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Update on Development

Multiple Signaling Pathways Control Tuber Induction in Potato

Stephen D. Jackson

Horticulture Research International, Wellesbourne, Warwick CV35 9EF, United Kingdom

Potatoes (tubers of *Solanum tuberosum*) are grown and eaten in more countries than any other crop, and in the global economy they are the fourth most important crop after the three cereals maize, rice, and wheat. Therefore, research into potato tuber initiation and development, which enables our understanding and possible manipulation of these processes, is of great relevance. In addition to improving the yield and quality of potato harvests and increasing resistance to pathogen infection, research is also directed at improving the nutritional content of the tuber, and "pharming" which is removing the starch in the potato tuber and instead producing organic compounds such as proteins that are too expensive or cannot be produced in bacterial or yeast culture systems.

Research on potatoes has many advantages in that they are easily transformable and therefore amenable to genetic manipulation, and can be propagated rapidly both in tissue culture and through cuttings. Also, microtubers can be induced to form in tissue culture and are used in experimental systems in some laboratories. Other laboratories have used stem cuttings as small models of the whole plant. Last but not least, the potato is very closely related to the tomato, for which there is a good genetic map. The main drawback to the use of potatoes in research is the fact that most potato species are polyploid, which means that classical genetic experiments cannot be performed.

What is a potato tuber? It is not formed from a root, as is often supposed, but from an underground stem called a stolon. In conditions that are noninductive for tuberization, e.g. LD, the stolons often grow upward and emerge out of the soil to form a new shoot (Fig. 1). In tuber-inducing conditions, e.g. SD, however, the stolons grow underground until the tip of the stolon swells to form the tuber. The swelling is caused when the stolon ceases to elongate and cells in the pith and cortex enlarge and divide transversely. Later, cells in the perimedullary region enlarge and divide in random orientations to form the bulk of the tissue of the mature tuber. If the plant is put back into noninducing conditions after a tuber has been formed, the plant loses its induced state, and after a lag of up to 2 weeks stolon growth may resume from the tuber. Stolon formation occurs in both tuber-inducing and noninducing conditions; however, the angle and amount of stolon growth has been correlated with the strength of the inductive signal. Very strong induction results in "sessile" tuber formation with no prior stolon growth (Fig. 2; Van den Berg et al., 1996).

Tubers can actually form on other parts of the plant above ground, normally from axillary nodes on the stem, and in specific circumstances they can even form from flowers (Ewing and Struik, 1992). These aerial tubers are usually formed only on injured or diseased plants, where translocation of assimilates below ground has been prevented, or in plants grown in very strong inducing conditions.

This *Update* cannot possibly summarize all of the knowledge available about potato tuberization, much of which can be found elsewhere (Li, 1985; Ewing and Struik, 1992, and refs. therein). Therefore, it will principally focus on the role of the environment and possible hormonal signals involved in the induction of tuberization rather than on the postinduction processes such as starch and storage protein accumulation that occur during tuber formation.

ENVIRONMENTAL AND HORMONAL FACTORS AFFECTING TUBERIZATION

There are many factors that affect tuber formation—even the bacteria living in the root zone are reported to have an influence—but nitrogen levels, temperature, and light have the greatest effect. Although the overall effects of various environmental factors are generally consistent, the genotype and physiological age and state of the plant (e.g. whether still attached to the mother tuber or derived from cuttings) can cause considerable variations in the degree to which a plant responds to a particular environmental stimulus. Analyses have been performed on a population segregating for the ability to tuberize under a specific set of conditions to try to identify quantitative trait loci that affect tuberization (Van den Berg et al., 1996).

High Nitrogen Levels Inhibit Tuberization

Elegant experiments by Krauss and co-workers (for review, see Krauss, 1985) demonstrated that tuberization could readily be manipulated by altering the supply of

* Corresponding author; e-mail stephen.jackson@hri.ac.uk; fax 44–1789–470552.

Abbreviations: JA, jasmonic acid; LD, long day(s); PHYA and PHYB, phytochrome A and B; SD, short day(s).

