'Elucidating the role and mechanism of the regulatory network of genes encoding dioxygenases potentially involved in plant adaptation to terrestrial environments'

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The colonization of land by plants represents one of the most groundbreaking evolutionary events in Earth's history, requiring profound transformations in their biochemical and regulatory pathways. In response to novel environmental pressures such as water deficiency and increased ultraviolet radiation, plants developed a wide spectrum of adaptive changes, including modifications in the biosynthesis and regulation of secondary metabolites.

This doctoral dissertation investigates the interactions between various *Arabidopsis thaliana* (thale cress) genotypes, selected abiotic and biotic environmental stresses, and the plant's secondary metabolism. Particular attention is given to coumarins – secondary metabolites derived from the phenylpropanoid pathway – which play an essential role in plant adaptation to unfavorable conditions. Coumarins are known for their ability to chelate iron (Fe), as well as their antioxidant and antimicrobial properties. By combining metabolomics, transcriptomics, phylogenetics, and reverse genetics approaches, this research provides new insights into plant responses to environmental stresses in the context of coumarin biosynthesis and phenylpropanoid pathway regulation.

The research is presented as a series of five thematically related publications. Three of these have been published in peer-reviewed scientific journals, while the remaining two are included as manuscripts in preparation. The first three publications (No. 1, 2, and 3) address the first research goal: to explore the correlation between coumarin accumulation, genotypic variation (natural accessions and mutants), and environmental factors, especially Fe availability and *Dickeya* spp. infection. The aim was to better understand how *A. thaliana* modulates coumarin metabolism in response to diverse environmental conditions, considering both genetic background and the nature of the applied stress.

The first study examined natural variation in the accumulation of simple coumarins (scopoletin, umbelliferone, esculetin) and their glycosylated forms across 28 *A. thaliana* accessions. UHPLC-MS metabolic profiling revealed significant intraspecific differences in coumarin profiles influenced by tissue type, growth conditions, and genotype (Perkowska *et al.,* 2021a). The second publication demonstrated that coumarin accumulation is modulated by Fe availability and correlates with resistance to *Dickeya* spp. – necrotrophic bacterial pathogens of agronomic importance, selected due to their virulence strategies and Fe-scavenging mechanisms. These findings suggest that coumarins may act as integral components in both nutrient acquisition and biotic stress defense (Perkowska *et al.*, 2021b). The third publication explored the relationship between gene expression dynamics and coumarin levels under specific environmental conditions. Based on previous coumarin content profiling, three genetically distinct accessions (Col-0, Est-1, and Tsu-1) were selected. To simulate contrasting conditions, plants were grown in liquid *in vitro* culture and in soil. Roots and shoots were harvested for parallel metabolite profiling (UHPLC-MS) and quantitative gene expression analysis (qRT-PCR) targeting the phenylpropanoid pathway. Selected genes were re-sequenced to identify polymorphisms potentially contributing to variations in protein biosynthesis and ultimately to metabolic diversity (Ihnatowicz *et al.,* 2024).

The first three publications presented in this thesis highlight how genetic diversity and environmental conditions shape coumarin biosynthesis in *A. thaliana*, revealing links between metabolite levels, phenylpropanoid gene expression, and adaptive potential. The fourth manuscript focuses on the functional characterization of *DOXC21-A*, its natural antisense transcript (NAT), and the paralog *DOXC21-B*. Both genes encode 2OGD enzymes of previously uncharacterized biological function. Phylogenetic analysis confirmed that these genes are conserved across multiple land plant lineages, suggesting an evolutionarily maintained function and highlighting their potential biological significance. Wild type and mutant plants were grown hydroponically under low pH (4.5) and osmotic stress (3% PEG), and transcriptomic, metabolomic, and phenotypic analyses were conducted. The results support a model in which DOXC21-A and its NAT form a regulatory unit that may redirect phenylpropanoid flux from lignin biosynthesis toward flavonoid production in response to stress, potentially involving phytohormonal pathways. A conserved miRNA-homologous sequence found within NAT suggests possible post-transcriptional regulation. The distinct phenotypes observed in *doxc21-a* and *nat* mutants suggest that, while both are involved in abiotic stress responses, they may operate through partially overlapping yet functionally non-redundant molecular pathways.

The study described in the fifth manuscript addresses the functional characterization of UGT79B9, a UDP-glucosyltransferase potentially involved in stress-induced glycosylation. Experiments conducted on *ugt79b9* knockout, overexpression and complementation lines revealed that UGT79B9 affects root exudates profiles and glycosylation of stress-responsive metabolites under Fe deficiency, osmotic stress, and drought. Untargeted metabolomic profiling uncovered several yet-unidentified m/z features associated with UGT79B9 mutation and PEG-induced osmotic stress.

In conclusion, the five articles comprising the presented doctoral thesis broaden the understanding of how *A. thaliana* integrates environmental signals with the regulation of specialized metabolism, with particular emphasis on coumarin biosynthesis. The findings uncover a complex network of interactions involving 2OGDs, UGTs, and NATs that collectively participate in the adaptive reprogramming of metabolic pathways. This research highlights the significance of natural genetic variation and regulatory plasticity in enhancing plant resilience to abiotic and biotic stressors.

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