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Variability of non-symbiotic and truncated hemoglobin genes from the genome of cultivated monocots

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Abbreviations: Hb, hemoglobin; MCL, maximum composite likelihood; nsHb, non-symbiotic hemoglobin; tHb, truncated hemoglobin

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Non-symbiotic (nsHb) and truncated (tHb) hemoglobins (Hbs) have been detected in a variety of land plants. The evolution of land plant nsHbs and tHbs at the protein level is well documented; however, little is known about the evolution of genes coding for these proteins. For example, the variability of the land plant *nshb* and *thb* genes is not known. Here, we report the variability of the *nshb* and *thb* genes from the genome of the cultivated monocots *Brachypodium distachyon*, *Hordeum vulgare* (barley), *Oryza glaberrima* (rice), *O. rufipogon* (rice), *O. sativa* (rice) var indica, *O. sativa* (rice) var japonica, *Panicum virgatum* (switchgrass), *Setaria italica* (foxtail millet), *Sorghum bicolor* (sorghum), *Triticum aestivum* (wheat), and *Zea mays* ssp. *mays* (maize) using sequence comparison and computational methods. Our results revealed that in cultivated monocots variability is higher in *nshb*s than in *thb*s, and suggest that major substitution events that occurred during the evolution of the cultivated monocot *hb*s were A→G and T→C transitions and that these genes evolved under the effect of neutral selection.

Non-symbiotic (nsHb) and truncated (tHb) hemoglobins (Hbs) are O_2-binding proteins that have been detected in a variety of land plants, ranging from primitive bryophytes to evolved angiosperms.[1-5] Phylogenetic analysis revealed that land plant *nshb* and *thb* genes apparently evolved from different ancestors. Also, sequence analysis showed that 2 classes of nsHbs exist in higher plants: class 1 and class 2 nsHbs (nsHb-1 and nsHb-2, respectively).[6,7] However, recent analysis revealed that apparently only nsHbs-1 exist in cultivated monocots, which diverged into clade I and clade II nsHbs (nsHb-I and nsHb-II, respectively) from a nsHb-1 ancestor.[8]

The evolution of land plant nsHbs and tHbs at the protein level is well documented.[3,4,8-10] However, little is known about the evolution of genes coding for these proteins. For example, the variability of the land plant *nshb* and *thb* genes is not known. Monocots are useful models for gene analysis because of the availability of several (fully or partially) sequenced genomes from these plants. Here, we report the variability of the *nshb* and *thb* genes from the cultivated monocots *Brachypodium distachyon*, *Hordeum vulgare* (barley), *Oryza glaberrima* (rice), *O. rufipogon* (rice), *O. sativa* (rice) var indica, *O. sativa* (rice) var japonica, *Panicum virgatum* (switchgrass), *Setaria italica* (foxtail millet), *Sorghum bicolor* (sorghum), *Triticum aestivum* (wheat), and *Zea mays* ssp. *mays* (maize). Our results revealed that in cultivated monocots, variability is higher in *nshb*s than in *thb*s and that these genes evolved under the effect of neutral selection.

Protein and gene sequences for nsHbs and tHbs from the above cultivated monocots were obtained from the GenBank (www.ncbi.nlm.nih.gov/genbank) and Phytozome (www.phytozome.org) databases as described by Rodríguez-Alonso and Arredondo-Peter[8] (**Tables S1** and **S2**) using the sequence of *O. sativa* nsHbs 1 to 5 and tHb (GenBank accession number AAK72229.1, AAK72228.1, AAK72230.1, AAK72231.1, ABN45744.1, and EEC80902.1, respectively) as probes. Pairwise sequence alignment was performed using the Needle program (http://www.ebi.ac.uk/Tools/psa/emboss_needle/). Differences between the aligned sequences were quantitated using a Phyton's script developed by one of the authors (Rodríguez-Alonso G). Values were normalized based on the number of aligned sequences. The maximum composite likelihood (MCL) matrix and GC content quantitation were obtained using the MEGA 5.0 program.[11] Testing of the neutral mutation hypothesis was performed by calculating the Tajima's

