

**Figure S1.** Schematic representation of the winter *VRN-H1* allele from cv. 'Strider' (AY750993) with positions of markers genotyped in this study indicated. Exons are denoted by black boxes. SNP1 (T-1,948/C), SNP2 (A-1,881/G) and SNP3 (T-1,655/C) are located in the promoter. SNP4 (T+14,567/C) and SNP5 (G+14,585/A) are located in intron VII, while SNP6 (C+14,828/G) is in the 3' UTR. Primer pair 1 assay for the absence of a deletion in intron I, which Fu et al. (2005) describe as characteristic of winter varieties; primer pair 2 assay for the presence of a 5.2 kb deletion within intron I, characteristic of the spring cv. 'Morex' (Fu et al. 2005); primer pair 3 (*HvBM5A*-exon2-F1 / *HvBM5A*-intron1-R13, sequences for which are presented in Table S1) assay for the presence or absence of a 42 bp InDel at the 3' end of intron I (first identified by von Zitzewitz et al. 2005). The positions of the MITE (grey box), Lolaog solo LTR (striped box) and the 'Morex' intron I deletion (boxed by dashed line) within intron I are indicated. All other intron I deletions are illustrated in Figure 2. Primers designed to assay for these additional intron I deletions were designed flanking these breakpoints, and are listed in Table S1.



**Figure S2.** Separation of PCR amplicons by agarose-gel electrophoresis, illustrating differentiation between *VRN-H1* haplotypes 1A (winter) / 2 (spring) and 5C (winter) using primers *HvBM5A*-intron1-F3 / Intr1/H/R3 (*VRN-H1* haplotype 1A/2 = 1,438 bp, haplotype 5C = 952 bp; all other spring alleles = no amplification). Haplotype 1A = 'Igri', haplotype 1B = 'Etu', haplotype 2 = 'Varunda', haplotype 3 = 'Dandy', haplotype 4 A = 'Optic', haplotype 4B = 'Prisma', haplotype 5A = 'Pohto', haplotype 5B = 'Oriol', haplotype 5C = 'Express', L = 1 kb ladder. Primer details listed in Table S1.

