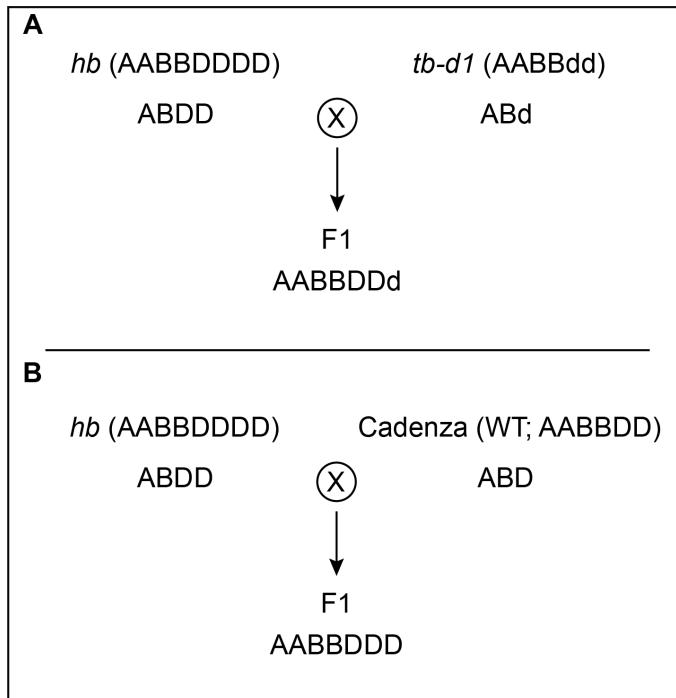
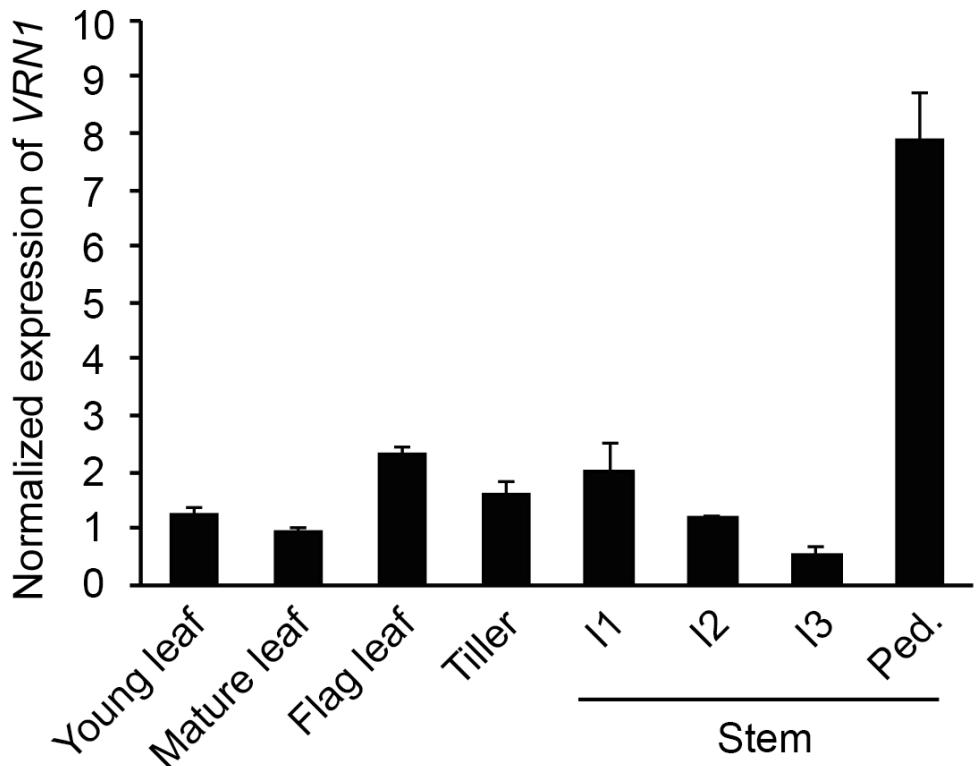


Supplementary Data



Supplementary Figure S1. Schematic of the *hb* × *tb-d1* cross, and the control *hb* × Cadenza cross. Schematic diagrams showing the crossing strategy of the *hb* line with either **(A)** *tb-d1* stop codon mutant (*Cad1721*) or **(B)** wild-type (Cadenza). A, B and D refer to *TB-A1*, *TB-B1* and *TB-D1* respectively, with ‘d’ referring to the nonsense allele of *TB-D1*. The diploid genotype is shown in brackets, and the gamete genotype is shown below. The F1 genotype is shown for the respective crosses.



Supplementary Figure S2. VRN1 tissue specific expression analysis.

Transcript analysis of *VRN1* in leaf, tiller and stem tissue. I1, internode 1; I2, internode 2; I3, internode 3; Ped., peduncle. Values are mean \pm s.e.m of 4 biological replicates.

