Supplementary Information for

Unleashing floret fertility in wheat through the mutation of a homeobox gene

Shun Sakuma, Guy Golan, Zifeng Guo, Taiichi Ogawa, Akemi Tagiri, Kazuhiko Sugimoto, Nadine Bernhardt, Jonathan Brassac, Martin Mascher, Goetz Hensel, Shizen Ohnishi, Hironobu Jinno, Yoko Yamashita, Idan Ayalon, Zvi Peleg, Thorsten Schnurbusch, Takao Komatsuda

Corresponding authors:
Shun Sakuma; ssakuma@tottori-u.ac.jp
Thorsten Schnurbusch; schnurbusch@ipk-gatersleben.de
Takao Komatsuda; takao@affrc.go.jp

This PDF file includes:

- Materials and Methods
- Figs. S1 to S9
- Tables S1 to S5
- References

Other supplementary materials for this manuscript include the following:

- Datasets S1 to S4

www.pnas.org/cgi/doi/10.1073/pnas.1815465116

Materials and Methods

QTL mapping

The QTL analysis was based on a population of 94 recombinant inbred substitution lines (RISLs) derived from a cross between durum wheat cv. Langdon (LDN) and the line DIC-2A, which harbors a copy of chromosome 2A inherited from wild emmer wheat accession FA-15-3 (ISR-A) in the genetic background of LDN (1). The phenotyping required an experiment, set out as a randomized complete block design with three replicates, and conducted in an insect-proof screen-house at the experimental farm of the Hebrew University of Jerusalem in Rehovot, Israel (34°47′ N, 31°54′ E; 54 m above sea level). The number of spikelets and the number of grains per spike were obtained from a sample of three spikes per plant, and these were used to derive the number of grains per spikelet. The linkage analysis and the construction of the genetic map used the evolutionary strategy algorithm included in the MultiPoint package (http://www.MultiQTL.com), as previously described (2). QTLs were detected using the general interval mapping function designed for RIL-selfing populations. Boot-strapping (10,000 replicates) was used to estimate standard deviations associated with the locus effect, its chromosomal position, its LOD score and the proportion of the phenotypic variation explained.

Fine mapping of *GNI-A1*

Three RISLs (RISL #4, #63 and #102), each of which harbored the DIC-2A allele of a marker defining the *GNI-A1* interval, were crossed to LDN and the hybrids allowed to self-pollinate to produce three independent F_2 populations. Recombinants within the target genomic region were detected by genotyping a sample of 1,006 F_2 progeny with the two microsatellite markers *Xgwm558* and *Xcfa2043*. In the following (F_3) generation, families (eight F_3 individuals per F_2 plant) were genotyped with *Xgwm558*, *Xcfa2043* and *Xhbg494*, and all homozygous recombinants were subsequently genotyped with 12 additional markers (***SI Appendix*, Table S4**), which target sequence polymorphisms between the wild and domesticated emmer wheat genome sequences (3). The phenotype of 17 F_4 homozygous recombinants was scored in the field. A split-plot, randomized complete block design experiment with five replicates was carried out in an insect-proof screen-house at Rehovot. Each 1 m ×1 m plot was sown with 20 plants of a single line, and the numbers of spikelets and grains per spike (and the derived number of grains per spikelet) were estimated from a sample of five spikes.

The phenotypic effect of the allelic status at *GNI-A1* was assessed in three field experiments, one conducted in 2015-16 in Rehovot (13 RISLs plus LDN and DIC-2A), one in Rehovot in 2016-17 and one in Ruhama (31º29' N, 34º42' E, 166m above sea level) (18 BC_1F_4 lines and LDN).

Transformation of wheat

As the transgene for including RNAi, a 323 bp fragment of *GNI-A1* was amplified (primer details given in ***SI Appendix*****, Table S4**), inserted into a pENTR D-TOPO vector (Thermo Fisher Scientific, Waltham, MA USA) and transferred via the LR recombination reaction into pANDA-b vector to allow it to be driven by the maize *Ubiquitin1* promoter (4). Transgenic wheat plants were produced by bombarding immature embryos of spring bread wheat variety Bobwhite S-26 (5). Transgene-positive plants were confirmed by PCR using vector-specific primers (***SI Appendix*****, Table S4**).

TILLING

The winter bread wheat cv. Kitahonami was used as the TILLING target, with mutations induced by irradiating mature grains with gamma rays (250 Gy). M_1 plants were grown in a greenhouse and all M_2 grains were harvested. DNA was extracted from the M_2 plants and used as the TILLING template. The primers used to amplify the key fragment of *GNI-A1* are given in ***SI Appendix*****, Table S4**. The phenotype of selected M_3 families was evaluated in the National Institute of Agrobiological Sciences (NIAS) experimental field in Tsukuba, Japan (36º01' N, 140º06' E).

Yield trials

Mutants of cv. Kitahonami in the M_4 generation were used in yield trials. M_4 seeds were collected from ~10 M_3 plants selected as either homozygous for the 105Y or for the 105N allele. The plants were grown at both the Kitami Agricultural Experiment Station (43º44'N, 143º43'W, Kitami, Hokkaido, Japan) and the Central Agricultural Experiment Station (43º3'N, 141º45'W, Naganuma, Hokkaido, Japan). At the former location, each entry was represented by four 5.4 m^2 plots (sowing density 200 grains/m^2), and at the latter by three 4.8 m^2 plots (same sowing density). The fertilizer regime at Kitami was 156 kg/ha N, 175 kg/ha P, 70 kg/ha K, and at Naganuma (respectively 140, 125 and 50 kg/ha).

RNA extraction and qRT-PCR

Immature spikes were developmentally staged by observation under a stereoscopic microscope (6). Total RNA was extracted using TRIzol (Invitrogen) and quantified using NanoDrop 1000 (Thermo Fisher Scientific). The resulting RNA was treated with RNase-free DNase (Takara Bio) to remove contaminating genomic DNA, and the cDNA first strand synthesized from a 25 ng aliquot of RNA using SuperScript III (Invitrogen). Transcript abundances were obtained by qRT-PCR using a StepOne Real-Time PCR System (Applied Biosystems) and a THUNDERBIRD SYBR qPCR Mix Kit (Toyobo) according to the manufacturers' protocols. The relevant primer sequences are detailed in in ***SI Appendix*, Table S4**. Each of the amplified gene fragments was cloned into pBluescript II KS (+) vector (Stratagene) and the resulting plasmids used to generate standard curves for the purpose of absolute quantification. Each qRT-PCR comprised at least three technical replicates, and each sample was represented by three biological replicates. The *Actin* gene was used as the reference sequence.

In situ mRNA hybridization

A 300 bp 3′-UTR segment of *GNI1* was amplified from cDNA prepared from immature wheat spikes using primers detailed in ***SI Appendix*, Table S4**. The amplicon was inserted into the pBluescript II KS (+) vector. Clones harboring the insert orientated oppositely were linearized by digestion with *Eco*RI, and treated with T3 RNA polymerase in order to generate both a sense and an antisense probe. The method used for *in situ* hybridization have been described elsewhere (7).

RNA-seq

RNA was extracted from immature inflorescences harvested at the white and green anther stages from plants of the cv. Kitahonami M_4 families using the TRIzol (Invitrogen) and treated with DNase I (Roche). The quality of the RNA was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies). RNA-seq libraries were prepared using the NEBNext Ultra^{TM} RNA Library Prep Kit for Illumina (New England Biolabs, Inc., Ipswich, MA, USA) and sequenced using a HiSeq 4000 devise (Illumina, Inc., San Diego, CA, USA). Estimates of transcript abundance were based on the number of transcripts per million reads (TPM). TPM was quantified by pseudo-alignment against the representative transcripts of high-confidence and low-confidence genes annotated on the IWGSC RefSeq V1 pseudomolecules (8) using Kallisto version 0.43.1 (9). Abundance estimates were imported into the R statistical environment (10). The identification of differential transcription was carried out using Limma-voom (11, 12). Differentially expressed genes (DEGs) were required to have $\log_2$ fold change ≥ 2 or ≤ -2 between contrasted conditions and an adjusted P-value ≤ 0.05 after Benjamini-Hochberg correction. Enrichment of gene ontology (GO) terms was analyzed with topGO (13) using the GO term assignment of IWGSC, 2018. The transcript data of *FT1* obtained from Wheat Expression Browser (http://www.wheat-expression.com; (14, 15)

Haplotype analysis

The panel of 72 tetraploid wheat accessions used for resequencing a 1,053 bp fragment of *GNI-A1* included 35 wild emmer, 14 domesticated emmer and 23 durum (***SI Appendix*, Table S2**). The relevant primers are detailed in ***SI Appendix*, Table S4**. Of the 72 entries, 30 were phenotyped with respect to the number of grains set per spikelet. Sequence alignments were performed using ClustalW as implemented in MEGA v7.0 software (16). A median-joining network (17) was constructed using DNA Alignment v1.3.3.2 and Network v5.0.0.1 software (Fluxus Technology) with default parameter settings (epsilon = 0; frequency . 1 criterion = inactive; The ratio of transversion:transition = 1:1; Criterion = Connection cost; External rooting = inactive; MJ square option = inactive). A panel of 210 European winter bread wheat cultivars (***SI Appendix*****, Table S3**) was similarly used for resequencing *GNI-A1*; their phenotype was recovered from archived data (18) (**Dataset S3**).

Phylogenetic analysis

A phylogenetic analysis was based on 139 entries, representing 35 Triticeae species (14 genera), with *Brachypodium* and *Bromus* used as the outgroup taxa (***SI Appendix*****, Table S5**). Sequence capture was performed as described elsewhere (19). For each taxon, a set of overlapping probes was designed to cover each sequence at least four times. The probe sequences are listed in **Dataset S4**. Sequences were assembled using Geneious v10.0.9 (20). For the diploid and autotetraploid taxa, reads were mapped simultaneously to barley *Vrs1* (AB478778) and *HvHox2* (AB490233) using the “Medium-Low Sensitivity” parameter. Only reads for which both sequences of a pair mapped were kept. For heterozygous diploid and allotetraploid entries, the haplotype phasing consisted of mapping, followed by *de novo* assembly, to obtain two alleles or homoeologs per locus, as previously described (21). Diploid heterozygous individuals were phased individual-wise. As a control, the two alleles recovered from each individual were used as references for mapping the reads from that individual. For allotetraploid entries, phasing was performed on the individual of a species showing the highest sequence coverage. The retrieved homoeologs were subsequently used as references (KU-8939, PI 428093). To control for potential chimeras the assemblies were manually inspected and their contigs aligned using Mafft v7.308 (22). For hexaploid wheat, the cv. Chinese Spring alleles of *GNI1* and *TaHox2* were used as references for read mapping. All assemblies were inspected for misalignments and coverage inconsistencies. Consensus sequences were called using the ‘Highest Quality' threshold, and multiple sequence alignment were performed using MAFFT, as implemented in GENEIOUS v10.2.3. The alignment was checked manually and trimmed to the coding sequences. The best-fit model of nucleotide substitution was selected using jModelTest2 (23). The Bayesian information criterion (24) selected K80 + G out of 24 tested models. Bayesian phylogenetic inferences were calculated using MrBayes v3.2.6 (25) mounted on Cyberinfrastructure for Phylogenetic Research (Cipres) Science Gateway v3.3 (26, 27). Two parallel Metropolis-coupled Monte Carlo Markov chain analyses with four chains were run for six million generations, sampling trees every 500 generations. Convergence of the runs was assessed applying a standard deviation of split frequencies threshold of 0.01. The continuous parameter values sampled from the chains were checked for mixing using Tracer v1.6 (http://beast.bio.ed.ac.uk/Tracer). A consensus tree was computed in MrBayes following burn-in of the initial 25% of trees. The consensus tree was visualized in Figtree v1.4.2

(http://tree.bio.ed.ac.uk/software/figtree), using the node representing the most recent common ancestor of *Brachypodium* and the Triticeae as the root.

