Plant Physiology **Supporting Information**

Article title: ***Photoperiod-H1* (*Ppd-H1*) controls leaf size in barley**

Authors: **Benedikt Digel, Elahe Tavakol, Gabriele Verderio, Alessandro Tondelli, Xu X, Luigi Cattivelli, Laura Rossini, Maria von Korff**

The following Supporting Information is available for this article:

Fig. S1. Principal component analysis (PCA) plot based on the first two principal axes with spring and facultative genotypes and *Ppd-H1* variants indicated in colour.

Fig. S2. Intra-chromosomal LD decay of markers pairs over all chromosomes as a function of genetic distance.

Fig. S3. Manhattan plots of GWAS for flowering date (FD), leaf length (LL) and leaf width (LW) calculated for Iran and Italy separately.

Fig. S4. Manhattan plots of GWAS for leaf length (LL) and leaf width (LW) with flowering time (FD) as a covariate.

Fig. S5. Size and flanking markers of *Ppd-H1* introgressions in three independent introgression lines.

Fig. S6. Heading date is delayed in the presence of the mutated *ppd-H1* allele under LDs.

Fig. S7. Leaf length and leaf emergence of Bowman/BW281 and Triumph/Triumph-IL.

Fig. S8. Variation at *Ppd-H1* does not affect the rate of leaf elongation.

Fig. S9. Leaf blade anatomy of the 5^{th} leaf emerging from the main shoot of SD grown plants.

Fig. S10. Variance explained by the first 10 principal components for the genetic diversity of the winter barley collection.

Table S1 Analysis of variance for flowering date (FD), leaf width (LW) and leaf length (LL).

Table S2. Genetic material and genotyping information **[provided as Excel table]**

Table S3. *Ppd-H1* haplotypes in the introgression lines

Table S4. Variation at *Ppd-H1* affects the phyllochron

Table S5. Variation at *Ppd-H1* does not affect the rate of leaf blade elongation

Table S6. Primers used for genotyping and Real Time qRT-PCR assays

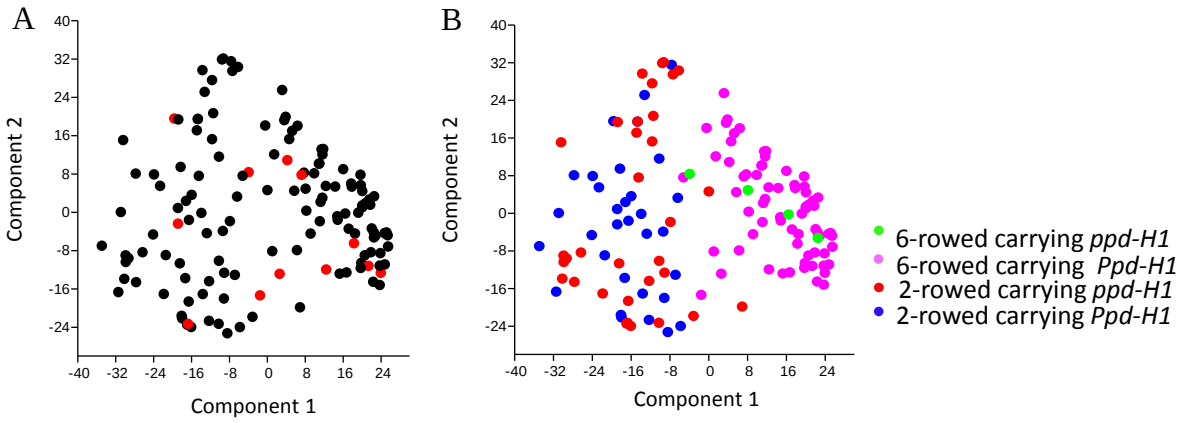

Fig. S1 Principal component analysis (PCA) plot of 138 barley cultivars based on the first two principal axes (component 1 = 12% and component 2 = 8%). A) Genotypes with a spring *Vrn-H1* allele or a deletion of the *Vrn-H2* locus are indicated in red. B) Genotypes are coloured according to row-type and *Ppd-H1* allele.

