

1 **New Phytologist Supporting Information**

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3 Article title **Sequence based mapping identifies a candidate transcription repressor**
4 **underlying awn suppression at the *B1* locus in wheat**

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11 The following supporting information is available for this article:

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14 tissue.

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26 **Table S7:** Geographic distribution of *B1* haplotypes.

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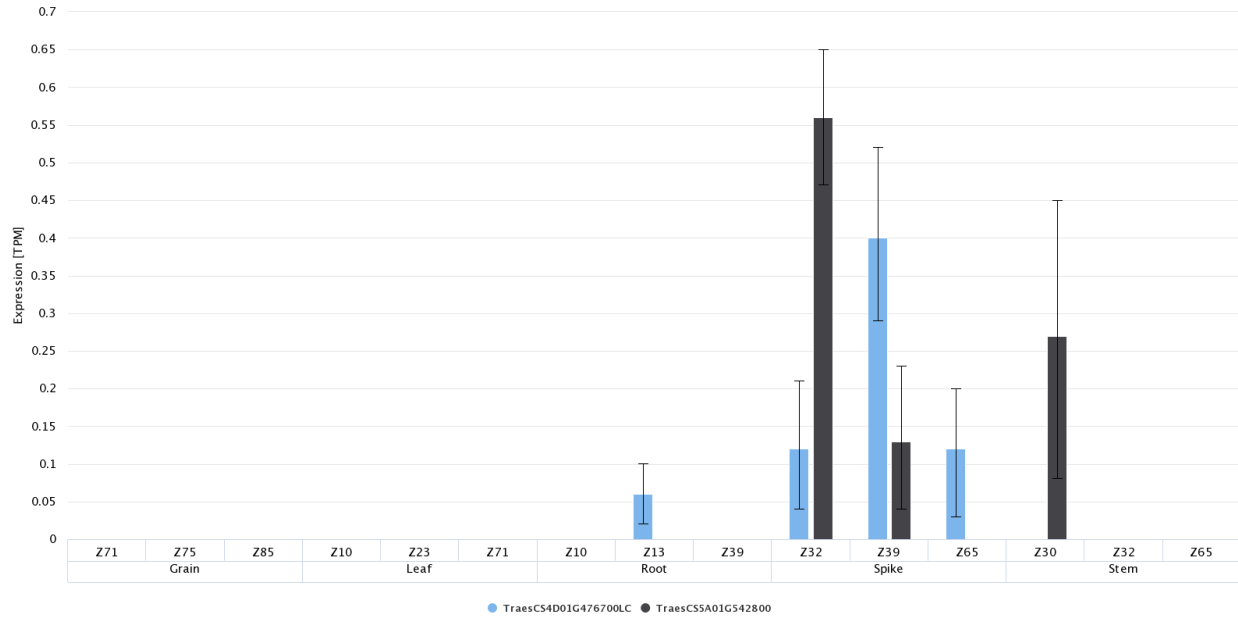
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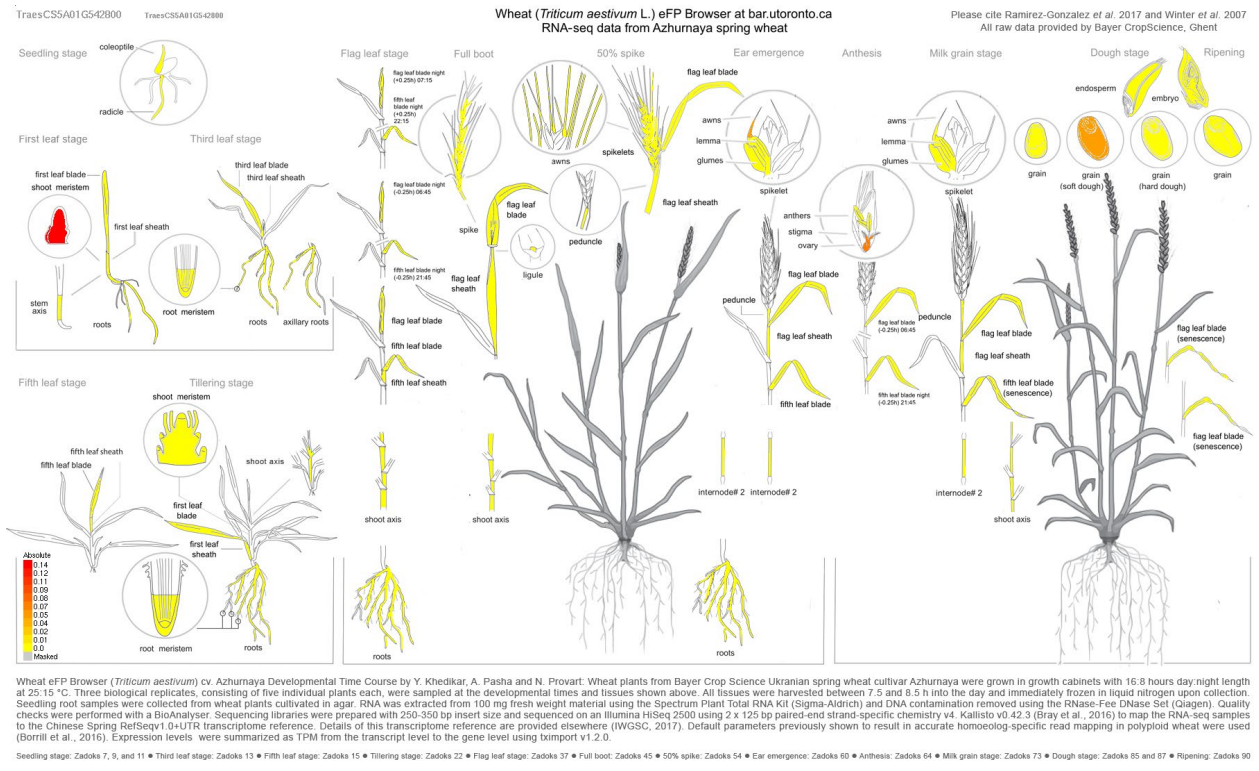
1 **Fig. S1.** Expression of *TraesCS5A02G542800* and *TraesCS4D01G476700LC* in different
 2 tissues. Expression is quantified in transcripts per million (TPM), representing raw reads scaled
 3 by gene length and total reads sequenced (Source: <https://wheat.pw.usda.gov/WheatExp/>).
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1 **Fig. S2.** Expression patterns of *TraesCS5A02G542800*. Wheat eFP browser output displaying
 2 regions with no or low expression of *B1* candidate gene *TraesCS5A02G542800* (yellow), and
 3 regions with higher expression (orange to red) in the awned bar spring wheat *Azhurnaya*. The
 4 *TraesCS5A02G542800* zinc finger protein is primarily expressed within the developing spike, as
 5 well as expressed in the awns, developing grain, and ovary. Source:
 6 (http://bar.utoronto.ca/efp_wheat/cgi-bin/efpWeb.cgi) (Ramirez-Gonzales *et al.*, 2018, Winter *et*
 7 *al.*, 2007).

