

Supplementary Materials

Figure S1. Identification of the *AtHFR1* transgenic lines and morphology of non-transgenic cv. Fielder (WT) and *AtHFR1* transgenic lines under different light-conditions, high-R/FR, low-R/FR, high-B or low-B conditions.

Figure S2. Relative expression level of *AtHFR1* in transgenic wheat lines.

Figure S3. Relative expression levels of *TaFT1*, *TaCO1* and *TaCO2*.

Figure S4. Grain length and width of the WT (cv. Fielder) and *AtHFR1* transgenic lines.

Figure S5. Morphology and chlorophyll contents of the WT (cv. Fielder) and *AtHFR1* transgenic lines (#498 and #511) under long-day condition for 20 days.

Table S1. Internode lengths of *AtHFR1* transgenic lines.

Table S2. Agronomic traits of *AtHFR1* transgenic lines.

Table S3. List of primers used in this study.

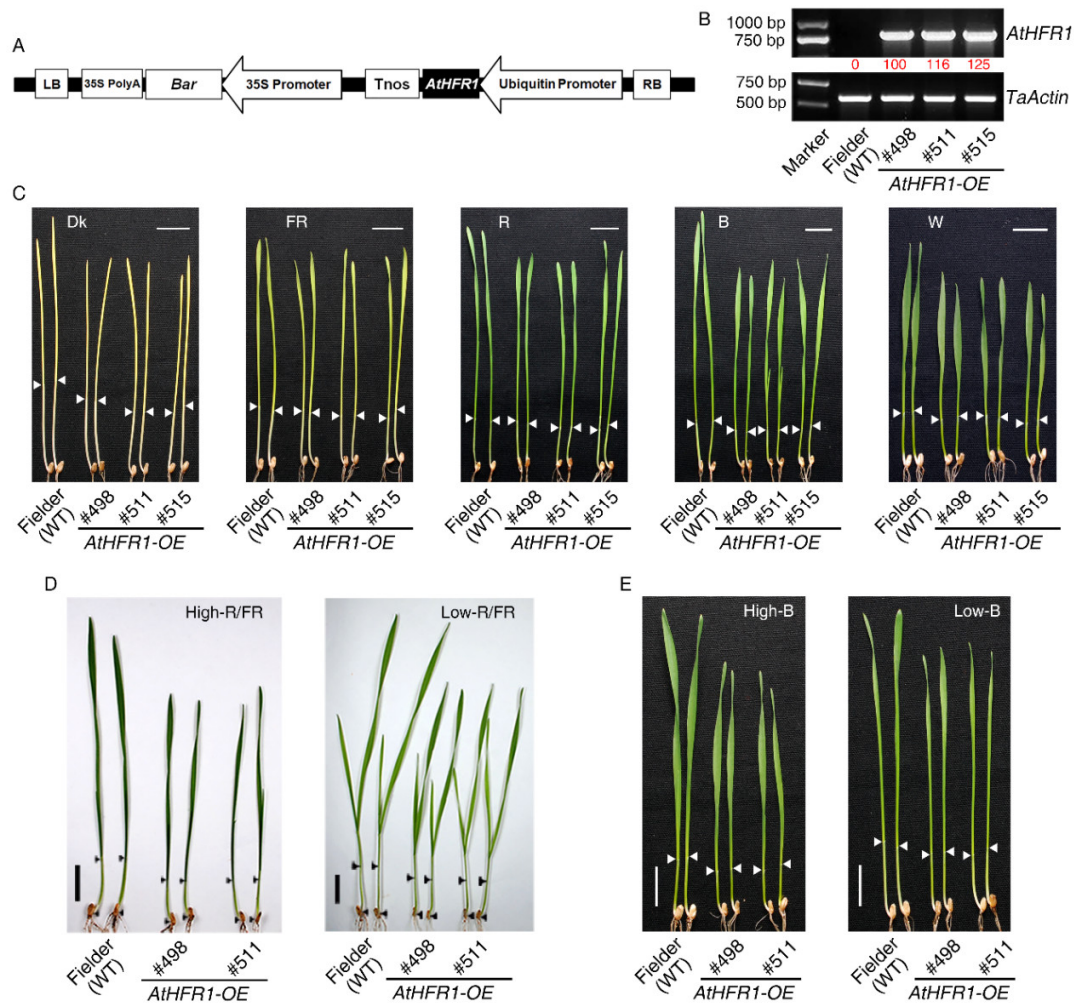


Figure S1. Identification of the *AtHFR1* transgenic lines and morphology of non-transgenic cv. Fielder (WT) and *AtHFR1* transgenic lines under different light-conditions, high-R/FR, low-R/FR, high-B or low-B conditions.

