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EFFECT OF TEMPERATURE ON GROWTH, PROTON EXTRUSION AND MEMBRANE POTENTIAL IN MAIZE COLEOPTILE SEGMENTS INCUBATED IN THE PRESENCE OF CHLORINATED AUXIN (4-CL-IAA)

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The effects of temperature (5-45 °C) on growth in the presence of 4-Cl-IAA and proton extrusion in maize coleoptile segments were studied. At some temperatures the membrane potential changes were also determined. It was found that in maize coleoptile segments growth in the presence of 4-Cl-IAA shows the maximal value at 25 °C. Simultaneous measurements of growth and external medium pH showed that temperature maxima of growth and acidification correlate for 4-Cl-IAA. The addition of chlorinated auxin to the incubation medium, at 25 °C, brought about an immediate hyperpolarisation of the membrane potential, which was 4-fold greater than at 30 °C. 4-Cl-IAA at 10 °C caused additional membrane depolarization apart from the one induced by low temperature. The results presented in this paper demonstrate that the temperature-induced changes in growth of maize coleoptile segments incubated in the presence of 4-Cl-IAA are, at least in part, mediated via a PM H^+-ATPase activity. These data also support our earlier hypotheses that chlorinated auxin induced a specific signal transduction pathway in maize coleoptile segments, which differ from one induced by IAA.

INTRODUCTION

Indole-3-acetic acid (IAA), the major and most abundant auxin in plants, exerts control over many processes of plant growth and development, including cell division and cell expansion, root initiation, tissue vascularization, gravitropic and phototropic responses, flowering, fruit ripening and abscission of leaves and fruit (Davies 2004). Although other auxins, such as 4-chloroindole-3-acetic acid (4-Cl-IAA), indole-3-butyric acid (IBA) and phenyl-acetic acid (PAA) have also been identified in plants (Müller-Ludwig 2000; Normanly 1997; Normanly et al. 1995) little is know about their physiological function. Chlorinated auxin, 4-Cl-IAA, was first identified in immature seeds of *Pisum sativum* (Gandar and Nitsch 1967; Marumo et al. 1968) and subsequently was also found in a number of plants, mainly members of the *Fabaceae* (Engvild 1975; Engvild 1980; Engvild et al. 1978; Engvild et al. 1980; Hofinger and Böttger 1979; Katayama et al. 1988). 4-Cl-IAA has been tested in many different bioassays, which have shown its exceptionally high biological activity, as compared to IAA (Ahmad et al. 1987; Böttger et al. 1978; Fischer et al. 1992; Hatano et al. 1987; Karcz et al. 1999; Karcz and Burdach 2002; Katayama 2000; Lekacz and Karcz 2006; Pless et al. 1984; Rescher et al. 1996). For example, in early experiments Böttger et al. found that 4-Cl-IAA was much more active, as compared to IAA, both for straight growth of the *Avena* coleoptile segments and for proton extrusion in stem protoplast suspensions of *Helianthus annuus* L. and *Pisum sativum* L. (Böttger et al. 1978). In turn, quite recently has been shown that in maize root segments 4-Cl-IAA is much more active than IAA in terms of the lowest concentrations needed to mediate redox activity and proton extrusion (Lekacz and Karcz 2006). 4-Cl-IAA's high auxin activity has been postulated to occur via reduced metabolism of 4-Cl-IAA, or a receptor and signal transduction pathway unique to 4-Cl-IAA (Karcz and Burdach 2002; Marumo et al. 1974; Rainecke et al. 1999).

In order to obtain much more information on high auxin activity of 4-Cl-IAA we have determined the temperature dependence of 4-Cl-IAA-induced growth and proton extrusion in maize coleoptile segments and compared these data with adequate ones recently published by us for IAA (Karcz and Burdach 2002; Karcz and Burdach 2007).

MATERIALS AND METHODS

Plant material

Seeds of maize (*Zea mays* L.) cv. K33xF2 were

soaked in tap water for 2 h, sown on wet lignin in plastic boxes and placed in a growth chamber at 27°C. The experiments were carried out with 10-mm long coleoptile segments cut from 4-day-old etiolated seedlings. The coleoptile segments with the first leaves removed were cut 3 mm below the tip of the seedlings.

