Pol. J. Environ. Stud. Vol. 20, No. 5 (2011), 1221-1225

Original Research

Temperature Dependence of Growth in Maize Seedlings and Excised Coleoptile Segments

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Received: 24 November 2010
Accepted: 12 April 2011

Abstract

The effect of temperature in the range 22-32ºC and 25-40ºC on elongation growth of maize seedlings and coleoptiles excised from them was studied, respectively. It was found that elongation of maize seedling's organs (root, mesocotyl and coleoptile) increased with increasing temperature in the range 22-32ºC. However, when coleoptile segments excised from seedlings grown at highest temperature (32ºC) were incubated at various temperatures (25, 30, 35, and 40ºC) and in the presence of IAA their elongation growth was always lower compared to segments excised from seedlings grown at 22 and 27ºC. Simultaneous measurements of elongation growth and external medium pH indicated that proton extrusion increased with increasing medium temperature from 25 to 35ºC. The results presented here show that the temperatures at which the maize seedlings were cultivated are crucial for temperature dependence of a coleoptile segment's elongation growth.

Keywords: auxin (IAA), elongation growth, medium pH, maize seedlings, temperature

Introduction

Plants exposed to considerable fluctuations in environmental temperatures demonstrate extensive physiological and biochemical changes that affect plant growth and development [1-3]. Despite numerous studies [4-6], the mechanisms underlying temperature-dependent plant growth are still poorly understood. It was established that temperature-dependent changes in growth can be mediated by the ability of the plant to respond to endogenous factors such as auxin [7-10]. For example, it was found that temperature-dependent plant growth correlated with level of free auxin (IAA) [11-12] and depended on auxin transport as well as IAA metabolism [10].

It has also been shown that indole-3-acetic acid (IAA) caused in maize coleoptile segments acceleration of elongation growth and proton extrusion [13-18], which are mediated by increases in either the activity or the amount of plasma membrane H^+-ATPase [19-20]. The correlation between IAA-induced proton efflux and acceleration of elongation growth is evidence for the acid growth theory, which explains the mechanism of auxin-induced growth [for rewiev see 21]. According to this theory, IAA-enhanced H^+ pumping lowers cell wall pH, activates pH-sensitive enzymes and proteins within the wall, and initiates cell-wall loosening and extension growth [21]. In our previous studies [9] we showed that endogenous growth of maize coleoptiles segments exhibited a clear maximum at 30ºC, whereas growth in the presence of IAA showed the maximum value in the range of 30-35ºC. Our results clearly indicated that temperature maxima for IAA-induced growth correlated with IAA-induced medium acidification and electrogenic activity of H^+-ATPase induced by this growth substance which, as we consider, suggested a pivotal role of plasma membrane H^+-ATPase in perception of extreme temperatures.

Despite significant progress in elucidation of IAA-induced elongation growth of plant cells [21-25], our

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knowledge concerning the mechanisms of IAA-induced growth at various temperatures is still limited. Taking into account the above, the main goal of the present study was to determine the temperature dependence of growth in maize seedlings and coleoptile segments excised from them.

Material and Methods

Plant Material and Growth Conditions

Seeds of maize (*Zea mays* L.) cv. KOKA were soaked in running tap water for 2 h and then sown on wet lignin in plastic boxes and placed for 4 days in growth chambers (Type MIR-553, Sanyo Electric Co., Japan) at 22, 27, and 32ºC. The experiments were performed with etiolated intact seedlings and 10-mm-long coleoptile segments excised from seedlings 3 mm bellow their tips. The first leaves were removed.

Chemicals

An aqueous stock solution (1 mM) of indole-3-acetic acid (Serva, Heidelberg, Germany) was prepared using the potassium salt of IAA. IAA at a final concentration of 10 μM was used.

