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Gibberellins and Subapical Cell Divisions in Relation to Bud Set and Bud Break in *Salix pentandra*

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Abstract. In young plants of *Salix pentandra*, a temperate zone deciduous woody species, elongation growth ceases and a terminal bud is formed at day lengths shorter than a critical length. This is the first step in dormancy development, making survival under harsh winter conditions possible. Early studies strongly indicate that gibberellin is involved in the photoperiodic control of bud set and bud break. GA_1 action was studied by application under short days to plants where cessation of shoot elongation had occurred, followed by subsequent anatomic investigations of shoot tips. Under short days the frequency of cell division decreased rapidly along with the earlier observed decrease in GA_1 levels. Application of GA_1 to short-day–induced terminal buds rapidly stimulated cell division in apices several days before visible shoot elongation in response to this treatment was observed. One day after GA_1 application a fourfold increase in cell division frequency in apices was observed, increasing to a maximum of sevenfold 2 days after application. Long-day treatment leading to induction of bud break after about 4–6 days was followed by slowly increasing frequency of cell divisions. In earlier studies of this species, short days and gibberellins had no effect on cell elongation. These data show that increased GA_1 content, by application or long-day treatment, results in increased frequency of mitosis. This strongly indicates that GA_1 affects stem elongation in connection with bud set and bud break primarily by affecting cell divisions in subapical tissues.

Key Words. Bud set—Bud break—Gibberellin—Growth cessation—Photoperiod—*Salix pentandra*

Temperate zone woody species survive normal summer-winter cycles by entering a dormant state well before freezing conditions occur. In young plants of such species exhibiting a free growth pattern, photoperiod length is a primary signal for dormancy induction (Nitsch 1957). Long days (LD) sustain growth, whereas photoperiods shorter than a critical length (SD) induce cessation of shoot elongation and formation of resting buds, a prerequisite for dormancy development (Junttila and Kaurin 1990, Weiser 1970). Both dormancy induction and breakage are gradual processes, and initially in the induction process buds are able to resume growth when returned to LD.

Previous studies of *Salix pentandra* and *Populus tremula* × *tremuloides* have shown involvement of gibberellins (GAs) in the regulation of elongation growth and growth cessation in woody species (Junttila and Jensen 1988, Junttila et al. 1991, Olsen et al. 1995a,b, 1997a,b). After exposure of a northern ecotype of *Salix* to 5 SD, although no visible signs of growth cessation were observed, levels of GA_1 were decreased by 50% within specific regions of the apex that normally contain maximum levels (Olsen et al. 1995b, 1997a). Bud break and resumption of growth after a dormant period were also found to be associated with increases in levels of gibberellins, especially GA_1. GA_1 then showed transiently elevated levels in subapical stem segments (Olsen et al. 1997a). Only certain GAs, such as GA_1, are able to replace LD effects and prevent growth cessation and induce new growth under SD (Junttila 1976, Junttila and Jensen 1988). Thus, inhibition of biosynthesis of GA_1, the GA active in stem elongation in woody plants (Junttila 1993, Junttila et al. 1991) appears to be an early step in induction of growth cessation and cold hardening.

There are several reports on GAs affecting cell elongation in internodes (see Jones 1983). In *Salix*, appearance of new internodes ceased within a period of 10–15 SD, but no effect of decreased GA_1 levels was found on

Abbreviations: LD, long days; SD, short days.

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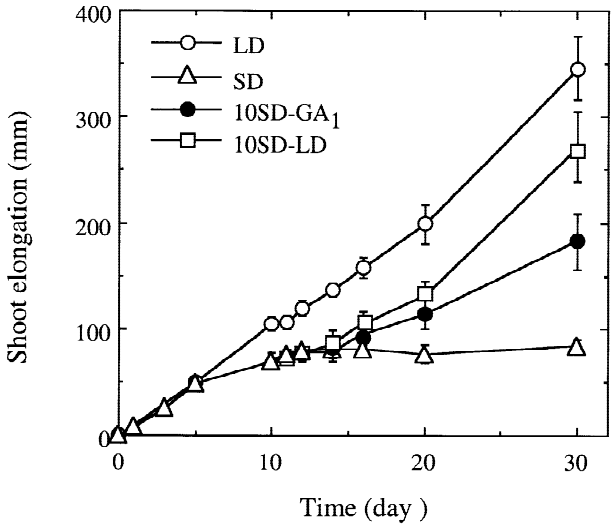

Fig. 1. Effect of day length (LD, long days of 24-h photoperiod; SD, short days of 12-h photoperiod) and apically applied GA_1 (10 μg per plant) on growth of young plants of *Salix pentandra*. Values are the means ± SE of measurements of seven individual plants.

elongation of cells in internodes already initiated at the start of the SD-treatment (Olsen et al. 1995b).

