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ORIGINAL PAPER

Characterization and precise mapping of a QTL increasing spike number with pleiotropic effects in wheat

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Abstract Tiller number (TN) and spike number per plant (SN) are key components of grain yield and/or biomass in wheat. In this study, an introgression line 05210, developed by introgression of chromosomal segments from a synthetic exotic wheat Am3 into an elite cultivar Laizhou953, showed a significantly increased TN and SN, but shorter spike length (SL) and fewer grain number per spike (GNS) than Laizhou953. To investigate the quantitative trait locus (QTL) responsible for these variations, the introgressed segments in 05210 were screened by SSR markers and one follow-up segregation population was developed from the cross 05210/Laizhou953. The population showed 3:1 segregation ratios for SN, SL and GNS, indicating that QTLs for these traits have been dissected into single Mendelian factors. Bulked segregation analysis showed that the markers located on the 4B introgressed segment were polymorphic between the two bulks. Therefore, they were further analyzed in the F_2 population to construct a linkage map. Three new QTLs, *QSn.sdau-4B*, *QSl.sdau-4B* and *QGns.sdau-4B*, were detected for SN, SL and GNS, respectively, which explained a large portion of the phenotypic variation (30.1–67.6%) for these traits with overlapping peaks. Correlation analysis and multiple-trait, multiple-interval mapping (MMIM) suggested pleiotropic effects of the QTL on SN, SL and GNS. Therefore, the QTL was designated as *QSn.sdau-4B*. By a progeny test based on F_3 families using SN, the QTL was mapped as a Mendelian factor to the proximal region of 4BL. It is a key QTL responsible for variation in spike number and size, which had not been reported previously. Thus, it is an important QTL for wheat to achieve high and stable biomass and grain yield. Dissection and mapping of this QTL as a Mendelian factor laid a solid foundation for map-based cloning of grain yield-related QTLs in wheat.

Abbreviations

TN	Tiller number
SN	Spike number per plant
SL	Spike length
GNS	Grain number per spike
IL	Introgression line
CIM	Composite interval mapping
MMIM	Multiple-trait, multiple-interval mapping
QTL	Quantitative trait loci
TGW	Thousand-grain weight
SNS	Spikelet number per spike
SSR	Simple sequence repeat
MTN	Maximum tiller number

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Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops worldwide, which feeds about 40% of the

world's population (Gupta et al. 2008). Given the current land usage, humans need to increase wheat production at an annual rate of 2%, to meet the growing demand imposed by the population (Gill et al. 2004). Breeding wheat cultivars with increased yield potential can contribute to meet at least half of the desired production increases and the remaining half can come through better agronomic and soil management practices (Reynolds and Borlaug 2006). Therefore, selection for high grain yield is an important focus in wheat breeding programs. Grain yield is a complex trait and usually controlled by a number of quantitative trait loci (QTL) with minor effects. It is influenced by environmental factors, which make it difficult to be manipulated and improved in breeding programs. The improvement is slow due to the lack of genetic information about the number, location and contribution of each QTL to the final expression of yield (Koebner and Snape 1999). Grain yield can be dissected into a number of components such as plant number per unit area, spike number per plant, grain number per spike, 1,000-grain weight (TGW) and other related traits such as spike length (SL) and spikelet number per spike (SNS; Cuthbert et al. 2008; Kato et al. 2000; Röder et al. 2008). However, some of them are less environmentally sensitive and have higher heritability than grain yield itself. Therefore, while mapping QTLs controlling grain yield, it is always important to obtain more information about QTLs governing these yield components (Kato et al. 2000; Kumar et al. 2007; Li et al. 2002; Röder et al. 2008; Yano and Sasaki 1997).

Studies on mapping QTLs (or genes) of yield components have been reported in wheat. Two genes responsible for tiller inhibition were mapped on 1AS and 3A (Kuraparthy et al. 2008; Spielmeyer and Richards 2004). Shah et al. (1999) mapped a significant QTL for TN on chromosome arm 3AL. Araki et al. (1999) detected QTLs for SNS on 4AS. Kato et al. (2000) detected a minor QTL for TN and SNS on 5A. Li et al. (2002) detected QTLs for TN on 6AS, 1DS and 2DS, and QTLs for SL on 1AL, 1BS, 4AL and 7AL. Börner et al. (2002) detected QTLs for SL on 1B, 4A and 5A, and QTLs for GNS on 4A and 7D. Huang et al. (2003) detected QTLs for TN on 1B, 2A, 2D, 3B, 4D, 5D, 6D and 7A from wild wheat relatives. Huang et al. (2004) detected QTLs for TN on 1B and 7A, and for GNS on 1D, 2A, 3D, 6A, 7A and 7D from elite winter cultivar. Narasimhamoorthy et al. (2006) detected QTL for TN and GNS on 3B and 3D, respectively. Kumar et al. (2007) detected coincident QTLs for TN on 3BL, 4AL and 6DL, QTLs for SL on 1BL, 2DS, 4AL and 5AL, QTLs for SNS on 2DS and 5AL, and QTLs for GNS on 1AL and 1BL in two populations. Cuthbert et al. (2008) detected QTLs for GNS on 1A, 2D, 3B, 5A and 7A.