Growth and pH Measurements

We have performed two kinds of growth experiments. The first, in which the lengths of roots, mesocotyls, and coleoptiles of 50 seedlings were measured with a ruler to the nearest 1 mm, and the second, in which elongation growth and proton extrusion in coleoptile segments were measured simultaneously in the same tissue [16-18]. The extension growth of a stack of 21 segments was measured by the shadow graph method, as previously described [17]. 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}). Medium flow was driven by peristaltic pump (Type Peri-Star PRO, World Precision Instruments, USA). Measurements of pH were done with the pH-meter CP-315 (Elmetron, Poland) and pH electrode OSH 10-10 (Metron, Poland). Temperature control of all solutions (25, 30, 35, and 40ºC) was obtained by immersing the elongation and pH measuring system in a thermostatically 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. Simultaneous measurements of elongation growth and pH were recorded every 15 min under the same conditions. All manipulations and growth measurements were carried out under dim green light.

Statistical Analysis

Data were analyzed using computer software by StatSoft, Inc. (2008). STATISTICA (data analysis software system), version 8.0. www.statsoft.com. Differences between individual treatments and control were analyzed using one-way ANOVA and LSD test. Statistical significance was $p<0.05$.

Results

The Elongation of Maize Seedling's Organs (Root, Mesocotyl, and Coleoptile) Increased with Increasing Temperature

The effect of temperatures (22, 27, and 32ºC) on the length of roots, mesocotyls, and coleoptiles of maize seedlings was determined (Fig. 1). When seedlings grew at high temperatures (27ºC or 32ºC) they exhibited an increase in organ length (roots, mesocotyls, and coleoptiles) compared to seedlings grown at 22ºC. For example, Fig. 1 shows that the length of a seedling's roots grown at 27 and 32ºC was by 26.8% and 64.4% greater compared to roots grown at 22ºC, respectively. At the same temperatures shoot growth (mesocotyl plus coleoptile) was 70% and 120% greater compared to 22ºC, respectively. These observations suggest that the growth of shoots is much more sensitive to higher temperatures than growth of roots.

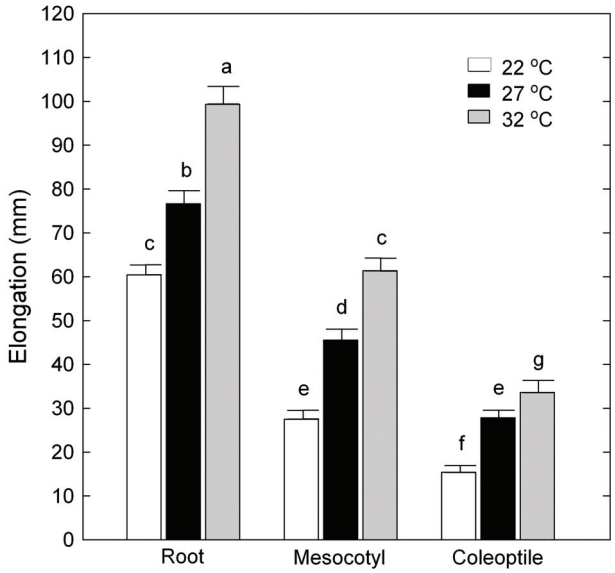

Fig. 1. The elongation growth of roots, mesocotyls, and coleoptiles of 4-day-old intact maize seedlings grown in the dark at 22, 27, and 32ºC. The differences between elongation growth of maize seedling's organs followed by the same letter are not significantly different from each other using the LSD-test ($p<0.05$), n=50. Bars indicate ±SE.

