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SHORT COMMUNICATIONS

Effect of Abscisic Acid on the Resistance of Cucumber Seedlings to Combined Exposure to High Temperature and Chloride

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Abstract—The effect of abscisic acid (ABA) on heat and salt resistance of cucumber seedlings exposed (consecutively or simultaneously) to high temperature and chloride was studied. Exogenous ABA proved to additionally increase the heat and salt resistance after both consecutive and simultaneous exposure of cucumber seedlings to 38∞N and NaCl. The involvement of this hormone in the common (nonspecific) mechanisms increasing plant resistance to the studied environmental factors is concluded.

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Numerous studies demonstrate that unfavorable environmental factors of different nature induce various structural and functional changes in plants that can increase or decrease the resistance to these factors depending on their dose (Chirkova, 2002). Under natural conditions, plants are often simultaneously exposed to several rather than a single environmental factor. Nevertheless, there are little data on the combined effect of two or more stress factors in numerous publications on plant resistance, although the pattern of plant response to independent and combined exposure to stress factors can differ quantitatively or even qualitatively (Rizhsky *et al*., 2002; Alexieva *et al*., 2003).

At the same time, it is common knowledge that some stress factors can increase plant resistance to other factors. This phenomenon called cross-adaptation has been established relatively long ago (Hale, 1969). For instance, heat shock can increase plant resistance to low temperature (Jennings and Salveit, 1994), salts (Kuznetsov *et al*., 1990), drought (Kuznetsov *et al*., 1999), and heavy metals (Talanova *et al*., 1996); while exposure to salts can consequently increase the resistance to low (Ryu *et al*., 1995) and high (Kuznetsov *et al*., 1990) temperatures. However, the mechanisms underlying cross-adaptation remain unclear (Sabehat *et al*., 1998).

It is commonly accepted that the hormonal system and, primarily, abscisic acid (ABA), so-called stress hormone, play an important role in the plant adaptive response to different stress factors (Kosakovskaya and Maidebura, 1989). This is confirmed, on the one hand, by a sharp increase in its endogenous level in plant tissues induced by unfavorable factors of different nature (Talanova *et al*., 1993; Cowan *et al*., 1997) and, on the other hand, by the capacity of exogenous ABA to increase plant resistance to different stress factors (Talanova *et al*., 1991; Gong *et al*., 1998). However, the role of ABA in the cross-adaptation of plants and the mechanisms underlying its protective effect in stress remain underexplored.

In this context, the goal of this work was to study the effect of ABA on the development of heat and salt resistance of cucumber seedlings after their combined (constitutive and simultaneous) exposure to high temperature and chloride.

MATERIALS AND METHODS

Experiments were carried out on seedlings of cucumber (*Cucumis sativus* L.) F1 hybrid Zozulya grown in filter paper rolls on Knop's solution with microelements (pH 6.2) in a climatic chamber at air temperature of 23–25°C, relative humidity of 60–70%, illumination of approximately 10 klx, and photoperiod of 14 h. One-week-old seedlings were separately or combinedly (consecutively or simultaneously) exposed to 38°C and 120 or 154 mM sodium chloride for 2–24 h. The temperature and NaCl concentrations were selected based on the previous experiments (Talanova *et al*., 1993). Prior to the exposure, a fraction of seedlings were placed in ABA solution (0.1 or 0.05 mM). Other experimental conditions were maintained constant.

The heat resistance of plants was evaluated from the temperature inducing death of 50% palisade cells in disks excised from the leaf (LT_{50}) after their heating in a water bath for 5 min (Aleksandrov, 1963). The temperature was maintained accurately within ±0.1°C. Cytoplasm coagulation was a marker of cell death. Salt resistance of cells was evaluated by the osmotic pressure of NaCl solution inducing death of 50% leaf palisade cells (LOP_{50}) (Balagurova *et al*., 2001). In this

