

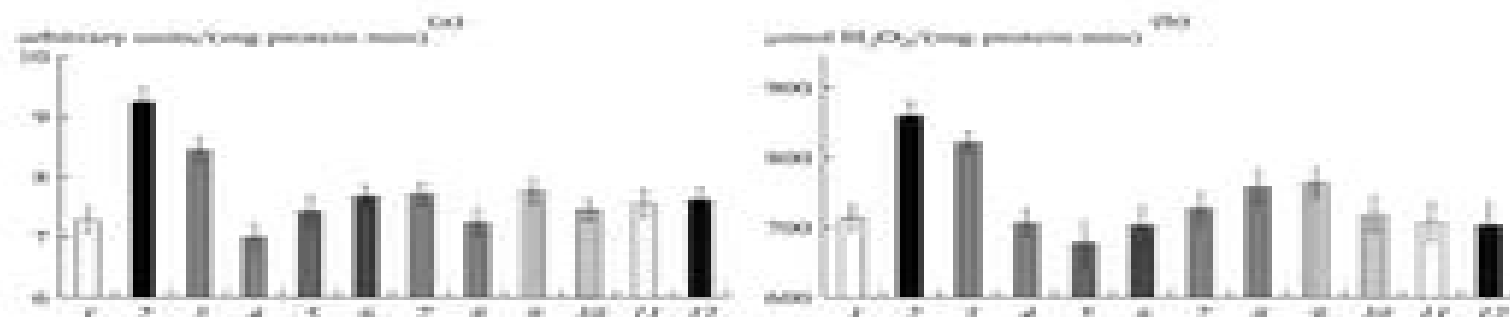


Fig. 3 The activity of SOD (a) and catalase (b) in wheat coleoptiles after treatment with BSs and/or other effectors. 1, control; 2, 24-epibrassinolide (10 nM); 3, 24-epibrassinolide (10 nM) + imidazole (1 μM); 4, imidazole (1 μM); 5, 24-epibrassinolide (10 nM) + EGTA (50 μM); 6, 24-epibrassinolide (10 nM) + imidazole (1 μM); 7, EGTA (50 μM); 8, 24-epibrassinolide (10 nM) + EGTA (50 μM); 9, 24-epibrassinolide (10 nM) + 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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Table of Contents Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8

1. Understanding the eBook Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
 - The Rise of Digital Reading Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
 - Advantages of eBooks Over Traditional Books
2. Identifying Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms

- Features to Look for in an Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
 - User-Friendly Interface
4. Exploring eBook Recommendations from Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
- Personalized Recommendations
 - Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8 User Reviews and Ratings
 - Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8 and Bestseller Lists
5. Accessing Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8 Free and Paid eBooks
- Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8 Public Domain eBooks
 - Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8 eBook Subscription Services
 - Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8 Budget-Friendly Options
6. Navigating Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8 eBook Formats
- ePub, PDF, MOBI, and More
 - Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8 Compatibility with Devices
 - Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8 Enhanced eBook Features
7. Enhancing Your Reading Experience
- Adjustable Fonts and Text Sizes of Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
 - Highlighting and Note-Taking Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8

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- Interactive Elements Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
8. Staying Engaged with Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
 9. Balancing eBooks and Physical Books Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
 10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
 11. Cultivating a Reading Routine Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
 - Setting Reading Goals Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
 - Carving Out Dedicated Reading Time
 12. Sourcing Reliable Information of Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
 - Fact-Checking eBook Content of Radiatsionnyi Monitoring Zhitelei I Ikh Produktov Pitaniia V Chernobylskoi Zone Belarusi Informatsionnyi Biulleten No 8
 - Distinguishing Credible Sources
 13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks

14. Embracing eBook Trends

- Integration of Multimedia Elements
- Interactive and Gamified eBooks

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