CHANGES IN CELL ULTRASTRUCTURE AND MORPHOLOGY OF *ARABIDOPSIS THALIANA* ROOTS AFTER COUMARINS TREATMENT

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ABSTRACT

The ultrastructure and morphology of roots treated with coumarin and umbelliferone as well as the reversibility of the coumarins effects caused by exogenous GA_3 were studied in *Arabidopsis thaliana*. Both coumarins suppressed root elongation and appreciably stimulated radial expansion of epidermal and cortical cells in the upper part of the meristem and in the elongation zone. The gibberellic acid applied simultaneously with coumarins decreased their inhibitory effect on root elongation and reduced cells swelling. Microscopic observation showed intensive vacuolization of cells and abnormalities in the structure of the Golgi stacks and the nuclear envelope. The detection of active acid phosphatase in the cytosol of swollen cells indicated increased membrane permeability. Significant abnormalities of newly formed cell walls, e.g. the discontinuity of cellulose layer, incorrect position of walls and the lack of their bonds with the mother cell wall suggest that coumarins affected the cytoskeleton.

KEY WORDS: *Arabidopsis thaliana*, coumarin, umbelliferone, acid phosphatase, gibberellin, ultrastructure of root cells.

INTRODUCTION

Coumarin and its derivatives are recognized as widespread allelochemicals, which are synthesized by a wide range of vegetative plant organs (Zobel and Brown 1989; Zobel and Brown 1991; Zobel and March 1993). Coumarins leached from leaves, seeds and fruits or exuded from roots into the soil (Fay 1977) have allelopathic effects on potential competitors. It was reported that the mixture of coumarins and furanocoumarins leached from *Heracleum laciniatum* L. fruits (Juntilla 1975) and bitter lupine (*Lupinus angustifolius* L.) seeds (Stobiecki et al. 1993) strongly inhibited lettuce (*Lactuca sativa* L.) seed germination and the growth of roots. The effectiveness of coumarin and its simple derivatives as root growth inhibitors has been studied in a few cases. Umbelliferone and scopoletin isolated from roots of *Heracleum lanatum* L. inhibited the growth of Chinese cabbage (*Brassica rapa* L.) roots (Shimomura et al. 1982). All the tested derivatives of coumarin were less active than the coumarin itself (Mayer and Poljakoff-Mayber 1963; Kupidłowska et al. 1994).

Coumarins not only act as growth retardants but they also induce the swelling of the apical part of the root

(Avers and Goodwin 1956; Svensson 1971; Jankay et al. 1976; Aliota et al. 1993; Kupidłowska et al. 1994). Both in roots and in stems swelling in the radial direction was observed only in epidermis and outer layers of cortical cells (Itoh 1976; Kupidłowska et al. 1994). Several lines of evidence suggest that gibberellins control radial expansion in the elongation zone of the root. It has been shown, that exogenous antigibberellins, such as: AMO 1618, ancymidol, paclobutrazol, cycocel; induced swelling of root in elongation zone with simultaneous reduction of longitudinal growth. The radial expansion of roots and reduction of their elongation were observed also in GA_3 deficient *d5* mutants of maize (Baluška, Parker and Barlow 1993) and in *gib1* mutants of tomato (Barlow, Brain and Parker 1991). The effects of antigibberellins (Tanimoto 1987; Tadeo et al. 1997) and mutations in GA_3 synthesis pathway (*gib 1*) (Butcher, Clark and Lenton 1990) was overcome by exogenously applied GA_3 .

Effects of gibberellin applied simultaneously with coumarin were studied on maize roots (Svensson 1972). In this plant coumarin-induced inhibition of longitudinal growth has not been counteracted by gibberellin.

The present paper describes the effects of simple coumarins, coumarin and umbelliferone on *Arabidopsis*

roots. Morphology, cell ultrastructure and the results of enzyme assays suggest that either an interaction of coumarins with the endomembranes or shifted differentiation as a secondary effect of the mitodepression, could be involved in the coumarins-induced abnormalities. The obtained results demonstrate that exogenous gibberellin reverses to some extent the effects of coumarins on both the longitudinal growth and swelling of the roots.

MATERIAL AND METHODS

Seeds of *Arabidopsis thaliana* L. Columbia ecotype were surface sterilized in 70% ethanol and then in commercial bleach supplemented with Tween 20 (one drop per 50 ml) for 15 min. After rinsing in sterile water, seeds were suspended in a small volume of sterile Murashige and Skoog (MS) (Murashige and Skoog 1962) medium and sowed on MS medium solidified with 0.8% agar and supplemented with 2.5 mM 2-(N-morpholino) ethanesulfonic acid (MES), 1% sucrose, pH adjusted to 5.8.

