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HIGH TEMPERATURE ALTERS THE GROWTH REACTION OF *POTTIA* PROTONEMATA

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ABSTRACT

Under gravistimulation, dark-grown protonemata of *Pottia intermedia* revealed negative gravitropism with a growth rate of approximately 28 $\mu m \cdot h^{-1}$ at room temperature (20 °C). In 7 days, the protonema formed a bundle of vertically oriented filaments. At an elevated temperature (30 °C), bundles of vertically growing filaments were also formed. However, both filament growth rate and amplitude of the gravicurvature were reduced. Red light (RL) irradiation induced a positive phototropism of most apical protonemal cells at 20 °C. In a following period of darkness, approximately two-thirds of such cells began to grow upward again, recovering their negative gravitropism. RL irradiation at the elevated temperature caused a partial increase in the number of protonemal cells with negative phototropism, but the protonemata did not exhibit negative gravitropism after transfer to darkness. The negative gravitropic reaction was renewed only when protonemata were placed at 20 °C. A dramatic decrease in starch amount in protonemal apical cells, which are sensitive to both gravity and light, occurred at the higher temperature. Such a decrease may be one of the reasons for the inhibition of the protonemal gravireaction at the higher temperature. The observation has a bearing on the starch-statolith theory.

INTRODUCTION

Because of their immobile way of life, plants are not able to avoid environmental influences. Gravity is one constant ecological factor, unlike light which significantly changes in nature. Nevertheless, the reaction of plants to changes of these factors is always influenced by the prevailing temperature conditions. While gravi- and phototropisms pose questions of great interest in the scientific world, the influence of temperature upon these growth responses is usually ignored. As is well known, temperature greatly affects plant growth and development, inducing a number of alterations to cellular components, including the extent of fatty acid unsaturation (Murata and Los, 1997), changes in the protein composition (Raison, 1973; Waters *et al.*, 1996), activation of ion channels (Gong *et al.*, 1997), and changes in sucrose and starch accumulation (Lafta and Lorenzen, 1995). Despite extensive research on the mechanisms of gravitropism, the primary sensors of gravity signals, as well as mechanisms of signal transduction, are not yet well known. Therefore, investigation of the influence of temperature on gravi- and phototropisms of moss protonemata will contribute to the solution of these questions.

MATERIAL AND METHODS

Stock cultures of *Pottia intermedia* protonemata were aseptically grown at 20 °C in Petri dishes filled with agar medium. These cultures were maintained in darkness for about 2 weeks following planting and then transferred into light conditions (16 h light / 8 h dark). Such conditions promoted branching. Protonemata were replanted and placed in darkness again, and the growth cycle repeated in order to maintain the culture. For experiments, protonemata were placed on fresh medium, and dishes were put in darkness in a vertical position for 7 days. Then, one group of dishes was turned 90° (gravistimulated) for 24 h or 48 h, and then fixed and photographed at these times. The second group was laterally illuminated with red light (RL, λ = 660 nm, 0.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 48 h. Protonemata from one half of this group were fixed and photographed immediately after illumination; the other half remained in darkness for 5 to 7 days, then fixed and photographed. Measurements of cell length and curvature amplitudes were made from the prints. For starch identification, protonemata were stained with solution of IK_2I (Jensen, 1962). All experiments were conducted at two temperatures, room (20.0 ± 1.5 °C) and elevated (30.0 ± 1.0 °C), and repeated 4 times. To observe starch content dynamics, protonemata grown for 1 week in darkness at 20 °C were exposed to 30 °C for different time intervals. In order to check the vitality of protonemata grown at high temperatures, a few dishes were transferred back to room temperature conditions and examined after 7 more days.

RESULTS

In darkness at room temperature (control), *Pottia* protonemata grew vertically upwards with growth rate of approximately 28 $\mu m \cdot h^{-1}$ creating more or less dense bundles. After 7 days of treatment at the elevated temperature, growth of protonemal filaments was significantly slower (about 35–40% of control) and there were fewer of them per bundle. After gravistimulation, protonemata bent upwards at both room and high temperatures. However, the bending rates were different depending on temperature conditions: protonemata curved upwards more slowly at the higher temperature (Figure 1).

Light microscope analysis of protonemal apical cells revealed a sharp decrease of starch content at the higher temperature (Figure 2). In control cells stained with IK_2I, numerous large starch grains were visible in subapical and intermediate zones (i.e., in zones of plastid sedimentation (Chaban, 1996)). After transferring protonemata into high temperature conditions, a gradual decrease in starch grain size and number was observed. In direct correlation with the period at high temperature, the grains became less dense, lighter in colour and were positioned further from the cell apex. Although some apical cells decreased in diameter, they maintained their tubular shape. When protonemata were returned to room temperature for 7 days, the apical cells reverted to their usual phenotype and increased gravicurvature rates were observed.

Irradiation of dark-grown protonemata with RL induced positive phototropic growth of most of the apical cells at both temperatures. It is characteristic, however, that some differences in protonematal growth and differentiation depended upon temperature. At room temperature, most filaments grew towards the light source, but a few (7%) grew in the opposite direction or vertically. Although dark-grown *Pottia* protonemata did not branch, extensive branching of protonemata was observed under RL. At 30 °C, the number of filaments which did not show positive phototropism was significantly increased (Figure 3). Moreover, protonemal growth rate decreased, and they did not branch.

When RL-irradiated protonemata were again transferred into darkness, most filaments started to bend upwards and in 5–7 days they had regained their vertical, negative gravitropic growth direction (Figure 4, unshaded columns). The rest of the cells, which did not renew negative gravitropism, stopped their