences in GA content were found during the diurnal day and night cycle in *Begonia* (Myster and others 1997). In WT sorghum, SD induce flowering and advance the GA_{20} and GA_1 maxima, an effect also observed in *phyB* mutants (ma_3^R) under LD (Lee and others 1998). Thus, phyB seems to control the daily regulation of GA_{20} biosynthesis in sorghum, and it would be of interest to know the effect of SD versus LD conditions, and of the phyB mutation on the daily expression of GA biosynthesis genes.

This kind of work has been carried out in *Solanum tuberosum* ssp. *andigena*, a species strictly dependent on SD for tuber formation. In this case, the expression of three genes encoding GA 20-oxidases (*StCA20ox1*, 2, and 3) show maxima at about 4 h after the lights were turned off. LD conditions, which prevent or delay tuberization, induce an additional peak of *StGA20ox1* and *StGA20ox3* transcripts in the leaves late at night, compared with plants grown under inductive SD conditions (Carrera and others 1999). *StGA20ox1* transcripts show a strong induction by the light-on signal, both in WT and anti-*PHYB* plants, but the levels in anti-*PHYB* were always higher than in WT plants (Jackson and others 2000). It is not known whether these changes of gene expression also correspond to diurnal changes in the GA content. Although some cycling of *StGA20ox1* transcripts is also observed, particularly in anti-PHYB plants, it is not clear whether this is the result of a circadian rhythm (Jackson and others 2000).

In pea leaves, the content of *PsGA20x1* transcripts also changes daily, increasing progressively during the 8 h dark period, remaining high during the first hours of the 16 h light period, and then decreasing progressively (Figure 5). When the plants were maintained in constant light after an 8 h dark period, the levels of *PsGA20x1* transcripts remained essentially unchanged (Figure 6), indicating that the observed changes may not be the result of circadian rhythm.

The physiological significance of the daily changes of GA contents and *GA20ox* transcript abundance is still unclear. In any case, the results presented strengthen again the critical importance of the light culture conditions and the time of day at which samples are collected for GA analysis (see also the section on Light Intensity).

Light Quality, Shade Avoidance and End-of-Day FR Treatment

Stem growth of green plants is modulated by the quality of light (absolute and relative intensity of

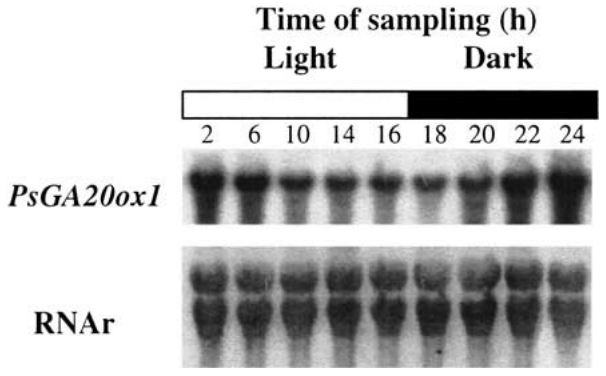

Figure 5. Daily changes of transcript levels of *PsGA20ox1* in pea leaves. The plants, grown under 16 h light (200 μmol m^{-2} s^{-1})/ 8 h dark photoperiod, were 14 days old at the time of the experiment. The RNA from third leaves was analyzed (about 20 μg total RNA per lane) at the specified times during the 16 h light and 8 h dark periods.

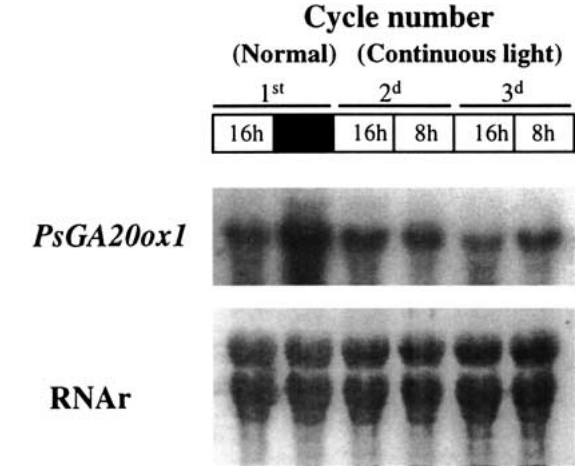

Figure 6. Transcript levels of *PsGA20ox1* in pea leaves during continuous light after a 16 h light/ 8 h dark cycle. The samples were taken at the end of the periods specified at each of three consecutive cycles. The plants, grown under 16 h light (200 μmol m^{-2} s^{-1})/ 8 h dark photoperiod, were 14 days old at the time of starting the experiment.