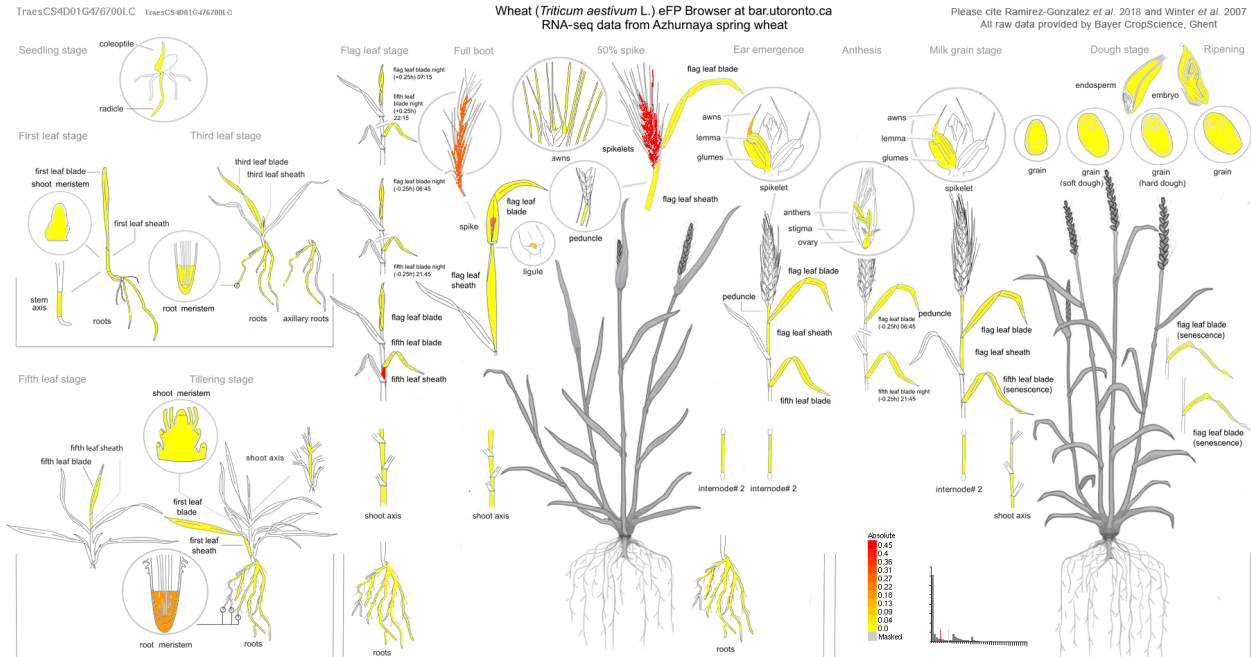
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1 **Fig. S3.** Expression patterns of *TraesCS4D01G476700LC*. Wheat eFP browser output
 2 displaying regions with no or low expression of *TraesCS4D01G476700LC* (yellow), and regions
 3 with higher expression (orange to red) in the awned spring wheat *Azhurnaya*.
 4 *TraesCS4D01G476700LC* is primarily expressed in the spike after the development of awn
 5 tissue. Source: (http://bar.utoronto.ca/efp_wheat/cgi-bin/efpWeb.cgi) (Ramirez-Gonzales *et al.*,
 6 2018, Winter *et al.*, 2007).

