# Redox activity monitoring in intact barley aleurone cells during gibberellic acid induced programmed cell death

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# Redox activity monitoring in intact barley aleurone cells during gibberellic acid induced programmed cell death

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Programmed cell death (PCD) has an important role in seed germination process and it is an essential component of plant defence mechanism against pathogens, hence it can influence the outcome of crop plant germination, yield, quality and interactions with pathogens [1, 2]. The cells in barley (*Hordeum vulgare*) aleurone layer play a key role in seed germination processes controlled by phytohormones such as gibberellic acid (GA) and abscisic acid (ABA). Even though evidence exists showing that reactive oxygen species play an important role in PCD and that there are significant changes in redox balance during GA induced PCD [5], the exact mechanisms of these processes are not fully elucidated in plant cells [3, 4].
To obtain a better understanding of redox activity related events during seed germination and PCD, it is important to be able to measure changes induced in intra and extracellular redox activity, preferably without destroying cell integrity.
Traditionally, redox activity in plant cells is evaluated using colorimetric assay by measuring enzyme activity from cell extracts after the cells of interest were isolated and lysed [5]. Electrochemical techniques have been successfully used to evaluate intracellular redox activity in living intact mammalian [6], yeast [7] and bacterial cells [8]. An amperometric detection system for probing intracellular redox activity in whole cells, based on the menadione (M)/ferricyanide double mediator system was developed and applied by Heiskanen et al. [9].
Given the need for redox activity probing in living plant tissues while keeping the cells intact as well as the capability of electrochemical redox activity assays, we propose the development of a non-destructive electrochemical assay for redox activity monitoring during GA-induced PCD in barley aleurone layers.
The method developed in this project allows intra- and extracellular, as well as membrane-associated redox activity measurements from intact plant tissue. A linear correlation was found between increased reducing capacity and number of live cells up to 48 h GA exposure. The increased reducing capacity of the cells during GA exposure is strongly related to their intracellular and/or membrane-related M reducing capacity. The M reducing capacity of the cells suggests the possible involvement of M-reducing flavoenzymes, such as quinone reductases, in GA related PCD.

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