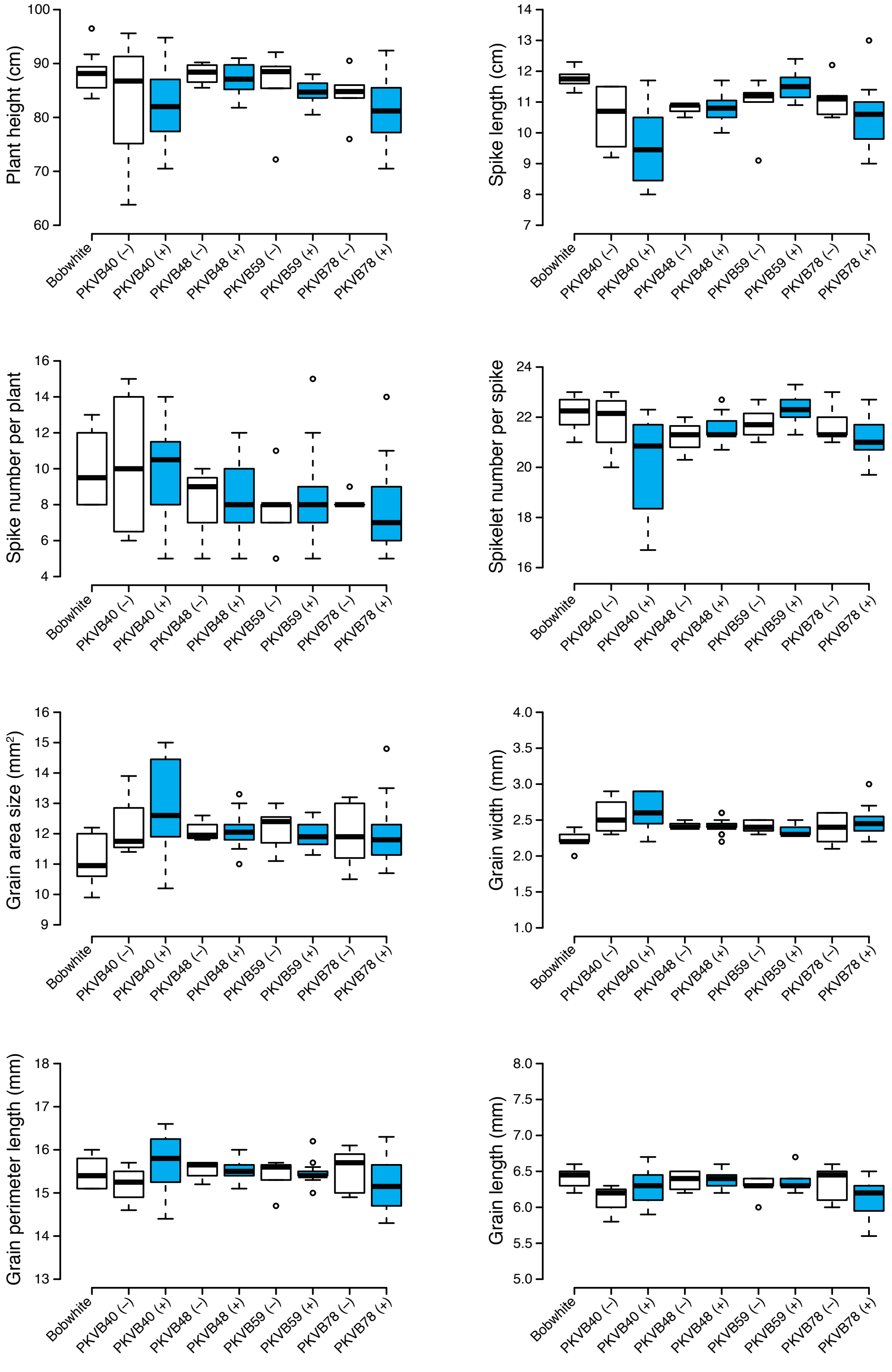


Fig. S1. The phenotype of cv. Bobwhite harboring a *GNI-A1* RNAi construct. White boxes indicate construct-negative plants and blue ones construct-positive plants.

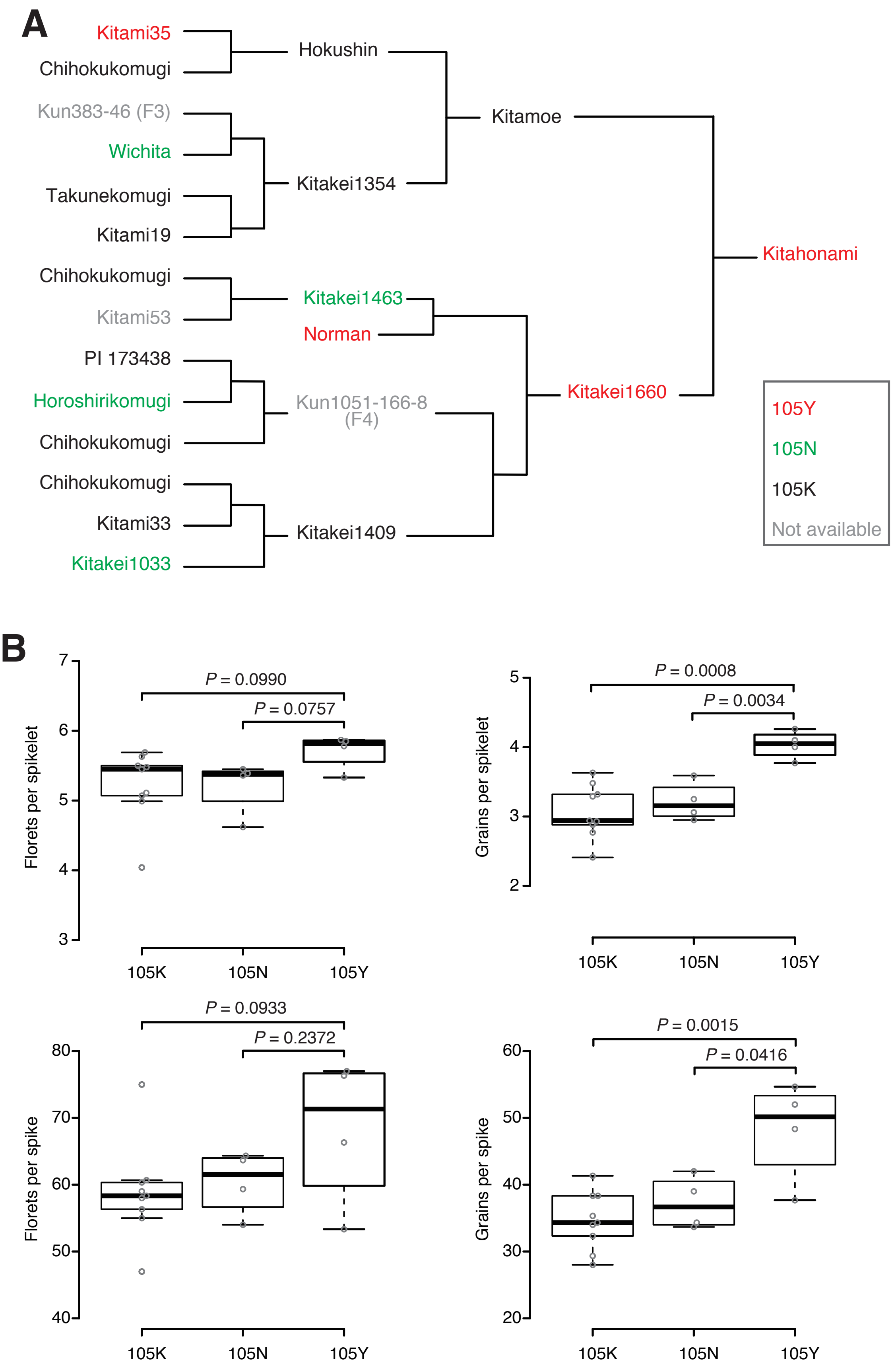


Fig. S2. The pedigree of cv. Kitahonami and their yield performance. (*A*) Genealogy of cv. Kitahonami, (*B*) variation in the number of florets per spikelet and the number of grains per spikelet in the progenitors of the cultivar.

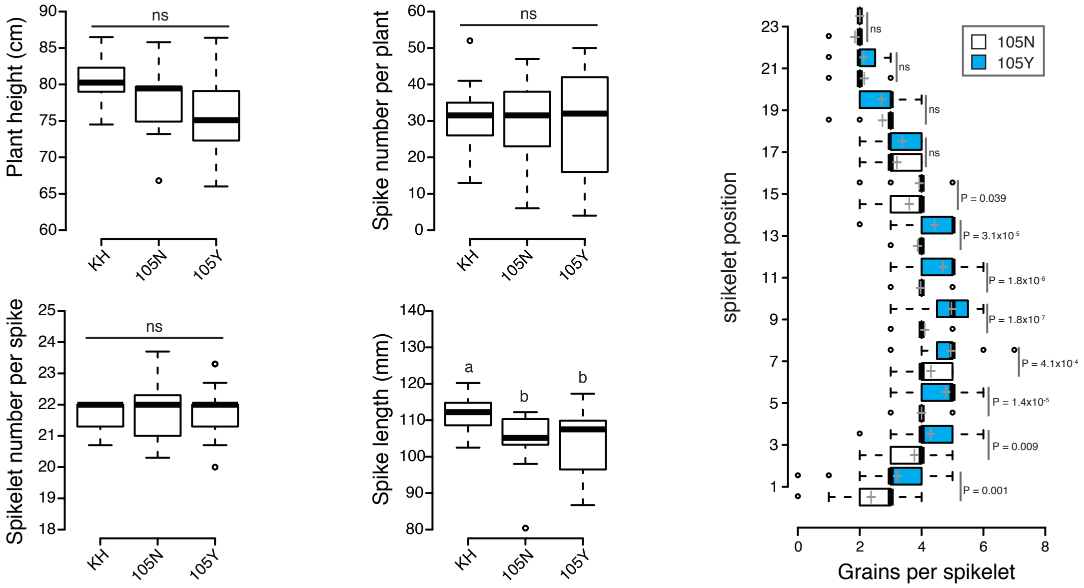


Fig. S3. The phenotype of *GNI-A1* TILLING mutants. Comparions were made between mutation homozygotes in the M_3 generation. 105N: the functional *GNI-A1* allele, 105Y: the reduced-function allele carried by cv. Kitahonami (KH). Box edges represent the 25% quantile and 75% quantile with the median values shown by bold lines. Whiskers indicate 1.5 times the interquartile range, and remaining data are indicated by circles.+: mean values. Differences between haplotype means analyzed using Tukey's Honest Significant Difference test or the Student' s *t*-test ($P \leq 0.05$). ns; non-significant.