different radiation wavelengths) perceived by the photoreceptors. Although apparently conflicting results can be found in the literature, there is evidence that modulation of stem elongation by light quality is mediated by the effect of phytochrome on both GA biosynthesis and GA response (Kamiya and García-Martínez 1999). For instance, over-expression of oat *PHYA* in hybrid aspen (Olsen and others 1997b) decreases the content of active GAs and results in a short phenotype that can be reversed by GA application, and over-expression of the antisense potato *PHYB* gene produces taller potato plants (Jackson and others 1996) with higher GA_1 content in the apical shoot (Martínez-García and others, this issue). Two *phyB* mutants, *ein* of *Brassica rapa* (Devlin and others 1997) and ma_3^R of *Sorghum bicolor* (Childs and others 1997) are taller than WT and contain higher levels of GA_1 (Beall and others 1991; Rood and others 1990). In the case of sorghum, however, it was found subsequently that the GA content fluctuates diurnally and that the *phyB* mutation alters the daily rhythm rather than the actual GA_1 content (Foster and Morgan 1995). In contrast, the *phyB* mutants of pea (*lv*) (Weller and others 2001), cucumber (*lh*) (López-Juez and others 1995) and *Arabidopsis* (Reed and others 1993), that also have an elongated phenotype, show no apparent differences in GA content compared with WT.

Light arriving at the surface of plants growing under or close to other plants has a lower R:FR ratio due to its enrichement in FR (Smith 1994). This light, therefore, increases the plant phytochrome ratio P_r:P_{fr}, which acts as a signal to stimulate stem growth (shade-avoidance syndrome) (Smith 1994). Treatment with FR at the end of the light period, the so-called ''end-of-day (EOD)-FR irradiation,'' also enhances stem elongation. *PhyB* mutants of different species grown in light have longer stems than WT plants and do not respond to FR. When these mutants are treated with inhibitors of GA biosynthesis they become shorter and do not elongate in response to FR. These results suggest that phyB is the main photoreceptor involved in the shade-avoidance syndrome and that the effect of FR is mediated by GAs. As in the case of de-etiolation, two alternative explanations have been proposed for the interaction of light (R/FR) and GAs in light-grown plants: (1) FR alters GA biosynthesis and catabolism, and (2) FR increases the sensitivity to endogenous GAs.

Pea. In pea, the response to FR is maintained in the GA-overproducing mutant *sln*, which is assumed to have saturating GA_1 levels (Weller and others 1994). On the other hand, the *lv* mutant (phyB deficient) lacks the EOD-FR response (Nagatani and others 1990), and its elongated phenotype in the light was negated when the levels of endogenous GA_1 were genetically reduced (Weller and others 1994). The level of GA_1 in the apical shoot, however, was not changed in *lv* compared with WT plants, though much lower contents of GA_{20} and higher of GA_8 were found (Weller and others 1994), indicating a higher flux through the GA biosynthesis pathway in the mutant.

Cucumber. Seedlings of the cucumber *lh* mutant show a response to the absence of phyB even in the presence of a saturating dose of GA_4 (López-Juez and others 1995; Moe and others 2001, Abstract presented at the 17th IPGSA Congress, Brno, unpublished). Application experiments of GA_4 (the active GA in cucumber) and GA_9 (the immediate precursor of GA_4) with an inhibitor of GA 3-oxidase and GA 2-oxidase are not consistent with phyB altering the turnover of GAs. Moreover, the level of GA_4 in entire seedlings was not clearly affected in the *phyB* mutant since it was higher than in WT only during the phase of sustained growth (López-Juez and others 1995). The authors proposed that GA_4 and phytochrome control cell elongation through separate mechanisms interacting near the terminal response.

Arabidopsis. Work with the *Arabidopsis ga1 phyB* double mutant has shown that the presence of a completely functional GA system is necesary for the full expression of the *phyB* mutant phenotype (Peng and Harberd 1997), an observation compatible with a role for phytochrome in GA metabolism and/or the response to GA. The phyB mutants of *Arabidopsis* elongate more that the WT in response to GA_3 and GA_4, indicating that the mutation increases the response to GA (Reed and others 1996). In the analysis of GA content in entire seedlings, no significant differences were found in the contents of GA_1, GA_8, and GA_{19}, but there was a 2-fold increase GA_{20} content in the mutant. Unfortunately, the effect of the *phyB* mutation on the content of GA_4 (the main active GA in *Arabidopsis*) and other GAs of the non-hydroxylation pathway (Reed and others 1996), as well as on the expression of GA biosynthesis genes is not known. It is therefore not possible yet to discard a possible effect of phytochrome on GA metabolism in *Arabidopsis.*