## Table S1

Primer name	Sequence 5' to 3'	Use
HvBM5A-promoter-F1	GCAATGCAGCACCTATGCCA	Amplify/sequence promoter
HvBM5A-promoter-R1	CGTTGCTGCGTGTGGGTTG	Amplify/sequence promoter
HvBM5A-promoter-F2	CATCGCATGATTCGACGCTG	Amplify /sequence promoter
HvBM5A-promoter-R2	GCGAGCGAGCGAGCGACCG	Amplify /sequence promoter
HvBM5A-exon1-F1	CTTCACCCAACCACCTGACAG	Amplify/sequence exon I
HvBM5A-exon1-R1	GCGGACGCAAATGTCACTTTT	Amplify/sequence exon I
HvBM5A-intronI-F1	GTTCTCCACCGAGTCATGGT	Amplify/sequence intron I
HvBM5A-intronI-R1	CGCTGGACGAGAATTATTGA	Amplify/sequence intron I
HvBM5A-intronI-F2	GGCGATGGTTCTTGACAAAG	Amplify/sequence intron I
HvBM5A-intronI-R2	TGAGTCGGTTATATGCAGGCTA	Amplify/sequence intron I
HvBM5A-intronI-F3	TTTGTCGGAACTACAACCTTCA	Amplify/sequence intron I
HvBM5A-intronI-R3	GTGTCAGCCTTACGCAAGAA	Amplify/sequence intron I
HvBM5A-intronI-F4	CCAGGGGACCCACAGTAGTA	Amplify/sequence intron I
HvBM5A-intronI-R4	TTTATGTGCACAGGATCTCCA	Amplify/sequence intron I
HvBM5A-intronI-F5	TAACCGTGCGAGATGACGTA	Amplify/sequence intron I
HvBM5A-intronI-R5	CCTTGAGTCATAGCCCACCA	Amplify/sequence intron I
HvBM5A-intronI-F6	CCACACATGCACAGTCACAC	Amplify/sequence intron I
HvBM5A-intronI-R6	AGAAGGTTACCCTGCGAACA	Amplify/sequence intron I
HvBM5A-intronI-F7	AGCACGAGAGGCGAAGATAG	Amplify/sequence intron I
HvBM5A-intronI-R7	GGGCGGAGTACTGCTACAAC	Amplify/sequence intron I
HvBM5A-intronI-F8	AATTTTGCCCGGCGTATAGT	Amplify/sequence intron I
HvBM5A-intronI-R8	GCTACCATTCCACAGCATCA	Amplify/sequence intron I
HvBM5A-intronI-F9	AGCATCACGGCGAAAGAG	Amplify/sequence intron I
HvBM5A-intronI-R9	CGGATGGTTCCTGAGGATATT	Amplify/sequence intron I
HvBM5A-intronI-F10	TCATGAGTGCTTGGAACGTC	Amplify/sequence intron I
HvBM5A-intronI-R10	TGCAGGTCACTCTTTGCATC	Amplify/sequence intron I
HvBM5A-intronI-F11	ATTGGAGGAGAAAGGCAACC	Amplify/sequence intron I
HvBM5A-intronI-R11	AAAGCCCGATGCTGAAAAC	Amplify/sequence intron I
HvBM5A-intronI-F12	CAGGATATTGAGCCCGAAGA	Amplify/sequence intron I
HvBM5A-intronI-R12	GCCAAGGAAAGTCCCTAACC	Amplify/sequence intron I
HvBM5A-intronI-F13	GGTCTGGATCGTGAGGAGAG	Amplify/sequence intron I
HvBM5A-intronI-R13	CCGTTCAAGAATTTTGTCCA	Amplify/sequence intron I
HvBM5A-exon2-F1	TCCCAAGAAAACTTGAACAACACCAG	Amplify/sequence exon II
HvBM5A-exon2-R1	ATTAGGTTACATCATTCGACCA	Amplify/sequence exon II
HvBM5A-intronII-F1	TCAGTTTCATGTGCTCTTTCTAG	Amplify/sequence intron II
HvBM5A-intronII-R1	GTGGTGCTTGTGGGTGGT	Amplify/sequence intron II
HvBM5A-intronII-F2	CACCTACGCGAGTCCATCTC	Amplify/sequence intron II
HvBM5A-intronII-R2	CAAACATGTCCACCTGGATT	Amplify/sequence intron II
HvBM5A-intronII-F3	AGAGGGAGGGGGGGGGGGGG	Amplify/sequence intron II
HvBM5A-intronII-R3	CCTGGAGCTCGAGTTACAAAA	Amplify/sequence intron II
HvBM5A-intronII-F4	AGCTTGTCCAAGGCTAACCA	Amplify/sequence intron II
HvBM5A-intronII-R4	TTCGCTGAACTTCTCTGCAA	Amplify/sequence intron II
HvBM5A-exon3-8-F1	AGCTTGTCCAAGGCTAACCA	Amplify/sequence exon III-VIII
$H_{\rm W}RM5A$ evon 3-8-R1	CCCCACCTCCAAACCAAAC	Amplify/sequence evon III_VIII
$H_{\rm V}BM5A$ T 1 048/C	CACACTCCAA ACTTTACCCA	SNP1 extension primer
$H_{V}BM5A = 1 - 1,948/C$		SNP2 extension primer
$H_{\rm W} BM5A = 1,661/G$	TTCTACAAACIGIGCAICIA	SNP2 extension primer
$H_{\rm M} BM5A = 1-1,004/C$		SND4 extension primer
$H_{V}BM5A = 1+14,507/C$		SNP5 extension primer
HyBM54 C 114,303/A		SND6 extension primer
11vDINJA-C+14,020/U	CCAGGCTGGCCG	Sivi o extension primer
HvBM5A-(CGCT)n-R	6FAM-CTGGCGGTTGATCTTGTTCT	(CGCT) <sub>n</sub> SSR, use with <i>HvBM5A</i> -exon1-F1
HvBM5A-intronI-F1m	TAGGCGCTAGAATACTTCGT	Haplotype 2A intron I InDel
HvFT1-F1	CACTCATCATCACCATTCAC	Amplify/sequence <i>HvFT1</i>
HvFT1-R1	TAGTCCGCTAGTGTATATCG	Amplify/sequence <i>HvFT1</i>

Table S1. Primer details for previously unpublished VRN-H1 genotype assays and sequencing of VRN-H1 and HvFT1. Fragments amplified using VRN-H1-promoter-F1 / VRN-H1-promoter-R1 and VRN-H1-exon3-8-F1 / VRN-H1-exon3-8-R1 were used as the template for SNaPshot<sup>®</sup> single base detection of SNP1/SNP2/SNP3 and SNP4/SNP5/SNP6, respectively. Non-complementary primer tails are underlined. Intron I deletions. HvBM5A-intronI-F1 / HvBM5A-intronI-R6 used to assay for haplotype 1B intron I deletions. HvBM5A-intron1-F1 / HvBM5A-exon1-R1 used to assay for haplotype 2 transposable element insertion; HvBM5A-intronI-F1 / HvBM5A-intronI-R9 used to assay for haplotype 3 intron I deletions. HvBM5A-intronI-F1m / HvBM5A-intronI-R13 used to assay for haplotype 4A intron I deletions. HvBM5A-intronI-F1 / HvBM5A-intronI-R7 used to assay for haplotype 5B/5C deletions. Previously published primers (Fu et al. 2005) were used to detect haplotype 4B and 5A deletions (Intr1/H/F1 / Intr1/H/R1) and the absence of intron I deletions (Intr1/H/F3 / Intr1/H/R3). HvBM5A-intronI-F3 used in combination with primer Intr1/H/R3 (5'-AAAGCTCCTGCCAACTACGA-3', Fu et al. 2005) to identify all known winter VRN-H1 alleles identified in this study (VRN-H1intronI-1A/5C assay). An 879 bp fragment from the VRN-H3 gene, HvFT1 (Yan et al. 2006) was amplified and sequenced in 'Xenia' using the primers shown.