Chemicals

4-chloroindole-3-acetic acid (Sigma, St Louis, USA) was dissolved in a small volume of isopropanol, and stored as a 0.1 mM stock solution. The final concentration of isopropanol in the incubation medium did not exceed 0.1% , which was shown to be without any effect on the elongation growth (Claussen et al. 1996). 4-Cl-IAA was used at its optimal concentration (1 μM) for elongation growth of maize coleoptile segments (Karcz et al. 1999).

Growth and pH measurements

The growth experiments were carried out in an apparatus, which allowed simultaneous measurements of elongation growth and pH of the incubation medium of maize coleoptile segments (Karcz et al. 1990; Karcz and Burdach 2002). The optical system used for growth measurements (shadow graph method) permitted the recording of longitudinal extension of a stack of 21 segments. The coleoptile segments (each 10 mm in length) were placed in the incubation medium of the following composition (control medium): 1 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$ and initial pH 5.8 – 6.0. The volume of the incubation medium in the growth and pH-measuring apparatus was 6.3 ml (0.3 ml segment^{-1}). It is noteworthy that in this apparatus the incubation medium flowed also through the lumen of the coleoptile cylinders (Karcz et al. 1995).

Measurements of pH were done with the pH-meter CP-315 (Elmetron, Poland) and pH electrode OSH 10-10 (Metron, Poland). Growth and pH were read every 15 min under the same conditions.

Temperature control of all solutions (5 – 45°C) was obtained by immersing the elongation and pH-measuring system in a termostatically controlled water bath. Prior to the addition of auxin to the incubation medium, the coleoptile segments were equilibrated for 2 hours at the desired temperature.

All manipulations and growth measurements were carried out under dim green light.

Electrophysiology

The electrophysiological experiments were performed on 10-mm long coleoptile segments. A standard electrophysiological technique was used to determine membrane potential, as previously described (Karcz and Burdach 2002; Stolarek and Karcz 1987). Briefly, the membrane potential (E_m) was measured by recording the voltage between a 3M KCl-filled glass micropipette (tip diameters less than 1 μm) inserted into the parenchymal cells and a reference electrode in the bathing medium containing the same composition as used in growth experiments. Micropipettes from borosilicate glass capillaries 1B150F-3 (World Precision Instrumentes, USA) were pulled on a vertical pipette puller L/M-3P-A (List-Medical, Germany). The microelectrodes were inserted into the cells under microscope by means of micromanipulator (Hugo Sachs Elektronik, Germany).The coleoptile segments were preincubated for 2 hours at the desired temperature in an intensively aerated bathing medium. After this period one coleoptile was transferred into a perfusion Plexiglass chamber, which contained the bathing medium at the desired temperature. Medium flow was driven by a peristaltic pump PP 1B-05A (Zalimp, Poland), which also allowed both the flow of the bathing medium at the desired temperature and the change to the bathing medium in the chamber (usually fourfold within less than 2 min).

RESULTS

Temperature dependence of growth in the presence of 4-Cl-IAA

Figure 1 shows the effect of temperature (5 – 45°C) on elongation growth of maize coleoptile segments incubated in the presence of 4-Cl-IAA. The segments were first preincubated over 2 h at the desired temperature, whereupon 4-Cl-IAA, at a final concentration of 1 μM, was added. As can be seen in Fig. 1 the maximal growth in the presence of 4-Cl-IAA was observed at 25°C. At this temperature, the growth of the segments was 110% greater compared with segments grown at 20°C. The temperatures in the range 5 – 10°C strongly inhibited segments elongation. At 45°C the elongation of the segments in the presence of 4-Cl-IAA was by 85% lower as compared to its maximal value at 25°C. In turn, at 20°C and 40°C the elongation of the segments after 420 min did not differ significantly.