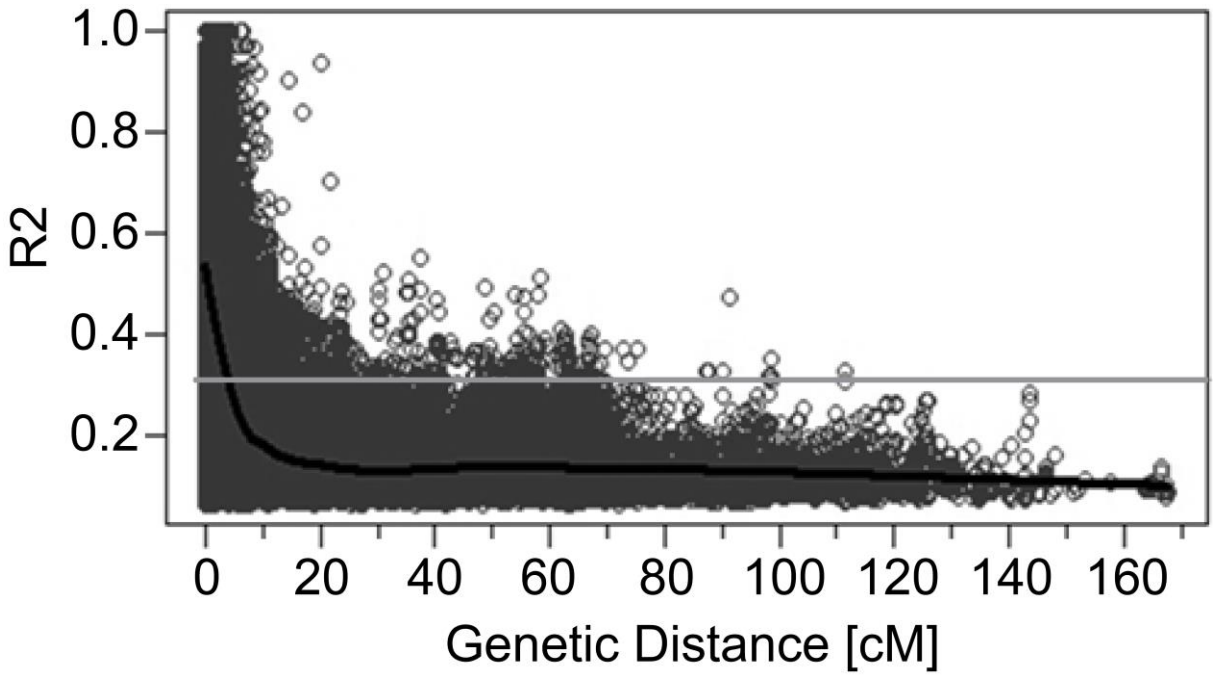

Fig. S2 Intra-chromosomal LD decay of markers pairs over all chromosomes as a function of genetic distance. The fitted LOESS curve (black line) illustrates the LD decay, and the horizontal line represents the critical r^2 value

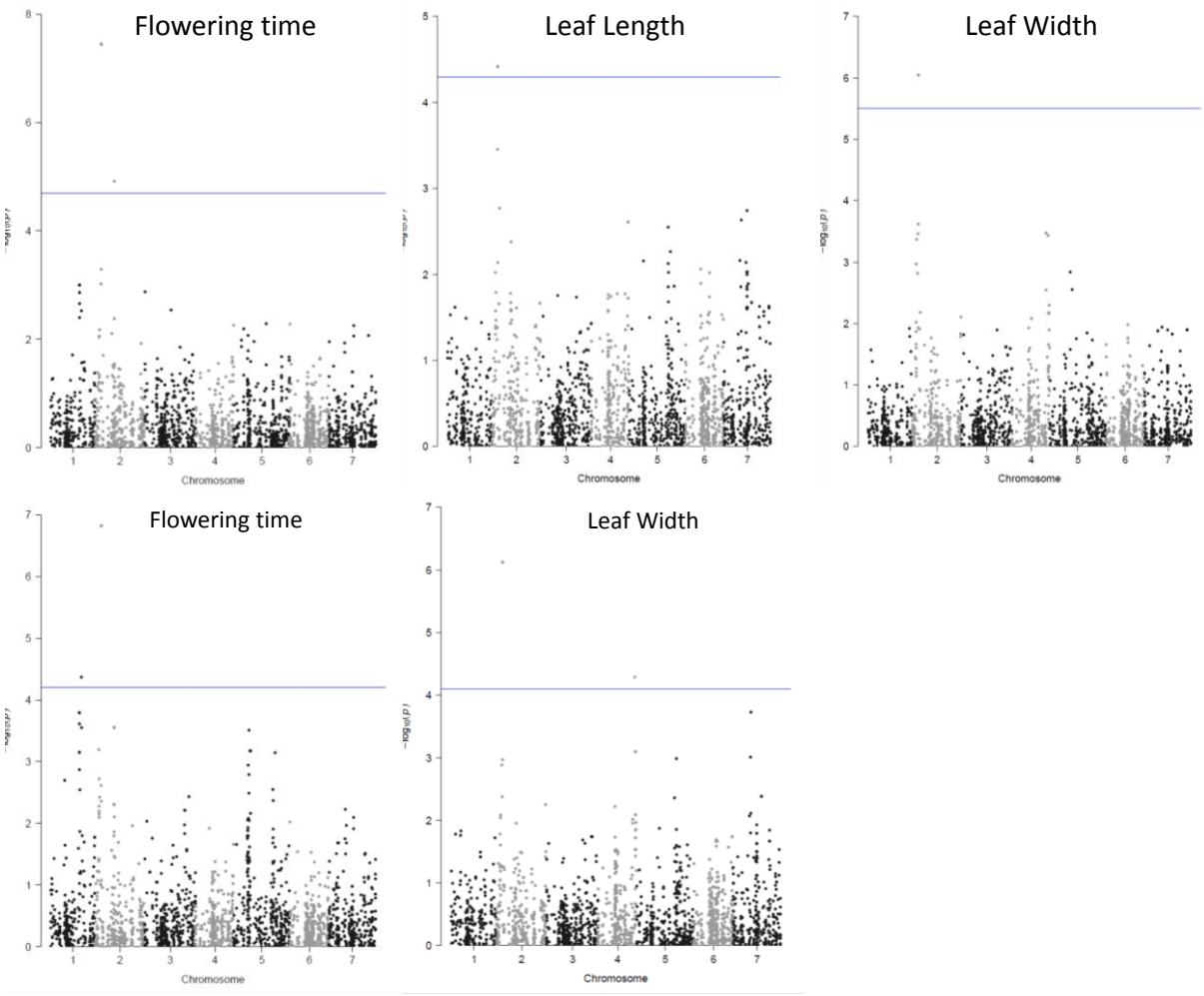

Fig. S3 Manhattan plots of GWAS for flowering date (FD), leaf length (LL) and leaf width (LW) scored in Iran (upper panel) and in Italy (lower panel) in the barley cultivar collection. The -$\log_{10}$ (p-values) from the association scans are plotted against the SNP marker positions on each of the seven barley chromosomes. The dashed horizontal line indicates the genome-wide significance threshold at FDR < 0.05.

