Article title: Interaction between row-type genes in barley controls meristem determinacy and reveals novel routes to improved grain
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Fig. S1 Spikes of Bowman and single vrs mutants before awn removal. Scale, 0.5 cm.

Fig. S2 Spikes of vrs3.f, vrs4, vrs5, vrs1 and Bowman at late awn primordium stage. Scanning electron microscopy of spikes at late awn primordium (AP) stage from intermedium alleles: (a) vrs3.f, (b) vrs4, (c,d) vrs5, (e) vrs1, (f) Bowman. Lemma and awns are false coloured to highlight the gradient in development along the intermdium spikes. Arrow in (d) shows variable lemma and awn development in vrs5. Arrowheads in (d-f) show differentiated stamen lobes in vrs5 lateral spikelets compared to vrs1 and Bowman. Scale, 100 µm. Bw, Bowman
Fig. S3  Spikes of vrs double mutants before awn removal. Genotypes from left to right: vrs1vrs3, vrs1vrs4, vrs1vrs5, vrs3vrs4, vrs3vrs5, and vrs4vrs5. Scale, 0.5 cm.
Fig. S4 Differential gene expression of meristem regulators in Bowman, \textit{vrs3vrs4} and \textit{vrs3vrs5} mutants. \textit{RAMOSA-ENHANCER2} (\textit{REL2}), \textit{INDETERMINATE SPIKELET1} (\textit{IDS1}), \textit{VRS2} and \textit{VRS4} transcript levels in developing spikes of \textit{vrs3vrs4} and \textit{vrs3vrs5} mutants and Bowman. Bar graphs show mean normalised expression (± SD) detected by qPCR (n=3). Significant differences to Bowman indicated by *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (student t-test). Bw, Bowman; mne, mean normalised expression. GP, glume primordium; LP, lemma primordium; SP, stamen primordium.
Fig. S5 Combined grain area from Bowman, single vrs mutants and double vrs mutants. Violin plots show grain area distribution from all grain including those from central spikelets, lateral spikelets, and (when present) additional spikelets. Grain harvested from the main culm and tallest tiller per individual. n = 10 individuals.
Fig. S6 Tiller outgrowth rate over time in Bowman and single *vrs* mutants and double *vrs* mutants. Graphs show duration and rate of tiller production in Bowman, single and double *vrs* mutants. Slope indicated by red lines showing rate of tiller production and goodness of fit indicated by R² correlation. Black circle symbols indicate window of active tiller outgrowth; dag, days after germination.
Fig. S7 Early tillering phase across Bowman and single vrs mutants (a) Tiller number was recorded at five days after germination (dag) and repeated at two to three day intervals until 23 dag (n=10 individuals/ genotype). Plants were grown in 7 cm pots under long day glasshouse conditions. Tiller number included tillers with visible spikes as well as ‘vegetative tillers’ without emerged spikes. Dots represent means (±SD). (b) Apex stage was determined at each time point in plants grown along side. VM, vegetative meristem; DR, double ridge; TSM, triple spikelet meristem; GP, glume primordium; LP, lemma primordium; SP, stamen primordium; AP, awn primordium; WA, white anther; GrA, green anther.

(a)

(b)

|       | 5dag | 7dag | 9dag | 11dag | 14dag | 16dag | 18dag | 20dag | 23dag | 35dag |
|-------|------|------|------|-------|-------|-------|-------|-------|-------|-------|
| Bw    | VD   | VD   | early| DR    | DR    | TSM   | GP/LP | LP/SP | SP    | AP    | WA/GrA|
| vrs1  | veg  | VD   | early| DR    | DR    | TSM   | GP/LP | LP/SP | SP    | AP    | WA/GrA|
| vrs2  | VD   | VD   | VD   | early| DR    | DR    | TSM   | GP/LP | LP/SP | SP    | AP    | WA/GrA|
| vrs3  | VD   | VD   | early| DR    | DR    | TSM   | GP/LP | LP/SP | SP    | AP    | WA/GrA|
| vrs3.f| VD   | VD   | early| DR/TSM| TSM  | TSM   | GP    | LP/SP | SP    | AP    | WA/GrA|
| vrs4  | VD   | VD   | early| DR    | DR    | TSM   | GP/LP | LP/SP | SP    | AP    | WA/GrA|
| vrs4.k| VD   | VD   | early| DR/TSM| TSM  | TSM   | GP    | LP/SP | SP    | AP    | WA/GrA|
| vrs5  | VD   | VD   | DR   | DR/TSM| TSM  | TSM   | GP    | LP/SP | SP    | AP    | WA/GrA|
Table S6 Spikelet parameters in single vrs mutants and Bowman compared by ANOVA Values are mean (±SD) from awned spikelets from n = 10 spikes per line. Means with the same letters are not statistically different from each other by ANOVA (p<0.05).

