

## QTL mapping of spike fertility index in bread wheat

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**Abstract:** Spike fertility index (SF) is a trait easily measured at maturity and strongly associated with the number of grains per unit area. In order to identify genomic regions involved in SF control, a biparental (Baguette 10 × Klein Chajá) population of 80 recombinant inbred lines (RIL) was used. Seven field trials were conducted to determine the SF BLUP value per RIL. RILs were genotyped using a commercial chip (Axiom® 35K SNP Wheat Breeder's Array, Affimetrix). A linkage map was constructed with 857 SNP markers, and SF QTL mapping was performed. The narrow-sense heritability of SF was 0.89. Three genomic regions (QTL) associated with SF were found on chromosomes 2D, 4A, and 7A. The proportion of genetic variation explained by these three QTL was 32%, with no significant epistatic interaction between QTL.

**Keywords:** Spike fertility index, QTL mapping, additive effects, BLUP.

### INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most important crops in the world. Given the current and future scenario of increased global demand for grains, breeding efforts must concentrate on improving grain yield (CIMMYT 2019). The identification of specific and efficient selection criteria, as well as advances in knowledge of the genetic and molecular basis of yield and yield components, will allow an increase in genetic gain.

The spike fertility index (SF), i.e., the number of grains per g of spike chaff [also termed “fruiting efficiency” (Ferrante et al. 2012)], has been widely proposed as a selection criterion in breeding programs (Slafer et al. 2015, Alonso et al. 2018, Fischer and Rebetzke 2018, Valvo et al. 2018, Gerard et al. 2019) mainly due to its association with number of grains per unit area (Abbate et al. 1998, Foulkes et al. 2015, Ferrante et al. 2017).

However, the method of reference for determination of the spike fertility index, first described by Fischer (1984), uses spike dry weight at anthesis, making it a destructive method that is highly sensitive to the exact phenological stage in which measurement is carried out (Fischer and Rebetzke 2018). Abbate et al. (2013) proposed the alternative of the spike fertility index measured at maturity (i.e., calculated with spike chaff weight at maturity), as a selection criterion in breeding programs. This trait has been then shown to have good association with NG (Alonso et al. 2018, Pradhan et al. 2019) and moderate to

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high heritability (Martino et al. 2015, Alonso et al. 2018, Pretini et al. 2020a), as well as transgressive segregation and a low genotype  $\times$  environment interaction (Martino et al. 2015, Mirabella et al. 2016, Alonso et al. 2018).

In addition, because it is a simpler, non-destructive method, it is ideal for use as a high-throughput measurement method for selection in early generations. Although there are no conclusive results of how accurate SF at maturity is in estimating SF at anthesis, Slafer et al. (2015) suggest that there are indications of a small overestimation of the SF at maturity, due to the fact that spike dry matter may increase from anthesis to maturity. In this respect, Pretini et al. (2020a) observed instability in this estimator in different environments. However, Abbate et al. (2013) found high association between these two indices ( $r > 0.7$ ), and Alonso et al. (2018), Fischer and Rebetzke (2018), and Pradhan et al. (2019) detected a positive association between SF at maturity and NG. Furthermore, Terrile et al. (2017) and Alonso et al. (2018) found that selection for high SF resulted in stable and positive genetic gain in grain yield.

Most important agronomic traits in cereals are quantitatively inherited and the genes underlying their variation have been difficult to detect (Neumann et al. 2011). Several studies carried out during the last  $\sim 20$  years have pursued identification of quantitative trait loci (QTL) of yield and yield-related traits in mapping populations (Börner et al. 2002, McCartney et al. 2005, Kumar et al. 2007, Neumann et al. 2011, Hussain et al. 2017). However, there is little information in the literature about mapping QTL for SF. The first evidence in the literature revealed QTL associated with SF at anthesis based on genome wide association studies (GWAS) in chromosomes 2A, 2D, 4D, 5A, and 7A (Basile et al. 2019, Gerard et al. 2019). As for SF at maturity, Guo et al. (2017) found a QTL on chromosome 2A, with a relatively small effect. Ramirez et al. (2018) reported significant yet small effects of *Ppd-B1* and *Ppd-D1* on SF at maturity that were independent from those on the flowering date. Recently, a GWAS conducted by Pradhan et al. (2019) allowed identification of 15 marker-trait associations (MTAs) with SF in chromosomes 1B, 3B, 4A, 6B, and 7D. Fourteen of these MTAs were located in four regions apparently involved in abiotic and biotic stress response pathways. Recently, Pretini et al. (2020b) validated two QTL for SF in a doubled haploid mapping population, derived from Baguette19 and BioINTA2002. Those QTL were located in chromosomes 3A and 5A. In summary, evidence of the existence of genomic regions significantly associated with SF at maturity is only fragmentary and it has been scarcely addressed through the use of biparental populations.