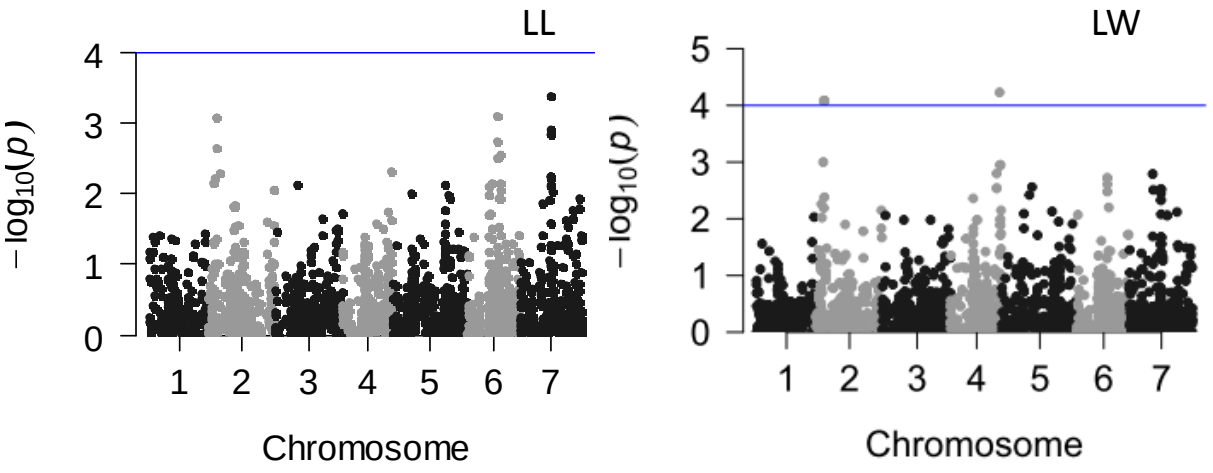

Fig. S4 Manhattan plots of GWAS for leaf length (LL) and leaf width (LW) with flowering time (FD) as a covariate based on data of the barley cultivar collection scored in both locations. The -$\log_{10}$ (p-values) from the association scans are plotted against the SNP marker positions on each of the seven barley chromosomes. The dashed horizontal line indicates the genome-wide significance threshold at FDR < 0.05.

