

# Isolation, sequencing of the *HvnHID* gene and its role in the purple-grain colour development in Tibetan hulless barley

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## Electronic Supplementary Material (ESM)

The authors are fully responsible for both the content and the formal aspects of the electronic supplementary material. No editorial adjustments were made.

Table S1. Primer sequences used in the present study

Gene	Description	Primer sequence (5'-3')
<i>HvnHID</i> (gene cloning)	forward-primer	CCCACGAACGACCAAACAC
	reverse-primer	AGGAACAACAATGGCGATAAAA
<i>HvnHID</i> (RT-qPCR)	forward-primer	CTCGTCCGCCAGTACAAGAG
	reverse-primer	CCTCCGTGGAAGTAGACGA
<i>HvnF3'H</i> (RT-qPCR)	forward-primer	CGTCAAGGGCGGCGACTTCGGGCTG
	reverse-primer	AAGGCCTCCTCCATGTTGAGCTTGC
<i>HvnDRF</i> (RT-qPCR)	forward-primer	AGAGAACTAGAAGAAGAGAAGG
	reverse-primer	GCCGTGAAGAGGTAATCC
<i>HvnANT1</i> (RT-qPCR)	forward-primer	AGGTGCACTGGCGGCCTCTTCTTCC
	reverse-primer	GCCAGGGCTCTCACGTCGTCCATCC
<i>HvnGT</i> (RT-qPCR)	forward-primer	GAAATACTTGGAGGTGCGCC
	reverse-primer	CGTCATGCACACCTTGTTCA
<i>18SrRNA</i> (internal reference)	forward-primer	CGGCTACCACATCCAAGGAA
	reverse-primer	GCTGGAATTACCGCGGCT

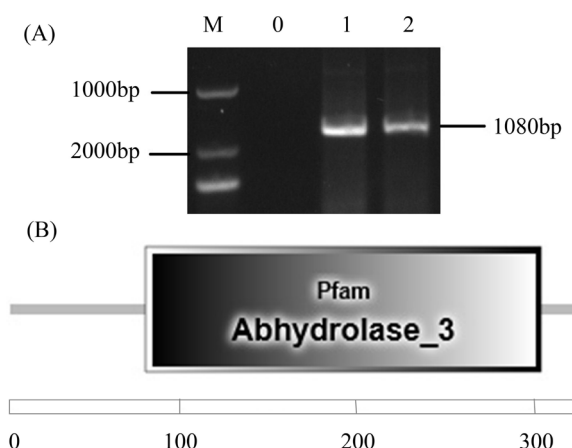


Figure S1. PCR product of the *HvnHID* gene (A) and conserved domains of the HvnHID protein (B)

M – DL2000; 0 – negative control; 1 – PCR product of the *HvnHID* gene in the variety Nerumuzha; and 2 – PCR product of the *HvnHID* gene in the variety Kunlun 10