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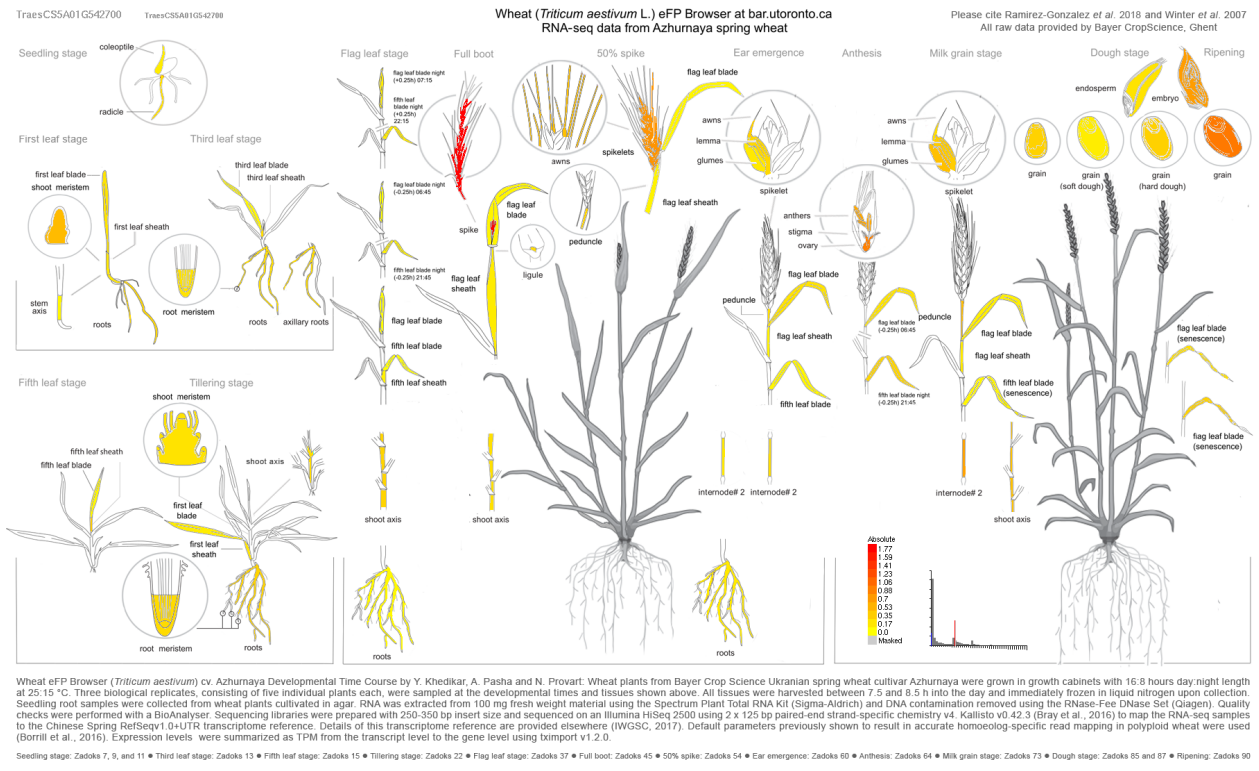
Wheat eFP Browser (*Triticum aestivum*) cv. Azhurnaya Developmental Time Course by Y. Khedkar, A. Pasha and N. Provart. Wheat plants from Bayer Crop Science Ukrainian spring wheat cultivar Azhurnaya were grown in growth cabinets with 16.8 hours day/night length at 25/15 °C. Three biological replicates, consisting of five individual plants each, were sampled at the developmental times and tissues shown above. All tissues were harvested between 7.5 and 9.5 h into the day and immediately frozen in liquid nitrogen upon collection. Seedling root samples were collected from wheat plants cultivated in agar. RNA was extracted from 100 mg fresh weight material using the Spectrum Plant Total RNA Kit (Sigma-Aldrich) and DNA contamination removed using the RNase-Free DNase set (Qiagen). Quality checks were performed with a BioAnalyser. Sequencing libraries were prepared with 250-350 bp insert size and sequenced on an Illumina HiSeq 2500 using 2 x 125 bp paired-end strand-specific chemistry v4. Kallisto v0.42.3 (Bray *et al.*, 2016) to map the RNA-seq samples to the Chinese Spring RefSeq v1.0+UTR transcriptome reference. Details of this transcriptome reference are provided elsewhere (IWGSC, 2017). Default parameters previously shown to result in accurate homoeolog-specific read mapping in polyploid wheat were used (Borrell *et al.*, 2016). Expression levels were summarized as TPM from the transcript level to the gene level using tximport v1.2.0.

