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The effect of decapitation on the levels of IAA and ABA in the lateral buds of *Betula pendula* Roth.

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Abstract

The level of IAA and ABA in lateral buds of birch shoots 24 h and 5 days after the decapitation of the apical bud was determined.

Twenty four hours after decapitation, when visible signs of outgrowth of lateral buds were not observed yet, an increase in the level of IAA and a decrease of ABA, as compared with the buds of non-decapitated shoots, was found.

Five days later, when lateral buds were in the period of intensive outgrowth, a decrease in the levels of IAA and ABA was observed.

It has been suggested that removing the source of auxin, by the decapitation of the apical bud makes possible the lateral buds to undertake the synthesis of their own auxin. It could lead to the decrease in the content of ABA. These all events could create suitable conditions for the outgrowth of lateral shoots.

Introduction

There is evidence that auxin, especially IAA synthesized in the apical part of the plant, can inhibit axillary bud growth and that the inhibitory effect of IAA is of an indirect character (Phillips 1975, Martin 1987). Tucker (1978) suggested that IAA induces or maintains high level of ABA in plant tissues which is responsible for the inhibition of bud growth. Similar suggestions concerning the role of ABA are found in the papers of Tucker and Mansfield (1972, 1973) on *Xanthium strumarium*, Eliasson (1975) on aspen and pea and those of Zieslin *et al.* (1978) on rose. These papers have not decided, however, whether the initiation of lateral bud outgrowth precedes or follows the decrease in ABA levels. So, for a better understanding of the mechanisms of apical dominance it is important to distinguish between the processes associated with the initiation of the lateral bud outgrowth that occurs early after decapitation from those associated with the subsequent outgrowth, occuring later. The aim of this work was to determine levels of IAA and ABA in lateral buds during two periods after decapitation: before and after initiation of the bud burst.

Material and Methods

Plant material

Experiments were performed with 15-year-old trees of *Betula pendula* Roth. In July of the years 1992-1993 shoots were decapitated just below the growing apical buds. For analyses 2 highest lateral buds of the shoots were gathered in two periods after decapitation: 1) after 24 h, when the buds did not exhibit yet any sign of growth and 2) 5 days after

decapitation, when two highest lateral buds released from apical control initiated the growth that could be measured by the increase in fresh weight. For the comparison, the axillary buds from nondecapitated shoots were also tested at the same time.

The samples were weighed then placed in 80 % methanol with the addition of the antioxidant BHT (2,6-Di-tert-Butyl-p-cresol) at the concentration of 10 $\mu g \cdot ml^{-1}$, and stored at -20 °C, until determination of the IAA and ABA levels. Each sample contained 1200 buds.

Determination of IAA and ABA

Homogenized samples of buds were extracted with 80 % methanol at 5 °C for 48 h. After methanol evaporation, the water residue was adjusted to pH 3 and extracted with diethyl ether. The ether fractions were evaporated to dry residue in nitrogen atmosphere and chromatographed. The purification of the extract by Polyvinylopyrrolidone (PVP, Polyclar AT) and Sephadex LH-20 column chromatography was conducted as described earlier (Galoch 1985). The quantitative determination of free IAA and ABA by gas chromatography was done according to Kulikowska *et al.* (1995, 1997). After methylation with N-methyl-N-nitroso-4-toluenesulfonamide IAA and ABA were determined on Shimadzu GC-14A equipped with a capilar column (0.32 mm diameter, 25 m long) packed with SE-54-DF 0.50. In all the cases the nitrogen carrier gas flow rate was 2 cm^3/min, the injector heaters were set at +260 °C and oven temperature was +180 °C.

The gas chromatographic analysis of each sample was repeated at least four times. The results were evaluated statistically by calculating LSD at P=0.05.

Results and Discussion

The level of free IAA and ABA was determined in lateral buds 24 hours and 5 days after decapitation.

The data in Fig.1 show that 24 h after decapitation the buds did not undertake the growth processes yet (fresh weight of buds from the intact and decapitated shoots was the same), whereas after 5 days the outgrowth of two highest lateral buds was observed.

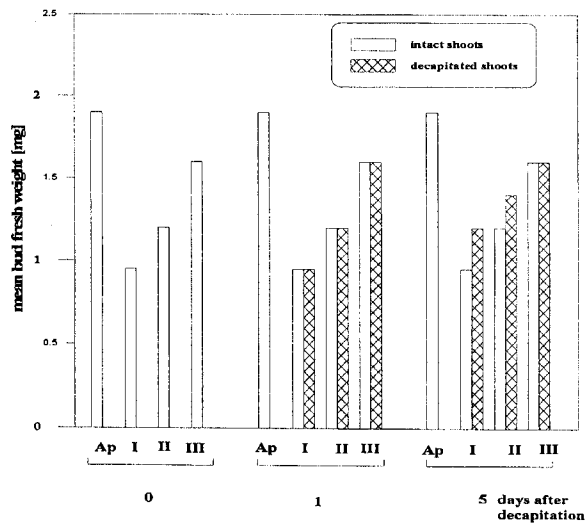

Fig. 1. Effect of decapitation on increase in fresh weight of lateral buds of birch. Ap - apical bud; 1, 2, 3 - successive lateral buds counting down from the shoot top.

