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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

Jorunn E. Olsen [1], Olavi Junttila [1] and Thomas Moritz [2]

[1] *Department of Plant Physiology and Microbiology, IBG, Univ. of Tromsø, N.9037 Tromsø, Norway*
[2] *Dept of Forest Genetics and Plant Physiology, Swedish Univ. of Agricultural Sciences, S-90183 Umeå, Sweden*

Initiation of dormancy in woody species is induced by a short photoperiod. In the early stages of dormancy development, growth can be initiated by long days. To study the possible involvement of gibberellins and indole-3-acetic acid in bud break in *Salix pentandra*, effects on levels of these plant hormones of transfer of seedlings in an early stage of dormancy to a growth-inductive long photoperiod was investigated. The bud break and initiation of growth correlated with a rapid transient increase in GA_1 in defined zones of shoot tips to levels twice of that in continuously elongating long day-grown control seedlings, followed by a rapid decrease to levels similar to in these control plants. Also, the contents of GA_{53}, GA_{19}, GA_{20}, GA_8 increased upon transfer from short to long days. Levels of indole-3-acetic acid showed a gradual decline under short days, and increased gradually upon transfer to long days up to a level of continuously elongating plants. The present data suggest an interaction between gibberellin and indole-3-acetic acid in the photoperiodic control of dormancy development and bud break in *S. pentandra*.

Key words: Bud break — Gibberellins — IAA — Indole-3-acetic acid — Photoperiod — *Salix pentandra*.

In young individuals of most temperate-zone woody species a long photoperiod is required for sustained shoot elongation, and cessation of growth and formation of resting buds are induced when the photoperiod is shorter than a critical length (Nitsch 1957). This is a prerequisite for cold hardening and induction of dormancy (Weiser 1970), which enable trees to survive under the normal summer-winter cycle. In seedlings of *Salix pentandra* growth has ceased and a terminal bud is formed after 2 weeks of a short photoperiod (SD) (Junttila 1976). Dormancy development is a gradual process, and initially buds are able to resume growth when returned to long days (LD) (Junttila 1976).

As the LD requirement for shoot elongation in *S. pentandra* can be substituted by certain exogenous gibberellins (GAs) (Junttila and Jensen 1988), GAs appears be involved in the photoperiodic control of shoot elongation. GA_1 seems to be the active GA (Junttila et al. 1991), and levels of this as well as other GAs decrease gradually under SD (Olsen et al. 1995a, b).

Indole-3-acetic acid (IAA) has also been suggested to be involved in control of shoot elongation. Auxin is well known to stimulate elongation in isolated stem segments (see Rayle and Cleland 1992), but the role of IAA in regulating stem extension of intact plants has for many years been unclear, as application experiments often have failed to give a continuous growth promotive effect (Rayle and Cleland 1992). However, there are consistent data showing correlations between endogenous IAA content and stem growth in studies of dwarf mutants compared with normal plants of pea (Law and Davies 1990, Behringer et al. 1992, McKay et al. 1994).

Continuous supply of exogenous IAA has also been shown to provide stimulation of shoot elongation in intact pea plants, with a particularly pronounced effect in dwarf seedlings (Hall et al. 1985, Behringer et al. 1992, Yang et al. 1993). Also, high levels of endogenous IAA after GA-application to various plant species have been reported (Law and Hamilton 1989, and refs therein), suggesting a GA-mediated enhancement of IAA biosynthesis. However, other data suggest other interactions between these hormones as well. Recently Yang et al. (1996) reported on promotive effects of exogenous GAs and IAA on elongation of dwarf mutants of pea containing low endogenous levels of both of these hormones. GA and IAA co-treatment stimulated a much greater elongation response than either treatment alone (Yang et al. 1996). In segments of azuki bean hypocotyls, GA_3 pretreatment promoted IAA-induced elongation, whereas GA_3 treatment without subsequent application of IAA did not exhibit any effect on the elongation of the segments (Kaneta et al. 1993).