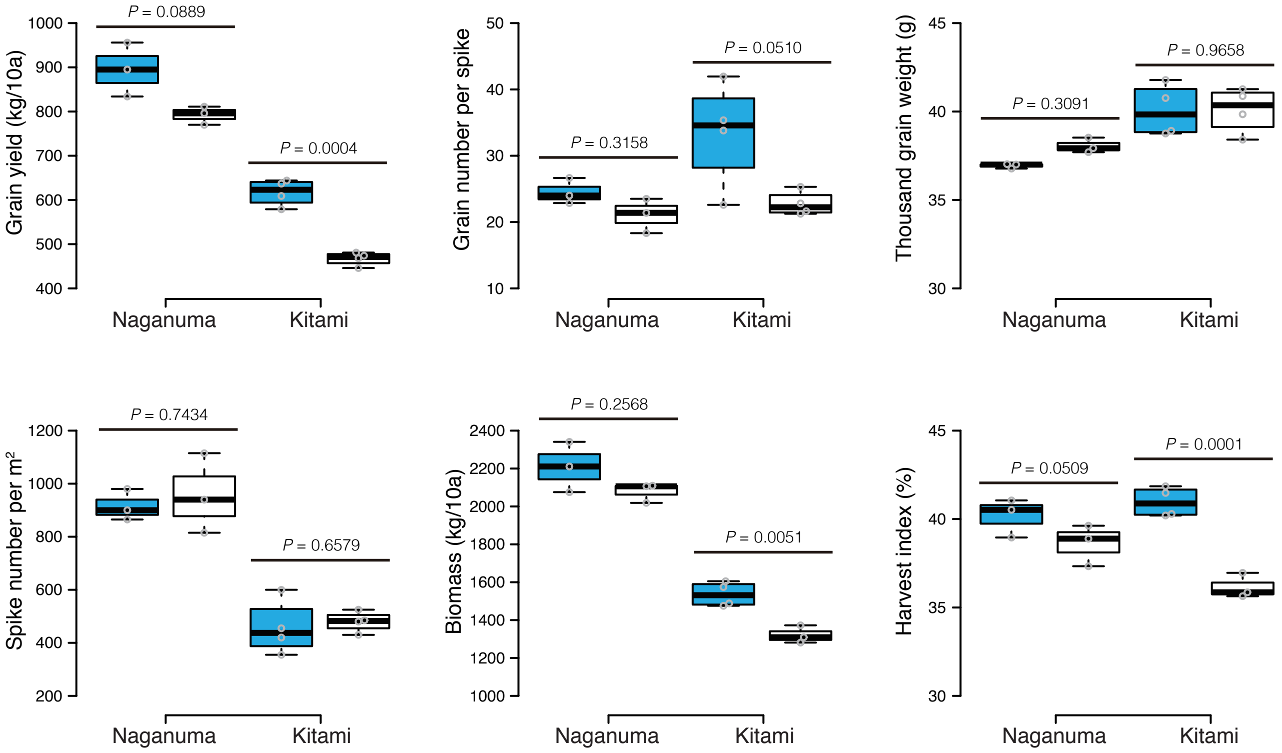
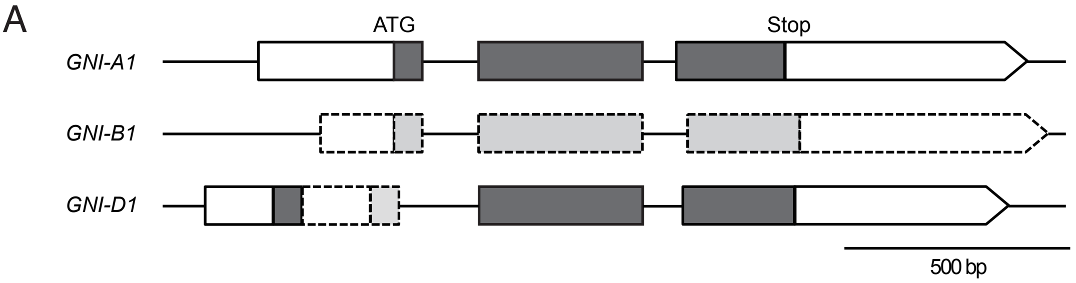


Fig. S4. Grain yield of the *GNI-A1* TILLING mutants under field conditions. The performance of M_4 generation lines was compared at two locations. The blue box indicates the 105Y allele and white box the 105N allele. The significance of differences between trait means was determined by Student' s *t*-test (*n*=4 in Kitami, *n*=3 in Naganuma).



B

```
VRS1        MDKHQLFDSSNVDTTFFAANGTAQGDTSKQRARRRRRRSARCGGGDGDGGEMDGGGDPKK   60
GNI-A1      MDNQQLFGSSYVDTPFFAANGTAQRES-QPKARRRRRRAARCGGGDGNGWEMDGGGDPKK   59
GNI-B1      MDNQQLFGSSYVDAPFFAANGTAQRES-QPKARRRRRKAARCGGGDGNGGEMDGGGDPKK   59
GNI-D1      MDNQQLFGSSYVDTSFFAANGTAQRES-QPKARRRRRRAARCGGGDGNGGEMDEGGDPKK   59

VRS1        RRLTDEQAEILELSFREDRKLETARKVYLAAELGLDPKQVAVWFQNRRARHKNKTLEEEF   120
GNI-A1      RRLTDEQAEMLELSFREERKLETGRKVYLAAELGLDPKQVAVWFQYRRARHKSKTLEQEF   119
GNI-B1      RRLTDEQAEMLELSFREERKLETGRKVYLAAELGLDPKQVAVWFQNRRARHKSKTLEQEF   119
GNI-D1      RRLTDEQAEMLELSFREERKLETGRKVYLAAELGLDPKQVAVWFQNRRARHKSKTLEQEF   119

VRS1        ARLKHAHDAAILHKCHLENELLRLKERLGATEQEVRRLRSAAGSHGASVDGGHAAGAVGV   180
GNI-A1      ARLKHAHDAAILHKCHLENEVLRLKEKLRATAEEVQRLRSAAGIH---ADGGDSAGAVSV   176
GNI-B1      TRLKHAHDADILHKCHLQNEVLRLKERLRATEEEVQRLRSAAGSHGASGDGGDAAGAVSV   179
GNI-D1      ARLKHAHDADILHKCHLENELLRLKEKLRATEEEVQRLRSAAGCHGASADGGDSAGAVSV   179

VRS1        CGGSPSSSFSTGTCQQQPGFSGADVLGRDDDLMMCVPEWFLA       222
GNI-A1      CGGSPRSSFSTGTCQQHPGFSGADVLGRDDDLMMCVPEWFFA       218
GNI-B1      CGGSPGSSFSTGTWQQHLGFSGANELGTDDDLMMCVPDCFFA       221
GNI-D1      CGGSPGSFFSTGTCQQHPGFSGADVLRPDDDLMMCVPEWFFA       221
```

Fig. S5. *GNI1* gene structure. (*A*) The structure of the three hexaploid wheat *GNI1* homoeologs. Exons shown as boxes, introns as lines. White boxes indicate UTRs and grey boxes coding regions. The transparent boxes in *GNI-B1* and *GNI-D1* indicate non-transcribed sequences. (*B*) The amino acid sequences of VRS1 and GNI1. The position of Tyr 105 (Y) in GNI-A1 is indicated.

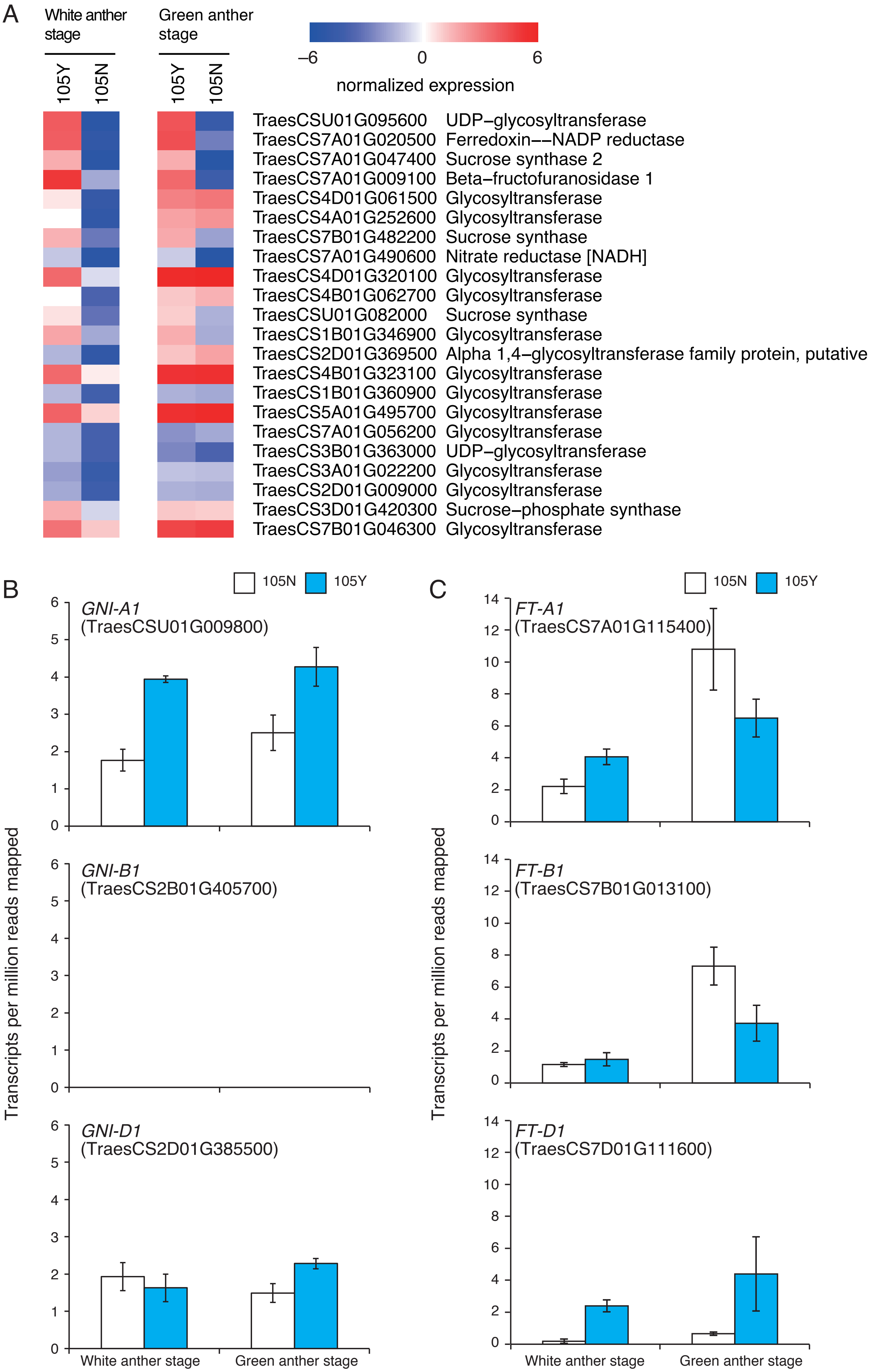


Fig. S6. Comparison of transcript level between 105Y and 105N alleles. (*A*) Heat map illustrating that carriers of the *GNI-A1* 105Y allele experience up-regulation of many genes implicated in nitrogen and sucrose metabolism. (*B* and *C*) The abundance of *GNI1* (*B*) and *FT1* (*C*) transcripts in carriers of the *GNI-A1* 105N and 105Y alleles, derived from RNA-seq. Values shown as mean±SD (n=4).