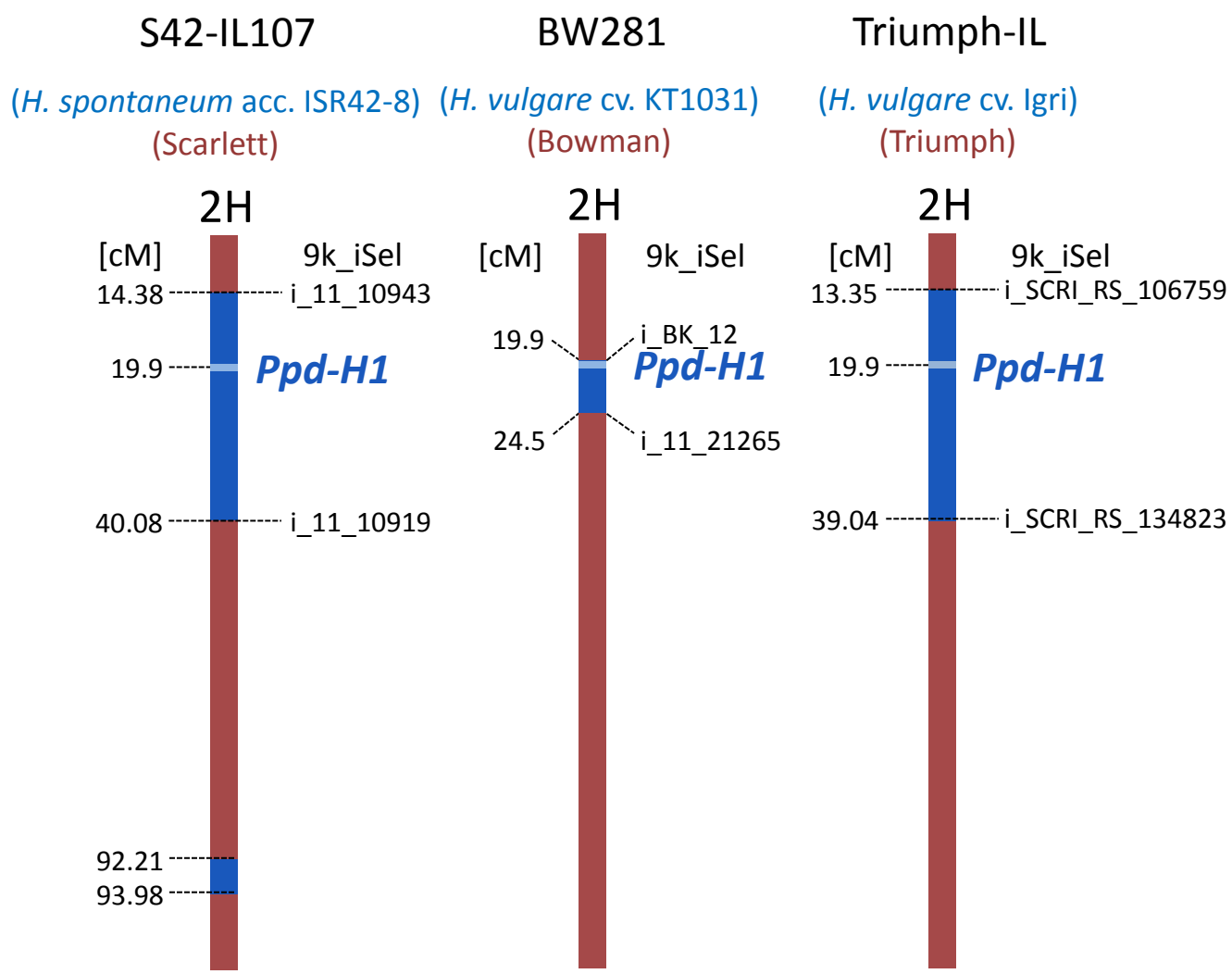

Fig. S5 Size and flanking markers of *Ppd-H1* introgressions on chromosome 2H.
Donor parents for the photoperiod-responsive *Ppd-H1* allele of the introgression lines and their respective spring barley background genotypes are indicated above each chart. Introgression lines were genotyped with the 9k iSelect chip from Illumina. Flanking marker positions of the introgressions are given relative to the POPSEQ map (Mascher *et al.*, 2013).

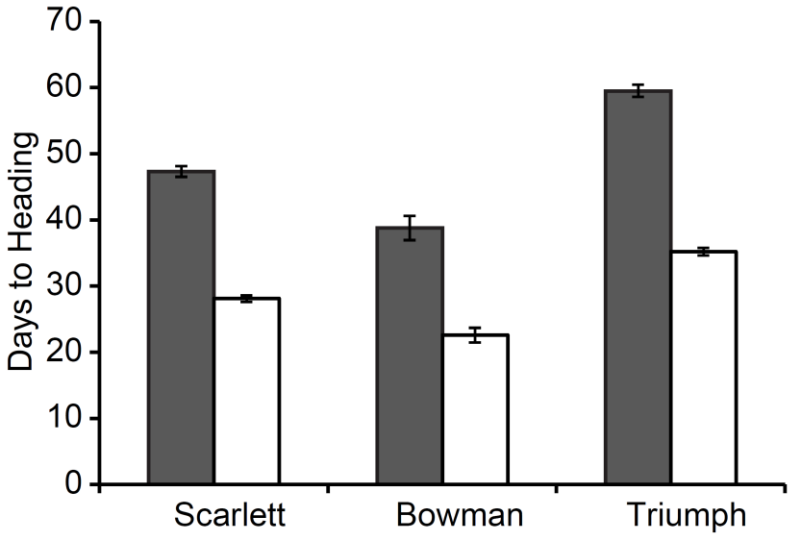

Fig. S6 Heading date is delayed in the presence of the mutated *ppd-H1* allele under LDs. Heading date of three LD grown spring barley genotypes with the mutated *ppd-H1* allele (grey bars) and introgression lines for the photoperiod responsive *Ppd-H1* allele (white bars). Bars indicate means with 95%-confidence intervals.

