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Ectopic expression of oat phytochrome A in hybrid aspen changes critical daylength for growth and prevents cold acclimatization

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Summary

Survival of temperate-zone tree species under the normal summer–winter cycle is dependent on proper timing of apical growth cessation and cold acclimatization. This timing is primarily based on the perception of daylength, and through evolution many tree species have developed photoperiodic ecotypes which are closely adapted to the local light conditions. The longest photoperiod inducing growth cessation, the critical photoperiod, is inherited as a quantitative character. The phytochrome pigment family is the probable receptor of daylength, but the exact role of phytochrome and the physiological basis for the different responses between photoperiodic ecotypes are not known. This report shows for the first time that over-expression of the oat phytochrome A gene (*PHYA*) in a tree significantly changes the critical daylength and effectively prevents cold acclimatization. While the critical daylength for elongation growth in the wild-type of hybrid aspen (*Populus tremula* × *tremuloides*) was approximately 15 h, transgenic lines with a strong expression of the oat *PHYA* gene did not stop growing even under a photoperiod of 6 h. Quantitative analysis of gibberellins (GA) as well as indole-3-acetic acid (IAA) revealed that levels of these were not down-regulated under short days in the transgenic plants expressing high levels of oat *PHYA*, as in the wild-type. These results indicate that photoperiodic responses in trees might be regulated by the amount of *PHYA* gene expressed in the plants, and that the amount of phytochrome A (phyA) affects the metabolism of GAs and IAA.

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Introduction

In the temperate zone, the special combination of light and temperature climate is one of the main limiting factors for tree growth. During evolution, trees have developed populations that are closely adapted to the local climatic conditions. Climatic responses in forest trees, as well as the existence of climatic ecotypes, have been thoroughly demonstrated and described, but our understanding of these responses, at the physiological or molecular level, is almost non-existant. In many woody species, the initiation of cold acclimatization and dormancy are synchronized with the end of the growth season and the onset of low temperatures in the autumn. The length of the photoperiod is an important primary signal for these growth responses (Nitsch, 1957; Vince-Prue, 1975). Long days (LD) sustain shoot elongation, whereas short days (SD) induce growth cessation and formation of terminal buds, resulting in cold acclimatization and bud dormancy (Weiser, 1970). Photoperiodic ecotypes differ in their response to daylength, and adaptation to various photoperiodic regimes is well described in all main temperate tree species (Håbjørg, 1978; Heide, 1974; Howe *et al.*, 1995; Junttila, 1989; Pauley and Perry, 1954; Vaartaja, 1959). The critical daylength for growth cessation is longer in northern populations than in southern populations, and varies also with altitude.

The photoreceptors phytochrome are generally believed to be involved in the detection of photoperiod (Sharrock, 1992; Smith, 1995). Phytochromes are dimers of approximately 124 kDa subunits, each carrying a covalently linked chromophore in the N-terminal. The chromophore of phytochromes consists of an open-chained tetrapyrrole, which persists in two main forms, interchangeable with each other, the red-light (R)-absorbing form (Pr) and the far-red-light (FR)-absorbing form (Pfr). Phytochrome receptors are encoded by a gene family (Quail, 1994). In *Arabidopsis*, the apoprotein is encoded by five genes, designed *PHYA*, *-B*, *-C*, *-D* and *E* (Clack *et al.*, 1994; Sharrock and Quail, 1989) and in tomato there are indications of 9 to 13 *PHY* genes (Hauser *et al.*, 1995). There is evidence indicating that the different phytochromes have distinct photosensory functions (Furuya and Schäfer, 1996; Quail *et al.*, 1995; Smith, 1995). So far, only phytochrome A (phyA) and B (phyB) have been studied thoroughly, although there is also evidence for involvement of other phytochromes in specific photomorphogenetic responses (Carabelli *et al.*, 1996; Devlin *et al.*, 1996). Work with mutants lacking phyA or phyB and transgenic over-expressors of the two genes

show that both types are involved in regulation of multiple growth events in plants (Smith, 1995). The function of phytochrome in photoperiodic perception is not well understood, although both phyA and phyB appear to be involved in the photoperiodic control of flowering in *Arabidopsis* (Amasino, 1996; Bagnall *et al.,* 1995). Studies of woody plants growing under different light regimes and night breaks with R or FR light have also indicated the importance of phytochrome for the regulation of photoperiodic responses in trees (D'Aoust and Hubac, 1986; Howe *et al.,* 1996; Nitsch, 1957; Vince-Prue, 1975). However, there are no published data on the relative importance of different phytochromes in short day-induced growth cessation in woody plants.

Light perception by phytochromes results in activation of signal transduction pathways leading to changes in gene expression that regulate physiological and developmental responses to light (e.g. Barnes *et al.,* 1997; Chamovitz and Deng, 1996). Plant hormones have also been suggested to have an important role in photomorphogenetic processes controlled by phytochrome, e.g. auxins (Behringer and Davies, 1992; Jones *et al.,* 1991) and brassinosteroids (Li *et al.,* 1996; Szekeres *et al.,* 1996). Several lines of evidence have shown the connection between phytochrome and the biosynthesis of gibberellins (GAs) and/or responsiveness to GAs (e.g. Boylan and Quail, 1989; Childs *et al.,* 1995; Jordan *et al.,* 1995; Lopez-Juez *et al.,* 1995; Reed *et al.,* 1996; Weller *et al.,* 1994). It has also been suggested that photoperiodic regulation of elongation growth in rosette plants is mediated by regulation of GA biosynthesis (Gilmour *et al.,* 1986; Zeevaart and Gage, 1993; Zeevaart *et al.,* 1990). Studies of *Salix pentandra* strongly suggest involvement of GA in the regulation of elongation growth and growth cessation in woody species (Junttila, 1990; Junttila and Jensen, 1988; Olsen *et al.,* 1995a, b). Olsen *et al.* (1995b) showed that, after exposure of seedlings of *Salix pentandra* to 5 short days, when visible signs of reduction in growth were not yet seen, the levels of GA_1 were decreased by 50% within a specific region of apex that normally contains maximum levels. As GA_1 is probably also the active GA for stem elongation in woody plants (Junttila *et al.,* 1991), inhibition of biosynthesis of GA_1, caused by short photoperiod, appears to be an early step in induction of growth cessation.

In the present paper, we show for the first time, by over-expression of oat *PHYA* in a temperate-zone deciduous tree, that phyA may be involved in the detection of photoperiod in trees. The over-expression results in changes in the critical daylength, and effectively prevents cold acclimatization. Furthermore, we also show that these changes are accompanied by changes in the levels of plant hormones, especially GAs, confirming the involvement of GAs in short day-induced growth cessation in trees.

Results

Screening of oat PHYA*-expressing lines*

When 22 independently transformed lines of hybrid aspen were grown in a phytotron at 15°C in continuous light from fluorescent tubes, all lines were characterized by reduced internode length compared with the wild-type (Figure 1a, b), and increased anthocyanine formation (data not shown). There were no differences in the numbers of axillary branches. Under continuous light, the elongation rate of the transformed lines with the shortest internodes was approximately 30% of that in wild-type. This difference was primarily due to the differences in internode lengths; no statistically significant differences in the number of nodes (leaves) were observed. However, the responses of the transformed lines to SD conditions (12 h photoperiod) were more significant. The wild-type and six of the 22 transgenic lines stopped growing and formed terminal buds under a 12 h photoperiod, while the 16 lines with the shortest internodes were still growing after 40 days under a short photoperiod.

Six of the transformed lines were selected for further experiments, representing short (lines 6 and 22), medium (13 and 24) and long (8 and 10) internodes. Northern analysis of RNA isolated from leaf tissue of these showed a strong expression of the oat *PHYA* gene in lines 6, 22, 24 and 13, although the relative expression levels in the different lines varied somewhat between individual analyses. No signal could be detected in lines 8 and 10 or in the wild-type (Figure 1c).