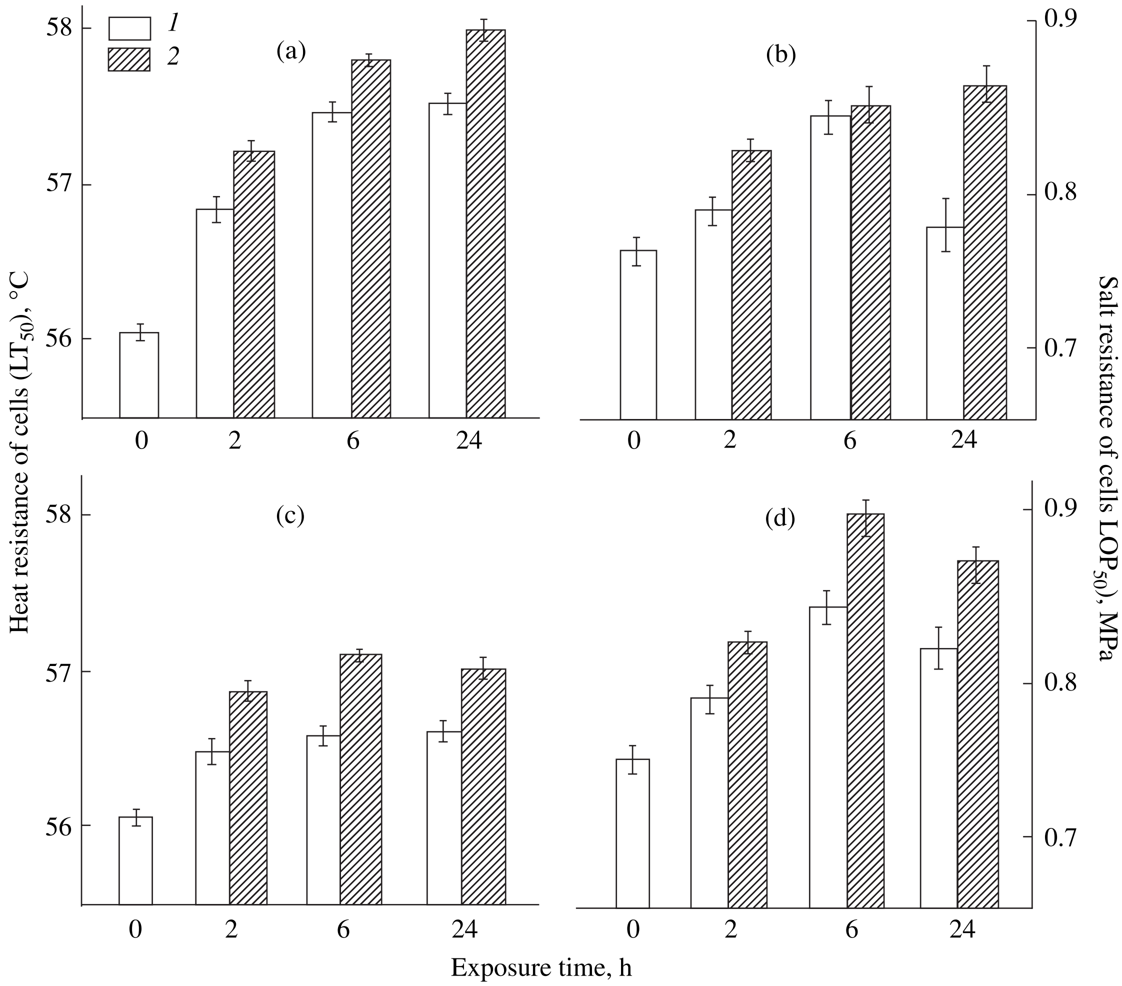

Fig. 1. Effect of ABA on heat and salt resistance of cucumber seedlings exposed to 38°C (a, b) or 154 mM NaCl (c, d); *1*, no ABA treatment; *2,* 0.1 mM ABA (for Figs. 1 and 2).

case, leaf cuttings were incubated in test salt solutions corresponding to the osmotic pressure from 0.5 to 1 MPa with a 0.05 MPa step. After 1 day, cell viability was evaluated using the same marker of cytoplasm coagulation.

The leaf cuttings were analyzed for heat and salt resistance under a light microscope MBI-15 (Russia) with a water immersion objective APO VI (40× or 70×).

Figures present arithmetic means and standard errors of the mean for three independent experiments each made in six replicates.