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Seedling stage: Zadoks 7, 9, and 11 • Third leaf stage: Zadoks 13 • Fifth leaf stage: Zadoks 15 • Tilling stage: Zadoks 22 • Flag leaf stage: Zadoks 37 • Full boot: Zadoks 45 • 50% spike: Zadoks 54 • Ear emergence: Zadoks 60 • Anthesis: Zadoks 64 • Milk grain stage: Zadoks 73 • Dough stage: Zadoks 85 and 87 • Ripening: Zadoks 90

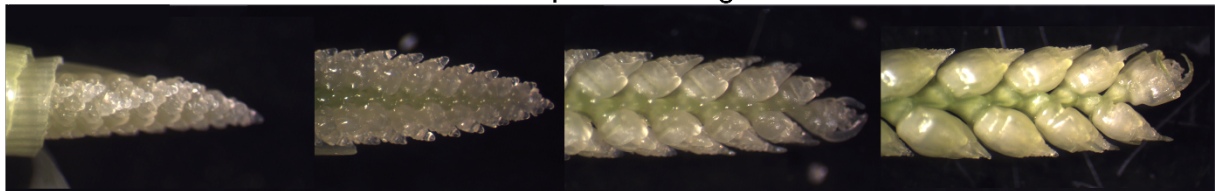
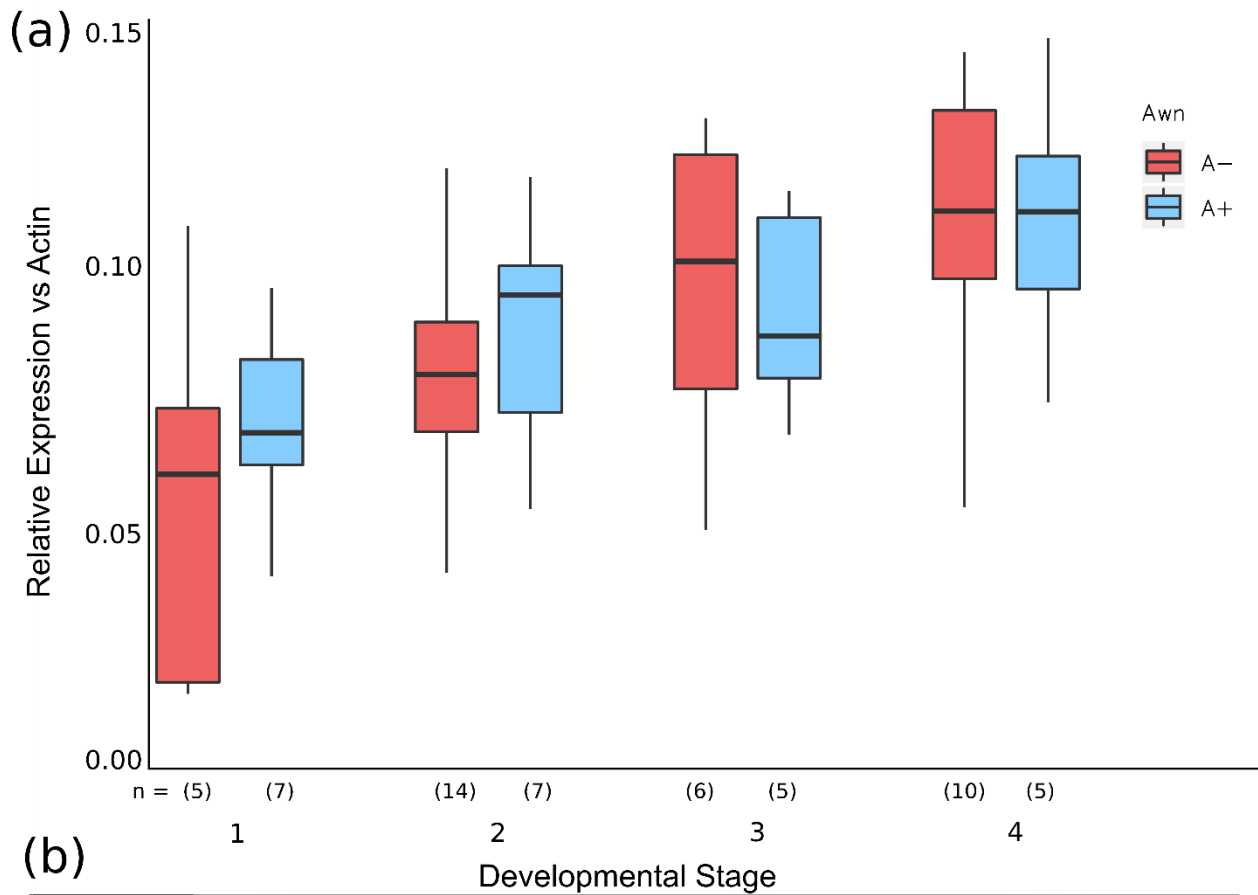
1 **Fig. S4.** Expression patterns of *TraesCS5A01G542700*. Wheat eFP browser output displaying
 2 regions with no or low expression of predicted gene *TraesCS5A01G542700* (yellow), and
 3 regions with higher expression (orange to red) in the awned spring wheat *Azhurnaya*.
 4 *TraesCS5A01G542700* is annotated as universal stress protein family with protein kinase
 5 domain and expressed in most tissues, with higher expression in the spike and in grain tissues
 6 after the development of awns. Source: (http://bar.utoronto.ca/efp_wheat/cgi-bin/efpWeb.cgi)
 7 (Ramirez-Gonzales *et al.*, 2018, Winter *et al.*, 2007).