Cell numbers per internode are affected by PHYA *over-expression*

There were no clear effects of oat *PHYA* expression on lengths of epidermal, cortical and pith cells in the transgenic lines (Figure 2). However, cell numbers per fully elongated internode were significantly affected in the lines with dwarf growth, i.e. most prominently in lines 6 and 22 (Figure 2). Cell numbers per internode for these were 40–65% of those in the wild-type. For lines 13 and 24, cell numbers were similar to the wild-type for epidermal cells, but 75–90% for pith and 75% for cortex. Lines 8 and 10 had cell numbers per internode similar to the wild-type in the cortex and pith, but slightly more cells in the epidermis.

Dwarf growth in PHYA *over-expressors correlates with reduced plant hormone contents*

The effect of oat *PHYA* expression on levels of GAs and indole-3-acetic acid (IAA) in apical leaf and stem tissues was investigated in plants grown under a 24 h photoperiod. Dwarf growth due to *PHYA* over-expression correlated with

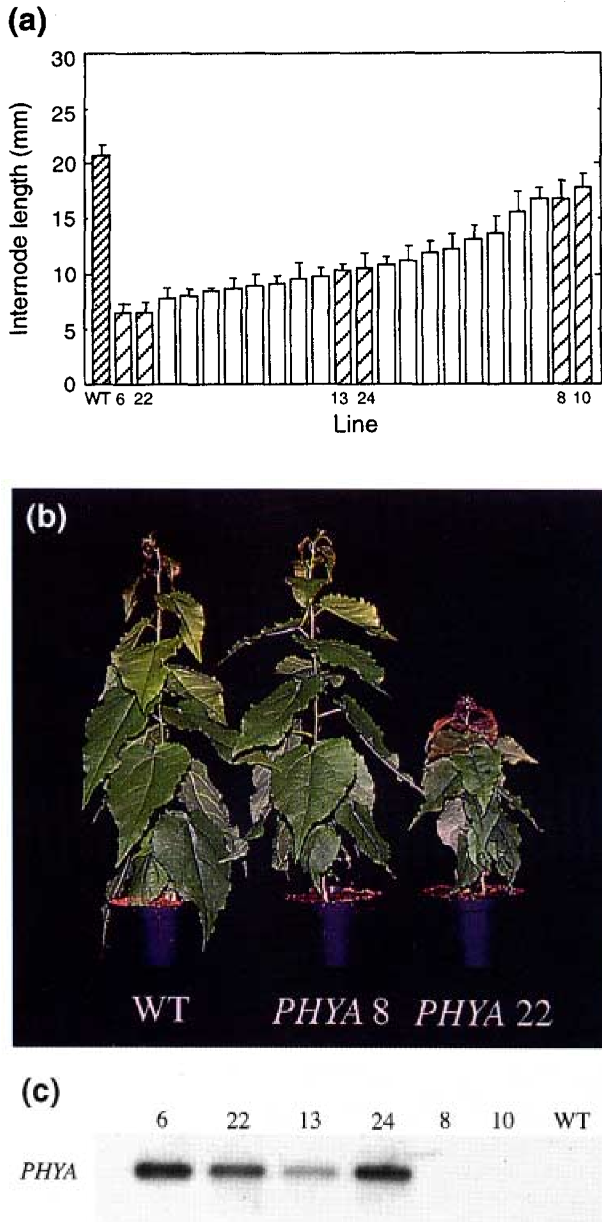

Figure 1. Reduction in internode lengths in hybrid aspen transformed with the oat *PHYA* cDNA (a), phenotype of wild-type and transgenic lines 8 and 22 (b), and Northern blot analysis of the oat *PHYA* in apical leaf tissue from selected transgenic lines (c).
Three fully grown internodes of each of seven individuals of each of 22 independently transformed lines and the wild-type were tested in continuous light. The six transgenic lines with numbers (a) were used in further experiments. The vertical bars represent SE.

low levels of GA_{19}, GA_{20} and GA_1 both in apical stem and leaf tissue (Figure 3). GA_{19} levels in lines 13, 24, 6 and 22 were 50–80% of those in the wild-type. Furthermore, lines 6 and 22 contained 20–40% and lines 13 and 24 contained 40–70% of the content of GA_{20} and GA_1 in the wild-type. Levels of IAA were also lower in the stem tissue of these four transgenic lines. In lines 6 and 22 and lines 13 and 24, 30–50% and 50–70%, respectively, of the IAA contents

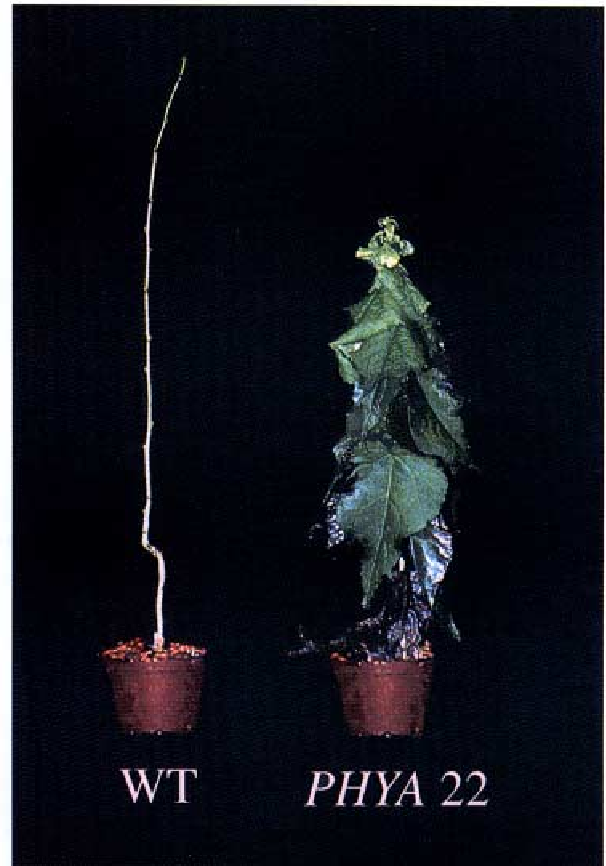

Figure 7. Effect of short day and low temperature on leaf abscission in WT of hybrid aspen and in the transgenic line 22 expressing the oat *PHYA* gene. After 7 weeks at 18°C in a 12 h photoperiod, plants were exposed for 3 weeks to 6°C followed by 6 weeks at 0.5°C, under a 11 h photoperiod.

in the wild-type were found (Figure 3). No differences in hormone levels between the upper and lower parts of the elongation zone were found (data not shown). The data presented are thus the mean of the two parts.

PHYA *over-expression has small effects on light quality responses*

Effects of the oat *PHYA* on light quality responses were tested, using R:FR ratios of 1.7 or 0.7 throughout the light period of 24 h of in one experiment, and end-of-day (EOD) treatments of 15 min of R or FR at the end of a 12 h photoperiod, as compared with no EOD treatments in another experiment. In all lines, a low R:FR ratio of 0.7, as well as a 15 min EOD FR treatment, stimulated stem elongation significantly within a couple of days, both in the wild-type and the transgenic lines, and up to twice as long internodes compared with other treatments were observed (Figure 4). No significant differences in the number of leaves were observed. The short internode phenotype of the transgenic lines expressing high levels of oat *PHYA* was persistent in the in the two R:FR ratios tested,

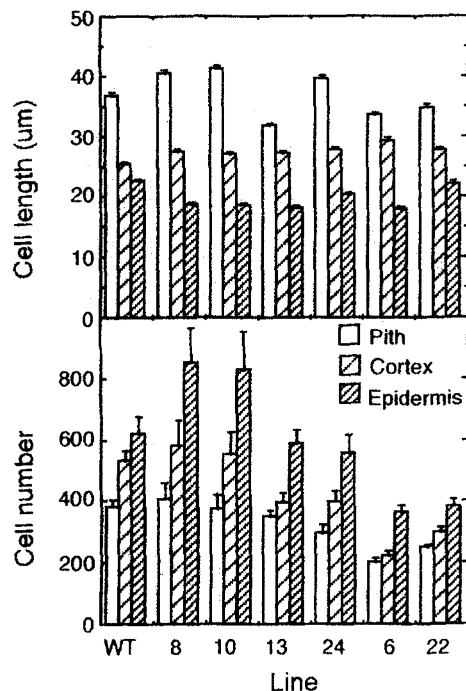

Figure 2. Cell lengths and cell numbers per internode as affected by expression of oat *PHYA* in transgenic lines of hybrid aspen.
The selected lines represented short (lines 6 and 22), medium (13 and 24) and long (8 and 10) internodes when grown in continuous light. Two fully elongated internodes were used for each of three plants per line. The vertical bars represent SE.

as well as in the EOD R treatment. This response was suspended by EOD FR, under which all transgenic lines showed similar internode lengths to the wild-type.