In these studies, QTLs for grain yield components were detected using F_2, RIL or DH populations. In such populations, multiple genetic factors on the whole genome segregate simultaneously; QTL can only be localized to a genomic region (confidential region) rather than to a locus and cannot be used for the fine mapping of a single QTL. Thus, it is still difficult to utilize the existing mapping results in the fine mapping and cloning of these QTLs (Zamir 2001). Advanced backcross (AB)-QTL analysis, proposed by Tanksley and Nelson (1996), has been used in detecting and transferring valuable QTLs from exotic lines into elite cultivars. Some introgression lines (ILs) harbor small chromosomal segments from the donor parents, and the recurrent parent genetic background has been used to map QTLs as Mendelian factors by blocking genetic background noise (Korff et al. 2004; Tian et al. 2006a, b; Xie et al. 2006, 2008; Xing et al. 2008; Zamir 2001). Since the first set of ILs has been constructed in tomato (Eshed and Zamir 1994), ILs have also been constructed in rice, *Brassica napus*, *Arabdopsis thaliana*, barley, maize and wheat (Chetelat and Meglic 2000; Howell et al. 1996; Korff et al. 2004; Liu et al. 2006; Pestsova et al. 2006; Ramsey et al. 1996; Sobrizal et al. 1999; Szalma et al. 2007). Several agronomically important QTLs have been dissected and mapped as single Mendelian factors and have been cloned in rice, tomato and wheat (Ashikari et al. 2005; Fridman et al. 2004; Uauy et al. 2006). Recently, a QTL in wheat increasing grain weight was mapped as a Mendelian factor by using ILs (Röder et al. 2008). However, there is yet no report of characterization and mapping of a QTL increasing SN as a single Mendelian factor.

To transfer and identify QTLs for grain yield components in wheat, we developed a set of ILs that contained donor segments of a synthetic wheat line Am3 (AABBDD) through continuous backcrossing in a Laizhou953 background (Liu et al. 2006). Am3 was synthesized from the cross of *Triticum carthlicum (AABB)/Aegilops tauschii (DD)*. One IL, 05210, showed significantly increased SN, shorter SL and fewer GNS than Laizhou953. The objectives of this study were to: (1) characterize and dissect the QTLs for SN, SL and GNS into single Mendelian factors; and (2) map the major QTLs for SN, SL and GNS in 05210 and elucidate the relationship among them.

Materials and methods

Plant materials

To produce ILs, an elite local-adapted winter wheat cultivar Laizhou953 was used as the recurrent parent and a synthetic wheat Am3 derived from a cross *Triticum carthlicum (AABB)/Aegilops tauschii (DD)* was used as the donor parent. After backcrossing and selfing, one IL, 05210, was selected from the BC_4F_4 generation. Based on

2 years of data collected at the Chinese Academy of Agricultural Sciences, Beijing, China and Shandong Agricultural University, Tai'an, China, this IL showed significantly increased TN at the seedling stage and increased SN, but shorter SL and fewer GNS at the adult stage than Laizhou953. To identify the QTL responsible for this variation, a new segregation population of 166 F_2 plants was developed from the cross 05210/Laizhou953. The F_2 individuals and F_2-derived F_3 progenies (40 seeds) were planted in the field at the experimental station, Shandong Agricultural University, Tai'an, China, in the fall of 2006 and 2007, respectively. The F_2 seeds were space planted at 8 cm apart in 3.2-m long rows with 30 cm between rows. The F_3 families were arranged in a randomized complete-block design with two replicates. Each F_3 family (40 seeds) was space planted in a single row as described for F_2. Parental lines were used as controls in each replicate. Standard cultivation practice was followed in both experiments.