In rosette plants, mitotic activity related to the bolting process that precedes flowering and that normally takes place under LD was induced by exogenous GAs under SD, when such plants normally stay in the rosette stage (Sachs 1965). Also cell division in intercalary meristems of rice were stimulated by exogenous GAs (Sauter et al. 1995).

Although the apical meristem proper is the origin of the initial cells of the stem, the subapical meristematic region is the site of formation and elongation of most cells that are to constitute the mature stem (Sachs 1965). The subapical stem tissue has been postulated to be the target tissue for endogenous GA_1. Previously, we have demonstrated a clear gradient in GA_1 along shoot tips of seedlings of *S. pentandra*, with the highest levels in the subapical region from 5–20 mm below the apical tip (i.e., twice the levels in other regions) (Olsen et al. 1995b). It was also demonstrated that a decrease in GA_1 in this specific region preceded visible signs of growth cessation. On LD-induced bud break, the GA_1 content increased rapidly but transiently in a zone of the stem 5–10 mm below the apical tip, with a gradient far more steep than in continuously elongating plants.

The aim of this investigation was to study the patterns of cell division in apices of *S. pentandra* as related to SD-induced dormancy and LD-induced bud break, as well as to address the mode of action of GA_1 in this respect. We show here that gibberellins and the photoperiod act by regulation of subapical cell divisions.

Materials and Methods

Plant Material and Growth Conditions

Seedlings of *Salix pentandra* L., ecotype from 69°39′N lat., were raised in fertilized peat at 18°C in a 24-h photoperiod (LD). 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), and extension of the photoperiod to 24 h was given with low-intensity light from incandescent lamps (10 μmol m^{-2} s^{-1}). The air humidity corresponded to a water vapor pressure deficit of 0.5 kPa.

Four weeks after sowing, when plants were about 5 cm tall, a subset of plants was transferred to a short photoperiod of 12-h light at 150 μmol m^{-2} s^{-1} (SD), whereas other plants were kept under LD conditions. After 10 days, the SD-treated seedlings had stopped growing and were about 10–12 cm tall, whereas the growing plants under LD were about 20 cm tall. A subset of the SD-exposed plants was then transferred back to LD. To another subset 10 μg GA_1 was applied per plant in 1-μL drops of 96% ethanol to the apical bud before further incubation under SD conditions. Parts of the experiments were performed twice.

Preparation of Tissue Sections and Microscopy

Plant shoot tips were harvested for microscopy in time courses of exposure to LD, SD, after application of GA_1 under SD, as well as after transfer from SD to LD. Samples of plants from the following treatments were harvested in two independent experiments: 0 LD, 5 SD, 10 SD, 20 SD, and 1, 2, and 4 days after GA_1 application to plants under SD (day 11, 12, and 14 in Figs. 1 and 3). Six plants were harvested per time point of each treatment in each experiment. Sequential 5-mm segments of the apical part of the stem of each seedling were fixed in FAA (37–40% formaldehyde/acetic acid/50% ethanol, 2:1:17, by vol.). Before embedding, the segments were dehydrated through an aqueous ethanol series; 50%, 70%, 95% and absolute ethanol, 45 min in each step, followed by incubation for 16 h under vacuum in a 50:50% mixture of absolute ethanol and historesin (Reichert-Jung. Cambridge Instruments GmbH, Heidelberg, Germany). Before the embedding proper in Historesin, the segments were incubated 24 h under vacuum in historesin. Teflon molds containing stem segments in historesin were covered by parafilm and left to polymerize for 24 h at 37°C.

The preparates were then sectioned longitudinally in 3 μm sections, which were stained with 1% toluidine blue, dissolved 1:1 (v/v) in 2.5% Na_2CO_2. Observations on dividing cells in cortex and pith of subapical stem tissues (i.e., occurrence of cells in mitosis in each 5-mm stem segment) were then performed. The apical dome itself was not included because it is the subapical area that gives rise to most of the mature stem.