PHYA *over-expression affects the critical photoperiod for cessation of apical growth*

The effect of the oat *PHYA* on the critical daylength for growth cessation was tested using photoperiodic treatments ranging from 6 to 18 h. As shown in Figure 5, the wild-type and lines 8 and 10 showed similar responses to photoperiod and had a critical daylength for growth of between 14 and 16 h. However, no sign of cessation of apical growth was observed in lines 6 and 22, even when exposed to a 6 h photoperiod. The lines 13 and 24 continued to grow under 8 h daylength, but started to show a response to a 6 h photoperiod after 49 days, suggesting that 6 h is close to a critical photoperiod for these transgenic lines (Figure 5). In fact, the growth rate of the lines 6, 13, 22 and 24 increased during the exposure to photoperiods of 8–14 h, being 19–28 and 63–80 mm week^{-1} for the 1st and the 7th week, respectively. Production of new leaves closely followed the time course of stem elongation (results not shown).

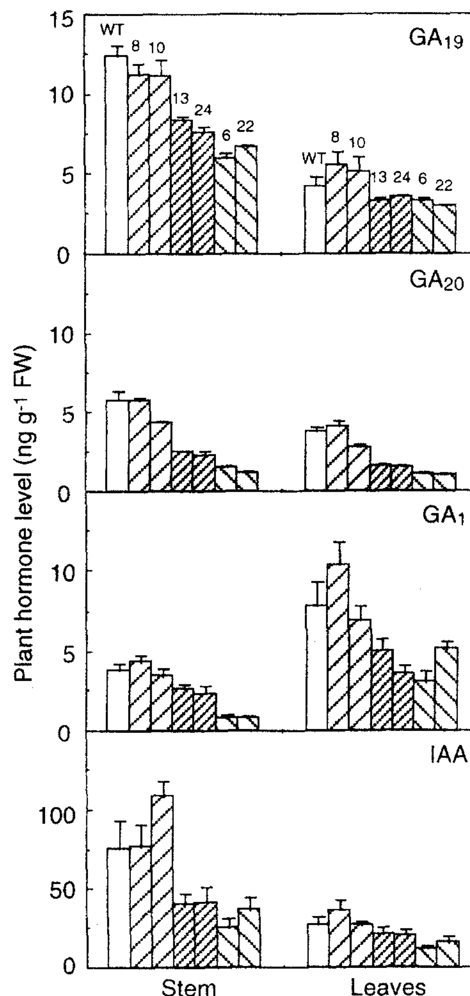

Figure 3. Levels of gibberellins (GAs) and indole-3-acetic acid (IAA) as affected by expression of the oat *PHYA* gene in transgenic lines of hybrid aspen grown under continuous light.
Samples from the elongating, apical part of the plants were analysed by combined GC/MS. The upper and lower portions of the elongation zone of each of three plants for each line were analysed separately. As no differences between the parts were detected, pooled data are shown. The vertical bars represent SE.

PHYA *over-expression affects regulation of plant hormone contents under short days*

The effect of the oat *PHYA* on the levels of GAs and IAA under short and long photoperiods of 10 h and 24 h, respectively, were investigated. In the wild-type and line 8, growth cessation occurring after 3–4 weeks under SD was accompanied by decreasing levels of GA_{53}, GA_{19}, GA_{20}, GA_1, GA_8 and IAA, compared with under LD (Figure 6). After 3–4 weeks of SD, levels of GA_{19}, GA_{20}, GA_1 and IAA commonly had decreased by 50–70%, and GA_{53} and GA_8 by 30–40%, compared with the start of the experiment. However, in line 22, in which growth did not cease under SD (Figure 5), the levels of GAs and IAA were similar under both LD and SD (Figure 6).

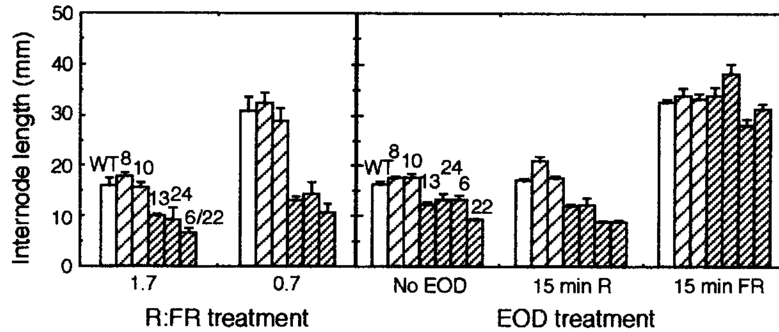

Figure 4. Effects of expression of the oat *PHYA* gene in hybrid aspen on light quality responses.
Three–eight plants per line were grown under R:FR ratios of 1.7 or 0.7 throughout the light period of 24 h in one experiment. In another experiment, 5–7 plants per line were given 15 min of R, FR or no EOD treatment. Vertical bars represent SE.

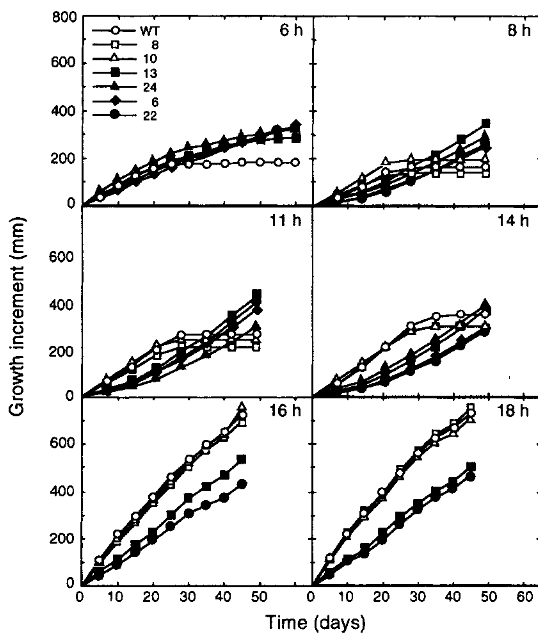

Figure 5. Cumulative shoot elongation in transgenic lines of hybrid aspen grown under different photoperiods as affected by over-expression of the oat *PHYA* gene.
The main photoperiod was 8 h (6 h), and photoperiod extensions were given as low-intensity light. There were 5–7 plants per line and photoperiod.

PHYA *over-expressing plants do not cold-harden*

Cessation of apical growth is generally a prerequisite for the development of cold acclimatization, suggesting that the transgenic plants over-expressing the oat *PHYA* might have lost the ability to cold acclimatize. When plants which had been exposed to short photoperiods at 18°C were transferred to a low temperature, 6°C followed by 0.5°C, and a 11 h photoperiod, the wild-type and lines 8 and 10 lost their leaves within 8–9 weeks, while the other lines retained most of their leaves (Figure 7). Thus, over-expression of the oat *PHYA* effectively prevented leaf abscission in hybrid aspen. This effect was reflected in the development of frost resistance. Stem and bud tissues of long day-grown plants of both the wild-type and all the six transgenic lines did not survive temperatures below about −4°C when tested under controlled freezing conditions with an early induction of ice nucleation (Junttila and Kaurin 1990). Exposure to short photoperiod at 18°C for 50 days increased the frost resistance in the wild-type and the transgenic lines 8 and 10 to LT_{50} −6 to −7°C, but did not have any effect on lines 6, 13, 22 and 24. Further, hardening treatments with successive lowering of the temperature resulted in a significant increase in frost resistance in wild-type and lines 8 and 10, but no increase of hardiness was observed in lines 6, 13, 22 and 24 (Table 1, Experiment I).