| Line | rachis nodes | spikelets/spike ( / node) | spikelets setting grain/spike ( / node) | empty awned spikelets/spike ( / node) | grain area (mm^2)\(^A\) | TGW (g) | Final tiller number |
|------|--------------|---------------------------|----------------------------------------|--------------------------------------|---------------------------|---------|-------------------|
| Bw   | 22.4 ±1.3    | 66.9 ±3.4b                | 19.1 ±2.5 a                            | 3.0 ±1.5 a                           | 27.2 ±1.5 b               | 56.5 ±4.6 c | 27 ±3.2 c         |
|      | b            | (3.0 ±0.0)b | (0.9 ±0.1)d | (0.1 ±0.1)d | (3.0 ±0.5)b | (0.6 ±0.1)a | (0.8 ±0.0)ab |
| vrs1 | 20.4 ±0.5    | 60.3 ±2.1bc               | 52.7 ±4.2 c                            | 7.7 ±4.2 b                           | 22.2 ±3.1 a               | 37.7 ±11 ab | 21.3±2.2 ab       |
|      | a            | (3.0 ±0.1)b | (2.6 ±0.2)c | (0.4 ±0.2)c | (3.0 ±0.5c) | (0.6 ±0.1)c | (0.8 ±0.0)ab |
| vrs2 | 23.6 ±0.5    | 76.3 ±3.1ab               | 14.6 ±2.9 a                            | 20 ±1.4 d                            | 24.2 ±3.1 a               | 48.1 ±7.6 bc | 36.8±2.8d         |
|      | b            | (3.1 ±0.2)b | (0.6 ±0.1)a | (0.8 ±0.0)a | (3.0 ±0.5b) | (0.6 ±0.1)a | (0.8 ±0.0)ab |
| vrs3 | 20.6 ±0.5    | 62.5 ±1.6bc               | 35.8 ±7.4 b                            | 8.75 ±4.3 bc                         | 23.2 ±4.1 a               | 39.6 ±13 ab | 22.5±2.6 b        |
|      | a            | (3.0±0.1)b | (1.7 ±0.4)b | (0.4 ±0.2)c | (3.0 ±0.5b) | (1.7 ±0.4)b | (0.4 ±0.2)cd |
| vrs4 | 19.6 ±1.1    | 78.4 ±6.6a                | 56.4 ±4.9 c                            | 13.4 ±4.6 c                          | 20.7 ±1.7 a               | 34.9 ±6.5 a | 21.6±2.1b         |
|      | a            | (4.0±0.3)a | (2.9 ±0.2)c | (0.7 ±0.3)b | (4.0±0.3a) | (2.9 ±0.2)c | (0.7 ±0.3)bc |
| vrs5 | 19.5 ±1.6    | 58.5 ±4.9c                | 34.5 ±3.2 b                            | 8.5 ±4.4 abc                         | 22.3 ±4.4 a               | 43.1 ±16 ab | 18 ±1.4a          |
|      | a            | (3±0.1)b | (1.8 ±0.3)b | (0.4 ±0.2)bc | (3±0.1)b | (1.8 ±0.3)b | (0.4 ±0.2)cd |

\(^A\)Averaged values for grains deriving from central, lateral and additional spikelets. Bw, Bowman

Methods S1 Supplemental methods for genotyping and qPCR
(1) KASP Genotyping. Each reaction contained 1 µl of 20 ng genomic DNA, 3 µl H2O, 4 µl 2x KASP master mix (LGC Genomics) and 0.11 µl allele-specific primers (LGC Genomics, listed below). Reactions were performed according to the following steps: 2 min at 20°C; 15 min at 94°C; 10 touch-down cycles of 20 sec at 94°C, 1 min at 62°C, with -0.7°C per cycle; 32 cycles of 20 s at 94°C, 1 min at 55°C, 2 min at 20°C. All assays were run on an ABI 3700 Step-One Plus Real Time PCR machine (Applied Biosystems). (2) VRS1 Genotyping. PCR amplicons (primers listed below) were purified with ExoSAP-IT™ PCR Product Cleanup Reagent (ThermoFisher Scientific) and sequenced using the BigDye Terminator version 3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems). Samples were sequenced on an ABI3730 and trimmed sequences were analyzed with Sequencher 5.2.3 software (GeneCodes). (3) Quantitative RT-PCR (qRT-PCR). The qRT-PCR for each gene was run in three (technical and biological) replicates and expression measured using TaqMan technology (Roche). Relative expression levels were calculated using two stable expressed internal reference genes, HvACTIN2 and PROTEIN PHOSPHATASE 2 (HvPP2A). Sequences of gene-specific oligonucleotides (listed below) and probe numbers were designed using Roche Universal Probe Library Design Center website.
Primer pair efficiency was tested by standard curve using a 1:5 dilution series over 5 dilution points. The qRT-PCR reaction was performed in 25 µl volume consisting of 2x Universal probe library master mix + ROX Fast Start TaqMan (Roche), 10 µM forward and reverse primer, 10 µM Universal probe library hydrolysis probe, and run in a 96-well plate on a ABI 3700 Step-One Plus Real Time PCR machine (Applied Biosystems) with the following conditions: 10 min at 95°C, 40 cycles of 15 s at 95°C, 1 min at 60°C using the comparative CT program. Statistical differences were assessed with a Student’s t-test (two-tailed).

