

Identification and validation of a QTL for spikelet number on chromosome arm 6BL of common wheat (*Triticum aestivum* L.)

Supplemental file 1

Table S1: Genetic markers used in this study

Marker	RefSeq v1.0 Pos	Locus	FAM primer (5'-3')	VIC primer (5'-3')	Common primer (5'-3')	Citation
KS0617_760354	Chr6B:491,950,112	<i>QSn.csu-6B</i>	tagctgtttaggcgtgtga	tagctgtttaggcgtgtgg	gcctaatacgattctgaattca	(Krasileva et al. 2017)
KS0617_760357	Chr6B:492,023,835	<i>QSn.csu-6B</i>	gcaaggtagtgatgataatcctcca	gcaaggtagtgatgataatcctccg	gctgctcttgataatttgcg	(Krasileva et al. 2017)
Bobwhite_c22638_135	Chr6B:496,988,214	<i>QSn.csu-6B</i>	aaactacgctcactagatgatgt	aaactacgctcactagatgatgc	ccaggtttgggcaagatg	(Wang et al. 2014)
KS0617_760427	Chr6B:502,486,272	<i>QSn.csu-6B</i>	cattggaagagctgtgcacc	cattggaagagctgtgcact	tcaccgctgcatagtccg	(Krasileva et al. 2017)
7AL_6666	Chr7A:673,419,048	<i>WAPO-A1</i>	gacaggaacgcgaacatggc	gacaggaacgcgaacatggg	cctcactcttcacgctgat	(Kuzay et al. 2019)
VRN-D3	Chr7D:68,416,693	<i>VRN-D3</i>	gccctcgccgaccatggga	ccctcgccgaccatgggg	gaagagcacgagcacgaagcgat	(Chen et al. 2010)
TaPpdBJ003	Chr2B:56,230,452	<i>PPD-B1</i>	N/A	cgtgaagagctagcgatgaaca	tgggcacgtaacacaccttt	(Beales et al. 2007)

Table S2: Association between *QSn.csu-6B* and spikelet number in eight HIFs in the Fort Collins 2020 environment. Each HIF is fixed for a single allele at the *WAP0-A1*, *VRN-D3*, and *PPD-B1* loci and segregating for at the *QSn.csu-6B* locus. There was no significant marker-trait association between *QSn.csu-6B* and heading date.

Family	Mean spikelet number	Effect size (spikelets)	<i>P</i> -value	Heading Date (Julian Days)	Heading Date (C.I. 95%)	<i>WAP0-A1</i>	<i>VRN-D3</i>	<i>PPD-B1</i>
COP6BHF361	20.2	0.352	0.0053	149.7	(149.6, 149.7)	<i>b</i>	<i>b</i>	<i>b</i>
COP 6BHF260	19.8	0.504	0.0006	149.3	(149.3, 149.4)	<i>a</i>	<i>b</i>	<i>b</i>
COP 6BHF146	19.7	0.352	0.0041	149.8	(149.7, 149.9)	<i>a</i>	<i>a</i>	<i>b</i>
COP 6BHF347	19.1	0.370	0.0012	149.7	(149.6, 149.7)	<i>b</i>	<i>b</i>	<i>b</i>
COP 6BHF337	19.1	0.200	0.0667	146.2	(146.1, 146.2)	<i>b</i>	<i>a</i>	<i>a</i>
COP 6BHF275	19.0	0.248	0.0215	148.2	(148.1, 148.2)	<i>b</i>	<i>a</i>	<i>b</i>
COP 6BHF365	19.0	0.344	0.0013	148.3	(148.3, 148.4)	<i>a</i>	<i>b</i>	<i>a</i>
COP 6BHF207	18.1	0.441	<0.0001	149.3	(149.3, 149.4)	<i>a</i>	<i>a</i>	<i>b</i>

Table S3. The log₁₀ (*P*) values for the peak markers identified from the 90K SNP assay (Wang et al. 2014). The genome-wide associations were performed using 298 genotypes from the HWWMAP and the BLUEs calculated for kernel width, spikelet number, thousand kernel weight, and heading date from the Greeley 2012 dryland, Greeley 2012 irrigated, Fort Collins 2013 dryland, and Fort Collins 2013 irrigated environments. Log₁₀ (*P*) values for all traits and markers can be found in supplemental data 1.

Marker	RefSeq v1.0 Pos	Locus	Kernel width	Spikelet number	Thousand kernel weight	Heading date
Bobwhite_c22638_135	Chr6B:496,988,214	<i>QSn.csu-6B</i>	3.358257	0.255466	3.686106	0.090588
IWA5913	Chr7A:674,276,906	<i>WAO-A1</i>	0.249613	7.642443	0.103143	0.748676
BS00063966_51	Chr7D:66,824,781	<i>VRN-D3</i>	0.181616	0.678392	0.003124	0.647817
Kukri_c16479_765	Chr2B:54,778,663	<i>PPD-B1</i>	0.676399	0.201718	0.183635	3.554004

Table S4. Correlations between heading date (HD), thousand kernel weight (TKW) and spikelet number (SNS) in the HWWAMP for four environments and calculated BLUEs across environments. Pearson's product moment correlation coefficient and *P*-value are shown. N = 299 for all environments and BLUEs.