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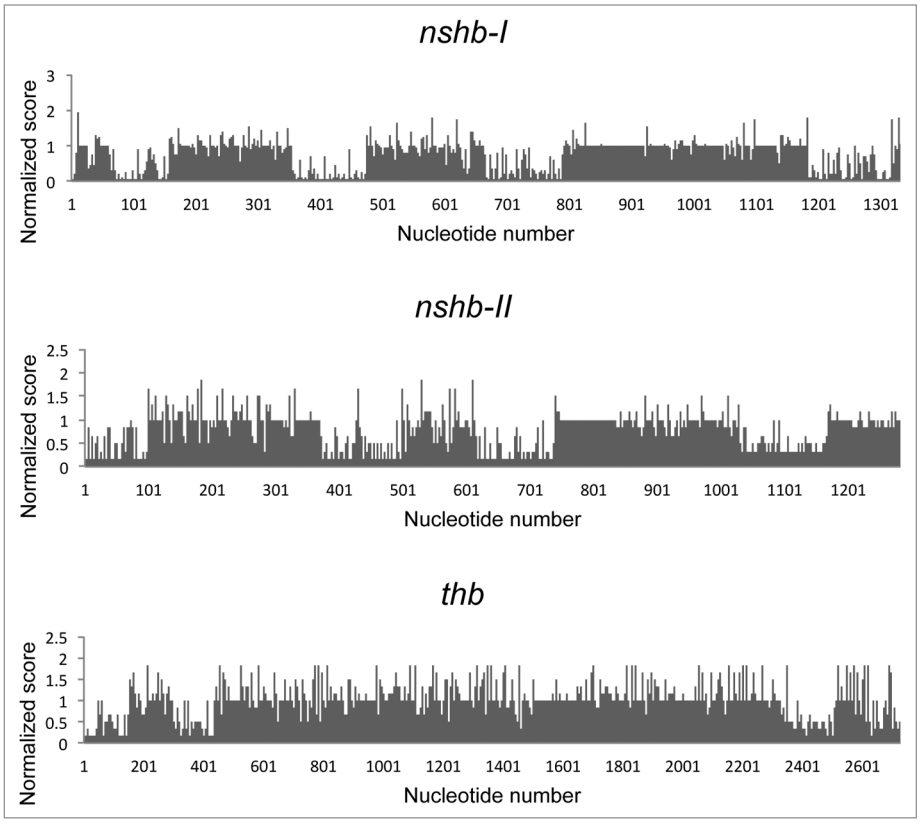

Figure 1. Variability of the *nshb-I*, *nshb-II* and *thb* genes from cultivated monocots. High and low score regions approximately correspond to introns and exons into the *nshb-I* and *nshb-II* genes, respectively.

		A	T	C	G	
nshb-I	A	-	2.69	4.57	30.72	R= 2.144
	T	3.4	-	10.97	5.22	
	C	3.4	6.45	-	5.22	
	G	20.6	2.69	4.57	-	
nshb-II	A	-	2.74	5.49	17.44	R= 1.463
	T	3.8	-	23.36	7.02	
	C	3.8	11.64	-	7.02	
	G	9.45	2.74	5.49	-	
thb	A	-	4.28	6.63	11.27	R= 1.077
	T	6.01	-	19.07	6.71	
	C	6.01	12.31	-	6.71	
	G	10.1	4.28	6.63	-	

Figure 2. Maximum composite likelihood (MCL) matrix for *nshb-I*, *nshb-II*, and *thb* genes from cultivated monocots. The *R* value indicates the transition/transversion ratio.

D value[12] using the DNAsp program (http://www.ub.edu/dnasp/).

Figure 1 shows that in cultivated monocot *nshb-I* and *nshb-II* genes the 3 introns interrupting land plant *nshb*s[1,4,13] are differentiated from exons because of the existence of high and low variability scores, respectively. This indicates that in cultivated monocot *nshb-I* and *nshb-II* genes, the variability of introns is higher than that of exons. In contrast, in cultivated monocot *thb* genes the variability score of exons and introns is similar. Quantitation of similarity between pairwise sequence alignments of cultivated monocot Hb proteins and exons from the *hb* genes showed that the average variability values (obtained from the similarity values reported in **Figures S1** and **S2**) are 30.36% for nsHbs, 25.52% for *nshb*s, 11.46% for tHbs, and 11.23% for *thb*s. This result indicates that in cultivated monocots, the average variability of nsHbs is higher than that of tHbs.

Direct quantitation of similarity from aligned sequences assumes that nucleotides exist approximately in the same proportion in the genomes of organisms and that point mutations occurred with the same frequency during the evolution of genomes. However, this is generally incorrect. Also, this method does not consider regressions. Thus, we generated MCL matrices to evaluate substitution rates for point mutations and regression frequencies into the cultivated monocot *hb* genes. **Figure 2** shows that the transition/transversion ratios (R) are 2.144 for *nshb-I*, 1.463 for *nsb-II*, and 1.077 for *thb* genes. This result suggests that major substitution events that occurred during the evolution of the cultivated monocot *hb*s were A→G and T→C transitions, and thus indicates that in these genes the GC content is high. Quantitation of the GC content in codons from exons of cultivated monocot *hb*s showed that GC content is slightly higher in *nshb*s than in *thb*s and that GC content in these genes is higher (~70–90%) in codon position 3 than in codon positions 1 and 2 (~55–60 and ~40%, respectively) (**Fig. 3**).