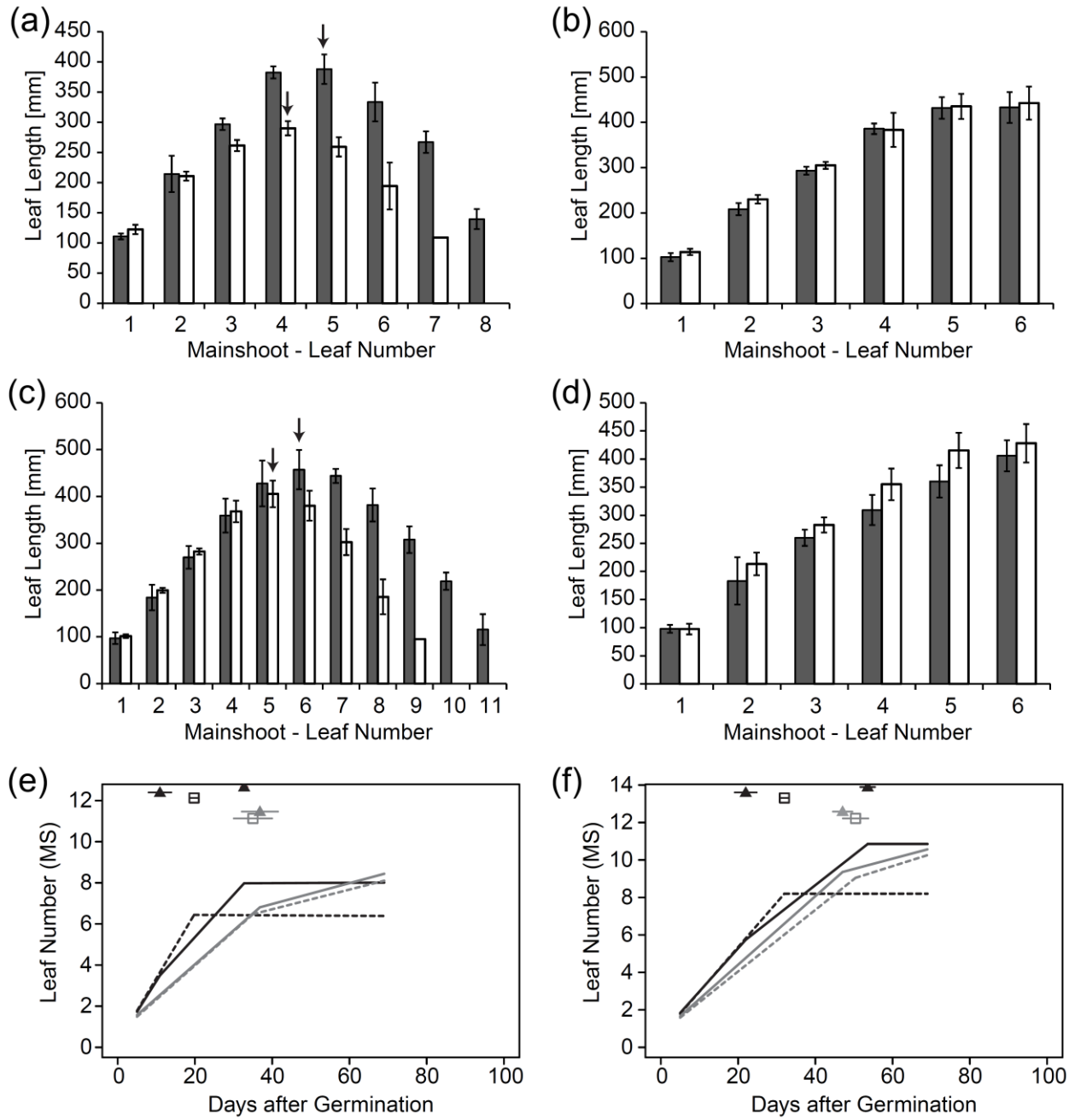

Fig. S7 Leaf length and leaf emergence of Bowman/BW281 and Triumph/Triumph-IL. **(a-d)** Analysis of leaf length of every leaf on the main shoot of plants grown under **(a, c)** LD and **(b, d)** SD conditions. Leaf length and leaf emergence is shown for the genotpyes **(a, b)** Bowman/BW281 and **(c, d)** Triumph/Triumph-IL with the spring barleys and introgression lines being represented by grey and white bars, respectively. Arrows indicate the longest leaf per genotype. Bars represent means with 95%-confidence intervals. **(e, f)** Number of leaves emerging from the leaf sheath per

time unit after germination in **(e)** Bowman/BW281 and **(f)** Triumph/Triumph IL, when spring barley genotypes (solid line, triangle) and introgression lines for the wild type *Ppd-H1* allele (dashed line, square) were grown under LDs (black) and SDs (grey). Breakpoints of the regression model and their 95%-confidence intervals are indicated for the different genotypes and conditions above the regression curves.