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1 **Fig. S5.** Expression of *TraesCS5A02G542700* in apical meristems of awned and awnless
 2 wheat. (a) Expression of *TraesCS5A02G542700* in apical meristems of awned (blue) and
 3 awnless (red) wheat plants at different developmental stages where 1 = younger meristems and
 4 4 = older meristems. Number of biological replicates (n) is given per group, with three technical
 5 replications per biological replicate. (b) Representative spikes at each developmental stage.



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1 **Table S1.** Positions and descriptions of KASP markers (NA where information non-applicable).

Name	SNP Position	Type	Source	SNP	Gene Id	Annotated Functional Domains
5A814	696993814	Intergenic	GBS	G/C	NA	NA
5A78871	697887148	Intron variant	Exome capture	G/A	TraesCS5A02G540400	Formyl transferase
5A208800	698208800	Intron variant	Exome capture	A/C	TraesCS5A02G541400	SNF2 family domain, Helicase conserved domain
BW8226_227	698508163	Missense exon 2	Illuminia SNP array (Mackay et. al 2014)	T/G	TraesCS5A02G542600	Sugar -and other - Transporter
5A13057	698513057	Intron variant	Exome Capture	T/G	TraesCS5A02G542700	Universal stress protein family, protein kinase
5A15019	698515019	Intron variant	Exome Capture	A/G	TraesCS5A02G542700	Universal stress protein family, protein kinase
5A16541	698516541	Missense exon 9	Sequence Alignment	A/G	TraesCS5A02G542700	Universal stress protein family, protein kinase
5A28417	698528417	Upstream	GBS	T/C	TraesCS5A02G542800	C2H2 Zinc Finger
5A29396	698529396	Downstream	Sequence alignment	G/A	TraesCS5A02G542800	C2H2 Zinc Finger
5A32641	698532641	Intergenic	Sequence alignment	Del	NA	NA
5A91913	698591913	Intergenic	Sequence alignment	C/T	NA	NA
5A10592	698610592	Intergenic	Sequence alignment	A/G	NA	NA

5A13482	698613482	Intergenic	Sequence alignment	AA/CT	NA	NA
5A14919	698614919	Intergenic	Sequence alignment	Del	NA	NA
5A30334	698630334	Synonymous variant	Exome capture	C/T	TraesCS5A02G542900	F-box-like, Armadillo -beta-catenin-like repeat
5A48195	702748195	Upstream	Exome capture	T/G	TraesCS5A02G548800	Helicase domain, P-loop_NTPase superfamily, EBV_NA3 super family
5A70348	705570348	Missense	Exome capture	C/G	TraesCS5A02G552800	Domain of unknown function - DUF 4220 -, DUF594

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1 **Table S2:** Sequences of KASP markers.

