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ABSTRACT BOOK



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### T51 Hemoglobins of vascular plants: from model plants to crops (and way back)

Manuel Becana<sup>1</sup>, I. Yruela<sup>1</sup>, Pilar Catalán<sup>2</sup>, Gautam Sarath<sup>3</sup>

1) Estación Experimental de Aula Dei, CSIC, Apartado 13034, 50080 Zaragoza, Spain 2) Escuela Politécnica Superior de Huesca, Universidad de Zaragoza, Spain 3) USDA-ARS, East Campus, University of Nebraska-Lincoln, Lincoln NE 68583, USA

The first hemoglobin discovered in plants was the leghemoglobin of soybean root nodules. This finding was extended to other symbiotic hemoglobins in nodules of legumes, *Parasponia* and actinorhizal plants such as *Casuarina* and *Myrica*. These hemoglobins provide O<sub>2</sub> to N<sub>2</sub>-fixing bacteria within nodules. With the advent of technology for massive genome sequencing and wide-scale transcriptomics, it has become clear that hemoglobins are ubiquitously expressed in plant organs, from seeds to flowers. Based on phylogenetic and biochemical analyses, three classes of Glbs can be distinguished in vascular plants: Glb1s have extreme O<sub>2</sub> affinity and are induced by hypoxia; Glb2s have moderate O<sub>2</sub> affinity and, with certain exceptions, are the evolutionary precursors of symbiotic hemoglobins; and Glb3s have low O<sub>2</sub> affinity and high sequence homology to bacterial truncated hemoglobins. The proteins also differ structurally. For example, Glb1s and Glb2s are typically hexacoordinate (two histidines coordinate heme iron) whereas Glb3s and leghemoglobins are penta-coordinate (only one histidine is involved in iron coordination), which in part explains the differences in O<sub>2</sub> affinity and reactivity towards physiological ligands. However, the functions of Glbs are not well defined. Several Glb1s are involved in modulating the level of nitric oxide (NO), a key signal molecule in plant biology, and there is evidence that some Glb2s and/or Glb3s can scavenge NO *in vivo* or act as O<sub>2</sub> transporters, perhaps in tissues with high metabolic activity.

Here we present data on the phylogeny and some possible functions of Glbs in model plants and representative crops, both monocots and dicots. Interestingly, monocots such as barley, rice and corn lack Glb2s, suggesting that this class of proteins appeared, probably as a result of gene duplication, after the early divergence of angiosperms. Monocots and dicots may contain multiple Glb1s that in some cases (barley, rice, soybean) have been biochemically characterized. Model plants for genetic and molecular biology studies are boosting the search for Glb functions. We have identified two genes in the model small grass *Brachypodium distachyon* (Bradi1g69320, Bradi2g19690) and in switchgrass (J01114, la04000). All these genes presumably encode Glb1s. In dicots there are ample differences in the number of Glbs even within model plants: *Arabidopsis thaliana* contains one Glb of each class, whereas the model legumes *Lotus japonicus* and *Medicago truncatula* have at least two Glb1s, probably one Glb2, two Glb3s and three (*Lotus*) or twelve (*Medicago*) leghemoglobins. We have used lines that overexpress or are knockout for specific Glbs of the dicot model plants to show a triple interaction among Glbs, NO and phytohormones. With the help of specific mutants, we are pursuing now the study of Glb functions in model legumes and expect to tackle soon similar studies of this challenging issue in *B. distachyon*.

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