Previously we have found that GAs appear to be involved in the photoperiodic control of dormancy induction in *S. pentandra*. In the present investigation we have addressed also the possible involvement of IAA in this, as well as of both IAA and GA in the release from a short day-induced dormancy.

Abbreviations: LD, long days of a 24-h photoperiod; SD, short days of a 12-h photoperiod.

Materials and Methods

Plant material and growth conditions—Seedlings of *Salix pentandra* L., ecotype from 69°39'N lat., were grown in fertilised peat at 18°C in a 24-h photoperiod (LD) as described earlier (Junttila and Jensen 1988). The main light period of 12 h had a minimum photon flux density of 150 μmol m^{-2} s^{-1} within 400–750 nm (Phillips TL 65W/83). The photoperiod was extended to 24 h (LD) with low-intensity light from incandescent lamps (10 μmol m^{-2} s^{-1}). The air humidity was controlled to give a water vapour pressure deficit of 0.5 kPa.

All seedlings were raised under LD for 4 weeks, i.e. to a size of about 5 cm. Plants were then exposed to a short photoperiod of 12 h light at 150 μmol m^{-2} s^{-1} (SD) for 15 days in order to induce growth cessation (see Junttila 1976), whereas control plants were kept continuously under growth-sustaining LD conditions. The LD-grown plants were then about 20 cm tall, whereas the SD-treated seedlings were 10–12 cm. Shoot length increment was then recorded after transfer from SD back to LD (corresponds to Day 0 in Fig. 1; seedlings were then 6 weeks old), as compared with the LD-grown pants (also 6 weeks old at day 0 in Fig. 1). There were 12 plants per treatment. The effect of exogenous IAA and 2,4-D on shoot elongation in plants exposed to 15 SD was also investigated. Auxins were dissolved in 95% ethanol, and applied in 1 μl drops to the apex at a dose of 0, 0.01, 0.1, 0.5 or 50 μg to each of 10–14 seedlings, and results were recorded 14 days after application and further exposure to SD.

In another experiment 7 sequential stem segments from 7 plants were harvested 7 days after transfer to LD following 15 SD. The stem segments were analysed individually for their GA content. In yet another experiment levels of IAA and GAs were compared in shoot tips in time courses for seedlings exposed to 15 SD followed by transfer to LD, as compared with under LD, and prolonged SD-exposure. For all photoperiodic treatments, 4 sequential 5 mm long stem segments were harvested from the apical part of the stem of each plant. Triplicate samples from 3 plants each were analysed for each photoperiodic treatment and harvest time. The most apical segment included the shoot apex, and in all cases as many folded leaves and leaf initials as practically possible were removed. All samples were harvested in the middle of the high-intensity light period.

Extraction and purification of GAs and IAA—The samples were extracted and purified according to Moritz and Olsen (1995). This involved homogenisation in liquid nitrogen, extraction in 500 μl of 80% aqueous MeOH containing 0.02% (w/v) sodium diethyl dithiocarbamate as an antioxidant, and [17, 17-$^2H_2$]GAs and [$^{13}C_6$]IAA as internal standards. (100 pg per sample of [17, 17-$^2H_2$]GAs; Prof. L.N. Mander, Research School of Chemistry, Australian Natl. Univ. Canberra, Australia, 1 ng per sample of [$^{13}C_6$]IAA; Cambridge Isotope laboratories, Woburn, Ma, U.S.A.), and purification by sequential use of aminopropyl (0.1 g) and C-18 (0.5 g) cartridges.

Gas chromatography-mass spectrometry—The samples were methylated, purified further on 0.1 g aminopropyl cartridges, trimethylsilylated and analysed by combined GC-selected reaction monitoring (SRM) using a JEOL-SX 102/102A tandem mass spectrometer (JEOL, Tokyo, Japan) as described earlier (Moritz and Olsen 1995). The reactions m/z 418 to m/z 375 and m/z 420 to m/z 377 for GA_{20}, m/z 448 to m/z 389 and m/z 450 to m/z 391 for GA_{53}, m/z 434 to m/z 375 and m/z 436 to m/z 377 for GA_{19}, m/z 506 to m/z 448 and m/z 508 to m/z 450 for GA_1, m/z 594 to m/z 448, m/z 596 to m/z 450 for GA_8, and m/z 261 to m/z 202 and m/z 267 to m/z 208 for IAA were recorded.