IAA-Induced Elongation Growth of Coleoptile Segments Decreased with Increasing Temperature of Seedlings Cultivation

Growth experiments described in this section were carried out with maize coleoptile segments excised from 4-day-old etiolated seedlings grown at 22, 27, or 32ºC. Subsequently, the segments were transferred into an apparatus that allowed simultaneous measurements of elongation growth and pH of the incubation medium (see "Material and Methods"). In this set-up the segments were incubated (in the dark in an intensively aerated medium) for 7 h at the desired temperatures (25, 30, 35, and 40ºC). IAA, at a final concentration of 10 μM was added after 2 h of a segment's preincubation. Fig. 2 shows the effects of temperature (25, 30, 35, and 40ºC) on IAA-induced growth of maize coleoptile segments excised from seedlings grown in the dark at 22, 27, or 32ºC. The experiments showed that the temperature dependence of IAA-induced elongation growth of coleoptile segments decreased with increasing temperature at which the seedlings grew (Fig. 2). This means that at the highest temperature (32ºC) at which the seedlings grew, the elongation growth of coleoptile segments incubated in the presence of IAA was always the lowest (Fig. 2).

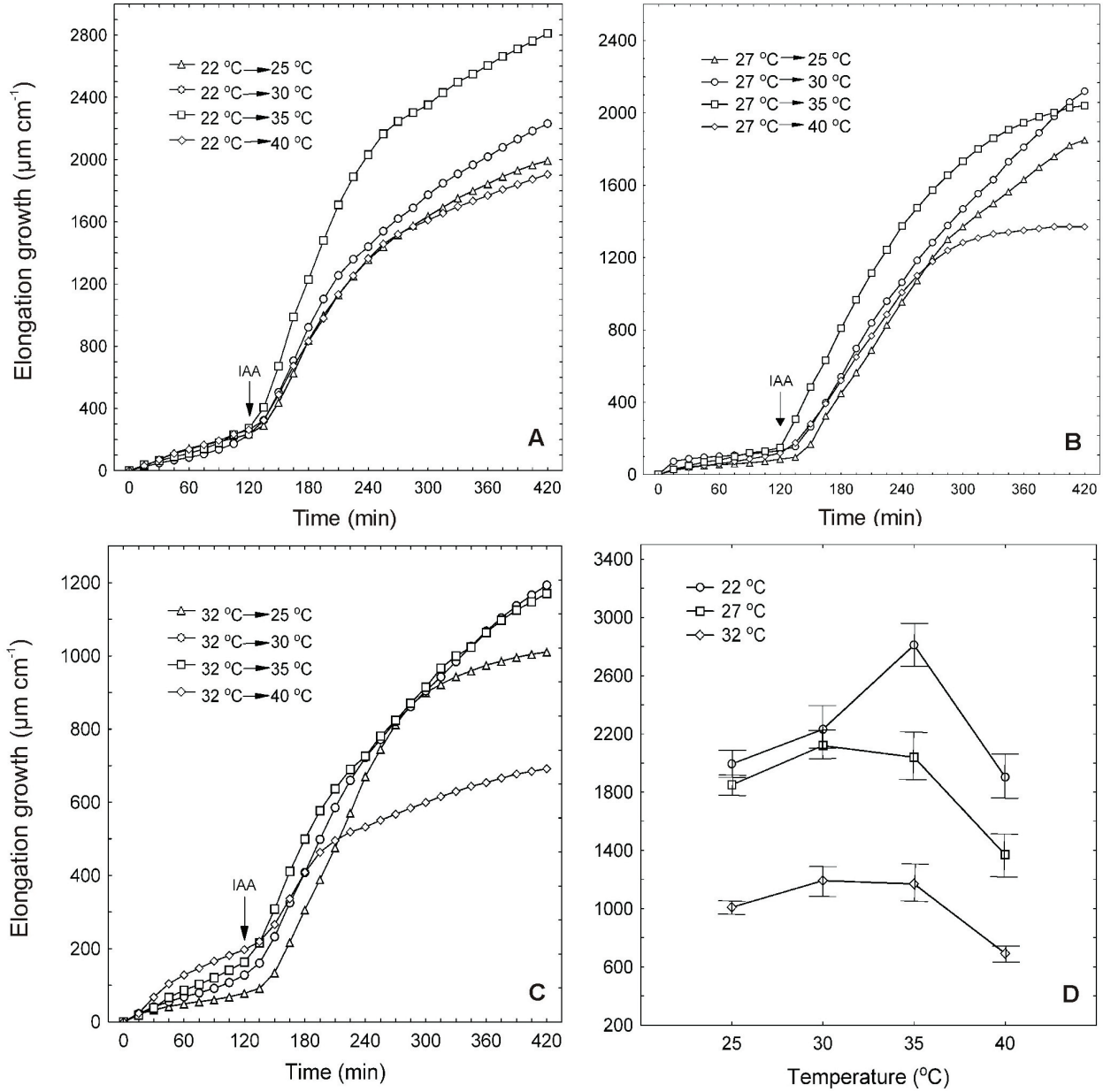

Fig. 2. Effect of temperature on growth of maize coleoptile segments incubated in the presence of auxin (IAA) (10 μM). Coleoptile segments were excised from seedlings grown at 22ºC (A), 27ºC (B), and 32ºC (C) and then transferred to different temperatures (25, 30, 35, and 40ºC). (D) Cumulative growth (measured over 420 min) of maize coleoptile segments incubated in the presence of auxin (IAA) (10 μM); the coleoptile segments were excised from maize seedlings grown at 22, 27, and 32ºC, and then transferred to medium at 25, 30, 35, and 40ºC. The growth of the stack of 21 segments, expressed as elongation (μm·cm^{-1}), was measured as described in "Material and Methods." After preincubation of the coleoptile segments (over 2 h at the desired temperature) in control medium, IAA was added (arrow). Values are means of 12 independent experiments. Vertical bars represent ±SE.