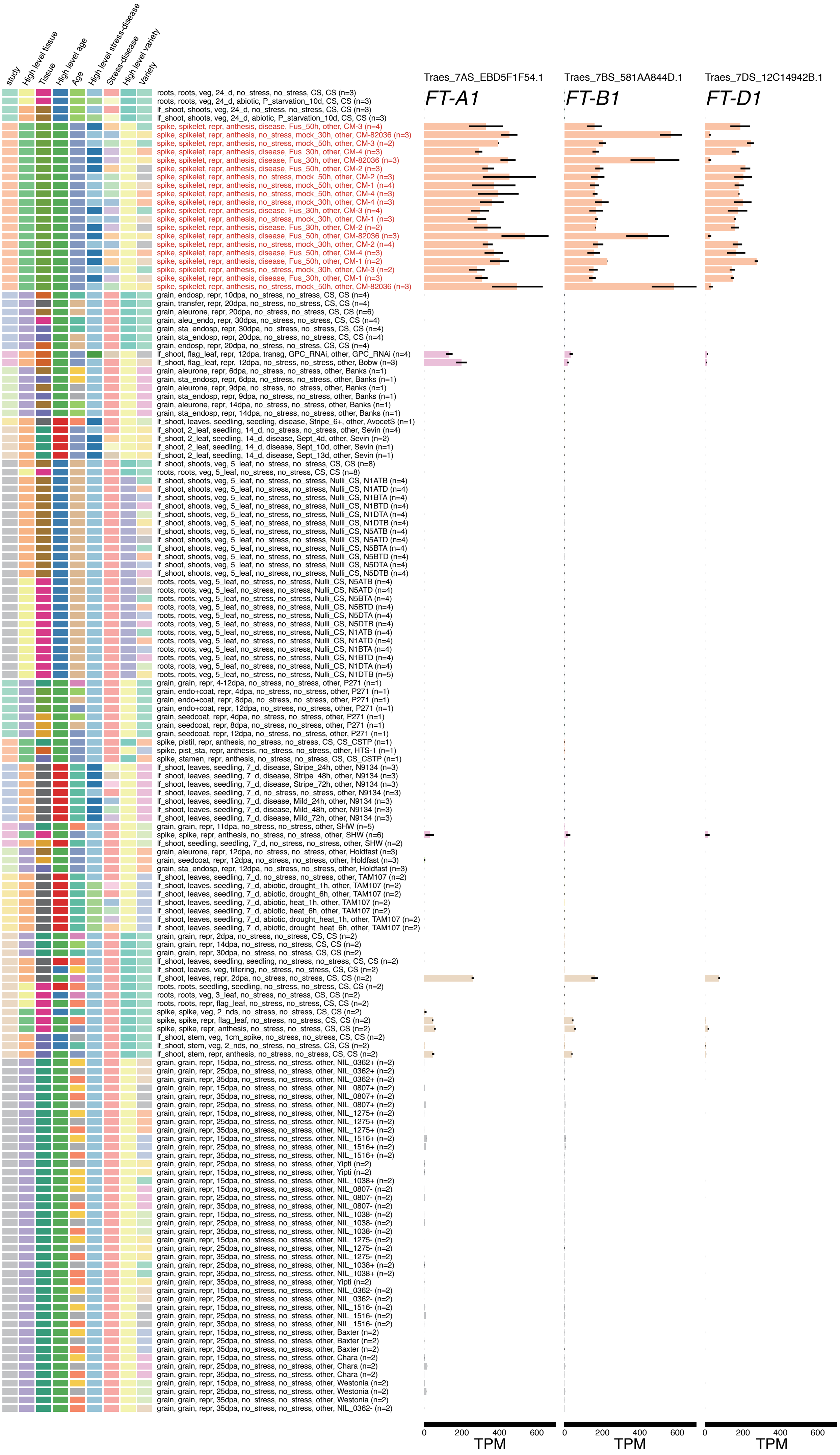


Fig. S7. *FT1* transcription in bread wheat. The abundance of transcripts is shown in the form transcripts per million reads (TPM). Data obtained from Wheat Expression Browser (http://www.wheat-expression.com).

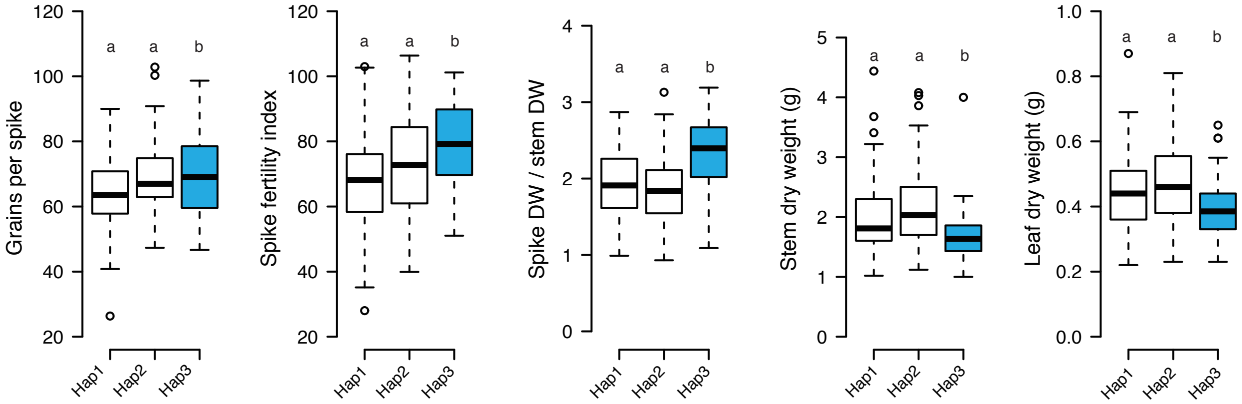


Fig. S8. The agronomic phenotype of the members of the winter bread wheat panel. The spike fertility index represents the ratio between the grain number per spike and the weight of the spike chaff. DW: dry weight. Letters are used to indicate where means differed from one another significantly ($P \leq 0.05$), as determined by Tukey's Honest Significant Difference test.

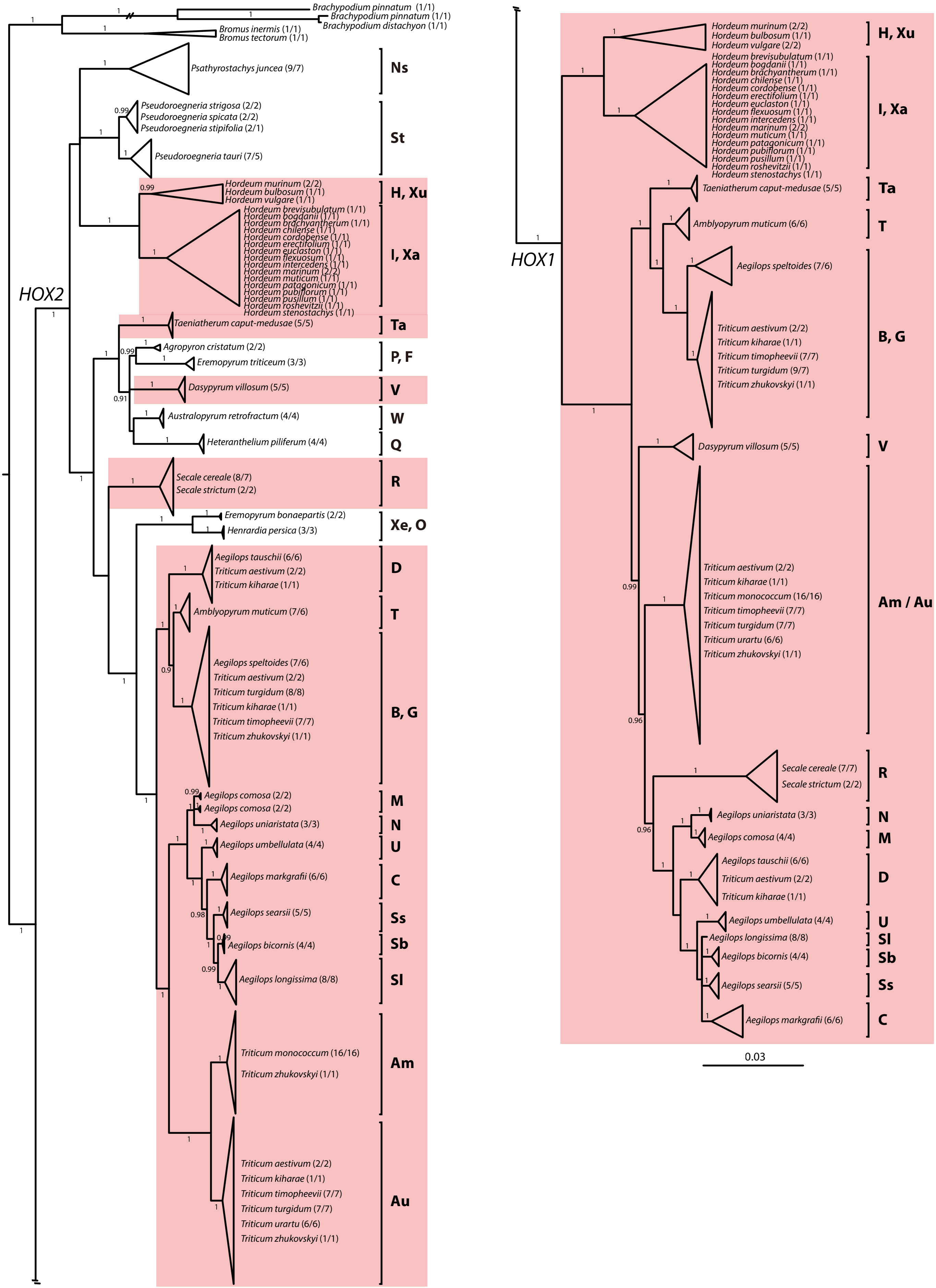


Fig. S9. The phylogeny of *HOX1* and *HOX2*. The multiple sequence alignment consisted of 361 sequences (1,496 nt) obtained from 51 Triticeae species belonging to 14 genera. *Brachypodium* and *Bromus* were used as the outgroup taxa. Support values appearing next to each node are given as a posterior probability when above 0.9. Clades were collapsed into triangles to reflect the main groupings. The area of the triangles reflects the genetic variation contained within the clade. The numbers appearing after the species names indicates the number of alleles present, followed by the number of accessions re-sequenced. Genomic groups are indicated to the right. Red boxes highlight taxa including homologs of both *HOX2* and *HOX1*. The *Ae. longissima* copy of *HOX1* did not cluster into a single clade, instead formed a polytomy with the ancestor of *Ae. bicornis*, *Ae. markgrafii*, and *Ae. searsii*.

Table S1. Genes lying within the genetic interval harboring *GNI-A1*.

Gene ID (Zabitan)	Start (bp)	End (bp)	Annotation
TRIDC2AG055740	626026540	626032109	autophagy related protein
TRIDC2AG055760	626312993	626323686	kinesin like protein
TRIDC2AG055770	626329188	626334644	BRCT domain-containing protein
TRIDC2AG055780	626566752	626567426	Lactoylglutathione lyase / glyoxalase I family protein
TRIDC2AG055790	626573695	626849477	NAD(P)H-quinone oxidoreductase subunit M
TRIDC2AG055810	**626823738**	**626838029**	**Homeobox-leucine zipper protein family**
TRIDC2AG055830	626843724	626859831	unknown function
TRIDC2AG055850	627403332	627414185	uncharacterized
TRIDC2AG055870	627918309	627928023	undescribed protein
TRIDC2AG055880	627933028	627936558	unknown function
TRIDC2AG055890	627943119	627944792	Cytochrome P450 superfamily protein
TRIDC2AG055900	628484338	628487746	Leucine-rich repeat (LRR) family protein
TRIDC2AG055910	628703514	628705838	GATA transcription factor
TRIDC2AG055920	628767384	628771868	SH3 domain-containing protein
TRIDC2AG055960	628878078	628879391	B-box zinc finger family protein
TRIDC2AG055980	629059513	629062826	undescribed protein
TRIDC2AG055990	629067624	629069433	COBRA-like protein 7
TRIDC2AG056000	630047055	630059045	Formin Homology 14
TRIDC2AG056020	630069315	630073680	Aldeyde de hydrogenase
TRIDC2AG056050	630341620	630346963	multiprotein bridging factor 1A
TRIDC2AG056060	630384017	630385673	Protein kinase superfamily protein
TRIDC2AG056070	630835927	630839606	Trihelix transcription factor GT-2
TRIDC2AG056100	631135440	631141632	sphingosine kinase 1
TRIDC2AG056120	631145110	631146719	Homeobox leucine zipper
TRIDC2AG056130	631215223	631217410	Ubiquitin-specific protease family C19-related protein
TRIDC2AG056140	631369996	631371886	Protein of unknown function, DUF584

Table S2. *GNI-A1* haplotype variation in tetraploid wheat.

