

# 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	ccaggtttgtggcaagatg	(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>WAP0-A1</i>	gacaggaacgcgaacatggc	gacaggaacgcgaacatggg	cctcacttccacgctgat	(Kuzay et al. 2019)
VRN-D3	Chr7D:68,416,693	<i>VRN-D3</i>	gccctcgtccgaccatggga	ccctcgtccgaccatgggg	gaagagcacgagcacgaagcgat	(Chen et al. 2010)
TaPpdBJ003	Chr2B:56,230,452	<i>PPD-B1</i>	N/A	cgtagaagagctagcgatgaaca	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 *WAPO-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.

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

**Table S3.** 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 S4:** 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. \* =  $P < 0.05$ , \*\*\* =  $P < 0.0001$ .

<b>BLUEs</b>	<b>Length</b>	<b>Width</b>	<b>TKW</b>
<b>SNS</b>	-0.163*	-0.266***	-0.184***
<b>Length</b>	-	0.454***	0.659***
<b>Width</b>	-	-	0.870***

**Table S5:** ANOVA *P*-values for the marker-trait association between the *QSn.csu-6B*, *WAP0-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.

Environment	N	<i>QSn.csu-6B</i>			<i>WAP0-A1</i>			<i>VRN-D3</i>			<i>PPD-B1</i>		
		Effect size	<i>P</i> -value	%R <sup>2</sup>	Effect size	<i>P</i> -value	%R <sup>2</sup>	Effect size	<i>P</i> -value	%R <sup>2</sup>	Effect size	<i>P</i> -value	%R <sup>2</sup>
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 S6.** 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>WAPO-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>WAPO-A1 : QSn.csu-6B</i>	2.87	0.086
<i>WAPO-A1 : VRN-D3</i>	3.69	0.052
<i>QSn.csu-6B : VRN-D3</i>	10.85	0.0009
<i>WAPO-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>WAPO-A1 : QSn.csu-6B : VRN-D3</i>	0.29	0.589
<i>WAPO-A1 : QSn.csu-6B : PPD-B1</i>	1.35	0.240
<i>WAPO-A1 : VRN-D3 : PPD-B1</i>	0	0.997
<i>QSn.csu-6B : VRN-D3 : PPD-B1</i>	13.28	0.0002
<i>WAPO-A1 : QSn.csu-6B : VRN-D3 : PPD-B1</i>	15.26	<0.0001

**Table S7:** 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

**Figure S1.** 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

