

## Article

# Mapping Quantitative Trait Loci for Yield Potential Traits in Wheat Recombinant Inbred Lines

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**Abstract:** Selecting wheat with favorable spike characteristics has been a popular breeding strategy to improve sink capacity and yield potential. In the present study, quantitative trait loci (QTLs) for yield potential traits were identified using a recombinant inbred line (RIL) population derived from the cross between Taejoong and Keumkang, two Korean wheat cultivars. A linkage map encompassing a total genetic length of 6544.8 cM was constructed using 838 single nucleotide polymorphisms from the 35K Axiom Wheat Breeder's Array. We detected eight QTLs for four yield potential traits that are consistently identified in at least two of the three environments, that is, one for days to heading date (*QDHD-1* on chromosome 7B), three for spike length (*QSL-1*, *QSL-2*, and *QSL-3* on chromosomes 1D, 5A, and 6A, respectively), one for tiller number (*QTN-1* on chromosome 5B), and three for length of center rachis (*QLCR-1*, *QLCR-2*, and *QLCR-3* on chromosomes 1B, 5B, and 6A, respectively). Notably, Taejoong contributed the alleles for long spike at all three spike length QTLs with the additive effects of 0.6 cm, 0.6 cm, and 0.9 cm at *QSL-1*, *QSL-2*, and *QSL-3*, respectively. No significant two-way or three-way interaction was observed among *QSL-1*, *QSL-2*, and *QSL-3*, indicating that pyramiding the Taejoong alleles at the three QTLs can increase spike length additively. While the Taejoong alleles at *QSL-1*, *QSL-2*, and *QSL-3* were associated with increased days to heading date, more kernels per spike, and reduced tiller number per plant, the extent of the pleiotropic effects were different among the three QTLs. Due to the limited number of molecular markers and mapping resolution, further work is required to narrow down the identified QTLs and characterize their effects more precisely. Our results would provide useful information for modulating spike characteristics and improving yield potential in wheat breeding programs.

**Keywords:** wheat; spike; yield; QTL; SNP



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## 1. Introduction

Improving grain yield is an important goal in wheat breeding to secure stable food production under changing climate and increasing world population [1,2]. Wheat grain yield is a complex trait involving a number of genes and their interactions which are largely influenced by various environmental factors including temperature, photoperiod, rainfall, and fertilization [3,4]. Therefore, studies on wheat yield generally focus on specific yield component traits such as spikes (or tillers) per unit area, kernels per spike, and kernel weight that are more stably inherited than the final grain yield [5,6]. While studies genetically dissecting a specific yield component trait can provide useful molecular tools for breeding programs, optimizing the balance between different yield components is also

critical as the yield component traits are interrelated and an increase in one component is frequently associated with a decrease in another, i.e., the yield improvement effect of an increase in kernels per spike can be canceled out by a decrease in kernel weight [7–11].

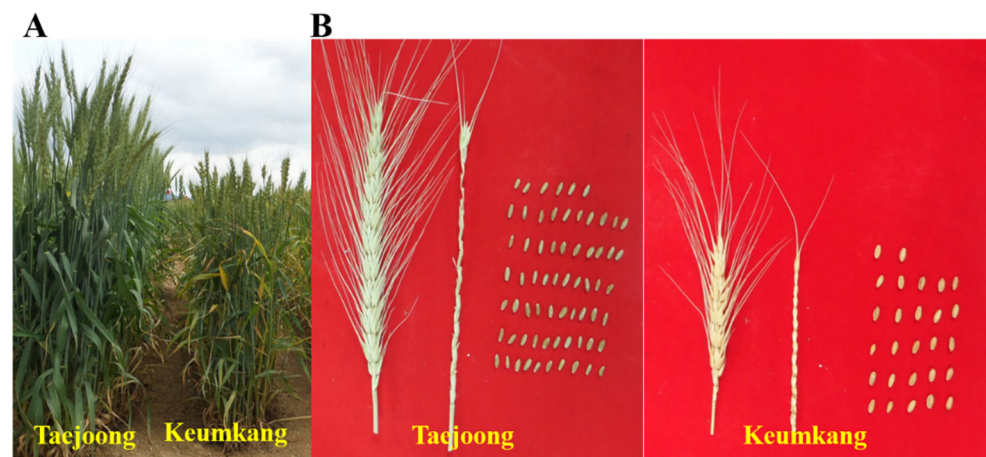
In many wheat breeding programs, kernels per spike has been a major target for improving yield as it often shows stronger positive correlation with the final grain yield compared with other yield components [12]. For example, the yield increasing trend of the major Argentinian wheat cultivars released between 1920 and 1990 was associated better with the increases in kernels per spike compared with those in spikes per unit area or grain weight [13]. Similarly, over 400 European winter wheat cultivars released since the 1950s showed continuous increases in kernels per spike over time [14]. At the International Maize and Wheat Improvement Center (CIMMYT), large spike wheat cultivars were developed by wide crossing between common wheat and accessions of *Agropyron elongatum* or *Triticum polonicum* to improve yield potential through increasing kernels per spike [15]. While these lines showed significant increases in both kernels per spike and kernel weight, the final grain yield fell short of expectations mainly due to the decrease in spikes per unit area [16,17].

In South Korea, a large spike wheat cultivar Taejoong was developed from the cross between the Chinese wheat Xian83 and the Korean cultivar Keumkang at the National Institute of Crop Science (NICS) [18,19]. In the regional yield trials conducted for three years (2014–2016) at the NICS, spike length and kernels per spike of Taejoong were 13.4 cm and 48, respectively, which were 76% longer and 66% more than those of Keumkang, respectively [19]. Taejoong also exhibited a ten-thousand kernel weight of 48.3 g, which was 5% heavier than Keumkang. Although Taejoong exhibited limited tillering capacity, i.e., 417 spikes per square-meter, which was 42% less than Keumkang, its final grain yield was moderately higher (13–21% higher depending on the cropping patterns) than Keumkang, mainly due to the superior spike characteristics in terms of kernel number and size [19]. As the genetic factors underlying the large spike characteristics of Taejoong remain unclear, we aimed to identify quantitative trait loci (QTLs) for yield potential characteristics of Taejoong using a recombinant inbred line (RIL) population derived from the cross between Taejoong and Keumkang and generate useful information for improving yield potential in wheat breeding programs.