Accession	Species	*GNI-A1*
PI 428018	*T. turgidum* ssp. *dicoccoides*	Hap01
28-6	*T. turgidum* ssp. *dicoccoides*	Hap01
PI 554583	*T. turgidum* ssp. *dicoccoides*	Hap01
PI 481521	*T. turgidum* ssp. *dicoccoides*	Hap01
PI 538626	*T. turgidum* ssp. *dicoccoides*	Hap01
PI 428014	*T. turgidum* ssp. *dicoccoides*	Hap01
12-3	*T. turgidum* ssp. *dicoccoides*	Hap01
18-60	*T. turgidum* ssp. *dicoccoides*	Hap01
mm5/4	*T. turgidum* ssp. *dicoccoides*	Hap01
24-39	*T. turgidum* ssp. *dicoccoides*	Hap01
10-209	*T. turgidum* ssp. *dicoccoides*	Hap01
PI 428092	*T. turgidum* ssp. *dicoccoides*	Hap01
Citr 17676	*T. turgidum* ssp. *dicoccoides*	Hap01
Rei-1	*T. turgidum* ssp. *dicoccoides*	Hap02
PI 428054	*T. turgidum* ssp. *dicoccoides*	Hap02
PI 428077	*T. turgidum* ssp. *dicoccoides*	Hap02
35-9	*T. turgidum* ssp. *dicoccoides*	Hap02
HR1-24	*T. turgidum* ssp. *dicoccoides*	Hap02
kh2/3	*T. turgidum* ssp. *dicoccoides*	Hap03
IG 46323	*T. turgidum* ssp. *dicoccoides*	Hap03
IG 45494	*T. turgidum* ssp. *dicoccoides*	Hap03
PI 538700	*T. turgidum* ssp. *dicoccoides*	Hap03
PI 503316	*T. turgidum* ssp. *dicoccoides*	Hap03
IG 116184	*T. turgidum* ssp. *dicoccoides*	Hap03
IG 46476	*T. turgidum* ssp. *dicoccoides*	Hap03
36-24	*T. turgidum* ssp. *dicoccoides*	Hap03
Citr 17675	*T. turgidum* ssp. *dicoccoides*	Hap03
TTD140	*T. turgidum* ssp. *dicoccoides*	Hap04
Am-1	*T. turgidum* ssp. *dicoccoides*	Hap04
PI 428069	*T. turgidum* ssp. *dicoccoides*	Hap05
13-B-53	*T. turgidum* ssp. *dicoccoides*	Hap06
Alm-1	*T. turgidum* ssp. *dicoccoides*	Hap06
ISR-A	*T. turgidum* ssp. *dicoccoides*	Hap06
Zabitan	*T. turgidum* ssp. *dicoccoides*	Hap06
8941	*T. turgidum* ssp. *dicoccoides*	Hap07
TTC 01 79	*T. turgidum* ssp. *dicoccum*	Hap07
G-929	*T. turgidum* ssp. *dicoccum*	Hap07
TTC 09 88	*T. turgidum* ssp. *dicoccum*	Hap07
PI 298586	*T. turgidum* ssp. *dicoccum*	Hap07
PI 197496	*T. turgidum* ssp. *dicoccum*	Hap07
TTC 02 80	*T. turgidum* ssp. *dicoccum*	Hap07
TTC 03 81	*T. turgidum* ssp. *dicoccum*	Hap07
PI94637	*T. turgidum* ssp. *dicoccum*	Hap07
PI94649	*T. turgidum* ssp. *dicoccum*	Hap07
TTC 10 89	*T. turgidum* ssp. *dicoccum*	Hap08
PI 626468	*T. turgidum* ssp. *dicoccum*	Hap08
PI 25415	*T. turgidum* ssp. *dicoccum*	Hap08
PI 225332	*T. turgidum* ssp. *dicoccum*	Hap08
PI 94633	*T. turgidum* ssp. *dicoccum*	Hap08
Gaza	*T. turgidum* ssp.*durum*	Hap08
LDN	*T. turgidum* ssp.*durum*	Hap09
kofa	*T. turgidum* ssp.*durum*	Hap09

Abu fashi	*T. turgidum* ssp.*durum*	Hap09
c-61	*T. turgidum* ssp.*durum*	Hap09
Svevo	*T. turgidum* ssp.*durum*	Hap09
Kyperounda	*T. turgidum* ssp.*durum*	Hap09
MG26427	*T. turgidum* ssp.*durum*	Hap09
Juljulith	*T. turgidum* ssp.*durum*	Hap09
Simhon	*T. turgidum* ssp.*durum*	Hap09
Dganit	*T. turgidum* ssp.*durum*	Hap09
Inbar	*T. turgidum* ssp.*durum*	Hap09
Noa	*T. turgidum* ssp.*durum*	Hap09
Durum 33	*T. turgidum* ssp.*durum*	Hap09
Durum 39	*T. turgidum* ssp.*durum*	Hap09
Eliav	*T. turgidum* ssp.*durum*	Hap09
Durum 74	*T. turgidum* ssp.*durum*	Hap09
Givati	*T. turgidum* ssp.*durum*	Hap09
Uzan	*T. turgidum* ssp.*durum*	Hap09
Abu fashi	*T. turgidum* ssp.*durum*	Hap09
m2787	*T. turgidum* ssp.*durum*	Hap09
JM-3989	*T. turgidum* ssp.*durum*	Hap09
m2034	*T. turgidum* ssp.*durum*	Hap09

Table S3. The membership of the winter bread wheat panel.

ID	Cultivar name	Country	Release year	*GNI-A1*
GW0061	Monopol	Germany	1975	Hap1
GW0215	Recital	France	1986	Hap3
GW0004	Alidos	Germany	1987	Hap1
GW0096	Zentos	Germany	1989	Hap1
GW0001	Actros	Germany	2005	Hap2
GW0037	Greif	Germany	1989	Hap3
GW0045	Kontrast	Germany	1990	Hap1
GW0003	Akteur	Germany	2003	Hap2
GW0007	Asketis	Germany	1998	Hap2
GW0025	Contra	Germany	1990	Hap3
GW0234	Hereward	United Kingdom	1991	Hap1
GW0008	Astron	Germany	1989	Hap2
GW0085	Tambor	Germany	1993	Hap1
GW0074	Ritmo	Germany	1993	Hap3
GW0086	Tarso	Germany	1994	Hap1
GW0009	Atlantis	Germany	1992	Hap2
GW0010	Batis	Germany	1994	Hap2
GW0012	Boomer	Germany	2005	Hap2
GW0013	Borneo	Germany	1997	Hap2
GW0015	Bussard	Germany	1990	Hap2
GW0019	Cardos	Germany	1998	Hap2
GW0146	Folio	France	1997	Hap1
GW0021	Centrum	Germany	2001	Hap2
GW0022	Certo	Germany	1999	Hap2
GW0136	Charger	France	1997	Hap3
GW0202	Versailles	France	1997	Hap3
GW0137	Craklin	France	1998	Hap1
GW0023	Cetus	Germany	2005	Hap2
GW0024	Compliment	Germany	2001	Hap2
GW0031	Ellvis	Germany	2002	Hap2
GW0035	Flair	Germany	1996	Hap2
GW0036	Format	Germany	2007	Hap2
GW0038	Haldor	Germany	1999	Hap2
GW0041	Impression	Germany	2005	Hap2
GW0044	Koch	Germany	2003	Hap2
GW0048	Limes	Germany	2003	Hap2
GW0050	Ludwig	Germany	1998	Hap2
GW0173	Ornicar	France	1998	Hap3
GW0029	Drifter	Germany	1999	Hap1
GW0052	Magnus	Germany	2000	Hap2
GW0054	Mandub	Germany	2003	Hap2
GW0056	Maverick	Germany	1999	Hap2
GW0057	Meteor	Germany	2006	Hap2
GW0058	Meunier	Germany	1998	Hap2
GW0059	Milvus	Germany	2004	Hap2
GW0011	Biscay	Germany	2000	Hap1
GW0075	Romanus	Germany	2000	Hap1
GW0101	History	Germany	2000	Hap1
GW0119	Autan	France	2000	Hap1
GW0178	Parador	France	2000	Hap1
GW0060	Mirage	Germany	2006	Hap2
GW0064	Naturastar	Germany	2002	Hap2

GW0065	Olivin	Germany	1999	Hap2
GW0068	Petrus	Germany	1996	Hap2
GW0069	Piko	Germany	1994	Hap2
GW0070	Potenzial	Germany	2006	Hap2
GW0071	Privileg	Germany	2004	Hap2
GW0124	Balance	France	2000	Hap3
GW0131	Caphorn	France	2000	Hap3
GW0164	Macro	United Kingdom	2000	Hap3
GW0239	Symbol	Denmark	2001	Hap1
GW0072	Quebon	Germany	2004	Hap2
GW0076	Schamane	Germany	2005	Hap2
GW0078	Skalmeje	Germany	2006	Hap2
GW0079	Skater	Germany	2000	Hap2
GW0081	Sokrates	Germany	2001	Hap2
GW0082	Solitär	Germany	2004	Hap2
GW0087	Tommi	Germany	2002	Hap2
GW0088	Toras	Germany	2004	Hap2
GW0027	Dekan	United Kingdom	2001	Hap3
GW0106	Andalou	France	2001	Hap3
GW0118	Aubusson	France	2001	Hap3
GW0129	Calisto	France	2001	Hap3
GW0151	Hamac	France	2001	Hap3
GW0193	Semafor	France	2001	Hap3
GW0198	Tapidor	France	2001	Hap3
GW0055	Manhattan	Germany	2002	Hap1
GW0084	SW Topper	Germany	2002	Hap1
GW0113	Allister	France	2002	Hap1
GW0144	Eveil	France	2002	Hap1
GW0181	PR22R20	France	2002	Hap1
GW0089	Toronto	Germany	1990	Hap2
GW0090	Transit	Germany	1994	Hap2
GW0093	Tulsa	Germany	2004	Hap2
GW0099	Dream	Germany	1999	Hap2
GW0026	Cubus	Germany	2002	Hap3
GW0095	Winnetou	Germany	2002	Hap3
GW0126	Bastide	France	2002	Hap3
GW0133	Catalan	France	2002	Hap3
GW0142	Equilibre	France	2002	Hap3
GW0165	Marcheval	France	2002	Hap3
GW0184	Quatuor	France	2002	Hap3
GW0188	Royssac	France	2002	Hap3
GW0102	Lindos		1994	Hap2
GW0116	Astuce	France	2004	Hap2
GW0125	Baltimor	France	1998	Hap2
GW0128	Brando	France	2000	Hap2
GW0134	Cezanne	France	1998	Hap2
GW0017	Campari	Germany	2003	Hap3
GW0018	Capnor	Germany	2003	Hap3
GW0066	Opus	Germany	2003	Hap3
GW0039	Hermann	Germany	2004	Hap1
GW0083	Striker	Germany	2004	Hap1
GW0094	Türkis	Germany	2004	Hap1
GW0100	Florett	Denmark	2004	Hap1
GW0105	Exotic	France	2004	Hap1