Nevertheless, extensive work has been carried out in a recombinant inbred line (RIL) population derived from two Argentinian cultivars (Baguette 10 and Klein Chajá) contrasting for SF and other yield-related traits. In this population, the mode of inheritance of SF (Martino et al. 2015, Mirabella et al. 2016, Alonso et al. 2018), the possibility of using SF as a selection criterion in breeding for grain yield (Alonso et al. 2018), and the existence of significant molecular marker-SF associations [in a preliminary study by Panelo et al. (2019)] have been established. Therefore, the objective of this study was to identify QTL associated with SF in this population, evaluated in Balcarce and Marcos Juárez, Argentina.

## MATERIAL AND METHODS

### Plant material

Quantitative trait loci mapping was conducted using a population of 80 RIL from the cross between 'Baguette 10' and 'Klein Chajá', both Argentinian semi-dwarf hard spring wheat varieties released in 2000. 'Baguette 10' (B10; pedigree ARCHE/GENIAL) has been classified as having high SF, whereas 'Klein Chajá' (KCJ; pedigree NINJING/3/BUC'S//H697/DKBL) has been classified as having low SF (Martino et al. 2015). Both cultivars have similar intermediate growth cycles and are well adapted to Argentinian wheat-growing areas. They show several differences in spike architecture: B10 has a compact, short, dense spike, with very thin glumes and rachis, whereas KCJ has a longer, laxer spike with a large number of spikelets and grains and a heavy chaff structure (Martino et al. 2015, Alonso et al. 2018).

### Phenotypic evaluation

Six field experiments were carried out at the Balcarce (BCE) Experimental Station (lat 37° 45' S; long 55° 18' W, alt 130 m asl) of the Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires Province, Argentina, and one experiment was carried out at the Marcos Juárez (MJ) Experimental Station (lat 32° 43' S; long 62° 06' W, alt 112 m asl), INTA, Córdoba Province, Argentina. General crop management of experiments 1-3 has been described in Alonso et al. (2018); the remaining experiments were conducted similarly except for the fact that no irrigation was applied. Specific information on each experiment is presented in Table 1.

**Table 1.** Field experiments carried out at Balcarce (BCE) and Marcos Juárez (MJ), Argentina, between 2013 and 2017

Exp.	Year	Location	No. of blocks	Sowing date	Plot description	Growing conditions
1	2013	BCE	2	June 27 <sup>th</sup>	Three 5-m length rows with 0.2 m between rows	Irrigated
2	2014	BCE	2	July 24 <sup>th</sup>	Seven 5-m length rows with 0.2 m between rows	Irrigated
3	2015	BCE	2	July 15 <sup>th</sup>	Seven 5-m length rows with 0.2 m between rows	Irrigated
4	2016	BCE	2	June 20 <sup>th</sup>	One 1-m length row	Rainfed
5	2016	BCE	2	July 10 <sup>th</sup>	One 1-m length row	Rainfed
6	2017	BCE	2	June 19 <sup>th</sup>	One 1-m length row	Rainfed
7	2017	MJ	1	June 25 <sup>th</sup>	Three 1-m length rows, with 0.2 m between rows	Rainfed

The heading, anthesis, and physiological maturity dates of each plot were registered in the field when 50% of the spikes reached those stages, using the Zadoks scale (Zadoks et al. 1974). Physiological maturity was determined as loss of green from the peduncle. As expected from previous data (Martino et al. 2015, Alonso et al. 2018), each of the phenological stages was concentrated within one week for >90% of the RILs and within 10 days for the entire population (data not shown). Weather conditions were recorded daily with a standard meteorological station located at each experimental station.

At maturity, 15-20 spikes were drawn at random from each plot. When seven-row plots were used, only the five central rows were sampled. Spikes were cut at the lowest spikelet level, counted, weighed, and threshed. Spike chaff dry weight (g) was calculated as the difference between total spike dry weight (i.e., before threshing) and total grain weight. Grains were counted using an electronic counter. Then, SF (grains g<sup>-1</sup>) was calculated as the quotient between number of grains and spike chaff dry weight (Abbate et al. 2013).

