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# Effect of Whole Plant and Local Heating on the ABA Content in Cucumber Seedling Leaves and Roots and on Their Heat Tolerance

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**Abstract**—The effects of heating at 38°C of whole cucumber (*Cucumis sativus* L.) seedlings or local heating of their shoots and roots on ABA content and heat tolerance of leaves and roots were investigated. During the initial period of the high-temperature treatment of whole seedlings, the ABA concentration in leaves and roots increased considerably. Local heating of the shoot or root resulted in an increase in the ABA concentration not only in the heated organ, but also in unheated seedling parts. A high-temperature treatment of the whole seedlings and the local treatment of shoots or roots caused an increase in the heat tolerance of leaf cells. The heat tolerance of root cells virtually did not change after heating of the whole seedlings or shoots, but decreased after heating of roots. The possible role of ABA in changing the heat tolerance of leaf and root cells by local heating of the seedling is discussed.

*Key words: Cucumis sativus - general and local heating - ABA - tolerance*

## INTRODUCTION

ABA belongs to the phytohormones that play an important role in protective and adaptive plant responses to unfavorable environmental conditions [1, 2]. ABA content in plants considerably increases under the effect of low and high temperatures [3–5], water deficit [3, 6], and high salinity [5, 7]. On the other hand, under normal conditions, exogenous ABA is capable of increasing the tolerance to a number of stress agents [7–9]. Moreover, in ABA-deficient mutants, cold acclimation can occur only in the presence of exogenous ABA [9].

When stress agents locally affect some plant parts or organs, they cause various changes in other plant organs. For instance, a high-temperature treatment of plant shoots or roots resulted in changes in the heat tolerance of cells not only in the heated organs, but also in those not directly subjected to heating [10]. On the basis of these data, it was suggested that a certain signal is formed in a heated plant organ; later, this signal is transmitted to unheated organs and exerts a complex of adaptive changes in these organs, which increase their heat tolerance [11]. However, particular metabolic and hormonal changes related to these effects are still unknown. Evidence on this matter concerning, in particular, cytokinins [12, 13], is fragmentary and calls for further investigation.

Taking into account the data mentioned above, the objective of this work was a comparison study of changes in the ABA content of cucumber leaves and roots, as well as in their heat tolerance, induced by a high-temperature treatment of the whole seedlings and local heating of its shoots or roots.

## MATERIALS AND METHODS

Cucumber (*Cucumis sativus* L., cv. Alma-Atinskii 1) seedlings were used in these experiments. The seedlings were grown for seven days in filter-paper rolls in a Knop nutrient solution supplemented with microelements, pH 6.2–6.4, at the air temperature of 25°C, a relative humidity of 60–70%, an illuminance of ca. 10 klx, and a photoperiod of 14 h. Subsequently, the whole seedlings and their shoots or roots were exposed to a temperature of 38°C in a specially designed device making it possible to maintain a predetermined temperature in the medium, in which a heated part of the seedling is located [11]. The duration of heating ranged from 5 min to 7 h. Unheated seedling portions were exposed to a temperature of 25°C.

Free ABA of leaves and roots was extracted and purified according to the method described earlier [14]. Plant material was fixed and ground in liquid nitrogen. ABA was first extracted with 80% ethanol at 4°C for 16 h and, after centrifugation of the extract and evaporation of ethanol, with diethyl ether, pH 2–3. ABA was methylated by diazomethane produced from nitrosomethylurea. The ABA concentration was determined by the ELISA technique [15].

The heat tolerance of leaf and root cells was assessed as $LT_{50}$, i.e., the temperature causing the death