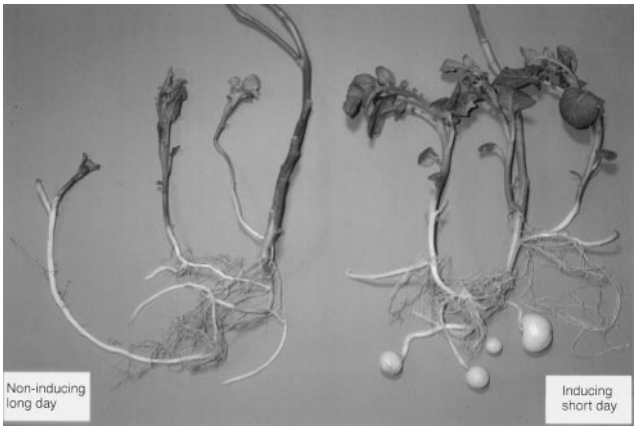

Figure 1. *Solanum demissum* plants grown in noninducing long-day (left) or inducing short-day (right) conditions.

nitrogen (either ammonium or nitrate ions) to the plant. Whereas previous studies had looked at the effect of high doses of nitrogen fertilizer on field-grown plants, Krauss and co-workers grew the plants in liquid media (hydroponic culture) in which the level of nitrogen supplied to the plant could be precisely controlled. They found that a continuous supply of between 1 and 3 mM nitrogen completely inhibited or severely delayed tuberization in otherwise inducing conditions. However, interrupting the nitrogen supply by putting the plants temporarily in nitrogen-free media for 4 to 6 d allowed tuberization to occur. If the plants are put into "excessive" nitrogen supply after they have started tuberizing, then tuber formation will cease and stolon growth may be resumed. Removal of the nitrogen from the media will then cause initiation of a second tuber from this stolon (secondary growth). Repeated cycles of high nitrogen/nitrogen withdrawal can result in the formation of "chain tubers," demonstrating that nitrogen levels play an important role in the control of tuber formation.

It is interesting that high nitrogen supply to the leaf did not prevent tuberization, even though the nitrogen content of the plants was comparable to those receiving high nitrogen through the roots. Furthermore, reducing nitrogen levels in normally noninducing conditions such as LD or high temperatures (see below) will not result in tuberization, indicating that nitrogen is probably not involved in the induction of tuberization but that it is able to repress tuber formation once induction has taken place.

It is not yet known how nitrogen levels cause the inhibition of tuberization, although there are reports that nitrogen withdrawal affects phytohormone levels, causing a reduction in GA levels and an increase in ABA levels (Krauss, 1985). An alternative hypothesis is that the ratio of carbohydrate to nitrogen is important. High levels of carbohydrates in the form of sugars and starch favor the formation of storage organs, i.e. tuberization, whereas high nitrogen levels are known to promote shoot and root growth that would utilize much of the available carbohydrate and thereby reduce the amount available for tuber formation. Observations consistent with this hypothesis have been made in in vitro tuberization experiments in which the inhibitory effect of increased nitrogen levels on tuberization were observed only at 2% Suc but not at higher concentrations (Koda and Okazawa, 1983), at which the high carbohydrate levels may be masking the effects of altering the nitrogen levels. The high Suc concentrations (up to 8%, w/v) often used to obtain uniform in vitro tuberization, along with the possible addition of other growth regulators, favor tuberization so much that the interpretation of results of in vitro experiments in soil-grown plants should be made with caution.

High Temperatures Inhibit Tuberization

High temperatures are inhibitory for tuberization in both short and long photoperiods, although the inhibitory effect is much greater in long photoperiods. High temperatures

Figure 2. The tuberization response of cuttings that have been induced to differing degrees. From left to right, noninduced (no stolon or tuber) to strongly induced (sessile tuber). Photo courtesy of E. Ewing (Cornell University, Ithaca, NY).