## 2. Materials and Methods

### 2.1. Plant Materials

A total of 94 F<sub>10</sub> RILs were produced from the cross between two Korean wheat cultivars, Taejoong and Keumkang. The main phenotypic feature of Taejoong is a large spike (Figure 1) [19,20]. The RIL population was cultivated in upland conditions at Jeonbuk National University (35°85' N 127°13' E) in Jeonju, South Korea, during three growth seasons (2017, 2018, and 2019). The seeds were sown in late October each year, and each plot consisted of three 2-m rows spaced 25 cm apart. Application of fertilizers was conducted as 50–70–50 kg/ha of N–P<sub>2</sub>O<sub>5</sub>–K<sub>2</sub>O, and weeds, disease, and insect pests were stringently controlled according to the standard wheat cultivation manual of the Rural Development Administration (RDA), South Korea [RDA. 2012, standard of research and analysis for agricultural technology].



**Figure 1.** Phenotype comparison of Taejoong and Keumkang: (A) plants at heading, and (B) spike, rachis, and kernels per spike after maturity.

### 2.2. Measurement of Yield Potential Traits

Major agronomic traits affecting yield potential were recorded as follows: days to heading date (DHD) as the number of days from sowing to spike exertion from the stem in 50% of a plot, culm length (CL) as the length from ground level to the base of the spike, spike length (SL) as the length of the spike excluding awn, number of tillers (TN) counted as the number of stems per plant, kernels per spike (KS) counted as the number of grains per spike, length of center rachis (LCR) as measured excluding flowerets, and the value of compactness (COM) as calculated according to the number of kernels per spike divided by spike length. CL, SL, and TN were measured from 20 plants randomly selected in each plot at maturity. KS, LCR and COM were determined from the main spikes of the same 20 plants.

### 2.3. Genotypic Analysis

Genomic DNA (gDNA) from the young leaves of the F<sub>10</sub> RIL population was extracted using a DNA extraction kit (Solgent, Daejeon, Korea) according to the manufacturer's instructions. Concentration of each gDNA was adjusted to 20 ng/μL with distilled water. The final concentration of gDNA was measured by Biodrop (Biodrop Ltd., Cambridge, UK). The 94 RILs were genotyped by DNA Link, Inc. (Seoul, Korea, <http://www.dnalink.com>) with Axiom™ 35K Wheat Breeder's Genotyping Array (ThermoFisherScientific, Applied Biosystems™, Waltham, MA, USA) in a 384-array plate format. Genotype calling was conducted by Affymetrix Power Tools (APT, release 2.11.1).

### 2.4. QTL Mapping and Statistical Analysis

A linkage map was constructed using the IciMapping program version 4.2 [21]. To select polymorphic single nucleotide polymorphisms (SNPs), the genotyping result of the 35,042 SNPs was converted by the "SNP" function of the program. Out of 35,042 SNPs, 2061 were polymorphic between Taejoong and Keumkang, the parental lines of the RIL population. Using the "BIN" function in IciMapping, SNPs with more than 10% missing data and/or significant segregation distortion (Chi squared test *P*-value < 0.001) were deleted. Finally, 838 SNPs were used to construct a linkage map by the "MAP" function. QTL mapping for yield potential traits (DHD, CL, SL, COM, KS, TN, and LCR) was performed by composite interval mapping. Significant QTLs were initially identified based on the threshold logarithm of odds (LOD) score of 3.0 in each year, and those consistently identified in at least two of the three years were declared as stable QTLs. Statistical analyses including ANOVA and correlation analysis were conducted using R (The R Project for Statistical Computing version 3.4.4, <https://www.r-project.org>).