Phenotypic evaluation

In spring of 2007, maximum tiller number (MTN), SN, SL and GNS of the 166 F_2 plants were individually evaluated. Self-pollinated seeds of these F_2 plants were harvested. In spring of 2008, SN, SL and GNS of 15–20 plants in each F_3 family were recorded in each replicate. Since MTN was the same as SN in F_2 data, it was not scored in the F_3 population. Phenotypic data of 149 F_3 families were obtained, while the remaining 17 families were not scored due to shortage of seedlings.

DNA extraction and molecular marker analysis

DNA was extracted from fresh leaves according to the CTAB method with minor modifications. The simple sequence repeat (SSR) marker information was obtained from the Web site http://wheat.pw.usda.gov. A total of 857 SSR primer pairs including GWM, GDM, BARC, CFD and WMC were used to detect the introgressed segment in 05210. PCR amplification was performed in 20 μl final volume reactions containing 50 ng template DNA, 0.2 μl *Taq* polymerase (5 $U\mu l^{-1}$, Promega), 2.0 μl 10× PCR buffer [500 mM KCl, 100 mM Tris–HCl (pH 9.0), 1% Triton X-100], 2.0 μl 25 mM $MgCl_2$, 0.75 μl dNTP (5 mM) and 0.1 μl of the forward and reverse oligonucleotide primers (10 μM).

Number, size of introgressed segments and linkage analysis

The number of the introgressed segments, length and the genome coverage of the introgressed segments in 05210 were calculated as described by Liu et al. (2006). The wheat SSR consensus linkage map (Somers et al. 2004) was used to estimate distances between markers, length of the introgressed segments and the overall genome size for genome coverage ratio calculations.

Bulked segregate analysis (BSA) was used to screen polymorphic SSR markers for a putative association between genotypic and phenotypic variation. The two bulks consisted of five F_2 lines with the most SN, and five lines with the least SN. The phenotype of the F_2 plants in both bulks were validated in their corresponding F_3 families. All polymorphic markers between 05210 and Laizhou953 were used to screen the two bulks (Table 2). The polymorphic markers between the two bulks were used to screen the F_2 population to construct the linkage map. Linkage analyses were performed by using JoinMap 3.0 software (Van Ooijen and Voorrips 2001).

Statistical analysis and QTL mapping

One-way ANOVA analysis was performed using SAS Version 9.0 (SAS Institute, Inc., Cary, NC) to compare differences between 05210 and Laizhou953 for MTN, SN, SL and GNS using the phenotypic data collected from 20 plants from each line. Spearman's rank correlation coefficients among SN, SL and GNS were calculated using MS Excel. The mean of each F_3 family was used to estimate the variance of the three traits. The linear model for the variance analysis was the following: $Yij = \mu + gi + rj + eij$, where μ is the overall mean, *Yij* is the phenotype of a specific genotype, *gi* is the fixed effect of genotypes, *rj* is the random effect of replicates, and *eij* is the error. The heritability of SN, SL and GNS was calculated by the formula $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$ where σ_g^2 is the genetic variance ($\sigma_g^2 = (\sigma_l^2 - \sigma_e^2)/r$), σ_l^2 is the variance of genotypes, r is the number of replicates, and σ_e^2 is the variance of genotype by replicate interaction (error).

Mapping of the QTLs for SN, SL and GNS was performed by two methods. First, composite interval mapping (CIM) was performed using trait means obtained for each $F_{2:3}$ families by using WinQTLCart 2.5 (Wang et al. 2005). LOD thresholds to declare significant QTLs were set at 2.2 based on 1,000 permutation tests (Churchill and Doerge 1994; Wang et al. 2005). Next, the genotypes of each F_2 plants for SN, SL and GNS were determined based on the segregation of SN in their F_3 progenies. SN was used because it showed the most significant difference between 05210 and Laizhou953 among the three traits. In F_3 progeny test, three different phenotypes of SN-homozygous lines with increased SN, segregating lines of both types and homozygous lines with fewer SN appeared clearly in most of the F_3 families. The homozygous lines with increased SN were considered as homozygotes for Am3 allele,

segregating lines as heterozygotes and homozygous lines with fewer SN as homozygotes for Laizhou953 allele. For the F_3 families that had difficulties in deciding the F_2 genotypes for SN, SL and GNS, additional analyses of variance were performed using the SAS Version 9.0 program to determine the F_2 genotype. Those F_3 families were compared to the two groups using contrasts of Laizhou953 and 05210. An F_2 plant was classified as an Am3 genotype if its F_3 progenies were significantly different from Laizhou953 ($P < 0.05$), but not significantly different from Am3, whereas an F_2 plant was classified as a Laizhou953 genotype if its F_3 progenies were significantly different ($P < 0.05$) from Am3, but not significantly different from Laizhou953. An F_2 plant with F_3 progenies significantly different from both Am3 and Laizhou953 ($P < 0.05$) was classified as heterozygote. Families that were not significantly different from both Am3 and Laizhou953 were not classified. Designation of QTLs for SN, SL and GNS detected in this study followed the International Rules of Genetic Nomenclature (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm). Accordingly, *QSn.sdau, QSl.sdau* and *QGns.sdau* were designated as the QTLs for SN, SL and GNS reported from Shandong Agricultural University, Tai'an, Shandong, China.

