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

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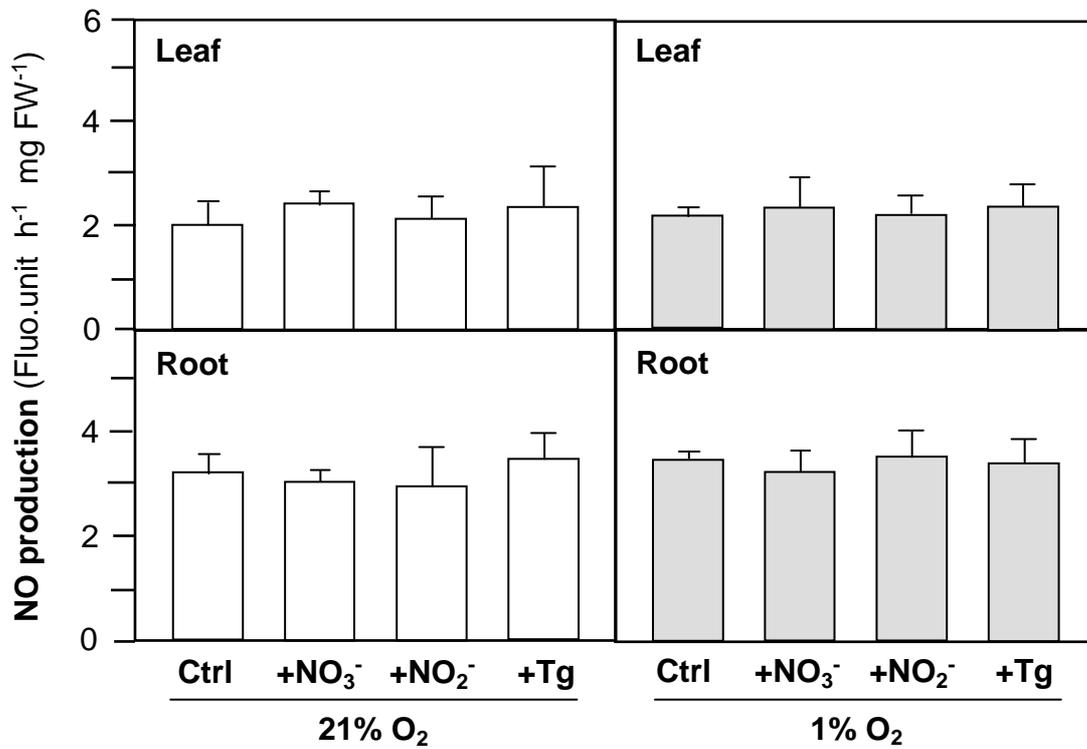


Figure S1: Effects of nitrate reductase effectors on *M. truncatula* leaf and root NO production. NO production, expressed as relative fluorescent units, was measured under either 21% or 1% O₂. Effector concentrations were 10 mM NaNO₃ (NO₃⁻), 1 mM NaNO₂ (NO₂⁻), and 1 mM sodium tungstate (Tg). Data are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

