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S-GLUTATHIONYLATION IN ARABIDOPSIS THALIANA

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The present work is included in a research project aimed at analyzing changes in the redox proteome and elucidating the role of protein nitrosylation and glutathionylation in Arabidopsis thaliana in response to Pseudomonas syringae. As a preliminary step to the glutathionylation studies, we are optimizing the methodology by using protein standards and leaf extracts. Leaf tissue was homogenized in PBS buffer instead of extracting the proteins by the usual precipitation methods (i.e. TCA-acetone-phenol) used with plant material. This was due to the difficulties in solubilising the pellet in a detergent-free solution. On one hand, a glutathion-biotinylation-agent was prepared as reported in Brennan et al. 2006 and Dixon et al. 2005 with some modifications. Protein samples were labelled with this agent and labelling was checked by Western blot using biotin and glutathione antibodies. On the other hand, experiments are being performed based on detecting in vivo and in vitro protein glutathionylation with glutathion antibody and identifying glutathionylated peptides through LC ESI ion trap MS analysis.