Pleiotropic effects of QTL was determined by MMIM using Qgene (http://coding.plantpath/ksu.edu/qgene), and LOD thresholds to claim a significant QTL with pleiotropic effects was set at 2.0 based on 1,000 permutation tests.

Results

Phenotypic differences between Laizhou953 and 05210

Stable and significant ($P < 0.001$) differences in MTN, SN, SL and GNS were observed between 05210 and Laizhou953 in both experiments conducted in Beijing and Tai'an locations (Table 1). MTN and SN of 05210 increased at 41–49.5% and 52.7–57.7%, while SL and GNS decreased at 16.6–17.4% and 14.9–15%, compared to Laizhou953 in the two locations, respectively. F_1 of 05210/Laizhou953 showed a similar phenotype as Laizhou953. Thus, the three traits were probably controlled by dominant QTLs with fewer MTN and SN, and longer spike and more GNS as dominant.

Introgressed segments in 05210

After screening 857 primer pairs between Am3 and Laizhou953, 152 SSR markers were found to be polymorphic. These markers were used to detect the introgressed segments in 05210 and 19 of them detected introgression of Am3 chromosomal segments. According to the wheat consensus linkage map (Somers et al. 2004), these markers represented 16 introgressed segments located on ten chromosomes with 1–4 segments on each of them. The length of the introgressed segment ranged from 0.5 to 49 cM (Table 2). The total introgressed segments length was 190.5 cM and covered about 7.4% of the Laizhou953 genome.

Variance and segregation of the F_2/F_3 population

Variation analysis showed that the effects of genotypes were significant ($P < 0.0001$ for SN; $P < 0.001$ for SL and GNS) for all the three traits. Heritability of the three traits ranged from 79.5 to 88.5% (Table 3).

The frequency distributions of SN, SL and GNS in the population were bimodal (Fig. 1a–c). Laizhou953 type and 05210 types were easily separated into two groups for these traits with higher peaks observed for Laizhou953 in all three traits. χ^2 test results indicated that the segregations of the three traits were in accordance with the 3:1 ratio, indicating that the three traits were controlled by single Mendelian factors and could be mapped as a single gene in the population (Table 4).

Map construction

Bulked segregation analysis identified four polymorphic markers, wmc657, gwm495, gwm113 and gwm513, between the two bulks, which were all located on

Table 1 Phenotypic differences of maximum tiller number, spike number per plant, spike length and grain number per spike between Laizhou953 and 05210 in Beijing and Tai'an

Traits	Maximum tiller number	Spike number per plant	Spike length (cm)	Grain number per spike
Laizhou953 (Beijing)	20.1	9.7	10.2	50
05210 (Beijing)	28.3*** (41%)	15.3*** (57.7%)	8.43*** (−17.4%)	42.5*** (−15%)
Laizhou953 (Tai'an)	19.8	9.3	9.8	48.4
05210 (Tai'an)	29.6*** (49.5%)	14.2*** (52.7%)	8.14*** (−16.6%)	39.2*** (−14.9%)
05210/Laizhou953 (Tai'an)	21.1	10.3	9.5	47.7

Numbers in brackets show the increasing or decreasing percentage compared to Laizhou953

*** Significance at $P < 0.001$ probability level

Table 2 Chromosome location and length of the introgressed Am3 segments in 05211

Chromosome location	Introgressed segments	Length (cM)	Homozygote or heterozygote
2A	Gwm339	10	z
	Wmc455	3.5	z
4A	Wmc617	5	z
5A	Wmc47	8.5	z
2B	Wmc332	13.5	z
3B	Gwm493	49	z
4B	Gwm513-wmc657-gwm113-gwm495	5	z
6B	Cfd13	13.5	h
	Wmc179	0.5	h
3D	Gwm152	13	z
	Barc71	24	z
4D	Wmc617	13.5	z
5D	Cfd18	5	z
	Cfd183	5	z
	Cfd13	12	z
	Barc154	9.5	z

z homozygote; *h* heterozygote

Table 3 Variance components and heritability for spike number per plant (SN), spike length (SL) and grain number per spike (GNS) in the F_2/F_3 population derived from the cross 05210/Laizhou953

