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Expression of non-symbiotic hemoglobin 1 and 2 genes in rice (*Oryza sativa*) embryonic organs

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Rice (*Oryza sativa*) contains five copies of the non-symbiotic hemoglobin (*hb*) gene, namely *hb1* to *hb5*. Previous analysis by RT-PCR revealed that rice *hb1* expresses in roots and leaves and *hb2* expresses in leaves. However, it is not known whether or not *hb1* and *hb2* express in rice embryonic organs. Here, we report the expression of *hb1* and *hb2* genes in rice embryonic organs using RT-PCR and specific oligos for Hb1 and Hb2. Our results indicate that *hb1* and *hb2* genes express in embryonic organs in rice growing under normal conditions. Specifically, *hb1* expresses in rice embryos and seminal roots, and *hb2* expresses in embryos, coleoptiles and seminal roots. These observations suggest that Hb1 and Hb2 coexist and function in rice embryonic organs.

Key words: embryonic organs, gene expression, hemoglobin, non-symbiotic, Oryza, rice

Abbreviations: Hb, hemoglobin; nsHb, non-symbiotic hemoglobin; RT-PCR, reverse transcriptase-polymerase chain reaction

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Non-symbiotic hemoglobins (nsHbs) are widespread in land plants. Nucleotide sequences coding for these proteins have been identified in plants ranging from primitive bryophytes to evolved angiosperms, including monocots and dicots.[1-3] In monocots, rice (*Oryza sativa*) contains five copies of the *nshb* gene, namely *hb1* to *hb5*.[4,5] Western blot analysis and immunolocalization by confocal microscopy using polyclonal anti-rice Hb1 antibodies revealed that nsHbs are localized in specific tissues of rice embryonic organs, such as the seed aleurone and scutellum, and of vegetative organs, such as the leaf schlerenchyma and root cap.[6,7] However, these analyses did not differentiate among individual nsHbs because detected signals could result from any one of the rice nsHbs (i.e., Hb1, Hb2, Hb3, Hb4 or Hb5), or from a combination of them. Previous analysis by RT-PCR using specific oligos revealed that rice *hb1* expresses in roots and leaves, *hb2* expresses in leaves, and *hb5* expresses in embryonic and vegetative organs.[5,8] Also, the analysis of an OsNSHB2 promoter fused to the *gus* reporter gene revealed tissue-specific expression of the rice *hb2* gene in roots, vasculature of young leaves, flowers and the pedicel/stem junction of transgenic Arabidopsis.[9] These observations suggest that Hb1, Hb2 and Hb5 coexist in rice leaves and roots. However, it is still not known whether or not *hb1* and *hb2* express in rice embryonic organs.

We analyzed the expression of *hb1* and *hb2* genes in rice embryonic organs using RT-PCR and specific oligos for Hb1 and Hb2. Seed germination, isolation of poly(A$^+$) RNA, reverse transcription and PCR amplification were performed as described by Arredondo-Peter et al.[8] The Hb1 and Hb2 transcripts are each approximately 550 bp in length. **Figure 1** shows that PCR fragments of the expected size for Hb1 were amplified from embryos and seminal roots, and for Hb2 were amplified from embryos, coleoptiles and seminal roots. These PCR fragments were cloned and sequenced, and the resulting sequences were identical to those of the rice Hb1 and Hb2 transcripts (Genbank accession number U76030.1 and U76031.1, respectively). This result indicates that *hb1* and *hb2* genes express in embryonic organs in rice growing under normal conditions. It was previously reported that

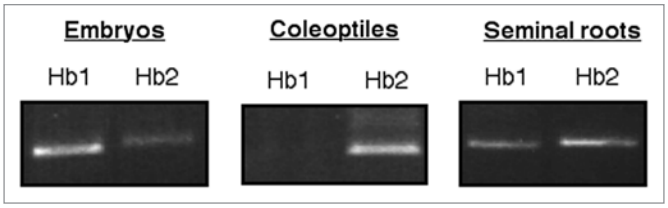

Figure 1. Expression analysis of *hb1* and *hb2* genes in rice embryonic organs. Amplification of the rice Hb1 and Hb2 transcripts was performed by RT-PCR using specific oligos for Hb1 and Hb2 and conditions described by Arredondo-Peter et al.[8]

Table 1. Expression of *nshb* genes in rice organs

nshb gene	Expression* in rice					References
	Embryonic organs			Vegetative organs		
	Embryos	Coleoptiles	Seminal roots	Leaves	Roots	
hb1	+	ND	+	+	+	This work; Arredondo-Peter et al.[8]
hb2	+	+	+	+	ND	This work; Arredondo-Peter et al.[8]
hb3	NA	NA	NA	NA	NA	
hb4	NA	NA	NA	NA	NA	
hb5	+	+	+	+	+	Garrocho-Villegas et al.[5]

*ND, not detected; NA, not analyzed.

hb2 apparently does not express in rice roots,[8] however in this work Hb2 transcripts were detected in rice seminal roots (**Fig. 1**). This observation suggests that expression of *hb2* is downregulated during root development.

The above observations together with those reported by Arredondo-Peter et al.[8] and Garrocho-Villegas et al.[5] suggest that Hb1, Hb2 and Hb5 coexist in rice embryonic and vegetative organs. Specifically, it is likely that Hb1, Hb2 and Hb5 coexist and function in rice embryos, seminal roots and leaves; Hb2 and Hb5 coexist and function in coleoptiles; and Hb1 and Hb5 coexist and function in roots (**Table 1**). Results reported here complement our knowledge of the expression of *nshb* genes in rice organs.

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