Monitoring intra- and extracellular redox capacity of intact barley aleurone layers responding to phytohormones

Redox regulation is important for numerous processes in plant cells including abiotic stress, pathogen defence, tissue development, seed germination and programmed cell death. However, there are few methods allowing redox homeostasis to be addressed in whole plant cells, providing insight into the intact in vivo environment. An electrochemical redox assay that applies the menadione-ferricyanide double mediator is used to assess changes in the intracellular and extracellular redox environment in living aleurone layers of barley (Hordeum vulgare cv. Himalaya) grains, which respond to the phytohormones gibberellic acid and abscisic acid. Gibberellic acid is shown to elicit a mobilisation of electrons as detected by an increase in the reducing capacity of the aleurone layers. By taking advantage of the membrane-permeable menadione/menadiol redox pair to probe the membrane-impermeable ferricyanide/ferrocyanide redox pair, the mobilisation of electrons was dissected into an intracellular and an extracellular, plasma membrane-associated component. The intracellular and extracellular increases in reducing capacity were both suppressed when the aleurone layers were incubated with abscisic acid. By probing redox levels in intact plant tissue, the method provides a complementary approach to assays of reactive oxygen species and redox-related enzyme activities in tissue extracts.

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