Effect of temperature on simultaneously with growth measured pH changes of the incubation medium

The pH-experiments described in this section were performed simultaneously with growth using the same tissue sample (as described in Materials and Methods). The data in Fig. 2 indicate that the coleoptile segments incubated within the first 2 h in auxin-free medium bro ught about an increase of medium pH to the level near neutral. When 4-Cl-IAA was added (after 2 h of preincubation) at temperature higher than 10°C a decrease of medium pH was observed. Addition of 4-Cl-

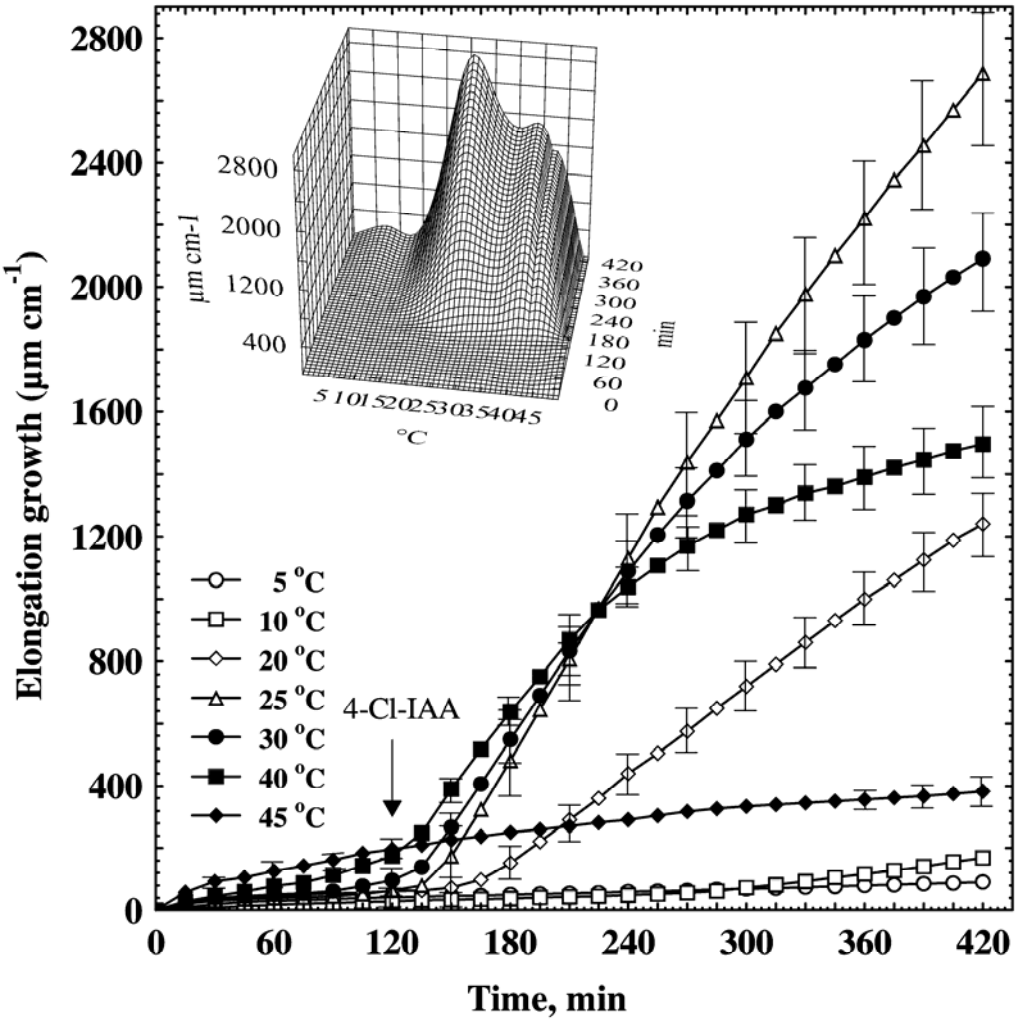

Fig. 1. Effect of temperature on growth of maize coleoptile segments in the presence of 4-Cl-IAA (1 µM). The growth of a stack of 21 segments, expressed as elongation (µm cm-1), was measured as described in Materials and Methods. After preincubation (over 2 h at the desired temperature) of the coleoptile segments in control medium, 4-Cl-IAA was added (arrow). The inset shows the temperature dependence of growth in the presence of 4-Cl-IAA as a function of time. Values are means of 11 independent experiments. Bars indicate ± SD.