Variety	<i>VRN-H1</i> haplotype	Recorded growth habit	Accession	Determined region
Arra	1B	Spring	EF591635	Promoter /partial 5'UTR / partial intron I/ exon II / partial intron II/ exon III – VIII, partial 3'UTR
Dandy	3	Spring	EF591636	Promoter /partial 5'UTR / exon I / intron I/ exon II / partial intron II/ exon III – VIII, partial 3'UTR
Golden Promise	5A	Spring	EF591637	Promoter /partial 5'UTR / exon I /intron I/ exon II / partial intron II/ exon III – VIII, partial 3'UTR
Golf	5A	Spring	EF591638	Promoter /partial 5'UTR / exon I / partial intron I/ exon II / partial intron II/ exon III – VIII, partial 3'UTR
Optic	4A	Spring	EF591639	Promoter /partial 5'UTR / exon I / intron I/ exon II / partial intron II/ exon III – VIII, partial 3'UTR
Pohto	5A	Spring	EF591640	Promoter /partial 5'UTR / exon I / partial intron I/ exon II / partial intron II/ exon III – VIII, partial 3'UTR
Prisma	4B	Spring	EF591641	Promoter /partial 5'UTR / exon I / intron I/ exon II / partial intron II/ exon III – VIII, partial 3'UTR
Triumph	4A	Spring	EF591642	Promoter /partial 5'UTR / exon I / partial intron I/ exon II / partial intron II/ exon III – VIII, partial 3'UTR
Igri	1A	Winter	EF591643	Promoter /partial 5'UTR / exon I / partial intron I/ exon II / partial intron II/ exon III – VIII, partial 3'UTR
Panda	1A	Winter	EF591644	Promoter /partial 5'UTR / exon I / intron I/ exon II / partial intron II/ exon III – VIII, partial 3'UTR
Pearl	1A	Winter	EF591645	Promoter /partial 5'UTR / exon I / partial intron I/ exon II / partial intron II/ exon III – VIII, partial 3'UTR
Oriol	5B	Spring	EF591646	Exon I / intron I / exon II
Express	5C	Winter	EF591647	Exon I / intron I / exon II
Xenia	1A	Spring	EF591648	Complete sequence *
Etu	1B	Spring	EF591649	Exon I / intron I / exon II
V arunda	2	Spring	EF591650	Exon I / intron I / exon II

**Table S2.** Accession numbers and further details for sequenced *VRN-H1* (*HvBM5A*) genomic regions. \* Sequence spans from -2,074 to +14,953, relative to the translation start site (+1 bp) in genomic sequence from the 'Strider' winter *vrn-H1* allele. The TA repeat region within the promoter could not be completely sequenced across.



**Table S3.** Haplotype groupings (boxed in black) based on polymorphic features identified by sequence comparison of the four genomic fragments amplified in this study with all publicly available genomic *H. vulgare* ssp. *vulgare VRN-H1* sequence (highlighted in grey). Their locations in the promoter (P), 5' UTR (5' UTR), intron I (In I), intron III (In III), exon IV (E IV), intron IV (In IV), intron VII (In VII) and 3' UTR (3' UTR) are indicated. The location of polymorphic features relative to the genomic sequence of the winter cv 'Strider' (AY750993), and their presence in amplicons 1 to 4 (A1 to A4) is indicated. GenBank accession numbers for previously published genomic sequences: 'Calicucima sib' (DQ492702; DQ492704), 'Dicktoo' (AY750994; AY785817; AY785828), 'Harrington' (AY785816), 'Igri' (AY785822 and sequenced in this study), 'Kompolti korai' (AY785824; AY866487), 'Morex' (AY785815; AY785826; AY750995; AY750995; AY758233), 'Nure' (AY785821), 'Oregon Wolfe Barley Dominant' (DQ492703; AY750996), 'Strider' (AY785823; AY750993), 'Tremois' (AY785819), 'Triumph' (AY866485 and sequenced in this study) and '88Ab536' (AY785818). Where no sequence is available for comparison, missing data is recorded as 'U'. Growth habit coding: S = spring, F = facultative, W = winter. InDel/SSR coding: InDel-1867: 1 = T , 2 = C, 3 = TC, 4 = TTC; InDel-1791: 1 = CATTA present, 2 = CATTA absent; InDel-1215: 1 = TCCTTT present, 2 = TCCTTT absent; SSR-1129: 1 = TAC present, 2 = TAC absent; SSR-1101: 1 = (T)\_6, 2 = (T)\_8; SSR-48: 1 = (CGCT)\_2 2 = (CGCT)\_4 3 = (CGCT)\_5; SSR+223: 1 = (C)\_6 2 = (C)\_7; InDel+10878: 1 = 42 bp InDel large allele, 2 = 42 bp InDel small allele.

**Table S4.** Complete raw genotype and phenotype dataset for all 429 barley varieties studied including ten *VRN-H1*-related and three *VRN-H2*-related polymorphisms, row number (where available), haplotype designation and predicted and recorded seasonal growth habit. This table is provided as a separate Excel spreadsheet.