

Accessory Publication

Supplementary methods

For the data reported in Table 2, *Datisca glomerata* plants were grown from seed in a greenhouse and inoculated as described in Berry *et al.* (2004). Hoagland's solution at half-strength was provided as a single soil drench just before inoculation, and then again at 4 and 7 weeks. Otherwise plants were watered with deionized water. Root nodules of *D. glomerata* were collected at 4 and 5 weeks after inoculation flash-frozen in liquid nitrogen, and stored at -80°C . For RNA purification, nodules from four plants per collection date were randomly selected; then equal nodule weights (1:1) from each collection date were combined. Two grams of the combined nodules with 3% PVPP were ground in liquid nitrogen with a mortar and pestle to a very fine powder. RNA was extracted from the ground nodule material using the Qiagen RNeasy Mini extraction kit (Qiagen, Hilden, Germany), according to manufacturer's instructions. Two additional DNase treatments were performed in solution, using successively DNaseI (Qiagen) and DNaseI (Roche, Mannheim, Germany). Absence of residual DNA was verified by PCR (40 cycles), using *nifH* primers. The quality of the RNA was confirmed with a Bioanalyzer (Agilent, Santa Clara, CA, USA). Primers were designed for each gene to be tested using PrimerThree software. For each RT-PCR reaction, 50 ng total RNA was amplified using the Qiagen OneStep RT-PCR kit, with the program: 50°C (30 min); 95°C (15 min); 35 cycles each of: 94°C for 30s; annealing for 30s at 55°C , or 57°C , or 60°C , depending on optimal T_m , extension; 72°C for 30s; then final extension 72°C (10min); store at 4°C . RT-PCR products were run on 1.5% agarose gel with EtBr ($1.5\ \mu\text{g}/50\ \text{ml}$), 60V, 2 h; imaged with the Red Imager (Alpha-Innotech, Cell Biosciences Inc, Santa Clara, CA, USA) and quantified using AlphaView. Replicate values were derived from the same ground nodule batch, but separate RNA purifications and PCR amplification runs.