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The Interaction of Gibberellins and Photoperiod in the Control of Potato Tuberization

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Abstract

Solanum tuberosum ssp. *andigena* plants require a short-day (SD) photoperiod for tuber formation, a process that is also affected by gibberellins (GAs). Grafting experiments have confirmed that the photoperiod is perceived in the leaves. Tuber formation, however, usually takes place in the underground stolons. In this review, photoperiod-dependent tuberization has been divided into five chronological events: SD photoperiod perception, short-term adaptive responses to SD conditions, generation and transport of tuber-inducing signal(s), tuber formation, and long-term adaptive responses to tuber growth. Within this frame of study, the interaction of GAs and photoperiod is revised. Similar to the flowering process in *Arabidopsis,* we suggest the existence of two independent pathways that control tuber formation: a photoperiod-dependent pathway and a GA-dependent pathway. Nevertheless, photoperiod-dependent tuber formation requires the action of GAs at specific stages to orchestrate this complex process of development.

Key words: Tuberization; Potato; Gibberellins; Photoperiod; Day-length; Flowering; *Solanum tuberosum* ssp. *andigena*

Introduction

Tuber formation in potato is affected by different environmental conditions including nitrogen levels, temperature, and light. Although the effect of these environmental factors is similar among the different potato species, the genotype can cause significant variations in the degree of the response to a specific environmental stimulus. For instance, photoperiod affects tuberization in all potato species, but short day (SD) conditions are a strict requirement for tuber formation only in some of them, including *Solanum demissum* and some lines of *Solanum tuberosum* ssp. *andigena* (reviewed in Jackson 1999).

Tuberization is also developmentally controlled. Even in the strict photoperiod-dependent species, plants do not tuberize until they reach a certain size or age (Ewing and Struik 1992). In addition, the endogenous levels of gibberellins (GAs) affect tuberization, as suggested by experiments in which GAs or inhibitors of GA-biosynthesis were applied. The data generated by several research groups support the idea that GAs are endogenous factors that inhibit tuberization. It is unclear, however, what the exact role of GAs is in regulating this process.

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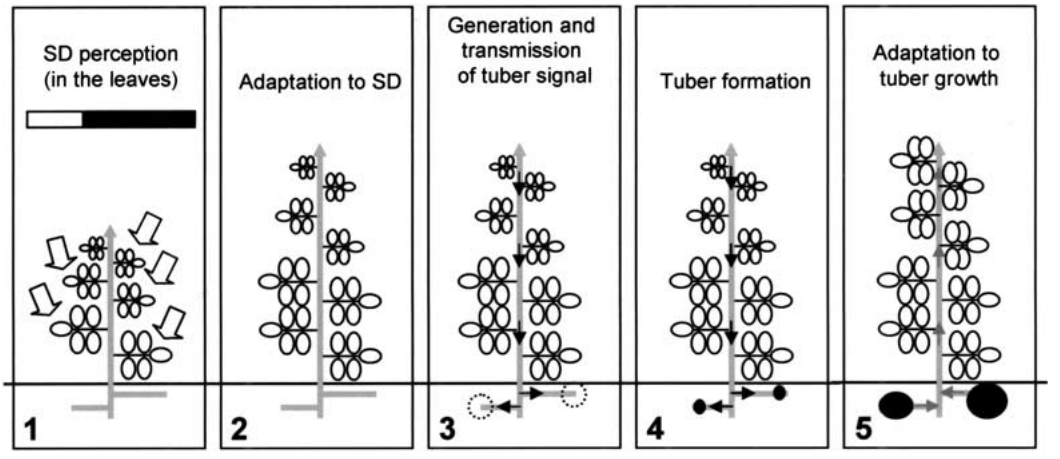

Figure 1. Diagram representing the five chronological steps in potato tuberization after transfer of the plants to SD inductive photoperiod conditions.

Some authors use the term 'tuberization' to include the whole sequence of events from stolon formation to tuber induction (Vreugdenhil and Struik 1989). Stolons are differentiated stems, usually arising as underground branches, that can develop into tubers upon induction. However, stolons may not necessarily develop into tubers, and tuber formation does not always require the previous development of stolons. For instance, under non-inductive long day (LD) conditions, *S. tuberosum* ssp. *andigena* plants produce stolons that will not develop into tubers unless transferred to SD. On the other hand, plants strongly induced to tuberize, like *S. tuberosum* ssp. *andigena* down-regulated in phytochrome B (phyB) expression, may form tubers directly on the main stem. Hence, we will refer to tuberization as tuber formation strictly. In this review we will analyze the role of GAs in five chronological events in tuberization (Figure 1): (1) perception of the SD photoperiod, (2) adaptive responses to SD photoperiod, (3) generation of tuber-inducing signals, (4) tuber formation, and (5) adaptive responses of the plant to tuber development and growth.