Results

Active shoot elongation in *Salix pentandra* under long photoperiods (Fig. 1) coincided with active cell division as shown for pith and cortical tissues of shoot tips. A subapical gradient in mitosis in shoot tips was observed (Fig. 2). The highest division frequencies were found in the uppermost 15 mm of the stem. Under SD treatment, when growth cessation takes place (Fig. 1), a rapid decrease in cell division frequency was observed (Fig 3). Already after 1 day of SD exposure, approximately 40% reduction in mitosis frequency was observed, and after

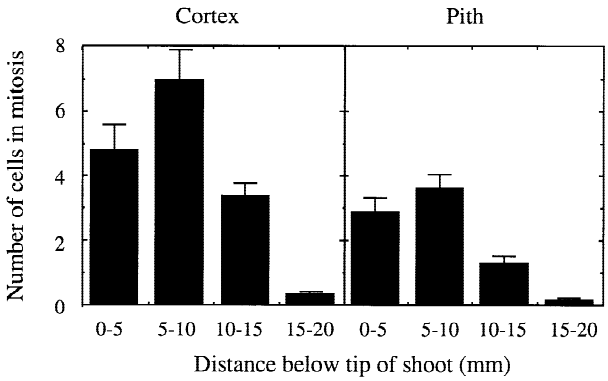

Fig. 2. Cell divisions in sequential apical stem segments of actively elongating young plants of *Salix pentandra* grown under long days of 24-h photoperiod. Values are the means of measurements of six individual plants. Vertical bars represent SE.

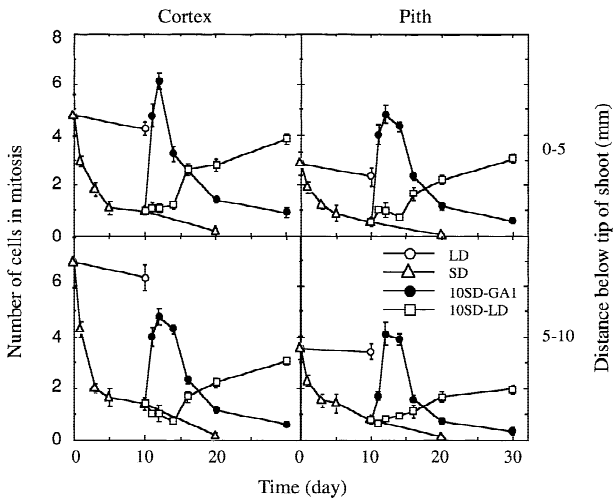

Fig. 3. Effect of day length (LD, long days of 24-h photoperiod; SD, short days of 12 h photoperiod) and apically applied GA_1 (10 μg per plant) on cell divisions in apical stem segments of young plants of *Salix pentandra*. For 0 LD, 5 SD, 10 SD, 20 SD and 1, 2, and 4 days after GA_1-application to plants under SD (days 11, 12, and 14), values are the means ± SE of measurements of six plants in each of two independent experiments. In the other cases values are the means ± SE of data from six plants.

10 SD when growth had came to a complete stop, cell divisions were reduced by 80–90%. A complete arrest in cell divisions was observed under further SD exposure. Transfer of plants from SD back to LD induced bud break within approximately 4 days, and normal growth was soon resumed (Fig. 1). This was followed by a slowly increasing frequency of cell division (Fig. 3). Application of 10 μg GA_1 per plant to SD-induced terminal buds also induced bud break after 4–5 days (Fig. 1). This treatment did within 1 day greatly stimulate cell divi-