Trait	Variation	*df*	SS	MS	*F* value	h^2 (%)
SN	Genotype	148	1,387.9	9.4	16.5***	88.5
	Replicate	1	1.5	1.5	2.5	
	Error	148	84.9	0.6		
SL	Genotype	148	143.3	0.97	8.1**	79.5
	Replicate	1	0.01	0.01	0.01	
	Error	148	18.1	0.12		
GNS	Genotype	148	7,229.8	48.85	9.7**	81.7
	Replicate	1	0.62	0.62	0.6	
	Error	148	742.9	5.02		

***, ** Significant at $P < 0.0001$ and $P < 0.001$ probability level

chromosome 4BL proximal to the centromeric region. After screening the population using the four markers, a linkage map was constructed with the total genetic distance of 21.9 cM (Fig. 3). The markers' order in the linkage group was the same as that in the consensus map of Somers et al. (2004).

CIM of the QTLs

Composite interval mapping (CIM) analysis located all three QTLs for the three traits to the same position in the linkage map with overlapping peaks (Table 5; Fig. 2).

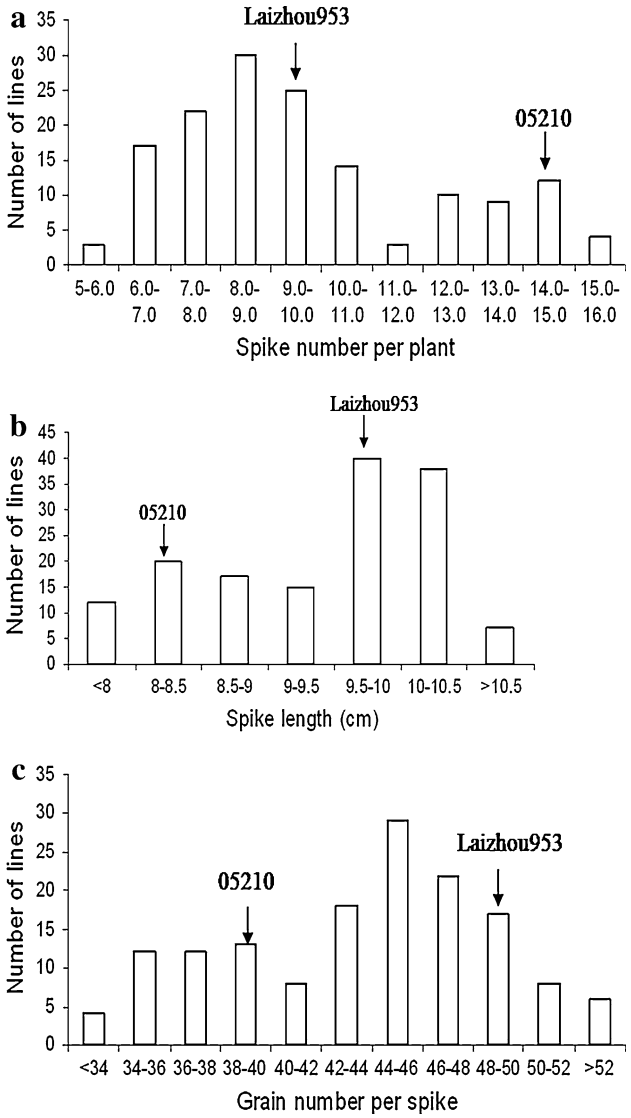

Fig. 1 Phenotypic distribution of spike number per plant (SN), spike length (SL) and grain number per spike (GNS) in the F_2/F_3 population of 05210/Laizhou953: **a** SN, **b** SL and **c** GNS

Table 4 Segregation of spike number per plant (SN), spike length (SL), and grain number per spike (GNS) in the F_2/F_3 population

Traits	F_2/F_3 segregation		χ^2 (3:1)	*P* value
	Laizhou953 type	05210 type		
SN	111	38	0.02	0.89
SL	103	46	3.4	0.1
GNS	108	41	0.49	0.48

These QTLs explained 30.1–67.6% of the phenotypic variation of the three traits, with *QSn.sdau-4B* explaining the largest (67.6%) phenotypic variation for SN. The QTLs were located at the same location in the linkage map. This suggests the existence of a single gene with pleiotropic