Environment	Trait	Correlation	<i>P</i> -value
Greeley 2012 Dry	TKW vs. SNS	-0.028	0.6340
	TKW vs. HD	-0.343	<0.0001
	SNS vs. HD	-0.107	0.0651
Greeley 2012 Irrigated	TKW vs. SNS	-0.408	<0.0001
	TKW vs. HD	-0.611	<0.0001
	SNS vs. HD	0.465	<0.0001
Fort Collins 2013 Dry	TKW vs. SNS	-0.255	<0.0001
	TKW vs. HD	-0.188	0.0011
	SNS vs. HD	0.194	0.0007
Fort Collins 2013 Irrigated	TKW vs. SNS	-0.207	0.0003
	TKW vs. HD	-0.198	0.0006
	SNS vs. HD	0.336	<0.0001
BLUEs	TKW vs. SNS	-0.260	<0.0001
	TKW vs. HD	-0.347	<0.0001
	SNS vs. HD	0.389	<0.0001

Table S5: Correlation between spikelet number (SNS), grain length, grain width, and thousand kernel weight (TKW) for the COP-RIL population in the Fort Collins 2017 environment based on BLUEs. Values indicate Pearson's product moment correlation. * = $P < 0.05$, *** = $P < 0.0001$.

BLUEs	Length	Width	TKW
SNS	-0.163*	-0.266***	-0.184***
Length	-	0.454***	0.659***
Width	-	-	0.870***

Table S6: ANOVA *P*-values for the marker-trait association between the *QSn.csu-6B*, *WAO-A1*, *Vrn-D3*, and *PPD-B1* loci and spikelet number in the COP-RIL population for Fort Collins 2017, Fort Collins 2019, Fort Collins 2020 and BLUEs.

		<i>QSn.csu-6B</i>			<i>WAO-A1</i>			<i>VRN-D3</i>			<i>PPD-B1</i>		
Environment	N	Effect size	<i>P</i> -value	%R ²	Effect size	<i>P</i> -value	%R ²	Effect size	<i>P</i> -value	%R ²	Effect size	<i>P</i> -value	%R ²
2017	211	0.808	<0.0001	12.9	0.921	<0.0001	14.5	0.46	0.0003	7.1	0.422	0.0013	6.3
2019	209	0.709	<0.0001	9.2	1.01	<0.0001	17.1	0.518	0.0006	5.1	0.331	0.0371	1.6
2020	215	0.679	<0.0001	11.6	0.713	<0.0001	11.8	0.576	<0.0001	8.9	0.42	0.0016	4.1
BLUEs	215	0.739	<0.0001	13.8	0.89	<0.0001	18.7	0.518	<0.0001	7.1	0.394	0.0031	3.6

Table S7. Sum of squares and *P*-values for the interaction terms calculated from the COP-RIL population from the Fort Collins 2017, 2019, and 2020 environments via a Tukey-Adjusted Type-2 ANOVA.

Term	Sum Sq.	P-value
<i>WAP0-A1</i>	119.8	<0.0001
<i>QSn.csu-6B</i>	83.25	<0.0001
<i>VRN-D3</i>	49.53	<0.0001
<i>PPD-B1</i>	18.94	<0.0001
Environment	535.39	<0.0001
<i>WAP0-A1 : QSn.csu-6B</i>	2.87	0.086
<i>WAP0-A1 : VRN-D3</i>	3.69	0.052
<i>QSn.csu-6B : VRN-D3</i>	10.85	0.0009
<i>WAP0-A1 : PPD-B1</i>	0.41	0.519
<i>QSn.csu-6B : PPD-B1</i>	0.8	0.364
<i>VRN-D3 : PPD-B1</i>	0.63	0.421
<i>WAP0-A1 : QSn.csu-6B : VRN-D3</i>	0.29	0.589
<i>WAP0-A1 : QSn.csu-6B : PPD-B1</i>	1.35	0.240
<i>WAP0-A1 : VRN-D3 : PPD-B1</i>	0	0.997
<i>QSn.csu-6B : VRN-D3 : PPD-B1</i>	13.28	0.0002
<i>WAP0-A1 : QSn.csu-6B : VRN-D3 : PPD-B1</i>	15.26	<0.0001

Table S8: Effect size and significance of spikelet number and heading date in the COP-RIL population from Fort Collins 2020.

Locus	Spikelet number		Heading date	
	<i>P</i> -value	Effect size (spikelets)	<i>P</i> -value	Effect size (days)
<i>QSn.csu-6B</i>	<0.0001	0.679	0.107	0.326
<i>WAP0-A1</i>	<0.0001	0.713	0.95	0.0135
<i>VRN-D3</i>	<0.0001	0.576	<0.0001	1.31
<i>PPD-B1</i>	0.001	0.420	<0.0001	1.16

Table S9: The frequency of individual lines containing pairs of positive effect alleles in the COP-RIL population (N = 215, $X^2 = 33.80$, P -value < 0.001).