These effects of *PHYA* over-expression on development of cold hardiness were confirmed in another experiment where the plants were grown at a 11 h 45 min photoperiod followed by 15 min EOD R or FR before hardening treatment (Table 1, Experiment II). EOD R treatment had no effect on frost resistance. In contrast, frost resistance was enhanced by EOD FR treatment both in the wild-type and in the transgenic lines. The effect of FR was statistically significant ($P = 0.0001$) in all freeze tests for the lines 6, 13, 22, and 24. Leaf abscission in the transgenic lines 6, 13, 22, and 24 was enhanced to some degree by EOD FR treatment, but no sign of bud set could be observed in these transgenic lines even after nine weeks of cold acclimatization.

Discussion

Further understanding of the role and function of phytochrome(s) in trees is of great interest in the understanding of mechanisms for adaptation to the climate. Such knowledge may lead to an improvement of forest trees for particular climatic conditions by breeding or genetic engineering. In this paper, we present data showing that the level of *PHYA* gene expression may affect short day-induced growth cessation and cold acclimatization in trees. In transgenic plants of hybrid aspen ectopically expressing the oat *PHYA*, the critical daylength for growth was

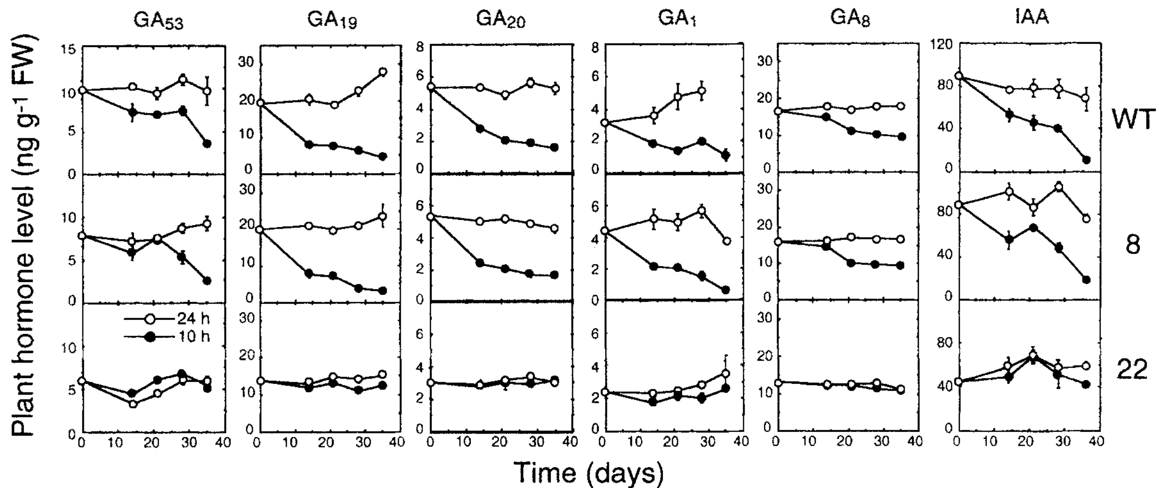

Figure 6. Time course study of levels of gibberellins (GA) and indole-3-acetic acid (IAA) under long-day conditions.
Two samples of each of the upper and lower part of the elongation zone consisting of stem tissue from three plants were analysed by GC/MS in each case. Pooled data for upper and lower parts are shown, as in Figure 4. The vertical bars represent SE.

changed, resulting in delayed growth cessation and prevention of cold acclimatization.

Correlation between dwarf growth, decreased levels of gibberellins and reduced cell numbers per internode

Transgenic lines over-expressing oat *PHYA* were characterized by reduced internode length compared to the wild-type when grown under fluorescent light (Figure 1). Reduced hypocotyle/internode elongation due to over-expression of *PHYA* has been reported in several herbaceous species, e.g. *Arabidopsis* (Boylan and Quail, 1991; Whitelam *et al.*, 1992), tobacco (Jordan *et al.*, 1995; Nagatani *et al.*, 1991) and rice (Casal *et al.*, 1996). The light-labile phyA has been suggested to be involved in FR-HIR responses in etiolated tissue, and light-stable phytochromes, such as phyB, in control of hypocotyl length in light-grown seedlings (Furuya and Schäfer, 1996; Smith, 1995). However, over-expressing *PHYA* constitutively under the CaMV 35S promoter results in continuous high levels of phyA, which also affects the elongation growth in light-grown plants. On the other hand, studies have shown that native phyA may also have a role in regulation of extension growth in light-grown seedlings (Johnson *et al.*, 1994).

The dwarf growth in *Populus* due to *PHYA* over-expression correlated with reduced levels of GAs (Figure 3). This is consistent with data reported for tobacco plants expressing high levels of the oat *PHYA* gene (Jordan *et al.*, 1995). In addition, the levels of IAA were also affected by *PHYA* over-expression, as the dwarfed plants had reduced IAA content (Figure 3). This is consistent with data from dwarf mutants of pea showing correlation between IAA contents and phytochrome-regulated shoot growth (Behringer and Davies, 1992).

In *Populus*, the dwarf growth was apparently not a consequence of reduced cell elongation, as no significant changes in cell lengths were found (Figure 2). However, numbers of cells per fully elongated internode were clearly decreased, indicating that cell divisions in stem tissue were affected by elevated phyA levels (Figure 2). In contrast, the dwarf response observed in 35S oat *PHYA* tobacco plants has been explained as a result of reduced cell expansion not cell division (Nagatani *et al.*, 1991). Although GAs have been found to affect cell elongation (Jones, 1983), it is also known that mitotic activity in the sub-apical region of the stem can be induced by GAs (Sachs, 1965; Sauter *et al.*, 1995). The partly contradictory results between tobacco and hybrid aspen are perhaps due to the complexity of the relationship between light and GA physiology/metabolism observed in different species. In *Thalpsi arvense*, it has been suggested that light conditions affect the degree of cell elongation, whereas GAs affect cell division, but not cell elongation (Metzger, 1988). It has also been suggested that a high amount of PfrB has an inhibitory effect on GA-induced growth in cucumber hypocotyl growth (Lopez-Juez *et al.*, 1995). These authors also observed that changes in levels of GAs correlated with changes in cell numbers rather than cell length. They suggested that this was not directly under phytochrome control, based on measurements on seedlings grown in white light with or without FR light. Our results suggests that levels of phyA affect both GA levels and cell numbers, at least in hybrid aspen, indicating that the low GA_1 levels lead to lower cell division activity in the sub-apical meristem. This shows further the complexity between light and GAs, and could indicate that different phytochromes have different effects on GA physiology and growth.

Table 1. Frost resistance (LT_{50}, °C; the median lethal temperature) in nodal stem segments of transgenic lines of hybrid aspen expressing the oat *PHYA* gene

Experiment and line	Light treatment before hardening	Hardening treatment		
		3 weeks at 6°C	3 weeks at 6°C + 3 weeks at 0.5°C	3 weeks at 0.5°C + 6 weeks at 0.5°C
Experiment I				
WT, 8, 10	8 h	-14 ± 0.9	<-27[a]	-
	11 h	-12 ± 0.4	<-27[a]	-
	14 h	-11 ± 1.0	-22 ± 0.7	-
13, 24, 22, 6	8 h	>-7.0[b]	>-7.0[b]	-
	11 h	>-7.0[b]	>-7.0[b]	-
	14 h	>-7.0[b]	>-7.0[b]	-
Experiment II				
WT, 8, 10	No EOD	-15 ± 0.5	-35 ± 1.5	<-50[a]
	EOD R	-14 ± 0.4	-37 ± 0.9	<-50[a]
	EOD FR	-20 ± 0.7	<-40[a]	<-50[a]
13, 24, 22, 6	No EOD	4 ± 0.3	-6 ± 0.7	-6 ± 0.3
	EOD R	-3 ± 0.2	-7 ± 0.6	-6 ± 0.3
	EOD FR	-9 ± 0.6	-14 ± 1.5	-17 ± 1.2

During cold acclimatization, plants were grown under a 10 h photoperiod (50 μmol m^{-2} sec^{-1}). Plants were then freeze tested, and injury was evaluted visually after thawing. In experiment I, plants were grown for 50 days under an 8, 11 or 14 h photoperiod before hardening. In experiment II, plants were grown for 40 days under an 11 h 45 min photoperiod followed by no light, 15 min of R, or 15 min FR light treatment. In both experiments, results are means ± SE of the different lines as indicated, and include samples from upper and lower parts of the stem. In experiment II, means of the visual evaluation and an ion leakage test are presented.
[a]Lowest temperature used in the test.
[b]Highest temperature used in the test.