### 3. Results

#### 3.1. Phenotype Evaluation of the RIL Population

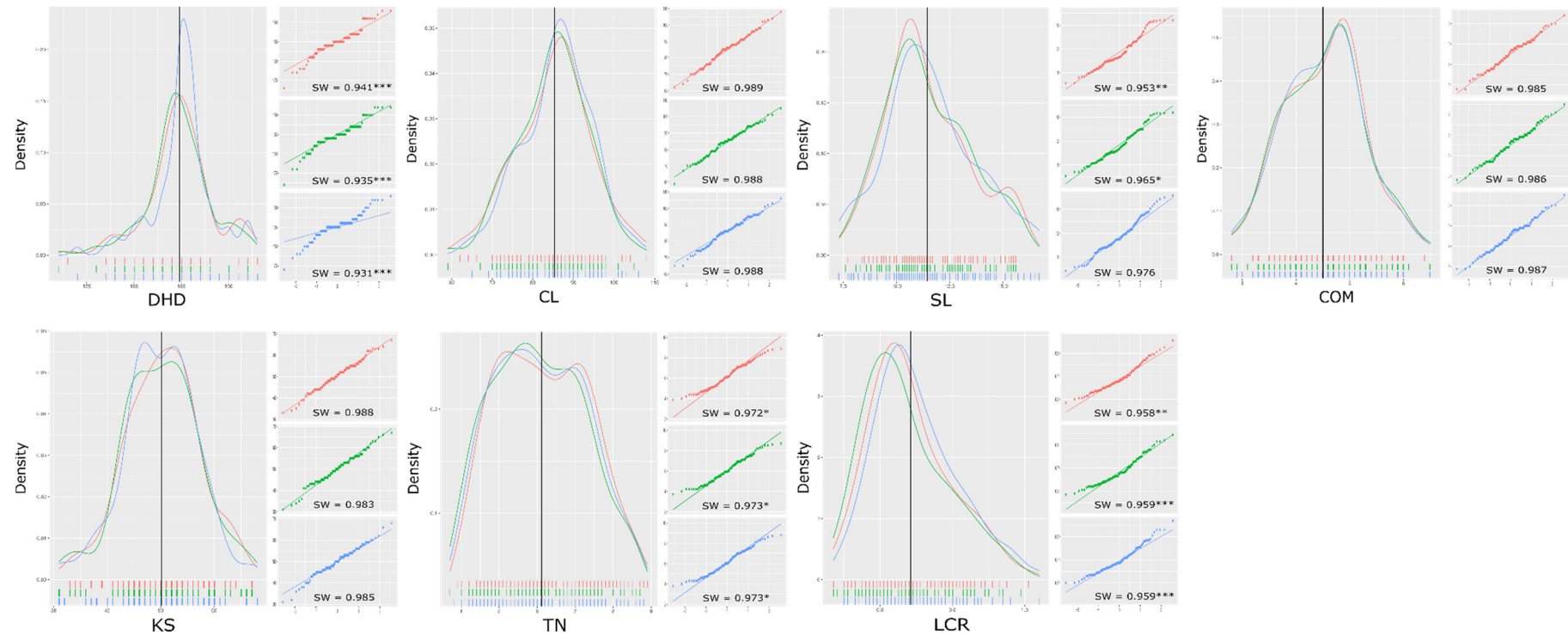
Descriptive statistics of the yield potential traits of the Taejoong  $\times$  Keumkang RILs are summarized in Figure 2 and Table S1. The average DHD (184.9 days) of the RILs was shorter than Taejoong (192.7 days) and longer than Keumkang (180.3 days), and its distribution was skewed toward late DHD. The distribution of CL was normal and the average (85.3 cm) was taller than Keumkang (66.1 cm) and similar to Taejoong (84.6 cm). The average SL (11.5 cm) was shorter than Taejoong (14.5 cm) and longer than Keumkang (8.4 cm), and its distribution was skewed toward short SL. The distribution of COM was normal and the average (4.5) was higher than both Keumkang (4.4) and Taejoong (4.2). The distribution of KS was normal and the average (50.1) was between Keumkang (36.7) and Taejoong (61.6). The average TN (6.1) was greater than Taejoong (5.1) and fewer than Keumkang (8.1), and its distribution was skewed toward a low value. The average LCR (0.68 cm) was shorter than Taejoong (0.75 cm) and similar to Keumkang (0.69 cm), and its distribution was skewed toward a low value.

Of 21 correlation pairs among the seven yield potential traits, nine were significant in all three years, 2017, 2018, and 2019 (Figure 3 and Figures S1–S3). Strong positive correlations were observed between SL and LCR ( $r = 0.69$  in all three years). KS showed moderate to low levels of positive correlations with COM ( $r = 0.45$ – $0.47$ ), SL ( $0.34$ – $0.40$ ), and DHD ( $r = 0.26$ – $0.30$ ). Relatively strong negative correlations were observed between COM and SL ( $r = -0.62$  to  $-0.57$ ), and COM and LCR ( $r = -0.54$  to  $-0.50$ ). TN was negatively, but weakly correlated with SL ( $r = -0.37$  to  $-0.33$ ), KS ( $r = -0.33$  to  $-0.29$ ), and LCR ( $r = -0.28$  to  $-0.25$ ). CL was not correlated with any of the six other yield potential traits, except for a weak positive correlation with SL ( $r = 0.21$ ) significant in only one of the three years (Figure S3).

#### 3.2. QTLs for Yield Potential Traits

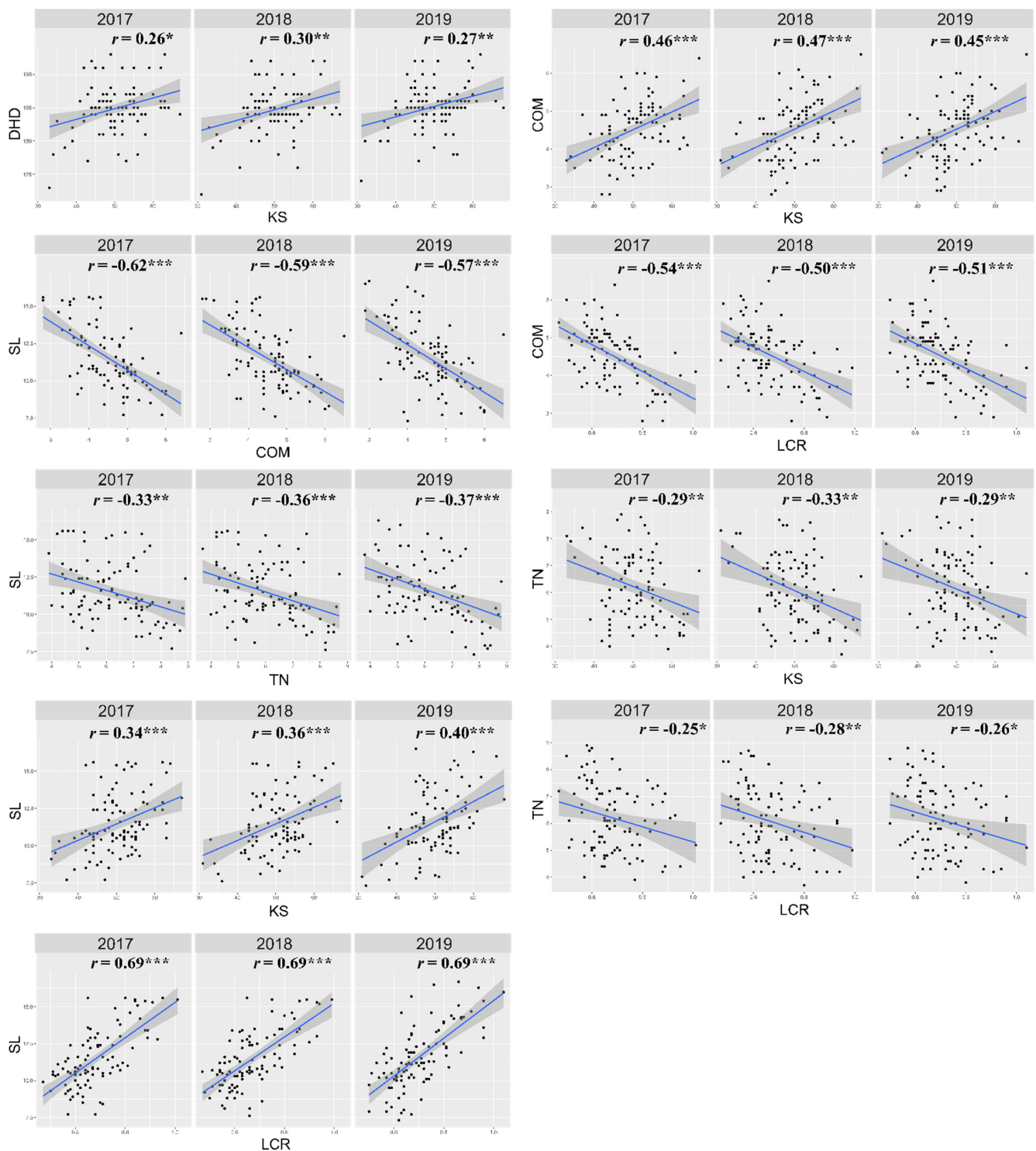
A linkage map spanning a total genetic length of 6544.8 cM was constructed using 838 SNPs selected from the 2061 polymorphic SNPs after excluding those with >10% missing data and/or significant segregation distortion (Chi squared test  $p$ -value < 0.001, Table S2). The number of SNPs polymorphic between Taejoong and Keumkang was relatively small (5.9% of the 35K SNPs) mainly due to the fact that Taejoong had been derived from the cross between Xian83 and Keumkang and shared similar genetic background with Keumkang.

