

## INVESTIGATING REDOX POTENTIAL WITH REDOX-SENSITIVE GFP INDICATORS IN REPRODUCTIVE TISSUES OF *A. thaliana*

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Redox homeostasis is a fundamental property of living cells and actively responds to cellular metabolism and external stimuli. In all cells, small molecules capable of thiol-disulphide exchange such as glutathione (GSH) are abundant and are thought to act as buffers in redox equilibrium [1]. Given the importance and the general interest in cellular redox processes, a non-invasive method that detects redox changes within living cells is highly desirable. So far, there is no general approach to the non-destructive measure of the redox state, and although several methods have been proposed for determining redox status *in vivo*, all have serious limitations.

The development of a redox-sensitive Green Fluorescent Protein (roGFP) construct generated by introducing redox reactive groups in places of the protein where a change in the oxidation state of the introduced group will modify the fluorescent properties, has allowed the dynamic monitoring of changes in cellular redox balance. When excited simultaneously at 405 nm and 488 nm, these probes show significant opposite changes in the emission spectra (505-530 nm), which allows the measurement of the relative redox values [2]. Successful application of roGFPs in the leaf epidermis or in the root cells has been already reported. Here we provide a protocol describing the application of roGFP to imaging redox state in pollen tubes [3], anthers and pistils of *Arabidopsis thaliana* by confocal laser scanning microscopy. The study was performed on CAD2 mutants, that are deficient in the first enzyme of glutathione biosynthetic pathway ( $\gamma$ -glutamylcysteine synthase,  $\gamma$ -ECS). In this way, we can simultaneously shed light on the role of GSH in cellular redox homeostasis in reproductive tissues of higher plants.

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