Phytochrome A might play a role in control of stem extension under different Pr/Pfr ratios

An important function of phytochromes involves the perception of changes in light quality, and the initiation of the shade-avoidance response. There is now conclusive evidence showing that light stable phytochromes such as phyB (e.g. Smith, 1995), as well as other novel phytochromes (Carabelli *et al.*, 1996; Devlin *et al.*, 1996) are involved in this perception. Different R:FR ratios affected plant height differently both in the wild-type of hybrid aspen and the oat *PHYA*-expressing lines (Figure 4), as is characteristic for the shade-avoidance response. However, the dwarf phenotype of the transgenics, relative to the wild-type, was sustained. The results are similar to observations in transgenic potato overproducing the oat *PHYA* (Heyer *et al.*, 1995), and the sustained growth can be explained by the presence of higher amounts of active phyA in the transformants. Although it cannot be excluded that phyA has a regulatory role in controlling stem extension under low Pr/Pfr ratios, it must be emphasized that mutants lacking phyA also show normal shade-avoidance response, which indicates that phyA is of minor importance in this regard (e.g. Smith, 1995).

End-of-day light treatments are a classical way of investigating phytochrome action. In our study, EOD FR resulted in transgenic lines with similar internode lengths as the wild-type plants (Figure 4). In contrast to the results of treatment with different R:FR ratios, the high levels of phyA in the transgenics could not sustain the growth inhibition. The EOD FR results are in agreement with results observed in other species transformed with oat *PHYA* (Casal and Sanchez, 1994; Casal *et al.*, 1995; McCormac *et al.*, 1993), and also show that the constitutive expression of oat *PHYA* during the dark period does not result in spontaneously formed Pfr levels high enough to inhibit the increased internode growth that was caused by reduced amounts of Pfr forms of phytochromes at the end of the day (Furuya and Schäfer, 1996; Pratt, 1995).

The critical photoperiod for growth and regulation of hormone levels is affected by PHYA *over-expression*

Strong expression of the oat *PHYA* gene in hybrid aspen was accompanied by insensitivity to a short photoperiod (Figure 5). The phyA over-expressors' inability to respond to the absence of light, i.e. short days, shows that high amounts of phyA interfere with the normal time measurement. There are several possible explanations for this, e.g. the total amount of PfrA in phyA over-expressors under SDs may be high enough to prevent growth cessation, or that there is an indirect effect on the measurements of daylength. Our results suggests that the gene dose of

PHYA can modify the photoperiodic regulation of apical growth and dormancy induction in such woody species. Bagnall *et al.* (1995) have shown that over-expression of the oat *PHYA* gene in *Arabidopsis*, a facultative long-day plant as far as flowering is concerned, results in early flowering under SDs, thus behaving as if they were under LDs, similar to hybrid aspen over-expressing the oat *PHYA* gene. Studies of a phyA-null mutant of *Arabidopsis* have shown the importance of phyA in detection of low-intensity incandescent light extension (Johnson *et al.*, 1994).

In temperate-zone trees, the photoperiodic response is clinic, i.e. the critical photoperiod for growth changes gradually with increasing northern latitude of origin of the plant. If the photoperiodic response in such trees is related to gene dosage of phytochrome genes, as suggested for other responses (Smith, 1995), different critical photoperiods could partly be related to quantitative aspects of phytochrome(s) in the plants.

Two main morphological processes are involved in the SD-induced cessation of apical growth in tree species such as *Populus*: initiation of bud scales and an inhibition of internode elongation that is associated with bud set (Howe *et al.*, 1995, 1996; Olsen *et al.*, 1995b). Over-expression of the oat *PHYA* obviously prevented these processes in hybrid aspen. On the other hand, over-expression of the oat *PHYA* reduced the internode length when the plants were grown under a continuous high irradiance. Different physiological mechanisms are probably involved in these growth processes, and they may even involve functions of different phytochromes (Howe *et al.*, 1996). Whereas involvement of phytochrome in the regulation of internode length is well documented (Smith, 1995), the influence of phytochrome on processes associated with bud set has not been investigated to the same degree.

The photoperiodic regulation of elongation growth has been suggested to be related to SD-induced block(s) in gibberellin biosynthesis (Gilmour *et al.*, 1986; Junttila and Jensen, 1988; Olsen *et al.*, 1995a; Zeevaart *et al.*, 1990). This is confirmed in the present study as the wild-type plants clearly show reduced GA levels after transfer to SD (Figure 6). This has also been shown in *Salix pentandra*, another temperate-zone woody species of the Salicaceae (Olsen *et al.*, 1995a,b).

The mulitifunctional enzyme GA 20-oxidase, which catalyses the stepwise conversion of GA_{53}, to GA_{20} via GA_{44} and GA_{19}, has been suggested to be photoperiodically regulated at the transcription level (Wu *et al.*, 1996). This suggests that there should be an accumulation of GA_{53}, GA_{44}, or GA_{19} under SD. In hybrid aspen, we could not detect increased levels of any GA metabolite under SD, but the GA_{53} level decreased more slowly under SD than the other GAs monitored (Figure 6). This might implicate a metabolic regulation of GA 20-oxidase under SD, but it also suggests that other GA biosynthetic steps might be under photoperiodic control, as has been shown in spinach (Zeevaart and Gage, 1993).

Over-expression of the *PHYA* gene in *Populus* was found to inhibit the SD-induced down-regulation of biosynthesis of the active gibberellin A_1 and thus prevent growth cessation. No changes in levels of any GAs were observed under SD (Figure 6). Our results confirm previous observations in *Salix pentandra* where the decline in GA levels appears to be an early step in induction of growth cessation and initiation of cold hardening in trees (Olsen *et al.*, 1995a,b). However, the GA levels at onset of the SD-induced growth cessation were similar to those in the continuously growing *PHYA* over-expressing dwarf plants. This demonstrates that GA content *per se* cannot be the only factor important in the photoperiodic regulation of growth and dormancy induction. Other important factors are probably reduction in GA concentration relative to the sites of GA action, as well as or rather than changes in sensitivity to GA under the course of the SD treatment. Further investigations on expression of genes involved in the biosynthesis of GAs could give more insight in the interaction between photoperiod, GA biosynthesis and growth.

Furthermore, the decline of IAA levels in wild-type plants observed under SD, as has also been observed in *Salix* (Olsen *et al.*, 1997), and the absence of this decline in the transgenic plants, raise important questions on the connection between GA- and IAA-dependent growth, and a role of IAA in light-mediated responses. The present results suggests that the *PHYA* gene can modify directly or indirectly the levels of GAs in two different ways: reduction during LD, resulting in a reduced internode length, and/or inhibition of the SD-induced block in biosynthesis of GA_1, preventing growth cessation.

PHYA *over-expression prevents cold hardening*

Over-expression of the oat *PHYA* effectively prevented leaf abscission (Figure 7) and development of cold hardiness in hybrid aspen exposed to hardening conditions of short photoperiods and low temperatures (Table 1, Experiment I). The inability of the transformants to respond to SD clearly shows the importance of apical growth cessation as a prerequisite for initiation of cold acclimatization in tree species with free growth pattern (Junttila and Kaurin, 1990; Weiser, 1970).

Frost resistance was enhanced by EOD FR treatment both in the wild-type and the transgenic lines (Table 1, Experiment II), indicating that phyA is not strongly involved in the perception of this treatment. Thus, high amounts of phyA do not appear to be able to inhibit the EOD FR response, which has been suggested to be mediated primarily by light-stable phytochromes, such as phyB (Bagnall *et al.*, 1995; Smith, 1995). Leaf abscission in the lines with strong expression of the oat *PHYA* was enhanced to some

degree by EOD FR treatment, but no sign of bud set could be observed. Thus, the enhancement of frost resistance by EOD FR (Table 1) was not directly related to the cessation of apical growth. In contrast, in *Cornus*, the enhancement of frost resistance by EOD FR treatment at the end of a long day was related to promotion of growth cessation (McKenzie *et al.*, 1974), suggesting that there can be species-specific differences in the response.