affect the partitioning of assimilates by decreasing the amount going to the tubers and increasing the amounts to other parts of the plant; similar effects are also observed in long photoperiods. It was established, by varying the temperature of the soil or the air, and thus treating different parts of the plant with different temperature regimes (high, 30°C–35°C; low, 17°C–27°C), that high temperatures given to the shoots had the greatest inhibitory effect on the induction to tuberize (as determined by tuberization of cuttings taken from the plants after the treatment). High soil temperature did not affect the production of the inducing signal but prevented stolons from developing into tubers (Ewing and Struik, 1992). At high soil temperatures stolons grow upward, and once they reach the soil surface and the cooler air, tuberization can occur. Hot weather can cause secondary growth of a tuber, i.e. resumption of stolon growth from the tuber, in a process known as heat sprouting. If the temperature becomes cooler after heat sprouting, then a new tuber will start to form at the stolon tip, forming a "chain tuber" in a manner similar to that obtained by cycles of alternating high/low nitrogen levels.

There is some evidence that the inhibitory effect of high temperatures is mediated through increased GA levels. Treating plants or cuttings with chloroethyltrimethylammonium chloride, an inhibitor of GA biosynthesis, overcame the inhibition of tuberization caused by high temperatures. This did not occur, however, if the plants had been disbudded, indicating that the increase in GA biosynthesis in response to high temperatures occurs in the buds and that this is the site of action of the chloroethyltrimethylammonium chloride. This is supported by measurements of GA activity, which indicated that higher temperatures caused higher levels of activity in the buds but not in the leaves and that this was associated with increased inhibition of tuberization (Menzel, 1983).

High Light and High Suc Promote Tuberization

Low light levels delay tuberization, and this has been shown with field-grown plants as well as with plants grown in controlled environments. The effects of low light intensities on growth resemble the effects of high temperatures, and the promotive effects of high levels of irradiance can ameliorate the inhibitory effects of high temperature. Menzel (1985) suggested that the effects of both temperature and irradiance may be mediated through the same control process, possibly involving GAs. Although low light intensities have been shown to increase the acidic GA levels in potato leaves (Woolley and Wareing, 1972), little evidence has been presented to refute the argument that the effect of low light levels on tuberization is due to reduced Suc levels as a direct consequence of lower photosynthetic rates.

As mentioned before, in vitro tuberization is highly dependent on Suc concentration (Xu et al., 1998), and Suc is known to induce several genes that are also induced in the potato tuber, e.g. patatin, proteinase inhibitor II, and ADP-Glc pyrophosphorylase. Xu et al. (1998) reported much higher levels of GA_1 (but not ABA) in the tips of stolons growing in media with 1% Suc, as opposed to 8% Suc, and suggested that Suc can modulate endogenous GA levels in the stolon tip. Perata et al. (1997) showed that Glc can repress both GA signaling and GA biosynthesis in barley embryos. It has also been reported that the reverse is true, i.e. that GAs can affect carbohydrate metabolism. GA_3 reduces the activity of ADP-Glc pyrophosphorylase and thus starch synthesis but increases the activity of UDPG pyrophosphorylase, an enzyme involved in the production of nucleotide sugars that can be used in cell wall synthesis (Mares et al., 1981).

Evidence supporting the role of Suc as an inducing signal includes the fact that an increase in leaf starch accumulation (and presumably, therefore, of Suc export from the leaf) can be detected after as few as 2 d in inducing conditions, and recent results show that Suc can repress phytochrome-mediated responses (Dijkwel et al., 1997). Increasing the level of Suc in the stolons by antisensing the ADP-Glc pyrophosphorylase and thus preventing starch formation in the tubers led to an increased number of tubers being initiated, even though they did not grow very large (Muller-Rober et al., 1992).