Vessels with seeds were placed in a growth chamber with temperature 21°C and a 16 hrs light/8 hrs dark cycle. Three days old seedlings were transferred onto media containing coumarins at 0.05 mM; 0.1 mM; 0.3 mM; 0.5 mM; 1.0 mM; and 1.5 mM. Three vessels, each containing 15 seedlings, were prepared for every combination of incubation period and coumarins concentration. Two simple coumarins were tested: coumarin and 7-hydroxycoumarin (umbelliferone). Some seedlings were transferred to the fresh MS medium and left to grow as the control. In order to examine whether the effect of coumarins on root growth can be reversed by exogenous gibberellin, the MS medium containing coumarin or umbelliferone supplemented with 0.1 mM or 0.3 mM gibberellic acid (GA₃) was also used. The 0.3 mM concentration of GA₃ proved effective and was chosen for further examination. Roots were treated either with 0.3 mM of GA₃ exclusively, or with 0.3 mM of GA₃ supplemented with 0.5 mM coumarin or 0.3 mM umbelliferone.

The diameter and length of roots were measured after 6; 24; 48; 72 and 96 hrs of incubation. Root diameter was measured using a light microscope with the scaled ocular. Measurements were taken at two levels: at the proximal edge of the meristem marked by the upper end of the root cap; and at the elongation zone just beneath the first root hairs. For roots with conspicuous swelling and root hairs appearing closely to meristem the diameter was measured at its maximal width. For the root length measurements the whole plants were placed between two glass slides and their image projected through an enlarger. The length was calculated from digitized images, based on the enlargement coefficient. Root length and diameter data were reported as a mean of 30 plants. Significance of differences between control and treatment data was evaluated by t-test at $P = 0.05$.

For the examination of cell ultrastructure apical fragments of roots after 48 hrs of treatment were prepared as described earlier (Kupidowska et al. 1994) except that Karnovsky's fixative was diluted twice. The active acid phosphatase (orthophosphoric-monoester phosphohydrolase EC 3.1.3.2) was localized using the modified Gomori's method (Gomori 1953). Root tips fixed in 1.6% glutaraldehyde were incubated for 1 hr at 37°C in a medium contain-

ing 50 ml of 0.05 M acetate buffer pH 5.0, 60 mg lead nitrate and 5 ml 3% sodium β -glycerophosphate. In this test lead phosphate is locally precipitated as the product of reaction catalyzed by the acid phosphatase. The control for this reaction was the material kept in a medium without sodium β -glycerophosphate. After incubation, the sections of roots were prepared as described earlier (Kupidowska et al. 1994). Observation and documentation were made with the aid of transmission electron microscope JEM 100B.

RESULTS

1. Changes in root morphology and directions of cell growth

Coumarin and umbelliferone caused an apparent change in the root morphology. The tips of roots treated with coumarin and umbelliferone were significantly thicker than those of the control (Fig. 1A). In the roots treated with coumarin the radially expanded zone began at the base of meristem and was uniform over the whole elongation zone (Fig. 1B). In the roots treated with umbelliferone it engulfed only a portion of the elongation zone adjacent to meristem (Fig. 1C). The root epidermal cells of the elongation zone, both atrichoblasts and trichoblasts, expanded radially (Fig. 1D). In the roots treated with coumarin and umbelliferone root hairs grew immediately above the meristematic zone.

The young lateral roots in treated plants appeared deformed. The swelling of root primordia in their elongation zone was apparent (Fig. 1E). Lateral roots were often initiated at very small distance from each other, which as they enlarged, made them form a shapeless proliferous tissue (Fig. 1F).

Coumarin and umbelliferone stimulated a concentration-dependent increase of root diameter. The measurements taken after 72 hrs of incubation showed that the largest diameters of both the meristematic and elongation zone were observed after the treatment with 0.5 mM coumarin (Fig. 2) or 0.3 mM umbelliferone (Fig. 3). These concentrations were chosen for further studies. For both compounds the concentration of 1 mM caused gradual necrotisation of roots after longer incubation periods which was probably responsible for the weaker growth reaction.

The longer was the period of incubation, the higher was the degree of radial expansion of root cells. The diameter increase was always more intensive in the elongation zone than at the meristem where even 6 hrs of incubation caused statistically significant increase (Fig. 4).

The observed increase of the diameter of root meristem treated with coumarin and umbelliferone was the result of radial growth of the dermatogen and periblem (Fig. 5A, B, C). The first indication that the cell structure of these layers changed was their more intense vacuolization. It was especially pronounced in roots treated with umbelliferone in which vacuolization of cells began immediately above the initial tiers (Fig. 5C). An early, in comparison with the control (Fig. 5A), vacuolization of the dermatogen and periblem was also visible in the roots treated simultaneously with coumarins and gibberellin (Fig. 5D). The change of the shape of the root epidermis and cortex cells was noticeable already in the elongation zone of the root and indicated that these cells grew differently from those of the control.

Fig. 1. The external morphology of *Arabidopsis thaliana* L. roots after 72 hrs (A-E) or 96 hrs (F) of treatment with 0.5 mM coumarin (B, D-F) or 0.3 mM umbelliferone (C). Bars = 100 μ m. RAM = root apical meristem, EZ = elongation zone, DIF = differentiation zone.

A – Control. Apical meristem and the elongation zone of the primary root; B – The root swollen within the elongation zone, with root hairs appearing just above the meristem; C – The swollen apical part of the root; D – Bulging epidermal cells at the elongation zone; E – A young lateral root, with the swollen base; F – Fused initials of the lateral roots.