Table 5 QTLs for spike number per plant (SN), spike length (SL), and grain number per spike (GNS) detected by composite interval mapping

Traits	QTLs	Position (cM)	Flanking marker	LOD	Additive effect	Dominant effect	R^2 (%)
SN	*QSn-sdau-4B*	16.5	Xwmc657-Xgwm113	15.5	3.2	2.3	67.6
SL	*QSl-sdau-4B*	16.5	Xwmc657-Xgwm113	6.7	−4.8	−3.8	30.1
GNS	*QGns-sdau-4B*	16.5	Xwmc657-Xgwm113	8.5	−0.7	−0.4	39.3

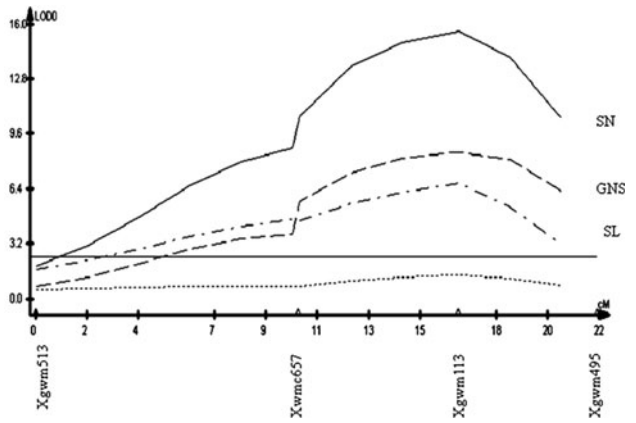

Fig. 2 QTL cartographer plot of the major QTL for spike number per plant (SN), spike length (SL) and grain number per spike (GNS) detected by composite interval mapping

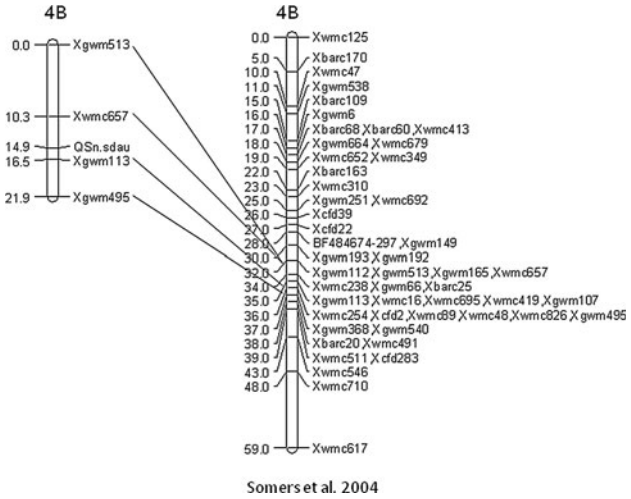

Fig. 3 Location of the QTL *QSn.sdau-4B* with pleiotropic effects for spike number per plant (SN), spike length (SL) and grain number per spike (GNS) (*left*) and the map position in the map of Somers et al. (2004) (*right*). The centromere was indicated by the *gray oval*

Table 6 Correlation coefficient among spike number per plant (SN), spike length (SL) and grain number per spike (GNS) in 05210/Laizhou953 population

	SN	GNS	SL
SN	1		
GNS	−0.66**	1	
SL	−0.75**	0.74**	1

** Significant at the 0.01 probability level

effects or a closely linked group of genes for the three traits.

Pleiotropic effects of the QTLs

Significant ($P < 0.01$) negative correlation was observed between SN and both SL and GNS (Table 6). One pleiotropy QTL was detected by MMIM analysis with an LOD value of 16.7 and 7.1 for joint effect and pleiotropic effects for the three traits, respectively, at the position of 16.0 cM of the linkage map. The result supported the existence of a QTL with pleiotropic effects. Thus, these traits were most likely controlled by one single QTL, designed as *QSn.sdau-4B*, hereafter.

Mapping of the QTL as a single gene

CIM and MMIM analyses determined the approximate location of the QTL between marker *Xwmc657* and *Xgwm495*, near *Xgwm113* (Fig. 2; Table 5). According to the F_3-deduced F_2 genotype, the major QTL was mapped as a single gene in the map between marker *Xwmc657* and *Xgwm113*, 4.6, and 1.6 cM away from the two markers, respectively (Fig. 3).