### Linkage map construction

Eighty RIL and the parents of the population were genotyped with the Axiom® 35K SNP Wheat Breeder's Array (Affimatrix) (Allen et al. 2017). For genotyping, genomic DNA was extracted from a single seedling leaf tissue according to Haymes (1996). Only those polymorphic SNPs showing less than 10% missing data and segregation distortion under 20% were considered for construction of the linkage map. In addition, independent genotyping of the population with two functional markers for the *Vrn-A1* (Yan et al. 2004) and *Rht-D1* (Ellis et al. 2002) genes was added to the analysis. Those markers that had identical or compatible segregation in the whole population were grouped using the merger.py software (<https://github.com/juanrescente/lmap>) in order to minimize the redundant information. Once the markers were grouped, the linkage map was constructed using the R/qtl software (Broman et al. 2003). Genetic distances between the markers were calculated based on the Haldane mapping function. Throughout the 21 wheat chromosomes, several SNPs were anchored to the Ref Seq 1.0 genome assembly (Appels et al. 2018). Relationships between the genetic and physical positions of the SNPs were then established.

### Statistical analysis and QTL detection

Linear mixed models were fitted for SF using the *lme* function from the *nlme* package (Pinheiro et al. 2013) of the R software (R Core Team 2015). The models included replications within environments (years and/or sowing dates within a year) and environments as fixed factors, and genotypes and the genotype × environment interaction as random factors. The critical level of significance used was 0.05. Variance components and narrow-sense heritability were estimated according to Alonso et al. (2018).

Quantitative trait loci analysis was conducted with Composite Interval Mapping (CIM) using QTL Cartographer software (Wang et al. 2012a). Best linear unbiased predictors (BLUP) for each RIL, obtained from the mixed model, were used in QTL analysis. Threshold was calculated with 500 permutations and a 0.05 critical level of significance. Up to ten markers showing the highest F value after the forward-backward stepwise regression analysis were added as cofactors in the CIM step [model 6, using a moving window size of 10 centiMorgan (cM) and a walking speed of 1 cM]. The most likely position of the QTL was determined as the point with the maximum logarithm of the odds (LOD) score. The confidence interval (CI) of each QTL was defined as the map interval corresponding to a LOD-2 decrease to each side of the LOD

peak. A linear fixed model was fitted to calculate the additive effect ( $a$ ) of each QTL. The model included QTL and QTL  $\times$  QTL interaction effects, and BLUPs were used as phenotypic values. The proportion of the genetic variation explained ( $R^2$ ) by all QTL was obtained from this model. The critical level of significance used was 0.05.

The physical position of QTL was considered to be that of the marker nearest the peak LOD score. Using the flanking sequence for each SNP marker, provided by the chip manufacturer (Axiom, Affimetrix), a local alignment was performed using the BLAST algorithm (Altschul et al. 1990) against the reference sequence IWGSC RefSeq v1.0 of the bread wheat genome (Appels et al. 2018) to verify its position.

Haplotype was constructed for each RIL using the marker associated with the peak of the maximum LOD-score of each QTL identified. Haplotype proportions were tested with the Chi-square test ( $p$  value = 0.05,  $df = 7$ ). Due to heteroscedasticity between haplotype groups, Welch's ANOVA test was carried out using *userfriendlyscience* package (Verboon et al. 2018) of the R software, and haplotype differences were estimated using the Games-Howell nonparametric test.

## RESULTS AND DISCUSSION

Mean SF in the RIL population was 98.3, 91.9, 89.5, 92.4, 89.5, 90.7, and 101.7 grains  $g^{-1}$  for experiments 1 to 7, respectively. In each environment, SF values showed a symmetric, bell-shaped distribution with a wide range of variation from  $\sim 55$  to  $\sim 135$  grains  $g^{-1}$  [partial data is available in Alonso et al. (2018) and Figure S1], whereas the parents showed SF values as expected from previously published data (Alonso et al. 2018 and Figure S1). The relative weight of variance components and heritability were also coincident with previous reports, as genetic, genetic  $\times$  environmental, and residual variances were 54.1, 9.3, and 67.2, respectively, and heritability was 0.89. These results are in line with previous studies, which showed that SF fit an oligogenic model comprising a few loci with relatively large effects and high heritability (Martino et al. 2015, Mirabella et al. 2016, Alonso et al. 2018); i.e., a high probability of a finite number of markers explaining a considerable proportion of the phenotypic variance. In this study we included data from seven environments, and SF was accurately determined and predicted with BLUPs, which increases the reliability of the QTL significance (Piepho et al. 2008, Segura et al. 2009, Sadok et al. 2013).