Of the 838 SNPs used for mapping, 233 (27.8%) were distributed on the A genome, 344 (41.1%) were on the B genome, and 261 (31.3%) were on the D genome. Among the 838 SNP markers, chromosome 1B had the greatest number of markers (86), whereas chromosome 6A had the smallest number of markers (9). Chromosome 5B showed the longest length (578.4 cM), whereas chromosome 4D showed the shortest length (113.1 cM). The average length between two adjacent markers were the longest on chromosome 6A (19.5 cM) and the shortest on chromosome 2B (4.1 cM).



**Figure 2.** Comparison of density and probability distribution of yield potential traits across three years. DHD, days to heading date; CL, culm length; SL, spike length; COM, compactness; KS, kernels per spike; TN, number of tillers; LCR, length of center rachis. Red, green, and blue colors indicate data from 2017, 2018, and 2019, respectively. A black vertical line indicates the mean value of the three years. SW indicates the  $P$ -value from the Shapiro–Wilk test, and an asterisk indicates significance (\*, \*\*, and \*\*\* at  $P \leq 0.05$ ,  $P \leq 0.01$ , and  $P \leq 0.001$  levels, respectively).





**Figure 3.** Correlation between yield potential traits in 94  $F_{10}$  recombinant inbred lines produced from the cross between Taejoong and Keumkang. DHD, days to heading date; CL, culm length; SL, spike length; COM, compactness; KS, kernels per spike; NS, number of spikes; LCR, length of center rachis. An asterisk indicates significance (\*, \*\*, and \*\*\* at  $p \leq 0.05$ ,  $p \leq 0.01$ , and  $p \leq 0.001$  levels, respectively).

We detected eight QTLs for four yield potential traits that are consistently identified in at least two of the three years (Table 1 and Figure 4). One QTL for DHD, *QDHD-1* on chromosome 7B was defined by *AX-94684729* and *AX-94474044* (within a 5.0 cM confidence interval) with the LOD scores of 3.90–4.24 explaining 11.8–13.7% of the DHD variation. Taejoong contributed the allele for late heading with the additive effects of 1.30–1.37 days. Three QTLs were identified for SL. *QSL-1* on chromosome 1D was defined by *AX-94390275* and *AX-94918964* (within a 5.0 cM confidence interval) with the LOD scores of 3.63–5.37 explaining 11.2–15.6% of the SL variation. *QSL-2* on chromosome 5A was defined by *AX-94831718* and *AX-94846616* (within a 16.0 cM confidence interval) with the LOD scores of 3.55–3.82 explaining 12.2–15.1% of the SL variation. *QSL-3* on chromosome 6A was defined by *AX-94411823* and *AX-94602621* (within a 13.5 cM confidence interval) with the LOD scores of 5.88–7.59 explaining 26.0–28.8% of the SL variation. Taejoong provided the alleles for long spike at all three QTLs with the additive effects of 0.59–0.72 at *QSL-1*, 0.61–0.62 at *QSL-2*, and 0.84–0.95 at *QSL-3*. One QTL for TN, *QTN-1* on chromosome 5B was defined by *AX-95176502* and *AX-94429067* (within an 18.0 cM confidence interval) with the LOD scores of 4.10–4.17 explaining 16.3–16.4% of the TN variation. Unlike SL, Keumkang provided the allele for high tillering with the additive effects between  $-0.75$  and  $-0.74$ . Three QTLs were identified for LCR. *QLCR-1* on chromosome 1B was defined by *AX-94414200* and *AX-94757158* (within a 3.0 cM confidence interval) with the LOD scores of 3.62–8.67 explaining 8.7–19.1% of the phenotypic variation. *QLCR-2* on chromosome 5B was defined by *AX-94904781* and *AX-95257493* (within a 7.0 cM confidence interval) with the LOD scores of 9.14–10.82 explaining 22.2–34.5% of the phenotypic variation. *QLCR-3* on chromosome 6A was defined by *AX-94411823* and *AX-94602621* (within a 17.5 cM confidence interval) with the LOD scores of 5.54–6.77 explaining 17.4–22.7% of the phenotypic variation. Taejoong provided the alleles for long LCR at *QLCR-2* and *QLCR-3* with the additive effects of 0.06–0.07 and 0.05, respectively, and Keumkang provided the allele for long LCR at *QLCR-1* with the additive effect between  $-0.05$  and  $-0.03$ . Only one QTL affected two or more traits, i.e., *QSL-3/QLCR-3* flanked by *AX-94411823* and *AX-94602621* on chromosome 6A. No stable QTL was detected for CL, COM, and KS.