Methods

Plant Material and Growth Conditions

The potato cultivar used in our experiments was *Solanum tuberosum* ssp. *andigena* line 7540 (Jackson and others 1996). Plants were propagated (1) by *in vitro* culture of single node stem cuttings in MS media (Murashige and Skoog 1962) supplemented with 2% sucrose, (2) from apical cuttings from adult plants in soil, or (3) from sprouts growing from stored tubers. Plants were grown as indicated elsewhere (Jackson and others 1996). After potting, plants were grown for about 6 weeks under greenhouse conditions (16 h light). When the plants were about 6 weeks old they were transferred to long day (LD) or short day (SD) chambers. LD chambers provide 16 h light and 8 h darkness. SD chambers provide 8 h light and 16 h darkness (Amador and others 2001). Plant height was measured as indicated in Figure 2.

Anthocyanin and Chlorophyll Extraction and quantification

Anthocyanins were extracted as described elsewhere (Schmidt and Mohr 1981). The absorbance at 535 (OD_{535}) and 650 nm (OD_{650}) was measured and anthocyanin accumulation was calculated as OD_{535}-OD_{650} per g of FW (Schmidt and Mohr 1981). Chlorophyll was extracted as described elsewhere (Jackson and others 1996). The OD_{645} and OD_{663} were measured and chlorophyll levels (in mg per g of FW) and chlorophyll a/b ratio were calculated (Chory and others 1991).

Gibberellin Content Analysis

Plant material was harvested and directly frozen in liquid nitrogen. GAs were extracted, purified, and quantified by GC-MS, as described elsewhere (Carrera and others 2000).

Results and Discussion

Perception of Photoperiodic Conditions

Grafting experiments performed in potato, tobacco, and other species demonstrated that leaves are the main site of perception of the photoperiodic condi-

