Both plant and bacterial nitrate reductase contribute to nitric oxide production in Medicago truncatula nitrogen-fixing nodules

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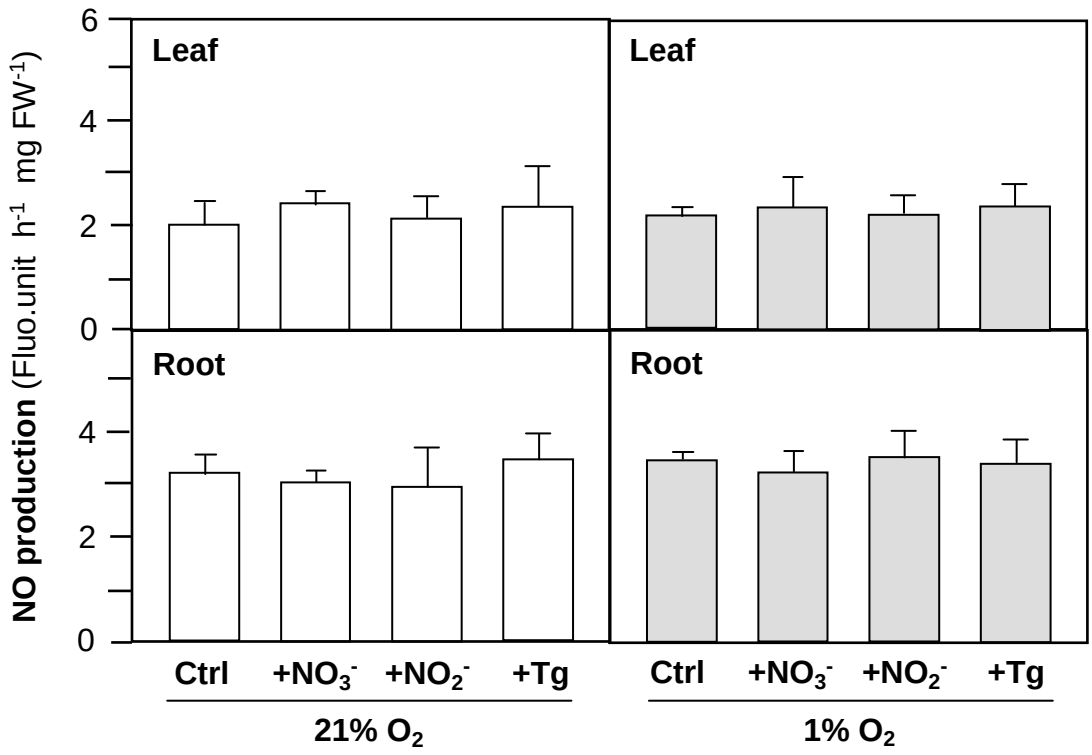


Figure S1: Effects of nitrate reductase effectors on *M. truncatula* leaf and root NO production. NO production, expressed as relative fluorescent units, was measured under either 21% or 1% O2. Effector concentrations were 10 mM NaNO3 (NO3-), 1 mM NaNO2 (NO2-), and 1 mM sodium tungstate (Tg). Data are the means ± SD of 2 independent experiments assayed in duplicates.

