

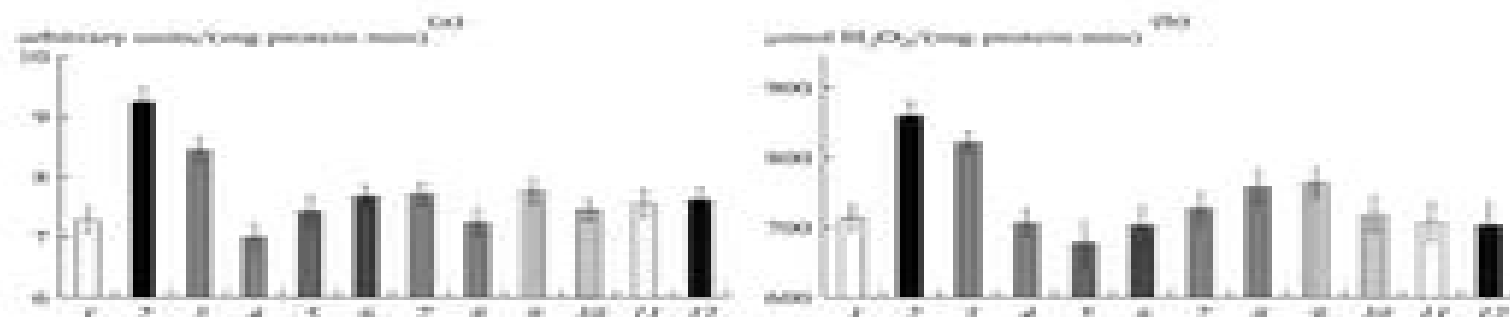


Fig. 3 The activity of SOD (a) and catalase (b) in wheat coleoptiles after treatment with BSs and/or other effectors.

1, control; 2, 24-epibrassinolide (10 nM); 3, 24-epibrassinolide (10 nM) + imidazole (1 μM); 4, imidazole (1 μM); 5, 24-epibrassinolide (10 nM) + EGTA (50 μM); 6, 24-epibrassinolide (10 nM) + imidazole (1 μM); 7, EGTA (50 μM); 8, 24-epibrassinolide (10 nM) + EGTA (50 μM); 9, 24-epibrassinolide (10 nM) + EGTA (50 μM) + nicotinicamide (1 nM); 10, nicotinicamide (1 nM); 11, 24-epibrassinolide (10 nM) + nicotinicamide (1 nM); 12, 24-epibrassinolide (10 nM) + nicotinicamide (1 nM). Incubation time of coleoptiles in the BS-solutions was 24 h; imidazole, EGTA, and nicotinicamide were immobilized into the incubation medium 1 h prior the immersion of BSs.

Activation of NADPH-oxidase and an increase in the production of ROS are apparently required for implementation of the stress-protective effects of BSs. Indeed, the increase in the activity of antioxidant enzymes induced by BSs was leveled by imidazole (the inhibitor of NADPH-oxidase) (Fig. 3). Imidazole also prevented the formation of an integrative reaction of coleoptiles to BS treatment, i.e. the increase in their thermal resistance (Tables). These effects of BSs were also reversed by EGTA and nicotinicamide, which prevent an increase in the concentration of calcium in cytosol (Fig. 3, Table). This effect of calcium antagonists may be due to both blocking of the activation of NADPH-oxidase and the formation of a ROS-mediated signal induced by BSs and the participation of calcium on other reactions, which do not deal with ROS generation. It is noteworthy that the BS-induced increase in the activities of SOD and catalase was obviously leveled by both EGTA and nicotinicamide, which inhibits cAMPK and prevents calcium outflow from intracellular compartments through the cADPR-dependent calcium channels (Fig. 7). It may be suggested that the BS-signal, which induces activation of these enzymes, depends on the calcium influx into the cytosol from both intercellular space and intracellular compartments. However, this suggestion needs experimental verification.

The obtained results bring us to the conclusion that ROS and calcium ions work as signaling messengers in the formation of BS-induced thermal resistance of wheat coleoptiles. Calcium ions may be involved in activation of NADPH-oxidase and transient activation of ROS production in wheat coleoptiles. Hence, BS-induced signaling, which is triggered by Ca^{2+} and

ROS, provides activation of stress-protective systems, particularly the antioxidant system, and an increase in the thermal resistance of plant cells.

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