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Critical study of coleoptile elongation controlled by IAA and ABA I. Growth kinetics and distribution

Claude Schlienger, Rose-Marie Hofer and Paul-Emile Pilet

Institute of Plant Biology and Physiology, University of Lausanne, 1005 Lausanne, Pl. de la Riponne, Switzerland

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The elongation rate of wheat coleoptiles, treated with IAA and ABA, was already affected during the first 8 hr of culture. The most sensitive zone of the material—for hormonal treatments—was first localized and then comparatively cultured both in situ and in vitro. Growth stimulation by IAA was nearly proportional to its concentration up to 10^{-4} M, while ABA always induced an significant inhibition.

The inhibitory effect of abscisic acid (ABA) on growth is now clearly established (see *15*), and its interactions with indolyl-3-acetic acid (IAA) have been studied using several parameters (*21, 22, 25*).

It was of interest to analyse the growth pattern of intact coleoptiles treated with IAA and ABA and to compare the results obtained with those given by a usual coleoptile segment test (*14, 24*).

Material and methods

Caryopses of *Triticum vulgare* L., cv. Probus were soaked in water for 2 hr, carefully washed and sown on moist filter paper, as previously described (*7*). After 72 hr (dark; 25±1°C), only seedlings with 20±1 mm coleoptiles were selected.

In situ experiments: The surface of the coleoptiles was carefully marked into 1 mm segments with India ink, and 20 intact seedlings were placed in Petri dishes on moist filter paper for 4 hr. Then, after careful washing, they were transferred (zero time) onto active solutions (still on filter paper).

In vitro experiments: According to a usual technique (*17, 19*), coleoptile segments of 5 mm in length were excised at 5 mm from the tip. After 4 hr incubation in water (zero time), they were immersed in active solutions.

Active solutions: Phosphate-citrate buffer solutions at pH 5.4 (*17*) containing 2% sucrose were used with or without IAA or ABA at several concentrations.

All active solutions and materials (Petri dishes, filters, etc.,) employed were previously sterilized. All manipulations were conducted under dim green light.

Changes of elongation were analyzed using a photographic technique (*26*). Each result thus represents the mean response of at least 60 measurements. Standard errors of this mean have been calculated and significant differences assessed by the *t*-test.

Abbreviations: IAA, indolyl-3-acetic acid; ABA, abscisic acid.

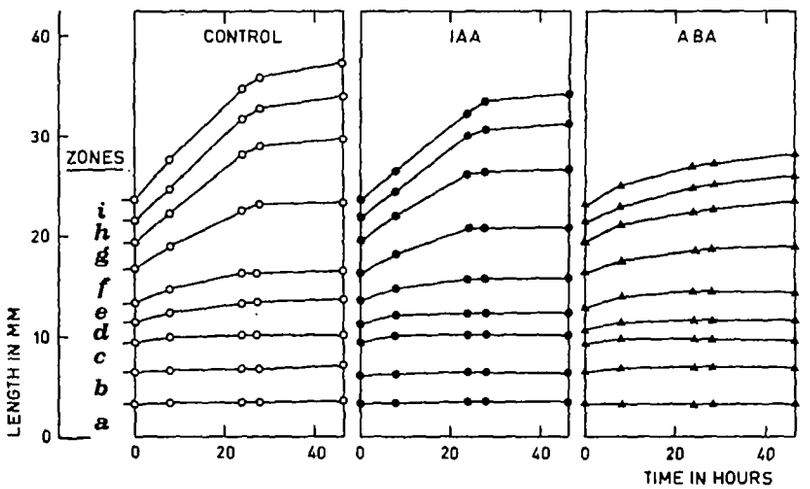

Fig. 1. *Comparative elongation in % for the different regions of intact coleoptiles, the seedlings being cultured 46 hr on buffer solution (control), with or without IAA (1×10^{-4} M) or ABA (3.8×10^{-6} M).*

Results and discussion

Two series of results will be successively discussed concerning growth of 1) coleoptiles from intact seedlings and 2) coleoptile segments.