Statistical analysis—Effect of photoperiod on levels of GAs and IAA, as averaged over all stem segments analysed, was tested by one-way analysis of variance ($P \leq 0.05$) followed by Fishers PLSD test, using a Statview program. As segments from a single plant are not independent, such statistical tests could not be used to test for differences between segments.

Results

Growth after transfer from short to long photoperiod or application of auxins—After 15 SD, elongation growth had ceased, and a terminal bud was formed (see Juntilla 1976). However, shoot elongation was then initiated upon transfer to LD (day 0, Fig. 1). After 4 LD bud break was observed, and from about day 9 the course of the growth increment was comparable with that of plants kept continuously under LD (Fig. 1). There was no effect on shoot elongation under SD of exogenous IAA or 2,4-D (doses from 0–50 μg) in plants exposed to 15 SD before application (data not shown).

Levels of GAs and IAA—Levels of GA and IAA in apical stem tissue were monitored under induction of growth upon transfer of seedlings from SD to LD (day 15, Fig. 2 and 3, corresponds to day 0, Fig. 1), compared with under SD and continuous LD (Fig. 2, 3). Under SD the levels of both IAA and GAs decreased significantly, i.e. 60–70% for IAA after 15 SD and between 20–60% for GAs. After exposure to SD for 24 days, their levels had decreased by 10–20% further. However, GA_{53} diverged from this pattern,

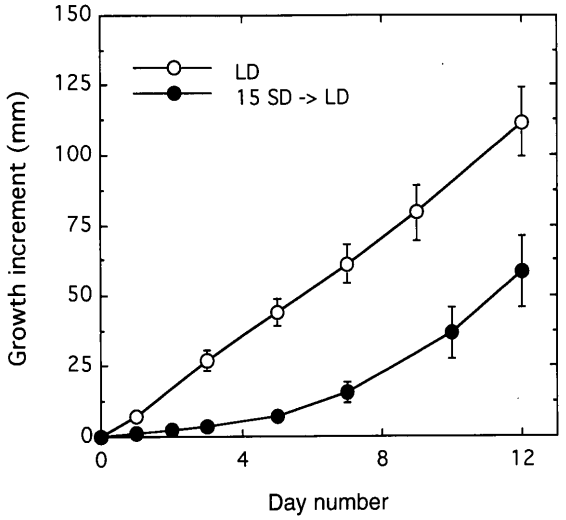

Fig. 1 Shoot elongation in seedlings of *S. pentandra* after transfer to continuous light (long days; LD) after a period of 15 short days of a 12 h photoperiod (SD), as compared with plants grown under continuous light. All plants were raised under LD for 4 weeks, and some seedlings were then exposed to 15 SD before transfer to LD (day 0). Other plants were kept further under SD, and others were kept continuously under LD throughout the experiment. Values are the means ± SE of 12 plants. There was no further growth in plants kept under SD.