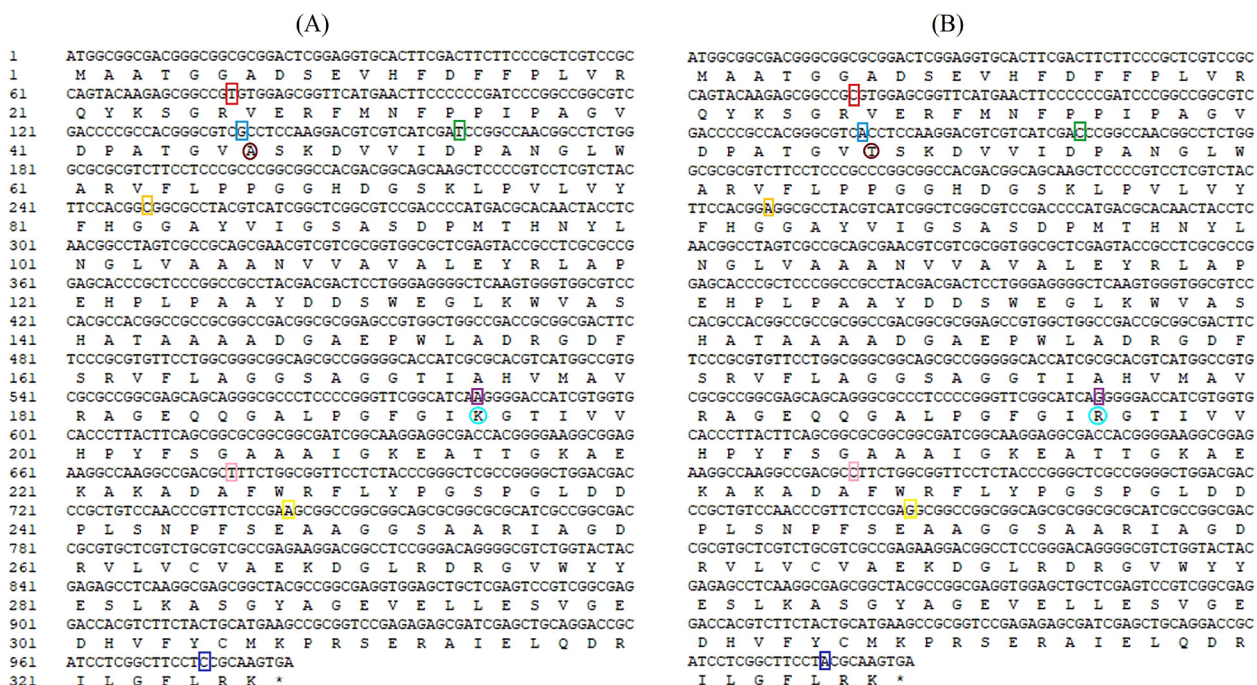


Figure S2. Coding sequence (CDS) and deduced amino acid sequence of the *HvnHID* gene amplified from the variety Nerumuzha (A) and the variety Kunlun 10 (B)

Coloured boxes indicate a nucleotide difference and coloured circles indicate an amino acid difference



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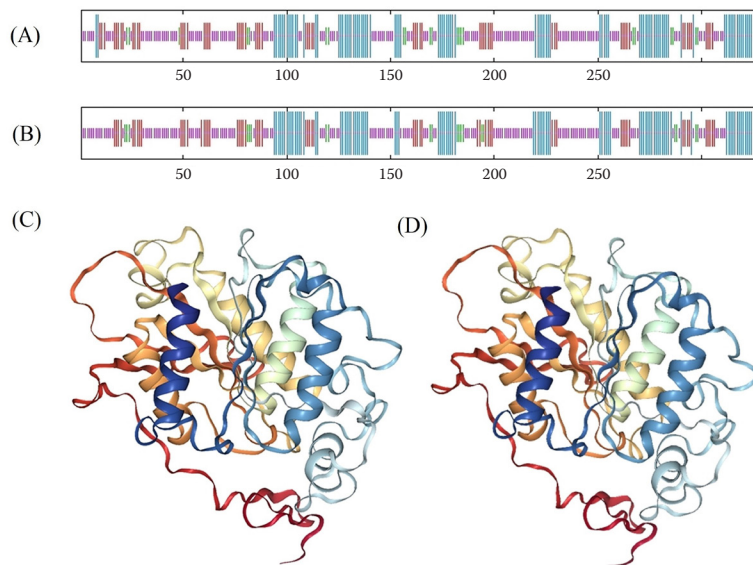


Figure S3. Predicted secondary structure of the HvnHID protein of the variety Nerumuzha (A) and the variety Kunlun 10 (B) and the tertiary structure of the HvnHID protein of the Nerumuzha (C) and Kunlun 10 (D) barley

In (A) and (B), the blue colour denotes an  $\alpha$ -helix, red denotes an extended chain, green denotes a  $\beta$ -turn, and orange denotes a random coil