Temperature Affects pH of the Incubation Medium of Coleoptile Segments

Temperature dependence of medium pH expressed as $\Delta[H^+]$, where $\Delta[H^+]$ means difference between H^+ concentration at 7 and 2 h, is shown in Fig. 3. Temperature maximum for IAA-induced medium acidification ($\Delta[H^+]$) was observed at 35ºC for coleoptile segments excised from seedlings grown at 27ºC. Simultaneous measurements of growth and external medium pH of maize coleoptile segments showed that proton extrusion increased with increasing medium temperature from 25 to 35ºC. Interestingly, the acidification of the external medium ($\Delta[H^+]$) observed here at 30ºC and 35ºC for segments excised from seedlings grown at 32ºC (the lowest IAA-induced growth) was higher than for segments excised from seedlings grown at 22ºC (the highest IAA-induced growth) (Fig. 3).

Discussion

The *Zea mays* L. is the C_4 plant for which optimal temperature for germination and early seedling's growth is in the range of 32 to 35ºC [26]. Because maize is a chilling-sensitive crop [27-29], environmental stresses such as low and high temperature can be detrimental to the rate of its growth and development [9, 27, for review see 30-32]. The results presented in this paper (Fig. 1) showed that when seedlings grew at high temperatures (27ºC or 32ºC), they exhibited an increase in organ growth (roots, mesocotyls, and coleoptiles) compared with seedlings grown at 22ºC. Interestingly, the growth of shoots was much more sensitive to higher temperatures than growth of roots. For example, the seedlings grown at 32ºC had coleoptiles 130% longer compared to seedlings grown at 22ºC (Fig. 1).

