

**CURRENT PLANT SCIENCE AND
BIOTECHNOLOGY IN AGRICULTURE**

Biological Nitrogen Fixation for the 21st Century

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editors**



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DIFFERENT OXYGEN CONCENTRATIONS IN LUPIN NODULE FUNCTIONING: GENETIC AND PHYSIOLOGICAL ASPECTS

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The effects of O₂ concentrations (1, 21, 40% applied for 1, 3 and 10d) on nitrogen activity (N₂ase), O₂ diffusion resistance (ODR), glycoprotein content, *enod2* expression and nodule structure of *Lupinus albus* L. plants have been studied. N₂ase and ODR were measured using a flow-through system installed in a greenhouse. When 1% O₂ was applied for 1d, N₂ase was completely inhibited. However as O₂ concentration was increased from 1% to 21%, nitrogenase activity was totally recovered. The recovery was only 30% after 3d of 1% O₂ application and it was completely lost after 10d. When 40% O₂ was applied during 1 and 3d a decrease in N₂ase was detected, but after 10d N₂ase was similar to the control. ODR increased highly with 3d of 40% O₂ and it was double than control after 10d. Glycoprotein content decreased along 1% O₂ treatment. In 40% O₂ treated plants an increase was observed after 3d but after 10d there were no significant differences to the control. Expression of *enod2* also diminished with 1% O₂ and its evolution with 40% O₂ followed a similar pattern to glycoprotein. A clear correlation between nodule functioning and structure was observed using different microscopies: At LM with 1% O₂ nodule cortex changed progressively into an aerenchyma tissue. At 3d, middle cortex cells in the 40% O₂ treatment presented an increase in size and their intercellular spaces (IS) were more occluded by glycoprotein (data confirmed by western-blot). Nodule structure was more similar to control after 10d of 40% O₂. At TEM, 1% O₂ bacteroids started to degrade after 3d, and after 10d bacteroids degradation was general. After 10d of 40% O₂ bacteroids were larger (15%) and contained numerous polyhydroxybutyrate grains. At Cryo-SFM, 1% O₂ infected cells appeared highly vacuolated. The sizes of the IS of infected cells in 40% and 21% O₂ were similar, although the volume of infected cells was bigger. So the IS volume/cell volume ratio diminished reducing O₂ diffusion into the infected cells.

In conclusion, 1% O₂ caused irreversible effects to nodule functioning and structure, however the maintenance of nitrogen fixation after 10d under 40% O₂ indicates that nodule was able to adapt to high oxygen concentrations by modification of structure and physiological and biochemical mechanisms.