IAA at low temperatures (5°C and 10°C) was practically not able to acidify external medium. At 20°C and temperatures higher than 25°C, 4-Cl-IAA-induced medium acidification, expressed as difference between H^+ concentration at 420 min and 120 min ($\Delta[H^+]$), was significantly lower as compared to 25°C (Fig. 2, inset).

Effect of temperature on 4-Cl-IAA-induced membrane potential (Em) changes

Before the electrophysiological experiments, the coleoptile segments were equilibrated for 2 hours at the desired temperature (10, 25 or 30°C). After insertion of the microelectrode into the parenchymal cell of coleoptile segment and stabilization of membrane potential (E_m), the bathing medium (auxin-free medium) was changed for a new one (at the same salt composition and temperature) containing 4-Cl-IAA at a final concentration of 1 µM. The membrane potential (E_m) of the parenchymal cells measured at 25°C, before being changed in response to 4-Cl-IAA, was -114.5 ± 10.1 mV (mean ± SD, n=12) (Fig. 3 A). The addition of 4-Cl-IAA to the incubation medium at 25°C caused a rapid hyperpolarisation of 25.1 ± 6.7 mV (mean ± SD, n=12).

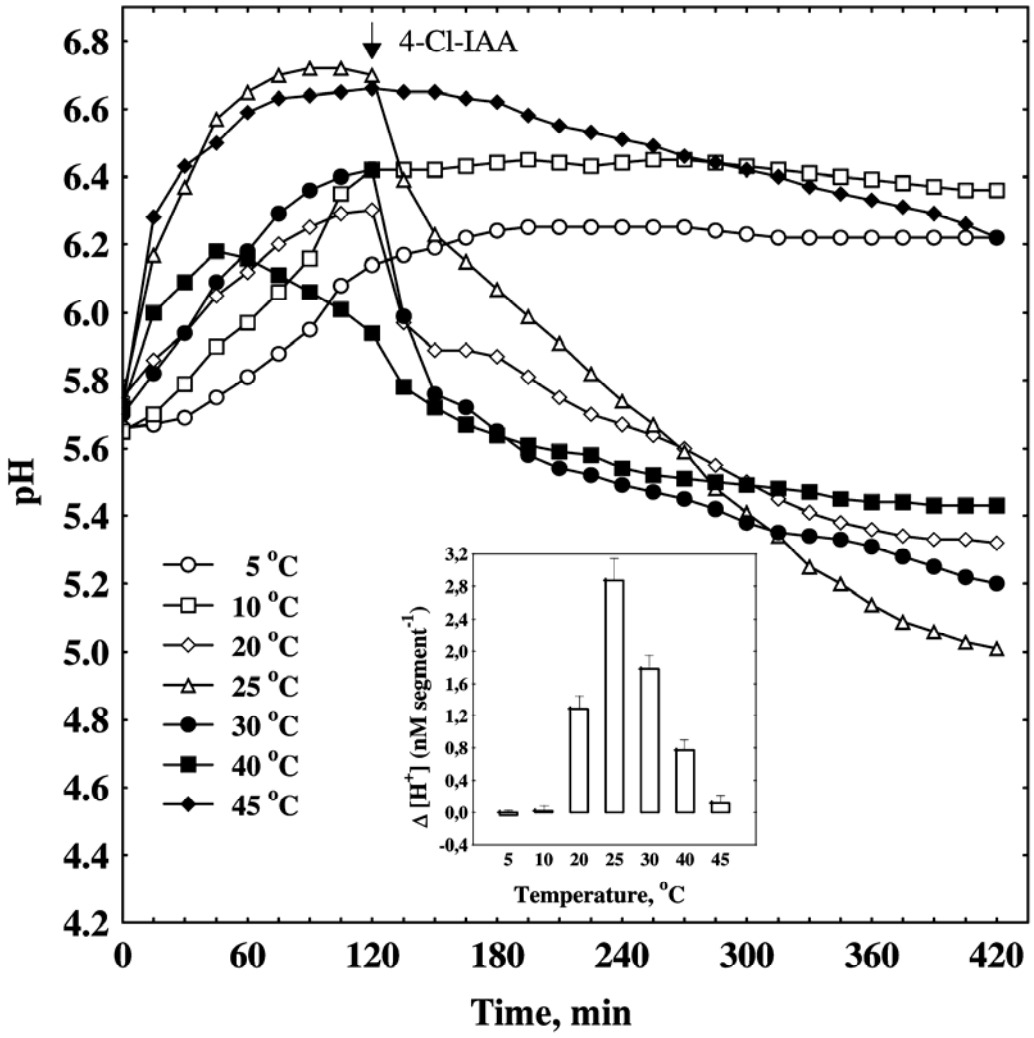

Fig. 2. Effect of temperature on 4-Cl-IAA-induced medium pH changes of maize coleoptile segments. Auxin was added after 2 h of segments preincubation at the desired temperature (arrow). Values for pH are means of 11 independent experiments performed simultaneously with growth using the same tissue sample (as described in Materials and Methods). The inset shows temperature dependence of medium pH expressed as Δ[H+] , where Δ[H+] means difference between H+ concentration ([H+]) at 420 min and 120 min.