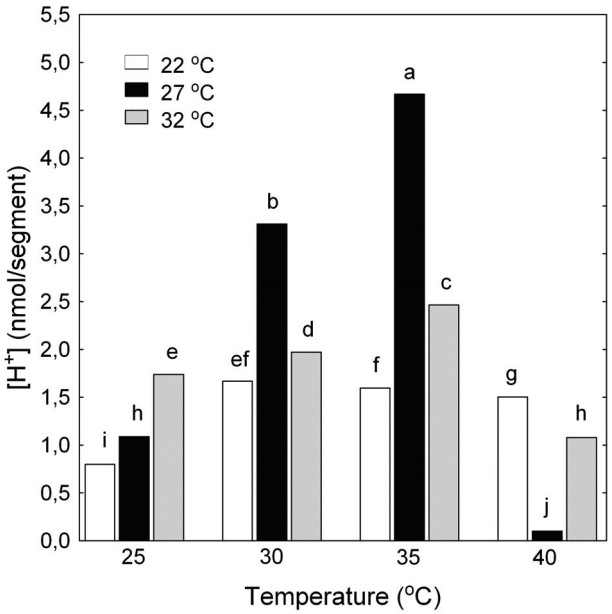

Fig. 3. pH changes of the incubation medium of maize coleoptile segments expressed as $\Delta[H^+]$, where $\Delta[H^+]$ means difference between H^+ concentration ($[H^+]$) at 420 min and 120 min. The coleoptile segments were excised from maize seedlings grown at 22, 27, and 32ºC and then transferred to the incubation medium at 25, 30, 35, and 40ºC. IAA, at a final concentration of 10 µM was added at 120 min. The differences between values of H^+ concentrations followed by the same letter are not significantly different from each other using the LSD-test ($p < 0.05$).

Apart from external factors like temperature, plant growth is also regulated by plant hormones, among which IAA plays a crucial role. The results presented in this paper (Fig. 2) clearly showed that the IAA-induced elongation growth of coleoptile segments excised from seedlings grown at 22ºC and subsequently incubated at 25, 30, 35, and 40ºC was always higher (Fig. 2 A) than the elongation growth of segments excised from seedlings grown at 27 and 32ºC (Fig. 2 B, C). The growth curves obtained for coleoptile segments excised from seedlings grown at 22, 27, and 32ºC are bell-shaped with maximum elongation at 30-35ºC (Fig. 2 D). This fact is in line with the finding that the optimal temperatures for germination and early growth of maize seedlings are in the range 32-35ºC [26]. Our findings suggest that under conditions of this study the elongation of seedling's coleoptiles was already saturated with the highest temperature (32ºC), and so after excision of segments these were not able to show such dramatic elongation (although still they were able to elongate slightly) (Fig. 2 C, D). However, the lowest temperature (22ºC) was not able to stimulate growth of seedling's coleoptiles so much and so after transfer to higher temperatures segments reacted by massive elongation (Fig. 2 A, D). Although it has not been directly studied here, a plausible interpretation for saturation of growth in coleoptiles of seedlings grown at higher temperatures is that, at least in part, it might be caused *via* an increased level of free IAA concentration. Such a possibility has previously been proposed by Gray et al. [11], who showed that temperature-induced hypocotyl elongation in *Arabidopsis* correlates with an increase in free IAA-concentration.

The results presented by Orbović and Poff [33] with *Arabidopsis thaliana* seedlings should also be mentioned. These authors reported that seedlings grown at 25ºC and transferred to both lower and higher temperatures showed a decrease in elongation rate. However, when the seedlings were grown at 11.5, 17.9, and 23.5ºC and subsequently transferred to temperatures in the range 4-38ºC, the growth curves were bell-shaped with maximum elongation rate (20-25ºC) corresponding to temperature optimum for this species. The results reported by Orbović and Poff [33] are well in line with our data, suggesting that the temperature optima for plant growth are characteristic for species. The results presented in this paper also showed that when coleoptile segments were excised from seedlings grown at 27ºC, the temperature maxima for IAA-induced elongation growth of coleoptile segments are in good agreement with maxima for IAA-induced medium acidification (Δ $[H^+]$) (Figs. 2D and 3), as has been shown in our previous work [9]. However, why coleoptile segments excised from seedlings grown at 22 and 32ºC acidified the incubation medium less than segments excised from seedlings grown at 27ºC is unclear and calls for additional experiments.

The results presented here show that the temperatures at which the maize seedlings were cultivated are crucial for temperature dependence of coleoptile segment elongation growth.

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