Coumarin and umbelliferone treatment inhibited root elongation, which completely ceased after 48 hrs of incubation (Fig. 6). The percentage of radial expansion was

shown as the function of the percentage of elongation inhibition so that the magnitude of root swelling could be compared with the rate of the inhibition of elongation. The

Fig. 2. Effect of various concentrations of coumarin on root diameter. The diameter of roots was measured at meristematic (M) and elongation (E) zone after 72 hrs of treatment. Bars represent $\pm$ s.e.; + marks differences significant at $P = 0.05$.

plots showing this relationship (Fig. 7) can be divided into two phases. The first one covers a 0-48-hour-period of incubation when coumarin is used; and a 0-72-hour-period when umbelliferone is used. Both coumarin and umbelliferone stimulate the radial expansion more effectively than they inhibit elongation for the first 48 or 72 hrs of incubation respectively. The fact that after this time both the graphs go down abruptly indicates that the radial growth is lower, whereas the inhibition of elongation more severe.

2. Effects of exogenous GA₃ on the root elongation and swelling

Roots treated with 0.3 mM GA₃ exclusively were about 40% longer than the control ones. Also their diameter was 7% to 16% smaller than that of the control roots. The counteraction of GA₃ on the elongation inhibition effect of both coumarins was noticeable during the first 24 hrs of simultaneous treatment. During this period gibberellin significantly reduced growth inhibition caused by coumarins making it

Fig. 3. Effect of various concentrations of umbelliferone on root diameter. The diameter of roots was measured at meristematic (M) and elongation (E) zone after 72 hrs of treatment. Bars represent $\pm$ s.e.; + marks differences significant at $P = 0.05$.

about 40% lower (Fig. 6). During longer than 24 hrs periods of incubation, GA₃ application did not cause any effect.

The first 24 hrs of incubation with coumarin and GA₃ resulted even in a slight increase in the root diameter in comparison with plants treated with coumarin only. The same period of incubation with umbelliferone and GA₃ resulted in the reduced root swelling (about 7% less in comparison with umbelliferone treatment) (Fig. 8). During the later stages of incubation, exogenous gibberellin almost completely inhibited the radial expansion of roots treated with both coumarins.

3. Changes in the ultrastructure of meristematic cells

Numerous secretory vesicles with osmiophilic content occurred in cytoplasm mainly along cell anticlinal walls perpendicular to root axis, in the dermatogen and perilem cells of the roots treated with coumarin or umbelliferone (Fig. 5E, F). Some abnormalities in the dictyosome structure, namely the hypertrophy of membranes and dilatation

Fig. 4. Changes in the diameter of roots treated with 0.5 mM coumarin or 0.3 mM umbelliferone. The diameter of roots was measured at meristem (M) and elongation (E). Control (white) M (hatched) E, coumarin (dark grey) M (cross-hatched) E, umbelliferone (black) M (white) E. Bars represent $\pm$ s.e.; + marks differences significant at $P = 0.05$.

Fig. 5. Cellular organization in root meristem and in the part of the elongation zone. Plerome (PL) and radially expanded cells of dermatogen (D) and periblem (PE) are visible in roots treated with 0.5 mM coumarin or 0.3 mM umbelliferone. Medial longitudinal sections of roots were stained with toluidin blue. A-D: bars = 50 μm.; E-F: bars = 0.5 μm. RAM = root apical meristem.

A – Control, 72 hrs; B – Coumarin, 72 hrs of treatment; C – Umbelliferone, 72 hrs of treatment; D – Coumarin and 0.3 mM GA₃, 72 hrs of treatment; E – Umbelliferone, 48 hrs of treatment, radially expanded periblem cell; F – The enlarged part of E, arrows indicate the secretory vesicles near the anticlinal cell wall, (CW), PM = plasma membrane.

Fig. 6. Inhibition of root elongation by 0.5 mM coumarin and 0.3 mM umbelliferone in the presence (filled symbols) and in the absence (empty symbols) of 0.3 mM GA₃ □ and ■ control, △ and ▲ coumarin, ○ and ● umbelliferone. Bars represent $\pm$ s.e. (not shown if smaller than symbols). Differences between control and coumarins treatment are significant by t-test at $P = 0.05$ except the case of 24 hrs treatment by coumarin.

Fig. 8. The effect of GA₃ addition on radial expansion of roots treated with 0.5 mM coumarin or 0.3 mM umbelliferone. GA₃ at 0.3 mM concentration was added to incubation media, diameter of roots was measured at elongation zone. □ control ■ GA₃, △ coumarin, ▲ coumarin + GA₃, ● umbelliferone, ○ umbelliferone + GA₃. Bars represent $\pm$ s.e. All differences between control and coumarins treatment are significant at $P = 0.05$.