In conclusion, these results demonstrate the importance of *PHYA* in the photoperiodic regulation of the acclimatization processes in *Populus*, and suggest that there could be a quantitative relationship between phytochrome and adaptation to photoperiodic conditions. These data also suggest that the actions of over-expressed *PHYA* on internode length and apical growth cessation have different physiological basis. Although an interaction between phyA and other phytochromes cannot be ruled out, our investigations show that phyA probably plays an important role in response to SD. Furthermore, over-expression of the *PHYA* gene was found to modify GA levels in different ways: reduction under continuous light, leading to a reduced internode length, and inhibition of SD-induced block in biosynthesis of the active gibberellin A_1 preventing growth cessation. These results also show a connection between phy-mediated growth and GAs.

Experimental procedures

Plant transformation

Standard techniques were used to construct recombinant DNA plasmids (Sambrook *et al.*, 1989). The oat *PHYA* cDNA clone (Boylan and Quail, 1989) was obtained from Dr Peter Quail as a subclone in plasmid pUC19. It was excised from this plasmid as a 3504 bp *Bam*HI/*Eco*RI fragment and inserted into plasmid pOK12, thus generating a *Bgl*II site on the 3′ end of the cDNA. The entire *PHYA* cDNA was then excised as a *Bam*HI/*Bgl*II fragment and cloned behind the 35S promoter in plasmid PCV702 (Walden, 1990). The resulting binary plasmid p35S:PHYA.kana was then transferred into stem segments of a clone of hybrid aspen (*Populus tremula* × *tremuloides*, clone T89) as described by Nilsson *et al.* (1992). Twenty-two independent transformants were obtained.

Plant growth conditions and growth experiments

Plants were multiplied by *in vitro* shoot culture on half-strength MS medium containing minerals and vitamins only (Murashige and Skoog, 1962). After root initiation, plants were potted in fertilized peat and cultivated in controlled environmental chambers in the Phytotron of the University of Tromsø, Norway, at 18°C (± 0.5°C) under a photoperiod of 24 h for 4 weeks before use in the experiments. In all experiments the photon flux density of the main light period was 150–200 µmol m^{-2} sec^{-1} at 400–750 nm (Phillips TL 65W/83 fluorescent tubes). In cases with daylength extensions, these were given as low-intensity light from incandescent lamps (10 µmol m^{-2} sec^{-1}, Osram). Humidity in the growth chambers was adjusted to give 0.5 kPa water-vapour pressure deficit. Plants were watered daily with a complete nutrient solution (Junttila, 1976). If not otherwise stated, plants were grown at 18°C.

Seven individual plants of each of the 22 transgenic lines and the wild-type, were screened at 15°C for their growth responses under LD and SD conditions of 24 h and 12 h high-intensity light photoperiods, respectively. Cumulative stem elongation and appearance of new leaves were recorded every 3rd–5th day over a period of 21 days under LD and 40 days under SD. At the end of the experiment, lengths of three fully elongated internodes were measured for each plant, i.e. numbers 6, 7 and 8 below an apical leaf of length of ~2 cm.

Six lines representing short (lines 6 and 22), medium (13 and 24) and long (8 and 10) internodes were selected for further experiments. Together with the wild-type, these lines were tested during a period of 40–60 days using photoperiodic treatments ranging from 6 to 18 h, with a main photoperiod of 8 h (6 h), and photoperiod extensions with low-intensity light. In all cases, the light period was terminated with 5 min incandescent light (2 µmol m^{-2} sec^{-1}). There were 5–7 plants per line and photoperiod.

The selected lines were also tested for their light quality responses. Three to eight plants per line were exposed to R:FR ratios of 1.7 (Phillips fluorescent tubes TLD84 and TLD33), or 0.7 (Phillips fluorescent tubes TLD84 and Phillips 75W incandent lamps), throughout the light period of 24 h (150–200 µmol m^{-2} sec^{-1}) for 14 days. Plants were also exposed to 15 min EOD R or FR treatments, 5–7 individuals per treatment. Plants were then grown for 40 days with a 11 h 45 min photoperiod followed by no light, 15 min of R (Philips fluorescent tubes TLD/15, 17 µmol m^{-2} sec^{-1} at 660 nm) or 15 min FR (GE lightening, 75 W incandescent lamps filtered through one layer of red, Röhm 502, and one layer of blue, Röhm 627, Plexiglas, 1.4 µmol m^{-2} sec^{-1}) EOD light treatment. Cumulative stem elongation and appearance of new nodes were recorded every 4th or 5th day, and lengths of three fully grown internodes (counted as above) were measured at the end of the experiments.

Cold-hardening experiments

Effect of *PHYA* over-expression on development of cold hardiness was tested in plants exposed to photoperiods of 8, 11 and 14 h for 50 days, as well as in plants given 15 min EOD R or FR treatments for 40 days (both experiments as described above). During cold acclimatization, plants were given a 10 h photoperiod with Philips TL 65W/840 fluorescent tubes, 50 µmol m^{-2} sec^{-1}. One or two nodal stem segments from the upper and lower parts of the stems were freeze-tested under controlled conditions using a freezing rate of 3°C h^{-1} down to −17°C and further at 10°C h^{-1}. The samples were removed at intervals of 4°C. After thawing overnight at 6°C, the samples were incubated at 18°C between moist paper towels and injury in various tissues was evaluated visually under a microscope. In the experiment with plants exposed to EOD treatments, survival was also evaluated by an ion leakage test (Dexter *et al.*, 1932). Internode segments of 3–10 mm were then incubated in 10 ml of distilled water for 12 h at room temperature. After measurement of the conductivity, the samples were autoclaved for 20 min at 120°C, and conductivity was measured again after an additional incubation for about 2 h.

Northern blot analysis

Poly(A)$^+$ RNA was isolated using magnetic beads (Dynabeads, Dynal, Oslo, Norway) from 0.2 g young leaf tissue according to the manufacturer's protocol. Approximately 3 µg of each sample

was loaded and separated on a formaldehyde–agarose gel and transferred to a nylon membrane (Hybond N, Amersham International, UK) according to Sambrook *et al.* (1989). Northern hybridizations were performed according to the Amersham protocol for Hybond N membranes. The 1066 bp *Hin*dIII fragment from the oat *PHYA* cDNA clone and an actin cDNA from *P. trichocarpa* (data not shown) were used as probes. The probes were randomly labelled with α[^{32}P] dCTP using the labelling kit Ready-To-Go (Pharmacia, Biotech Inc., USA) and purified on a NICK Column (Pharmacia, Uppsala, Sweden). Membranes were hybridized at 65°C and 60°C respectively, and the last wash was performed in 0.1 $\times$ SSPE, 0.1% SDS at 65°C or 1 $\times$ SSPE, 0.1% SDS at 60°C for the actin probe. The membranes were then exposed to X-ray film (Hyperfilm–MP, Amersham International, UK).

Cell length determinations

Internodes 6 and 8 (counted as above) were harvested from each of three individual plants of each of the lines 6, 8, 10, 13, 22 and 24 and wild-type, grown under a long photoperiod of 24 h, fixed in FAA (37–40% formaldehyde:acetic acid:50% ethanol, 2:1:17, by volume), dehydrated through a standard tertiary butyl alcohol series, imbedded in paraffin (Johansen, 1940) and sectioned longitudinally in 10 µm sections. Sections were stained with 0.05% toluidine blue. Lengths of 75 cortical and pith cells as well as of 30 epidermal cells were determined for each internode, as well as the number of cells per internode.

