

Figure 1. Diversity of structures and possible functions of plant hemoglobins. Pentacoordinate indicate that the 5th position of the heme is coordinated to a histidine residue (proximal His), whereas the 6th position is free to bind physiological ligands such as O₂ and NO. Hexacoordinate indicates that both the 5th and 6th positions are coordinated to amino acid residues (the proximal and distal His). However, hexacoordinate hemoglobins also bind to O₂ and, in fact, the distal His stabilizes the binding, so that they have much higher O₂ affinity than pentacoordinate hemoglobins. Symbiotic hemoglobins are pentacoordinate and derived evolutionarily from class 2 Glbs, and transport O₂ at a low and steady concentration to the endosymbiont (rhizobia in legume nodules or frankiae in actinorhizal nodules).

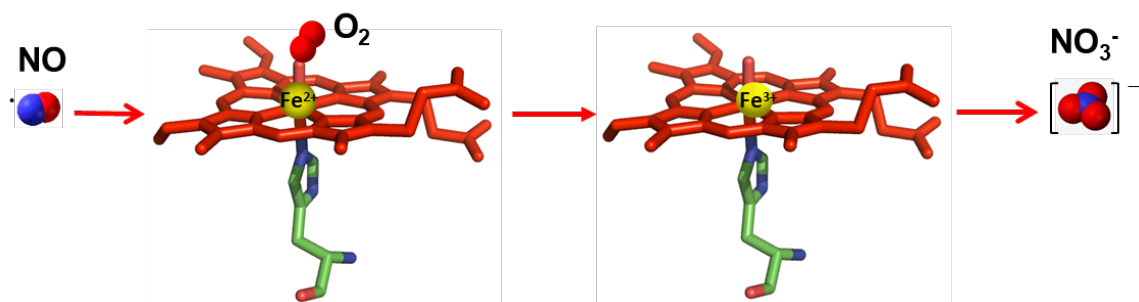


Figure 2. Scheme showing the nitric oxide dioxygenase (NOD) activity of the oxyferrous form (Fe²⁺O₂) of hemoglobins. This form has Fe²⁺ in the heme and a bound O₂ molecule. Then, the heme binds NO, which reacts with O₂ to produce nitrate, leaving the hemoglobin in its ferric (Fe³⁺) form.

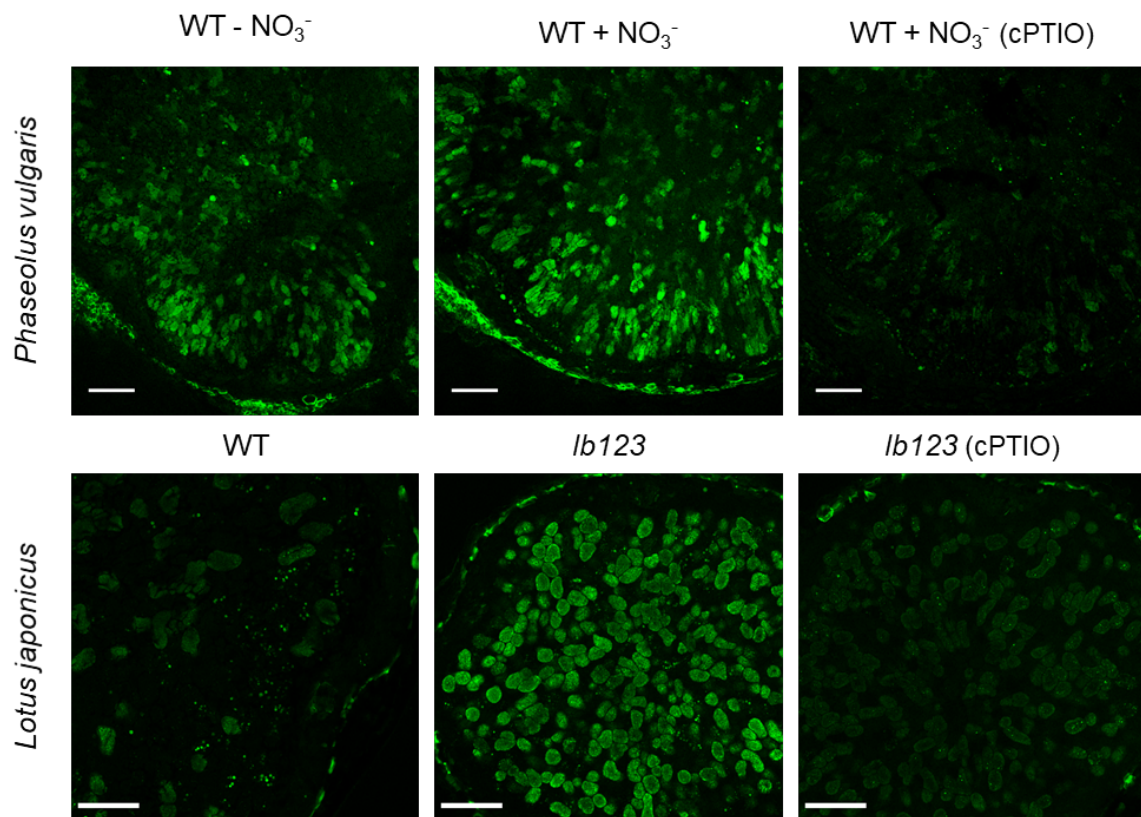


Figure 3. Production and localization of NO in nodule sections with the fluorescent dyes DAF-2 diacetate (upper panels) and DAF-FM diacetate (lower panels) and visualized by confocal microscopy. In common bean nodules note a higher green fluorescence (NO production) in nodules of WT plants treated with nitrate compared with untreated nodules. In *L. japonicus* nodules note that the nodule of the *lb123* mutant displays a much higher signal than the WT nodule. Nodule sections of both legumes incubated with the NO scavenger cPTIO show a drastical inhibition of NO production. Bars: upper panels (200 μ m), lower panels (150 μ m).