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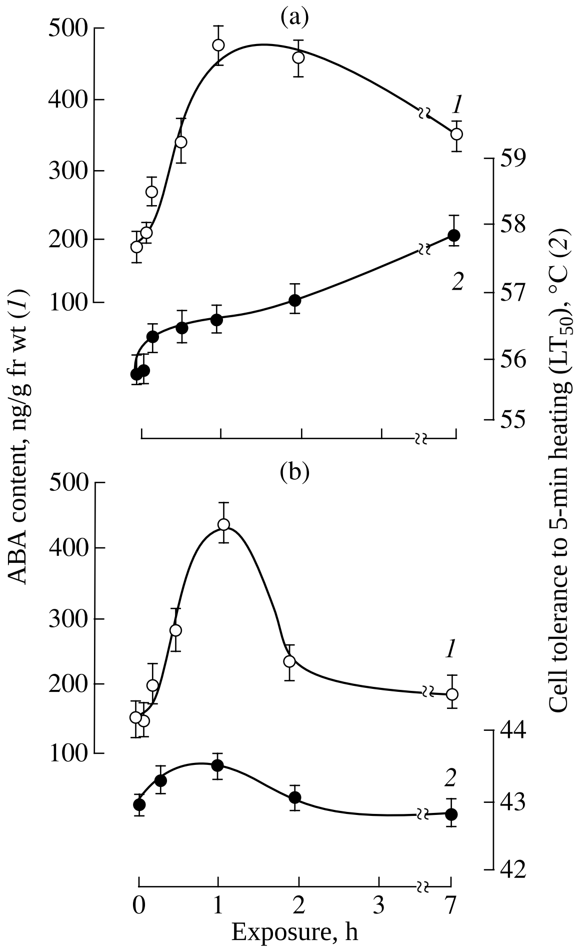


**Fig. 1.** Effect of heating of cucumber seedlings on the ABA content in (a) leaves and (b) roots and their heat tolerance.
Whole seedlings were heated at 38°C.

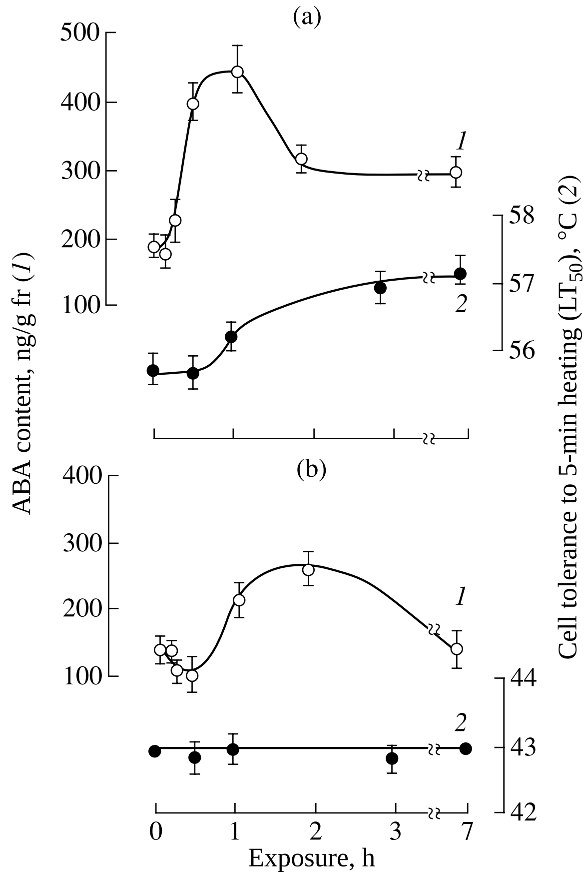


**Fig. 2.** Effect of local heating of cucumber shoots on the ABA content in (a) leaves and (b) roots and their heat tolerance.
Temperature of heating was 38°C.

of 50% of the palisade cells in ca. 0.3 $cm^2$ leaf segments or the epidermal cells of ca. 0.5-cm-long root segments. Plant material was heated for 5 min in a water thermostat (Institute of Biology, Karelian Research Center, Russia) using a temperature range with 0.4°C intervals [16]. The predetermined temperature was maintained with an accuracy of ±0.1°C. The coagulation of cytoplasm in leaf cells and the loss of the capability of root cell cytoplasm to plasmolysis in 1 M sucrose were used as the criteria of death of the respective cell types. For microscopic investigation of leaf and root segments, an MBI-15 light microscope (LOMO, Russia) equipped with an APO VI (40× or 70×) aqueous immersion objective was used.

Mean values from five independent experiments performed in six to eight biological replications and their standard errors are presented in the figures.

