Supporting Information

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Quantitative PCR

RNA Extraction and cDNA Synthesis. Entire aerial tissues from vegetative seedlings were collected at 7 d postgermination (dpg) and just after the reproductive transition at 14 dpg. Spikes at 21 dpg and 28 dpg were harvested along with the lower 5 mm of the internode. Spikes also were harvested at 35 dpg. Tissue was ground with a mortar and pestle in liquid nitrogen. Pulverized powder was resuspended in TriReagent (Sigma) and centrifuged for 10 min to pellet out fibrous debris. RNA extraction was performed according to the manufacturer's instructions with an additional chloroform extraction after isopropanol precipitation. Concentration was determined using a NanoDrop spectrophotometer, and RNA integrity was checked using a Bioanalyzer 2100 (Agilent Technologies). Only RNA with a RNA Integrity Number above 8 was used for cDNA synthesis. cDNA was generated with SuperScript II RT (Life Technologies) using a 3:1 mix of oligo dT:Random Hexamer primers. Quantitative PCR (qPCR) intron-spanning primers and probes for the TaqMan system were designed using the Roche Universal Probe Assay design center website (www.roche-applied-science.com/shop/CategoryDisplay?catalogId=10001&tab=&identifier=Universal+Probe+Library&langId=-1&storeId=15006). Reactions were set up with FastStart TaqMan (Roche) and were executed on the Applied Biosystems StepOnePlus machine.

Determination of Primer Efficiency. Each primer pair was validated with a seven-step serial-dilution series of barley spike cDNA. Reactions were run in 96-well plates on the StepOnePlus machine (Applied Biosystems) using the Quantitation–Standard Curve method program on Standard Ramp Speed (2-h run), as shown in the table below:

Primer pair	Efficiency, %	R^2
HvACTIN UP129	98.862	0.999
26S UP19	108.5	0.996
HvPPA2 UP7	99.523	0.996
HvAP2 UP70	101.013	0.998

qPCR Reaction Set-Up. The qPCR reaction set-up was as follows: Roche Fast Start TaqMan Mix, 12.5 µL; Universal Probe, 0.25 µL; primer L, 0.25 µL; primer R; 0.25 µL; water, 6.75 µL; 1:25 cDNA,5 µL, to a final volume of 25 µL.

Run Parameters. Three or four independent biological replicates for each genotype and time point were run in 96-well plates in triplicate on the StepOnePlus machine (Applied Biosystems) using the Quantitation–Comparative CT (ΔΔCT) method program at the Standard Ramp Speed (2-h run). Multiple reference genes (*HvACTIN2*, *HvPPA2*, and *26S* rRNA) were run on the same plate as *HvAP2* for each sample.

Cycle Conditions. The cycle conditions were as follows: holding step, 10 min at 95 °C; cycle step 1, 15 s at 95 °C; cycle step 2, 1 min at 60 °C for 40 cycles.

Data Analysis. Baselines and threshold Ct values were inspected manually within the StepOnePlus software and were adjusted if required. Average Ct values from each biological replicate were exported and put into an Excel Spreadsheet. To determine the best reference gene for these RNA samples, threshold Ct values of all three reference genes were input into Refinder (www.leonxie.com/referencegene.php), which applies four computational methods (Normfinder, BestKeeper, GeNorm, and the comparative ΔCt) to produce a comprehensive stability value for each gene. Through this method, *HvACTIN2* (*HvACT2*) and *HvPPA2* were resolved as the best reference genes, as shown below:

Reference gene	Stability
HvACT2	1.19
26S rRNA	2.28
HvPPA2	1.86

Averaged biological replicates from each time point were compared between genotypes with *Zeo1.b* values standardized against Bowman (Bw) values so that a ratio of *HvAP2* expression *Zeo1.b*/Bw was derived.