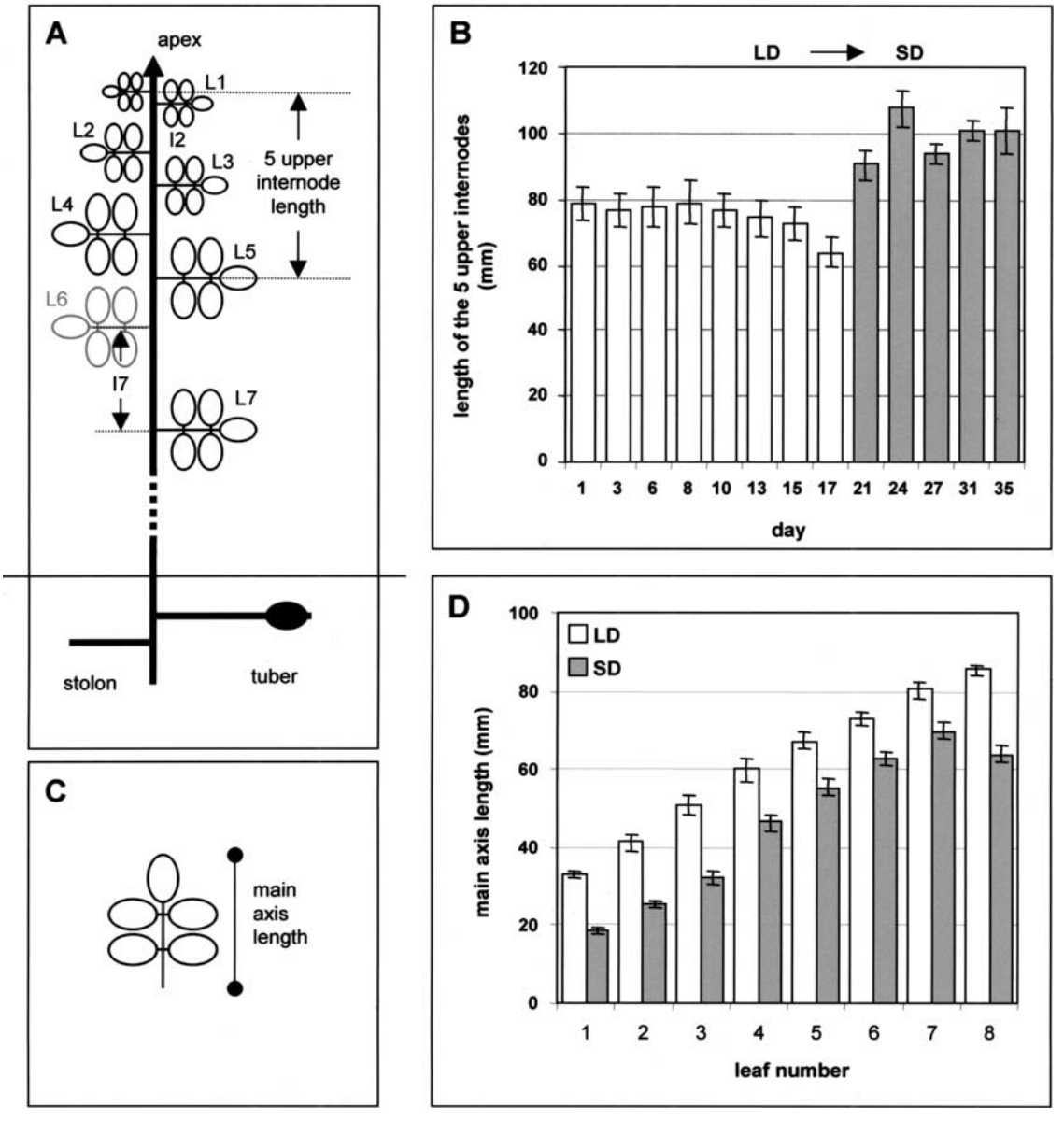

Figure 2. Morphological changes of the aerial parts of potato plants growing under LD or SD. (**A**) Diagram of the aerial parts of potato plants and the region measured in **B**. I, internodes; L, leaf. Numbering starts in the apical part of the plant. L7 refers to the leaf located at the base of 17. (**B**) Stem length of potato plants growing under LD after transfer to SD. Stem length was first recorded when the plants were about 6-weeks old (they had about 12 nodes) (day 1). Until day 18, plants were growing under LD (white boxes), and afterwards transferred to SD (grey boxes). (**C**) Diagram of the leaf axis length measured in D. (**D**) Main axis of different age leaves from plants grown differentially for 3 weeks under LD or SD. Values in B and D are means of 6 plants ± SE.

tions controlling tuberization (in potato) and flowering (in tobacco and other species) (King and Zeevaart 1973; Kumar and Wareing 1973; Lang and others 1977). The involvement of the photoreceptors phytochromes (phys) in photoperiod perception was deduced from classical studies in which the inhibitory effect of long days (LD) on tuberization was mimicked in SD by giving a night break (NB) light treatment in the middle of the dark period. The quality of the light used in the NB revealed that red light was more effective than light of other wavelengths. Moreover, the inhibitory effect of the red light NB was abolished by an immediate far-red light treatment, providing evidence for the involvement of the phys in this process (Batutis and Ewing 1982). Of all the phy family members, only the role of phyB has been assessed in the photoperiod control of potato tuberization. Transgenic

plants with reduced levels of phyB (anti-phyB plants) lost their photoperiod dependence and tuberized constitutively under both LD and SD conditions (Jackson and others 1996).

Phytochrome, and specifically phyB, also plays a role in the photoperiodic control of flowering, in both SD- and LD-plants. In rice, a facultative SD plant, and in *Arabidopsis*, a facultative LD plant, a deficiency in the synthesis of the chromophore required for phy function results in early flowering (Goto and others 1991; Izawa and others 2000). In *Sorghum bicolor*, a SD plant, and in *Arabidopsis*, *phyB* loss-of-function mutations also cause early flowering (Childs and others 1997; Koornneef and others 1998; Lin 2000). The photoperiodic control of flowering is thought to be mediated by the interaction of a clock mechanism or circadian rhythm with the mechanisms involved in photoperiod perception. GAs are not known to have a role in the specific step of photoperiod perception, a process directly carried out by the photoreceptors, cryptochromes and phys. So far, other photoreceptors or clock mechanisms that regulate flowering in *Arabidopsis* have not been investigated for their involvement in the control of potato tuberization.

Adaptive Responses to the Inductive SD Photoperiod

Immediately after transfer of the plants from LD to SD conditions, a series of short-term developmental and morphological changes take place. Some of these changes probably result from an adaptation of the plant to the reduction in light hours whereas others are related to the generation of the tuber-inducing signal (see next section). At present, it is not known whether both types of changes take place independently, are closely related, or are consequences, of each other.

As a way to illustrate some of these short-term changes, we measured stem and leaf elongation in potato plants shortly after transfer from LD to SD. As a measure of stem elongation, we recorded the length of the 5 upper (younger) internodes (5UI; Figure 2A). In plants transferred to SD, the 5UI are about 20–30% longer than those of plants growing under LD (Figure 2B). This increase in stem elongation is detectable as early as 3 days after transfer to SD (plants were transferred to SD on d 18) (Figure 2B). After 3 weeks of growing under SD conditions, the length of the 5UI still remained about 20–30% longer than the length of the corresponding region from LD plants (data not shown).

Leaf growth is another adaptive response to changes in day length. For simplification, we measured the length of the leaf main axis as an indication of its growth (Figure 2C), as other features of the leaves, like leaflet area, followed a similar pattern (data not shown). In plants growing for 3 weeks under SD, leaf length was about 20–40% smaller than in plants kept under LD, at all the stages of development (Figure 2D). These changes in leaf growth are also short-term, although the overall differences become more obvious the longer the plants have been under SD, as a result of the higher number of leaves affected.