Supplementary Table S1. Oligonucleotide sequences used in qRT-PCR assays.

| Gene | Gene ID | Forward (sense) sequence | Reverse (anti-sense) sequence |
|------------------------------|--------------------|--------------------------|-------------------------------|
| <i>TB-B1</i> | TraesCS4B02G042700 | ACCCGCCAACCAAAGC | CATCCGGTTCTTTCCCTAGT |
| <i>TB-D1</i> | TraesCS4D02G040100 | CACCCGCCAATCCAAAAC | ATCCGGTTCTTTCCCTCGT |
| <i>Traes_6DS_BE8B5E56D.1</i> | TraesCS6D02G145100 | CATGCTCTGGGATTATCCAT | CTGGATCATTCCGGTGC |
| <i>TaRPA-15</i> | | GCACACGTGCTTGAGATAAG | GCCCTCAAGCTAACCTAACT |

Supplementary Table S2. Oligonucleotide sequences used in KASP marker assays.

| Gene | WT allele oligonucleotide (FAM) | Alternate allele oligonucleotide (HEX) | Common allele | Source |
|---------------|--|---|-----------------------|----------------------|
| <i>TB-B1</i> | GAAGGTGACCAAGTTCATGCT-TTCCTTGCGGCTTGGT | GAAGGTGGAGTCAACGGATT-TTCCTTGCGGCTTGGA | CGGCGAGGGGAAGAAGCA | Dixon et al. 2018 |
| <i>Rht-B1</i> | GAAGGTGACCAAGTTCATGCT-CCCATGGCCATCTCCAGCTG | GAAGGTGGAGTCAACGGATT-CCCATGGCCATCTCTAGCTA | TCGGGTACAAGGTGCAGGGCG | Simon Griffiths, JIC |
| <i>Rht-D1</i> | GAAGGTGACCAAGTTCATGCT-CGTGGCGCAGAAGCTGG | GAAGGTGGAGTCAACGGATT-CGTGGCGCAGAAGCTGT | TCCCCATGGCCATCTCG | Simon Griffiths, JIC |

Supplementary Table S3. *TB-B1* alleles of 8-way MAGIC lines investigated in this study.

| MEL ID | TB-B1 genotype |
|--------|----------------|
| 002-1 | <i>TB-B1b</i> |
| 002-3 | <i>TB-B1b</i> |
| 002-5A | <i>TB-B1a</i> |
| 002-8 | <i>TB-B1a</i> |
| 004-2 | <i>TB-B1a</i> |
| 004-3 | <i>TB-B1a</i> |
| 005-7 | <i>TB-B1a</i> |
| 005-8A | <i>TB-B1b</i> |
| 006-3 | <i>TB-B1b</i> |
| 008-1 | <i>TB-B1a</i> |
| 008-2 | <i>TB-B1a</i> |
| 008-3 | <i>TB-B1a</i> |
| 010-1b | <i>TB-B1a</i> |
| 010-3 | <i>TB-B1a</i> |
| 010-5 | <i>TB-B1a</i> |
| 011-1b | <i>TB-B1b</i> |
| 011-2a | <i>TB-B1a</i> |
| 011-3 | <i>TB-B1b</i> |
| 011-4 | <i>TB-B1a</i> |
| 012-4 | <i>TB-B1a</i> |
| 014-7 | <i>TB-B1a</i> |
| 015-3 | <i>TB-B1b</i> |
| 015-8 | <i>TB-B1a</i> |
| 016-4 | <i>TB-B1b</i> |
| 017-4 | <i>TB-B1b</i> |
| 017-7 | <i>TB-B1b</i> |
| 020-3 | <i>TB-B1a</i> |
| 020-7 | <i>TB-B1a</i> |
| 023-5 | <i>TB-B1a</i> |
| 024-2 | <i>TB-B1a</i> |
| 024-5 | <i>TB-B1a</i> |
| 025-1d | <i>TB-B1a</i> |
| 025-1e | <i>TB-B1a</i> |
| 025-1f | <i>TB-B1a</i> |
| 026-1b | <i>TB-B1b</i> |
| 029-2b | <i>TB-B1b</i> |
| 032-2 | <i>TB-B1b</i> |
| 033-5 | <i>TB-B1a</i> |
| 033-7 | <i>TB-B1a</i> |
| 034-7 | <i>TB-B1a</i> |
| 035-1 | <i>TB-B1a</i> |
| 036-7 | <i>TB-B1a</i> |
| 036-8 | <i>TB-B1b</i> |
| 043-1 | <i>TB-B1b</i> |
| 043-2 | <i>TB-B1a</i> |
| 043-3 | <i>TB-B1a</i> |
| 043-5 | <i>TB-B1b</i> |
| 043-6 | <i>TB-B1b</i> |