RESULTS

Exposure of cucumber seedlings to 38°C for 2 h increased the heat resistance of leaf cells, which further increased after a 6 h exposure and remained constant later (24 h) (Fig. 1a). At the same time, a short-term (2–6 h) exposure of plants to high temperature increased the salt resistance of leaf cells (Fig. 1b). Exogenous ABA further increased heat and salt resistance of seedlings not only in the initial period of exposure to 38°C but also during the whole experiment (Figs. 1a, 1b).

Salt stress conditions increased salt resistance of leaf cells in cucumber seedlings, which was accompanied by their increased heat resistance (Figs. 1c, 1d). Exogenous ABA notably increased salt and heat resistance of seedlings exposed to NaCl as compared to those not treated with the hormone (Figs. 1c, 1d).

Thus, heat and salt resistance of leaf cells increased in the initial period (first 2–6 h) of cucumber exposure to high temperature or chloride, while exogenous ABA further increased it.

Combined (consecutive) exposure of cucumber seedlings to high temperature and sodium chloride also gradually increased their salt resistance (Fig. 2a). Salt resistance of the seedlings heated to 38°C for 4 h became relatively high and continued to increase for some time (about 2 h) after their incubation in NaCl. Upon further exposure to sodium chloride, it gradually decreased to baseline. In this experiment, seedlings pretreated with ABA demonstrated a more considerable

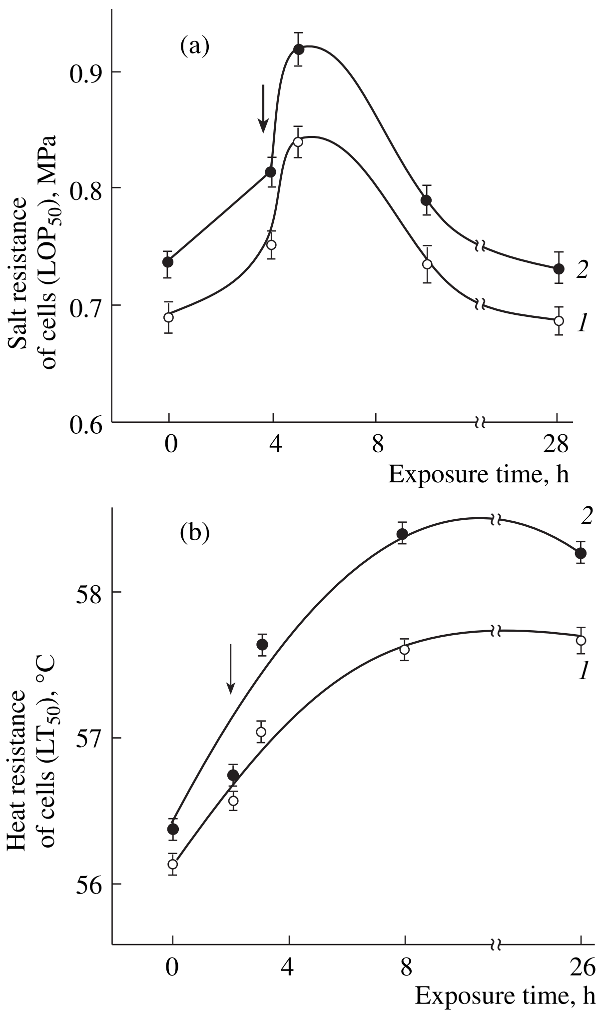

Fig. 2. Effect of ABA on heat and salt resistance of cucumber seedlings consecutively exposed to 38°C and 154 mM NaCl; (a) 38°C + NaCl; (b) NaCl + 38°C; arrow indicates the onset of exposure to NaCl (a) were 38°C (b).

increase in salt resistance as compared to untreated ones. Consecutive exposure of seedlings to high temperature and NaCl both with and without ABA temporarily increased their salt resistance. Nevertheless, comparison of data shown in Figs. 1d and 2a demonstrates that its maximum level in both cases was higher than in seedlings exposed to high temperature alone.

