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Nitrogen-Regulated Theanine and Flavonoid Biosynthesis in Tea Plant Roots: Protein-Level Regulation Revealed by Multiomics Analyses

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Title

Nitrogen-Regulated Theanine and Flavonoid Biosynthesis in Tea Plant Roots: Protein-Level Regulation Revealed by Multiomics Analyses

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Abstract

Theanine and flavonoids (especially proanthocyanidins) are the most important and abundant secondary metabolites synthesized in the roots of tea plants. Nitrogen promotes theanine and represses flavonoid biosynthesis in tea plant roots, but the underlying mechanism is still elusive. Here, we analyzed theanine and flavonoid metabolism in tea plant roots under nitrogen deficiency and explored the regulatory mechanism using proteome and ubiquitylome profiling together with transcriptome data. Differentially expressed proteins responsive to nitrogen deficiency were identified and found to be enriched in flavonoid, nitrogen, and amino acid metabolism pathways. The proteins responding to nitrogen deficiency at the transcriptional level, translational level, and both transcriptional and translational levels were classified. Nitrogen-deficiency-responsive and ubiquitinated proteins were further identified. Our results showed that most genes encoding enzymes in the theanine synthesis pathway, such as CsAlaDC, CsGDH, and CsGOGATs, were repressed by nitrogen deficiency at transcriptional and/or protein level(s). While a large number of enzymes in flavonoid metabolism were upregulated at the transcriptional and/or translational level(s). Importantly, the ubiquitylomic analysis identified important proteins, especially the hub enzymes in theanine and flavonoid biosynthesis, such as CsAlaDC, CsTSI, CsGS, CsPAL, and CsCHS, modified by ubiquitination. This study provided novel insights into the regulation of theanine and flavonoid biosynthesis and will contribute to future studies on the post-translational regulation of secondary metabolism in tea plants.