Cowpea. The cowpea (*Vigna sinensis* L.) epicotyl, which elongates significantly in response to both light extension with low intensity tungsten light (which contains relatively low R:FR ratio), and to the EOD-FR treatment (García-Martínez and others 1987; Martínez-García and Garcia-Martínez 2000), has been used to investigate the role of GAs in FR-stimulated stem elongation. Early work showed that treatment of the seedlings with paclobutrazol abolished the effect of FR (García-Martínez and others 1987), and that the response of these seedlings to FR was recovered when treated with GA_{20}, or GA_1 (Martínez-García and García-Martínez 1992a). The higher response of the epicotyl to applied GA_1 after FR irradiation was constant in seedlings up to 11 days-old, whereas the response to FR of non-GA_1-treated epicotyls decreased progressively and was almost none at day 11 (Martínez-García and García-Martínez 1992b). This indicates that the EOD-FR response of cowpea depends on the level of endogenous GA_1. This hypothesis is supported by the observation that the content of GA_1 in the epicotyl decreases progressively with age, parallel to the response to FR (Martínez-García and García-Martínez 2000). FR enhances the amount of $[^3H]GA_1$ produced from applied $[^3H]GA_{20}$ as well as the amount of applied $[^3H]GA_1$ that remains unmetabolized in the epicotyl (Martínez-García and García-Martínez 1995, 2000). The content of GA_1, was higher in epicotyls of FR-treated than R-treated explants or entire seedlings, whereas it was not altered in the leaves (Martínez-García and García-Martínez 2000). These results suggest that phytochrome may control stem elongation in light-grown seedlings by regulating the inactivation of GA_1 by 2β-hydroxylation. This regulation may take place in the epicotyl itself because FR has to be perceived by the epicotyl (not by the leaves) (García-Martínez and others 1987) to modulate elongation, and no changes in the content of GAs occur in the leaves after EOD-FR treatment (Martínez-García and García-Martínez 2000). Determining the effect of EOD-FR on the expression of *GA2ox* genes will be necessary in order to test this hypothesis.

Tobacco. Overproduction of oat *PHYA* in transgenic tobacco results in shorter plants with reduced GA levels (Jordan and others 1995). In these plants, the shade-avoidance response, which depends on phyB, is suppressed as expected (because phyA and phyB play antagonistic effects on stem elongation in response to FR), causing proximity-conditional dwarfing (Robson and others 1996).

The *ATH1* gene is a light-regulated homeobox transcription factor that is derepressed in the *cop1* and *det1* photomorphogenic *Arabidopsis*, mutants (Quaedvlieg and others 1995). Transgenic *Arabidopsis* and tobacco plants with deregulated *ATH1* expression (antisense and overexpressing) are specifically affected in phyB-mediated responses (like R-induced de-etiolation and shade avoidance response). The over-expression of *ATH1* in tobacco significantly reduces the levels of GA_{20} and GA_1 and produces a dwarf phenotype that can be rescued by applied GA_3. This suggests that *ATH1* is a positive mediator of a phyB-specific signal transduction cascade that regulates the final steps of GA biosynthesis (Proveniers and others 2001, Abstract presented at the 17th IPGSA Congress, Brno, unpublished).

Tuber Formation

Evidence that GAs play a role in the control of potato tuberization came originally from the observations that the application of GA_3 inhibits tuber initiation, whereas the application of inhibitors of GA biosynthesis (Ewing and Struik 1992; Jackson and Prat 1996), or the blockage of GA biosynthesis in the *ga1* mutant (Van den Berg and others 1995) promotes tuber initiation. This hypothesis is also supported by the following observations: (1) The content of GA_1 (the endogenous active GA) in the stolons (underground stems), which is high when they are elongating, decreases when the stolons start to swell after transferring to inductive (high sucrose concentration) conditions (Xu and others 1998); (2) The photoperiod controls tuber formation, which is generally enhanced or absolutely dependent (as in *Solanum demissum* and *S. tuberosum* ssp. *andigena*) on SD conditions (Jackson 1999), and an additional peak of transcript content for *StGA20ox1* (one of the three *GA20ox* genes isolated from potato that is strongly expressed in leaves) and *StGA20ox3* is observed in WT potato *andigena* under non-inductive LD conditions (Carrera and others 1999). (3) Over-expression of *StGA20ox1* in potato *andigena* produces taller plants that require more SD to tuberize, whereas antisense *StGA20ox1* potato plants, which contain decreased levels of GA_{20} and GA_1 in the shoot, are shorter and tuberize earlier (Carrera and others 2000).