Fig. 7. Radial expansion plotted as a function of elongation inhibition in roots treated with ▲ coumarin and ● umbelliferone. Both values are expressed in % of the control. The diameter of roots was measured at the elongation zone.

of the central part of Golgi cisternae (Fig. 9B) were observed. Dilatation occurred also in other parts of the endomembrane system, i.e. in ER cisternae (Fig. 9F) and in the nuclear envelope. The latter resulted in local swelling of the perinuclear space (Fig. 9A). The dilatation of ER cisternae was one of the modes of the vacuole formation, especially in the cells of the peribem. Autophagy, through gradual digestion of portions of cytoplasm sequestered by smooth ER (Fig. 9D), was presumably another way of vacuole biogenesis.

Some significant abnormalities were noticed in the cell walls, i.e. their structure and localization, especially in the material treated with coumarin. The walls between sister cells were sometimes only fragmentary (Fig. 9C), the bond between the newly-formed wall and the mother cell wall often did not exist (Fig. 9E).

The structure of the walls was also abnormal. Their thickness was not uniform and discontinuities occurred in

the central fibrillar layer (Fig. 9H). In some cases the wall morphology indicated that disturbances during its maturation took place after cytokinesis: the fibrillar part of the wall was thin and wavy (Fig. 9G).

4. Localization of active acid phosphatase in meristematic cells

No electron dense lead phosphate precipitates were found in the reaction's background material incubated in the medium without the substrate (Fig. 10A). The active enzyme was found in the older meristematic cells of the control roots. The precipitates of lead phosphate indicating the presence of active phosphatase were always detectable inside confined membranous structures. They mainly occurred in provacuoles (Fig. 10B), in the membranes of degraded organelles inside the autophagic vacuoles or in the tonoplast (Fig. 10C). In the meristems of the roots treated with coumarin and umbelliferone the active acid

Fig. 9. Ultrastructure of meristematic cells of roots treated with 0.5 mM coumarin or 0.3 mM umbelliferone for 48 hrs. Bars = 0.5 μ m.

A – Coumarin, extension of the perinuclear space (arrow) and dilated cisterna of ER, NU = nucleus; B – Coumarin, Golgi stack with dilated central parts of the cisternae; C – Coumarin, discontinuous cell wall between the sister cells; D – Umbelliferone, autophagic vacuole containing remnants of membranes in multilamellar arrangement and the cytoplasmic debris; E – Coumarin, the cell wall formed as a result of disturbed cytokinesis. Arrow indicates a very thin part of the cell wall (CW) lacking the regular connection with the mother cell wall; F – Coumarin, the vacuole formed from the dilated part of the ER cisterna in dermatogen cell; G – Coumarin, periblem cell. Arrows indicate very thin and wavy arranged part of cell wall. H. Coumarin, discontinuity of the fibrillar layer of the cell wall (CW) visible in the periblem cell.

Fig. 10. Localization of active acid phosphatase in meristematic cells of the control roots and those treated for 48 hrs with 0.5 mM coumarin and 0.3 mM umbelliferone. Bars = 0.5 μ m.

A – No precipitates are visible in the control sample incubated in the medium without the substrate; B – Control, enzyme located in the provacuole of dermatogen cell; C – Control, enzyme located in the tonoplast and in the lumen of the autophagic vacuole; D – Coumarin, enzyme located in the provacuole and in the cytosol (arrows) of dermatogen cell; E – Coumarin, enzyme located in the secretory vesicles near the cell wall (CW) and in the cytosol (arrows) of the dermatogen cell; F – Umbelliferone, enzyme located on the surface the cell wall (CW) and in the cytosol (arrowheads) of periblem cells.

phosphatase was found in the cells of the dermatogen and periblem (except the cells of the plerome). Here the enzyme was also present in the provacuoles (Fig. 10D), the secretory vesicles (Fig. 10E) and the cell wall (Fig. 10F), but in contrast to the control, the active acid phosphatase was detected also in the cytosol of the cells treated with both coumarins (Fig. 10D, E, F).

DISCUSSION

Coumarin and umbelliferone significantly affect the morphology of *Arabidopsis* roots.

The effect of coumarin and umbelliferone on root elongation is presumably drawn by two different processes, each one dominating during one of the two incubation

stages. Initially, (0-48 hrs of incubation) coumarins affect the rate and direction of cell enlargement. During a later period of incubation (48-96 hrs), the influence of allelochemicals on the mitotic activity of apical meristem prevails: the mitodepression causes decrease of root elongation, most distinct between 48th and 72th hour of incubation. Coumarin and umbelliferone are known to be strong antimitotics (Podbielkowska et al. 1993).

Coumarins depressed the mitotic activity of meristem but cell differentiation was not inhibited, hence the wave of differentiation was moved toward the root apex. It is difficult to separate such a shifted differentiation (the secondary effect of mitodepression) from the immediate effect of coumarins on the cell ultrastructure, especially on the endomembrane system. In roots treated with both compounds strong vacuolization of meristematic cells was noted, particularly in the dermatogen and periblem, even in the close vicinity of initials. Similar effect was discovered in roots of other plants treated with the methyl derivatives of coumarin (Kupidłowska et al. 1995) and furocoumarins (Kupidłowska et al. 1994). Precocious vacuolization could be a manifestation of earlier differentiation, which is additionally supported by the significant reduction of the distance between the zone of lateral root formation and the meristem as well as by the appearance of root hairs immediately above the apex. The latter effect was also observed in *Phleum pratense* roots treated with coumarin although growth of root hairs was completely inhibited after treatment with scopoletin, an antimitotic stronger than coumarin (Avers and Goodwin 1956).