Testing of the neutral mutation hypothesis for the evolution of cultivated monocot *hb* genes was performed by estimating the Tajima's D value.[12] Results showed that the Tajima's D value for cultivated monocot *nshb* and *thb* genes is 0.39 and –0.272, respectively, and that $P > 0.1$ for both estimations. Thus, these estimations are not statistically different from values expected in a neutralist model. These results suggest that cultivated monocot *nshb* and *thb* genes evolved under the effect of neutral selection.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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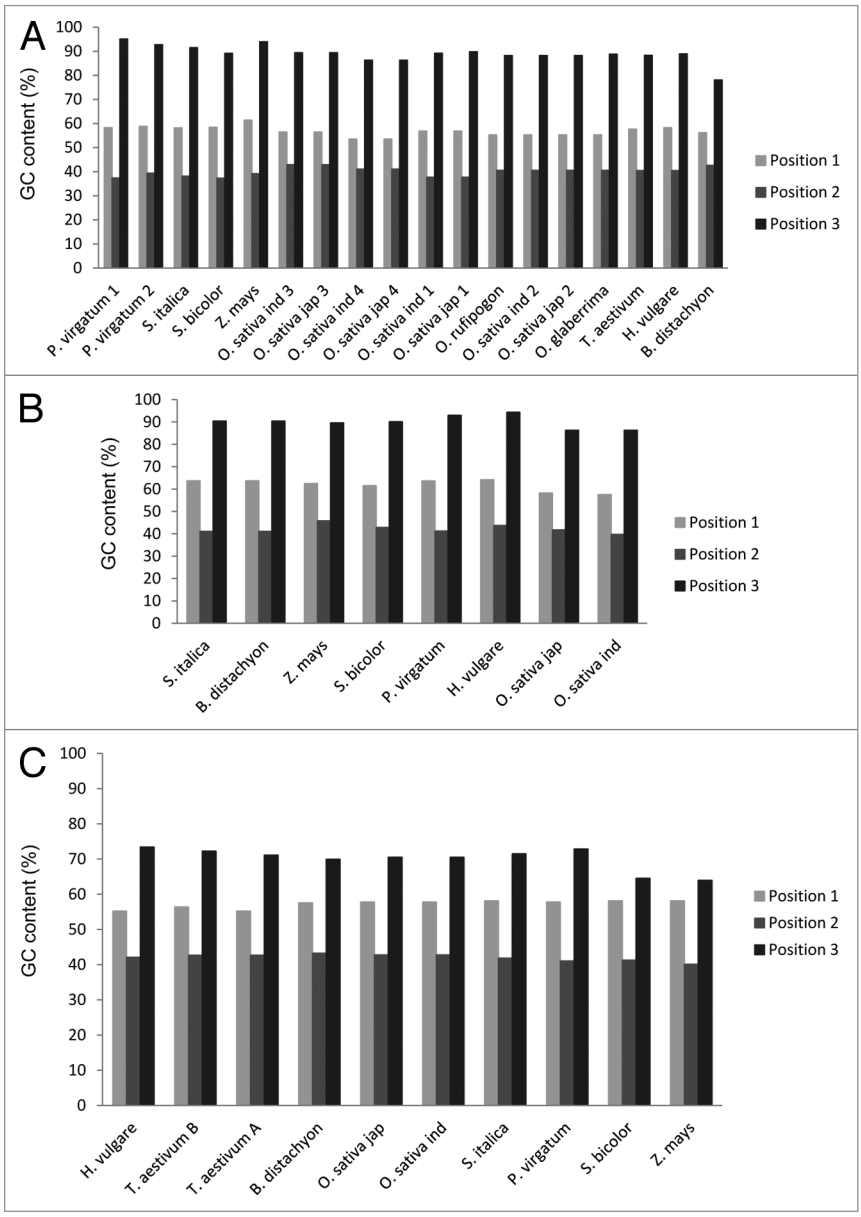
Figure 3. GC content in codon positions 1, 2, and 3 from the cultivated monocot *nshb-I* (**A**), *nshb-II* (**B**), and *thb* (**C**) genes.



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Supplemental Materials

Supplemental materials may be found here: www.landesbioscience.com/journals/CIB/article/27496

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