## RESULTS

The experiments showed that, within 15 min after the onset of heating of the whole cucumber seedling at 38°C, the free ABA content in cotyledonary leaves increased considerably (Fig. 1a, curve *1*). An increase in the ABA content continued for 1.5 h after the onset of heating. Subsequently, its content decreased somewhat, but remained rather high up to the end of the experiment. Within 15 min after the onset of heating at 38°C, the heat tolerance of leaf cells markedly increased, and this increase persisted for a further 7 h of the experiment (Fig. 1a, curve *2*).

Within 0.5–1 h after the onset of heating of the whole seedling, the ABA content in roots increased drastically. However, after 2 h, its content decreased to almost the initial value (Fig. 1b, curve *1*). In this case, the heat tolerance of roots changed to a far lesser extent than that of leaves; its small increase was recorded within 0.5–1 h after the onset of heating (Fig. 1b, curve *2*).

Local heating of cucumber seedling shoots resulted in an increase in the ABA content by ca. 2.5-fold within 0.5–1 h after its onset (Fig. 2a, curve *1*). During the subsequent hour, the ABA content decreased, but this value still considerably exceeded its initial content. Within 1 h after the local heating of the shoot, the heat tolerance of leaf cells markedly increased and within 7 h

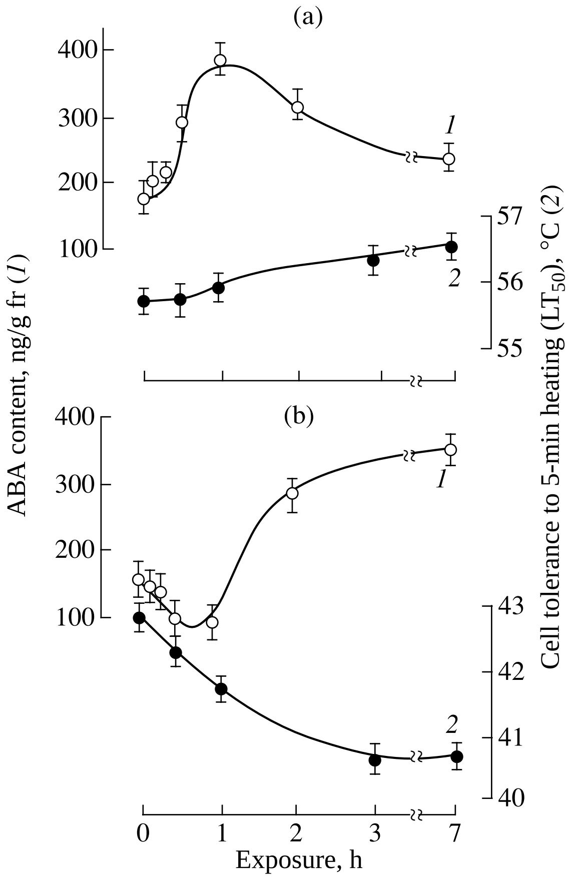


**Fig. 3.** Effect of local heating of cucumber roots on the ABA content in (a) leaves and (b) roots and their heat tolerance. Temperature of heating was 38°C.

after heating achieved its maximum (Fig. 2a, curve *2*). The ABA content in roots almost doubled 1–2 h after the onset of shoot heating, but decreased to the initial level after 7 h of heating (Fig. 2b, curve *1*). Shoot heating did not significantly affect root heat tolerance (Fig. 2b, curve *2*).

In the case of local heating of the cucumber roots at 38°C, the leaves and roots differed from each other in the pattern of the changes in their ABA content. In particular, within 0.5–1 h after the onset of heating, the leaf ABA content increased (Fig. 3a, curve *1*); however, this increase was lower than that induced by heating of a whole seedling (Fig. 1a) or shoot (Fig. 2a). The peak of ABA content was followed by its gradual decrease. In contrast, within the first hour of heating, the ABA content in roots decreased, and its increase was recorded only during the subsequent 2–7 h of heating (Fig. 3b, curve *1*). Within one to three hours after the onset of local heating of roots, the heat tolerance of leaves increased and virtually did not change afterwards (Fig. 3a, curve *2*), while the heat tolerance of roots gradually decreased as the heating duration increased (Fig. 3b, curve *2*).