SD Promote Tuberization

The potato is a short-day plant, although the critical night length for tuberization and the strength of the photoperiodic response varies with different genotypes (Snyder and Ewing, 1989). Potato species such as *S. demissum* and *S. tuberosum* ssp. *andigena* are qualitative short-day plants that require daylengths of 12 h or less to tuberize, and because of their strict photoperiodic response, they are often used in experiments on photoperiodic effects on tuberization. With photoperiodic responses it is actually the length of the dark period rather than the light period that is important, i.e. a SD has a long night and vice versa. This is illustrated by the fact that interrupting an inducing long night with a light treatment (night break) will prevent tuberization, whereas a dark treatment in the middle of a long light period will have no effect (Fig. 3A). SD promote higher rates of photosynthesis per unit leaf dry weight and

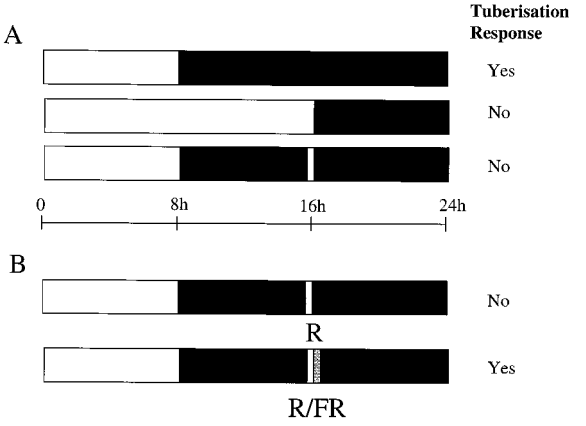

Figure 3. A, Tuberization response of *S. tuberosum* ssp. *andigena* to different photoperiodic treatments. White boxes, Lights on; black boxes, lights off. B, Tuberization response to night breaks of red (R) and far-red (FR) light.

more starch accumulation in the leaf during the day. Assimilate export from leaves was also found to be higher in SD than in LD (Lorenzen and Ewing, 1992). These effects were observable fairly soon (2 d) after the short-day treatment started and preceded tuber initiation, which is usually observed after 1 to 2 weeks.

The principal site of perception of the photoperiodic signal is in the leaves. Photoperiodic responses can be readily observed in single leaf cuttings (Ewing and Wareing, 1978), where it was shown that increasing the number of SD shifted growth from aboveground meristems to those below ground, where stolons and tubers were formed. The effect of photoperiod on tuberization appears to be mediated at least in part by GA application, which prevents or delays tuberization in inducing SD, whereas inhibiting GA biosynthesis using inhibitors such as ancymidol will allow tuberization to proceed in normally noninducing LD (Jackson and Prat, 1996).

Reports of the effects of photoperiod on tuberization in vitro are inconsistent, with some studies finding that LD rather than SD are more favorable for tuberization. Apart from the high levels of Suc, the growth regulators added to the media (some of which are inhibitors of GA biosynthesis) may be complicating the picture. In addition, some studies were performed with leafless stem sections or even stolons, which, without any expanded leaves, cannot be expected to exhibit a strong photoperiodic response.

PHYB Is Involved in the Photoperiodic Response

Interrupting a long dark period with a night break of red light inhibits tuberization and this inhibition can be partially reversed by a subsequent treatment with far-red light (Fig. 3B; Batutis and Ewing, 1982). This photoreversibility is a defining characteristic of responses mediated by phytochrome. There are at least five different types of phytochrome identified in tomato, and because potato and tomato are so closely related, it can safely be assumed that equivalent types are present in potato. Using an antisense approach in the short-day *S. tuberosum* ssp. *andigena* to obtain plants with reduced phytochrome levels enables the roles of different phytochromes in the photoperiodic control of tuberization to be studied (because *S. tuberosum* ssp. *andigena* is tetraploid, it is not possible to screen for mutants in which phytochrome genes are knocked out, as has been done with tomatoes and Arabidopsis). To date, only the role of PHYB has been reported (Jackson et al., 1996). Reduced levels of PHYB in transgenic antisense *S. tuberosum* ssp. *andigena* plants enables them to tuberize in both SD and LD, whereas wild-type plants form only stolons and do not tuberize in LD (Fig. 4). Tubers form on the antisense plants with little or no stolon formation, even in continuous light, reflecting a strongly induced state of these plants to tuberize (Ewing and Wareing, 1978; Ewing and Struik, 1992). Thus, the antisense plants have lost the inhibitory effect on tuberization caused by LD; in other words, PHYB appears to play a role in inhibiting tuberization in LD.