GW0121	Azimut	France	2004	Hap1
GW0161	Kleber	France	2004	Hap1
GW0200	Toisondor	France	2004	Hap1
GW0141	Epidoc	France	2006	Hap2
GW0147	Forban	France	2001	Hap2
GW0149	Graindor	France	2006	Hap2
GW0150	Grisby	France	2001	Hap2
GW0155	Incisif	France	2005	Hap2
GW0159	Intense	France	2001	Hap2
GW0160	Isengrain	France	1997	Hap2
GW0002	Akratos	Germany	2004	Hap3
GW0040	Heroldo	Germany	2004	Hap3
GW0080	Sobi	Germany	2004	Hap3
GW0109	Acienda	France	2004	Hap3
GW0111	Alcazar	France	2004	Hap3
GW0156	Inoui	France	2004	Hap3
GW0167	Mendel	France	2004	Hap3
GW0187	Rosario	France	2004	Hap3
GW0006	Anthus	Germany	2005	Hap1
GW0014	Brilliant	Germany	2005	Hap1
GW0030	Elegant	Germany	2005	Hap1
GW0047	Leiffer	Germany	2005	Hap1
GW0051	Magister	Germany	2005	Hap1
GW0145	Exotic	France	2005	Hap1
GW0158	Intact	France	2005	Hap1
GW0162	Levis	France	1998	Hap2
GW0169	Nirvana	France	2001	Hap2
GW0171	Octet	France	2007	Hap2
GW0172	Ordeal	France	1998	Hap2
GW0174	Orpic	France	1998	Hap2
GW0175	Orvantis	France	2000	Hap2
GW0091	Tuareg	Germany	2005	Hap3
GW0110	Aguila	France	2005	Hap3
GW0115	Arobase	France	2005	Hap3
GW0132	Caribou	France	2005	Hap3
GW0166	Maxyl	France	2005	Hap3
GW0168	Mercato	France	2005	Hap3
GW0177	Paledor	France	2005	Hap3
GW0062	Mulan	Germany	2006	Hap1
GW0077	Skagen	Germany	2006	Hap1
GW0097	Zobel	Germany	2006	Hap1
GW0122	Azzuro	France	2006	Hap1
GW0170	Nuage	France	2006	Hap1
GW0204	Inédit	France	2006	Hap1
GW0240	Torkil	Denmark	2006	Hap1
GW0180	PR 22 R 28	France	2000	Hap2
GW0182	Pulsar	France	1998	Hap2
GW0183	Pytagor	France	2002	Hap2
GW0185	Raison	France	2006	Hap2
GW0194	Seyrac	France	2006	Hap2
GW0201	Trocadero	France	2001	Hap2
GW0207	Expert	France	2007	Hap2
GW0209	H05581A	France		Hap2
GW0049	Lucius	Germany	2006	Hap3

GW0053	Manager	Germany	2006	Hap3
GW0092	Tukan	Germany	2006	Hap3
GW0114	Arack	France	2006	Hap3
GW0130	Campero	France	2006	Hap3
GW0148	Garcia	France	2006	Hap3
GW0152	Haussmann	France	2006	Hap3
GW0157	Instinct	France	2006	Hap3
GW0176	Paladain	France	2006	Hap3
GW0186	Richepain	France	2006	Hap3
GW0196	Sogood	France	2006	Hap3
GW0203	CCB Ingénio	France	2006	Hap3
GW0205	CCB Préférence	France	2006	Hap3
GW0033	Esket	Germany	2007	Hap1
GW0073	Retro	Germany	2007	Hap1
GW0224	Iridium	France	2007	Hap1
GW0210	H04438	France		Hap2
GW0221	Altigo	France	2007	Hap2
GW0223	Boisseau	France	2007	Hap2
GW0236	Senat	Denmark	2000	Hap2
GW0237	Smuggler	Denmark		Hap2
GW0042	Inspiration	Germany	2007	Hap3
GW0043	Jenga	Germany	2007	Hap3
GW0046	Kranich	Germany	2007	Hap3
GW0206	Intérêt	France	2007	Hap3
GW0208	Bueno	France	2007	Hap3
GW0218	Bermude	France	2007	Hap3
GW0222	Bagou	France	2007	Hap3
GW0103	Julius	Germany	2008	Hap1
GW0238	Solist	Denmark	2000	Hap2
GW0241	Trintella	Denmark		Hap2
GW0235	Portland	Denmark		Hap1
GW0252	Globus	Austria	2003	Hap2
GW0259	Corsaire	Czech Republic	1999	Hap2
GW0098	Cliff	Denmark		Hap3
GW0107	LOCH 3754 LP 232.1.03	Germany		Hap3
GW0225	Andi			Hap3
GW0242	Tuscan	Denmark		Hap3

Table S4. Primers used in this study.

Gene	5'- sequence -3'	Vector
Fine mapping		
Xhuj005	AAGAAGCTTGTCTCAGTTCCTG	
	GCGGCAAGGCGAAGGTAA	
Xhuj006	AGGGTACTACTGCTGGCTCT	
	CCACAGCGTCACAATCAGAT	
Xhuj007	GCTTACGGGAGGGAGTATGAG	
	AATCATACGACAACACCCGATG	
Xhuj008	ATGTCCATCAATTGCCTTGG	
	TGGCATCTCATGGTGACCTA	
Xhuj009	AAGGAGCTGACGTACGTGGT	
	TGGTAGAGCCCGAATCTGTC	
Xhuj010	CCCGATGGAGATGAATGAAC	
	GGGCAGATCCACATATCACC	
Xhuj011	TCAGCCTCATGCTCAACATC	
	GTCGGTCATCTCCACAGGTT	
Xhuj012	GGAGCCCTCCATTCGTCAAA	
	CGCGCCAGATATAAGCACCA	
Xhuj013	CAGCAAATGACCCAATCAGA	
	GGTCCAAGACTGACTGTTGCT	
Xhuj014	CCCAGAGACTTCCTTGCATT	
	GGATTGGCGCTTCAAAGTAA	
Xhuj015	CACTTCTGCTGGCAAGATCA	
	CCACATCATCTCCCTTAGGC	
Xhuj016	GGGAACTTGAGCTAGGGGAG	
	TGATCTCAAAACACCCACCC	
RNAi experiment		
GNI-A1	CACCTGGTTTTTCGCATGAATTAGC	pENTR D-TOPO
	CCAAAGATTAAAATCGCCGGA	
Transgene	GCCGACGCGAAGCGGGTAGAT	
	CCAAAGATTAAAATCGCCGGA	
Transgene	GCGCGTTGGCGGTAACAAGAA	
	CCAAAGATTAAAATCGCCGGA	
Transgene	GTCTGCACCATCGTCAACC	
	GAAGTCCAGCTGCCAGAAAC	
qRT-PCR		
GNI-A1	ACAAAATAGGCGCTATAGCTGCTC	
	CGGGACAGATGATTTCTAGAGGTT	
GNI-B1	ACAAAATAGGTGCGTTAATTG	
	GGTATTTCCTGATTCTGCAGC	
GNI-D1	GCTATGCTATGGCTGCATGC	
	GATTTAGCGGCGGCCTTTTC	
Actin	AAGTACAGTGTCTGGATTGGAGGG	
	TCGCAACTTAGAAGCACTTCCG	
In situ hybridization		
GNI1	TGGTTTTTCGCATGAATTAGC	pBluescript II KS(+)
	CCAAAGATTAAAATCGCCAGA	
Resequencing		
GNI-A1	AGTCTCCAAAATTAAGTGGCAT	
	TGCCATTAATACACACTCTCCA	
GNI-B1	GTATTTGTCAAAAAAGAGAGTCGG	
	AGTTATATTAGAACACAAGGGCAT	
GNI-D1	AGGAGCGCAAGCTGGAGACT	
	GATTTAGCGGCGGCCTTTTC	
For sequencing	CCTGGCTCGGAAAGCACCTA	
	GGAGGGAGAGAGACCTAGGC	
	TCCAACCATCCCTTCTCTTCC	
	GGATGGTAACGGCTGGGAGA	
	AAGGAGAAGCTGAGAGCGAC	
	CCGATAGCAGCGTGTTCGAG	

Table S5. List of Triticeae and outgroup species used in this study.

Line	Species	Subspecies / variety / cultivar	Genomes	Ploidy level (measured)	Country of origin	Material source / Reference
PI 222050	*Psathyrostachys juncea* (Fisch.) Nevski		Ns	2×	Afghanistan	USDA, Idaho, USA
PI 565055	*Psathyrostachys juncea* (Fisch.) Nevski		Ns	2×	Russian Federation	USDA, Idaho, USA
PI 565077	*Psathyrostachys juncea* (Fisch.) Nevski		Ns	2×	China	USDA, Idaho, USA
PI 565080	*Psathyrostachys juncea* (Fisch.) Nevski		Ns	2×	Russian Federation	USDA, Idaho, USA
PI 595135	*Psathyrostachys juncea* (Fisch.) Nevski		Ns	2×	China	USDA, Idaho, USA
PI 598613	*Psathyrostachys juncea* (Fisch.) Nevski		Ns	2×	Kazakhstan	USDA, Idaho, USA
PI 619487	*Psathyrostachys juncea* (Fisch.) Nevski		Ns	2×	Mongolia	USDA, Idaho, USA
PI 632532	*Pseudoroegneria spicata* (Pursh) A. Löve		St	2×	USA	USDA, Idaho, USA
PI 635993	*Pseudoroegneria spicata* (Pursh) A. Löve		St	2×	USA	USDA, Idaho, USA
PI 325181	*Pseudoroegneria stipifolia* (Czern. ex Nevski) A. Löve		St	2×	Russian Federation	USDA, Idaho, USA
PI 499638	*Pseudoroegneria strigosa* (M. Bieb.) A. Löve	ssp. *aegilopsides* (Drobow) A. Löve	St	2×	China	USDA, Idaho, USA
W6 14049	*Pseudoroegneria strigosa* (M. Bieb.) A. Löve		St	2×	Russian Federation	USDA, Idaho, USA
PI 228389	*Pseudoroegneria tauri* (Boiss. & Balansa) A. Löve	ssp. *libanotica* (Hack.) A. Löve	St	2×	Iran	USDA, Idaho, USA
PI 330688	*Pseudoroegneria tauri* (Boiss. & Balansa) A. Löve	ssp. *libanotica* (Hack.) A. Löve	St	2×	Iran	USDA, Idaho, USA
PI 401274	*Pseudoroegneria tauri* (Boiss. & Balansa) A. Löve	ssp. *libanotica* (Hack.) A. Löve	St	2×	Iran	USDA, Idaho, USA
PI 401322	*Pseudoroegneria tauri* (Boiss. & Balansa) A. Löve		St	2×	Iran	USDA, Idaho, USA
PI 401333	*Pseudoroegneria tauri* (Boiss. & Balansa) A. Löve		St	2×	Iran	USDA, Idaho, USA
H3140a	*Hordeum vulgare*	ssp. *spontaneum* (K. Koch) Asch. & Graebn.	H	2×	Cyprus	NordGen, Alnarp, Sweden
H3878	*Hordeum bulbosum* L.		H	2×	Italy	NordGen, Alnarp, Sweden
BCC 2002	*Hordeum murinum* L.		Xu	2×		
H1012	*Hordeum murinum* L.	ssp. *leporinum* (Link) Arcangeli	Xu	4×	–	NordGen, Alnarp, Sweden
H2024	*Hordeum pusillum* Nutt.		I	2×	USA	NordGen, Alnarp, Sweden
H1941	*Hordeum intercedens* Nevski		I	2×	USA	NordGen, Alnarp, Sweden
H2148	*Hordeum euclaston* Steud.		I	2×	Uruguay	NordGen, Alnarp, Sweden
H2127	*Hordeum flexuosum* Nees ex Steud.		I	2×	Uruguay	NordGen, Alnarp, Sweden
H6479	*Hordeum muticum* J.		I	2×	Argentina	NordGen,