Preincubation of maize coleoptile segments over 2 h at 30°C brought about hyperpolarisation of the E_m by 15.1 mV, as compared to the E_m at 25°C (Fig. 3 A). If 4-Cl-IAA was added to the segments after 2 h preincubation at 30°C, the hyperpolarisation of the E_m was significantly lower (6.6 ± 4.3 mV mean ± SD, n=12) as compared to the hyperpolarisation induced in the presence of 4-Cl-IAA at 25°C (25.1 mV). Preincubation of maize coleoptile segments (over 2 h) at 10°C caused depolarisation of E_m to the level of –67.6 ± 8.9 mV (mean ± SD, n=12). The addition of 4-Cl-IAA at this low level of E_m caused an additional membrane depolarisation by 15 – 17 mV (Fig. 3 B).

DISCUSSION

It is well established that indole-3-acetic acid (IAA) causes in maize coleoptile segments acceleration of elongation growth, enhancement of proton extrusion and transient depolarisation followed by a slow hyperpolarisation of membrane potential (Claussen et al. 1996; Felle et al. 1991; Karcz et al. 1990; Karcz et al.

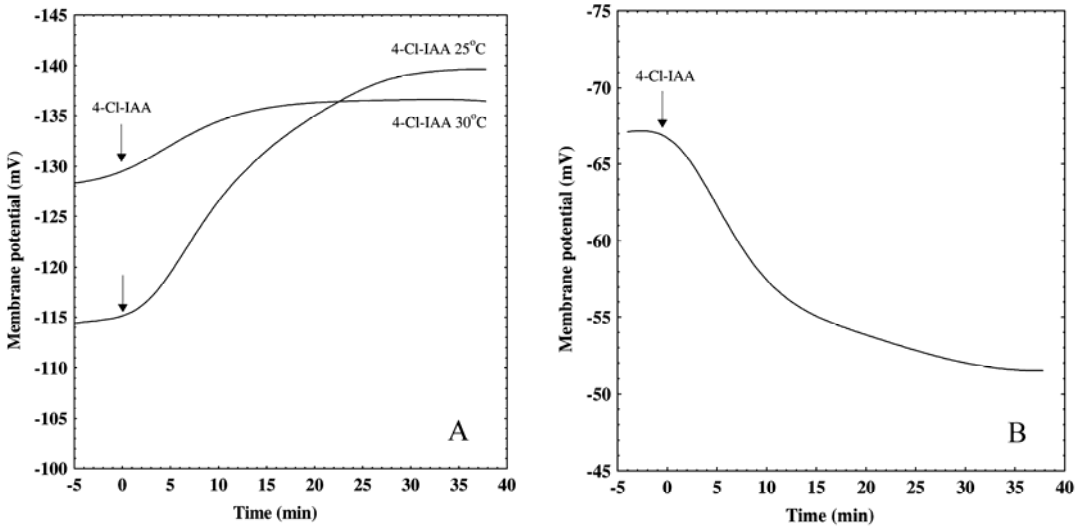

Fig. 3. Effect of temperature on 4-Cl-IAA-induced changes in membrane potential (Em) of parenchymal coleoptile cells. At time 0 (arrow) the control medium was changed for a new one, at the same temperature and salt composition, containing in addition 4-Cl-IAA. Representative curves are shown. Adequate mean value are indicated in text. (A) 4-Cl-IAA added at 25ºC and 30ºC, respectively; (B) 4-Cl-IAA added at 10ºC.