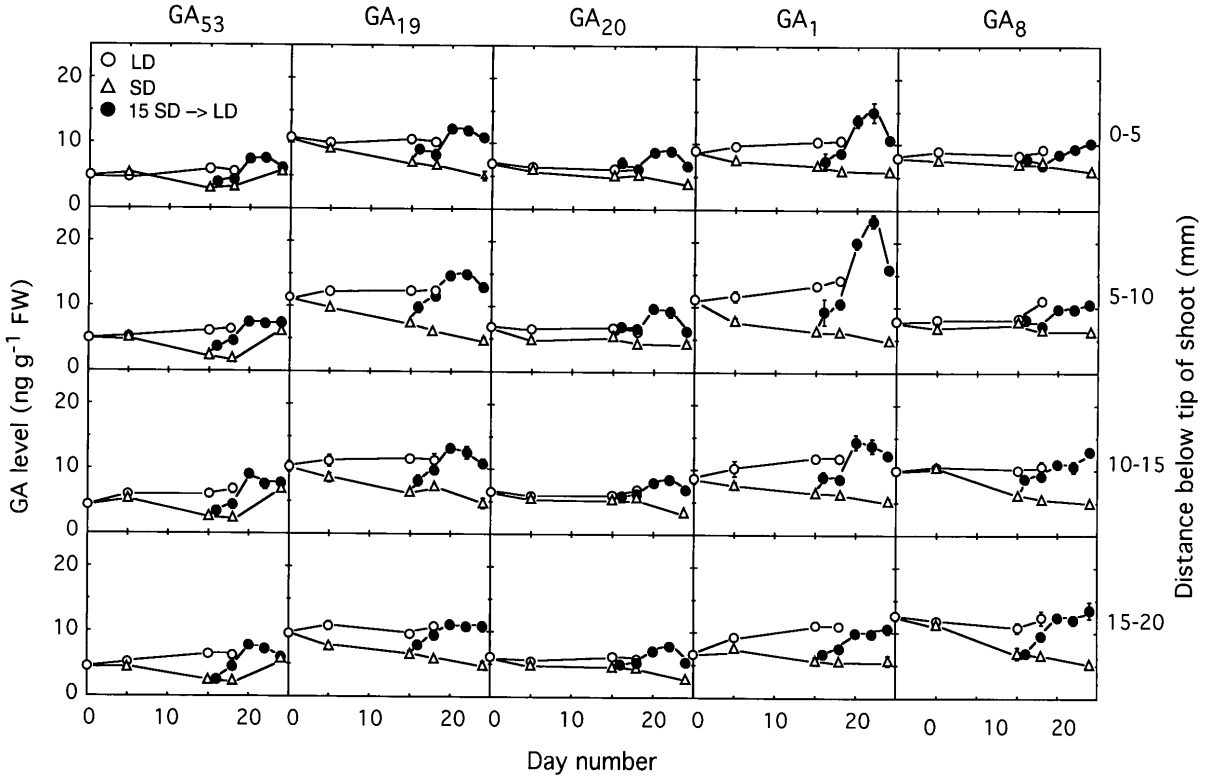

Fig. 2 Effect on levels of gibberellins (GAs) of transfer of predormant *S. pentandra* seedlings to continuous light (long days; LD) after a period of 15 short days of a 12 h photoperiod (SD), as compared with plants grown under continuous LD or exposed further to SD. Day 0 refers to the start of the SD-treatement. Four sequential 5 mm long stem segments were harvested in each case, and values are the means ± SE of 3 samples consisting of 3 plants each.

after having decreased by 50–60% after 15–18 SD, it increased significantly to a level similar to under LD at 24 SD.

Recording of GA levels in sequential 5 mm long segments of the apical 35 mm of the stem at day 7 after transfer from SD to LD, showed that bud break and initiation of growth was correlated with steep gradients especially of GA_{20} and GA_1 in shoot tips (Fig. 4). Though there were small differences in absolute GA levels between independent experiments, these observations were confirmed in the time course experiment which included the uppermost 20 mm of the stem (Fig. 2). The highest levels of GA_{20} and GA_1 were found 5–10 mm below the apical tip, and the amounts decreased downwards to ca. 20 mm below the shoot tip, from where GA contents were similar.

Initiation of growth was correlated with a rapid increase in the active GA_1. The first signs of bud burst at day 4 after transfer to LD coincided with an accumulation of GA_1 up to a level of that in continuously elongating plants (Fig. 2). Thereafter GA_1 increased rapidly to a maximum at day 7 after transfer to LD. The greatest increase was observed from 5–10 mm below the apical tip, i.e. up to a level twice of that in continuously elongating plants, and 4-fold higher than at the time for the transfer to LD. In the segments above and immediately below this, the increases in GA_1 were 2-fold relative to after 15 SD, and from 15 to 20 mm 1.5-fold. From day 7 to day 9 after transfer, GA_1 declined rapidly down to levels comparable to those in plants grown under continuous light. This coincided with the time point when growth was again comparable to in these control plants. For GA_{53}, GA_{19}, GA_{20} there were also significant increases to levels higher than those in plants kept continuously under LD, i.e. with maximum 5–7 LD after transfer to LD. Thereafter significant decreases in all GAs to levels similar to those under continuous LD were observed.