Name	Allele	Sequence
5A814	A1	GAAGGTGACCAAGTTCATGCTAAATTCCTGCTAGACACTCGTGAG
	A2	GAAGGTCGGAGTCAACGGATTAATTCCTGCTAGACACTCGTGAC
	C	GTTTGCGCGAATCCTGCAGCGAA
5A78871	A1	GAAGGTGACCAAGTTCATGCTGTGCCGCCTCCATTCCCAC
	A2	GAAGGTCGGAGTCAACGGATTTCGTGCCGCCTCCATTCCCAC
	C	CCGTCGAACAGGTGTCGGAGC
5A208800	A1	GAAGGTGACCAAGTTCATGCTCCTTCCCCGCTGCCCCGA
	A2	GAAGGTCGGAGTCAACGGATTTCCTTCCCCGCTGCCCCG
	C	GTGCTCAAGGAGAAGCTAAGAGGTA
5A13057	A1	GAAGGTGACCAAGTTCATGCTGGTCGATCCATCCATTTCATCATGA
	A2	GAAGGTCGGAGTCAACGGATTGTCGATCCATCCATTTCATCATGC
	C	CCGTTTCATGTTCTAGACTGAATCTGAAT
5A15019	A1	GAAGGTGACCAAGTTCATGCTATGAACATCTTCATTGAAACTATATTTACACA
	A2	GAAGGTCGGAGTCAACGGATTGAACATCTTCATTGAAACTATATTTACACG
	C	GAACCCTGCAATTAACCAAAAACAAAAGCTA

5A28417	A1	GAAGGTGACCAAGTTCATGCTTGCGGCCGCTCAG
	A2	GAAGGTCGGAGTCAACGGATTGCATGCTTGCGGCCGCTCAA
	C	GACAGTAATAATGCTGCAGTAGATGTGTA
5A29396	A1	GAAGGTGACCAAGTTCATGCTGCCTAGAGACAAAAATAAAGTTATTATTATTC
	A2	GAAGGTCGGAGTCAACGGATTGGCCTAGAGACAAAAATAAAGTTATTATTATTT
	C	ACCATTGCAATTATAGCACCAAGATATAAA
5A32641	A1	GAAGGTGACCAAGTTCATGCTAGCTACGGGCCCACTTRGACA
	A2	GAAGGTCGGAGTCAACGGATTCTACGGGCCCACTTRGACG
	C	CCTGCGGGGCTCCCAGCAA
5A91913	A1	GAAGGTGACCAAGTTCATGCTCACATGCAACACACCACTTGTCA
	A2	GAAGGTCGGAGTCAACGGATTACATGCAACACACCACTTGTCTG
	C	TTCACACTCCTACTTCCCCAGGTT
5A613482	A1	GAAGGTGACCAAGTTCATGCTTTCCAGCAAAGTTGGAAGTACAATTTA
	A2	GAAGGTCGGAGTCAACGGATTCCAGCAAAGTTGGAAGTACAATTTT
	C	TAGTAAAGCGCGTCCAACCCTTCTA
5A614989	A1	GAAGGTGACCAAGTTCATGCTGATTAAGATATTCAATTTTGGATTTGATTCATA

	A2	GAAGGTCGGAGTCAACGGATTAAGATATTCAATTTTGGATTTGATTCATG
	C	GATCAAAATTGAAAGCGTTGATCTGGTCAA
5A610592	A1	GAAGGTGACCAAGTTCATGCTATACACGGCTTCCACAATTAGTTGT
	A2	GAAGGTCGGAGTCAACGGATTACACGGCTTCCACAATTAGTTGC
	C	CCCTAACTAACATATAGCCATGTGCAAT
5A30334	A1	GAAGGTGACCAAGTTCATGCTCTTCGAGTGGCAAGCGCAAC
	A2	GAAGGTCGGAGTCAACGGATTCTCTTCGAGTGGCAAGCGCAAT
	C	CCATGTGGGTTGGTCTCAGGGAT
5A48195	A1	GAAGGTGACCAAGTTCATGCTGGTTTCTTCAGAAAATGGAGGTCGA
	A2	GAAGGTCGGAGTCAACGGATTGTTTCTTCAGAAAATGGAGGTCGC
	C	CTTCATTGATGACCTCCCCATCTTTATAT
5A70348	A1	GAAGGTGACCAAGTTCATGCTGCGGTCTTGCAGGATGCTGAC
	A2	GAAGGTCGGAGTCAACGGATTGCGGTCTTGCAGGATGCTGAG
	C	GGGGTAGCCATACTTCTCTATAGAT
BW8226_22 7	A1	CGTCCATGGAGTCGTTCCCTCAAT
	A2	GTCCATGGAGTCGTTCCCTCAAG
	C	GTGGTACACGTCCGGGAAGAAT

- 1 **Table S3.** Haplotypes and awn status of sequenced lines. Observed haplotypes and geographic origin of accessions selected for
- 2 sequencing of B1 and the surrounding region.

Name	Haplotype	Origin	Awns
SS_MPV-57	Hap8	Virginia	-
LA-95135	Hap1	Louisiana	+
AR05055-1-1	Hap3	Arkansas	+
Echinoides	Hap3	Afghanistan	+
Ideal	Hap3	Denmark	+
Sandu 22	Hap6	Romania	+
Tadzyksaja 13	Hap8	Tajikstan	-
Winter Bearded	Hap8	UK	-
IWA 8613594	Hap8	Iran	-
IWA 8606785	Hap3	Iran	+

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1 **Table S4.** Sequences of RT-qPCR markers. Sequences for qPCR primers used for quantifying gene expression.