PHYB also appears to be involved in the photoperiodic control of flowering, especially in short-day plants. The

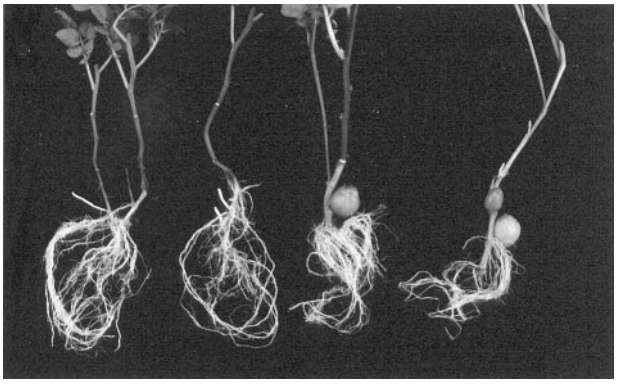

Figure 4. Tuberization response of two wild-type (left) and two antisense *PHYB* (right) potato plants grown in LD. Tuber formation occurred only on the antisense *PHYB* plants. Notice the lack of stolon formation.

ma_3^R mutant of *Sorghum bicolor* is now known to be a *phyB* mutant, and this mutant has a reduced sensitivity to photoperiod in its flowering response. Even in the long-day plant Arabidopsis, flowering is earlier in the *phyB* mutant than in wild type. PHYA has also been shown to be involved in the photoperiodic control of flowering in Arabidopsis, and it is very likely that it will be involved in other responses controlled by photoperiod, such as tuberization in potato.

Apart from the influence of PHYB, there are other similarities between the flowering and tuberization processes. Like tuberization, flowering is also affected by nitrogen levels, temperature, and light levels; indeed, they may even share the same transmissible signals (see below).

TRANSMISSIBLE SIGNALS ARE INVOLVED IN THE CONTROL OF TUBERIZATION

Photoperiodic perception occurs in the leaf. Some sort of signal must therefore be produced in response to the photoperiodic stimulus that is then transmitted from the leaves of the plant to the underground stolons, where tuber formation occurs. Such a signal can be transmitted across a graft union, as was demonstrated in experiments by Gregory (1956) and Chapman (1958). In these experiments leaves from potato plants that were induced to tuberize caused noninduced stocks onto which they were grafted to tuberize, even though after grafting they were maintained in noninducing conditions. Furthermore, the signal produced in leaves of tobacco plants that have been induced to flower is similar to or the same as the signal that induces tuberization in potato plants. Grafting leaves from tobacco plants induced to flower onto potato plants maintained in noninducing conditions led to tuberization of the potato plants, whereas grafted leaves from noninduced tobacco plants did not cause tuberization (Table I). Thus, the processes leading to the production of this signal in response to an inducing photoperiod appear to be similar in potato and tobacco for tuberization and flowering, respectively. Similar results have been obtained with leaves of induced sunflowers, which were able to cause tuberization of

Table I. *Results of grafting scions from different photoperiodic tobacco species onto potato stocks*

The grafted plants were kept in LD or SD. Mammoth is a short-day species, Xanthi is a day-neutral species, and Sylvestris a long-day species. (Summarized from Ewing, 1995.) +, Tuberization occurred; −, tuberization did not occur.

Tobacco Scion	SD		LD	
	Tobacco scion flowering	Potato stock tuberizing	Tobacco scion flowering	Potato stock tuberizing
Mammoth	+	+	−	−
Xanthi	+	+	+	+
Sylvestris	−	−	+	+

Jerusalem artichoke stocks, and therefore the phenomenon does not seem to be restricted to tobacco and potato plants.