1999; Karcz and Burdach 2002; Kutschera and Schopfer 1985a; Lüthen et al. 1990). In turn, it was also shown that in maize, which does not contain chlorinated auxins naturally (Hofinger and Böttger 1979), 4-Cl-IAA is much more active in growth stimulation compared with IAA (Fischer et al. 1992; Karcz et al. 1999; Karcz and Burdach 2002). Recently, we have also found that in contrast to IAA, 4-Cl-IAA at 1 µM caused an immediate hyperpolarisation of the membrane potential, which was 2-fold greater than for IAA (Karcz and Burdach 2002). These findings and the data obtained in experiments with re-addition of IAA and 4-Cl-IAA were used by us to formulate the hypothesis that 4-Cl-IAA's strong auxin activity in maize coleoptile segments is caused via a reduced metabolism of 4-Cl-IAA or a specific signal transduction pathway for this auxin (Karcz et al. 1999; Karcz and Burdach 2002). A receptor and signal transduction pathway unique to 4-Cl-IAA was also early proposed for pea pericarp growth (Rainecke 1999).

The results presented in this paper showed that the growth of maize coleoptile segments in the presence of 4-Cl-IAA exhibits a clear maximum (2685.9 ± 214.8 µm cm^{-1}; mean ± SD, n=11) at 25°C (Fig. 1). This observation shows that 4-Cl-IAA and IAA differ in terms of temperature maxima; growth in the presence of IAA exhibited the maximal value (2134 ± 192.6 and 2053.01 ± 177.6 µm cm^{-1}; mean ± SD, n=11) in the range 30 – 35°C (Karcz and Burdach 2007). It is noteworthy that 4-Cl-IAA and IAA-induced growth at 30°C did not differ significantly (2091,2 ± 167,3 µm cm^{-1}; mean ± SD, n=11 for 4-Cl-IAA and 2134 ± 192,6 µm cm^{-1}; mean ± SD, n=11 for IAA (Karcz and Burdach 2007), suggesting that at higher temperatures 4-Cl-IAA is less effective in growth stimulation of maize coleoptile segments than IAA. Simultaneous measurements of growth and external medium pH of maize coleoptile segments showed that 4-Cl-IAA at 25°C was not only much more active in the stimulation of growth, as compared to other temperatures, but also effectively acidified external medium. This observation is consistent with the "acid growth theory" of auxin action, which postulates the striking correlations between the auxin-induced H^+ extrusion and the auxin-induced extension growth (Hager 2003).

At presence, there is no doubt that the slow membrane hyperpolarisation observed in the presence of IAA is a consequence of stimulation of proton extrusion by a H^+-ATPase (Felle et al. 1991; Hedrich et al. 1995; Rück et al. 1993). Although the kinetics of the IAA and 4-Cl-IAA-induced membrane potential changes of maize coleoptile cells are different it is suggested that a rapid hyperpolarisation of the membrane potential in the presence of chlorinated auxin is a result of a stimulated proton extrusion through the H^+-ATPase (Karcz and Burdach 2002). Incubation of maize coleoptile segments over 2 h at 30°C caused hyperpolarisation of the E_m by 15.1 mV, as compared to 25°C. Such hyperpolarisation of the E_m at 30°C may reflect the direct effect of high temperatures on activation of the H^+-ATPase. The addition of 4-Cl-IAA to the bathing medium at 30°C significantly decreased the 4-Cl-IAA-induced membrane

hyperpolarisation, as compared to 25°C (Fig. 3A). This observation suggests that the hyperpolarisation of E_m recorded here at 30°C and in the presence of chlorinated auxin are not additive processes. In contrast to high temperature (30°C), preincubation of maize coleoptile segments over 2 h at 10°C caused depolarisation of the E_m to the level of -67.6 mV (Fig. 3B). In turn, addition of 4-Cl-IAA to the incubation medium at 10°C brought about additional membrane depolarisation by 15-17 mV. Similar effects at 30°C and 10°C were recently observed by us in the presence of IAA (Karcz and Burdach 2007).