Figure S1

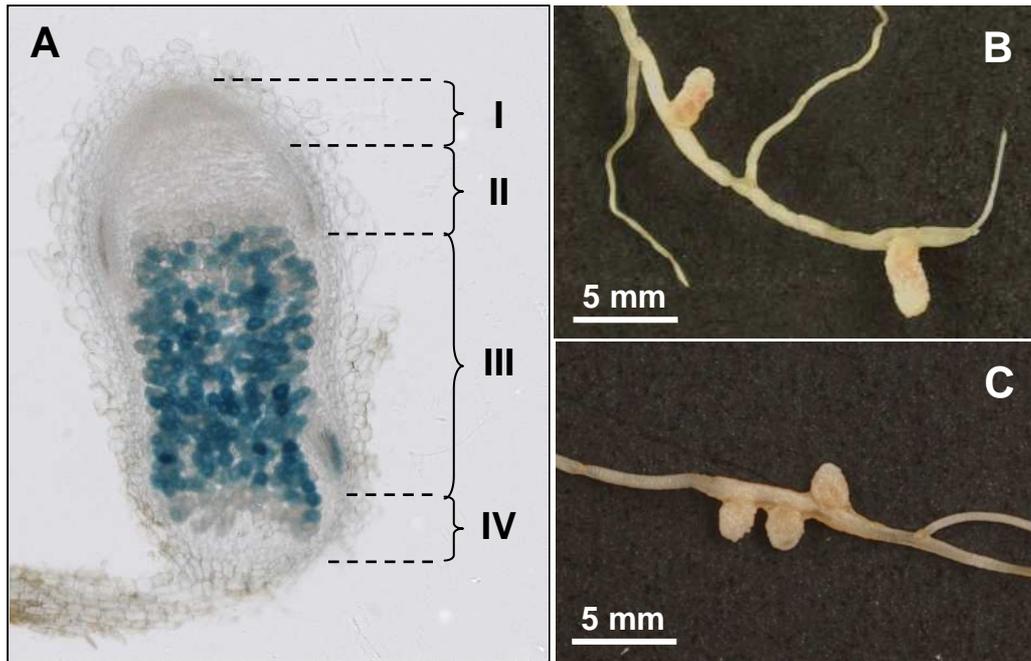


Figure S2: Histochemical analysis of *MtNCR001* expression in nodules and phenotype of *nr1/nr2* mutant nodules. A, *ProMtNCR001*:GUS constructs were used to generate transgenic roots as described in material and methods section. GUS activity was only detected in the N₂-fixing zone (zone III) of the nodule. I, meristematic zone; II, infection zone; III, N₂-fixing zone; IV, senescence zone. *M. truncatula* nodules of *ProMtNCR001*:GUS (B), and *ProMtNCR001*:*nr1/nr2* (C) four weeks after inoculation with 2011 *S. meliloti* strain.

Figure S2

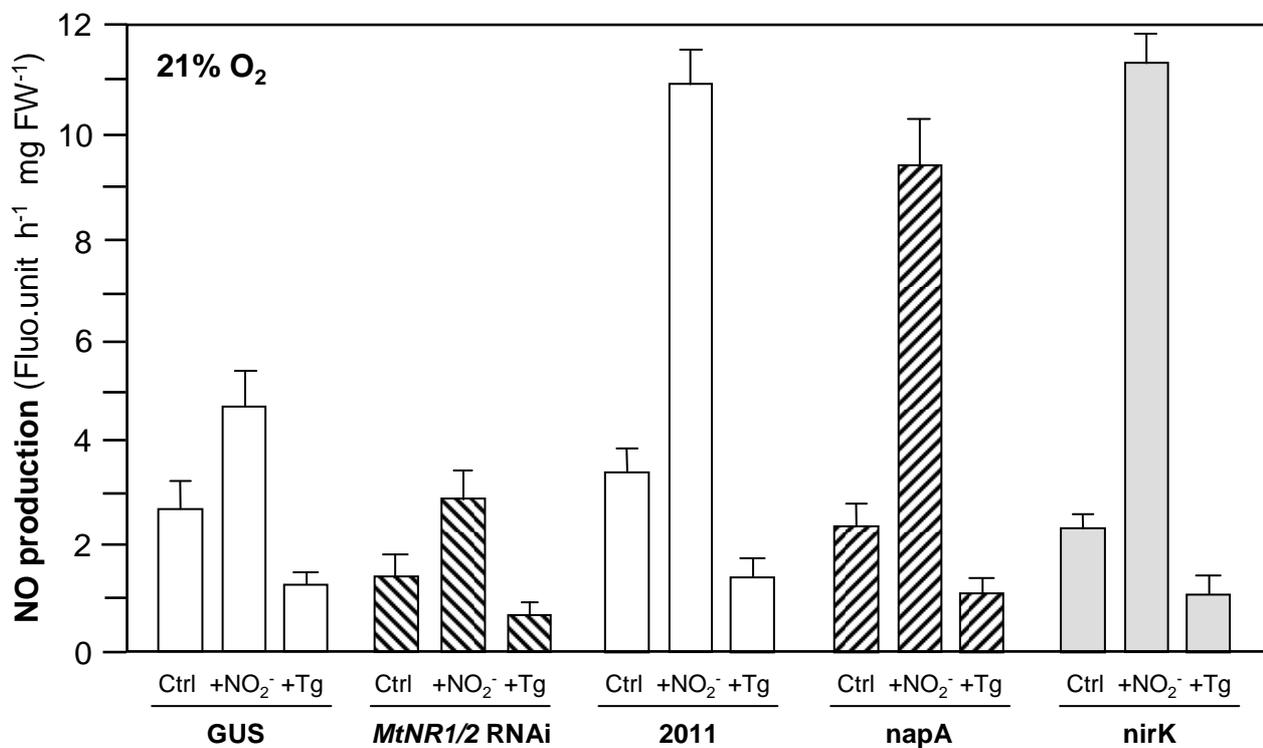


Figure S3: NO production by *M. truncatula* GUS and *nr1/nr2*, and by *S. meliloti* 2011, *napA* and *nirK* nodules under 21% O₂. *M. truncatula* control (GUS) and MtNR1/2 RNAi plants were inoculated with *S. meliloti* 2011 strain. NO production is expressed as relative fluorescent units. Effector concentrations 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