(A) Information of recombinant plasmid *pBCXUN-AtHFR1*. (B) Semi-RT-PCR analysis for *AtHFR1* expression in the WT and *AtHFR1* transgenic lines (#498, #511, and #515). Numbers under lanes indicate the relative band intensities to quantify *Actin* and are normalized for each panel. (C) Morphology of the WT and the *AtHFR1* transgenic lines (#498, #511, and #515). Seedlings were grown under dark (Dk), far-red (FR, $10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), red (R, $15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), blue (B, $15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) or white (W, $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) light condition for 7 d. Bar = 2 cm. (D) Morphology of the Fielder and the *AtHFR1* transgenic lines (#498 and #511) under high-R/FR (R, $96 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; FR, $21 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; B, $15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) or low-R/FR (R $12 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; FR $105 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; B $15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 7 d. Bar = 2 cm. (E) Morphology of the Fielder and the *AtHFR1* transgenic lines (#498 and #511) under high-B ($50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) or low-B ($2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 7 d. Bar = 2 cm. Triangles in (C), (D) and (E) are showing the positions of coleoptile.

Related to Figure 1.

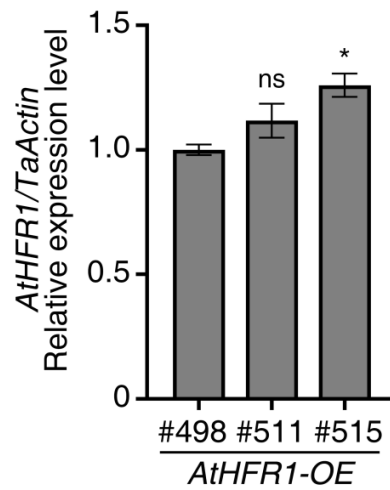


Figure S2. Relative expression levels of *AtHFR1* in transgenic wheat lines.

Seedlings grown under long-day condition for 8 days were used to analyze *AtHFR1* expression level. Data are means \pm SEs, $n = 3$. ns indicates no significant difference and asterisks denote significant differences of #498, #511, or #515 with the WT according to ANOVA (* $P < 0.05$; ** $P < 0.01$).

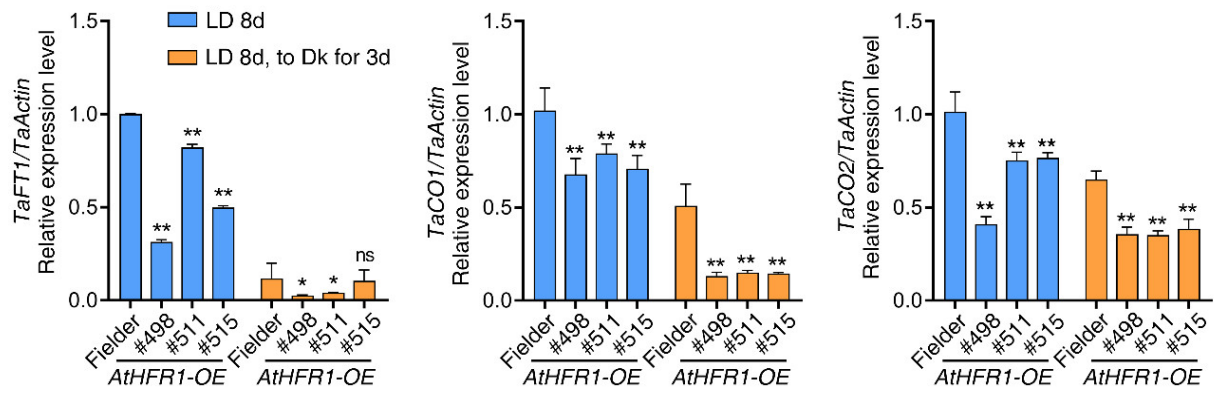


Figure S3. Relative expression levels of *TaFT1*, *TaCO1* and *TaCO2*.

Seedlings were grown under long-day conditions for 8 days, and then transferred to the dark for 3 days to eliminate the effect of photoperiod on gene expression. Data are means \pm SEs, $n = 3$. Asterisks denote significant differences of #498, #511, or #515 with the WT according to ANOVA (ns indicates no significant difference; * $P < 0.05$; ** $P < 0.01$).