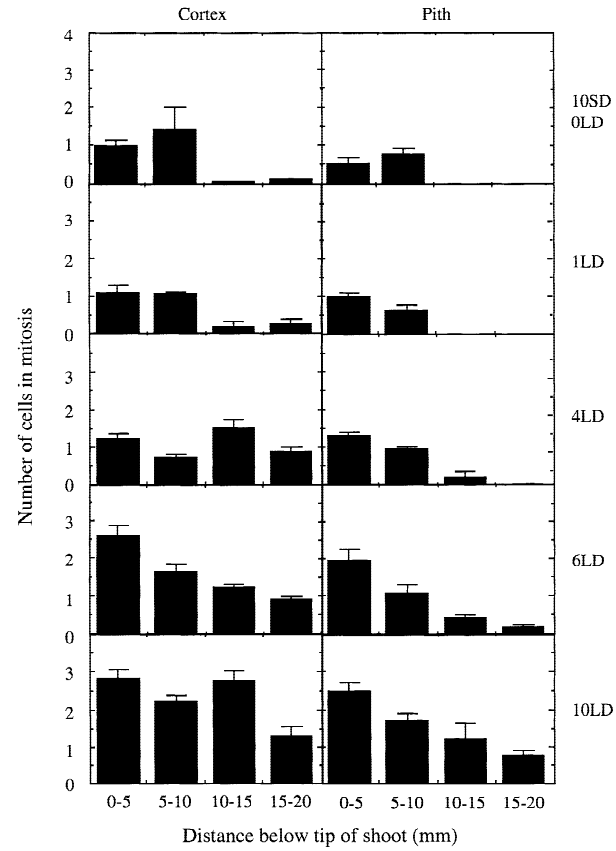

Fig. 4. Effect of transfer to long days (LD, 24-h photoperiod) on cell divisions in sequential stem segments of young plants of *Salix pentandra* exposed to 10 short days (SD) of 12-h photoperiod. Values are the means of measurements of six individual plants. Vertical bars represent SE.

sions in shoot tips (Fig. 3), which was several days before visible bud break and shoot elongation was observed. One day after GA_1 application, a fourfold increase in cell division frequency in apices was observed, increasing to maximum of sevenfold 2 days after application. Thereafter mitosis frequency decreased. After transfer from SD to LD, similar gradients in cell divisions as observed under continuous LD conditions (Fig. 2) were soon established in cortex and pith tissues of shoot tips (Fig. 4).

Discussion

Further understanding of the role of factors controlling induction and breakage of dormancy in trees is of great importance for increasing our knowledge about mechanisms of adaptation to climate. Growth cessation, the first step in dormancy induction, is known to be controlled by photoperiod in most tree species of the tem-

perate zone (Håbjørg 1978, Heide 1974, Junttila 1976, Nitsch 1957, Sylven 1940). From previous studies we know that gibberellins play an important role in photoperiodic growth control of trees (Junttila and Jensen 1988, Moritz 1995, Olsen et al. 1995 a,b, Olsen et al, 1997a,b). In this respect no effect of photoperiod and gibberellin on cell elongation has been found (Olsen et al. 1995a). We show here that photoperiod affects the frequency of cell division in subapical tissues below the eumeristem. In actively growing plants, a gradient of cells in mitosis was observed in the uppermost 20 mm of the stem (Figs. 2, 4). This is in accordance with the hypothesis that GA_1 acts by controlling cell divisions in the subapical meristematic region, as the gradient of cell divisions coincides with a similarly demonstrated gradient in active gibberellin GA_1 content in shoot tips (Olsen et al. 1995a, 1997a). The rapid reduction in cell division frequency under SD preceding growth cessation also correlates well with the previously shown lowered levels of gibberellins in shoot tips of *S. pentandra* seedlings that precedes visible growth cessation and bud set (Olsen et al. 1995a,b, Olsen et al 1997a). In *Populus tremula* × *alba* transformed with a fusion between the promoter of the cell cycle–regulating cyclin 1 gene from *Arabidopsis* and the coding sequence of β-glucuronidase, reduced β-glucuronidase activity was found in dormant plants compared with those undergoing growth (Rohde et al. 1997).

That GA_1 affects subapical cell divisions is confirmed here by the dramatic and rapid effect of GA_1 application to plants that had undergone growth cessation in response to a short photoperiod (Fig. 3). Transfer of plants in which bud set has occurred, from SD to growth-inductive LD, has earlier been shown to induce a rapid stimulation of GA_1 biosynthesis that precedes bud break (Olsen et al. 1997a). The fact that increased GA_1 content precedes visible budbreak, and similarly, that the reduction in GA_1 levels precedes bud set, confirms the importance of GA_1 as one causal factor in the control of bud set and bud break.

In conclusion, this study shows that bud set and bud break are associated with cessation and resumption of cell divisions, respectively, in the subapical region of shoot tips and that GA_1 affects stem elongation primarily by affecting cell divisions in subapical tissues.

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