Both short-term changes in leaf growth and stem elongation occur before any detectable tuber formation (the first tubers appeared normally not before 15 days after the transfer to SD). However, the lack of molecular markers for early events in tuberogenesis makes it difficult to establish whether these changes are concomitant with or precede tuber initiation.

Stem and leaf main axis length are traits related to endogenous GA levels or responsiveness to these hormones (Amador and others 2001). However, it remains to be established whether these photoperiod-induced changes are regulated by GAs. An increment in stem $GA_{1/4}$ levels or responsiveness might explain the higher stem elongation under SD, and a reduction in leaf $GA_{1/4}$ levels or responsiveness might explain the reduced length of the leaf main axis. It has been shown that GA activity decreases in leaves exposed to SD, and as few as 2 days under SD cause a decline in the GA activity of *andigena* leaves (Railton and Wareing 1973). Furthermore, in the same cultivar GA activity was found to be lower in leaves and stems from SD-grown compared with LD-grown plants (Machácchová and others 1998). However, we need to be cautious with this information, because GA activity refers to data from bioassays, which do not discriminate between bioactive GAs and their precursors (which can be metabolized to bioactive GAs in the plants used for the bioassay). Moreover, the material harvested for analyses corresponded to adult leaves and nodes from the central region of the stem that already had stopped growing (Machá chová and others 1998). Advances in GA quantification techniques by GC-MS have led to the important finding that GA levels are locally regulated at the organ level (Martínez-García and others 2000) and even within an organ (O'Neill and others 2000). Therefore, our physiological observations would suggest that after SD transfer of the plants, high levels of $GA_{1/4}$ within the stems may account for the higher stem elongation and lower levels of $GA_{1/4}$ in the leaves for their altered growth. Moreover, such changes in GA distribution would not

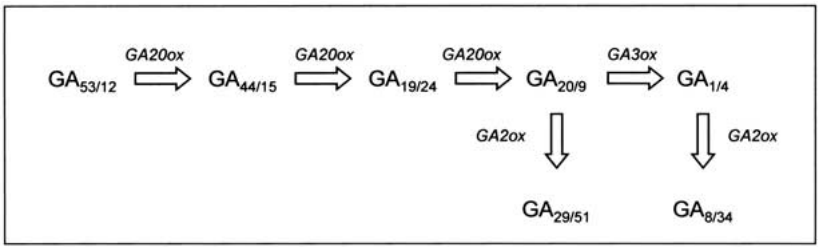

Figure 3. Diagram of the last reactions in the GA-biosynthesis pathway in potato plants.

preclude the reported reduction of GA activity in leaves or stems (Railton and Wareing 1973; Machá chová and others 1998).

To examine further the proposed role of GAs in these short-term responses, mRNA levels of *GA20ox, GA3ox,* and *GA2ox* in LD- and SD-grown plants were analyzed. These genes encode the enzymes involved in the final steps of GA biosynthesis (see Figure 3). RNA analyses indicate that genes involved in GA metabolism are tightly regulated by both development and photoperiod (data not shown). However, quantification of GAs separately in the leaves, stems, and stolons of wild-type plants growing under SD and LD conditions would be necessary to confirm these observations and clarify whether the observed changes in the expression of *GA20ox, GA3ox, and GA2ox* reflect local changes in GA contents in response to photoperiod. Our data suggest that GA levels have an important role in the short-term adaptive responses of potato plants to photoperiod conditions, the altered morphology of SD plants being very likely mediated by local changes in GA contents.

Generation of the Tuber-inducing Signal

After perception of the inductive photoperiodic conditions in the leaves, some sort of signal must be produced and transmitted from these organs to the responsive tissues, usually the underground stolons, where tuber formation begins. This was best shown with grafting experiments in which leaves from potato plants induced to tuberize (grown under SD conditions) caused non-induced plants onto which they were grafted to tuberize, even though these plants were maintained under non-inducing conditions after grafting (reviewed in Ewing and Struik 1992).

Grafting experiments also showed that phyB is involved in the production of a transmissible inhibitor of tuberization in the aerial part of the plants, since wild type (wt) plant stocks (which contain the stolons) were induced to tuberize in LD when grafted with a scion (the shoot) from an anti-phyB plant. However, in these experiments, tuberization did not occur if some leaves were left on the wt stock plant. The reciprocal grafting of a wt scion onto an anti-phyB stock resulted in the inhibition of tuberization on the antisense stock under LD. These results indicate that an inhibitor of tuberization does exist in the leaves of non-induced plants, and that phyB would be involved in the production of that inhibitor under non-inducing LD conditions (Jackson and others 1998). Moreover, these and other grafting experiments indicate that the tuberizing signal is complex in nature and probably the result of a balance between positive and negative components (Ewing and Struik 1992; Jackson 1999).