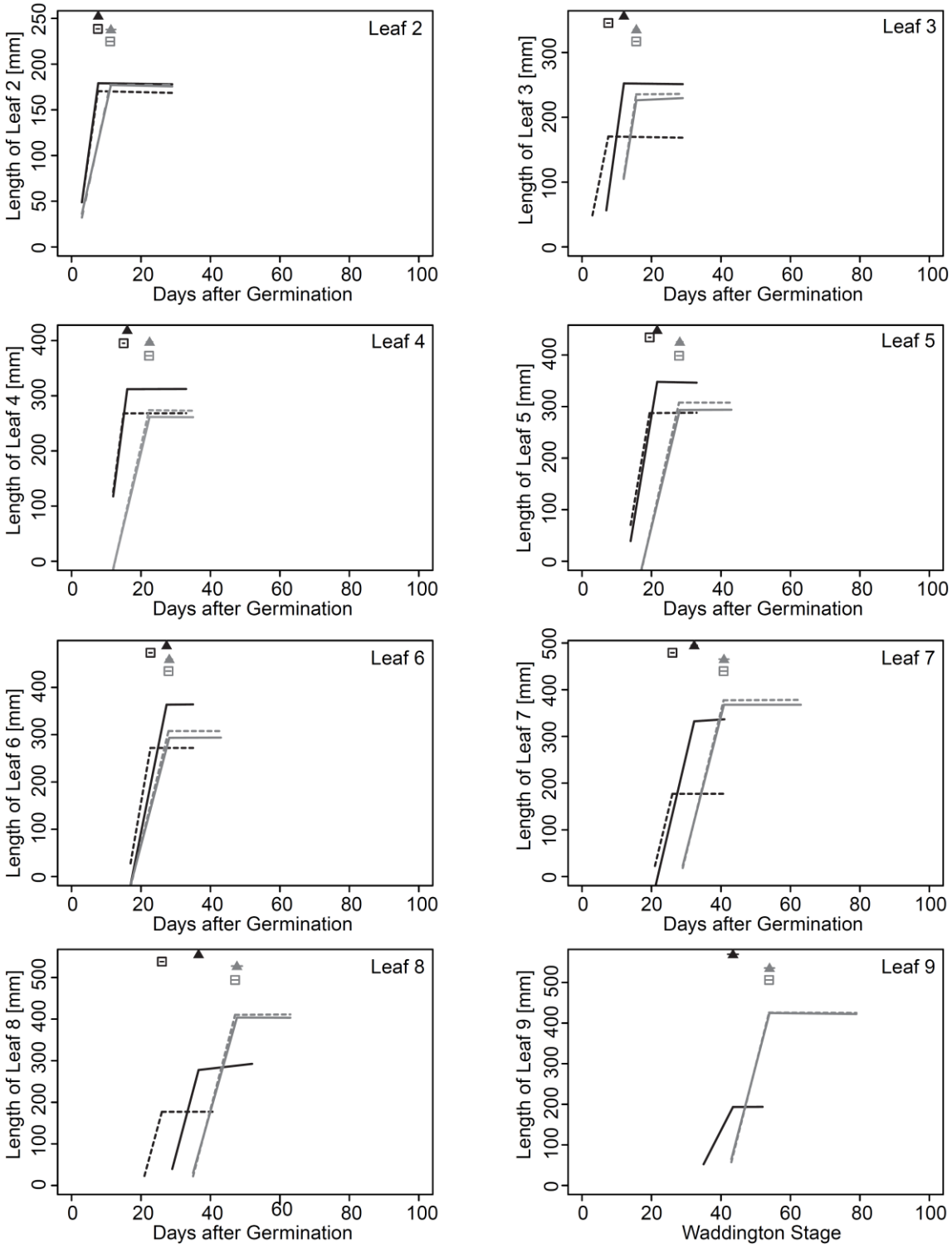

Fig. S8 Variation at *Ppd-H1* does not affect the rate of leaf elongation. Leaf length was measured every three days under LDs (black) and every four days under SDs (grey) in Scarlett (solid line, triangle) and S42-IL107 (dashed line, square). Breakpoints of the regression model and their 95%-confidence intervals are indicated for the different genotypes and conditions above the regression curves.

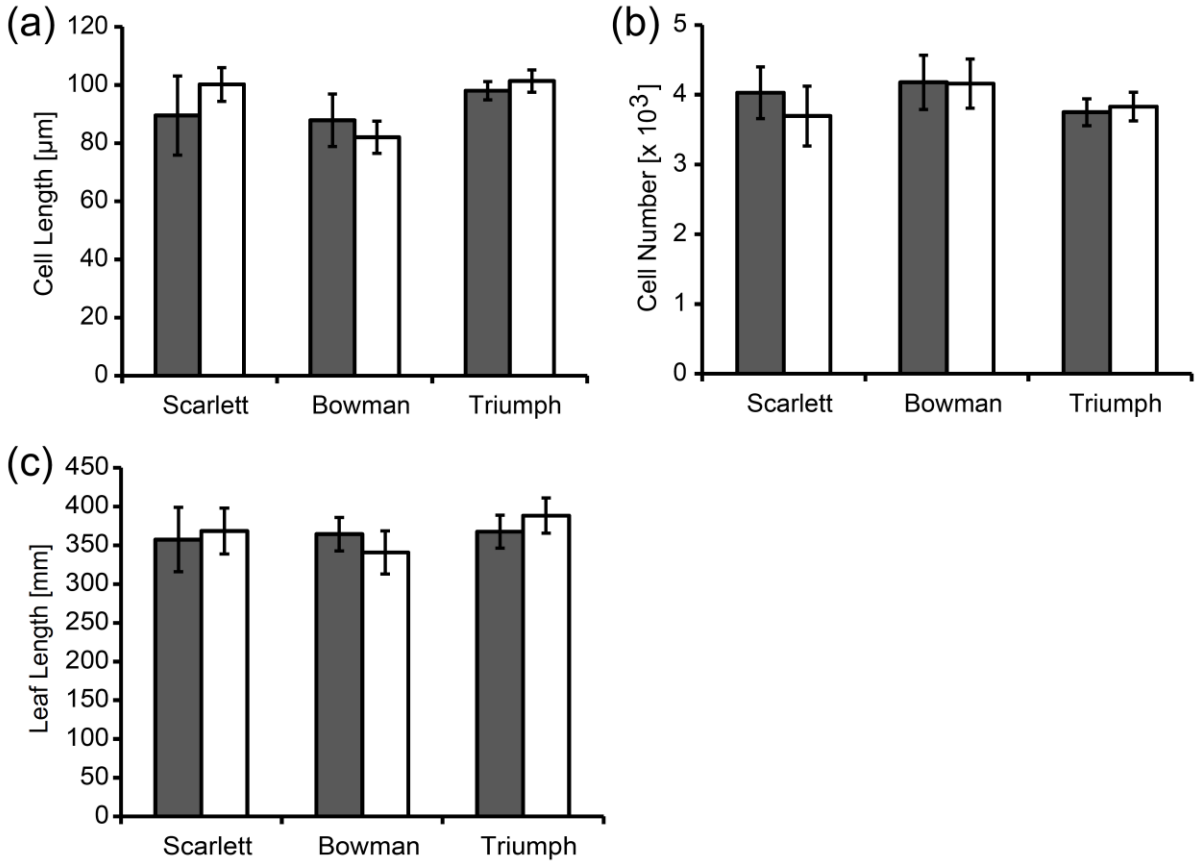

Fig. S9 Leaf blade anatomy of the 5th leaf emerging from the main shoot of SD grown plants. **(a)** Cell length, **(b)** estimated cell number and **(c)** final leaf blade length of spring barley genotypes with the mutated *ppd-H1* allele (grey bars) as compared to their respective ILs with the photoperiod responsive *Ppd-H1* allele (white bars). Bars represent means +/- 95%-confidence intervals (n=5).