On the other hand, it can not be ruled out that vacuolization of meristematic cells and abnormalities of dictyosomes and ER occurring in the treated material resulted from interactions of coumarins with cellular membranes. Strong vacuolization, protrusions of the plasma membrane and dilatation of ER cisternae were also observed in mouse fibroblasts of TCTC line treated with xanthotoxin. The electron paramagnetic resonance technique was applied to prove the presence of xanthotoxin in the hydrophobic region of the lipid bilayer of cell membranes (Gawron 1992). Both coumarin, i.e. a lactone of *cis-o*-hydroxycinnamic acid, and its derivative umbelliferone, being hydrophobic compounds are likely to interact with membranes in the same way. Brefeldin A (BFA), another macrocyclic hydrophobic lactone binds with the lipid bilayer of the membrane in a similar way as xanthotoxin (Lanubile, Piro and Dalessandro 1997). Interactions of BFA with phospholipids also change the topological and functional organization of cellulose synthase complex (Piro et al. 1999), which directly results in the inhibition of cellulose synthesis. The radial expansion of cells in the elongation zone of the *Arabidopsis* root, similar to this taking place after treatment with coumarin and umbelliferone, is a secondary effect of BFA (Baskin and Bivens 1995).

In differentiated cells intracellular acid phosphatase was usually localized in the vacuoles (Duff, Sarath and Plaxton 1994). At all the steps of the vacuolar system formation, phosphatase was kept rigorously in the exoplasmic space surrounded by a membrane impermeable for the enzyme (Marty 1978). In roots, the extracellular acid phosphatase can typically occur also in the cell walls before it is secreted into the soil (Goldstein 1992; Duff et al. 1994; Gilbert et al. 1999). The distribution of active acid phosphatase,

marker of lysosomal structures in the cell, in the control material of the present study was standard for this class of enzymes. In meristematic cells the high activity of acid phosphatase was demonstrated in provacuoles and developing autophagic vacuoles. The striking feature of the distribution of phosphatase in cells of roots treated with coumarins was the fact that the enzyme was detected also in the cytosol. The release of acid phosphatase to the cytosol, which was also shown in the fibroblasts exposed to xanthotoxin in the darkness (Gawron 1992), indicates the increase in membrane permeability.

Coumarin and umbelliferone apparently change the direction of cell growth in the elongation zone. In *Arabidopsis* the root diameter is set by the radial expansion of cells in the apical zone of the meristem and by the absence of radial growth of cells in the elongation zone (Baskin et al. 1992, 1995). Thus any treatment that stimulates the radial swelling of the cells within the elongation zone simultaneously changes the direction of growth of these cells. Growth direction of the cell is known to be controlled by the synthesis, directed transportation and deposition of components of the wall, as well as by interactions between the cellulose microfibrils and matrix components (Carpita and Gibeault 1993; Hauser, Morikami and Benfey 1995). Coumarin was found to be a specific inhibitor of cellulose synthesis (Hara et al. 1973; Hogetsu, Shibaoka and Shimokoriyama 1974). This may be one of the reasons why this mode of the growth control is lost in cells of the roots treated with coumarin. Other inhibitors of the cellulose synthesis, i.e. the aforementioned BFA and 2,6-dichlorobenzonitrile (DCB), stimulate in a similar fashion the radial expansion of cells in *Arabidopsis* roots (Baskin and Bivens 1995). However, in the case of umbelliferone it is not known whether it affects cellulose synthesis.

The radial swelling of cells occurs presumably not only due to the decrease of cellulose content in the cell walls. The molecular analysis of cellulose biosynthesis in the *rswl Arabidopsis* mutant (Arioli et al. 1998) and *cobra* mutant (Schindelman et al. 2001) proved that the loss of ability to crystalize cellulose microfibrils was sufficient to initiate the swelling of root cells. At the same time the synthesis of β -1,4 glucan, hence total cellulose content in the cell wall, remained unaffected. This suggests that interaction of coumarins with the cellular membranes may result not only in higher membrane permeability but also in dysfunction of cellulose synthase complex localized in the plasma membrane.

Coumarins, especially the umbelliferone, as well as oryzalin and taxol (Morejohn and Foskett 1991; Shibaoka 1994; Baskin et al. 1994) stimulated the maximal radial expansion of cells on the boundary between the meristematic and elongation zones. Passing through this distal part of elongation zone is a transitional phase in cell ontogeny in which more intensive isodiametric growth starts after mitosis cessation (Baluška, Kubica and Hauskrecht 1990). The onset of rapid elongation at the end of this zone is thought to be actomyosin dependent (Baluška et al. 1997). This is confirmed by the fact that cytochalasin B stimulates the radial growth of cells in the *Arabidopsis* roots (Baskin and Bivens 1995). The abnormally shaped and positioned cell walls demonstrated in this study, and the swelling of the cells, prove that both coumarins may cause some dysfunction of F-actin cytoskeleton. It has been previously

reported that the bond between growing cell plate and the adhesion zone, the latter determined by the preprophase band (PPB), is dependent on actin microfilaments (Mineyuki and Gunning 1990; Endle et al. 1998). If the cell plate fuses to the mother wall beyond the area marked by PPB, the growing new cell wall retains its irregular wavy shape (Mineyuki, Marc and Palevitz 1991).