Alteration of endogenous GA levels by application of ancymidol, a GA-biosynthesis inhibitor, can overcome the strict SD requirement for tuberization, resulting in plants that produce tubers after about 3 weeks of treatment under LD (Jackson and Prat 1996). However, there are no data on the tuberization time of ancymidol-treated plants transferred to SD. In the dwarf *ga1* mutant of *S. tuberosum* ssp. *andigena,* which is thought to be partially blocked in the 13-hydroxylation of GA_{12}-aldehyde to GA_{53} (van den Berg and others 1995), reduction in the endogenous GA levels results in plants that tuberize after several months growing under LD non-inductive conditions. However, these plants tuberize very early after transfer to SD inductive conditions (less than 1 week, compared with 3–4 weeks for wt plants). Changes in the expression of *GA20ox* (encoding GA 20-oxidase, the enzyme catalyzing the synthesis of the immediate precursors of bioactive GAs; see Figure 3), in transgenic plants correlated inversely with the tuberization time: reduced *GA20ox* levels resulted in semi-dwarf plants that tuberize early under SD, and increased *GA20ox* levels resulted in tall plants that tuberize late under SD. In both cases, transgenic plants do exhibit a strict requirement for SD in order to tuberize (Carrera and others 2000). These results indicate that these plants respond normally to photoperiod. They also show that although GAs

have a role in inhibiting photoperiod-dependent tuberization, by themselves they are unable to overcome the requirement imposed by day length.

The GA and photoperiod signals are additive in controlling tuberization time, which suggests the existence of two independent pathways that control tuber formation in potato: a photoperiod-dependent pathway and a GA-dependent pathway. These pathways for control of tuberization probably coexist and overlap with endogenous regulatory mechanisms that control some aspects of GA metabolism involved in the short-term adaptive morphological responses to the reduced day-length (data not shown).

Over-expression of *GA20ox* under the control of a leaf-specific promoter delays tuberization as does over-expression under the control of the constitutive CaMV 35S promoter (Carrera and others 2000). Two alternative and non-exclusive explanations may account for this observation: (1) a direct negative effect of GAs in the production of the tuber-inducing signal in the leaves or in inhibiting its transport to stolons; and (2) an indirect effect of the increased production of $GA_{20/9}$ in the leaves and its high mobility within the plant. In the latter case, the high levels of leaf-produced $GA_{20/9}$, the immediate precursors of the bioactive GAs, could readily reach the responsive stolons where, once metabolized into $GA_{¼}$, would delay tuber formation (see next section). The generation of transgenic plants with altered levels of expression of the genes that control the metabolism of bioactive GAs (encoding GA 3β- or 2β-hydroxylases) under organ-specific promoters might help to select the correct mechanism from the proposed alternatives.

Tuber Formation

After the arrival of the tuber-inducing signal, changes in the pattern of development at the stolon tips (ST) or the buds in isolated organs (leaf-node cuttings) result in tuber formation or tuberogenesis. Using isolated organs, the role of GAs in tuberogenesis was studied by adding GA-biosynthesis inhibitors or the bioactive $GA_{4/7}$ in the culture media. These experiments indicated a main regulatory role of GAs in controlling tuber formation, the reduction of GA levels having a positive effect and the addition of bioactive GAs an inhibitory effect on tuber initiation (Perl and others 1991; Vreugdenhil and others 1994; Xu and others 1998). Moreover, data on GA endogenous levels showed that active GA_1 content strongly decreased in the ST prior to development of the tubers (Xu and others 1998). The changes in GA_1 levels were much more dramatic in the apical part compared with the rest of the stolon, precisely in the region that will develop the tuber (Xu and others 1998). Therefore, GAs seem to play an important inhibitory role in tuberization, shifting growth away from tubers towards stolons (Ewing and Struik 1992).

During early stages of tuberogenesis, differentiation of the ST into a tuber occurs by a change in the plane of cell division and expansion. Later on, there is an accumulation of starch and a specific set of proteins (Park and others 1985). The initial changes at the ST are probably controlled by ''tuber-identity genes,'' whose action involves the switch of the fate of the ST-responsive cells from elongating stolon to swelling tubers. Unfortunately, nothing is known about the nature of these genes. The identification of the ''tuber-identity genes'' will be needed to test their activity and the role of GAs and other factors in their expression.