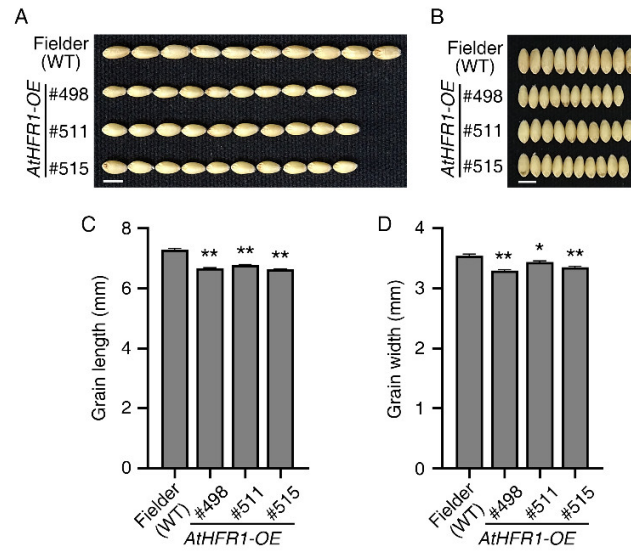


Figure S4. Grain length and width of the WT (cv. Fielder) and *AtHFR1* transgenic lines.

Grain morphology showing grain length (A) and grain width (B) and quantification of grain length (C) and grain width (D) of the WT and *AtHFR1* transgenic lines (#498, #511, and #515). Data are means \pm SEs, $n = 15$. Asterisks denote significant differences of #498, #511, or #515 with the WT according to ANOVA (* $P < 0.05$; ** $P < 0.01$).

Related to Figure 4.

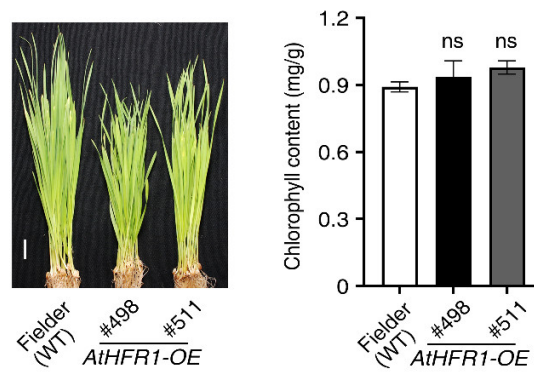


Figure S5. Morphology and chlorophyll contents of the WT (cv. Fielder) and *AtHFR1* transgenic lines (#498 and #511) under long-day condition for 20 days.

Data are means \pm SEs, $n \geq 5$. ns indicates no significant difference of #498 or #511 with Fielder according to ANOVA.

Related to Figure 6.

Table S1. Internode lengths (cm) of *AtHFR1* transgenic lines.

			<i>AtHFR1-OE</i>		
Fielder (WT)			#498	#511	#515
Internode (top to bottom)	spike	13.1 ± 1.28	12.07 ± 0.76 *	12.1 ± 1.25 *	13.11 ± 1.21
	1st	37.15 ± 4.96	27.14 ± 1.88 **	26.43 ± 3.46 **	28.79 ± 4.32 **
	2nd	25.16 ± 1.68	19.26 ± 1.26 **	20.52 ± 1.25 **	20.53 ± 3.03 **
	3rd	19.3 ± 2.47	14.48 ± 1.54 **	15.71 ± 2.43 **	16.78 ± 2.22 **
	4th	13.3 ± 2.81	10.1 ± 1.15 **	9.38 ± 3.03 **	10.63 ± 1.8 **
	5th	8.62 ± 3.64	4.37 ± 1.42 **	3.82 ± 1.82 **	4.16 ± 1.34 **

Materials were planted in field (near Zhengzhou, $\approx 34.9^\circ$ N, $\approx 113.6^\circ$ E) from Oct. 2019 to May 2020. After harvest, Internode lengths were measured. Data are means \pm SDs, Asterisks denote significant differences of #498, #511, or #515 with the WT according to ANOVA (* $P < 0.05$; ** $P < 0.01$).

Table S2. Agronomic traits of *AtHFR1* transgenic lines.

	Fielder (WT)	<i>AtHFR1</i> -OE		
		#498	#511	#515
Plant height (cm)	116 ± 4.87	87.66 ± 2.30 **	87.11 ± 3.86 **	93.24 ± 3.96 **
Spikes per plant	22.45 ± 6.81	35.67 ± 10.36 **	34.41 ± 7.66 **	34.28 ± 7.85 **
Heading data (d)	181.1 ± 1.78	192.2 ± 0.73 **	190.8 ± 1.24 **	189.2 ± 1.49 **
Thousand-gain weight (g)	42.88 ± 4.96	30.27 ± 2.38 **	36.58 ± 2.50 *	35.91 ± 3.16 *
Grain number per spike	64.41 ± 6.37	59.59±3.89 *	61.35 ± 3.42	60.76 ± 5.77
Grain yield per plant (g)	39.23 ± 3.61	46.92 ± 4.17 **	49.57 ± 7.12 *	47.84 ± 6.0 *
Grain length (mm)	7.381 ± 0.08	6.45 ± 0.1 **	6.45 ± 0.06 **	6.357 ± 0.11 **
Grain width (mm)	3.45 ± 0.11	3.186 ± 0.04 **	3.286 ± 0.06 *	3.243 ± 0.05 **
Length of flag leaf (mm)	235.2 ± 12.95	156.6 ± 8.43 **	160.3 ± 7.04 **	163.4 ± 6.49 **
Width of flag leaf (mm)	22.06 ± 1.12	17.69 ± 0.6 **	16.5 ± 0.63 **	15.19 ± 0.75 **