The photoperiod dependence of S. *tuberosum* ssp. *andigena* is lost in anti-*PHYB* potato lines, which tuberize equally well under SD, LD, and SD plus night break conditions (Jackson and others 1996). This means that phyB produces an inhibitor under LD (non-inducing conditions) which blocks tuber formation. This graft-transmissible inhibitor is produced in the leaves as a result of LD and is absent in anti-*PHYB* plants (Jackson and others 1998). However, anti-*PHYB* potatoes are taller than WT plants and contain higher transcript levels of *StGA20ox1* in the leaves (Jackson and others 2000) and of GA_1 (2- to 5-fold) in the shoot (Martínez-García and others, 2002 this issue). This raises an apparent paradox: anti-*PHYB* potato plants have reduced sensitivity to photoperiod and produce tubers under LD while having higher content of GA_1 in the apical shoot (which should prevent tuber formation). It has been suggested that this could be explained if one of the roles of phyB is to enhance the transport of either a GA or a regulator of GA biosynthesis to the stolons under LD, and that in the absence of transport it would accumulate in the leaves and thereby stimulate shoot elongation (Kamiya and García-Martínez 1999). An alternative hypothesis is that a reduction of phyB level in potato plants constitutively switches on both the short-term responses to SD (for example, GA-dependent elongated shoot) and the photoperiod-dependent pathway to tuberization (in response to a tuber-inducing signal from the leaves) (Martínez-García and others, this issue). The quantification of GAs, and the expression of different genes of GA biosynthesis and photoperiod molecular markers (for example, PHOR1, Amador and others 2001), separately in the leaves and stolons of WT and anti-*PHYB* potatoes, should help to clarify the role of GAs in the mechanism of tuber formation regulated by phytochrome.

As well as inducing tuberization in potato, SD also up-regulates the expression of *PHOR1*, a gene isolated recently that encodes a protein with homology to the *Drosophila* segment polarity protein armadillo (Amador and others 2001). Antisense *PHOR1* plants are semidwarf, tuberize earlier, and produce more tubers than controls; they also have increased levels of endogenous GAs (mostly GA_{20} and GA_{29}) and are less responsive to applied GA_3, whereas lines over-expressing *PHOR1* are more responsive to GA. The application of GA_3 induces rapid migration of PHOR1-GFP protein to the nucleus in tobacco BY2 cells. The results support the idea that the photoperiodic control of tuberization may not be explained simply by regulation of GA levels, and suggest that PHOR1 is a component of the GA signaling pathway involved in the mechanism that integrates both photoperiodic and GA signals (Amador and others 2001).

Conclusions and Prospects

There is clear evidence that some effects of light on plant growth and development are mediated by the modification of active GA levels. This includes the physiological processes of germination, de-etiolation, and the control of stem growth by light intensity and photoperiod. In all these cases, changes of both transcript levels of genes encoding enzymes of GA biosynthesis (mainly GA 20-oxidase and GA 3-oxidase) and of contents of active GAs have been shown in different species. Light quality conditions (for example, R/FR ratio, EOD-FR treatment) that modulate stem elongation have been found to affect GA metabolism and content in some cases (cowpea), but not in others (pea, cucumber, *Arabidopsis*). Because it has been shown that meaningful changes in GA content take place in localized tissues and

may in many cases, such as in *Arabidopis*, be difficult to analyze, some of these discrepancies may be resolved by collecting appropriate material for analysis, or by investigating the effect of light on the activity of promoters of biosynthetic genes using appropriate reporter genes. Nevertheless, results of application experiments and work with phytochrome mutants strongly suggest that light quality alters plant responses to GAs.

There are three areas of research that are expected to give results of interest in the next years leading to a clarification of light-GA interactions. First, it is necessary to define the specific role of the different photoreceptors (phytochromes and blue light) and wavelengths involved in each of the processes regulated by light and GAs; see for example, the case of *Arabidopsis* seed germination, where the expression of two *GA3ox* genes is regulated by R through different phytochromes (section on Seed Germination). Second, we need to identify the intermediates in the signal transduction cascade (for example, ATH1 of *Arabidopsis*), and their function. Third, to understand the effect of light on the response to GA it will be necessary to know whether and how the levels, localization, and function of factors involved in the GA signal transduction cascade are affected by light characteristics (as shown for PHOR1 in potato).

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