Adaptive Responses of the Plant to the Growth and Development of Tubers

Tuber induction and growth is followed by overall long-term morphological changes throughout the plant: leaves become larger and thinner, axillary branching is suppressed, flower buds abort more frequently, stem growth stops, and senescence is hastened (Steward and others 1981; Ewing and Struik 1992). These changes take place sequentially, some of them requiring weeks to develop. We have measured some of these responses in wt plants grown differentially under LD and SD. After around 3 weeks under inductive SD conditions, when tubers become visible, plants become paler as a result of changes in the level of pigments such as chlorophylls and anthocyanins. Chlorophyll levels in the leaves under SD were about 40% lower than those from LD-grown plants (Figure 4A). In contrast, the ratio of chlorophyll a/b levels remained unaffected by the photoperiod conditions (Figure 4B). Anthocyanin levels in the SD leaves also decreased to about 20–25% of those in LD leaves (Figure 4C). Concomitant with these changes, there is an inhibition of growth of the stem and leaf main axis (Figure 5A) (Ewing and Struik 1992), and an increase in leaf expansion (Figure 5A), which are likely to be associated with the senescence process. This inhibition of plant elongation might be the result of a reduction in GA levels, since it has been shown that exogenous application of GAs delays the onset of senescence (Menzel 1985). Taken together, these observations suggest that a reduction in GA levels may account for some of the long-term adaptive responses of potato plants to tuber growth.

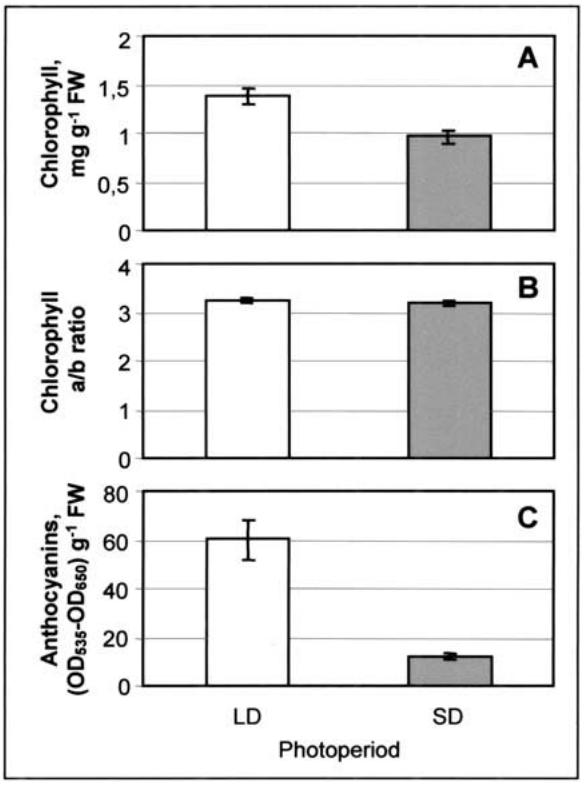

Figure 4. Chlorophyll and anthocyanin contents in leaves from potato plants growing under LD or SD for 3 weeks. Plants were about 6 weeks-old when transferred to LD and SD growth cabinets. Values are means of 3 samples ± SE.

However, not all the observed responses correlate with low levels of GAs. For instance, low levels of chlorophylls usually correlate with high rather than low GA contents. Other hormones that regulate senescence, like cytokinins (Gan and Amasino 1995; Ori and others 1999), might act together with GAs in these long-term adaptive responses. Recently, a cross-talk between auxin content and GA metabolism has been demonstrated in pea (Ross and others 2000; Ross and O'Neill 2001; Ross and others 2002, this issue). Although no data are available in potato, it would be interesting to study whether these hormones might be part of the mechanisms that underlie the local control of GA metabolism as part of the overall long-term responses of the potato plant to photoperiod and tuber growth.

The Paradox of the Anti-phyB Potato Plants

As already mentioned, reduced levels of phyB in transgenic antisense *S. tuberosum* ssp. *andigena* plants enables them to tuberize in both SD and LD conditions (Jackson and others 1996). In these plants the photoperiodic control of tuber formation is abolished, which implies that phyB is involved in the inhibition of tuberization under LD. Anti-phyB plants also display other phenotypic alterations that resemble those of slender mutants or wt plants treated with saturating doses of GAs (Jackson and Prat 1996): leaves are paler, because chlorophyll levels in anti-phyB plants are reduced compared with those of wt, and internodes are longer than those of wt plants (Jackson and others 1996). Indeed, the quantification of endogenous levels of GAs indicated that anti-phyB plants had a higher GA_1 content in the aerial part than wt plants (Table 1), a result consistent with the phenotypic traits mentioned above. Anti-phyB plants also had higher contents of GA_{20} and GA_8 and lower contents of GA_{29} than wt plants (Table 1), which suggests that phyB negatively regulates GA 3β-hydroxylation in the leaves and shoots of wt plants. Leaves from transgenic anti-phyB plants have increased levels of *GA20ox* expression (Jackson and others 2000), which might contribute to the observed increased levels of GA_{20} and GA_1 (Table 1). Whether there is also a concerted alteration in the expression levels of other GA-biosynthetic genes is under study. Taken together, these data reveal that phyB action affects GA levels and metabolism in potato leaves and shoots similarly to what has been observed in other processes and plant systems (reviewed in García-Martínez and Kamiya 1999; García-Martínez and Gil 2001). The paradox comes from the known fact that high levels of GAs are associated with inhibition of tuberization; hence, the constitutive tuberization phenotype of anti-phyB plants looks apparently inconsistent with a general increase in GA levels in these lines.

