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**SHORT COMMUNICATION**

# Endogenous abscisic acid content in cucumber leaves under the influence of unfavourable temperatures and salinity

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## Abstract

**Endogenous abscisic acid (ABA) content was measured in leaves of *Cucumis sativus* L. under the influence of hardening (low and high) temperatures and salinity. The rise in cold and heat resistance of the seedlings was accompanied by a considerable increase in the ABA level in the leaves. Chloride salinity also brought about a rise in the ABA content. The data indicate that ABA may induce resistance when the plants are exposed to several stresses.**

Key words: *Cucumis sativus*, ABA, cold and heat hardening, salinity.

## Introduction

The response of plants to stress involves complex physico-biochemical changes including the balance between endogenous phytohormones, lowering the levels of some and raising others. For instance, ABA content increases under the influence of dehydration (Wright, 1978), salinity (Walker and Dumbroff, 1981), cold (Chen *et al.*, 1983) and heat (Daie and Campbell, 1981). In addition, exogenous ABA increases the resistance of plants to cold and heat (Rikin *et al.*, 1976; Titov *et al.*, 1985). These data support the possibility that this hormone actively participates in the adaptive responses of the plants to different stress factors.

On the basis of these findings we have carried out a comparative investigation of the effects of several stresses (low and high temperatures, and salinity) on the endogenous ABA content of cucumber leaves.

## Materials and methods

### *Plant material*

Seedlings of cucumber (*Cucumis sativus* L. cv. Alma-Atinsky 1) were grown for 7 d in rolls of filter paper on half-strength Knop nutrient solution (pH 6.2–6.4) at 25 °C, relative humidity 60–70%, illumination 10 klx, and photoperiod 14 h. Cold hardening was then carried out for 5 d at 10 °C, and heat hardening for 1 d at 38 °C. All other environmental conditions remained constant. Seedlings were exposed to salinity by placing them in 0.9 or 1.6% NaCl solutions for 24 h.

In some experiments seedlings were treated with actinomycin D (ACT, 1.6 mmol $dm^{-3}$) and cycloheximide (CH, 2.8 mmol $dm^{-3}$), inhibitors of RNA and protein synthesis, respectively. Treatment with inhibitors was carried out 1 d before cold and heat hardening via the roots.

Cold and heat resistance of the seedlings was estimated as the temperature at which 50% of the parenchymic cells died ($LT_{50}$) either after freezing leaf portions in a thermoelectrical microrefrigerator for 40 min (Drozdov *et al.*, 1976) or after heating in an aquatic thermostat for 5 min (Alexandrov, 1963). The viability of the cells after freezing and heating was determined on the basis of chloroplast destruction and cytoplasm coagulation. The values of resistance represent the mean of leaf portions from six plants. The response of seedlings to salinity was estimated by their survival.

### *Analysis of ABA*

The ABA content in the cotyledonary leaves was analysed by enzyme-linked immunosorbent assay (Weiler, 1982) using the modification of Kudoyarova *et al.* (1986). Leaves were frozen in liquid nitrogen, homogenized with 80% ethanol and extracted overnight at 4 °C. The ethanol extract was then centrifuged and evaporated to an aqueous fraction from which ABA was extracted using ethyl ether. ABA was methylated by ethereal diazomethane and evaporated to dryness. After evaporation it was taken up with 80% ethanol. The amount of ABA was determined by solid-phase immuno-assay as described by

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Abbreviations: ACT = actinomycin D; CH = cycloheximide.

Kudoyarova *et al.* (1986). Specificity of the antibodies used in this study was described by Ivanov *et al.* (1989). The ABA concentration in the samples was calculated by reference to standard curves. The values of one experiment represent the means of eight wells of the microtitration plates per extract.

All experiments were carried out at least three times. The data given in the figures are the means of all experiments.

## Results and discussion

Cold hardening of cucumber seedlings at 10 °C for 5 d caused a significant increase in their cold resistance. Moreover, this increase was recorded within the first 24 h. Henceforth, the resistance continued to increase, reaching a maximum after 3–5 d (Fig. 1A). During heat hardening of the seedlings an increase in heat resistance was recorded within 1–2 h at 38 °C. Thereafter it increased for 8 h and sustained this level (Fig. 1C).

The temperature resistance increase of seedlings during the process of cold and heat adaptation was accompanied by a significant increase in the ABA content of the cotyledonary leaves (Fig. 1B, D). Moreover, both at cold and heat hardening the initial increase in ABA content took place rapidly—during the first few hours of hardening. Five hour heat-hardening and 4 d cold-hardening both led to a decrease in the quantity of ABA in the cotyledonary leaves which gradually returned towards the initial level. Thus, the increase in both cold and heat resistance of seedlings was associated with a transient rise in ABA content.

