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ORIGINAL ARTICLE

# A major QTL simultaneously increases the number of spikelets per spike and thousand-kernel weight in a wheat line

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## Abstract

***Key message*** **A novel and stably expressed QTL *QSNS.sicau-SSY-7A* for spikelet number per spike in wheat without negative effects on thousand-kernel weight was identified and validated in different genetic backgrounds.**

**Abstract** Spikelet number per spike (SNS) is an important determinant of yield in wheat. In the present study, we combined bulked segregant analysis (BSA) and the wheat 660 K single-nucleotide polymorphism (SNP) array to rapidly identify genomic regions associated with SNS from a recombinant inbred line (RIL) population derived from a cross between the wheat lines S849-8 and SY95-71. A genetic map was constructed using Kompetitive Allele Specific PCR markers in the SNP-enriched region on the long arm of chromosome 7A. A major and stably expressed QTL, *QSNS.sicau-SSY-7A*, was detected in multiple environments. It was located in a 1.6 cM interval on chromosome arm 7AL flanked by the markers *AX-109983514* and *AX-109820548*. This QTL explained 6.86–15.72% of the phenotypic variance, with LOD values ranging from 3.66 to 8.66. Several genes associated with plant growth and development were identified in the interval where *QSNS.sicau-SSY-7A* was located on the 'Chinese Spring' wheat and wild emmer reference genomes. Furthermore, the effects of *QSNS.sicau-SSY-7A* and *WHEAT ORTHOLOG OFAPO1*(*WAPO1*) on SNS were analyzed. Interestingly, *QSNS.sicau-SSY-7A* significantly increased SNS without negative effects on thousand-kernel weight, anthesis date and plant height, demonstrating its great potential for breeding aimed at improving grain yield. Taken together, these results indicate that *QSNS.sicau-SSY-7A* is a promising locus for yield improvement, and its linkage markers are helpful for fine mapping and molecular breeding.

## Abbreviations

| | |
|---|---|
| SNS | Spikelet number per spike |
| BSA | Bulked segregant analysis |
| SNP | Single-nucleotide polymorphism |
| RIL | Recombinant inbred line |
| KNS | Kernel number per spike |
| TKW | Thousand-kernel weight |
| QTL | Quantitative trait loci |
| KASP | Kompetitive allele-specific PCR |
| BLUP | Best linear unbiased prediction |
| AD | Anthesis date |

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PTN Productive tiller number
PH Plant height
SL Spike length
KL Kernel length
SSY S849-8/SY95-71 (216 $F_6$ lines including two parents)
SCN S849-8/CN16 (217 $F_6$ lines including two parents)
S83 S849-8/3642 (227 $F_6$ lines including two parents)

## Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important and widely grown food crops in the world, accounting for 20% of the global caloric intake and a quarter of cereal production (Ding et al. 2022a). Kernel number per spike (KNS), thousand-kernel weight (TKW) and spike number per unit area are three components of yield and are closely related to spike traits such as spikelet number per spike (SNS), sterile spikelet number and grain weight per spike. Increasing either SNS or grain weight per spike and reducing the sterile spikelet number could significantly increase crop yield.

SNS is determined by the number of lateral spikelet meristems produced by the spike meristems before it transforms into the terminal spikelet. This trait is influenced by various factors including the duration of spike development, temperature, nitrogen nutrition level and plant spacing (Zhang et al. 2018; Ma et al. 2019). Since it is a stable quantitative trait with high heritability, analyzing the genetic basis of SNS at the quantitative trait loci (QTL) or gene level can provide insights into the role of this trait in yield formation (Chen et al. 2020, 2022c). A few genes associated with SNS in wheat have been identified. For example, *TaSPL14* may influence SNS by interacting with the ethylene response gene *EIN3-LIKE 1* (*TaEIL1*), ethylene response transcription factor 2.11 (TaRAP2.11) and ethylene response transcription factor 1 (TaERF1) (Cao et al. 2021). Through fine mapping, *WAPO1* on chromosome 7AL of wheat was identified as a candidate gene that positively regulates SNS (Kuzay et al. 2019). *WFZP* plays an important role in the spikelet meristem and axillary meristem (Komatsu et al. 2003). A *WFZP-D* single mutant shows significant increases in the SNS and KNS, while maintaining the normal spikelet structure (Li et al. 2021b). Overexpression of *TaAGL6*, a member of the MADS-box gene family, increases the SNS and thus KNS in common wheat (Kong et al. 2022). Overexpression of *TaCol-B5* increases SNS and spikelet length (SL), thus significantly increasing wheat yield (Zhang et al. 2022).

Studies of SNS have mainly focused on genetic mapping, and the development of markers is limited owing to the complexity of the wheat genome. To date, QTLs for SNS have been detected on almost all 21 pairs of chromosomes of wheat (Wolde et al. 2019; Yao et al. 2019; Isham et al. 2021; Li et al. 2021a; Cao et al. 2022; Katz et al. 2022). These studies have revealed the regulatory mechanism of SNS at the QTL level, providing a basis for fine mapping and map-based cloning of promising loci. It is noteworthy that the effects of these QTLs on other yield-related traits differ. For example, the favored allele of *qSnps-7D* had no effect on TKW (Cao et al. 2022). Both *QSns.sau-2D* and *QSns.sau-2SY-7A* can increase SNS, but significantly reduce TKW (Ma et al. 2019; Ding et al. 2022a). *QTsn/Fsn.cib-3D* had no effect on TKW (Li et al. 2021a). In rice, *OsMADS1-OsMADS17-OsAP2-39* participates in the regulatory network involved in grain yield. Downregulating *OsMADS17* or *OsAP2-39* can simultaneously increase grain number and weight (Li et al. 2023). Therefore, it is necessary to identify loci/genes that can increase SNS without negative effects on other agronomic traits such as TKW for molecular breeding.

