Opportunistic Recognition & Interaction Between Plant NCR Peptides & Rhizobia SPSs Affect Plant Immunity & Symbiotic Specificity

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Alfalfa expresses significantly distinct sets of genes in response to infection by different rhizobia strains at the below-species level (i.e., biotype or strain). However, differences in the transcriptomic profiles of two alfalfa cultivars nodulated by a single rhizobium strain have been largely unexamined. In this study, the comparative RNA-seq analysis of two alfalfa cultivars, *Medicago sativa* cv. Gannong No. 3 and cv. Gannong No. 9 inoculated with one *Ensifer meliloti* strain LL2, with varying in symbiotic performance, was conducted, followed by a hub gene interaction network construction based on weighted gene co-expression network analysis (WGCNA). The G9-LL2 symbiotic system showed better nodule-formation, nitrogen-fixing, and growth characteristics than the G3-LL2 system. Compared with the uninoculated control (CK), the LL2-inoculated G9 plants (10053) produced more differentially expressed genes (DEGs) than the LL2-inoculated G3 plants (7112). A group of 227 genes displayed completely distinguished expression in G9 ($6.63 < \log2(FC) < 15.45$) and G3 ($-3.05 < \log_2(FC) < 12.05$), which are primarily involved in encoding nodule-specific cysteine-rich peptides (NCRs), nodulin, and leghemoglobin. Although genes with predicted roles in nitrogen metabolism were primarily upregulated, and almost all of those in ubiquitin-mediated proteolysis and plant-pathogen interaction were suppressed, interestingly, a consistently higher expression level measured by $\log_2(FC)$ was observed in G9 plants. Hub gene interaction networks showed that the NCRs, late nodulin, and genes related to plant immunity (TIR-NBS-LRR, defensin, thioredoxin, thionine, and polygalacturonase) regulated other genes at the source node positions. After the successful initiation of nodulation in both alfalfa cultivars G3 and G9 by *E. meliloti* strain LL2, G9 achieved preferable outcomes of rhizobia-alfalfa symbiosis by equilibrating the antagonism and compatibility of plant immunity. It elevated PTI and suppressed defense and ETI, as well as enhancing nitrogen fixation and utilization efficiency by inducing the expression of genes encoding NIN, NFH1, LysM-RLK, LRP, NCRs, nodulin, and leghemoglobin. Hub genes predominantly underlying the highly specific rhizobia-alfalfa symbiosis, positively governed by NCRs and fine-tuned immune antagonism, comprise NCRs, late nodulin, and TIR-NBS-LRR. These findings provide insights into the genetic mechanisms underlying the modification and efficient utilization of semi-compatible and incompatible rhizobia resources.

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