

A WEIGHTED GENE CO-EXPRESSION NETWORK ANALYSIS (WGCNA) APPROACH TO UNCOVER N-FORMS INTERACTIONS IN ARABIDOPSIS

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In soil, three main N forms are present, as native or applied sources (ammonium, nitrate and urea) and, at least for a short time, plants are exposed to a combination of all of them. To date limited information are available concerning the molecular events in response to their interactions useful to improve the comprehension and optimization of N nutrition in plants. In this respect, Arabidopsis seedlings were exposed to low N (1 mM) by using different inorganic N forms such as nitrate (N), ammonium (A) or ammonium nitrate (AN) alone or in combination with Urea (N+U, A+U and AN+U) for 7 days. Afterwards, phenotypic (root morphology, chlorophyll, and carotenoids content as well as nitrogen use efficiency (NUE) and transcriptomic (RNA-seq) analysis on whole seedling were performed. The A+U combination reduced shoot and root biomass, primary and lateral root length, lateral root number as well as NUE. By contrast, plants exposed to N+U and AN+U combinations exhibited an increase of the above parameters. Transcriptomic analysis revealed 477, 218 and 206 differentially expressed genes (DEGs) in N+U vs N, A+U vs A and AN+U vs AN, respectively. In addition, two transcription factors belonging to NAC and WRKY families, involved in root development and architecture, were specifically expressed with U co-supply only. The Weighted Gene Co-expression Network Analysis (WGCNA) revealed four functional modules (turquoise, brown, yellow and black) correlated to some phenotypic traits. In particular, the turquoise module, including 182 genes, enriched in response to chemicals GO terms, was positively to carotenoids content, whereas brown module, including 84 genes, almost enriched in photosynthesis GO terms, displayed positive correlation with shoot biomass and NUE. By contrast, negative correlations were observed in the yellow module (81 genes, GO: oxidation-reduction and organonitrogen compound biosynthetic processes) with root architecture traits as well as in the black module (77 genes, GO: response to abiotic stimulus) with shoot biomass. In addition, the module eigengene-based intramodular connectivity measure was considered to select hub genes within the modules correlated to NUE and related traits such as the root architecture. The transcription factor jumonji (JMJ), involved in hormonal control via epigenetic regulation of gene expression, represented the hub in the brown module, whereas LHCB4.1 (light harvesting complex photosystem II) as well as AAT1 (cationic amino acid transporter) were identified as hub genes in the yellow module. In conclusion, our results provide novel insights into the specific phenotypic responses in Arabidopsis exposed to inorganic N-forms in combination with urea. Transcriptomics and WGCNA allowed us to identify hub genes as references for functional analysis on the molecular mechanisms controlling N nutrition in plants.