As it was shown, exogenous GA₃ stimulated root elongation of control plants and simultaneously reduced their diameter at the elongation zone. Gibberellin applied to intact root system has very variable effects and, as shown in many reports, the growth response to exogenous GA₃ is an individual feature of plant species (Tanimoto 1987, 1988 and refs. therein). In the present experiment, simultaneous coumarins and GA₃ treatment significantly reduced the inhibition of root elongation subsequent to cell longitudinal growth (during first 24 hrs of incubation). This is in disagreement with Svensson report (Svensson 1972). However, in his experiment the growth was measured only after 4-days incubation, when the inhibition of the root elongation resulted mainly from the mitodepression. GA₃ also impeded the radial expansion of cells. The data obtained from 24 hrs coumarin-treated roots are inconsistent with this tendency but this probably results from the fact that different populations of roots were measured after each incubation period.

The directions of growth of root apex cells are well known to be controlled by the interaction of phytohormones (gibberellin among others) with cytoskeleton (Shibaoka 1994; Baluška, Volkmann and Barlow 1999). The deficit of gibberellin stimulates the radial growth of root cells at elongation zone, and is associated with changes in the cortical microtubule orientation. The application of gibberellin re-establishes the arrangements of cortical microtubules and the polarity of cell growth (Baluška, Parker and Barlow 1993). Gibberellin also regulates the bundling of microtubules (Simonds, Setterfield and Brown 1983; Mita and Shibaoka 1984; Akashi and Shibaoka 1987), which in turn distinguishes the organization of microtubules in the proximal part of elongation zone from their organization in the central part and impedes the radial expansion of cells. The weaker reaction of roots to coumarin and umbelliferone in the presence of exogenous gibberellin suggests that both allelochemicals affect microtubules and actin microfilaments. Thus, the mechanism of the influence of coumarins on growth may also be linked with structure, alignment and dynamics of cortical cytoskeleton.

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LITERATURE CITED

- AKASHI T., SHIBAOKA H. 1987. Effect of gibberellin on the arrangement and the cold stability of cortical microtubules in epidermal cells of pea internodes. *Plant Cell Physiol.* 28: 339-348.
- ALIOTTA G., CAFIERO G., FIORENTINO A., STRUMIA S. 1993. Inhibition of radish germination and root growth by coumarin and phenylpropanoids. *J. Chem. Ecol.* 19: 175-183.
- ARIOLI T., PENG L., BETZNER A.S., BURN J., WITTKKE W., HERTH W., CAMILLERI C.H., HOFTE H., PLAZINSKI J., BIRCH R., CORK A., GLOVER J., REDMOND J., WILLIAMSON R.E. 1998. Molecular analysis of cellulose biosynthesis in *Arabidopsis*. *Science* 279: 717-720.
- AVERS C., GOODWIN R.H. 1956. Studies on roots. IV. Effects of coumarin and scopoletin on the standard root growth pattern of *Phleum pratense*. *Am. J. Bot.* 43: 612-620.
- BALUŠKA F., KUBICA S., HAUSKRECHT M. 1990. Postmitotic „isodiametric” cell growth in the maize root apex. *Planta* 181: 269-274.
- BALUŠKA F., PARKER J.S., BARLOW P.W. 1993. A role of gibberellic acid in orienting microtubules and regulating cell growth polarity in the maize root cortex. *Planta* 191: 149-157.
- BALUŠKA F., VITHA S., BARLOW P.W., VOLKMANN D. 1997. Rearrangements of F-actin arrays in growing cells of intact maize root apex tissue: a major developmental switch occurs in the postmitotic transition region. *Eur. J. Cell Biol.* 72: 113-121.
- BALUŠKA F., VOLKMANN D., BARLOW P.W. 1999. Hormone-cytoskeleton interactions. In: *Biochemistry and Molecular Biology of Plant Hormones*. Libbenga K.L., Hall M., Hooykas P.J.J. (eds) Elsevier Publishing, Leiden.
- BARLOW P., BRAIN P., PARKER J.S. 1991. Cellular growth in roots of a gibberellin-deficient mutant of tomato (*Lycopersicon esculentum* Mill) and its wild type. *J. Exp. Bot.* 42: 339-351.
- BASKIN T.J., BETZNER A.S., HOGGART R., CORK A., WILLIAMSON R.E. 1992. Root morphology mutants in *Arabidopsis thaliana*. *Austr. J. Plant Physiol.* 19: 427-438.
- BASKIN T.J., WILSON J.E., CORK A., WILLIAMSON R.E. 1994. Morphology and microtubule organisation in *Arabidopsis* roots exposed to oryzalin or taxol. *Plant Cell Physiol.* 35 (6): 935-942.
- BASKIN T.J., BIVENS N.J. 1995. Stimulation of radial expansion in *Arabidopsis* roots by inhibitors of actomyosin and vesicle secretion but not by various inhibitors of metabolism. *Planta* 197: 514-521.
- BUTCHER D.N., CLARK J.A., LENTON J.R. 1990. Gibberellins and the growth of excised tomato roots: comparison of *gib-1* mutant and wild type and responses to applied GA₃ and 2s, 3s paclobutrazol. *J. Exp. Bot.* 41: 715-722.
- CARPITA N.C., GIBEAUT D.M. 1993. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J.* 3: 1-30.
- DUFF S.M.G., SARATH G., PLAXTON W.C. 1994. The role of acid phosphatases in plant phosphorus metabolism. *Physiol. Plant.* 90: 791-800.
- ENDLE M.-C.H., STOPPIN V., LAMBERT A.M., SCHMIT A.-C. 1998. The growing cell plate of higher plants is a site of both actin assembly and vinculin-like antigen recruitment. *Eur. J. Cell Biol.* 77: 10-18.
- FAY P., DUKE W.B. 1977. An assessment of allelopathic potential in *Avena* germplasm. *Weed Sci.* 25: 224-228.
- GAWRON A. 1992. Investigation on 8-methoxypsoralen interactions with the cell membranes (in Polish). M. Skłodowska-Curie University, Lublin.
- GILBERT G.A., KNIGHT J.D., VANCE C.P., ALLAN D.L. 1999. Acid phosphatase activity in phosphorus deficient white lupine roots. *Plant Cell and Environ.* 22: 801-810.
- GOLDSTEIN A.H. 1992. Phosphate starvation inducible enzymes and proteins in higher plants. In: *Inducible Plant Proteins*, Wray J.L. (ed) Cambridge Univ. Press, Cambridge, pp. 25-44.
- GOMORI G. 1953. *Microscopic Histochemistry. Principles and Practice*. Univ. of Chicago Press, Chicago, pp. 37-224.
- HARA M., UMETSU N., MIYAMOTO C.H., TAMARI K. 1973. Inhibition of the biosynthesis of plant cell wall materials, especially cellulose biosynthesis by coumarin. *Plant Cell Physiol.* 14: 11-28.