The nature of this transmissible signal is not known but it is thought to be hormonal for a number of reasons. It moves through the phloem both acropetally and basipetally but is prevented from doing so by "heat girdling" of the stem, which results in tubers forming from axillary buds above the site of girdling but not below on the stolons. Such a signal may have more than one component, e.g. an inducing substance that increases under inductive conditions and/or an inhibitory substance that decreases under inductive conditions. As mentioned above, PHYB is involved in the inhibition of tuberization in LD rather than the induction of tuberization in SD, since removal of PHYB results in tuberization in both LD and SD. Tuberization of the antisense *PHYB* plants in LD could be caused by a reduction in the levels of an inhibitor or by the production of an inducing substance in normally noninducing LD. That PHYB is involved in the production of a transmissible signal(s) has been shown by grafting experiments in which a wild-type *S. tuberosum* ssp. *andigena* plant could be induced to tuberize in LD by grafting on a shoot from an antisense *PHYB* plant but not by a graft from another wild-type plant (Fig. 5; Jackson et al., 1998). Tuberization of such graftings does not occur, however, if some leaves are left on the wild-type stock plant. Furthermore, with the reciprocal grafting of a wild-type shoot grafted onto an antisense *PHYB* plant, tuberization of the antisense plant that would normally occur in LD is inhibited by the wild-type graft. These results indicate that an inhibitor of tuberization exists in the leaves of wild-type potato plants in LD and that the lower levels of PHYB in the antisense plants has led to reduced levels of this inhibitor, thus allowing tuberization to occur in LD. PHYB thus appears to be involved in the production of the inhibitor in noninducing LD.

GAs INHIBIT TUBERIZATION AND PLAY A ROLE IN THE CONTROL BY PHOTOPERIOD

As already mentioned, nitrogen levels, temperature, and light intensity are all thought to have an effect on GA levels. Photoperiod also has an effect; in many species levels of GAs are higher in LD than in SD. It has been shown, for example, that levels of GA-like activity decrease in leaves of *S. tuberosum* ssp. *andigena* plants upon transfer from LD or night-break conditions to SD (Machackova et al., 1998). Although other studies of GA_{12}-aldehyde metabolism in *S. tuberosum* ssp. *andigena* plants grown in SD and LD did not find any difference between the photoperiods (Van den Berg et al., 1995), the step controlled by photoperiod could lie before GA_{12}–aldehyde in the biosynthetic pathway. Certain steps in the GA biosynthetic pathway are known to be affected by photoperiod, as has been shown in spinach and pea. In spinach bolting is prevented in SD by a lower activity of GA 20-oxidase, which results in less GA_{20} and GA_1. In pea senescence is prevented in SD by an increased production of GA_{53} from GA_{12}-aldehyde.

GAs inhibit tuberization and appear to play a role in the photoperiodic control of tuberization by preventing tuberization in LD. A dwarf mutant of *S. tuberosum* ssp. *andigena* that is able to tuberize in LD as well as in SD has been shown to have a partial block in its GA biosynthetic pathway (Van den Berg et al., 1995). Furthermore, wild-type *S. tuberosum* ssp. *andigena* plants treated with ancymidol, an inhibitor of GA biosynthesis, will tuberize in LD (Jackson and Prat, 1996). This ancymidol treatment of wild-type plants resulted in sessile tuber formation, with little or no stolon formation, in a manner very similar to the formation of tubers on the antisense *PHYB* plants. These results indi-

Figure 5. Graftings of wild-type and antisense *PHYB* plants maintained in LD. A wild-type scion grafted onto a wild-type stock (left) did not tuberize, whereas an antisense *PHYB* scion could induce a wild-type stock to tuberize in the long-day conditions (right).

cate that a decrease in GA levels may be involved in the photoperiodic induction of tuberization in potato plants and that the reduced levels of PHYB in the antisense plants may lead to reduced levels of, or sensitivity to, GA, therefore enabling them to tuberize in LD.

Whereas the observations mentioned above may appear to contradict reports of increased GA levels or sensitivity in *phyB* mutants such as the *Brassica ein*, *Sorghum* ma_3^R, Arabidopsis *phyB*, and cucumber *lh* mutants, there is a range of different biologically active GAs affecting different responses. It is known that the 3β-hydroxylated GA_1 is the most active GA with respect to stem elongation, and there is evidence from *Lolium* that non-3β-hydroxylated GAs are more active in promoting flowering than 3β-hydroxylated GAs (Evans et al., 1994). Reduced levels of PHYB may not, therefore, be causing a general reduction in the levels of all GAs, but only in specific ones, which would alter the relative levels of different GA species. By affecting the expression or activity of one or more enzymes involved in the GA biosynthetic pathway, PHYB could, for example, change the ratio of 3β-hydroxylated to non-3β-hydroxylated GAs and thus change the development of the plant away from stem elongation and vegetative growth and toward flowering and reproductive growth. PHYA has also been shown to affect GA levels; overexpressing PHYA in tobacco results in reduced GA levels and a dwarfed phenotype. Thus, it may be the case that both PHYA and PHYB affect photoperiodic responses such as tuberization and flowering by modifying GA metabolism/response.