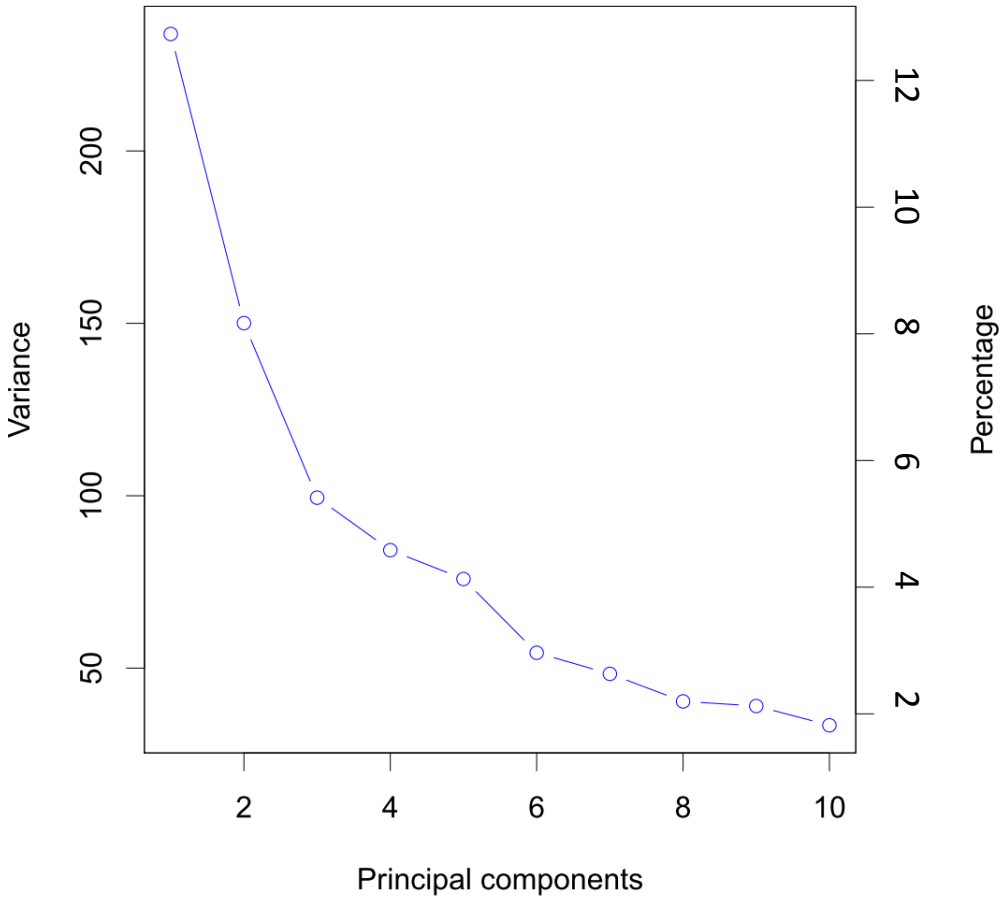

Fig. S10 Variances and percentage of overall variance explained by the first 10 principal components. The first 3 components of the PCA explain collectively 24.1% of the phenotypic variation.

Table S1: Analysis of variance for flowering date (FD), leaf width (LW) and leaf length (LL).

	FD							LW							LL						
Factor	DF	SS[a]	MS[b]	F value	Sig	R^{2c}	R^2_{Ppd-H1}	DF	SS	MS	F value	Sig	R^2	R^2_{Ppd-H1}	DF	SS	MS	F value	Sig	R2	R^2_{Ppd-H1}
A. Full interaction model																					
Genotype (G)	137	11119	81	44	***	0.82		137	2538	19	14	***	0.31		137	113501	829	8.2	***	0.80	
Environment (E)	1	384	384	209	***	0.03		1	4736	4736	3618	***	0.58								
Replicate(E)	4	128	32	17	***	0.01		4	22	6	4	**	0.00		2	1700	850	8.4	***	0.01	
G*E	129	993	8	4	***			137	174	1	1	ns									
Residuals	513	943	2					537	703	1					269	27230	101				
B. Partitioning G+GE using Ppd-H1																					
Ppd-H1	1	2563	2563	41	***		0.23	1	137	137	8	**		0.05	1	7337	7337	9.4	**		0.06
Residual Genotype	136	8556	63	8	***			136	2401	18	14	***			136	106164	781	7.7	***		
Ppd-H1*Env	1	21	21	3	ns			1	4	4	3	ns									
Residual Gen*Env	128	972	8	4	***			136	170	1	1	ns									

[a] Sums of squares, MS= means square, [c]R^2 war calculated by dividing the sums of squares of the respective factor by the overall variation for the trait, $R^2_{Ppd-H1} = SS_{Ppd-H1} / SS_g$

Table S3 *Ppd-H1* haplotypes in the introgression lines

SNP[a]	SNP[b]	Alleles	Position in AY943294.1	iSelect Marker	part of the gene	AA change	Bowman	Scarlett	Triumph	BW821	ISR42-8 S42-IL107	Igri Trium-ph-IL	Winter barley collection:SNP associated with increase in LW and LL	Winter barley collectionSNP associated with decrease in LW and LL
SNP12	1	G-C	51	BK_16	exon PRR-domain	Gln to His	G	G	G	C	C	C	G	C
SNP41	12	C-A	1843	12_30870	exon	Asn to Lys	C	C	C	A	A	A	C	A
SNP68	19	G-A	2721	12_30871 and BK_14	exon	Thr to Ala	A	A	A	G	G	G	A	G
SNP79 *	22	G-T	3081	nd	exon CCT-domain	Trp to Gly	T	T	T	G	G	G	T	G
SNP81	23	G-A	3114	BK_15	exon CCT-domain	Thr to Ala	A	A	A	G	G	G	A	G

[a] Number of polymorphism according to Jones et al. (2008), [b] number of polymorphism according to Turner et al. (2005), * SNP associated with flowering time in Turner et al. (2005).