- HAUSER M.-T., MORIKAMI A., BENFEY P.N. 1995. Conditional root expansion mutants of *Arabidopsis*. Development 121: 1237-1252.
- HOGETSU T., SHIBAOKA H., SHIMOKORIYAMA M. 1974. Involvement of cellulose synthesis in actions of gibberellin and kinetin on cell expansion. Gibberellin-coumarin and kinetin-coumarin interactions on stem elongation. Plant Cell Physiol. 15: 265-272.
- ITO T. 1976. Microscopic and submicroscopic observation of the effects of coumarin and colchicine during elongation of pine seedlings. Plant Cell Physiol. 17: 367-384.
- JANKAY P., MULLER W. 1976. The relationships among umbelliferone, growth and Peroxidase levels in cucumber roots. Am. J. Bot. 63: 126-132.
- JUNTILLA O. 1975. Allelopathy in *Heracleum laciniatum*: inhibition of lettuce seed germination and root growth. Physiol. Plant. 24: 446-470.
- KUPIDŁOWSKA E., KOWALEC M., SULKOWSKI G., ZOBEL A.M. 1994. The effect of coumarins on root elongation and ultrastructure of meristematic cell protoplast. Ann. Bot. 73: 525-530.
- KUPIDŁOWSKA E., BIENIAK B., RUCHIRAWAT A., ZOBEL A.M. 1995. Influence of methyl derivatives of coumarin on mitotic activity and ultrastructure of meristematic cells of *Allium cepa* root tips. Phytomedicine 2: 63-69.
- LANUBILE R., PIRO G., DALESSANDRO G. 1997. Effect of brefeldin A on the synthesis and transport of cell wall polysaccharides and proteins in pea root seedlings. J. Exp. Bot. 48: 1925-1933.
- MARTY F. 1978. Cytochemical studies on GERL, provacuoles and vacuoles in root meristematic cells of *Euphorbia*. Proc. Natl. Acad. Sci. USA 75: 852-856.
- MAYER A.M., POLJAKOFF-MAYBER A. 1963. Coumarins and their role in growth and germination. In: Plant Growth Regulation. Roberts E.H. (ed). Iowa University Press, pp. 735-749.
- MINEYUKI Y., GUNNING B.E.S. 1990. A role of preprophase bands of microtubules in maturation of new cell walls and a general proposal for the functional of PPB sites in cell division in higher plants. J. Cell Sci. 97: 527-537.
- MINEYUKI Y., MARC J., PALEVITZ B.A. 1991. Relationship between the preprophase band, nucleus and spindle in dividing *Allium* cotyledon cells. J. Plant Physiol. 138: 640-649.
- MITA T., SHIBAOKA H. 1984. Gibberellin stabilizes microtubules in onion leaf sheath cells. Protoplasma 119: 100-109.
- MOREJOHN L.C., FOSKET D.E. 1991. The biochemistry of compounds with antimicrotubule activity in plant cells. Pharm. Therap. 51: 217-230.
- MURASHIGE T., SKOOG F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.
- PIRO G., MONTEFUSCO A., PACODA D., DALESSANDRO G. 1999. Brefeldin A: a specific inhibitor of cell wall polysaccharide biosynthesis in oat coleoptile segments. Plant Physiol. Biochem. 37: 33-40.
- PODBIELKOWSKA M., KUPIDŁOWSKA E., WAŁĘZA M., DOBRZYŃSKA K., LOUIS S.A., KEIGHTLEY A., ZOBEL A.M. 1994. Coumarins as antimitotics. Int. J. Pharm. 32: 262-273.
- SCHINDELMAN G., MORIKAMI A., JUNG J., BASKIN T.I., CARPITA N.C., DERBYSHIRE P., MC CANN M., BENFEY P. 2001. COBRA encodes a putative GPI-anchored protein, which is polarly localized and necessary for oriented cell expansion in *Arabidopsis*. Gen. Develop. 15: 1115-1127.
- SHIBAOKA H. 1994. Plant hormone-induced changes in the orientation of cortical microtubules: alteration in the cross-linking between microtubules and the plasma membrane. Ann. Rev. Plant Physiol. (Plant Mol. Biol.) 45: 527-544.
- SHIMOMURA H., SASHIDA Y., NAKATA H., KAWASAKI J., ITO Y. 1982. Plant growth regulators from *Heracleum lanatum*. Phytochemistry 21: 2213-2215.
- SIMMONDS D., SETTERFIELD G., BROWN D.L. 1983. Organization of microtubules in dividing and elongating cells of *Vicia hajastana* Grossh. in suspension culture. Eur. J. Cell Biol. 32: 59-66.
- STOBIECKI M., CIESIOLKA D., PERETLAKOWICZ M., GULEWICZ K. 1993. Phenolic compounds isolated from bitter lupine seeds and their inhibitory effects on germination and seedling growth of lettuce. J. Chem. Ecol. 19: 325-338.
- SVENSSON S.B. 1971. The effect of coumarin on root growth and root histology. Physiol. Plant. 24: 446-470.
- SVENSSON S.B. 1972. A comparative study of the changes in root growth induced by coumarin, auxin, ethylene, kinetin and gibberellic acid. Physiol. Plant. 26: 115-135.
- TADEO F.R., GOMEZ-CADENAS A., BEN-CHEIKH W., PRIMOMILLO E., TALON M. 1997. Gibberellin-ethylene interaction control radial expansion in citrus roots. Planta 202: 370-378.
- TANIMOTO E. 1987. Gibberellin-dependent root elongation in *Lactuca sativa*: recovery from growth retardant-suppressed elongation with thickening by low concentration of GA_3 . Plant Cell Physiol. 28: 963-973.
- TANIMOTO E. 1988. Gibberellin regulation of root growth with change in galactose content of cell walls in *Pisum sativum*. Plant Cell Physiol. 29: 269-280.
- ZOBEL A.M., BROWN S.A. 1989. Histochemical localization of furanocoumarins in *Ruta graveolens* shoots. Can. J. Bot. 67: 915-921.
- ZOBEL A.M., BROWN S.A. 1991. Furanocoumarin concentrations in fruits and seeds of *Angelica archangelica*. Environ. Exp. Bot. 31: 447-452.
- ZOBEL A.M., MARCH R.E. 1993. Autofluorescence reveals different histological localisations of furanocoumarins in fruits of some Umbelliferae and Leguminosae. Ann. Bot. 71: 251-255.