This study was carried out with genotypic data from 80 individuals. The genetic linkage map consisted of 368 loci on the 21 chromosomes of bread wheat and spanned 3674 cM (Table S1). These loci included 857 SNP and two functional markers corresponding to the *Rht-D1* and *Vrn-A1* genes. Linkage maps per chromosome are shown in Figure S2. The fact that the functional markers *Rht-D1* and *Vrn-A1* were correctly mapped in the genome validates the mapping procedure. The use of mapping populations of reduced size, as was the case in the present study, may allow detection of QTL with major effects, but limits the detection of additional, small, yet real QTL (Beavis 1998, Vales et al. 2005, Cavalcanti et al. 2012, Wang et al. 2012b). Schön et al. (2004) recommend the use of a conservative threshold, such as the one used here, if the aim of a study is to identify a few large QTL controlling a limited proportion of the genetic variance. On the other hand, the number of SNP markers on each chromosome does not allow restriction of QTL to smaller map distances (Li et al. 2010).

The elucidation of the molecular and genetic basis of yield-related traits can contribute not only to understanding how yield is determined, but also to the development of technologies for speeding up the selection process, leading to high yielding cultivars. In this study, we detected three QTL associated with SF, a trait closely linked to number of grains per unit area, which is the main yield component in bread wheat. Three QTL for SF were detected in chromosomes 2D, 4A, and 7A (Table 2); the latter two have not been previously reported. All three QTL showed high stability across seven environments, which spanned differences in temperature, water regime, solar radiation, and photoperiod. The proportion of genetic variation explained by these three QTL was 32%, with no significant epistatic interaction between QTL.

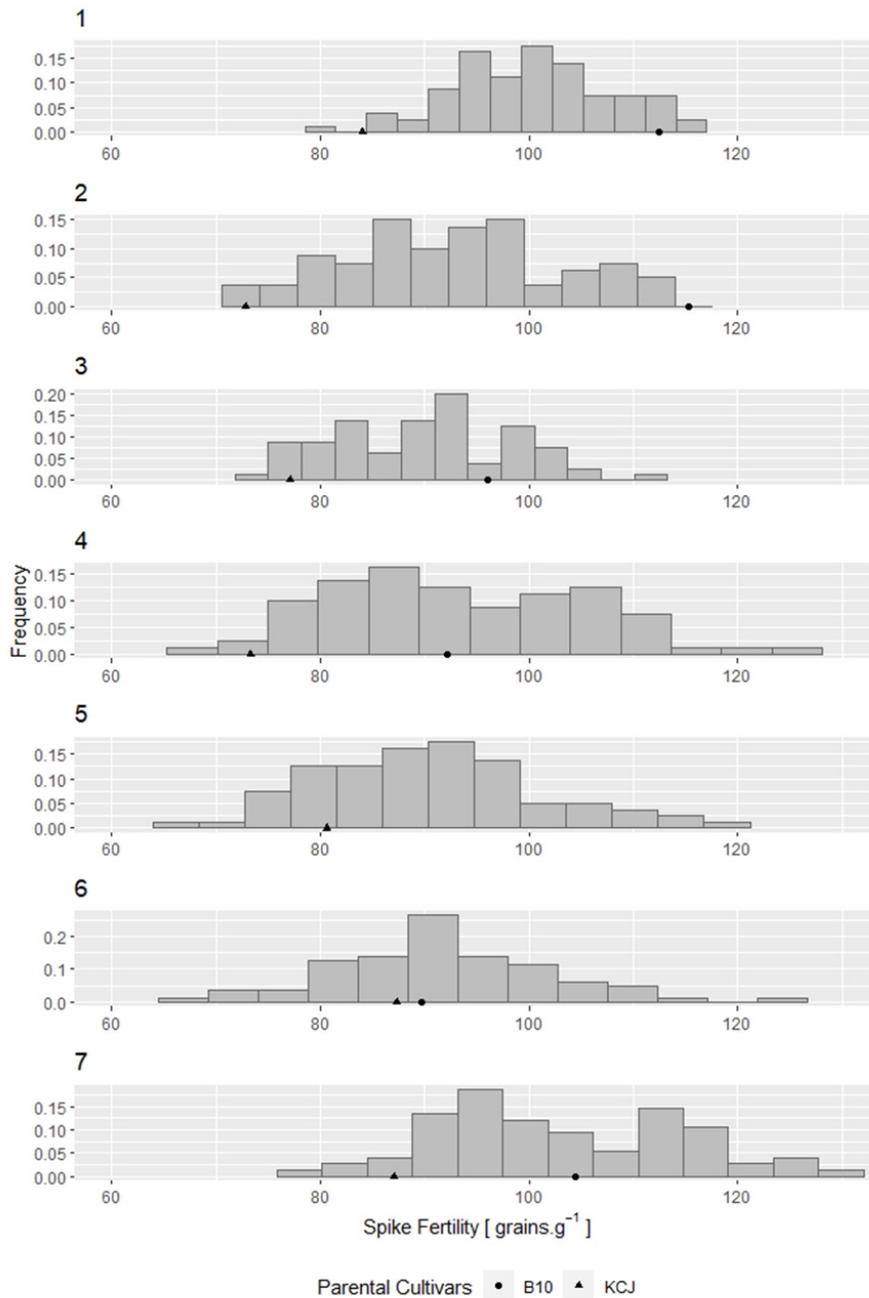
The QTL on chromosome 2D (referred to as  $Q_{sf.bfe.2DL}$ ) had an additive effect of 7.44 grains  $g^{-1}$ , expressed as the difference from the population mean. The donor of the SF-increasing allele was B10 (i.e., the parent with higher SF). The confidence interval of this QTL was 9 cM, and the physical position of the SNP marker associated with the peak of the maximum LOD score was  $\sim 648$  Mbp (Table 2).