**Table 1.** Quantitative trait loci (QTLs) for yield potential traits identified from the Taejoong  $\times$  Keumkang recombinant inbred lines.

QTL <sup>a</sup>	Year	Chromosome	Position (cM)	Left Marker	Right Marker	Interval (cM) <sup>b</sup>	LOD <sup>c</sup>	PVE (%) <sup>d</sup>	Add <sup>e</sup>
<i>QDHD-1</i>	2017	7B	172.0	<i>AX-94684729</i>	<i>AX-94474044</i>	5.0	3.90	11.8	1.37
	2019						4.24	13.7	1.30
<i>QSL-1</i>	2017	1D	268.0	<i>AX-94390275</i>	<i>AX-94918964</i>	5.0	3.63	11.2	0.59
	2018						5.37	15.6	0.72
	2019						4.04	15.1	0.62
<i>QSL-2</i>	2018	5A	208.0	<i>AX-94831718</i>	<i>AX-94846616</i>	16.0	3.82	12.2	0.62
	2019						3.55	15.1	0.61
<i>QSL-3</i>	2017	6A	175.0	<i>AX-94411823</i>	<i>AX-94602621</i>	13.5	7.59	26.0	0.88
	2018						7.25	28.8	0.95
	2019						5.88	28.8	0.84
<i>QTN-1</i>	2017	5B	269.0	<i>AX-95176502</i>	<i>AX-94429067</i>	18.0	4.17	16.3	$-0.75$
	2018						4.10	16.4	$-0.74$
	2019						4.15	16.4	$-0.75$

Table 1. Cont.

QTL <sup>a</sup>	Year	Chromosome	Position (cM)	Left Marker	Right Marker	Interval (cM) <sup>b</sup>	LOD <sup>c</sup>	PVE (%) <sup>d</sup>	Add <sup>e</sup>
QLCR-1	2017	1B	330.0	AX-94414200	AX-94757158	3.0	8.67	19.1	−0.05
	2018						3.62	8.7	−0.03
	2019						3.75	8.7	−0.04
QLCR-2	2017	5B	419.0	AX-94904781	AX-95257493	7.0	9.14	22.2	0.06
	2018						10.82	34.5	0.07
	2019						9.73	30.3	0.07
QLCR-3	2018	6A	171.0	AX-94411823	AX-94602621	17.5	5.54	17.4	0.05
	2019						6.77	22.7	0.05

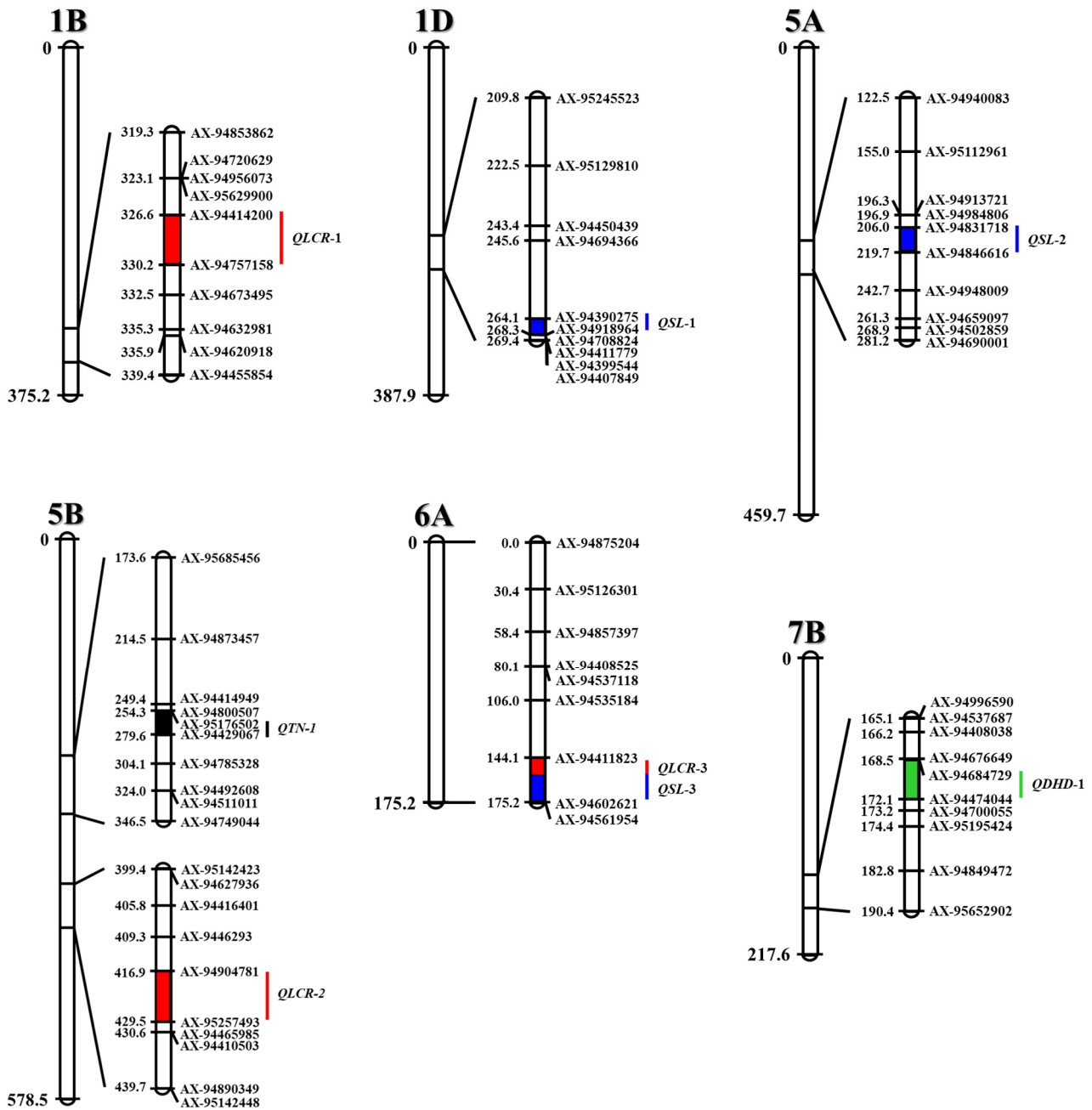
<sup>a</sup> DHD, days to heading date; SL, spike length; TN, number of tillers; LCR, length of center rachis. <sup>b</sup> Interval means 2-LOD confidence interval. <sup>c</sup> Logarithm of the odds. <sup>d</sup> Phenotypic variance explained. <sup>e</sup> Additive effect of allele substitution. The units are those of the corresponding traits. A positive sign indicates that the Taejoong allele increased the trait value.