Upon transfer from short to LD, levels of IAA increased gradually up to those in plants kept under continuous LD (Fig. 3). There were also small differences in IAA content in different positions along the longitudinal axis of the stem, though far from as pronounced as for GA_1.

Discussion

The present study, which focuses upon release from an early stage of dormancy resulting from prolonged SD-exposure of *S. pentandra* seedlings, demonstrates clear correlations between LD-induced bud break (Fig. 1), and

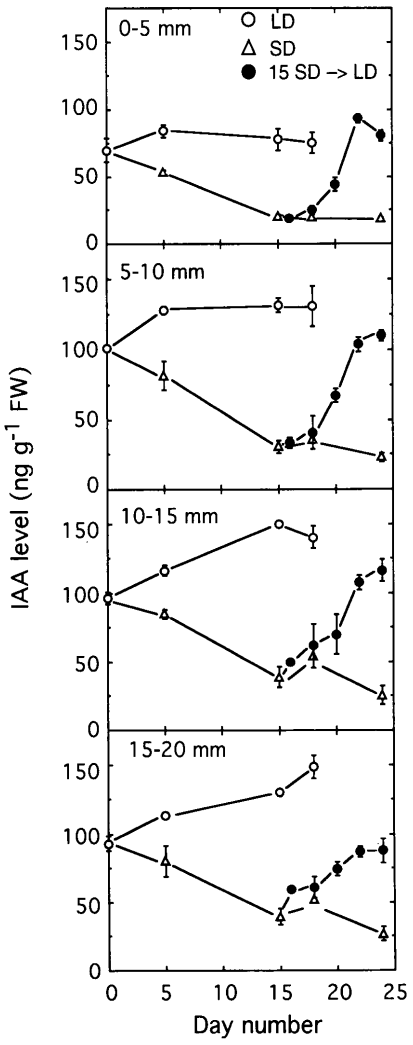

Fig. 3 Effect on levels of indole-3-acetic acid (IAA) of transfer of predormant *S. pentandra* seedlings to continuous light (long days; LD) after a period of 15 short days of a 12 h photoperiod (SD), as compared with plants grown under continuous LD or exposed further to SD. Day 0 refers to the start of the SD-treatement. Four sequential 5 mm long stem segments were harvested in each case, and values are the means±SE of 3 samples consisting of 3 plants each.