Nevertheless, not all the altered traits in the aerial parts of anti-phyB plants can be mimicked by high levels of GAs. For instance, anthocyanin levels are reduced in both the slender anti-phyB plants and the dwarf *ga1* mutant, compared with wt plants (Figure 6). Leaf main axes are shorter in anti-phyB plants in a similar manner to the *ga1* mutant and anti-GA20ox plants, compared with wt plants (see Figure 3 in Jackson and Prat 1996; Amador and others 2001). These trials also change with photoperiodic conditions (Figure 2D and 4C). Therefore, an alternative interpretation of the anti-phyB potato phenotypes is that these plants growing under LD have features that are characteristic of wt plants growing under SD (Figure 2 and 4). In other words, a reduction of phyB levels in potato plants constitutively switches on the short-term responses to SD, as well as the photoperiod-dependent pathway to tuberization. Consistent with this hypothesis, the aerial part of these plants displays an

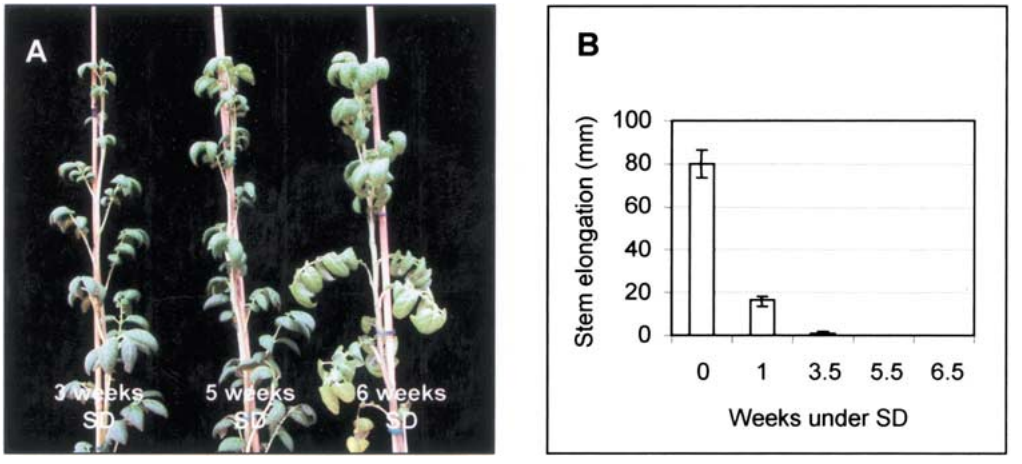

Figure 5. Long-term changes of the aerial parts of potato plants growing under SD. (**A**) Aspect of the apical part of the plants grown for 3, 5, and 6 weeks under SD. At this stage all the plants had tubers. Note the progressive yellowing of the leaves and leaflet expansion. (**B**) Influence of the time (in weeks) growing under SD conditions on stem elongation. Elongation was measured 1 week after initial recording of the 5 upper internode length. Values are means of at least 4 plants ± SE.

Table 1. Effect of Reduced phyB Levels on the Contents of GAs (ng·g^{-1} FW) in Shoots

	GA contents (ng·g^{-1} FW)								
	EXP. A						EXP. B		
	Ap			L+St			Ap+L+St		
	wt	α-4	α-10	wt	α-4	α-10	wt	α-4	α-10
GA_{44}	1.04	0.3	tr.	0.22	0.18	tr.	n.d.	n.d.	n.d.
GA_{19}	3.74	1.62	1.10	0.70	0.54	0.50	1.0	1.1	1.5
GA_{20}	13.3	15.4	16.4	5.9	6.0	6.2	3.5	4.5	3.9
GA_{29}	22.7	17.9	18.3	33.3	24.5	21.9	16.2	12.3	15.2
GA_1	0.32	0.70	0.64	0.12	0.68	0.58	0.4	1.7	0.8
GA_8	12.4	13.3	12.0	2.4	6.0	6.6	8.1	13.6	14.0

n.d., not determined; tr., traces.
Wild type (wt) and two transgenic anti-phyB lines (α-4 and α-10) plants were grown under LD conditions until 5-leaf stage. Apex (Ap), and leaves plus stems (L+St) until L4 were harvested either separately (EXP. A) or together (EXP. B) for GA extraction and quantification.

elongated phenotype, whereas the underground stolons respond to the tuber-inducing signal that is constitutively produced in the leaves (Jackson and others 1998). Tuber formation would switch on the long-term adaptive responses (senescence) to tuber growth, resulting in a reduction of chlorophyll and anthocyanin levels in these plants. This hypothesis might be confirmed by analyzing the expression of genes responsive to photoperiodic conditions, like *PHOR1* (Amador and others 2001) or other markers isolated in our laboratory. Therefore, whereas the alteration of the SD-inductive pathway (such as in the transgenic anti-phyB plants) results in plants impaired in their photoperiod responses, the alteration of the GA-pathway (such as in the *ga1* mutant, and the transgenic anti-GA20ox or anti-PHOR1 plants) promotes tuber formation but does not abolish the photoperiodic control of tuber induction (van der Berg and others 1995; Carrera and others 2000; Amador and others 2001).