## DISCUSSION

The results obtained made it possible to demonstrate differences in the effects of general and local heating on heat tolerance and ABA content in leaves and roots. Both general (Fig. 1a) and local (Fig. 2a) heating of leaves brought about an increase in the ABA content and the heat tolerance of these organs. Therefore, the ABA content in leaves is related to their heat tolerance. A high rate of ABA accumulation after the onset of heating demonstrates that this hormone is involved in the development of leaf heat tolerance. Later, however, the content of ABA in leaves decreased, but their heat tolerance nonetheless continued to increase. Thus, the increase in ABA content was transient, and the increase in heat tolerance was steady: this increase continued when ABA content decreased. Evidently, ABA manifested itself in leaves as a trigger, which turned on the increase in heat tolerance; subsequently, heat tolerance was developed independently of the hormone content.

In contrast to leaves, heating of roots increased their ABA content but did not affect the heat tolerance of roots (Fig. 1b) or decrease it (Fig. 3b). This implies that roots are far less capable of heat adaptation as compared to leaves and that ABA does not induce this process in roots.

The response of leaves to root heating was compared to the response of roots to leaf heating. It was shown that the local heating of roots (Fig. 3a) brought about the same changes in leaves as the heating of the leaves themselves. These changes consisted in a transient increase in ABA content and a gradual increase in heat tolerance. Therefore, locally heated roots sent a certain signal to the shoot, and this effect was accompanied by a decrease in the heat tolerance of the roots themselves.

Local heating of leaves did not increase the heat tolerance of roots, but, at the same time, did not decrease it (Fig. 2); it was accompanied by an increase in the ABA content in roots. However, in the case of a combined heating of roots and leaves, leaf heating prevented a decrease in the heat tolerance of roots and maintained it at an approximately constant level (Fig. 1b). Evidently, heated leaves sent a signal to the roots aimed at their adaptation. However, this adaptation was less than that in leaves and manifested itself only in the stabilization of heat tolerance rather than in its increase.

The present results are consistent with earlier evidence for the changes in ABA content in particular plant organs in response to the effect of high (or low) temperature on other plant organs. In particular, within five hours after burning the fourth leaf of tomato plants with an open flame, the ABA concentration increased not only in this leaf, but also in the second one not subjected to heating [17]. Besides, within 10 min after a short-term (4 s) chilling (4°C) of maize seedling shoots, the ABA content in the distal part of roots increased [18].

Our data and those published earlier demonstrate that the local effect of temperature brings about very rapid and considerable changes in the content of ABA in plants, which evidently have an operative mechanism for changing the content of this hormone. The ABA concentration in plants is known to be determined by a balance between its synthesis (and/or its import) and degradation (and/or its efflux) [19]. Because ABA is synthesized both in leaves and roots [19], its increased content in these organs found here as a result of general and local heating can be related to an increase in ABA formation. Moreover, the ABA synthesized in roots can be rather rapidly transported into leaves with the xylem transpiration stream [20, 21], and this process can be related to a local heating of roots. However, when the rate of ABA transport from the site of its biosynthesis is small, for instance, during its translocation from the shoots into roots, a rapid increase in the ABA content in roots is likely to be brought about due to hydrolysis of bound forms [22].

It was suggested that, under stress conditions caused, first of all, by water deficit, ABA acted as a long-distance signal coming from the roots to the leaf in the xylem vessels [20]. However, in our experiments, the time intervals between the onset of heating and an increase in heat tolerance were small, and this fact seems to exclude the possibility of information transfer from one organ to another by hormonal signals, particularly in the direction from the shoot to the roots. The function of a long-distance signal after the local temperature effect is more likely to be performed by an electrical [18, 23, 24] or hydraulic impulse [18] preceding a hormonal response.

In summary, it can be concluded that a rapid increase in the ABA content in cucumber leaves caused not only by their direct heating but also by root heating represents one of the factors involved in the development of leaf heat tolerance. Moreover, rapid changes in the ABA content observed in the seedling organs, which are spatially remote from the site of a local effect of unfavorable temperature, provide support for the important role of this hormone in the integration of protective and adaptive responses in the whole plant.

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