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Title: Real-time monitoring of plastid-induced oxidation of the cytosolic glutathione pool.

Abstract: Metabolic changes in chloroplasts trigger retrograde signals to feedback information to nuclei. One proposed signal is reactive oxygen species (ROS), high concentrations of which may cause oxidation of glutathione (GSH) to glutathione disulfide (GSSG). An imbalance of the GSH/GSSG pool is considered an important marker for stress responses. While the use of Grx1-roGFP2 enables in vivo visualization of the GSH redox potential at subcellular level, microscope-based approaches are limited by sample throughput and long-term recordings. Here, we implemented a plate reader-based system that enables monitoring the GSH/GSSG dynamics in a high-throughput manner in intact samples exposed to stress over several hours. The use of compartment-targeted versions of Grx1-roGFP2 together with specific inhibitors of electron transport and the non-selective herbicide methyl viologen (MeV), revealed a more sensitive GSH pool in plastids compared to the cytosol upon MeV treatments. In addition, we show that the MeV-induced GSH oxidation in the cytosol depends exclusively on ROS released from chloroplasts.