Conclusions

As discussed above there are many similarities between flowering and tuberization. For instance, nitrogen levels, temperature, light quantity and quality, and assimilate levels have similar effects on flowering and tuberization, two developmental processes that are otherwise very different (Ewing

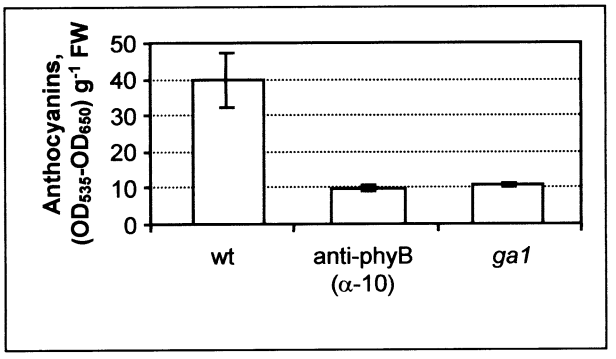

Figure 6. Anthocyanin contents in leaves from wt, anti-phyB (α-10 line) and *ga1* potato plants. Plants were grown in the greenhouse (LD) for about 10 weeks. Anti-phyB plants already had tubers. Values are the means of 5 samples ± SE.

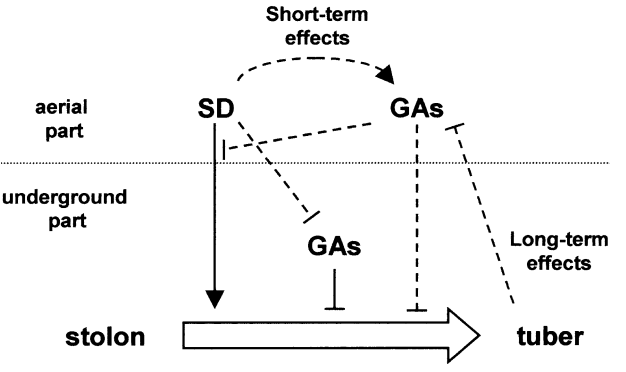

Figure 7. Proposed interaction between gibberellins (GAs) and inductive SD photoperiod in the control of potato tuberization. In the aerial parts of the plant, SD conditions are perceived and a tuber-inducing signal is generated and transported to the responsive stolons (continuous line, arrow), where tuber induction takes place. The GAs also have a role inhibiting the change of stolon into tuber (continuous line, T-bar). The proposed interactions between both signals or pathways are indicated with discontinuous lines as positive (arrow) or negative (T-bar) factors.

and Struik 1992; Bernier and others 1993; Jackson 1999). The analogy between these two processes suggests the possibility of the existence of several genetic pathways controlling tuberization in potato (Figure 7), similar to what has been shown for flowering in *Arabidopsis* (Koornneef and others 1998; Levy and Dean 1998; Piñeiro and Coupland 1998; Lin 2000). If this is the case, the existence of at least an autonomous pathway involved in potato tuberization can be hypothesized. This pathway would act coordinately with the GA and photoperiod pathways in controlling the conversion of stolons into tubers. The use of comparative genetics might help to identify some of the potato components of these pathways, their convergence points, and their role in regulating the expression of the ''tuber-identity genes.'' Furthermore, the physical separation of the leaves and the responsive stolons make potato a useful system to investigate the site of action of the as yet unidentified signaling components, and to study their possible interaction with the GA pathway.

In plants, GA levels and sensitivity are probably continuously modulated depending on the organ, developmental stage, and environmental conditions. The results generated in our laboratory clearly support an important role for GAs in photoperiodically controlled potato tuberization. These hormones appear to act at different times in this complex regulatory process, and to play a role in the adaptive changes associated with the LD to SD transfer. GAs may be involved in the generation or transport of the tuber inducing signals, in the tuberogenesis process ''per se'' and later on in the adaptive responses of the plant to the tuber sinks (Figure 7). The action of GAs appears to happen through local modulation of GA levels: whereas in the stolon tips, a decrease in GA_1 has been associated with tuber formation (Xu and others 1998), higher levels of bioactive GAs might be responsible for the initial increase in stem elongation after SD transfer. A similar mechanism has been observed in other species and, at least in some cases, it is achieved through local changes in GA_1 levels, which are mediated by a fine control of GA metabolism (Martínez-García and others 2000; O'Neill and others 2000). This points to GAs as important regulatory elements controlling the integral and coordinated growth of plants.

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