Quantification of gibberellins and indole-3-acetic acid

Apical plant portions were processed in liquid nitrogen to a homogenous powder, of which samples of 25–50 and 50–100 mg were analysed for their content of gibberellins (GAs) and indole-3-acetic acid (IAA), respectively, by GC/MS-selected reaction monitoring (SRM) using a JEOL SX/SX102A four-sector mass spectrometer, with the configuration B_1E_1-B_2E_2 (JEOL; Tokyo, Japan). [$^2H_2$]-GAs (0.1–0.2 ng) and [$^{13}C_6$]-IAA (1–2 ng) were used as internal standards. GAs were analysed as described by Moritz and Olsen (1995), and IAA according to Edlund *et al.* (1995) with minor modifications. For purification of IAA, 60 mg XAD-7 resin (Sigma Chemical company, St Louis, MO, USA) was used per sample, from which IAA was eluted with 2 $\times$ 2 ml dichloromethane.

Samples were from the elongating zone, and were divided in two, the upper part consisting of four distinguishable internodes as well as the internode initials above, the lower part consisting of two partially elongated internodes. In one experiment, hormone levels were compared in the wild-type and the selected transgenic lines grown under a 24 h photoperiod. Stem samples and their corresponding leaves from three plants per line were analysed separately. In a time course experiment, apical stem portions (upper and lower) from plants grown under 10 or 24 h photoperiods (main light period of 10 h) were analysed. Two samples consisting of stem tissue from three plants were analysed for each stem part in each case.

Statistical analysis

Growth data were subjected to analyses of variance, followed by Fishers PLSD test.

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References

Amasino, R.M. (1996) Control of flowering time in plants. *Curr. Opin. Genet. Devel.* **6,** 480–487.

Bagnall, D.J., King, R.W., Whitelam, G.C., Boylan, M.T., Wagner, D. and Quail, P.H. (1995) Flowering responses to altered expression of phytochrome in mutants and transgenic lines of *Arabidopsis thaliana* (L) Heynh. *Plant Physiol.* **108,** 1495–1503.

Barnes, S.A., McGrath, R.B. and Chua, N.-H. (1997) Light signal transduction in plants. *Trends Cell Biol.* **7,** 21–26.

Behringer, F.J. and Davies, P.J. (1992) Indole-3-acetic acid levels after phytochrome-mediated changes in stem-elongation rate of dark- and light-grown *Pisum* seedlings. *Planta,* **188,** 85–92.

Boylan, M.T. and Quail, P.H. (1989) Oat phytochrome is biologically active in transgenic tomatoes. *Plant Cell,* **1,** 765–773.

Boylan, M.T. and Quail, P.H. (1991) Phytochrome A over-expression inhibits hypocotyl elongation in transgenic *Arabidopsis. Proc. Natl Acad. Sci. USA,* **88,** 10 806–10 810.

Carabelli, M., Morelli, G., Whitelam, G. and Ruberti, I. (1996) Twilight-zone and canopy shade induction of the Athb-2 homeobox gene in green plants. *Proc. Natl Acad. Sci. USA,* **93,** 3530–3535.

Casal, J.J. and Sanchez, R.A. (1994) Over-expression of oat phytochrome gene differentially affects stem growth responses to red/far-red ratio signals characteristic of sparse of dense canopies. *Plant Cell Environ.* **17,** 409–417.

Casal, J.J., Sanchez, R.A., Boylan, M., Vierstra, R.D. and Quail, P.H. (1995) Is the far-red-absorbing form of *Avena* phytochrome A that is present at the end of the day able to sustain stem-growth inhibition during the night in transgenic tobacco and tomato seedlings? *Planta,* **197,** 225–232.

Casal, J.J., Clough, R.C. and Vierstra, R.D. (1996) High-irradiance responses induced by far-red light in grass seedlings of the wild type or over-expressing phytochrome A. *Planta,* **200,** 132–137.

Chamovitz, D.A. and Deng, X.W. (1996) Light signaling in plants. *Crit. Rev. Plant Sci.* **15,** 455–478.

Childs, K.L., Lu, J.L., Mullet, J.E. and Morgan, P.W. (1995) Genetic regulation of development in *Sorghum bicolor.* 10. Greatly attenuated photoperiod sensitivity in a phytochrome-deficient sorghum possessing a biological clock but lacking a red light-high irradiance response. *Plant Physiol.* **108,** 345–351.

Clack, T., Mathews, S. and Sharrock, R.A. (1994) The phytochrome apoprotein family in *Arabidopsis* is encoded by five genes: the sequences and expression of PHYD and PHYE. *Plant Mol. Biol.* **25,** 413–427.

D'Aoust, A.L. and Hubac, C. (1986) Phytochrome action and frost hardening in black spruce seedlings. *Physiol. Plant.* **67,** 141–144.

Devlin, P.F., Halliday, K.J., Harberd, N.P. and Whitelam, G.C. (1996) The rosette habit of *Arabidopsis thaliana* is dependent upon phytochrome action: novel phytochromes control internode elongation and flowering time. *Plant J.* **10,** 1127–1134.

Dexter, S.T., Tottingham, W.E. and Graber, L.F. (1932) Investigations of the hardiness of plants by measurement of electrical conductivity. *Plant Physiol.* **7,** 63–78.

Edlund, A., Eklöf, S., Sundberg, B., Moritz, T. and Sandberg, G. (1995) A microscale technique for GC–MS measurements of pg amounts of indole-3-acetic acid in plant tissues. *Plant Physiol.* **108,** 1043–1047.

Furuya, M. and Schäfer, E. (1996) Photoperception and signalling of induction reactions by different phytochromes. *Trends Plant Sci.* **1**, 301–307.

Gilmour, S.J., Zeevaart, J.A.D., Schwenen, L. and Graebe, J.E. (1986) Gibberellin metabolism in cell-free extracts from spinach leaves in relation to photoperiod. *Plant Physiol.* **82**, 190–195.

Håbjørg, A. (1978) Photoperiodic ecotypes in Scandinavian trees and shrubs. *Meld. Nor. Landbrukshogsk.* **57**, 1–20.

Hauser, B.A., Cordonnier-Pratt, M.-M., Daniel-Vedele, F. and Pratt, L.H. (1995) The phytochrome gene family in tomato includes a novel subfamily. *Plant Mol. Biol.* **29**, 1143–1155.

Heide, O.M. (1974) Growth and dormancy in Norway spruce ecotypes (*Picea abies*). Interaction of photoperiod and temperature. *Physiol. Plant.* **30**, 1–12.

Heyer, A.G., Mozley, D., Landschutze, V., Thomas, B. and Gatz, C. (1995) Function of phytochrome A in potato plants as revealed through the study of transgenic plants. *Plant Physiol.* **109**, 53–61.

Howe, G.T., Hackett, W.P., Furnier, G.R. and Klevorn, R.E. (1995) Photoperiodic responses of a northern and southern ecotype of black cotton wood. *Physiol. Plant.* **93**, 695–708.

Howe, G.T., Gardner, G., Hackett, W.P. and Furnier, G.R. (1996) Phytochrome control of short-day-induced bud set in black cottonwood. *Physiol. Plant.* **97**, 95–103.

Johansen, D.A. (1940) *Plant Microtechnique.* New York: McGraw-Hill Book Company.

Johnson, E., Bradley, M., Harberd, N.P. and Whitelam, G.C. (1994) Photoresponses of light-grown *phyA* mutants of *Arabidopsis. Plant Physiol.* **105**, 141–149.

Jones, A.M., Cochran, D.S., Lamerson, P.M., Evans, M.L. and Evans, J.D. (1991) Red light regulated growth. I. Changes in abundance of indoleacetic acid and a 22-kilodalton auxin-binding protein in the maize mesocotyl. *Plant Physiol.* **97**, 352–358.

Jones, R.L. (1983) The role of gibberellins in plant cell elongation. *Crit. Rev. Plant Sci.* **1**, 23–47.

Jordan, E.T., Hatfield, P.M., Hondred, D., Talon, M., Zeevaart, J.A.D. and Vierstra, R.D. (1995) Phytochrome A over-expression in transgenic tobacco – Correlation of dwarf phenotype with high concentrations of phytochrome in vascular tissue and attenuated gibberellin levels. *Plant Physiol.* **107**, 797–805.

Junttila, O. (1976) Apical growth cessation and shoot tip abscission in *Salix. Physiol. Plant.* **38**, 278–286.