Studies of the effect of GAs on in vitro tuberization have shown that concentrations of GA_1 vary throughout the stolon, with the highest concentration located in the stolon tip. The stolon tip is also where the greatest differences in GA_1 concentrations were observed between inducing (8% Suc) and noninducing (1% Suc) conditions (Xu et al., 1998). It was also shown that, by putting the cuttings in alternating low and high GA-containing media, chain tubers could be induced to form in vitro. GAs are known to promote cell elongation in meristematic tissue, and GA_3 has been shown to cause microtubules and microfibrils to become orientated transversely to the cell axis, resulting in longitudinal cell expansion (Shiboaka, 1993) and thus stolon elongation (Fujino et al., 1995). Reducing the levels of GA will result in the microtubules and microfibrils becoming orientated longitudinally (as has been shown to occur during treatment with uniconazol, a GA biosynthesis inhibitor; Shiboaka [1993]), thus allowing lateral cell expansion and division.

NO CLEAR ROLE HAS BEEN DEFINED FOR ANY OTHER PLANT HORMONE

To date there is little evidence that shows a role for any other hormone in the control of tuber induction. Numerous measurements have been made on auxin and cytokinin levels, but the results have been inconsistent. ABA levels have been shown to be affected by photoperiod with up to 4-fold higher levels being measured in *S. tuberosum* ssp. *andigena* plants in inducing SD conditions as opposed to noninducing long-day or short-day plus night-break conditions (Machackova et al., 1998). ABA levels in shoots and roots of potato have also been shown to be affected by nitrogen levels (Krauss, 1985). However, as an ABA-deficient mutant of potato, *Droopy* is able to tuberize (Quarrie, 1982), and it is clear that ABA is not an essential component of the tuberization stimulus. The promotive effects of ABA on tuberization, both in soil-grown plants and in vitro, appear to be due to the antagonistic effects of ABA and GA (Xu et al., 1998). Such antagonism could be at the level of cortical microtubules, where ABA was shown to promote longitudinal arrays of microtubules and was able to reverse the effect of GA_3 on microtubule orientation (Shiboaka, 1993).

Ethanol extraction of induced potato leaves led to the identification and isolation of an acidic substance that showed tuber-inducing activity in vitro. This substance, called tuberonic acid, was found to be structurally related to JA, which also showed similar levels of tuber-inducing activity in vitro (Koda et al., 1991). JA itself, when repeatedly sprayed on noninduced *S. tuberosum* ssp. *andigena* plants, and taken up and transported throughout the plant in sufficient quantities to induce a systemic wound response (an established role of JA in plants), did not result in tuberization (Jackson and Willmitzer, 1994). No differences in the endogenous levels of JA were observed in leaflets of photoperiodic *S. demissum* plants grown in SD and LD. Furthermore, application of salicylhydroxamic acid, an inhibitor of one step in the JA biosynthetic pathway, did not prevent tuberization in short-day conditions (Helder et al., 1993). These results indicate that differences in the levels of JA itself do not control tuberization. This does not exclude the possibility that tuberonic acid or other JA-related compounds may be able to cause tuberization in noninductive conditions, but as yet there are no reports of these compounds having been tested on soil-grown plants. JA may promote tuberization in vitro by disrupting cortical microtubules of the cells and thus allowing their lateral expansion (Matsuki et al., 1992). Consequently, JA may act in a manner similar to that proposed for ABA (see above) and exert its effect principally by antagonizing the effect of GA on microtubule orientation.