	Presl					Alnarp, Sweden
H1816	*Hordeum chilense* Brongn.		I	2×	Chile	NordGen, Alnarp, Sweden
H6460	*Hordeum cordobense* Bothmer, N. Jacobsen & Nicora		I	2×	Argentina	NordGen, Alnarp, Sweden
H1780	*Hordeum stenostachys* Godr.		I	2×	Argentina	NordGen, Alnarp, Sweden
H4014	*Hordeum bogdanii* Wilensky		I	2×	Pakistan	NordGen, Alnarp, Sweden
H10070	*Hordeum roshevitzii* Bowden		I	2×	Russia	NordGen, Alnarp, Sweden
H10227	*Hordeum brevisubulatum* (Trin.) Link		I	2×	Tajikistan	NordGen, Alnarp, Sweden
H3317	*Hordeum brachyantherum*	ssp. californicum (Covas & Stebbins)	I	2×	USA	NordGen, Alnarp, Sweden
H1150	*Hordeum erectifolium* Bothmer, N. Jacobsen & R.B .Jørg.		I	2×	Argentina	NordGen, Alnarp, Sweden
H1358	*Hordeum patagonicum*	ssp. mustersii (Nicora) Bothmer, Giles & N.Jacobsen	I	2×	Argentina	NordGen, Alnarp, Sweden
BCC 2028	*Hordeum pubiflorum* Hook. f.	ssp. *pubiflorum*	I	2×	Argentina	International Barley Core Collection, IPK
H121	*Hordeum marinum* Huds.		Xa	2×	Greece	NordGen, Alnarp, Sweden
BCC 2006	*Hordeum marinum* Huds.	ssp. *marinum*	Xa	2×	Spain	International Barley Core Collection, IPK
GRA 2570	*Taeniatherum caput-medusae* (L.) Nevski	ssp. *crinitum* (Schreb.) Melderis	Ta	2×	Kazakhstan	IPK, Gatersleben, Germany
PI 220589	*Taeniatherum caput-medusae* (L.) Nevski	ssp. *crinitum* (Schreb.) Melderis	Ta	2×	Afghanistan	USDA, Idaho, USA
PI 251387	*Taeniatherum caput-medusae* (L.) Nevski	ssp. *crinitum* (Schreb.) Melderis	Ta	2×	Iran	USDA, Idaho, USA
PI 561095	*Taeniatherum caput-medusae* (L.) Nevski	ssp. *crinitum* (Schreb.) Melderis	Ta	2×	Turkey	USDA, Idaho, USA
GRA 1126	*Taeniatherum caput-medusae* (L.) Nevski		Ta	2×	Tajikistan	IPK, Gatersleben, Germany
PI 494615	*Agropyron cristatum* (L.) Gaertn.		P	2×	Romania	USDA, Idaho, USA
PI 598631	*Agropyron cristatum* (L.) Gaertn.		P	2×	Kazakhstan	USDA, Idaho, USA
GRA 2250	*Eremopyrum triticeum* (Gaertn.) Nevski		Ft	2×	Kazakhstan	IPK, Gatersleben, Germany
PI 502364	*Eremopyrum triticeum* (Gaertn.) Nevski		Ft	2×	Russian Federation	USDA, Idaho, USA
W6 26631	*Eremopyrum triticeum* (Gaertn.) Nevski		Ft	2×	Kazakhstan	USDA, Idaho, USA
GRA 1020	*Dasypyrum villosum* (L.) P. Candargy		V	2×	Italy	IPK, Gatersleben,

						Germany
GRA 1027	*Dasypyrum villosum* (L.) P. Candargy		V	2×	Bulgaria	IPK, Gatersleben, Germany
PI 368884	*Dasypyrum villosum* (L.) P. Candargy		V	2×	Turkey	USDA, Idaho, USA
W6 19414	*Dasypyrum villosum* (L.) P. Candargy		V	2×	Bulgaria	USDA, Idaho, USA
W6 7300	*Dasypyrum villosum* (L.) P. Candargy		V	2×	Greece	USDA, Idaho, USA
PI 531553	*Australopyrum retrofractum* (Vickery) A. Löve		W	2×	Australia	USDA, Idaho, USA
PI 533013	*Australopyrum retrofractum* (Vickery) A. Löve		W	2×	Australia	USDA, Idaho, USA
PI 533014	*Australopyrum retrofractum* (Vickery) A. Löve		W	2×	Australia	USDA, Idaho, USA
PI 547363	*Australopyrum retrofractum* (Vickery) A. Löve		W	2×	Australia	USDA, Idaho, USA
PI 314152	*Heteranthelium piliferum* (Banks & Sol.) Hochst.		Q	2×	Uzbekistan	USDA, Idaho, USA
PI 401351	*Heteranthelium piliferum* (Banks & Sol.) Hochst.		Q	2×	Iran	USDA, Idaho, USA
PI 401353	*Heteranthelium piliferum* (Banks & Sol.) Hochst.		Q	2×	Iran	USDA, Idaho, USA
PI 401354	*Heteranthelium piliferum* (Banks & Sol.) Hochst.		Q	2×	Iran	USDA, Idaho, USA
PI 618662	*Secale cereale* L.	ssp. *afghanicum* (Vavilov) K. Hammer	R	2×	Armenia	USDA, Idaho, USA
PI 618665	*Secale cereale* L.	ssp. *ancestrale* Zhuk.	R	2×		USDA, Idaho, USA
PI 618669	*Secale cereale* L.	ssp. *rigidum* Vavilov & Antropov	R	2×	Turkey	USDA, Idaho, USA
PI 618671	*Secale cereale* L.	ssp. *segetale* Zhuk.	R	2×	Turkey	USDA, Idaho, USA
PI 253957	*Secale cereale* L.		R	2×	Afghanistan	USDA, Idaho, USA
R 1027	*Secale cereale* L.		R	2×	Italy	IPK, Gatersleben, Germany
KU-S-4309	*Secale cereale* L.	ssp. *afghanicum* (Vavilov) K. Hammer	R	2×		Kyoto Univ., Kyoto, Japan
R 1108	*Secale strictum* C. Presl	ssp. *kuprijanovii* (Grossh.) K. Hammer	R	2×	Kazakhstan	IPK, Gatersleben, Germany
R 853	*Secale strictum* C. Presl	ssp. *strictum* (Grossh.) K. Hammer	R	2×	Italy	IPK, Gatersleben, Germany
PI 203442	*Eremopyrum bonaepartis* (Spreng.) Nevski		Xe	2×	Turkey	USDA, Idaho, USA
PI 222956	*Eremopyrum bonaepartis* (Spreng.) Nevski		Xe	2×	Iran	USDA, Idaho, USA
PI 401347	*Henrardia persica* (Boiss.) C.E. Hubb.		O	2×	Iran	USDA, Idaho, USA
PI 577112	*Henrardia persica* (Boiss.) C.E. Hubb.		O	2×	Turkey	USDA, Idaho, USA
PI 577113	*Henrardia persica*		O	2×	Turkey	USDA,

	(Boiss.) C.E. Hubb.					Idaho, USA
KU-6-1	*Aegilops markgrafii* (Greuter) K. Hammer	*Aegilops caudata* ssp. *polyathera* Boiss.	C	2×	–	Kyoto Univ., Kyoto, Japan
KU-6-2	*Aegilops markgrafii* (Greuter) K. Hammer	*Aegilops caudata* ssp. *typica* L.	C	2×	Syria	Kyoto Univ., Kyoto, Japan
AE 1381	*Aegilops markgrafii* (Greuter) K. Hammer		C	2×	Greece	IPK, Gatersleben, Germany
PI 254863	*Aegilops markgrafii* (Greuter) K. Hammer		C	2×	Iraq	USDA, Idaho, USA
PI 542208	*Aegilops markgrafii* (Greuter) K. Hammer		C	2×	Turkey	USDA, Idaho, USA
PI 596287	*Aegilops markgrafii* (Greuter) K. Hammer		C	2×	Turkey	USDA, Idaho, USA
AE 1069	*Aegilops tauschii* Coss.	ssp. *tauschii* var. *meyeri* (Griseb.) Tzvelev	D	2×	Syrian Arab Republic	IPK, Gatersleben, Germany
46800	*Aegilops tauschii* Coss.		D	2×	Turkey	ICARDA, Aleppo, Syria
49116	*Aegilops tauschii* Coss.		D	2×	Iran	ICARDA, Aleppo, Syria
AE 956	*Aegilops tauschii* Coss.		D	2×	Tajikistan	IPK, Gatersleben, Germany
KU-20-10	*Aegilops tauschii* Coss.		D	2×	Iran	Kyoto Univ., Kyoto, Japan
AE 1255	*Aegilops comosa* Sm.	ssp. *comosa* (Boiss.) Eig	M	2×	Greece	IPK, Gatersleben, Germany
AE 1378	*Aegilops comosa* Sm.	ssp. *comosa* var. *comosa*	M	2×	Greece	IPK, Gatersleben, Germany
AE 783	*Aegilops comosa* Sm.	ssp. *heldreichii* (Boiss.) Eig	M	2×	Greece	IPK, Gatersleben, Germany
PI 276970	*Aegilops comosa* Sm.	var. *comosa*	M	2×	Greece	USDA, Idaho, USA
AE 157	*Aegilops uniaristata* Vis.		N	2×	Former Soviet Union	IPK, Gatersleben, Germany
AE 680	*Aegilops uniaristata* Vis.		N	2×	Italy	IPK, Gatersleben, Germany
PI 276996	*Aegilops uniaristata* Vis.		N	2×	Turkey	USDA, Idaho, USA
PI 487231	*Aegilops speltoides* Tausch	ssp. *ligustica* (Savign.) Zhuk.	S	2×	Syrian Arab Republic	USDA, Idaho, USA
PI 486264	*Aegilops speltoides* Tausch	ssp. *ligustica* var. *ligustica* (Savign.) Bornm.	S	2×	Turkey	USDA, Idaho, USA
AE 1064	*Aegilops speltoides* Tausch	ssp. *speltoides* (Savign.) Bornm.	S	2×	Syrian Arab Republic	IPK, Gatersleben, Germany
AE 900	*Aegilops speltoides* Tausch	ssp. *speltoides* (Savign.) Bornm.	S	2×	Iraq	IPK, Gatersleben, Germany
PI 542245	*Aegilops speltoides* Tausch	var. *speltoides*	S	2×	Turkey	USDA, Idaho, USA
KU-7856	*Aegilops speltoides* Tausch		S	2×	Iraq	Kyoto Univ., Kyoto, Japan
AE 106	*Aegilops bicornis* Jaub. & Spach	var. *bicornis*	Sb	2×	Russian Federation	IPK, Gatersleben, Germany
AE 1079	*Aegilops bicornis* Jaub. & Spach	var. *mutica* (Asch.) Eig	Sb	2×	Jordan	IPK, Gatersleben, Germany
AE 788	*Aegilops bicornis* Jaub. & Spach		Sb	2×	Libya	IPK, Gatersleben,