Coleoptiles were marked into 9 zones (a to i). Fig. 1 shows the growth pattern over 46 hr for the most stimulating IAA concentration (10^{-4} M) and for an inhibiting ABA concentration (3.8×10^{-6} M). Elongation was found to be significant only for about 24 hr. From these data, the growth velocity of each zone was calculated (Table 1): as can be seen, it increased progressively from the base to the apex of the coleoptile (a to h). Elongation was already affected by IAA and ABA treatments during the first 8 hr.

In order to determine the most sensitive zone for such a hormonal treatment,

Table 1 *Growth velocity in μm per hour (during 8 hr) for several regions of coleoptiles from intact seedlings in presence or absence of IAA and ABA*

Regions (see Fig. 1)	Control	IAA [a]	ABA [a]
a	12.5± 8.5	10.0± 8.2	6.3± 4.6
b	51.3± 9.4	22.5±12.2	17.5± 8.0
c	90.0±15.0	81.3±22.2	76.3±26.3
d	140.0±24.6	102.0±25.6	95.0±25.1
e	202.0±25.8	162.0±23.5	142.5±23.9
f	296.3±40.2	216.3±27.4	185.0±33.0
g	363.8±36.5	302.5±37.6	241.3±37.2
h	412.5±42.0	346.3±51.6	248.8±40.8
i	50.3± 5.8	36.3± 5.8	26.3± 3.0

[a] Most stimulating IAA concentration: 1×10^{-4} M.
Inhibiting ABA concentration: 3.8×10^{-6} M.

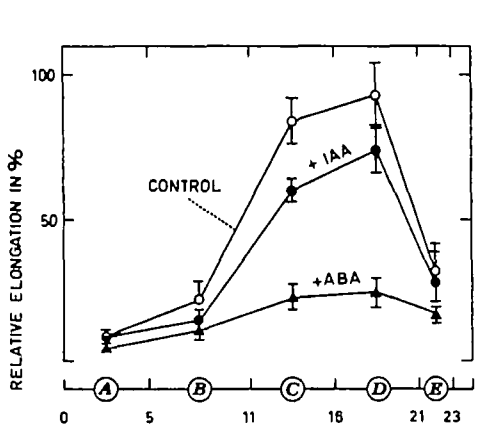

Fig. 2.

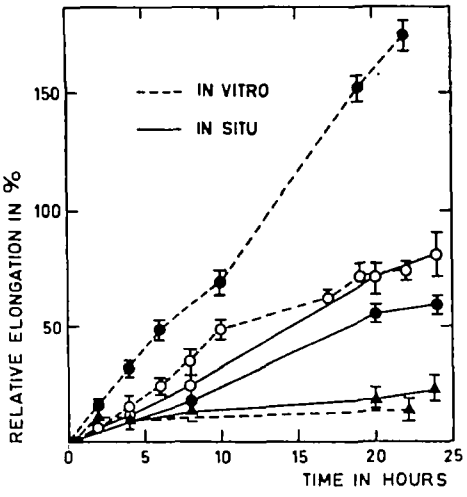

Fig. 3.

Fig. 2. *Relative elongation* (±*standard error*) *after 24 hr of intact coleoptiles treated with or without IAA* (1×10^{-4} M) *or ABA* (3.8×10^{-6} M). Regions: A (0–5 mm); B (5–11 mm); C (11–16 mm); D (16–21 mm); E (21–23 mm). Length respectively at 0 hr (L_0) and at 24 hr (L_{24}).

Fig. 3. *Comparative elongation* (±*standard error*) *of zone C* (11–16 mm *from the base*) *for intact coleoptiles* (*in situ*) *or for coleoptile segments* (*in vitro*). Control: ○; IAA at 1×10^{-4} M: ●; ABA at 3.8×10^{-6} M: ▲.

relative elongation (after 24 hr) of 5 zones (A to E) was measured (Fig. 2). A significant maximum of growth was found in both regions C and D, as previously reported (*18*). It was noticed that, for treated coleoptiles, the optimal growth inhibition was also observed in these regions. Under the present conditions, the ABA effect was found to be three times greater than the IAA effect, although the ABA concentration was about twenty five times lower. In the other zones, no significant difference was found for the elongation between treated coleoptiles and the controls.