	<i>VRN-D3b</i>	<i>PPD-B1b</i>	<i>WAP0-A1b</i>
<i>QSn.csu-6Bb</i>	0.31	0.23	0.21
<i>VRN-D3b</i>	-	0.18	0.13
<i>PPD-B1b</i>	-	-	0.13

Figure S1: Tree diagram representing spikelet number for allelic combinations of *PPD-B1*, *WAP0-A1*, *VRN-D3*, and *QSn.csu-6B* in the COP-RIL population. Each node of the tree includes mean spikelets per spike $\pm$ the standard error. Significant pairwise comparisons between allelic pairs are denoted by * = $P < 0.05$.

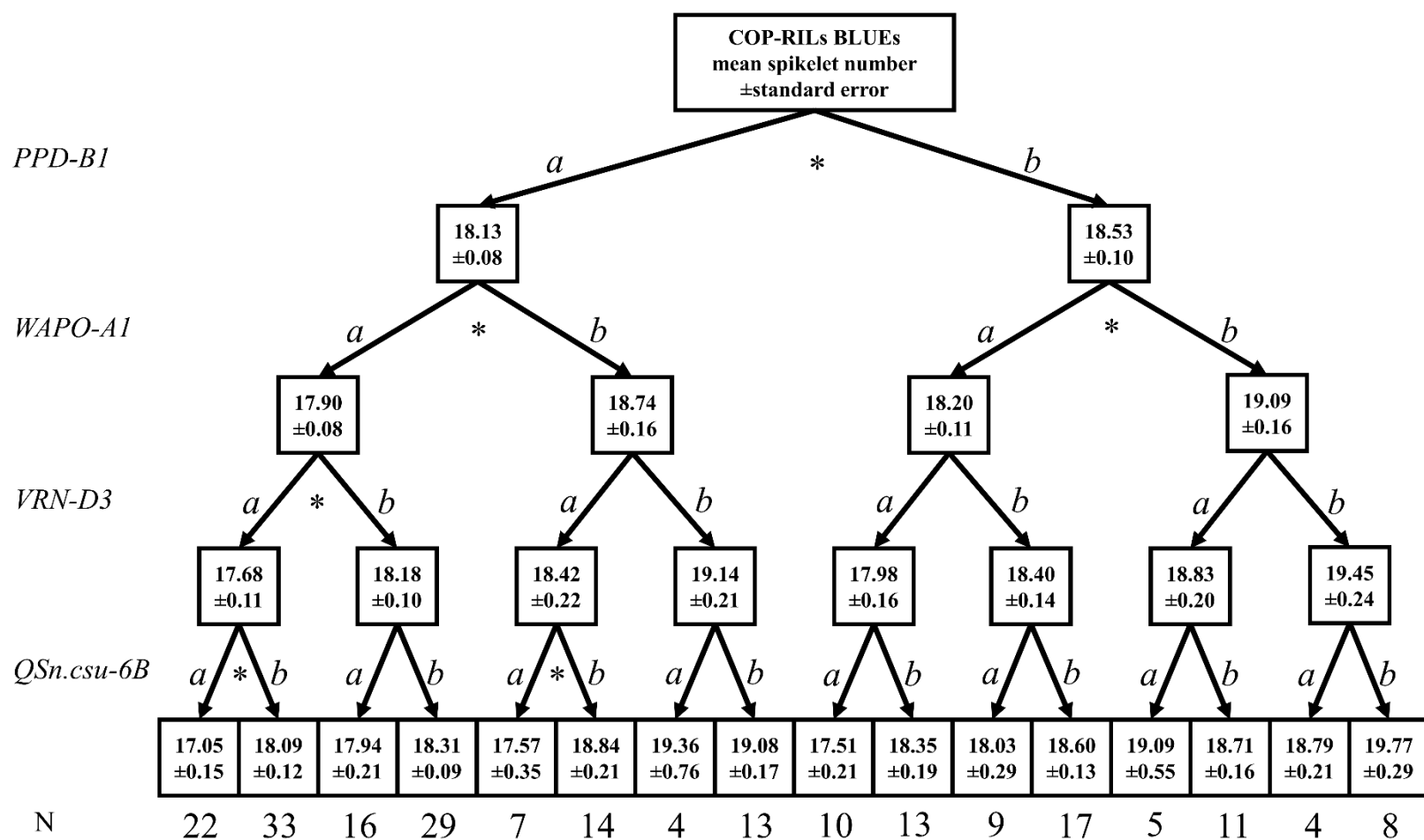


Figure S2. Mean spikelet number by heading date for the COP-RIL population in the Fort Collins 2020 environment. The X-axis is heading date in Julian Days, Y-axis is mean spikelet number and each line is represented by a color point denoting the allele present. Spikelet number and heading date are positively correlated ($r = 0.37$, $CI95 = 0.25-0.48$, $P < 0.0001$). Each scatter plot represents a linear regression for each allele of a given loci of interest and the shaded area is the 95 % confidence level interval of the regression