Discussion

To get an insight into the genetic factors underlying agronomically important traits in wheat, a set of ILs with Am3 segment in a Laizhou953 background was developed by continuous backcrossing (Liu et al. 2006). An assay of agronomic important traits for this set of ILs has been conducted. Since each IL contains fewer donor segments in the near-isogenic background of Laizhou953, high-resolution mapping of QTLs as Mendelian factors is possible in many ILs by constructing a segregating population through a cross between an IL and Laizhou953. In this study, 05210 showed a stable and significantly increased MTN and SN, a shorter SL and fewer GNS compared to Laizhou953 (Table 1). Therefore, it was crossed to Laizhou953 to construct a segregating population. The segregations of SN, SL and GNS were in accordance with the 3:1 ratio, suggesting that single Mendelian factors control the three

traits. To map QTLs for the three traits, the introgressed donor segments in 05210 were screened with SSR markers, and 7.4% of the Am3 genome coverage ratio was detected in 05210. The ratio is higher than the theoretical value (3.13%) in the BC_4 generation because background selection was not conducted during the backcrossing process. The elimination of most of the donor genetic materials can effectively block the genetic background noise and make the phenotypes express more stably. This enables us to dissect the QTLs as single Mendelian factors. Furthermore, detection of the introgressed segments in 05210 allowed focusing on segments harboring the QTLs for the target traits.

The key factor to be used to dissect and map the QTL as a single Mendelian factor is the large phenotype difference between 05210 and Laizhou953 caused by the introgression of the donor segments harboring the QTL in 05210, especially with respect to SN. Among the three traits, the most significant difference in SN was observed between the two parents (increase 52.7–57.7%) and the QTL responsible for SN explained the largest portion of phenotypic variation (67.7%). This made it possible to determine the genotype of each of the F_2 plants based on phenotypes of corresponding F_3 families and also to map the QTL as a single Mendelian factor in the linkage map (Fig. 3). Theoretically, the homozygous Am3 and Laizhou953 genotypes of F_2 plants will produce homozygous F_3 families. Thus, homozygous F_3 families will be non-segregating phenotypes having either increased or fewer SN, while the heterozygous F_2 plants will produce segregating F_3 families. In this study, most F_3 families displayed the expected phenotypes. For few F_3 families that could not be clearly classified based on phenotypic data, ANOVA analysis was conducted to determine their genotypes. To date, QTLs for SN, SL and GNS have not been reported to be located on 4BL; therefore, the QTL reported in this study is a new QTL for the three traits. The dissection and mapping of the QTL as a Mendelian factor will greatly facilitate fine mapping, marker-assisted breeding and map-based cloning of the QTL.

In a former study (Liu et al. 2006), putative QTLs for plant height, SN, SL and GNS were detected on 4B represented by marker *Xgwm113* by comparison of the phenotype variation between a set of ILs and Laizhou953. Considering the fact that the *Rht-B1* locus was also located near the centromeric region on 4BS (Huang et al. 2003, 2004; Quarrie et al. 2005), we deduced that the QTL might be the *Rht-B1* locus. In the same backcrossing population, we identified another IL 05210, which showed a significantly increased SN, but a shorter SL and fewer GNS compared to Laizhou953. However, it showed the same plant height as Laizhou953, which excluded the hypothesis that this QTL was *Rht-B1*. By developing a segregation population, a QTL for SN with pleiotropic effects to SL and GNS was detected and precisely mapped as a Mendelian factor by three different methods. CIM positioned the QTL at 16.5 cM in the linkage map, between the marker interval of *Xwmc657* and *Xgwm495*, which is the same position of *Xgwm113* in the linkage map (Fig. 2). Similar result was obtained in MMIM analysis where it showed a pleiotropic QTL located between *Xwmc657* and *Xgwm113*, at the position of 16 cM in the linkage map. When the QTL was scored as a qualitative trait based on the phenotypic data from F_3 families, the QTL was mapped between *Xwmc657* and *Xgwm113,* 1.6 cM away from *Xgwm113*. Mapping of the QTL at a similar position by all three independent methods probably showed the presence of a real QTL for these traits in this region. The slight difference in the QTL location in this study can be caused by different mapping methods. *Xgwm113* is the nearest marker to the QTL; thus, it is the most useful marker for marker-assisted selection and fine mapping of this QTL.