| | |
|--------|---------------|
| 044a-1 | <i>TB-B1b</i> |
| 045-1b | <i>TB-B1b</i> |
| 046-1 | <i>TB-B1b</i> |
| 046-2 | <i>TB-B1b</i> |
| 049-2 | <i>TB-B1a</i> |
| 052-7 | <i>TB-B1a</i> |
| 056-1 | <i>TB-B1b</i> |
| 056-4 | <i>TB-B1a</i> |
| 057-1a | <i>TB-B1a</i> |
| 059-1b | <i>TB-B1a</i> |
| 060-1b | <i>TB-B1b</i> |
| 060-3 | <i>TB-B1b</i> |
| 062-2 | <i>TB-B1a</i> |
| 062-3 | <i>TB-B1a</i> |
| 064-1b | <i>TB-B1a</i> |
| 070-1c | <i>TB-B1b</i> |
| 071-1b | <i>TB-B1b</i> |
| 072-1a | <i>TB-B1b</i> |
| 072-1c | <i>TB-B1b</i> |
| 072-1d | <i>TB-B1b</i> |
| 077-3 | <i>TB-B1a</i> |
| 083-1 | <i>TB-B1b</i> |
| 084-3 | <i>TB-B1b</i> |
| 086-3 | <i>TB-B1b</i> |
| 086-7 | <i>TB-B1b</i> |
| 088-1 | <i>TB-B1a</i> |
| 088-3 | <i>TB-B1a</i> |
| 088-6 | <i>TB-B1a</i> |
| 089-4 | <i>TB-B1a</i> |
| 090-1a | <i>TB-B1a</i> |
| 090-2c | <i>TB-B1a</i> |
| 095-1 | <i>TB-B1a</i> |
| 096-6 | <i>TB-B1a</i> |
| 097-1 | <i>TB-B1b</i> |
| 097-2 | <i>TB-B1b</i> |
| 099-4 | <i>TB-B1b</i> |
| 101-7 | <i>TB-B1a</i> |
| 103-1 | <i>TB-B1a</i> |
| 104-4 | <i>TB-B1b</i> |
| 105-1 | <i>TB-B1a</i> |
| 114-8 | <i>TB-B1b</i> |
| 119-1 | <i>TB-B1a</i> |
| 119-2 | <i>TB-B1b</i> |
| 119-4 | <i>TB-B1b</i> |
| 119-5 | <i>TB-B1b</i> |
| 120-3 | <i>TB-B1a</i> |
| 122-2b | <i>TB-B1a</i> |
| 122-4 | <i>TB-B1a</i> |
| 123-1 | <i>TB-B1a</i> |
| 126-2 | <i>TB-B1b</i> |
| 128-3 | <i>TB-B1a</i> |
| 128-4 | <i>TB-B1a</i> |

| | |
|---------|---------------|
| 129-3 | <i>TB-B1a</i> |
| 129-6 | <i>TB-B1a</i> |
| 130-4 | <i>TB-B1a</i> |
| 132-4 | <i>TB-B1a</i> |
| 139-6 | <i>TB-B1a</i> |
| 139-7 | <i>TB-B1a</i> |
| 140-1b | <i>TB-B1a</i> |
| 140-3 | <i>TB-B1a</i> |
| 145-2 | <i>TB-B1a</i> |
| 146-1b | <i>TB-B1a</i> |
| 152-2a | <i>TB-B1b</i> |
| 154-3 | <i>TB-B1a</i> |
| 156-4 | <i>TB-B1b</i> |
| 158-4 | <i>TB-B1b</i> |
| 158-7 | <i>TB-B1b</i> |
| 159-8 | <i>TB-B1a</i> |
| 161-2 | <i>TB-B1a</i> |
| 168-1b | <i>TB-B1b</i> |
| 170-3 | <i>TB-B1a</i> |
| 170-8 | <i>TB-B1a</i> |
| 179-2 | <i>TB-B1b</i> |
| 182-3b | <i>TB-B1a</i> |
| 183-1 | <i>TB-B1a</i> |
| 185-2 | <i>TB-B1b</i> |
| 186-1b | <i>TB-B1a</i> |
| 187-2a | <i>TB-B1b</i> |
| 189-3 | <i>TB-B1a</i> |
| 190-4 | <i>TB-B1b</i> |
| 191-4 | <i>TB-B1a</i> |
| 191-6 | <i>TB-B1a</i> |
| 196-4 | <i>TB-B1b</i> |
| 198-2 | <i>TB-B1b</i> |
| 198-3 | <i>TB-B1b</i> |
| 199-3 | <i>TB-B1b</i> |
| 201-4 | <i>TB-B1b</i> |
| 201-5 | <i>TB-B1b</i> |
| 202-2 | <i>TB-B1a</i> |
| 202-4 | <i>TB-B1b</i> |
| 203-8 | <i>TB-B1b</i> |
| 204-4 | <i>TB-B1a</i> |
| 206a-1a | <i>TB-B1b</i> |
| 207-6 | <i>TB-B1a</i> |
| 207-7 | <i>TB-B1a</i> |
| 208-6 | <i>TB-B1a</i> |

MEL = MAGIC Elite Line from the 8-way winter wheat population, NIAB UK. MEL is the prefix for each line.