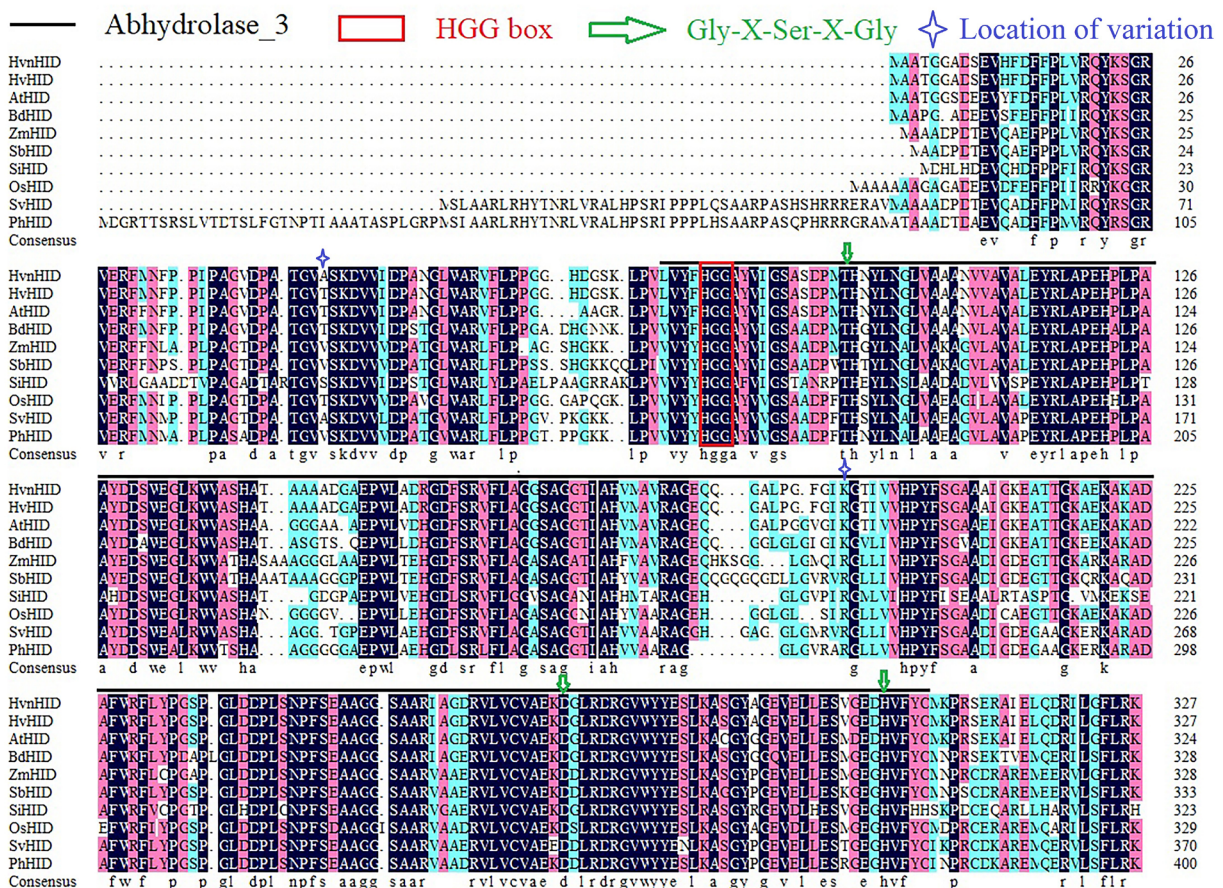


Figure S4. Sequence alignment of the HID proteins from the hulless barley and other gramineous plants

Hvn – hulless barley; Hv – *Hordeum vulgare*; At – *Aegilops tauschii*; Bd – *Brachypodium distachyon*; Zm – *Zea mays*; Sb – *Sorghum bicolor*; Si – *Setaria italica*; Os – *Oryza sativa*; Sv – *Setaria viridis*; Ph – *Panicum hallii*; the black line indicates the Abhydrolase\_3 domain, the red box indicates the HGG motif, the stars indicate the Gly-X-Ser-X-Gly sequences, and purple four-pointed stars indicate location of variants