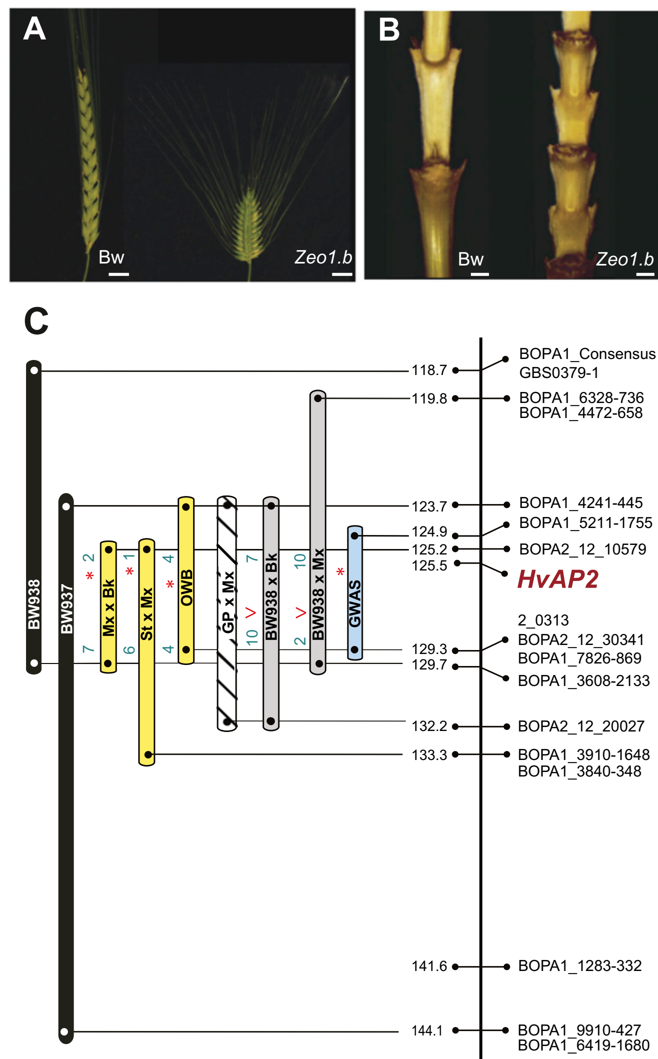

Fig. S1. *Zeo* phenotype and locus determination. (*A*) Mature spikes from the *Zeo1.b* line (BW938) are almost twice as dense as those from its nearly isogenic parent (cv. Bowman). (*B*) Stripping spikelets from the main axis of mature spikes reveals the extreme reduction in *Zeo1.b* rachis internode length compared with Bowman. The rachis internode length phenotype is effectively synonymous with spike density. (*C*) Mapping the *Zeo1* locus on chromosome 2H. SNP names are from the Illumina 9K SNP array, and positions in cM are from the reference Morex × Barke map. Introgressions previously identified in Bowman introgressed lines associated with *Zeo1* or dense spikes after SNP genotyping (3,072 SNPs) (1) are shown by black bars. Intervals identified by F2 population mapping are represented by gray or yellow bars. The blue bar denotes intervals defined by the genome-wide association scan for spike density. The hatched bar represents the quantitative trait loci interval identified in the Golden Promise × Morex population. For clarity, only markers that are polymorphic between cv, Bowman and Bowman isolines with a dense spike regulated by *HvAP2* are shown on the consensus map. Map positions for *HvAP2* based on the number of recombinants are indicated by asterisks for Steptoe × Morex (2), Oregon Wolfe Barley (3), and Morex × Barke, and the position of *Zeo1.b* in two F2 mapping populations is indicated by ">." The position of *HvAP2* on the barley consensus map, based on Morex × Barke, is derived from the genome-wide association scan and is indicated by an asterisk. (Scale bars, 5 mm in *A*; 1 mm in *B*.)

1. Close TJ, et al. (2009) Development and implementation of high-throughput SNP genotyping in barley. *BMC Genomics* 10:582.
2. Kleinhofs A, et al. (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. *Theor Appl Genet* 86:705–712.
3. Costa JM, et al. (2001) Molecular mapping of the Oregon Wolfe Barleys: a phenotypically polymorphic doubled-haploid population. *Theor Appl Genet* 103:415–424.

A

WAP2A^Q
TaAP2-L1
TaAP2-L2
WAP2D
HvAP2-Q
BdRAP2-7-like
ZmIDS
Os03g0818800
OsAP2-like
AtTOE1
OsAP2D23-like
HvAP2-2L
BdRAP2-7-like 2
HvAP2-1L
BdAP2-like 2
ZmGLOSSY15
AtTOE3
AtAP2
ZmAP2/EREBP
ZmAP2/EREBP 2
OsSHAT1
BdAP2-like
HvAP2
TaAP2-B
TaAP2-A
TaAP2-D
AtSNZ

B

1. HvAP2
2. gi|241983258|dbj|AK331198.1|
3. TaAP2-B (BAN05521.1)
4. TaAP2-A (BAN05520.1)
5. TaAP2-D (BAN05522.1)

Fig. S2. Phylogenetic analysis of *AP2* genes. Analysis of *AP2* genes in Poaceae genera and in *Arabidopsis thaliana*. (*A*) A neighbor-joining tree of *HvAP2* in *Hordeum vulgare* and putative orthologs from *Triticum aestivum* (*Ta*), *Zea mays* (*Zm*), *Brachypodium distachyon* (*Bd*), *Oryza sativa* (*Os*), and *Arabidopsis thaliana* (*At*). *AtSCHNARCHZAPFEN* (*AtSNZ*), an *AtAP2*-like gene, was used as the outgroup. (*B*) Protein sequence alignment for *Zeo1* and the closest *TaAP2* candidate, AK331198.1, and *TaAP2* orthologs from each chromosome (B, A, and D). Amino acids are shaded by similarity; dark shading i indicates high similarity between sequences, and light shading indicates low similarity.

Fig. S3. Comparison of Bowman (Bw) and *Zeo1.b* lodicules at anthesis. Expanded lodicules are associated with noncleistogamy (open flowering), whereas unexpanded lodicules are associated with cleistogamy (closed flowering). (*A*) Bowman spikelet with lodicules dissected away show expansion. (*Inset*) Bowman spikelet with lemma removed showing position of lodicules (arrow). (*B*) *Zeo1.b* lodicules dissected away lack expansion. (*Inset*) Equivalently staged *Zeo1.b* spikelet with lemma removed showing position of lodicules (arrow). (*C*) Fully expanded Bowman lodicule. (*D*) Unexpanded *Zeo1.b* lodicule. (Scale bars, 0.5 mm.)

Other Supporting Information Files

Dataset S1 (XLSX)