Table S4 Variation at *Ppd-H1* affects the phyllochron

Photoperiod	Genotype	Leaf No.	Phyllochron[1] [days]	95%-CI
Long Day	Bowman	1 - 3	3.4	2.7 - 4.0
		4 - 8	4.9	4.7 - 5.1
	BW281	1 - 7	3.2	2.8 - 3.5
	Triumph	1 - 6	4.3	4.1 - 4.6
		7 - 11	6.2	5.9 - 6.5
	Triumph-IL	1 - 7	4.2	4.0 - 4.4
Short Day	Bowman	1 - 7	6.1	5.4 - 6.8
		8 - 9	19.7	12.2 - 27.3
	BW281	1 - 7	6.0	5.2 - 6.8
		8 - 9	20.9	11.6 - 30.1
	Triumph	1 - 9	5.5	5.2 - 5.7
		10 - 11	18.2	12.1 - 24.3
	Triumph-IL	1 - 9	6.1	5.9 - 6.3
		10 - 11	15.4	9.7 - 21.0

[1] Phyllochron was calculated as the leaf emergence rate^{-1} from the slopes of the linear segments of the regression lines presented in Fig. 4C, Fig. 3E and F.

Table S5 Variation at *Ppd-H1* does not affect the rate of leaf blade elongation

Photoperiod	Genotype	Leaf No.	Leaf Blade Elongation[1] [mm*day^{-1}]	95%-CI
Long Day	Scarlett	2	27.7	25.4 - 30.1
		3	38.7	36.5 - 40.9
		4	48	40.2 - 55.8
		5	40.4	38.5 - 42.4
		6	36.7	35.2 - 38.2
		7	31.6	28.5 - 34.7
		8	31.4	28.2 - 34.6
		9	16.7	14.0 - 19.4
	S42-IL107	2	26.5	23.7 - 29.4
		3	33.2	31.4 - 35.0
		4	47.6	42.0 - 53.1
		5	39.6	37.2 - 42.1
		6	42.5	38.7 - 46.3
		7	30.7	27.3 - 34.1
Short Day	Scarlett	2	16.9	13.6 - 20.2
		3	33.3	24.8 - 41.8
		4	26.2	24.2 - 28.3
		5	27.9	25.5 - 30.4
		6	22.6	18.4 - 26.8
		7	29.1	25.1 - 33.0
		8	29.6	26.0 - 33.2
		9	32.8	29.2 - 36.3
	S42-IL107	2	17.9	15.1 - 20.6
		3	35.6	27.9 - 43.4
		4	28	26.2 - 29.8
		5	30.1	28.0 - 32.2
		6	24.5	21.2 - 27.8
		7	30.5	27.4 - 33.7
		8	32	29.2 - 34.9
		9	34	31.1 - 36.8

[1] Rate of leaf blade elongation calculated from the slopes of the regression line segments presented in Fig. 5a and Fig. S4.

Table S6 Primers used for genotyping and Real Time qRT-PCR assays

Gene Name	GenBank ID	Marker Name	Primer Sequence	Reference
Genotyping				
Vrn-H1	AY785826.1	HvBM5A-intronI-F3b HvBM5A-intronI-R3b	5'-CTTGCATGTGTTGTCGGTCT 5'-GCTGGGACAAGACTCTACGG	Cockram et al. 2009
		HvBM5A-intronI-F1 HvBM5A-TE-R1	5'-GTTCTCCACCGAGTCATGGT 5'-AGAGATGGAGGCATGGAGCA	
		HvBM5A-exon2-F1 HvBM5A-exon2-R	5'-TCCCAAGAAAACTTGAACAACACCAG 5'-ATTAGGTTACATCATTCGACCA	
Vrn-H2	DQ492699.1	HvZCCT.HcF HvZCCT.HcR	5'-CACCATCGCATGATGCAC 5'-TCATATGGCGAAGCTGGAG	Karsai et al. 2005
Ppd-H1	AY970705.1		5'-ATGCGAATGGTGGATCGGC 5'-TATAGCTAGGTGCGTGGCG	Turner et al. 2005
Expression Analysis				
Actin	AK362208.1		5'-CGTGTTGGATTCTGGTGATG 5'-AGCCACATATGCGAGCTTCT	Campoli et al. 2012
FT1	DQ100327.1		5'-GGTAGACCCAGATGCTCCAA 5'-TCGTAGCACATCACCTCCTG	Campoli et al. 2012
BM3	AJ249143.1		5'-GCCGTCACCAGCACAAGCAA 5'-CCCCATTCACCCTGTAGCAAAGA	Sasani et al. 2009
Vrn-H1	AY785826.1		5'-CTGAAGGCGAAGGTTGAGAC 5'-TTCTCCTCCTGCAGTGACCT	Campoli et al. 2012
BM8	AJ249146.1		5'-GCACAGCAGCCGACACCTA 5'-TGCCTTTGGGGGAGAAGACG	Sasani et al. 2009