While the debate continues about whether JA or related compounds are involved in inducing tuberization in soil-grown plants (Koda, 1997), another compound, called coronatine, with at least 1000-fold greater in vitro tuber-inducing activity than JA, has been discovered (Koda et al., 1996). Coronatine is a phytotoxin isolated from *Pseudomonas syringae* and, in addition to its tuber-inducing activity, has been shown to induce cell expansion in tissue from potato tubers (at a concentration of 1:100 of that required by JA to produce the same effect). The ability of coronatine to induce tuberization of soil-grown plants maintained in noninducing conditions remains to be tested.

WHAT IS THE SIGNAL TRANSDUCTION PATHWAY?

The signal transduction pathway(s) is only beginning to be elucidated (Fig. 6), and there is good evidence that shows the involvement of phytochrome in the response to photoperiod. PHYB is known to affect GA levels and/or response, and this is probably the mechanism by which

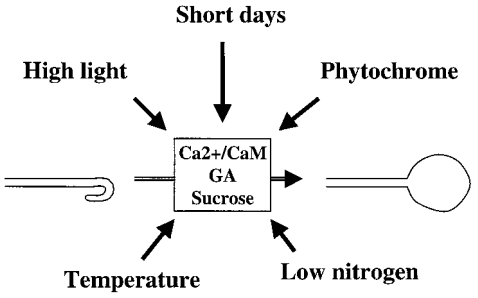

Figure 6. Environmental factors and signaling molecules affecting the induction of tuberization. The transduction pathway still has to be defined.

tuberization is affected in the PHYB-deficient antisense plants. Photoperiod, however, is also known to affect the production and export of Suc, another signaling molecule. Whether this effect of photoperiod is mediated through phytochrome is not yet known, although there is known to be a close interaction between Suc and light signaling pathways, with Suc being able to repress phytochrome-mediated responses.

There is also evidence that indicates the involvement of Ca^{2+}/calmodulin at some stage downstream in the induction pathway. Studies with single leaf cuttings have shown that including Ca^{2+} chelators together with a Ca^{2+} ionophore in the liquid medium prevented tuberization, but tuberization occurred when the cuttings were transferred to Ca^{2+}-containing medium. Calmodulin antagonists were also found to inhibit tuberization of the cuttings (Balamini et al., 1986). Transgenic plants overexpressing a potato calmodulin isoform, PCM1, were found to be inhibited in their tuberization response (Poovaiah et al., 1996). These plants exhibit a phenotype reminiscent of GA-treated plants. Such results suggest that Ca^{2+} and calmodulin are somehow involved in the tuberization process, which may not be surprising since they have been shown to be involved in at least one phytochrome signal transduction pathway. Furthermore, a Ca^{2+}-dependent protein kinase has been isolated from potatoes and shown to increase in activity at the onset of in vitro tuberization, implying that more than one Ca^{2+}-signaling pathway may be involved in the induction of tuberization.

The identification of a tuber-inducing signal remains an elusive goal. Although the majority of opinions favor a mechanism whereby the relative levels of two or more factors (inducing and inhibitory) determine whether tuberization occurs, so far the only strong candidate for the inhibitor that has been identified is GA. All environmental conditions and other hormones that have an effect on tuberization appear to affect GA levels or to antagonize the effects of GA, e.g. on microtubule orientation.

In addition to controlling microtubule orientation, GA levels may control carbohydrate metabolism and direct Suc utilization toward storage (tuber formation at low GA) or cell wall synthesis (continued stolon growth at high GA). At the same time Suc may exert a positive influence (thereby being classified as a tuber-inducing signal) by regulating endogenous GA levels and responses in the stolon tip. Conditions such as high light or short photoperiods, which lead to high Suc levels, would thus also cause a reduction in GA levels and promote tuberization. A high level of photoassimilate was once thought to be an inducing signal for the formation of tubers, but this was later modified to incorporate the effect of nitrogen, and the ratio of carbohydrate to nitrogen was proposed to be the important factor. This may eventually turn out to be the case if indeed Suc reduces endogenous GA levels, whereas nitrogen (along with other noninducing conditions such as high temperatures) increases them.

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