Similar data were obtained in experiments with consecutive exposure of seedlings to chloride and high temperature. Preliminary exposure of plants to NaCl for 2 h increased their heat resistance after the subsequent exposure to 38°C (Fig. 2b). ABA pretreatment further increased their heat resistance.

Simultaneous exposure of seedlings to two factors (38°C and NaCl) also notably increased their heat resistance (Fig. 3). After a 2 h exposure, heat resistance of reached the maximum and remained constant later. Exogenous ABA further increased heat resistance of

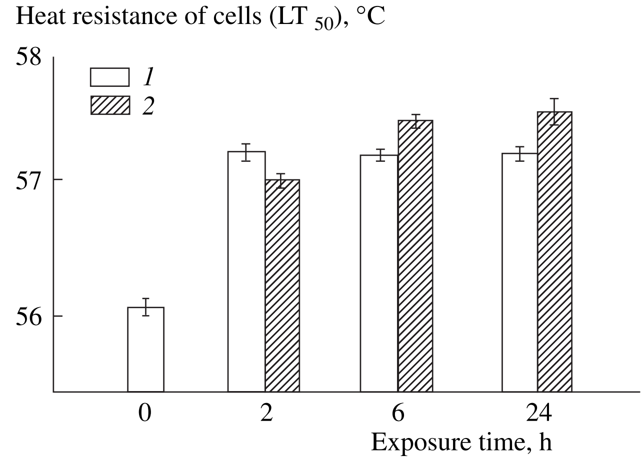

Fig. 3. Effect of 0.05 mM ABA on heat resistance of cucumber seedlings simultaneously exposed to 38°C and 120 mM NaCl; *1*, 38°C + NaCl; *2*, 38°C + NaCl + ABA.

seedlings simultaneously exposed to salt and high temperature stress for 6 and 24 h.

DISCUSSION

Our experiments on the response of cucumber seedlings separately and combinedly exposed to high temperature and salt demonstrated that each of these factors notably increased heat and salt resistance of leaf cells. However, the resistance dynamics in the case of separate and combined (particularly, simultaneous) exposure to high temperature and salt differed. In particular, heat resistance of seedlings combinedly exposed to salt stress and high temperature was much higher compared to sodium chloride alone (Figs. 1c, 2b). Note also that heat resistance after a 2 h exposure to two stress factors was higher compared to 38°C alone but became notably lower after a longer exposure (Figs. 1a, 3).

Exogenous ABA additionally increased heat and salt resistance after both separate and combined exposure to these unfavorable factors. Importantly, ABA treatment of plants exposed to either heat or salt stress further increased both heat and salt resistance. This clearly indicates the involvement of this hormone in the general (nonspecific) mechanisms underlying the resistance to stress factors of different nature.

Cross adaptation of plants demonstrated in this work and the involvement of ABA in this process can be attributed to different mechanisms of nonspecific resistance. Such mechanisms include the induction of antioxidant systems (Mittler, 2002), which can underlie ABA-induced increase in heat resistance of plants (Gong *et al*., 1998). Under conditions of high temperature and salt stress, ABA can activate antioxidant enzymes such as superoxide dismutase, glutathione transferase (Brilkina, 2002), and glutathione peroxidase (Gueta-Dahan *et al*., 1997).

Another possible mechanism to increase nonspecific resistance induces the expression of groups of genes and synthesis of stress proteins including heat shock proteins (Sabehad *et al*., 1998). The involvement of ABA in this mechanism is confirmed by the ABA-induced expression of the same genes in plants and accumulation of the corresponding mRNAs and proteins as after salt stress (Chen and Plant, 1999; Xiong *et al*., 1999). At the same time, plants seem to have both ABA-dependent and ABA-independent mechanisms to induce gene expression after exposure to high temperature and salt stress (Wei *et al*., 2000).

Finally, ABA can be involved in the control of the synthesis of proline (Hare *et al*., 1999) and osmotine (Grillo *et al*., 1995), which have protective and osmoregulatory effects on plants exposed to salt and high temperature stress (Kusnetsov and Shevyakova, 1997).

The above considerations suggest that the adaptation and cross-adaptation of cucumber seedlings to high temperature and chloride stress are mediated by ABA, which can trigger the development of increased resistance.

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