On chromosome 4A, the QTL  $Q_{sf.bfe.4AL}$  showed an additive effect of 2.44 grains  $g^{-1}$ , with B10 as the donor parent. Located at  $\sim 472$  Mbp, the confidence interval of  $Q_{sf.bfe.4AL}$  comprised 5.4 cM. A third QTL,  $Q_{sf.bfe.7A}$  was detected in chromosome

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7A donated by B10.  $Q_{sf,bfe.7A}$  showed an additive effect of 1.74 grains  $g^{-1}$ , and a greater confidence interval (15.1 cM) in comparison with the remaining two QTL. Regarding its physical position, the SNP marker associated with the peak of maximum LOD was located in  $\sim 119$  Mbp.

Basile et al. (2019) also detected SF QTL in the latter two regions. One of the QTL was located in the vicinity, but not in the same position, of the newly reported  $Q_{sf,bfe.4AL}$ . Indeed, the QTL reported by Basile et al. (2019) on chromosome 4A (at 600 Mbp) was located around 200 Mbp from  $Q_{sf,bfe.4AL}$ . The QTL on chromosome 7A, on the other hand, appears



**Figure S1.** Frequency histogram of spike fertility index in the B10xKCJ RIL population (B10/KCJ) as evaluated in seven experiments (1 to 7).

to colocalize with  $Q_{sf,bfe,7A}$  detected in this study. Guo et al. (2017) proposed two candidate genes for SF and other associated traits, named *CONSTANS4 (CO4)* and *Six-rowed spike 1 (Vrs1)*, located in chromosome 2AL. *Vrs1*, also known as *GNI-A1*, has recently been associated with the number of fertile flowers and grains per spikelet traits (Sakuma et al. 2019, Golan et al. 2019). However, considering the genetic positions of markers associated with *CO4*, this QTL would not be homoeologous to  $Q_{sf,bce,2DL}$ . The genetic position of *Vrs1* has not yet been defined in the IWGSC RefSeq v1.0 reference sequence of the bread wheat genome (Appels et al. 2018). As for the QTL mapped by Gerard et al. (2019) on chromosome 2D, the physical position of the associated marker (Kukri\_rep\_c68068\_95) is 641.1 Mb, which is very close to AX-94501170 and AX-95232269 (648 Mb), the SNP marker associated with the peak of maximum LOD of  $Q_{sf,bce,2DL}$  detected in the present study. Gerard et al. (2019) measured SF (fruiting efficiency - FE) at anthesis, not at maturity, as it was done in this study. Abbate et al. (2013) have shown the existence of differences between FE at anthesis and at maturity. Therefore, the QTL detected by Gerard et al. (2019) may not colocalize with  $Q_{sf,bce,2DL}$ . None of the QTL found by Pretini et al. (2020b) in chromosomes 3A (685.12 Mbp) and 5A (461.49 Mbp) were present in the B10xKJ RIL population.