### Primers

| Gene   | Mutant Line (Allele) | Primer Pair 5'—3' | Expected Fragment Size |
|--------|----------------------|-------------------|------------------------|
| HvVRS1 | BW898 (vrs1.a)       | Forward ACAGGCAACAGAACAACCCTACC, Reverse CAAGAACGGGAGAGAACCCTACC | 696 bp |

### VRS1 Genotyping

| Gene   | Mutant Line (Allele) | Primer Pair 5'—3' |
|--------|----------------------|-------------------|
| HvVRS1 | BW898 (vrs1.a)       | Forward ACAGGCAACAGAACAACCCTACC, Reverse CAAGAACGGGAGAGAACCCTACC |
| Gene   | accession number     | Primer Pairs (5'--->3')                                      | UPL # | Efficiency (%) | R²  |
|--------|----------------------|------------------------------------------------------------|-------|----------------|-----|
| HvACT2 | HORVU1Hr1G002840.4   | Forward GCGAGGTGTCTGGGTCTCTTCT Reverse ACATGGCAAGGAACCTTGAGAAA | 129   | 97.5           | 0.999 |
| HvPP2A | HORVU5Hr1G109430.2   | Forward CGTCGCATCATGATCAAGTG Reverse CGAGGTGAGTAACACGATG   | 11    | 99.7           | 0.998 |
| HvVRS1 | HORVU2Hr1G092290.6   | Forward CCCATAAAAATAGCCGAGATAGC Reverse AGTTTCTGCCGATCTTGAA  | 70    | 98.0           | 0.969 |
| HvVRS2 | HORVU5Hr1G081450.1   | Forward CAACATCGGTGTGTCACTG Reverse GGGAACAGGCGTACAGG     | 9     | 98.6           | 0.929 |
| HvVRS3 | HORVU1Hr1G051010.5   | Forward CACTTCTTTTATGAGTGACGAAA Reverse CAGAAGAAGTTTCACCGCAGA | 101   | 106           | 0.975 |
| HvVRS4 | HORVU3Hr1G016690.1   | Forward GTGAACGCCACATTGACCAT Reverse GTGATCCCTACCTATCTCT    | 77    | 99.0           | 0.999 |
| HvVRS5 | HORVU4Hr1G007040.1   | Forward ACCATTCCTCCCCCTCATT Reverse GCACCGGCACCGCAGAGTGA   | 31    | 95.2           | 0.997 |
| HvIDS1 | HORVU5Hr1G112440.1   | Forward GACCTGCGGTGCCAGATGACTA Reverse CAGAAAAAGCGCCGCGGAAGTGA | 9     | 100           | 0.974 |
| HvREL2 | HORVU0Hr1G008690.1   | Forward AAGACGATGACAACTTTATATG Reverse AGGATGGAATGCGAAGAAAAG | 77    | 95.6           | 0.993 |
| BM3    | HORVU0Hr1G003020.3   | Forward CGAGGATATACCTATGGGCTGAA Reverse CGCACTTTGACCAATGCGACT | 164   | 99.5           | 0.929 |
| BM8    | HORVU2Hr1G063800.7   | Forward TCTCGGGCGATGTTGCTCTACT Reverse AAGTTGCAATTGCGATGTTG  | 156   | 106           | 0.975 |
| HvLOG1 | HORVU5Hr1G124750.1   | Forward CCGGTCTTCTTTATGAAATC Reverse AACTCTCTCCTGTCCTCATCG   | 76    | 92.7           | 0.955 |
| HvCKX2 | HORVU3Hr1G027460.1   | Forward TGTCGATGATGCTTGG Reverse TCCTCGCAAACCCATGAGTATG   | 25    | 99.5           | 0.996 |