In conclusion, the results presented in this paper demonstrate that: (1) growth of maize coleoptile segments in the presence of 4-Cl-IAA has a clear maximum at 25°C (Fig. 1); (2) temperature maxima of acidification and growth correlate for 4-Cl-IAA; (3) 4-Cl-IAA-induced electrogenic activity differ qualitatively and quantitatively at high (30°C) and low (10°C) temperatures.

The results suggest that the temperature-induced changes in growth of maize coleoptile segments incubated in the presence of 4-Cl-IAA are, at least in part, mediated via a PM H^+-ATPase activity. Taking into account that growth in the presence of 4-Cl-IAA and IAA (Karcz and Burdach 2007) differ in terms of temperature maximum the obtained data support our earlier hypotheses that chlorinated auxin induces a specific signal transduction pathway in maize coleoptile cells.

REFERENCES

Ahmad A, Anderson AS, Engvild KC (1987) Rooting, growth and ethylene evolution of pea cuttings in response to chloroindole auxins. *Physiol Plant*, 69, 137-140.

Böttger M, Engvild KC, Soll H (1978) Growth of Avena coleoptiles and pH drop of protoplast suspensions induced by chlorinated indoleacetic acids. *Planta*, 140, 89-92.

Claussen M, Lüthen H, Böttger M (1996) Inside or outside? Localization of the receptor relevant to auxin-induced growth. *Physiol Plant*, 98, 861-867.

Davies PJ (2004) *Plant hormones. Biosynthesis, Signal Tranduction, Action!* Kluwer Academic Publishers, Dordrecht.

Engvild KC (1975) Natural chlorinated auxins labelled with radioactive chloride in immature seeds. *Physiol Plant*, 34, 286-287.

Engvild KC (1980) Simple identification of the neutral chlorinated auxin in pea by thin layer chromatography. *Physiol Plant*, 48, 435-437.

Engvild KC, Egsgaard H, Larsen E (1978) Gas chromatographic-mass spectrometric identification of 4-chloroindolyl-3-acetic acid methyl ester in immature green peas. *Physiol Plant*, 42, 365-368.

Engvild KC, Egsgaard H, Larsen E (1980) Determination of 4-chloroindole-3-acetic acid methyl ester in Lathyrus, Vicia and Pisum by gas chromatography-mass spectometry. *Physiol Plant*, 48, 499-503.

Felle H, Peters W, Palme K (1991) The electrical response of maize to auxins. *Biochim Biophys Acta*, 1064, 199-204.

Fischer C, Lüthen H, Böttger M, Hertel R (1992) Initial transient growth inhibition in maize coleoptiles following auxin application. *J Plant Physiol*, 141, 88-92.

Gandar JC, Nitsch C (1967) *Isolement de l'ester methylique d'un acide chloro-3- indolyacetique a partir de graines immatures de pois Pisum sativum* L C R Acad Sci (Paris). Ser. D 265, 1795-1798.

Hager A (2003) Role of the plasma membrane H+-ATPase in auxin-induced elongation growth: historical and new aspects. *J Plant Res*, 116, 483-505.

Hatano T, Katayama M, Marumo S (1987) 5,6-dichloroindole-3-acetic acid as a potent auxin: ist synthesis and biological activity. *Experientia*, 43, 1237-1239.

Hedrich R, Bregante M, Dreyer I, Gambale F (1995) The voltage-dependent potassium-uptake channel of corn coleoptiles has permeation properties different from other K+ channels. *Planta*, 197, 193-199.

Hofinger M, Böttger M (1979) Identification by GC-MS of 4-chloroindolylacetic acid and its methyl ester in immature Vicia faba seeds. *Phytochemistry*, 18, 653-654.

