ABSTRACT

LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY BASED METABOLITE PROFILING IN REVERSE GENETIC INVESTIGATIONS OF WOUNDING AND PATHOGEN STRESS RESPONSES IN *ARABIDOPSIS THALIANA*

By

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Investigations of gene functions have relied on two strategies known as forward genetics and reserve genetics. Both strategies are most powerful when phenotypes are described explicitly, and global phenotype profiling has emerged as a set of vital methodologies for functional genomics. This dissertation presents development and application of liquid chromatography/mass spectrometry (LC/MS) methods for metabolomic profiling for investigations of plant responses to wounding and pathogen stress. In the first study, liquid chromatography/time-of-flight mass spectrometry was used for nontargeted profiling of metabolites in leaves of wild type and *O*-methyltransferase (OMT) knockout mutants. *O*-methyltransferase (OMT) methylates the hydroxyl groups on phenols and produces methylated products. One of these mutants, *omt1*, exhibited metabolic phenotypes distinct from wild type plants, with notable accumulation of 5-hydroxyferuloyl malate in the mutant. After inoculation with pathogen *Pseudomonas syringae* DC3000, wild type plants exhibited five-fold greater bacterial counts relative to the *omt1* mutant. Profiling of metabolites in control and pathogen-infected *omt1* mutant and wild type plants pointed to several aspects of stress biochemistry associated with this specific mutation. Most notable was evidence of conversion of 5-hydroxyferuloyl malate to a reactive quinone metabolite in the pathogen-

infected *omt1* mutant. The quinone metabolite was converted to glutathione (GSH) conjugates *in vivo* in mutant plants. Further support for antimicrobial properties of the quinone metabolites came from *in vitro* screening of synthetic substances against *P. syringae*. Inhibition of bacterial growth was observed at low concentrations for 5-hydroxyferulic acid, its quinone, and the glutathione conjugate of the quinone, but not for the methylated analog sinapic acid. Though glutathione conjugation of quinones has been considered a detoxification step, the persistence of antagonism to *P. syringae* growth with the quinone-glutathione conjugate suggests this class of metabolites may undergo redox cycling, catalyzing formation of superoxide and hydrogen peroxide, as has been observed in studies of quinone toxicity in animals. Though some polyphenols have been documented in earlier studies to exhibit antimicrobial properties, roles for specific metabolites or metabolic genes have remained elusive. Furthermore, the *omt1* mutant displayed a more pronounced initial burst in levels of jasmonic acid, suggestive of crosstalk between polyphenol metabolism and jasmonate signaling.

The second part of this dissertation research has focused on development of ultraperformance LC (UPLC)-MS/MS methodology for rapid screening of multiple phytohormones in a single analysis. A fast, sensitive, and selective UPLC MS/MS method was developed for quantifying an assortment of jasmonic acid metabolites including bioactive amino acid conjugates, and an assortment of related phytohormones including salicylates, auxin, abscisic acid, and other oxylipins. These experimental approaches have been applied to study interactions among different metabolic pathways involved in gene regulation and signal transduction in wounding and pathogen stress.