Figure S1

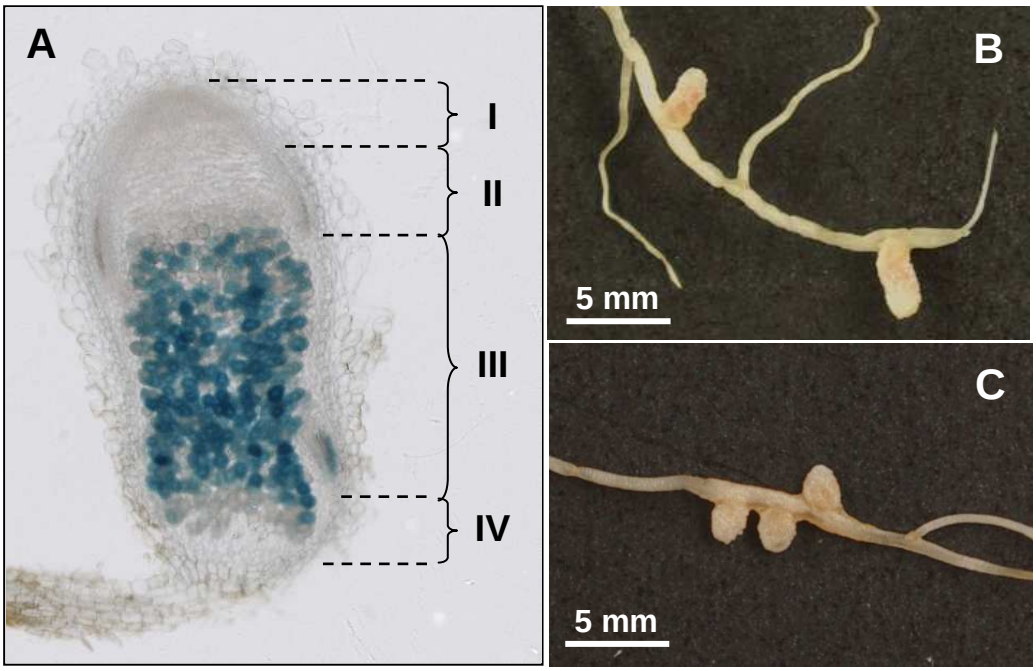


Figure S2: Histochemical analysis of *MtNCR001* expression in nodules and phenotype of *nr1/nr2* mutant nodules. A, Pro*MtNCR001*:GUS constructs were used to generate transgenic roots as described in material and methods section. GUS activity was only detected in the N2-fixing zone (zone III) of the nodule. I, meristematic zone; II, infection zone; III, N2-fixing zone; IV, senescence zone. *M. truncatula* nodules of Pro*MtNCR001*:GUS (B), and Pro*MtNCR001*:*nr1/nr2* (C) four weeks after inoculation with 2011 *S. meliloti* strain.