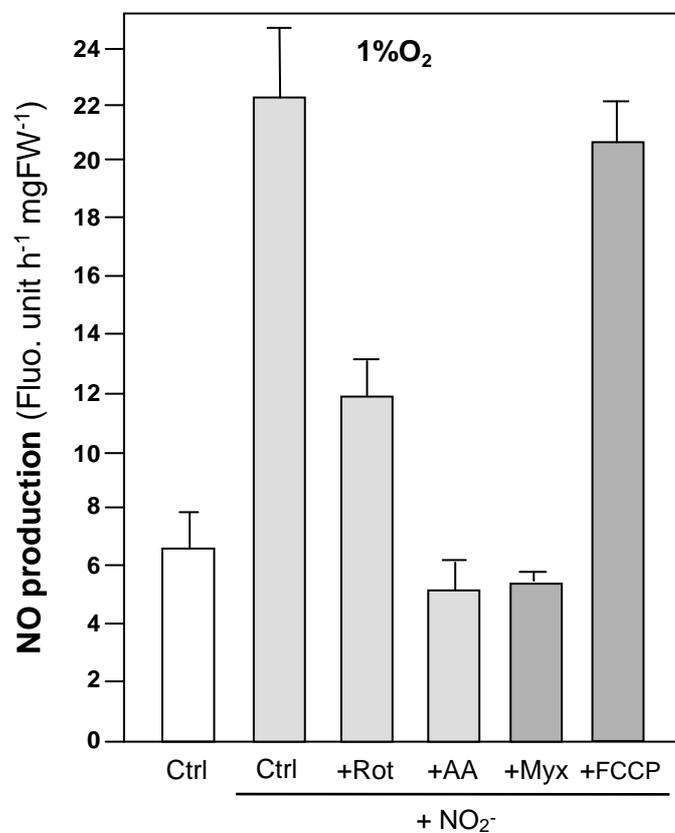


Figure S4: Effects of electron transfer chain effectors on NO production of *M. truncatula*/*S. meliloti* nodules in the presence of nitrite. *M. truncatula* wild type plants were inoculated with *S. meliloti* 2011 strain. NO production, expressed as relative fluorescent units, was measured under 1% 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

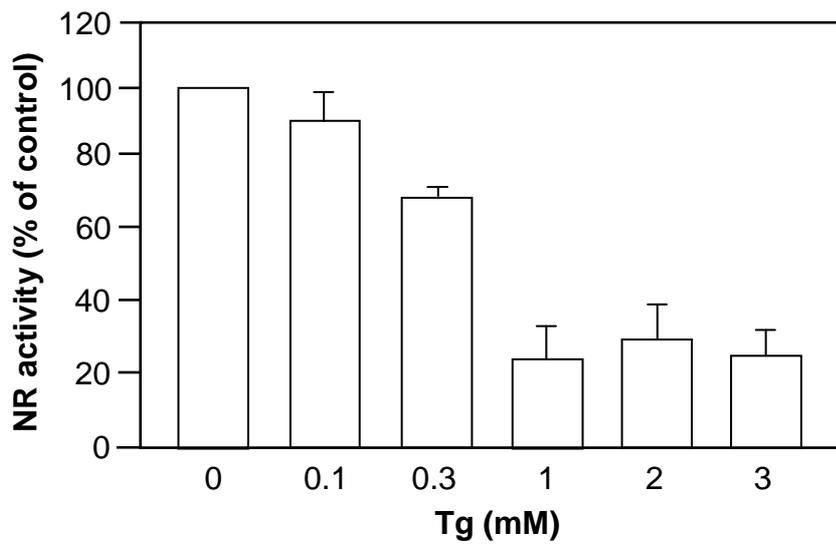


Figure S5: Effects of NaTg on nodule nitrate reductase activity. Nodule protein extracts were preincubated for 15 min at ambient temperature with NaTg before NR activity measurement. Data are the means \pm SD of 3 independent experiments.

