

## **Determination of ros generation rates in plant mitochondria in vitro using fluorescent indicators: Non-specific effects of inhibitors of terminal oxidases**

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### **Abstract**

© Y.R. Abdrakhimova, I.M. Andreev, A.G. Shugaev, 2015, published in *Fiziologiya Rastenii*, 2015. A possibility of quantitative analysis of the ROS production by mitochondria isolated from etiolated winter wheat (*Triticum aestivum* L., c v. Mironovskaya 808) seedlings was studied by following hydrogen peroxide accumulation in medium of organelle incubation using a new highly sensitive test-system including the fluorogenic indicator Amplex Red and horseradish peroxidase. The rates of the process were about 100 pmol H<sub>2</sub>O<sub>2</sub>/(mg protein min) under state 3 conditions; they were almost doubled after mitochondria transition into state 4, and further increased by more than 50% after the addition of alamethicin, the pore-forming antibiotic inducing the organelle inner membrane permeabilization for low-molecular-weight compounds, H<sub>2</sub>O<sub>2</sub> molecules among them. Experimental evidence is presented indicating that the classical inhibitors of the terminal oxidases of the plant mitochondrial respiratory chain, such as cyanide, salicylhydroxamic acid, and propyl gallate, can inactivate the ROS-detecting test-systems in vitro because of interactions with their functional components. These results are discussed in comparison with literature data obtained by similar test-systems and indicate that there are considerable limitations for obtaining reliable experimental evidence of the antioxidant role of alternative cyanide-resistant oxidase in plant mitochondria.

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### **Keywords**

Alamethicin, Amplex Red, Fluorescent indicators, Mitochondria, Reactive oxygen species, *Triticum aestivum*