Relative growth of portion C (coleoptiles from intact seedlings: in situ) was compared to the elongation of similar segments immersed in the test solution (in vitro). From the results given in Fig. 3, it can be seen that there is no significant difference between either the two controls or the in situ IAA treatment. In contrast, the segments treated in vitro showed a greater "sensitivity" to auxin. The ABA treatment induced a similar inhibition for both segments. Compared to the

Table 2 *IAA and ABA effect on relative growth* (*in situ and in vitro measurements*) *of coleoptile segments in relation with time*

Time in hr	In situ		In vitro	
	IAA	ABA	IAA	ABA
4	69.6±7.0	71.3±6.8	195.8±18.5	57.6±5.1
24	73.6±6.2	27.2±2.9	251.3±24.9	19.2±2.1

Each value is expressed in % of the control.
Respective IAA and ABA concentrations: 1×10^{-4} M and 3.8×10^{-6} M.

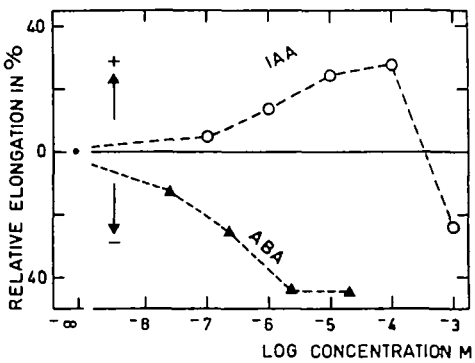

Fig. 4. *Relative growth in % of coleoptile segments treated with IAA and ABA at different concentrations (from 0 to 10^{-3} M).* Positive and negative values show respectively stimulation and inhibition.

control (Table 2), the IAA effect remained quite constant after 4 hr of culture, while the ABA effect became larger, as incubation time increased.

As previously observed (*19*), there is a clear correlation between the IAA concentration (in the test solution) and the growth promotion of the coleoptile segments from 10^{-7} M to 10^{-4} M of IAA. In Fig. 4, the results indicate a strong inhibition for a higher concentration (10^{-3} M) of IAA. But, under the present conditions, ABA was found to inhibit elongation in every case, and the effect remained unchanged for every concentration tested over 3.8×10^{-6} M.

The presence, in intact wheat coleoptiles (*4*, *18*) of IAA-oxidases and peroxidases, with a minimal activity in zones C and D (Fig. 2), for which a maximal elongation was reported, may explain, at least partly, the small growth changes (Fig. 1) caused by IAA (treatment in situ). It is well established that the movement of endogenous auxin is basipetal in coleoptiles (*8*) and acropetal in roots (*20*), consequently polar (*3*, *9*, *11*). For exogenous IAA, the present data indicated a similar situation (Fig. 3). In fact, IAA applied to the roots showed no significant effect on the elongation of coleoptiles, but, as can be seen in Fig. 3, the in vitro uptake of IAA was linear with time (*6*). Growth stimulation (Fig. 4) was nearly proportional to the log of auxin concentration up to 10^{-4} M (*6*), but a decrease in elongation was observed for a higher concentration of IAA (*1*).

In contrast, ABA induced significant growth inhibition in each coleoptile portion, for every tested concentration (*10*, *16*, *25*) for both in vivo and in vitro treatments. The last point could be explained by the fact that ABA is possibly not formed (*15*) by the coleoptile tissues, which was of course not the case for IAA.

As there exists in coleoptile segments, characterized by the absence of mitosis (*2*), a proportionality between growth and auxin-induced H^+-excretion (*5*), treatment in vitro either with IAA or acid pH may have a similar effect on cell wall structure (*12*), promoting a relase of xyloglucan (*13*). In contrast, ABA, which may antagonize—at least for the root (*23*)—any direct or non direct effects on proton extrusion, is known to have a strongly inhibitory effect on increase in the cell volume (*26*).

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