### 3.3. Effects of QSL-1, QSL-2, and QSL-3 on Spike Length and Other Yield Potential Traits

To evaluate the main effects of the three QTLs for SL and their interactions on yield potential traits, three-way factorial ANOVAs of QSL-1, QSL-2, and QSL-3 were carried out for each trait with the year (2017, 2018, and 2019) as a random factor (Table 2). *Spike length (SL)*: The main effects of QSL-1, QSL-2, and QSL-3 were highly significant for SL. Compared to the RILs carrying the Keumkang allele at each QTL, those carrying the Taejoong allele exhibited significantly longer spike, i.e., 1.2, 1.2, and 1.8 cm longer for QSL-1, QSL-2, and QSL-3, respectively. Notably, there was no significant two-way or three-way interaction among the three QTLs, indicating that pyramiding of the Taejoong alleles at QSL-1, QSL-2, and QSL-3 would increase SL additively. While the average SL of the RILs carrying the Keumkang alleles at all three QTLs was 9.7 cm, those carrying the Taejoong allele at one of the three QTLs exhibited the average SL of 10.6–11.1 cm (Figure 5). The RILs carrying the Taejoong alleles at two of the three QTLs exhibited the average SL of 11.8–12.5 cm. The RILs carrying the Taejoong alleles at all three QTLs exhibited the longest average SL of 13.8 cm. *Days to heading date (DHD)*: QSL-1 and QSL-2 had significant main effects on DHD, with the Taejoong alleles for long spike being associated with late heading. The RILs carrying the Taejoong allele at QSL-1 headed 1.0 day later than those carrying the Keumkang allele. Similarly, the RILs carrying the Taejoong allele at QSL-2 headed 0.8 days later than those carrying the Keumkang allele. The main effect of QSL-3 was not significant for DHD. While there was no significant two-way interaction, the three-way interaction was significant and explained 4.7% of the DHD variance. *Culm length (CL)*: While the main effects of QSL-1 and QSL-2 were not significant for CL, that of QSL-3 was highly significant. The RILs carrying the Taejoong allele at QSL-3 were 3.9 cm taller than those carrying the Keumkang allele. The QSL-2 × QSL-3 interaction and the three-way interaction were significant for CL, but explained limited levels (3.0% and 2.3%, respectively) of the CL variance. *Compactness (COM)*: The main effects of QSL-1, QSL-2, and QSL-3 were highly significant for COM, with the RILs carrying the Keumkang allele exhibiting more compact spike than those carrying the Taejoong allele. No significant two-way or three-way interaction was observed. *Kernels per spike (KS)*: QSL-1 and QSL-3 had significant main effects on KS. Compared to the RILs carrying the Keumkang allele at QSL-1, those carrying the Taejoong allele had 2.8 more kernels per spike. Similarly, those carrying the Taejoong allele at QSL-3 had 2.8 more kernels per spike than those carrying the Keumkang allele. Two-way or three-way interactions were not significant, indicating that the Taejoong alleles at QSL-1 and QSL-3 would increase KS additively. *Tiller number (TN)*: The main effects of QSL-1, QSL-2, and QSL-3 were significant for TN, with the RILs carrying the Keumkang allele exhibiting more tillers than those carrying the Taejoong allele. The QSL-1 × QSL-2 interaction and



the  $QSL-2 \times QSL-3$  interaction were significant for TN, explaining the limited level (3.6% and 1.6%, respectively) of the TN variance. *Length of center rachis (LCR)*: The main effects of  $QSL-1$ ,  $QSL-2$ , and  $QSL-3$  were highly significant for LCR, with the RILs carrying the Taejoong allele exhibiting longer LCR than those carrying the Keumkang allele. The  $QSL-1 \times QSL-2$  interaction and the  $QSL-1 \times QSL-3$  interaction were significant, explaining the limited level (2.0% and 1.4%, respectively) of the LCR variance.