ZMIANY ULTRASTRUKTURY KOMÓREK
I MORFOLOGII KORZENIA *ARABIDOPSIS THALIANA*
TRAKTOWANEGO KUMARYNAMI

STRESZCZENIE

Badano wzrost i ultrastrukturę korzeni traktowanych dwoma kumarynowymi kolinami, kumaryną i umbeliferonem, oraz antagonistyczny efekt egzogennej gibereliny (GA₃). Obydwie koliny hamowały wzrost elongacyjny korzenia i stymulowały radialny wzrost komórek ryzodermisy i kory pierwotnej w dystalnej strefie merystemu i w strefie elongacyjnej. GA₃ podany jednocześnie z kumarynami zmniejszał zarówno intensywność hamowania elongacji korzenia, jak i radialną ekspansję komórek. W korzeniach traktowanych kumarynami wykazano intensywną wakuolizację komórek merystematycznych i zakłócenia w strukturze diktyosomów. Stwierdzono także, w przeciwieństwie do kontroli, obecność aktywnej kwaśnej fosfatazy w cytosolu, co wskazuje na wzrost przepuszczalności endomembran. W post-mitotycznych komórkach wykazano nieprawidłowe umiejscowienie nowo utworzonych ścian komórkowych, brak ich połączenia ze ścianą komórki macierzystej oraz nieciągłość fibrylarnej warstwy szkieletowej, co sugeruje dysfunkcję cytoszkieletu.

SŁOWA KLUCZOWE: *Arabidopsis thaliana*, kumaryna, umbeliferon, kwaśna fosfataza, giberelina, ultrastruktura komórek.