Materials were planted in field (near Zhengzhou, $\approx 34.9^\circ$ N, $\approx 113.6^\circ$ E) from Oct. 2019 to May 2020. The agronomic traits were investigated according to the method described in 4.3. Data are means \pm SDs. Asterisks denote significant differences of #498, #511, or #515 with the WT according to ANOVA (* $P < 0.05$; ** $P < 0.01$).

Table S3. List of primers used in this study

Gene	Primer	Sequence (5'→3')	Use	Reference
<i>AtHFR1</i> (At1g02340)	AtHFR1-F1	ATGTCGAATAATCAAGCTTTC	Gene cloning and vector construction	
	AtHFR1-R1	TCATAGTCTTCTCATCGCATG		
	AtHFR1-828R	AGGAACCAAACCGTGAAG	RT-PCR identification of transgenic lines	
	AtHFR1-28F	TTGGGATGGAGAAACGAC		
<i>TaACTIN</i> (AB181991.1)	TaAct-F2	CTATCCTTCGTTTGGACCTTG	As an internal control of RT-PCR	
	TaAct-R2	CGGGACCAGACTCATCGT		
	Ta-Act F	TACTCCCTCACAACAACC	As an internal control of real-time PCR	
	Ta-Act R	GCTCCTGCTCATAATCAAG		
<i>TaCAB</i> (XM_044587543.1)	TaCAB-QF1	AAGGTGAAGGAAATCAAGAAC	Marker gene for photomorphogenesis	
	TaCAB-QR1	ACCCTTACCAGTGACGAT		
<i>TaCHS</i> (XM_044598705.1, XM_044526325.1)	TaCHS-QF1	ACTACTACTTCAGGGTCACC	Marker gene for photomorphogenesis	
	TaCHS-QR1	ACTTCTCGCACATCCTCT		
<i>TaFT1</i> (DQ890162.1)	TaFT-F3	CAGCAGCCCAGGGTTGAG	Marker genes for heading	Yan et al., 2006
	TaFT-R3	ATCTGGGTCTACCATCACGAGTG		
<i>TaCO1</i>	CO1-AB-F3	CACATCAGAGTGGTTATGC	Marker genes for heading	Chen et al., 2014
	CO1-AB-R3	GGACTGGACCGTATTGTC		
<i>TaCO2</i>	CO2-AB-SYB-F4	AAGGGTGTGAGTGTGTAG	Marker genes for heading	Chen et al., 2014
	CO2-AB-SYB-R4	GATATGTCATTGCTGATGGAAG		
<i>TaSGR</i> (XM_044526410.1, XM_044533918.1, XM_044542480.1)	TaSGR-QF	CGTCCACTGCCACATCTCCG	Real-time PCR	
	TaSGR-QF	CACGAACGCCTTCAGAACCAC		

<i>TaPIL13</i> (XM_044530914.1, XM_044530915.1)	PIL13-QF PIL13-QR	GACCTCTTCTCGGAGATC TTCGCTGCTTATCATCCT	Real-time PCR
<i>TaPIL15-1B</i> (XM_044531643.1, XM_044531644.1)	PIL15-1B-QF PIL15-1B-QR	GTCGGAGAGGAGGAGAAG ACGCCTTGTCATCTTGT	Real-time PCR
<i>TaPIL15-1D</i> (XM_044592408.1, XM_044592409.1)	PIL15-1D-QF PIL15-1D-QR	GAGAAGGGACCGAATCAA GCCTTGTCGATCTTGTTG	Real-time PCR

Reference

- Yan, L., Fu, D., Li, C., Blechl, A., Tranquilli, G., Bonafede, M., Sanchez, A., Valarik, M., Yasuda, S. and Dubcovsky, J. (2006) The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. *Proc. Natl. Acad. Sci. USA*, **103**, 19581-19586.
- Chen, A., Li, C., Hu, W., Lau, M.Y., Lin, H., Rockwell, N.C., Martin, S.S., Jernstedt, J.A., Lagarias, J.C. and Dubcovsky, J. (2014) Phytochrome C plays a major role in the acceleration of wheat flowering under long-day photoperiod. *Proc. Natl. Acad. Sci. USA*, **111**, 10037-10044