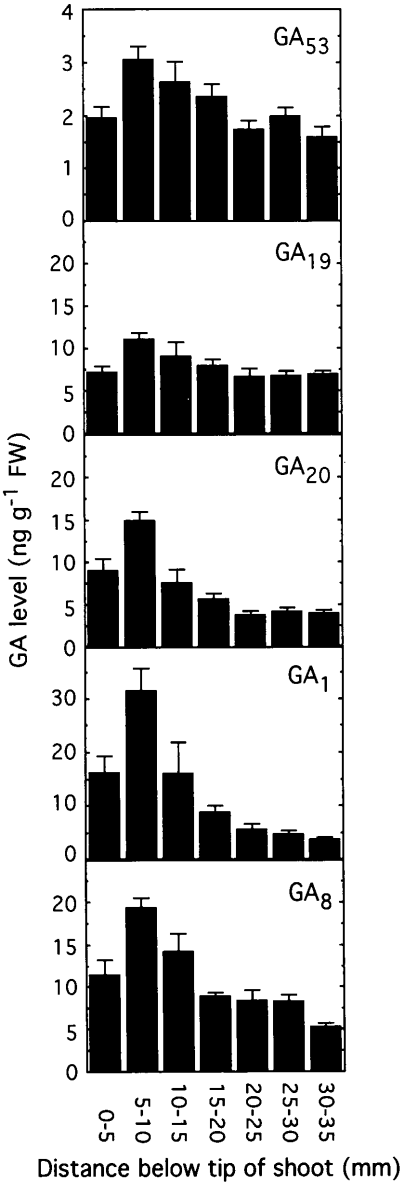

Fig. 4 Levels of gibberellins (GAs) 7 days after transfer of predormant *S. pentandra* seedlings to continuous light (long days; LD) after a period of 15 short days of a 12 h photoperiod (SD). Seven sequential 5 mm long stem segments were harvested, and values are the means±SE of measurements from 7 individual plants.

localised, transiently elevated levels of GAs, especially pronounced for GA_1 (Fig. 2, 4). This gradient of GA_1 within apices was far more steep than in continuously elongating plants, probably reflecting the initiation of renewed shoot elongation in a narrow zone of the stem. In *S. pentandra*, exogenous GA_1 and GA_{20} are both able to mimic LD conditions, and induce bud break and new growth under SD (Junttila and Jensen 1988). The prevailing data on GAs relative to the events involved in bud break in *S. pentandra*, are thus suggestive of a causal effect of GA_1. The temporary high level of GA_1 in *S. pentandra* disappeared as elongation growth was similar to that of continuously elongating seedlings. It is possible that the levels of GA_1 are down-regulated as soon as growth has resumed.

Though the amounts of GA_{53} decreased the first 18 days under SD-treatment, an accumulation of this GA was observed under further SD exposure (Fig. 2). This accumulation could partly reflect decreased conversion to GA_{44} and other GAs along the metabolic route to GA_1, and/or decreased activity of conversion to other derivatives of GA_{53}, such as 2β-OH GA_{53}, which we have tentatively identified by full-scan GC-MS in *S. pentandra* (J.E. Olsen and T. Moritz, unpublished results). The accumulation of GA_{53}

might be indicative of a photoperiodic control point under prolonged SD conditions. Photoperiodic control of metabolism of GA_{53} has been observed for spinach (Gilmour et al. 1986). This step, as well as the subsequent conversions to GA_{19}, have been shown to be catalysed by a GA20-oxidase in *Cucurbita, Arabidopsis* and *Spinacia* (Lange et al. 1994, Phillips et al. 1995, Wu et al. 1996). In spinach a photoperiodic control of the expression of this enzyme was recently demonstrated (Wu et al. 1996). Also, this enzyme activity has been shown to be down-regulated by bio-active GAs in a type of feed-back regulation (Hedden and Crocker 1992). Thus, this enzyme appears to be a key-enzyme in the regulation of GA metabolism. However, the initial decline in GA_{53} under SD in *S. pentandra*, prior to the accumulation under further SD-exposure, makes the picture more unclear in this case so far.

The levels of IAA decreased rapidly under SD. Upon transfer to a growth-inductive long photoperiod, the IAA increased gradually up to levels similar to those in continuously elongating plants at the time when growth had fully resumed. Unlike the situation for GA_1, IAA did not increase above the levels of the continuously long-day grown control plants. Furthermore, as opposed to with certain GAs (Junttila 1976, Juntilla and Jensen 1988), auxins were not able to substitute for the LD requirement for shoot elongation in *S. pentandra* under SD. This was the case for doses from 0.01 μg to 50 μg of IAA and 2,4-D. Though a GA-mediated enhancement of IAA-biosynthesis involved in the photoperiodic control of LD-induced bud break is possible, GAs does apparently also play other important roles, since auxin was not able to sustistute for the LD requirement for shoot elongation in *S. pentandra* under SD.

In conclusion, it appears that GA_1 is involved in the mediation of the photoperiodic control of dormancy and bud break in *S. pentandra*. It also seems apparent from the present correlations between growth and lack of growth, and higher and lower levels of IAA respectively, that IAA is also a necessary component involved in the photoperiodic control of dormancy and release, though exogenous auxin could not induce bud break, under SD.

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