Identification of new active compounds and gene functions using non-

targeted metabolome analysis

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Small molecule glycosylation in plants is crucial for the biosynthesis of secondary metabolites and the regulation of several signaling molecules and defense compounds. Here, the highly stress responsive but previously uncharacterized glycosyltransferase UGT76B1 was selected for functional characterization. Non-targeted metabolomic analyses of knockout and overexpression lines using ultra-high resolution Fourier-transform ion cyclotron mass spectrometry (FT-ICR-MS) led to the *ab initio* identification of isoleucic acid as a UGT76B1 substrate. It was conjugated *in vitro* by the recombinant enzyme and it enhanced resistance to bacterial *Pseudomonas* infection when applied exogenously, which was in accordance with the observed phenotypes of knockout and overexpression lines. The results presented provide a potential application of ILA as a plant protective agent and highlight the great potential of using high resolution metabolomic analysis to identify new active compounds and reveal novel gene functions without any other prior knowledge.