The effect of the *Ppd* genes, reported by Ramirez et al. (2018) as being associated with SF, could not be evaluated in this population because their parents, B10 and KJ, are monomorphic for these genes (Vanzetti et al. 2013).

**Table S1.** Distribution of 857 SNP markers used for linkage map construction. Twenty-one bread wheat chromosomes are listed. Marker#/Loci#: number of markers and loci assigned to each chromosome; total length in centiMorgan (cM) of each chromosome; average and maximum spacing of loci in cM

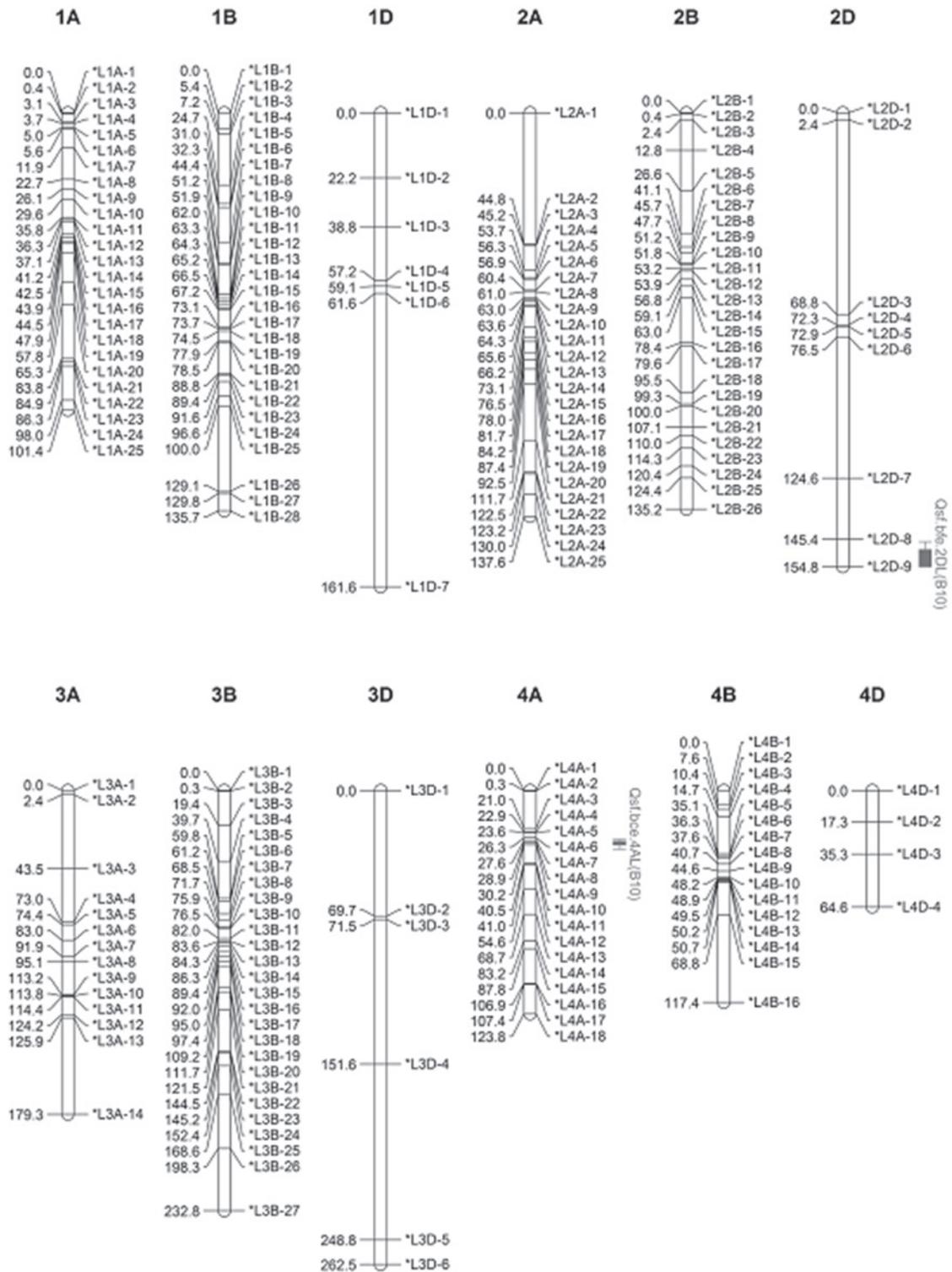
Chr	Marker#	Loci#	length (cM)	ave.spacing	max.spacing
1A	64	25	101.4	4.2	18.5
1B	58	28	135.7	5.0	29.1
1D	29	7	161.6	26.9	100.0
2A	75	25	137.6	5.7	44.8
2B	61	26	135.2	5.4	15.9
2D	29	9	154.8	19.4	66.4
3A	33	14	179.3	13.8	53.4
3B	55	27	232.8	9.0	34.5
3D	19	6	262.5	52.5	97.2
4A	26	18	123.8	7.3	20.7
4B	26	16	117.4	7.8	48.6
4D	6	6	161.2	21.5	29.3
5A	67	36	252.7	32.2	72.1
5B	82	38	190.3	5.1	32.4
5D	19	7	253.9	42.3	146.4
6A	40	19	160.3	8.9	49.4
6B	51	14	139.2	10.7	45.7
6D	16	9	246.2	30.8	136.0
7A	68	24	179.7	7.8	30.3
7B	26	13	179.8	15.0	77.7
7D	4	4	185.0	61.7	100.0
UNL	3		100.0	50.0	90.0
Overall	857	368	3674.3	10.5	146.4