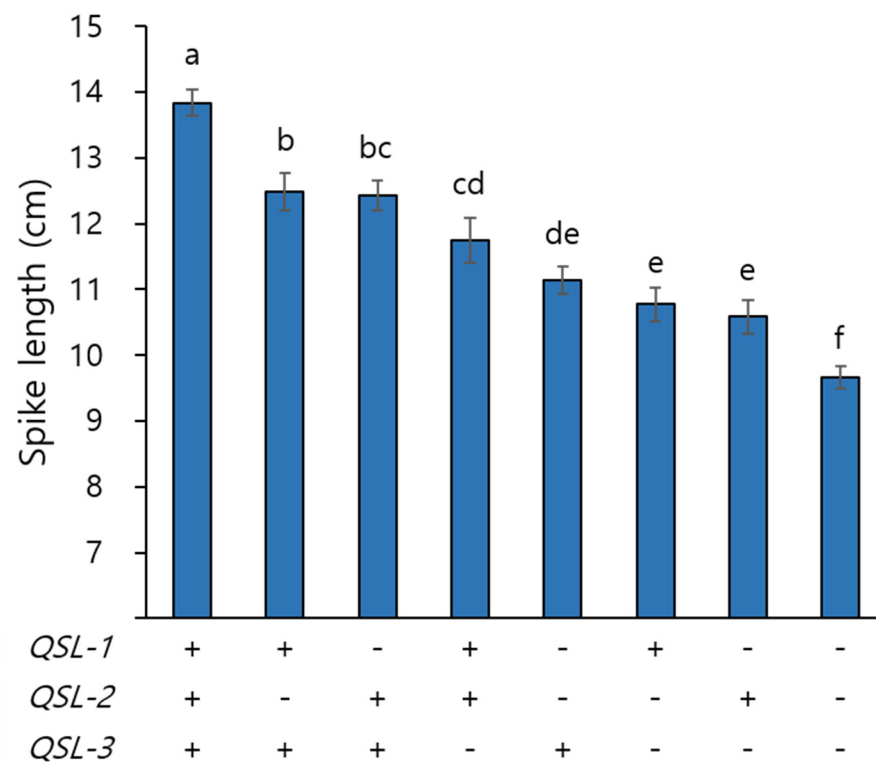


**Figure 4.** SNP-based QTL mapping for yield potential traits in 94  $F_{10}$  recombinant inbred lines produced from the cross between Taejoong and Keumkang. SL, spike length; DHD, days to heading date; TN, number of tillers; LCR, length of center rachis.

**Table 2.** Three-way ANOVAs of *QSL-1*, *QSL-2*, and *QSL-3* for different yield potential traits.

Trait <sup>a</sup>	Allele <sup>b</sup>	Main Effect <sup>c</sup>			Interaction <sup>d</sup>			
		<i>QSL-1</i>	<i>QSL-2</i>	<i>QSL-3</i>	<i>Q1</i> × <i>Q2</i>	<i>Q1</i> × <i>Q3</i>	<i>Q2</i> × <i>Q3</i>	<i>Q1</i> × <i>Q2</i> × <i>Q3</i>
SL (cm)	T	12.2	12.2	12.5				
	K	11.0	11.0	10.7				
	<i>P</i> -value	****	****	****	ns	ns	ns	ns
	PVE (%)	20.2	15.2	18.6				
DHD (no.)	T	185.5	185.4	185.1				
	K	184.5	184.6	184.9				
	<i>P</i> -value	****	**	ns	ns	ns	ns	***
	PVE (%)	5.7	2.6					4.7
CL (cm)	T	84.4	84.5	87.4				
	K	86.5	86.4	83.5				
	<i>P</i> -value	ns	ns	****	ns	ns	**	**
	PVE (%)			6.5			3.0	2.3
COM (no./cm)	T	4.4	4.3	4.3				
	K	4.6	4.7	4.7				
	<i>P</i> -value	****	****	****	ns	ns	ns	ns
	PVE (%)	5.4	7.5	5.9				
KS (no.)	T	51.7	50.6	51.7				
	K	48.9	50.0	48.9				
	<i>P</i> -value	****	ns	**	ns	ns	ns	ns
	PVE (%)	6.0		3.6				
TN (no.)	T	5.7	5.8	6.0				
	K	6.4	6.3	6.1				
	<i>P</i> -value	****	**	*	***	ns	*	ns
	PVE (%)	8.3	2.6	1.6	3.6		1.6	
LCR (cm)	T	0.71	0.73	0.73				
	K	0.66	0.64	0.64				
	<i>P</i> -value	****	****	****	**	**	ns	ns
	PVE (%)	9.9	21.6	11.9	2.0	1.4		

<sup>a</sup> SL, spike length; DHD, days to heading date; CL, culm length; COM, compactness; KS, kernels per spike; TN, number of tillers; LCR, length of center rachis. <sup>b</sup> Mean values of the recombinant inbred lines (RILs) carrying the Taejoong (T) allele and the Keumkang (K) allele are indicated for each QTL. An asterisk indicates significance (\*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , \*\*\*  $P \leq 0.001$ , and \*\*\*\*  $P \leq 0.0001$ ; ns: not significant) from the three-way factorial ANOVA with the year (2017, 2018, and 2019) as a random factor and the three QTLs as fixed factors. Phenotype variance explained (PVE) is indicated only for significant effects. <sup>c</sup> *QSL-1*, *QSL-2*, and *QSL-3* were represented by the markers *AX-94918964*, *AX-94831718*, and *AX-94602621*, respectively. <sup>d</sup> *Q1*, *Q2*, and *Q3* indicate *QSL-1*, *QSL-2*, and *QSL-3*, respectively.



**Figure 5.** Spike length of the RILs carrying different allele combinations of *QSL-1*, *QSL-2*, and *QSL-3*. The symbol “+” indicates the Taejoong allele for a long spike while “-” indicates the Keumkang allele for a short spike. *QSL-1*, *QSL-2*, and *QSL-3* were represented by the markers AX-94918964, AX-94831718, and AX-94602621, respectively. Different letters above the bars indicate that there was significant difference from Duncan’s multiple range test at  $P < 0.05$ . Error bars indicate standard errors.

#### 4. Discussion

##### 4.1. Potential Use of the Spike Length QTLs in Breeding

Improving yield potential has been one of the most important goals in wheat breeding [22–25]. As kernels per spike is strongly correlated with the final grain yield, selecting breeding lines with large spike has been a popular strategy for increasing sink capacity and improving yield potential [12,26]. To genetically dissect the large spike characteristics of the Korean wheat cultivar Taejoong, we identified three major QTLs for spike length, *QSL-1*, *QSL-2*, and *QSL-3*, using  $F_{10}$  RILs which were derived from the Taejoong  $\times$  Keumkang cross and genotyped with high density SNPs. At all three QTLs, the Taejoong alleles for long spike were associated with greater number of kernels per spike. Therefore, the SNPs linked to *QSL-1*, *QSL-2*, and *QSL-3* would provide useful molecular tools to develop breeding lines with increased sink capacity through marker assisted selections. *QSL-1*, *QSL-2*, and *QSL-3* are especially promising as they exhibited no significant two-way or three-way interactions on spike length and kernels per spike, indicating that pyramiding the Taejoong alleles at these QTLs would additively increase sink capacity (Table 2, Figure 5).