Name	Sequence
Actin-SYBR-F1 (Li <i>et al.</i> , 2013)	ATGGAAGCTGCTGGAATCCAT
Actin-SYBR-R1 (Li <i>et al.</i> , 2013)	CCTTGCTCATAACGGTCAGCAATAC
B1_24_F	GGAGATGGAAGAGGGGCTCGAT
B1_8_R	TTGAAGCTGCGTGAGCAGTAGG
2700_exon9_F	CGGAACTGGGTCTAATGGGACT
2700_exon9_R	CTTGATTGAAATGCTTGGTACTGGAGG

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- 1 **Table S5.** SNPs significantly associated with awn suppression. Genome position and LOD scores of markers significantly associated
- 2 with presence or absence of awns in association mapping panel of 640 soft red winter wheat lines. Most significant marker in the
- 3 region is noted in bold font.

SNP	maf	FDR Adjusted P-values
S5A_681455268	0.163964	0.000321
S5A_684942630	0.320721	3.43E-07
S5A_685141655	0.368468	7.79E-06
S5A_690417237	0.083784	1.56E-09
S5A_693326887	0.227027	0.000183
S5A_695581531	0.451351	2.75E-08
S5A_696479493	0.477477	0.000153
S5A_696993814	0.446847	1.11E-08
S5A_697552811	0.266667	1.56E-09
S5A_697590026	0.252252	7.37E-08
S5A_698003176	0.283784	3.21E-06
S5A_698127281	0.285586	6.38E-07
S5A_698225912	0.263964	1.05E-07
S5A_698320453	0.257658	2.35E-08
S5A_698528417	0.404505	7.19E-57
S5A_699803948	0.308108	2.32E-16
S5A_700181210	0.252252	2.63E-09
S5A_700435349	0.097297	6.16E-14
S5A_702633797	0.223423	4.09E-05

S5A_702908675	0.227027	0.001581
S5A_703364169	0.222523	0.001969
S5A_704142049	0.233333	0.003892
S5A_705293051	0.100901	3.16E-09
S5A_705351694	0.102703	1.11E-08
S5A_705365208	0.097297	1.56E-09
S5A_706065949	0.276577	8.60E-06
S5A_706487909	0.407207	1.70E-08
S5A_706574241	0.384685	1.33E-08
S5A_706673002	0.338739	2.87E-07
S5A_706705101	0.285586	1.94E-06

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1 **Table S6.** QTL results for yield components in LxM population. Location of significant QTL and estimated effects for thousand kernel
 2 weight (TKW), estimated test weight, spikelets per spike (SPS), kernel width (K Width), kernel length (K Length), kernel area (K Area)
 3 from data collected in the field in 2018 or in the greenhouse (GH) in 2017. Data was collected in the field in Raleigh in 2018 for most
 4 traits, and in Raleigh and Kinston, North Carolina in 2018 for spikelets per spike.

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Trait	Exp.	Chr	LOD	Peak Marker	cM	Mean	Effect	SE
TKW (g)	Field	4D	33.76	RhtD1	13	27.68	-2.56	0.19
TKW (g)	Field	3D	7.4	S3D_476608044	219	27.68	1.09	0.21
TKW (g)	Field	5A	6.51	Awns	269	27.68	0.88	0.17
TKW (g)	Field	7A	4.22	S7A_71631668	82	27.68	0.79	0.19
TKW (g)	GH	4D	23.24	RhtD1	12	28.78	-3.53	0.33
TKW (g)	GH	2D	8.1	PpdD1	60	28.78	1.99	0.32
TKW (g)	GH	5A	4.06	S5A_698528417	269	28.78	1.47	0.31
Test Weight (g)	Field	4D	33.66	RhtD1	12	7.91	-0.42	0.03
Test Weight (g)	Field	7A	6.58	S7A_672150738	187	7.91	0.18	0.03

Test Weight (g)	Field	5A	5.56	S5A_698528417	269	7.91	0.15	0.03
SPS	Field	7A	16.89	S7A_673996636	186	20.4	-0.51	0.05
SPS	Field	2D	9.11	PpdD1	59	20.4	0.41	0.06
SPS	Field	5A	8.44	S5A_698528417	269	20.4	-0.37	0.05
SPS	Field	4D	6.99	RhtD1	14	20.4	0.35	0.06
SPS	Field	2B	5.47	S2B_600741549	108	20.4	0.24	0.05
SPS	Field	4B	4.31	S4B_34025485	54	20.4	0.21	0.05
SPS	GH	2D	25.12	PpdD1	60	17.7	1.07	0.09
SPS	GH	4D	10.6	RhtD1	12	17.7	-0.77	0.09
SPS	GH	2B	9.08	S2B_642534505	110	17.7	0.65	0.09
SPS	GH	5A	5.27	S5A_698528417	269	17.7	-0.48	0.09
SPS	GH	5B	5.1	S5B_517797845	85	17.7	-0.66	0.13
K Width (mm)	Field	4D	35.13	RhtD1	12	2.98	-0.09	0.01