Karcz W, Burdach Z (2002) A comparison of the effects of IAA and 4-Cl-IAA on growth, proton secretion and membrane potential in maize coleoptile segments. *J Exp Bot*, 53, 1089-1098.

Karcz W, Burdach Z (2007) Effect of temperature on growth, proton extrusion and membrane potential in maize (Zea mays L.) coleoptile segments. *Plant Growth Reg*, 52, 141-150.

Karcz W, Lüthen H, Böttger M (1999) Effect of IAA and 4-Cl-IAA on growth rate in maize coleoptile segments. *Acta Physiol Plant*, 21, 133-139.

Karcz W, Stolarek J, Lekacz H, Kurtyka R, Burdach Z (1995) Comparative investigation of auxin and fusicoccin-induced growth and H+-extrusion in coleoptile segments of Zea mays L. *Acta Physiol Plant*, 17, 3-8.

Karcz W, Stolarek J, Pietruszka M, Małkowski E (1990) The dose-response curves for IAA induced elongation growth and acidification of the incubation medium of Zea mays coleoptile segments. *Physiol Plant*, 80, 257-261.

Katayama M (2000) Synthesis and biological activities of 4-Chloroindole-3-acetic acid and its esters. *Biosci Biotechnol Biochem*, 64, 808-815.

Katayama M, Thiruvikraman SV, Marumo S (1988) Localization of 4-chloroindole-3-acetic acid in seeds of Pisum sativum and its absence from all other organs. *Plant Cell Physiol*, 29, 889-891.

Kutschera U, Schopfer (1985a) P Evidence against the acid-growth theory of auxin action. *Planta*, 163, 483-493.

Lüthen H, Bigdon M, Böttger M (1990) Reexamination of the acid growth theory of auxin action. *Plant Physiol*, 93, 931-939.

Lekacz, H, Karcz W (2006) The effect of auxins (IAA and 4-Cl-IAA) on the redox activity and medium pH of Zea mays L. root segments. *Cell Mol Biol Lett*, 11, 376-383.

Marumo S, Hattori H, Abe H, Munakata K (1968) Isolation of 4-chloroindolyl-3-acetic acid from immature seeds of Pisum sativum. *Nature*, 219, 959-960.

Marumo S, Hattori H, Yamamoto A (1974) *Biological activity of 4-chloroindolyl-3-acetic acid. Plant Growth Substances* 1973 Tokyo: Hirokawa Publishing Co. 419-428.

Müller-Ludwig J (2000) Indole-3-butyric acid in plant growth and development. *Plant Growth Reg*, 32, 219-230.

Normanly J (1997) Auxin metabolism. *Physiol Plant*, 100, 431-442.

Normanly J, Slovin JP, Cohen JD (1995) Rethinking auxin biosynthesis and metabolism. *Physiol Plant*, 107, 323-329.

Pless T, Böttger M, Hedden P, Grabe J (1984) Occurence of 4-Cl-indoleacetic acid in broad beans and correlation of its levels with seed development. *Plant Physiol*, 74, 320-323.

Reinecke DM, Ozga JA, Ilić N, Magnus V, Kojić-Prodić B (1999) Molecular properties of 4-substituted indole-3-acetic acids affecting pea pericarp elongation. *Plant Growth Reg*, 27, 39-48.

Reinecke DM (1999) 4-Chloroindole-3-acetic acid and plant growth. *Plant Growth Reg*, 27, 3-13.

Rescher U, Walther A, Schiebl C, Klämbt D (1996) In vitro binding affinities of 4-chloro-, 2-methyl, 4-methyl, and 4-ethylindoleacetic acid to auxin-binding protein 1 (ABP1) correlate with their growth-stimulating activities. *J Plant Growth Regul*, 15, 1-3.

Rück A, Palme K, Venis MA, Napier RM, Felle HH (1993) Patch-clamp analysis establishes a role for an auxin binding protein in the auxin stimulation of plasma membrane current in Zea mays protoplasts. *Plant J*, 4, 41-46.

Stolarek J, Karcz W (1987) Effects of UV-C radiation on membrane potential and electric conductance in internodal cells of Nitellopsis obtusa. *Physiol Plant*, 70, 473-478

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