**Table 2.** QTL for spike fertility index detected in the B10xKJ RIL population using BLUPs

QTL	cM <sup>a</sup>	LOD <sup>b</sup>	CI <sup>c</sup>	Allele Donor	AE <sup>d</sup> (grains g <sup>-1</sup> )	Physical position	Best SNP marker <sup>e</sup>
$Q_{sf,bfe,2DL}$	154.8	4.95	145.8; 154.8	B10	7.44	~648 Mbp	AX-94501170 AX-95232269
$Q_{sf,bfe,4AL}$	29.2	5.54	27.2; 32.6	B10	2.44	~472 Mbp	AX-94674955
$Q_{sf,bfe,7A}$	72.2	4.91	66.0; 81.1	B10	1.74	~119 Mbp	AX-94523322

<sup>a</sup> Position of the LOD score peak in centiMorgan (cM). <sup>b</sup> LOD score value at the LOD score peak. <sup>c</sup> LOD-2 confidence intervals in cM. <sup>d</sup> AE: Additive Effect: Difference from the population mean. <sup>e</sup> SNP marker associated with the LOD score peak.

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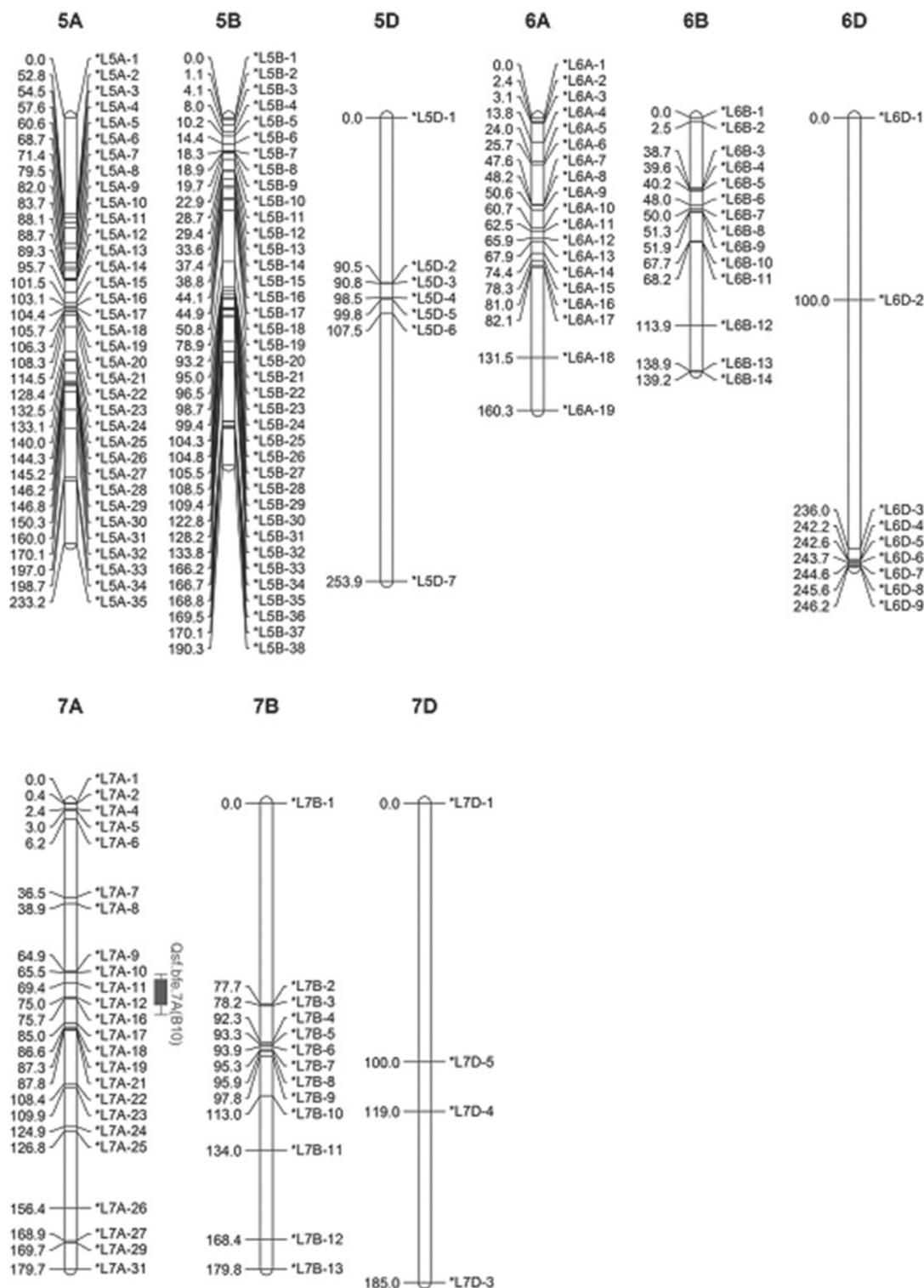


Figure S2. Linkage map of the B10xKCJ RIL population constructed using 857 SNP markers. Vertical boxes show QTL for SF. The error bars indicate the QTL confidence interval (LOD-2 decrease to each side of the LOD peak).

**Table 3.** Average increase in spike fertility index (SF, grains g<sup>-1</sup>) with respect to the population mean as analyzed by the haplotype group at three QTL

Haplotype group <sup>a</sup>	N <sup>b</sup>	Average SF increase (grains g <sup>-1</sup> ) <sup>c</sup>	
BBB	5	4.9	a
ABB	12	4.7	ab
BAB	10	4.3	ab
BBA	12	3.8	ab
BAA	9	2.8	abc
ABA	12	-3	abc
AAA	8	-3.8	bc
AAB	12	-4.4	c