Nevertheless, caution is required as the effect of increased kernels per spike is frequently compensated for by decreased tiller number per unit area [27–30]. Our results showed that the Taejoong alleles for long spike at *QSL-1*, *QSL-2*, and *QSL-3* are also associated with reduced tiller number, and that the three QTLs exhibit different levels of tradeoffs between kernels per spike and tiller number. For example, while the effect of the Taejoong allele increasing kernels per spike was similar between *QSL-1* and *QSL-3*, the effect of the Taejoong allele decreasing tiller number was nearly seven times greater at *QSL-1* compared with that at *QSL-3* (Table 2). While further investigation is required to characterize the effects of the spike length QTLs on other yield potential traits under

different environments and genetic backgrounds, the current results suggest that *QSL-3* is likely more beneficial than *QSL-1* in terms of optimizing the balance between kernels per spike and tiller number. Our results also illustrate the importance of dissecting individual genetic factors underlying yield potential traits and characterizing their effects on other yield components in order to provide breeding programs with useful molecular tools for optimizing the balance among different yield components.

#### 4.2. Comparison of *QSL-1*, *QSL-2*, and *QSL-3* with Previously Reported Spike Length QTLs

Using the IWGSC RefSeq v1.0 wheat reference genome information [31], we compared the locations of *QSL-1*, *QSL-2*, and *QSL-3* with other spike length QTLs identified from previous studies. Interestingly, the *QSL-2* region flanked by the markers *AX-94831718* (503.8 Mb) and *AX-94846616* (640.2 Mb) on chromosome 5A overlapped with many previously reported spike length QTLs, that is, *QSL.caas-5AL* near the marker *JD\_c15758\_288* (595.4 Mb) identified from the Zhou 8425B × Chinese Spring RIL population [32], *Qsl.cau-5A.4* near *BS00096756\_51* (538.8 Mb) identified from the Yumai 8679 × Jing411 RIL population [33], *qSl-5A.3* flanked by *AX-110071854* (478.6 Mb), *AX-111139819* (541.3 Mb) identified from the Kenong 9204 × Jing411 RIL population [34], and *QSL-5A.4* peaking near *BS00066143\_51* (533.1 Mb) identified from the Yanzhan 1 × Hussar RIL population [35]. While *QSL.caas-5AL* was detected in only one out of three tested environments [32], *Qsl.cau-5A.4*, *qSl-5A.3*, and *QSL-5A.4* were stably detected in seven (out of nine), eight (out of eight), and two (out of eight) different environments, respectively [33–35]. These QTLs explained 1.9% (*QSL-5A.4*) to 20.6% (*qSl-5A.3*) of the spike length variation in the relevant RIL populations, and their additive effects ranged from 0.27 cm (*qSl-5A.3*) to 0.62 cm (*QSL-5A.4*) [32–35], which were smaller than or similar to that (0.61–0.62 cm) of *QSL-2* (Table 2). While further work is required to narrow down each QTL and determine whether *QSL-2* from the present study and the other four previously reported QTLs represent the same gene or not, our study and the previous reports suggest the *QSL-2* region as an important locus affecting spike length in different genetic backgrounds and environments. Although a few spike length QTLs have been previously reported on chromosomes 1D and 6A, that is, *QSL-1D* and *QSL-6A* from the NongDa3331 × Zang 1817 RIL population [36] and *QSL.czm-6A* from the MD233 × SS8641 doubled haploid population [37], we were unable to precisely compare the chromosomal locations of them with *QSL-1* and *QSL-3* from the present study.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2073-4395/11/1/22/s1>, Figure S1: Distribution and correlation of yield potential traits in 94 F10 recombinant inbred lines produced from the cross between Taejoong and Keumkang in 2017. T and K indicate Taejoong and Keumkang, respectively. M (black dia-mond) indicates the average value of each trait. DHD, days to heading date; CL, culm length; SL, spike length; COM, compactness; KS, kernels per spike; TN, number of tillers; LCR, length of center rachis. An asterisk indicates significance ( , \* , and \*\*\* at  $P \leq 0.05$ ,  $P \leq 0.01$ , and  $P \leq 0.001$  levels, respectively). Figure S2: Distribution and correlation of yield potential traits in 94 F10 recombinant inbred lines produced from the cross between Taejoong and Keumkang in 2018. T and K indicate Taejoong and Keumkang, respectively. M (black dia-mond) indicates the average value of each trait. DHD, days to heading date; CL, culm length; SL, spike length; COM, compactness; KS, kernels per spike; TN, number of tillers; LCR, length of center rachis. An asterisk indicates significance ( , \* , and \*\*\* at  $P \leq 0.05$ ,  $P \leq 0.01$ , and  $P \leq 0.001$  levels, respectively). Figure S3: Distribution and correlation of yield potential traits in 94 F10 recombinant inbred lines produced from the cross between Taejoong and Keumkang in 2019. T and K indicate Taejoong and Keumkang, respectively. M (black dia-mond) indicates the average value of each trait. DHD, days to heading date; CL, culm length; SL, spike length; COM, compactness; KS, kernels per spike; TN, number of tillers; LCR, length of center rachis. An asterisk indicates significance ( , \* , and \*\*\* at  $P \leq 0.05$ ,  $P \leq 0.01$ , and  $P \leq 0.001$  levels, respectively). Table S1: Descriptive statistics of the yield potential traits in the Taejoong × Keumkang recombinant inbred lines. Table S2: SNP-based genetic map for QTLs related to yield potential traits in 94 F10 recombinant inbred lines produced from the cross between Taejoong and Keumkang.

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