Figure S2

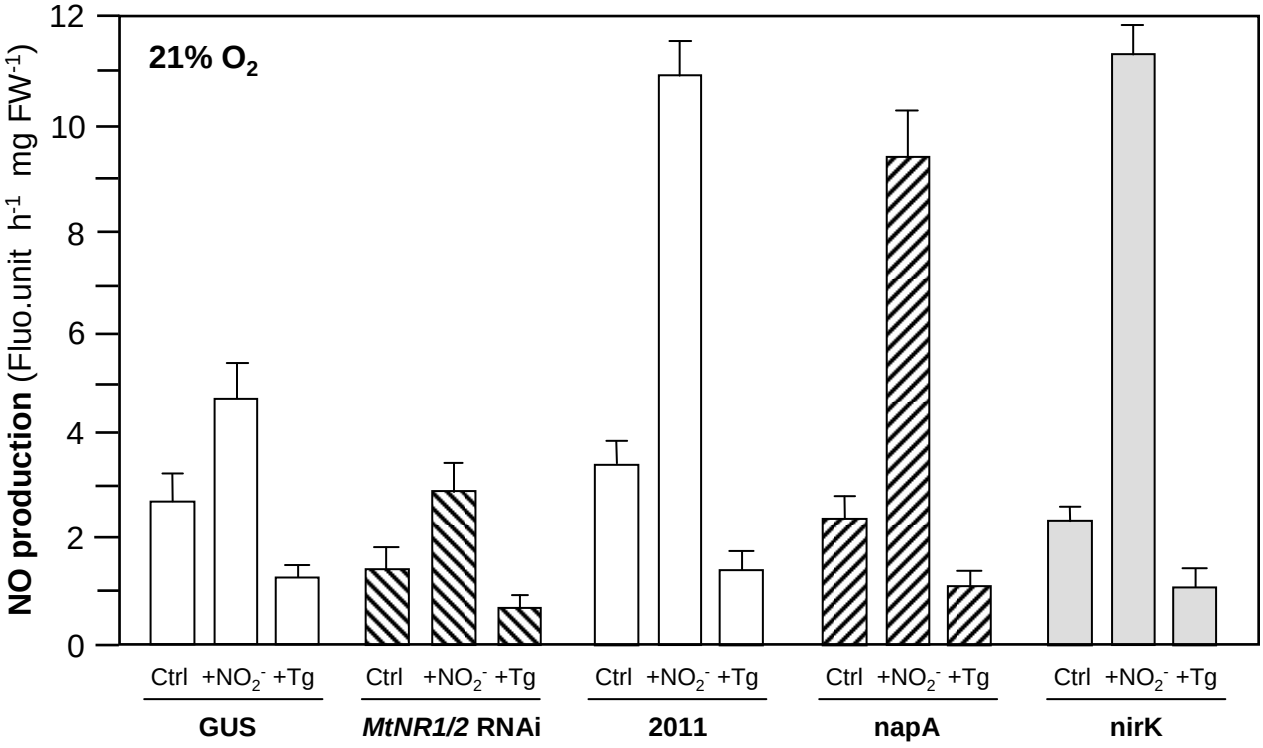


Figure S3: NO production by *M. truncatula* GUS and *nr1/nr2* , and by *S. meliloti* 2011, *napA* and *nirK* nodules under 21% O2. *M. truncatula* control (GUS) and MtNR1/2 RNAi plants were inoculated with *S. meliloti* 2011 strain. NO production is expressed as relative fluorescent units. Effector concentrations were 1 mM NaNO2 (NO2-), and 1 mM sodium tungstate (Tg). Data are the means ± SD of 2 independent experiments assayed in duplicates.

Figure S3

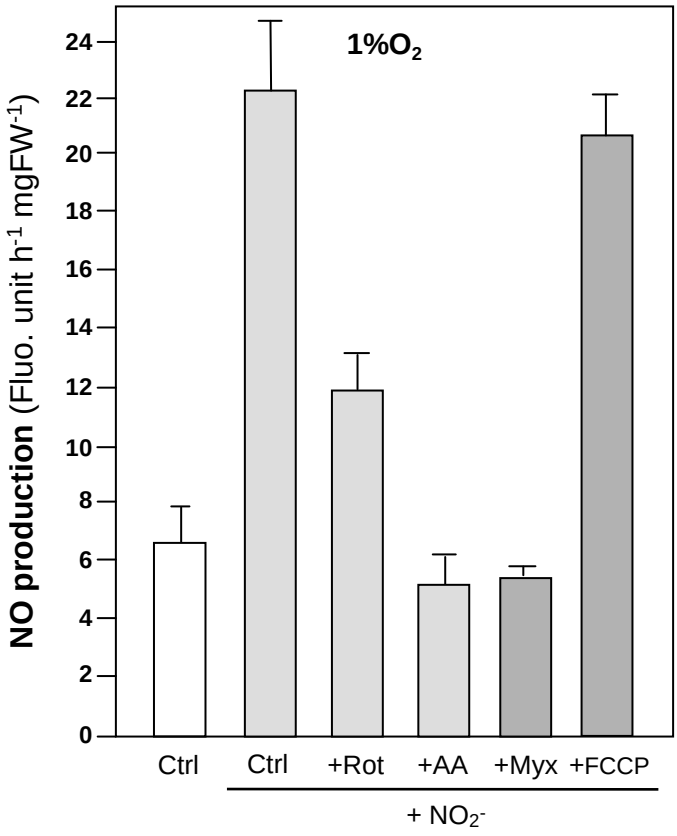


Figure S4: Effects of electron transfer chain effectors on NO production of *M. truncatula/S. meliloti* nodules in the presence of nitrite. *M. truncatula* wild type plants were inoculated with *S. meliloti* 2011 strain. NO production, expressed as relative fluorescent units, was measured under 1% O2. Effector concentrations were 1 mM NO2-, 10 µM rotenone (Rot), 25 µM antimycin A (AA), 25 µM myxothiazol (Myx), and 10 µM FCCP. Data are the means ± SD of 3 independent experiments assayed in duplicates.