An increase in both the content (A) and in the concentration (B) of IAA occured 24 hours after decapitation (Fig. 2). The participation of auxin in the control of outgrowth of lateral buds was the subject of many investigations, and the views on the role of IAA in the apical domination were changing (Phillips 1975, Brenner *et al.* 1987, Martin 1987, Cline 1991). There are data showing that correlatively inhibited lateral buds are not able to synthesize its own auxin, because the auxin produced by the dominant bud reaches lateral organs and prevents the formation of native IAA in them. The ability of lateral buds to synthesize auxin appears only some time after decapitation (Thimann and Skoog 1934, Phillips 1971). Suboptimal concentration of this hormone may be the cause of the inhibition of lateral buds. This assumption is confirmed by the authors who showed that the level of auxin in the buds released from the correlative inhibition, by decapitation, was higher than in the inhibited buds (Thomas 1972, Jablanowić and Neškowić 1977, Hillman *et al.* 1977, Harrison and Kaufmann 1984, Gocal *et al.* 1991). Also, the increase in the level of IAA after decapitation observed in this work probably resulted from undertaking the synthesis of this hormone by the lateral buds released from the apical dominance.

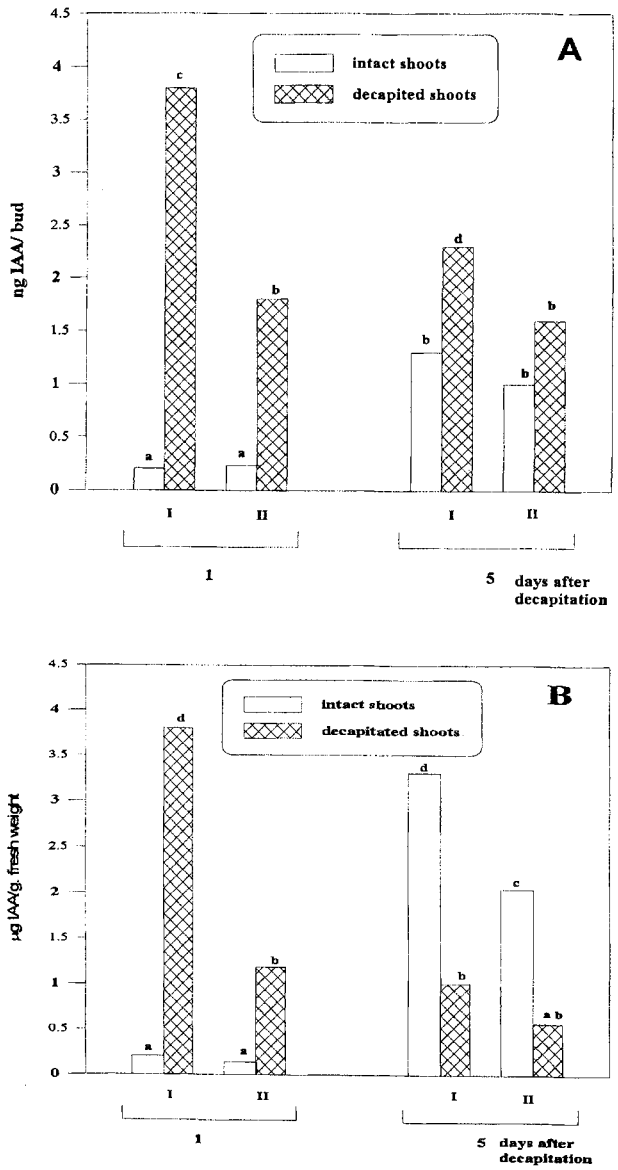

Fig.2. Effect of decapitation on content (A) and concentration (B) of endogenous IAA in the lateral buds of birch. I, II - successive lateral buds counting from the shoot top. Means denoted by the same letters are not significantly different from each other.

Since the analyses were conducted in the period preceding the appearance of distinct signs of outgrowth it may be assumed that undertaking the synthesis of IAA by lateral buds is one of the conditions necessary for the initial stage of overcoming the correlative inhibition.