The plants responded to chloride salinity as well as to low and high temperature hardening by accumulation of ABA in the cotyledonary leaves (Fig. 2). Moreover, at various levels of salinity (0.9% or 1.6% NaCl) the increase in ABA content was quite rapid and substantial. It should be mentioned that there was no change in the seedlings' survival during this period. Longer exposure of seedlings to saline conditions decreased their survival as well as the ABA level in the leaves. Thus, although different in nature, temperature (low and high) and salinity caused similar changes in the ABA level of cucumber seedlings.

As ABA accumulation under unfavourable conditions, e.g. water stress, can be blocked by protein synthesis inhibitors (Quarrie and Lister, 1984) we studied the influence of ACT and CH on ABA levels during heat hardening. Treatment of seedlings with the inhibitors prevented much of the increase in heat resistance (Fig. 3A). In addition, CH and ACT blocked ABA accumulation in the cotyledonary leaves during hardening (Fig. 3B). Thus, in the presence of CH, the ABA level 1 h after the beginning of hardening was about 30%, and after 6–24 h only 10% of the control (hardening without inhibitors). When treated with ACT, the ABA content was 50–60% of that of the control. It seemed that ACT and CH were preventing protein synthesis in the plants

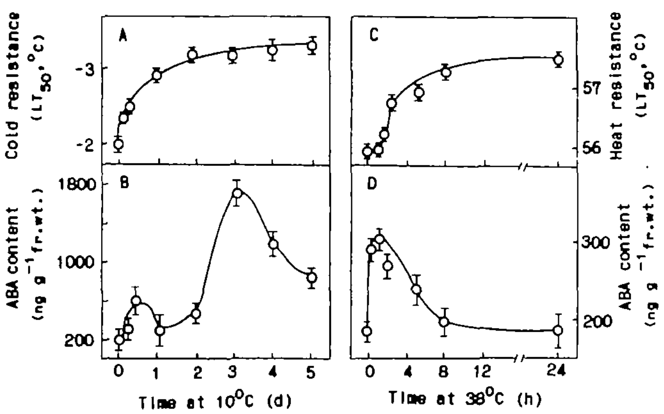

**Fig. 1.** Effect of cold (A, B) and heat (C, D) hardening on cold (A) and heat (C) resistance of cucumber seedlings and the ABA content (B, D) in their cotyledonary leaves. Bars are standard errors.

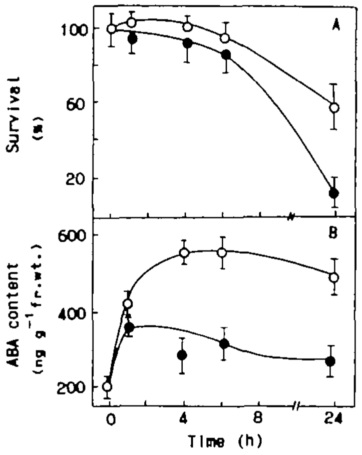

**Fig. 2.** Effect of NaCl (○, 0.9%; ●, 1.6%) on survival of cucumber seedlings (A) and ABA content (B) in their cotyledonary leaves. Bars are standard errors.

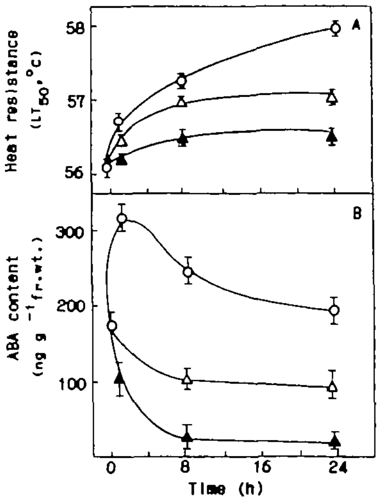

**Fig. 3.** Effect of ACT (△, 1.6 mmol dm$^{-3}$) and CH (▲, 2.8 mmol dm$^{-3}$) on cucumber seedling heat resistance (A) and leaf ABA content (B) during heat (38 °C) hardening. Control seedlings were hardened without inhibitors (○). Bars are standard errors.

and, in addition, blocking the ABA accumulation directly or indirectly.

As ABA accumulation occurred in the initial period of stress it could be inferred that an increased resistance to unfavourable temperatures and salinity was caused by an increase in the levels of this hormone. It is possible that the ABA accumulation could be one of the trigger mechanisms for the process of adaptation. The regulating role of ABA in the plant could take place both at the membrane level and at the level of the protein synthesis apparatus (Kefeli *et al.*, 1989). Nevertheless, the precise mechanisms of ABA functioning under various types of stresses remain to be determined. Moreover, the extent to which an increase in ABA levels under the influence of stress is connected with ABA release from a bound state (or cell compartments), or with the distribution of ABA between the plant's organs or with stimulation of ABA synthesis is not yet known.

We conclude from our study that the response of plants to several stresses and the formation of higher resistance are connected with alteration of the ABA level. It is possible that ABA can induce a common (unspecific) plant resistance.

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