

## Article Addendum

# A novel non-symbiotic hemoglobin from oak

## Roles in root signalling and development?

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The cellular and molecular adaptations of non-model woody species to environmental changes are still poorly understood. We have cloned and characterised a novel non-symbiotic hemoglobin from oak roots (*QpHb1*) which exhibits a specific cellular distribution in the root. The *QpHb1* gene is strongly expressed in the protoderm and the protoxylem cells in two *Quercus* species (*Q. petraea* and *Q. robur*) with contrasting adaptive potential to drought and flooding. The constitutive expression of *QpHb1* in both oak species in specific root tissues combined with the reported presence of nitric oxide in the same tissues and its potential for protein S-nitrosylation could support a role for non-symbiotic hemoglobins in signalling changes in the root environment and/or in controlling some aspects of root development.

### Introduction

The genus *Quercus* (oak) includes over 300 woody species, widespread in the northern hemisphere, where it represents the dominant vegetation of temperate forests.<sup>1</sup> Among these, two sympatric predominant European oak species, pedunculate and sessile oak, are known to display different ecological requirements. The two species generally cohabit in forest ecosystems; however, sessile oak is found more frequently on well drained soils, whereas pedunculate oak can populate poorly drained sites.<sup>2</sup> This differential spatial distribution is due to the higher tolerance of pedunculate oak for soil waterlogging.

In an attempt to identify pertinent markers to discriminate between the two species, we have cloned and characterised a class I non-symbiotic hemoglobin gene. This class of Hbs has a high O<sub>2</sub> affinity and is induced under hypoxic conditions.<sup>3,4</sup> However, because

of an extremely low O<sub>2</sub>-dissociation constant, class I non-symbiotic Hbs may in fact participate in the regulation of cellular nitric oxide (NO) levels thus improving the redox and/or energy homeostasis of plant cells during hypoxia.<sup>5,6</sup>

### Non Symbiotic Hb Shows Differential Distribution in Oak

We have recently showed that *QpHb1* expression exhibits organ specificity in sessile oak.<sup>7</sup> We also found that in pedunculate oak (Fig. 1) there is a similar spatial distribution of expression, with a decreasing gradient from roots to leaves. However overall, transcripts are more abundant in pedunculate oak and more specifically in the roots. The fact that ns-Hb is strongly expressed in both species under normal growth conditions, suggests a constitutive role for this protein.

The cellular localisation of *QbHb1* in sessile oak roots, as evidenced by in situ hybridization, indicates that hemoglobin transcripts are most abundant in the protoderm and the protoxylem cells. A very similar pattern of expression was also found in pedunculate oak under control conditions (Fig. 2). What could be the significance of such a localisation? Could the constitutive expression of *QpHb1* play a role in root development?

### A Role for ns-Hb in Root Development and Signalling?

The localisation of ns-Hb in cells undergoing differentiation towards xylem elements but not in protoxylem cells, suggests a specific role for ns-Hb in the development of root xylem tissues. Interestingly, plant cells which are just predetermined to irreversibly *trans*-differentiate in xylem elements, show a burst in NO production.<sup>8</sup> This burst is observed when the cells reach a “point of no return” and undergo programmed cell death (PCD), essential to xylem development. The NO/Hb couple is well known to detoxify NO to nitrate via a NAD(P)H dependant mechanism, and thus ns-Hb could play a predominant role for root cell survival by regulating NO levels.<sup>9-11</sup> Other authors have also shown a co-localisation of ns-Hb with xylem cells during xylem differentiation in rice.<sup>12</sup> It would be extremely interesting to confirm a spatio-temporal co-localisation of ns-Hb expression with the NO burst responsible for protoxylem differentiation.

NO has also been associated with cell signalling through S-nitrosylation of proteins. This post-translational process permits a conformational change of target proteins which can modify their

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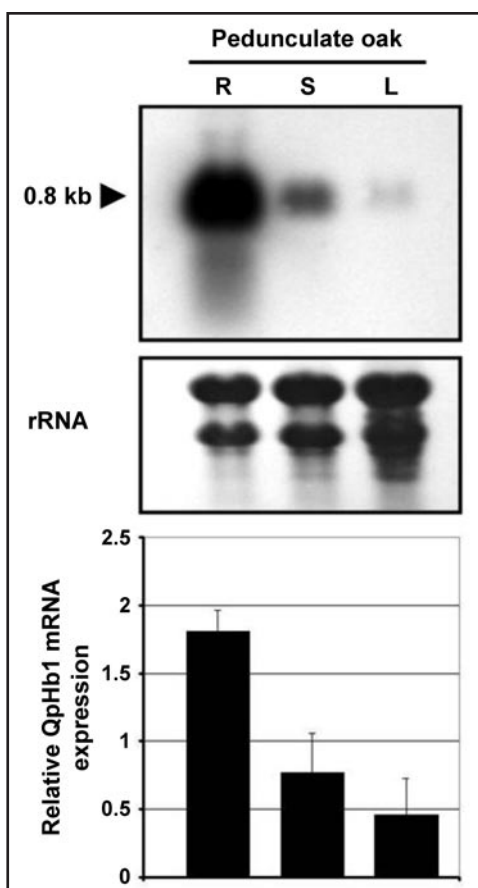


Figure 1. Transcript pattern of the *QpHb1* gene in vegetative organs of pedunculate oak (*Quercus robur*). Total RNAs were extracted from roots, stems and leaves of seedlings grown under control conditions for 5 weeks. R, roots; S, shoot; L, leaves.

activity.<sup>13,14</sup> Recent data suggest that this process is widespread in plants and can be used in regulating various cell processes. Our data showing that ns-Hb is strongly expressed in the root protoxylem could also indicate that the regulation of S-nitrosylation via NO detoxification by ns-Hb or the direct S-nitrosylation of ns-Hb could be key features of root-to-shoot signalling. Indeed, the xylem serves as a direct route between the root and the shoot for various signalling molecules. The constitutive presence of ns-Hb in this tissue may thus serve as a “standby surveillance system” for rapid signalling of changes in the root environment.

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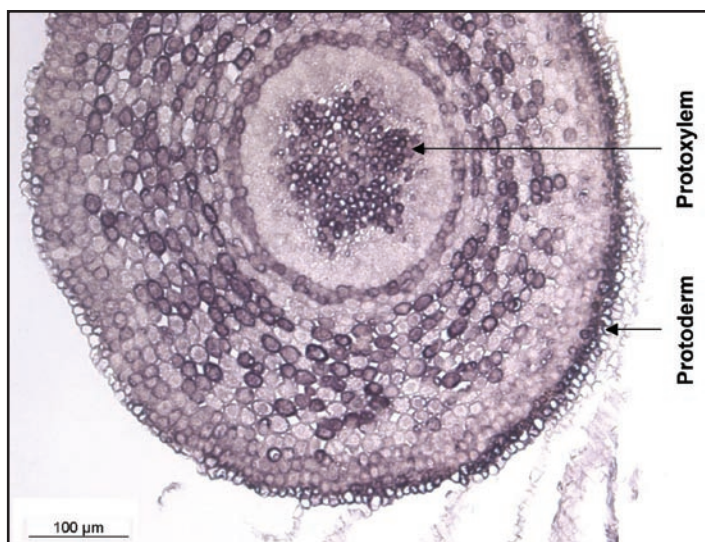


Figure 2. In situ *QpHb1* expression in a cross section realized at 800  $\mu\text{m}$  from the tip of the root cap of *Quercus robur* grown for 5 weeks under control conditions.

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