<sup>a</sup> Group defined by allelic constitution at QTL  $Q_{sf,bfsc-2DL}$ ,  $Q_{sf,bfsc-4AL}$ , and  $Q_{sf,bfsc-7A}$  (from left to right). A = allele from KCJ; B = allele from B10. <sup>b</sup> N = number of RILs per group. <sup>c</sup> Different letters indicate significant differences ( $p < 0.05$ ).

The results of haplotype analysis are shown in Table 3. The haplotype proportions corresponded with the expected values ( $\chi^2 = 0.71$ ). The best performing group, BBB (i.e., the one with B10 alleles at all three QTL), showed an average increase of 4.9 grains g<sup>-1</sup> in SF with respect to the overall mean, whereas group AAB (i.e., with two KCJ alleles and one B10 allele) decreased average SF by 4.4 grains g<sup>-1</sup> compared with the overall mean. The haplotype group BBB, as well as all three groups carrying at least two B alleles, showed significantly higher SF increases than did group AAB. The AAA group was significantly different only from BBB. These results show the positive effect of B10 alleles on SF compared to the presence of KCJ alleles.

The present study provides information on genomic regions controlling SF in bread wheat. Peak SNP markers (Table 2) may be used to develop fine mapping populations in order to detect candidate genes which control the trait and to design strategies of marker-assisted selection for a complex quantitative trait such as grain yield.

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