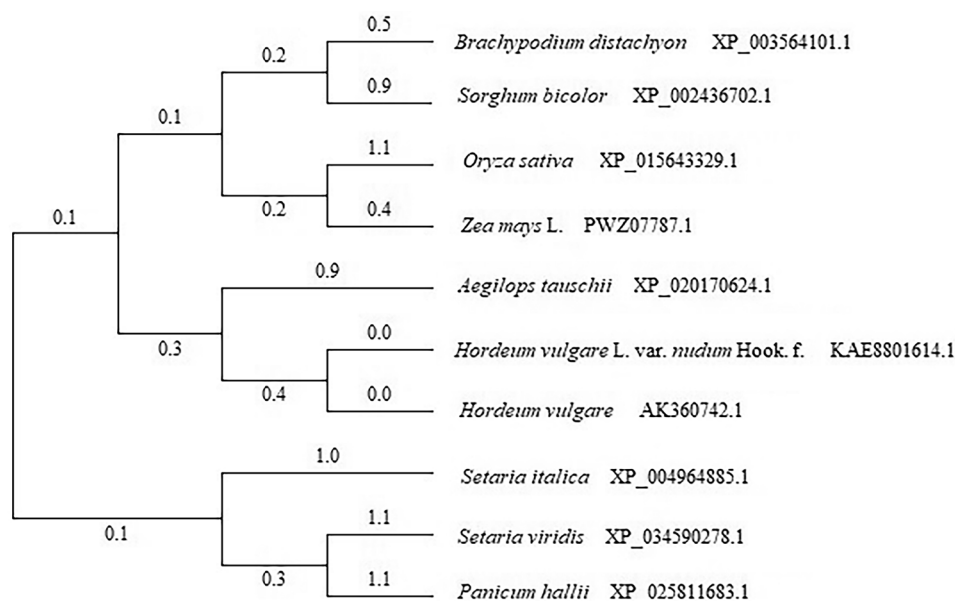
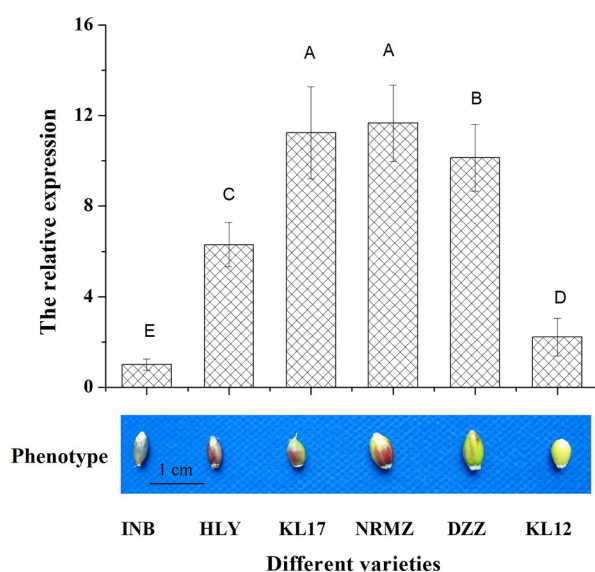
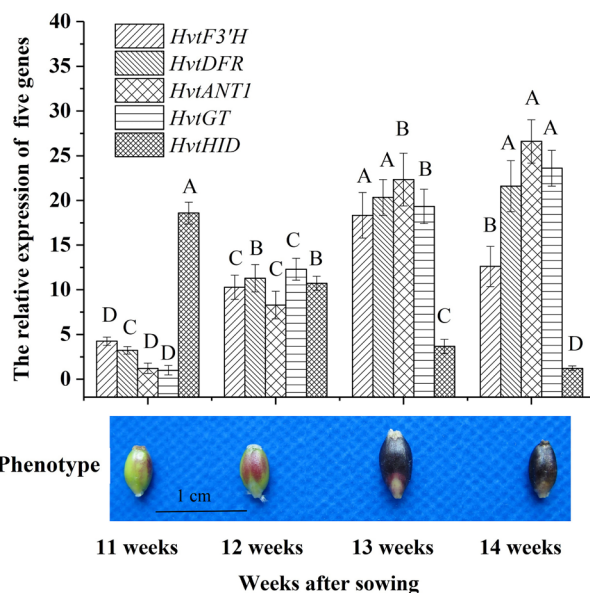


Figure S5. Phylogenetic tree of the HID proteins from the hulless barley and other gramineous plants

Figure S6. Expression level of the *HvHID* gene in the seeds of the coloured hulless barley varieties at the early-mid stage of grain filling

INB – INB0N-7; HLY – Heilaya; KL17 – Kunlun 17; NRMZ – Nerumuzha; DZZ – Dazhangzi; KL12 – Kunlun 12; the columns represent the mean value of three replicates and the bars represent the standard deviation; different capital letters indicate  $P < 0.01$ ; the horizontal line in the phenotype is the scale

Figure S7. Expression level of the *HvHID* gene in the seeds of the purple-grained hulless barley during the seed colour development

Seeds were sampled at the 11, 12, 13, and 14 weeks after sowing; different capital letters indicate  $P < 0.01$ ; the horizontal line in the phenotype is the scale

Figure S8. The role of the *HvnHID* gene in the anthocyanidin biosynthesis