

Functional Analysis of Nodulation-Related Genes in soybean

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Objectives

MDH, a sole carbon source for nodule, was shown to be dramatically up-regulated in supernodulating mutant, SS2-2, compared with wild type soybean. To elucidate the function of MDH in supernodulation, loss of function mutants were generated using methods of RNAi and *Agrobacterium rhizogenes*-mediated hairy root transformation.

Materials and Methods

◆ Materials

- Plant : Sinpaldal2, SS2-2 (supernodulating mutant)
- Bacteria : *Agrobacterium* strain - *Agrobacterium rhizogenes* K599
: Rhizobium strain - *Bradyrhizobium japonicum* USDA110

◆ Methods

- Vector construction : pENTR/D-TOPO (Cloning vector), pB7GWIWG2(I) (RNAi vector)
- *Agrobacterium*-mediated transformation : Freeze-Thaw method
- Induction of Hairy roots : Pricking hypocotyl below cotyledon with a needle with *A. rhizogenes* K599

Results and Discussions

Soybean malate dehydrogenase (MDH) is the nodule protein expressed exclusively in supernodulating mutant and grafting indicated the exclusive expression of the MDH is determined by root not by shoot as expected from supernodulating. The functional analysis of the genes was performed by MDH RNAi (MDHi) construction following transformation of soybean hairy root with *Agrobacterium rhizogenes* K599.

The root and nodule transformed with *A. rhizogenes* without any of RNAi genes showed the same genetic traits as those of the parent in either wild type or supernodulation.

Moreover, the length of the transgenic hairy roots of MDHi SS2-2 transformed by *A. rhizogenes* (ArK-MDHi) were longer than those of the empty vector controls and the nodule numbers were increased with a depression of MDH gene expression in SS2-2.

Subsequently, proteome analysis for nodule in hairy root with MDHi is performed to see any alteration of in expression of MDH at the level of protein.

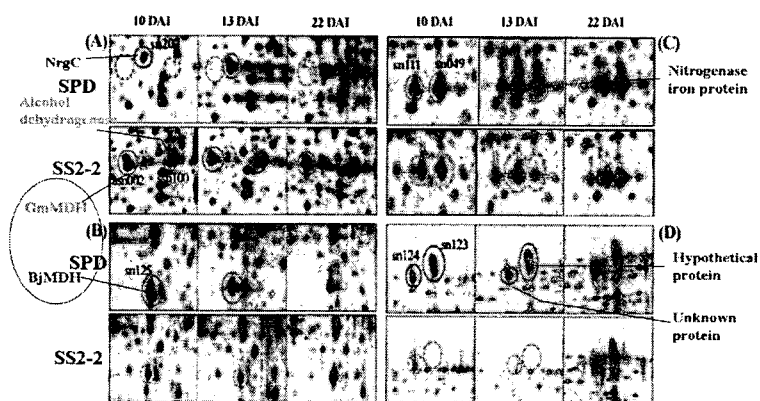


Figure 1. Proteins regulated differentially in between SPD (wild type, top) and SS2-2 (supernodulating mutant, bottom) during nodule development. Red circle indicates up-regulated protein and blue circle indicates down-regulated. MDH, Malate dehydrogenase.

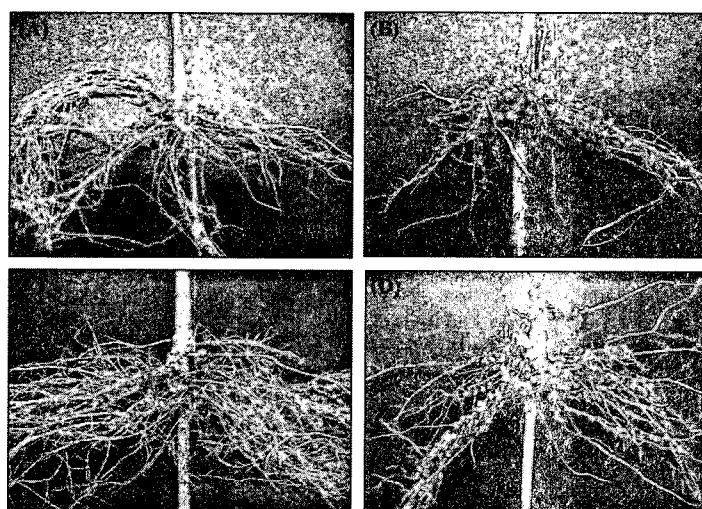


Figure 2. Nodulation in the Hairy roots of SPD and SS2-2 with an inoculation of *B. japonicum* USDA110 at 22DAI after hairy root induction by *A. rhizogenes* K599 with a MDH RNAi (MDHi). (A), SPD with *A. rhizogenes* K599; (B), SS2-2 with *A. rhizogenes* K599; (C), SPD with *A. rhizogenes* K599-MDHi; (D), SS2-2 with *A. rhizogenes* K599-MDHi.

Table 1. Comparison of nodule numbers in the MDH-RNAi gene transferred hairy root in SPD and SS2-2. Nodule and Root numbers are averages of 17 over plants.

	SPD		SS2-2	
	Hairy root Length (cm)	Nodule number per Hairy root	Hairy root Length (cm)	Nodule number per Hairy root
ArK599	10.7 ± 8.9	3.4 ± 2.8	5.6 ± 2.6	8.1 ± 4.3
ArK-MDHi	9.3 ± 3.6	5.4 ± 3.8	8.3 ± 3.9	19.3 ± 9.8