K Width (mm)	Field	6A	5.75	S6A_115348315	69	2.98	0.04	0.01
K Width (mm)	Field	5A	5.56	S5A_73816555	58	2.98	0.03	0.01
K Width (mm)	Field	3D	4.98	S3D_496955493	229	2.98	0.03	0.01
K Width (mm)	Field	7A	4.43	S7A_71631668	81	2.98	0.03	0.01
K Length (mm)	Field	3D	13.58	S3D_298356803	206	6.28	0.1	0.01
K Length (mm)	Field	5A	9.48	Awns	269	6.28	0.08	0.01
K Length (mm)	Field	1D	7.7	S1D_253081308	82	6.28	-0.08	0.01
K Length (mm)	Field	3B	5.67	S3B_576228566	104	6.28	-0.06	0.01
K Length (mm)	Field	6B	5.01	S6B_687553875	95	6.28	0.05	0.01
K Length (mm)	Field	1B	4.72	S1B_639043667	40	6.28	0.05	0.01
K Area (mm ²)	Field	4D	20.41	RhtD1	14	13.97	-0.55	0.05
K Area (mm ²)	Field	3D	8.73	S3D_298356803	205	13.97	0.33	0.05

K Area (mm ²)	Field	5A	6.08	S5A_73816555	58	13.97	0.28	0.05
K Area (mm ²)	Field	6A	5.34	S6A_115348315	68	13.97	0.27	0.05

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- 1 **Table S7.** Geographic distribution of *B1* haplotypes. Number of lines having observed haplotypes in spring and winter wheat
- 2 germplasm based on geographic region of origin for accessions. For each haplotype, left column represents number of awnless lines
- 3 (A-) and the right column the number of accessions having awns (A+).

Region	Growth habit	Hap1		Hap2		Hap3		Hap4		Hap5		Hap6		Hap7		Hap8		Total
		A-	A+	A-	A+	A-	A+	A-	A+	A-	A+	A-	A+	A-	A+	A-	A+	
North America	Spring	5	47	-	-	-	-	-	-	-	-	-	7	-	-	35	-	94
	Winter	-	15	-	-	-	-	-	-	-	-	-	1	-	-	11	-	27
Central America	Spring	1	61	-	1	-	2	-	-	-	-	-	-	1	-	20	-	86
South America	Spring	3	191	-	4	-	4	-	1	-	-	-	-	1	-	58	1	263
	Winter	-	5	-	-	-	1	-	-	-	-	-	-	-	-	29	-	35
Western Europe	Spring	5	81	-	1	-	2	-	1	-	1	-	3	3	-	119	-	216
	Winter	-	3	-	-	-	1	-	-	-	-	-	4	-	-	107	-	115
Eastern Europe	Spring	2	46	-	-	-	3	-	3	-	-	-	10	1	-	48	-	113
	Winter	-	42	-	-	-	-	-	-	-	-	-	31	-	-	72	2	147
South Asia	Spring	39	58	2	2	-	3	-	-	-	-	-	-	-	-	12	-	116
East Asia	Spring	2	43	-	-	-	2	-	1	-	-	-	-	-	-	13	-	61
	Winter	-	15	-	-	-	-	-	-	-	-	-	-	-	-	4	-	19
Central Asia	Spring	15	193	-	2	-	13	-	-	-	9	-	1	1	-	70	-	304
	Winter	1	45	-	-	-	8	-	-	-	-	-	5	-	-	28	1	88
Caucuses	Spring	4	52	-	-	-	1	-	-	-	2	-	-	-	-	20	-	79
	Winter	1	11	-	-	-	-	-	-	-	-	-	-	-	-	4	-	16
Middle East	Spring	6	156	-	3	-	2	-	-	-	-	-	-	1	-	38	4	210
	Winter	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	2
North Africa	Spring	5	104	-	1	-	1	-	-	-	-	-	2	-	-	30	10	153
Sub-Saharan Africa	Spring	8	149	1	3	-	1	-	1	-	-	-	-	1	-	79	-	243
	Winter	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2
Oceania	Spring	-	6	-	-	-	-	-	-	-	-	-	-	2	-	38	-	46
	Winter	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	4
Total		97	1324	3	17	-	44	-	7	-	12	-	65	11	-	841	18	2439

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2 **References**

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