						Germany
KU-5786	*Aegilops bicornis* Jaub. & Spach		Sb	2×	Egypt	Kyoto Univ., Kyoto, Japan
AE 1078	*Aegilops longissima* Schweinf. & Muschl.	ssp. *longissima* (Eig) K. Hammer	Sl	2×	Jordan	IPK, Gatersleben, Germany
AE 417	*Aegilops longissima* Schweinf. & Muschl.	ssp. *longissima* (Eig) K. Hammer	Sl	2×	Israel	IPK, Gatersleben, Germany
AE 906	*Aegilops longissima* Schweinf. & Muschl.	ssp. *longissima* (Eig) K. Hammer	Sl	2×	Israel	IPK, Gatersleben, Germany
AE 133	*Aegilops longissima* Schweinf. & Muschl.	ssp. *sharonensis* var. *major* (Eig) K. Hammer	Sl	2×		IPK, Gatersleben, Germany
PI 604141	*Aegilops longissima* Schweinf. & Muschl.		Sl	2×	Israel	USDA, Idaho, USA
TA 1921	*Aegilops longissima* Schweinf. & Muschl.		Sl	2×	Jordan	Kansas State Univ., Kansas, USA
KU-4-1	*Aegilops longissima* Schweinf. et Muschl.		Sl	2×	Israel	Kyoto Univ., Kyoto, Japan
KU-5-2	*Aegilops sharonensis* Eig		Sl	2×	Israel	Kyoto Univ., Kyoto, Japan
AE 1075	*Aegilops searsii* Feldman & Kislev e× K. Hammer		Ss	2×	Jordan	IPK, Gatersleben, Germany
AE 1083	*Aegilops searsii* Feldman & Kislev e× K. Hammer		Ss	2×	Syrian Arab Republic	IPK, Gatersleben, Germany
KU-14655	*Aegilops searsii* Feldman & Kislev e× K. Hammer		Ss	2×	Israel	Kyoto Univ., Kyoto, Japan
PI 599142	*Aegilops searsii* Feldman & Kislev e× K. Hammer		Ss	2×	Jordan	USDA, Idaho, USA
PI 599148	*Aegilops searsii* Feldman & Kislev e× K. Hammer		Ss	2×	Syrian Arab Republic	USDA, Idaho, USA
01C2100106	*Amblyopyrum muticum* (Boiss.) Eig.	var. *loliacea* (Jaub. & Sp.) Eig	T	2×	Turkey	Genebank Res. Inst. Crop Prod., Prague, Czech Republic
PI 560122	*Amblyopyrum muticum* (Boiss.) Eig.		T	2×	Turkey	USDA, Idaho, USA
PI 560124	*Amblyopyrum muticum* (Boiss.) Eig.		T	2×	Turkey	USDA, Idaho, USA
PI 560125	*Amblyopyrum muticum* (Boiss.) Eig.		T	2×	Turkey	USDA, Idaho, USA
PI 560126	*Amblyopyrum muticum* (Boiss.) Eig.		T	2×	Turkey	USDA, Idaho, USA
PI 636562	*Amblyopyrum muticum* (Boiss.) Eig.		T	2×	Turkey	USDA, Idaho, USA
AE 153	*Aegilops umbellulata* Zhuk.	ssp. *umbellulata* var. *umbellulata*	U	2×	Iran	IPK, Gatersleben, Germany
AE 740	*Aegilops umbellulata* Zhuk.		U	2×	Turkey	IPK, Gatersleben, Germany
AE 811	*Aegilops umbellulata* Zhuk.		U	2×	Azerbaijan	IPK, Gatersleben, Germany
KU-8-1	*Aegilops* umbellulata Zhuk.		U	2×	Turkey	Kyoto Univ., Kyoto, Japan
BGRC 20518	*Triticum monococcum* L.	ssp. *aegilopoides* (Link) Thell.	A	2×	Balkans	IPK, Gatersleben, Germany

ID 379	*Triticum monococcum* L.	ssp. *aegilopoides* (Link) Thell.	A	2×	Lebanon	Kansas State Univ., Kansas, USA
PI 272520	*Triticum monococcum* L.	ssp. *aegilopoides* (Link) Thell.	A	2×	Hungary	USDA, Idaho, USA
PI 427451	*Triticum monococcum* L.	ssp. *aegilopoides* (Link) Thell.	A	2×	Turkey	USDA, Idaho, USA
PI 427620	*Triticum monococcum* L.	ssp. *aegilopoides* (Link) Thell.	A	2×	Turkey	USDA, Idaho, USA
PI 427627	*Triticum monococcum* L.	ssp. *aegilopoides* (Link) Thell.	A	2×	Turkey	USDA, Idaho, USA
KU-101-1	*Triticum monococcum* L.	ssp. *aegilopoides* (Link) Thell.	A	2×	–	Kyoto Univ., Kyoto, Japan
BGRC 43454	*Triticum monococcum* L.		A	2×	Germany	IPK, Gatersleben, Germany
BGRC 43459	*Triticum monococcum* L.		A	2×	Switzerland	IPK, Gatersleben, Germany
PI 119423	*Triticum monococcum* L.		A	2×		USDA, Idaho, USA
TRI 13061	*Triticum monococcum* L.		A	2×	Italy	IPK, Gatersleben, Germany
TRI 13612	*Triticum monococcum* L.		A	2×	Georgia	IPK, Gatersleben, Germany
KU-104-2	*Triticum monococcum* L.		A	2×	–	Kyoto Univ., Kyoto, Japan
KU-104-5	*Triticum monococcum* L.		A	2×	–	Kyoto Univ., Kyoto, Japan
TRI 17824	*Triticum urartu* Thumanjan e× Gandilyan	var. *spontaneoalbum* Tumanian e× Dorof. & Filat.	A	2×	Lebanon	IPK, Gatersleben, Germany
TRI 18407	*Triticum urartu* Thumanjan e× Gandilyan	var. *spontaneoalbum* Tumanian e× Dorof. & Filat.	A	2×	Syrian Arab Republic	IPK, Gatersleben, Germany
PI 428184	*Triticum urartu* Thumanjan e× Gandilyan		A	2×	Turkey	USDA, Idaho, USA
PI 428317	*Triticum urartu* Thumanjan e× Gandilyan		A	2×	Iran	USDA, Idaho, USA
PI 428320	*Triticum urartu* Thumanjan e× Gandilyan		A	2×	Lebanon	USDA, Idaho, USA
KU-199-12	*Triticum urartu* Thumanjan e× Gandilyan		A	2×	Turkey	Kyoto Univ., Kyoto, Japan
TRI 15127	*Triticum turgidum* L.	ssp. *carthlicum* (Nevski) A. Löve & D. Löve	BA	4×	Georgia	IPK, Gatersleben, Germany
KU-8812	*Triticum turgidum* L.	ssp. *dicoccoides* (Koern. e× Aschers. & Graebn.) Thell	BA	4×	Iraque	Kyoto Univ., Kyoto, Japan
PI 352322	*Triticum turgidum* L.	ssp. *dicoccoides* (Koern. e× Aschers. & Graebn.) Thell	BA	4×	Lebanon	USDA, Idaho, USA
PI 41025	*Triticum turgidum* L.	ssp. *dicoccoides* (Koern. e× Aschers. & Graebn.) Thell	BA	4×	Russian Federation	USDA, Idaho, USA
PI 428018	*Triticum turgidum* L.	ssp. *dicoccoides* (Koern. e× Aschers. & Graebn.) Thell	BA	4×	Turkey	USDA, Idaho, USA

PI 428093	*Triticum turgidum* L.	ssp. *dicoccoides* (Koern. e× Aschers. & Graebn.) Thell	BA	4×	Israel	USDA, Idaho, USA
TRI 3428	*Triticum turgidum* L.	ssp. *polonicum* (L.) Thell.	BA	4×	Turkey	IPK, Gatersleben, Germany
TRI 6243	*Triticum turgidum* L.		BA	4×	Iran	IPK, Gatersleben, Germany
01C0202037	*Triticum timopheevii* (Zhuk.) Zhuk.		GA	4×	SUN	Genebank Res. Inst. Crop Prod., Prague, Czech Republic
IG 119456	*Triticum timopheevii* (Zhuk.) Zhuk.		GA	4×	Syrian Arab Republic	ICARDA, Aleppo, Syria
KU-1820	*Triticum timopheevii* (Zhuk.) Zhuk.		GA	4×	Former Soviet Union	Kyoto Univ., Kyoto, Japan
KU-1966	*Triticum timopheevii* (Zhuk.) Zhuk.		GA	4×	Turkey	Kyoto Univ., Kyoto, Japan
KU-8561	*Triticum timopheevii* (Zhuk.) Zhuk.		GA	4×		Kyoto Univ., Kyoto, Japan
KU-8939	*Triticum timopheevii* (Zhuk.) Zhuk.		GA	4×	Turkey	Kyoto Univ., Kyoto, Japan
PI 352264	*Triticum timopheevii* (Zhuk.) Zhuk.		GA	4×	Armenia	USDA, Idaho, USA
TRI 7258	*Triticum zhukovskyi* Menabde & Ericzjan		GAA	6×	Georgia	IPK, Gatersleben, Germany
01C0204240	*Triticum kiharae* Dorof. & Migush.		GAD	6×		Genebank Res. Inst. Crop Prod., Prague, Czech Republic
BK 2011	*Triticum aestivum* L.	cv. Chinese Spring	BAD	6×		Dr. Benjamin Kilian, IPK
PI 83402	*Triticum aestivum* L.	ssp. *sphaerococcum* (Percival) Mac Key	BAD	6×	China	USDA, Idaho, USA
Outgroup						
AH_2011	*Brachypodium pinnatum* (L.) P. Beauv.			4×		Prof. Andreas Houben, IPK
W6 21403	*Bromus inermis* Leyss.			4×	Mongolia	USDA, Idaho, USA
GRA 1085	*Bromus tectorum* L.			4×	Libya	IPK, Gatersleben, Germany

References

1. Joppa LR (1993) Chromosome engineering in tetraploid wheat. *Crop Science* 33(5):908-913.
2. Golan G, Oksenberg A, & Peleg Z (2015) Genetic evidence for differential selection of grain and embryo weight during wheat evolution under domestication. *J Exp Bot* 66(19):5703-5711.
3. Avni R*, et al.* (2017) Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. *Science* 357(6346):93-97.
4. Miki D & Shimamoto K (2004) Simple RNAi vectors for stable and transient suppression of gene function in rice. *Plant Cell Physiol* 45(4):490-495.
5. Pellegrineschi A*, et al.* (2002) Identification of highly transformable wheat genotypes for mass production of fertile transgenic plants. *Genome* 45(2):421-430.
6. Kirby EJM & Appleyard M (1981) *Cereal development guide* (Cereal Unit, Kenilworth) 1st Ed p 80.
7. Komatsuda T*, et al.* (2007) Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. *Proceedings of the National Academy of Sciences* 104(4):1424-1429.
8. International Wheat Genome Sequencing C*, et al.* (2018) Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science* 361(6403).
9. Bray NL, Pimentel H, Melsted P, & Pachter L (2016) Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol* 34(5):525-527.
10. R Core Team (2015) R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing).
11. Law CW, Chen Y, Shi W, & Smyth GK (2014) voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome biology* 15(2):R29.
12. Smyth GK (2005) Limma: linear models for microarray data. *Bioinformatics and computational biology solutions using R and Bioconductor*, (Springer), pp 397-420.
13. Alexa A & Rahnenfuhrer J (2010) topGO: enrichment analysis for gene ontology. *R package version* 2(0).
14. Ramirez-Gonzalez RH*, et al.* (2018) The transcriptional landscape of polyploid wheat. *Science* 361(6403).
15. Borrill P, Ramirez-Gonzalez R, & Uauy C (2016) expVIP: a Customizable RNA-seq Data Analysis and Visualization Platform. *Plant Physiol* 170(4):2172-2186.
16. Kumar S, Stecher G, & Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol* 33(7):1870-1874.
17. Bandelt HJ, Forster P, & Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 16(1):37-48.
18. Guo Z*, et al.* (2017) Genome-wide association analyses of 54 traits identified multiple loci for the determination of floret fertility in wheat. *New Phytol* 214(1):257-270.

19. Bernhardt N, Brassac J, Kilian B, & Blattner FR (2017) Dated tribe-wide whole chloroplast genome phylogeny indicates recurrent hybridizations within Triticeae. *BMC Evol Biol* 17(1):141.
20. Kearse M*, et al.* (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12):1647-1649.
21. Brassac J & Blattner FR (2015) Species-Level Phylogeny and Polyploid Relationships in Hordeum (Poaceae) Inferred by Next-Generation Sequencing and In Silico Cloning of Multiple Nuclear Loci. *Systematic biology* 64(5):792-808.
22. Katoh K & Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30(4):772-780.
23. Darriba D, Taboada GL, Doallo R, & Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9(8):772.
24. Schwarz G (1978) Estimating the Dimension of a Model. *Ann. Statist.* 6(2):461-464.
25. Ronquist F & Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19(12):1572-1574.
26. Escobar JS*, et al.* (2011) Multigenic phylogeny and analysis of tree incongruences in Triticeae (Poaceae). *BMC Evol Biol* 11:181.
27. Mason-Gamer RJ (2013) Phylogeny of a genomically diverse group of *Elymus* (Poaceae) allopolyploids reveals multiple levels of reticulation. *PLoS One* 8(11):e78449.