Figure S5

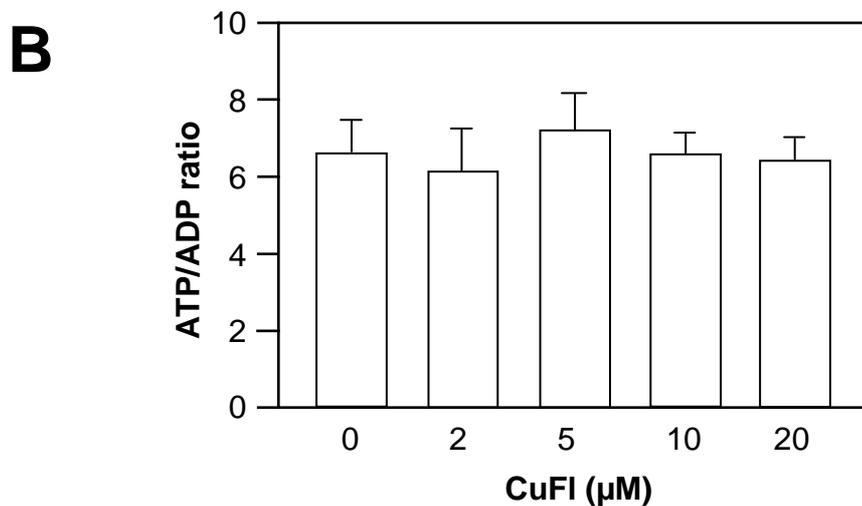
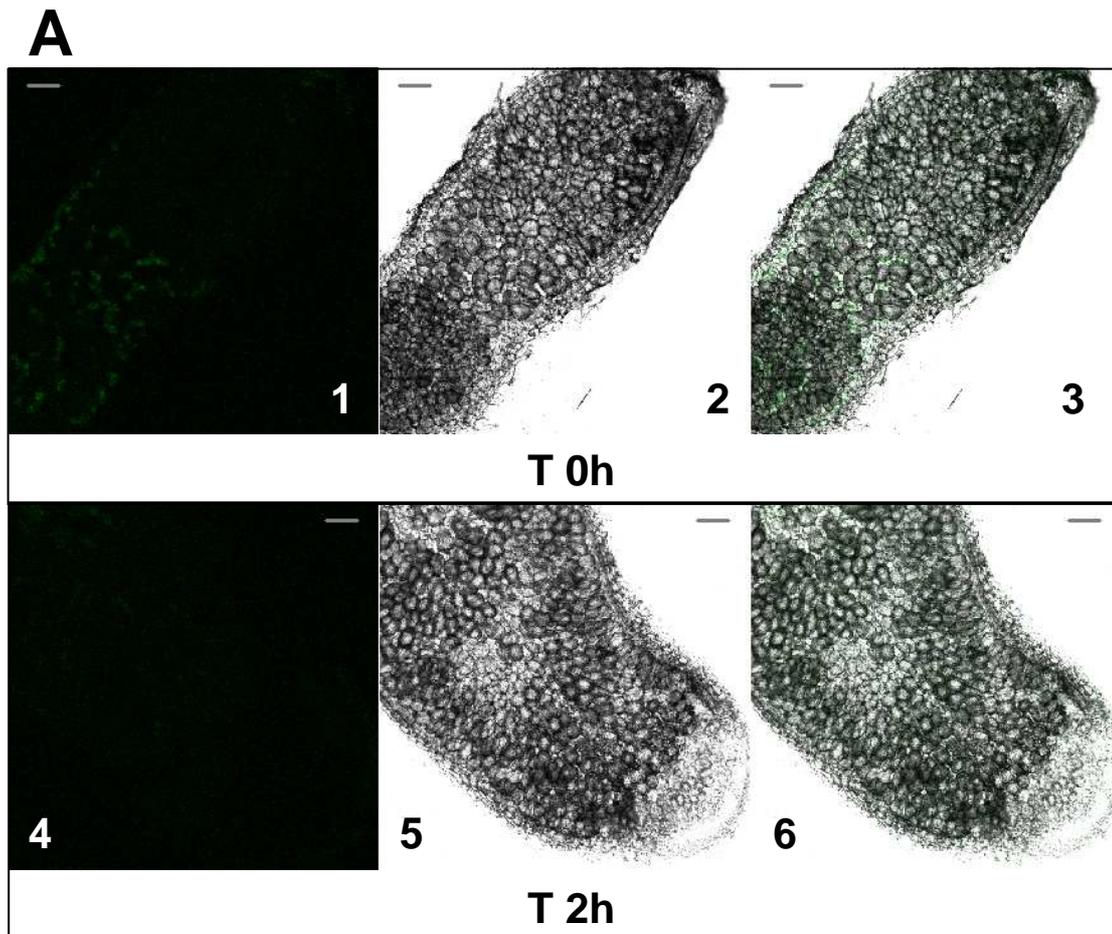


Figure S6: Histochemical analysis and toxicity test of CuFL treated nodules. A, CuFL detection of NO after incubation (0 and 2 h) of entire nodules with 5 μM CuFL. Nodules were then cut into 100 μm thick slices and analyzed with a Zeiss LSM 500 confocal laser microscope; 1 and 4, fluorescence-field images; 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