Figure S4

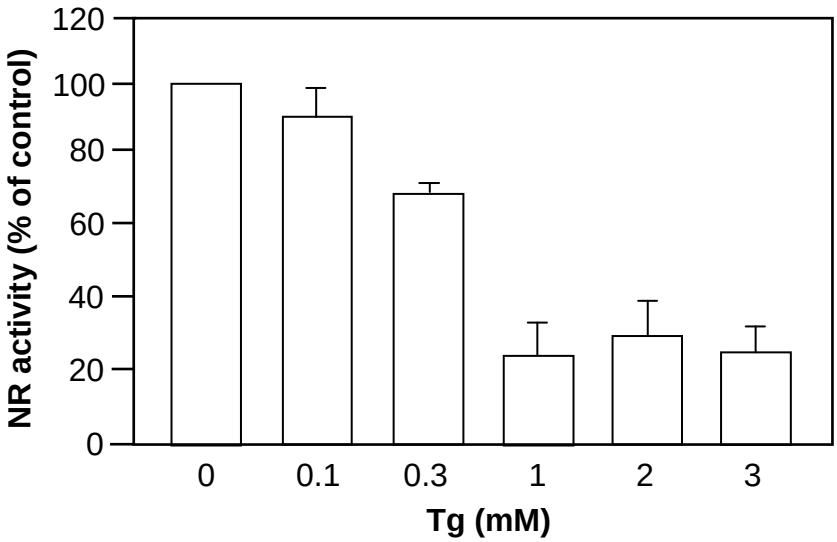
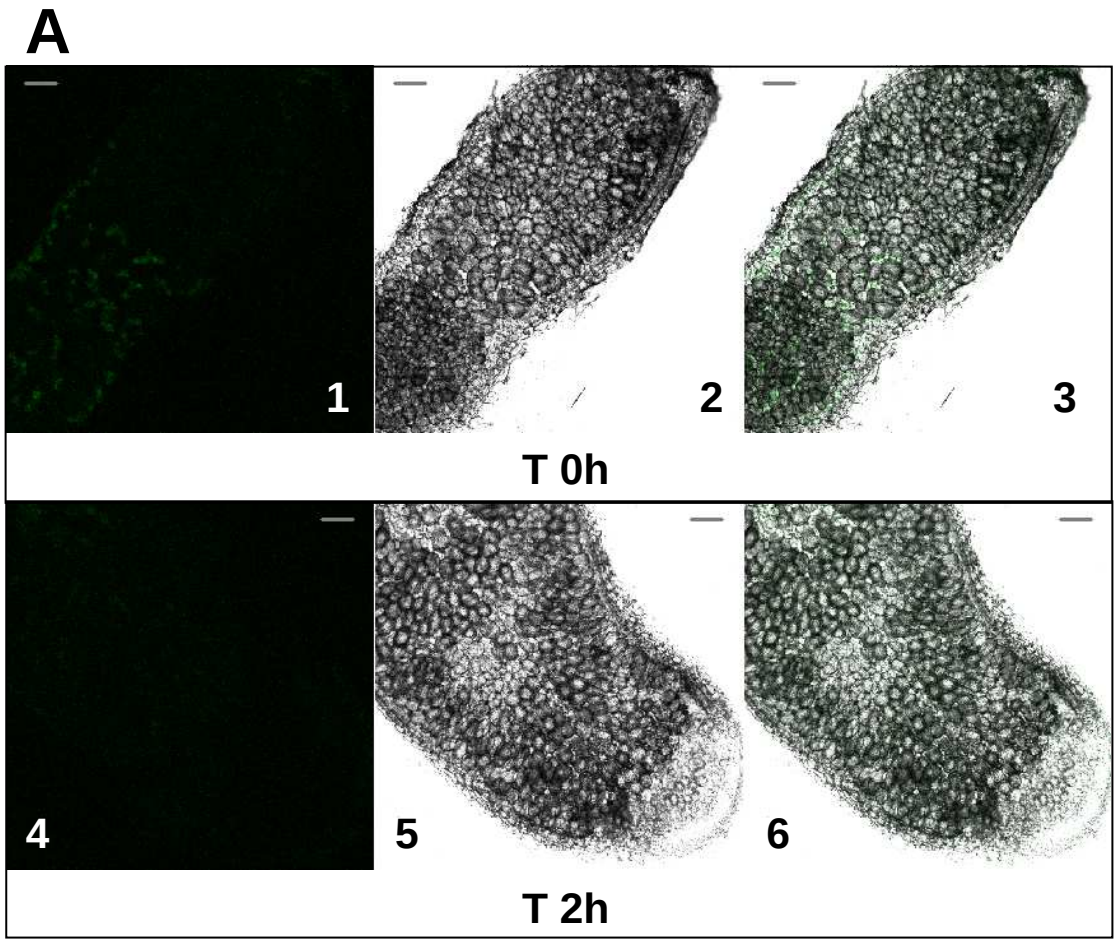


