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ARABIDOPSIS THALIANA LEAF DISULFIDE PROTEOME

Eleonora Egidi, Sira Echevarria-Zomeño, Ana M. Maldonado Alconada, Inmaculada Redondo, Jesús V. Jorrín Novo

Agricultural and Plant Biochemistry and Proteomics Research Group,
Dpt. of Biochemistry and Molecular Biology, University of Córdoba. Córdoba, Spain

Reactive oxygen species (ROS) are generated as a consequence of the cellular metabolism (e.g. photosynthesis), and their production rate increase in response to biotic or abiotic stresses. The overproduction of ROS shifts the redox status of the cell to oxidizing conditions, which causes thiol-disulfide transitions of Cys residues and other redox changes in proteins. Such modifications mediate stress sensing, triggers signalling cascades and activates the programmed cell death and other defence related processes. The methodology used to identify this transitions occurring *in vivo* is presented. The protocol has been modified from the previously reported by Lee et al. (2004; Electrophoresis 25:532-541), and include the following steps: i) blocking of free thiols by alkylation; ii) reduction of disulfide cysteines to sulfhydryl groups; iii) purification by thiol affinity chromatography; iv) separation by 1-D, SDS-PAGE; v) band cutting and trypsin digestion; vi) MS of the tryptic fragments and protein identification. This methodology will be used to analyze changes in the *Arabidopsis thaliana* leaf thiol-disulfide proteome in response to the infection with the bacterium *Pseudomonas syringae*.