A completely different situation exists 5 days after decapitation when the buds have already undertaken the outgrowth. The concentration of IAA in them is decreased in comparison to non-decapitated control shoots.

The analyses of ABA (Fig. 3) showed the drop of this inhibitor in lateral buds, both 24 h and 5 days after decapitation. The fact that removing an apical bud causes the lowering of the content of ABA before the appearance of distinct signs of outgrowth of lateral buds released from the correlative inhibition, suggests that this hormone may play a role of a correlative inhibitor (see Tucker 1978).

The experiments with exogenous ABA application confirm the inhibiting influence of this hormone on lateral buds growth (among others: Arney and Mitchell 1969, Bellandi and Dörffling 1974, Galoch 1987). The experiments devoted to endogenous ABA, however, have not supplied univocal data. In *Vicia faba* a decrease in the ABA content in lateral buds has been detected 6h after decapitation (Everat-Bourboloux and Charnay 1982). Similarly, Gocal *et al.* (1991) have stated in *Phaseolus* buds 4 hours after decapitation, that auxin content increased five times, while ABA content decreased nearly 30 %. On the other hand, White and Mansfield (1977) found no difference in ABA content in lateral buds of pea following decapitation. Also, Knox and Wareing (1984) did not find a change in ABA content in decapitated plants until after the lateral buds began to grow out.

The increase in the levels of IAA (Fig. 2) and the decrease of ABA (Fig. 3) in lateral buds after decapitation and before the visible signs of outgrowth of these organs allow us to consider the mechanism of apical dominace within the indirect auxin inhibition theory. It seems, that among others, the important role of auxin in apical dominance consists in inducing or maintaining high levels of ABA in the regions of initiation of lateral buds growth. The auxin diffusing from apical bud prevents at the same time IAA synthesis in lat-

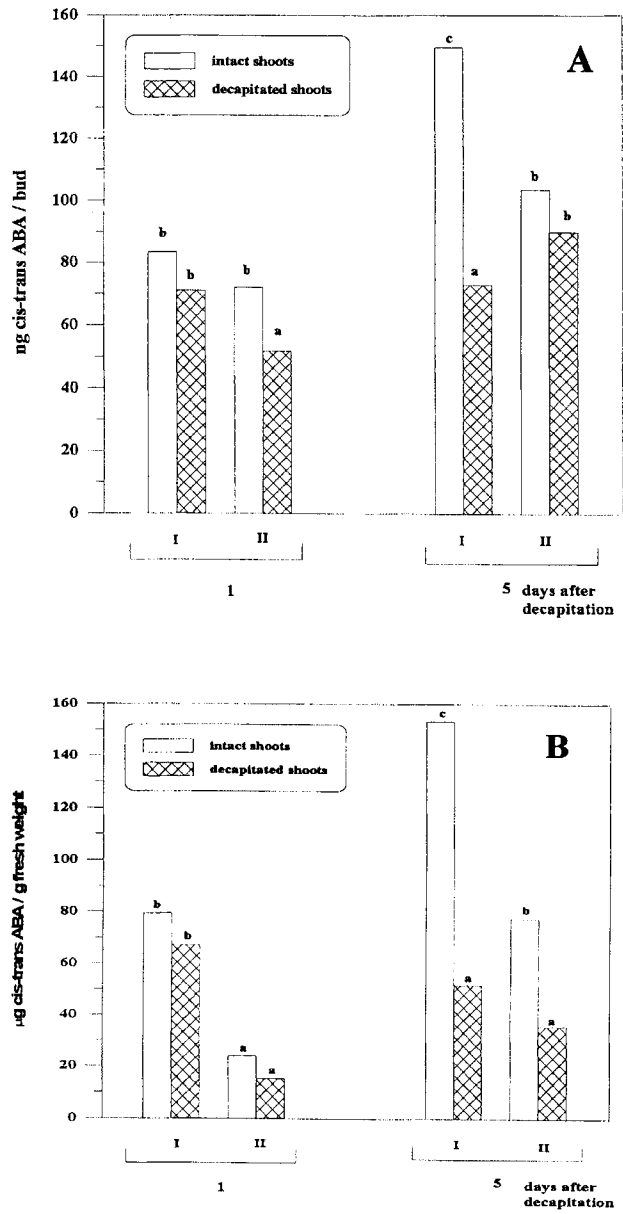

Fig 3. Effect of decapitation on content (A) and concentration (B) of endogenous ABA in lateral buds of birch.
I, II - successive lateral buds counting from the shoot top.
Means denoted by the same letters not significantly different from each other.

eral ones. Removing IAA source by decapitation makes it possible to undertake the synthesis of their own auxin by lateral buds. It could also lead to the decrease in ABA content. These events could create suitable conditions for the outgrowth of lateral organs.

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