Figure S5: Effects of NaTg on nodule nitrate reductase activity. Nodule protein extracts were preincubated for 15 min at ambiant temperature with NaTg before NR activity measurement. Data are the means ± SD of 3 independent experiments.

Figure S5



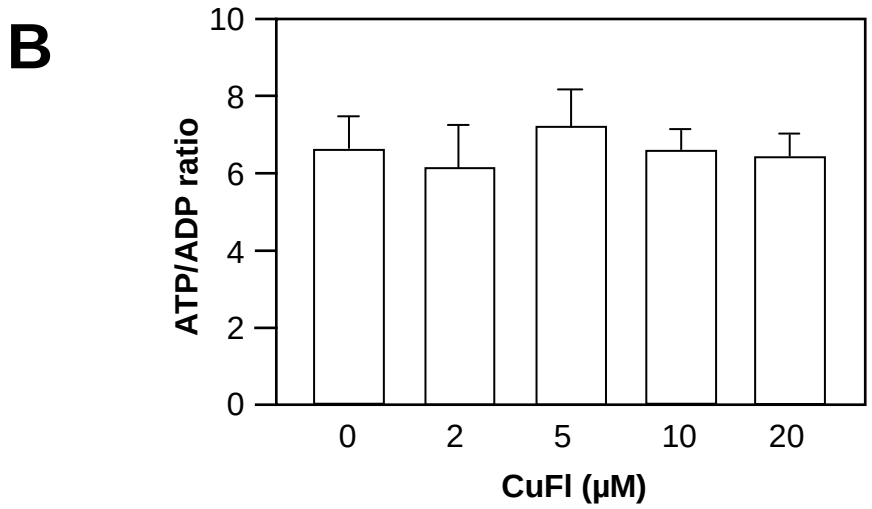


Figure S6: Histochemical analysis and toxicity test of CuFl treated nodules. A, CuFL detection of NO after Incubation (0 and 2 h) of entire nodules with 5 µM CuFL. Nodules were then cut into 100 µM thick slices and analyzed with a Zeiss LSM 500 confocal laser microscope; 1 and 4, fluorescence-field images; 2 and 5, bright-field images; 3 and 6, merged images. B, nodules were incubated for 2h in the presence of various CuFL concentrations. Adenine nucleotides were then extracted and measured with a luminometer (see Materials and methods).

Figure S6