Junttila, O. (1989) Physiological responses to low temperature. *Ann. Sci. For.* **46**, 604–613.

Junttila, O. (1990) Gibberellins and the regulation of shoot elongation in woody plants. In *Gibberellins* (Takahashi, N., Phinney, B.O. and MacMillan, J., eds) Berlin: Springer Verlag, pp. 199–210.

Junttila, O. and Jensen, E. (1988) Gibberellins and photoperiodic control of shoot elongation in *Salix. Physiol. Plant.* **74**, 371–376.

Junttila, O. and Kaurin, Å. (1990) Enviromental control of cold acclimatization in *Salix pentandra. Scand. J. For. Res.* **5**, 195–204.

Junttila, O., Jensen, E. and Ernstsen, A. (1991) Effects of prohexadione (BX-112) and gibberellins on shoot elongation in *Salix. Physiol. Plant.* **83**, 17–21.

Li, J., Nagpal, P., Vitart, V., McMorris, T. and Chory, J. (1996) A role for brassinosteroids in light-dependent development of *Arabidopsis. Science,* **272**, 398–401.

Lopez-Juez, E., Kobayashi, M., Sakurai, A., Kamiya, Y. and Kendrick, R.E. (1995) Phytochrome, gibberellins, and hypocotyl growth – a study using the cucumber (*Cucumis sativus* L) long hypocotyl mutant. *Plant Physiol.* **107**, 131–140.

McCormac, A.C., Wagner, D., Boylan, M., Quail, P., Smith, H. and Whitelam, G. (1993) Photoresponses of transgenic *Arabidopsis* seedlings expressing introduced phytochrome B-encoding cDNAs: evidence that phytochrome A and phytochrome B have distinct photoregulatory functions. *Plant J.* **4**, 19–27.

McKenzie, J.S., Weiser, C.J. and Burke, M.J. (1974) Effects of red and far red light on the initiation of cold acclimatization in *Cornus stolonifera* Michx. *Plant Physiol.* **53**, 783–789.

Metzger, J.D. (1988) Gibberellins and light regulated petiole growth in *Thlapsi arvense. Plant Physiol.* **86**, 237–240.

Moritz, T. and Olsen, J. (1995) Comparison between high-resolution selected ion monitoring, selected reaction monitoring and four-sector tandem mass spectrometry in quantitative analysis of gibberellins in milligram amounts of plant tissue. *Anal. Chem.* **67**, 1711–1716.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bio-assay with tobacco tissue cultures. *Physiol. Plant.* **15**, 473–479.

Nagatani, A., Kay, S.A., Deak, M., Chua, N.H. and Furuya, M. (1991) Rice type I phytochrome regulates hypocotyl elongation in transgenic tobacco seedlings. *Proc. Natl Acad. Sci. USA,* **88**, 5207–5211.

Nilsson, O., Aldén, T., Sitbon, F., Little, C.H.A., Chalupka, V., Sandberg, G. and Olsson, O. (1992) Spatial pattern of cauliflower mosaic virus 35S promoter–luciferase expression in transgenic hybrid aspen trees monitored by enzymatic assay and non-destructive imaging. *Transgenic Res.* **1**, 209–220.

Nitsch, J.P. (1957) Photoperiodism in woody plants. *Proc. Am. Soc. Hort. Sci.* **70**, 526–544.

Olsen, J.E., Jensen, E., Junttila, O. and Moritz, T. (1995a) Photoperiodic control of endogenous gibberellins in seedlings of *Salix pentandra. Physiol. Plant.* **93**, 639–644.

Olsen, J.E., Junttila, O. and Moritz, T. (1995b) A localised decrease of GA_1 in shoot tips of *Salix pentandra* seedlings precedes cessation of shoot elongation under short photoperiod. *Physiol. Plant.* **95**, 627–632.

Olsen, J.E., Junttila, O. and Moritz, T. (1997) Long-day induced bud break in *Salix pentandra* is associated with transiently elevated levels of GA_1 and gradual increase in indole-3-acetic acid. *Plant Cell Physiol.* **38**, 536–540.

Pauley, S.S. and Perry, T.O. (1954) Ecotypic variation of the photoperiodic responce in *Populus. J. Arnold. Arbor.* **35**, 167–188.

Pratt, L. (1995) Phytochromes: differential proporties, expression patterns and molecular evolution. *Photochem. Photobiol.* **61**, 10–21.

Quail, P.H. (1994) Phytochrome genes and their expression. In *Photomorphogenesis in Plants,* 2nd Edn (Kendrick, R.E. and Kronenberg, G.H.M., eds). Amsterdam: Kluwer Academic Press, pp. 71–103.

Quail, P.H., Boylan, M.T., Parks, B.M., Short, T.W., Xu, Y. and Wagner, D. (1995) Phytochromes: photosensory perception and signal transduction. *Science,* **268**, 675–680.

Reed, J.W., Foster, K.R., Morgan, P.W. and Chory, J. (1996) Phytochrome B affects responsiveness to gibberellins in *Arabidopsis. Plant Physiol.* **112**, 337–342.

Sachs, R.M. (1965) Stem elongation. *Ann. Rev. Plant Physiol.* **16**, 73–96.

Sambrook, J., Fritsch, E. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sauter, M., Mekhedov, S.L. and Kende, H. (1995) Gibberellin promotes histone H1 kinase activity and the expression of cdc2 and cyclin genes during the induction of rapid growth in deepwater rice internodes. *Plant J.* **7**, 623–632.

Sharrock, R.A. (1992) Plant photoperception: the phytochrome

system. In *Development: the Molecular Genetic Approach* (Russo, U.E.A., Brody, S., Cove, D. and Ottolenghi, S., eds). Berlin: Springer Verlag, pp. 194–205.

Sharrock, R.A. and Quail, P.H. (1989) Novel phytochrome sequences in *Arabidopsis thaliana*: structure, evolution, and differential expression of a plant regulatory phot receptor family. *Genes Devel.* **3**, 1745–1757.

Smith, H. (1995) Physiological and ecological function within the phytochrome family. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46**, 289–315.

Szekeres, M., Nemeth, K., Koncz Kalman, Z., Mathur, J., Kauschmann, A., Altmann, T., Redei, G.P., Nagy, F., Schell, J. and Koncz, C. (1996) Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in *Arabidopsis*. *Cell,* **85**, 171–182.

Vaartaja, O. (1959) Evidence of photoperiodic ecotypes in trees. *Ecol. Monogr.* **29**, 91–111.

Vince-Prue, D. (1975) *Photoperiodism in Plants*. London: McGraw-Hill.

Walden, R., Koncz, C. and Schell, J. (1990) The use of gene vectors in plant molecular biology. *Methods Mol. Cell Biol.* **1**, 175–194.

Weiser, C.J. (1970) Cold resistance and injury in woody plants. *Science,* **169**, 1269–1278.

Weller, J.L., Ross, J.J. and Reid, J.B. (1994) Gibberellins and phytochrome regulation of stem elongation in pea. *Planta,* **192**, 489–496.

Whitelam, G.C., McCormac, A.C., Boylan, M.T. and Quail, P.H. (1992) Photoresponses of *Arabidopsis* seedlings expressing an introduced oat phyA cDNA: persistence of etiolated plant type responses in light-grown plants. *Photochem. Photobiol.* **56**, 617–621.

Wu, K., Li, L., Gage, D.A. and Zeevaart, J.A.D. (1996) Molecular cloning and photoperiod-regulated expression of gibberellin 20-oxidase from the long-day plant spinach. *Plant Physiol.* **110**, 547–554.

Zeevaart, J.A.D. and Gage, D.A. (1993) *ent*-Kaurene biosynthesis is enhanced by long-day plants *Spinacia oleracea* L. and *Agrostemma githago* L. *Plant Physiol.* **101**, 25–29.

Zeevaart, J.A.D., Talon, M. and Wilson, T.M. (1990) Stem growth and gibberellin metabolism in spinach in relation to photoperiod. In *Gibberellins* (Takahashi, N., Phinney, B.O. and MacMillan, J., eds). Berlin: Springer Verlag, pp. 273–279.