Regarding the QTL for plant height, we believe that it is not located on the introgressed donor segment in 05210. Introgressed segment screening and mapping of the region showed that the introgressed 4B segment in 05210 extended to marker *Xgwm495* on the distal end on 4BL (Table 2; Fig. 3). This marker was not detected in the ILs harboring the 4B introgressed segment in the former study. This segment also extended to marker *Xgwm513* on the proximal end, which was also detected in most of the ILs containing the 4B introgressed segment used in the former study. With respect to plant height, 05210 and these ILs were significantly different. QTL for plant height was probably located in the region between *Xgwm513* and *Xgwm6*, which span over a large distance across the centromere to the short arm of 4B in the map of Somers et al. (2004); 05210 probably contained a smaller segment between marker *Xgwm513* and *Xgwm6* than the ILs used in the former study. We have not delimited the exact point due to lack of polymorphic markers in the region. However, by developing more ILs in the same backcross population harboring different introgressed segment on 4B, we successfully dissected the QTL for plant height, SN, SL and GNS, which was linked together and detected as one QTL in the former study into two QTLs. A new QTL for SN with pleiotropic effects to SL and GNS independent of *Rht-B1* was precisely mapped in this study.

Pleiotropic effects refer to the fact that a single gene affects two or more distinct and seemingly unrelated traits. Pleiotropy is one of the most commonly observed attributes of genes, with broad implications in genetics, evolution, development, aging and disease. Genes of high pleiotropy are expected to be under strong stabilizing selection, because they affect multiple traits (Hodgkin 1998). There are some reports of QTLs showing pleiotropic effects in

crops. Shimizu et al. (2008) detected a QTL (*qREP-6*) in rice with pleiotropic effects for root elongation and TN under phosphorus deficiency. Xie et al. (2008) identified a QTL with pleiotropic effects for TGW, spikelets per panicle, grains per panicle, panicle length, spikelet density, heading date and plant height in rice. Tian et al. (2006a, b) mapped a QTL (*gpa7*) with pleiotropic effects for five panicle traits (panicle length, primary branches per panicle, secondary branch per panicle, grains on primary branches and grains on secondary branches). QTL increasing grain protein content (*Gpc-B1)* in wheat also showed pleiotropic effects to senescence (Uauy et al. 2006). These results suggest that pleiotropic effects exist widely in crops. In these studies, pleiotropic effects were analyzed only by correlation or ANOVA analysis or by the peak overlap of IM or CIM mapping (Shimizu et al. 2008; Xie et al. 2006, 2008). In this study, three different analysis methods indicated the existence of the pleiotropic effects of the QTL. First, CIM analysis showed the perfectly overlapping peaks for the three traits (Fig. 2). Secondly, correlation analysis showed significantly high correlation among the three traits (Table 6). Finally, pleiotropic effects were detected by MMIM analysis. All three methods showed the coincident results indicating pleiotropic effects of the QTL. Pleiotropy also causes side effects on adaptations of different traits. This could be because a genetic change beneficial to one trait may be deleterious to another (He and Zhang 2006; Otto 2004). In this study, QTL *QSn-sdau-4B* increased SN significantly, but decreased SL and GNS, which could be the side effects of the QTL. This QTL probably plays an important role in plant development; characterization and cloning of this QTL may facilitate the understanding of the molecular mechanism of pleiotropic effects that will aid in future improvement of wheat.

Spike number per plant (SN) is one of the most important components of grain yield and/or biomass of wheat, as it determines the population density of wheat in a field. Wheat cultivars can be divided into large- and multi-spike types. Large-spike type cultivars have larger spike, a longer SL and more GNS, but a fewer SN and a lower population density than those with the multi-spike type. Compared to large-spike type, multi-spike type cultivars usually have a more stable and high grain yield. This is true in regions with different stresses such as drought and salt, because an individual plant of multi-spike type cultivars can form more TN and SN easily than the large-spike type. A larger population in unit area will greatly contribute to grain yield and biomass (Reynolds et al. 2007; Tian et al. 2006a, b). Laizhou953 is a typical large-spike type cultivar, while 05210 is a typical multi-spike type line. Transfer of the 4BL segment from Am3 to Laizhou953 changed it from a large-spike type to a multi-spike type line. Therefore, the QTL located on this segment is a key gene or a closely linked gene family controlling the transition of the two types. Tillers are formed by the outgrowth of axillary buds that are located within the leaf axils on the basal internodes (Spielmeyer and Richards 2004). Thus, we speculate that *QSn.sdau-4B* probably can induce the axillary meristem to form more axillary bud and thus more tillers. This QTL has the largest effect on increasing SN; therefore, it is an important QTL that can be used in breeding programs. Fine mapping and cloning of this QTL would be highly valuable for the understanding of population and grain yield in wheat. The dissection and mapping of this QTL as a Mendelian factor laid a solid foundation for map-based cloning of this QTL.

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