Bulked segregant analysis (BSA) is a convenient and rapid method for identifying markers in genomic regions associated with target traits by screening and collecting samples with extreme phenotypic differences (Wu et al. 2017). Previous studies have shown that the gene/locus associated with a target trait could be rapidly located within a small interval by combining the 660 K SNP array and BSA (Winfield et al. 2016; Zou et al. 2016; Qu et al. 2022; Xie et al. 2022).

In this study, we used a 660 K SNP array combined with BSA to identify a locus associated with SNS and then constructed a genetic map for mapping in a mapping population containing 214 $F_6$ recombinant inbred lines (RILs). The positive allele at the newly identified QTL can significantly increase SNS and TKW simultaneously. The effect of this major QTL was further validated in different genetic backgrounds.

## Materials and methods

### Plant materials

Three RIL populations were used, including S849-8/SY95-71 (216 $F_6$ lines including two parents, SSY), S849-8/CN16 (217 $F_6$ lines including two parents, SCN) and S849-8/3642 (227 $F_6$ lines including two parents, S83). The SSY population was used for QTL mapping, and the other two were used for validation. S849-8 (Chuan04 pin4/YunB58863-2) is a stable wheat line with a high SNS and TKW and a good plant architecture (Qu et al. 2022). SY95-71 has an excellent root system (Chen et al. 2022a) and tiller number (Liu et al. 2020). Chuannong 16 is a commercial variety with multiple tillers (Liu et al. 2018). 3642 is also an important advanced

breeding line. All materials were provided by the Triticeae Research Institute of Sichuan Agricultural University.

## Phenotypic evaluation

SSY RILs were planted in six environments: Wenjiang (103° 51′ E, 30° 43′ N), Chongzhou (103° 38′ E, 30° 32′ N) and Ya'an (103° 0′ E, 29° 58′ N) in 2020–2021 (2021WJ, 2021CZ and 2021YA) and 2021–2022 (2022WJ, 2022CZ and 2022YA) in Sichuan province, China. A randomized complete block design with two replications was adopted at Wenjiang and Chongzhou during the 2020–2021 and 2021–2022 growing seasons. SCN and S83 populations were planted at Chongzhou in 2020–2021. Fifteen kernels of each line were planted in a single row of 1.5 m with 0.1 m between plants and 0.3 m between each row (Ma et al. 2020). All field management was performed according to local standard practices. In particular, nitrogen and potassium phosphate were applied at 80 kg/ha and 100 kg/ha, respectively. Commercial herbicides, insecticides and fungicides were applied preventively monthly after tillering to ensure that each line has a sufficient number of healthy plants for phenotypic identification. Anthesis date (AD) was calculated from the planting date to the date when half of the plants for a given line flowered. Productive tiller number (PTN) was defined as the number of branches capable of producing spikes in a single wheat plant. Plant height (PH) was measured from the base of the plant to the top of the main spike (not including awns). Spike length (SL) was measured as the length from the base to the top of the main spike (excluding awns) for each plant. SNS was measured by counting the number of spikelets per main spike. The data for kernel length (KL) and TKW were retrieved from a previous study (Qu et al. 2022). The data for SNS, PH, PTN, SL and AD in 2021 were obtained from the same previous study (Qu et al. 2022). In this study, data for SNS, PH, PTN, SL and AD in 2022 were added and the BLUP was recalculated. Detailed environmental information on agronomic traits of the three RIL populations is listed in Table S1.

## Statistical analysis

IBM SPSS Statistic 26 (SPSS, Chicago, IL, USA; https://en.wikipedia.org/wiki/SPSS) was used for descriptive statistics, Student's *t*-test ($p < 0.05$), correlation analysis and analysis of variance (ANOVA). SAS V8.0 (SAS Institute, Cary, NC, USA; https://www.sas.com) was used for best linear unbiased prediction (BLUP) and to estimate the broad-sense heritability ($H^2$) of SNS from different environments. Q–Q polts and boxplots were drawn using Origin 2018 (https://www.originlab.com). The ANOVA of multi-environmental trials module in QTL IciMapping (Version 4.1, based on ICIM, http://www.isbreeding.net) was used for ANOVA.

## BSA and wheat 660 K SNP array analysis

Two phenotypically contrasting pools for the 660 K analysis were constructed based on SNS data from 2021WJ, 2021CZ and 2021YA. According to the method of Qu et al. (2022), the screening conditions are shown in Fig. S1. (1) The SNS phenotype values for each line from three environments in 2021 were arranged in descending order. According to the numerical arrangement results based on SNS, the lines ranked in the top 50 (more SNS group) and bottom 50 (few SNS group) in each environment were screened. Subsequently, the line numbers in a given extreme group that appeared in at least two environments were counted and pooled. The new pools were referred to as the group with more SNS and the group with less SNS. (2) The SNS phenotype data in three environments from each line were combined to calculate the average value. Then, according to the SNS in descending order, the first/last 30 lines were screened. Finally, 23 shared lines (SNS > 27.01) were obtained as the 'more pool' (MP) after comparison with the group with more SNS from (1) and the first 30 lines from (2). Similarly, 24 lines (SNS < 21.86) were obtained and named the 'few pool' (FP) after comparing with the group with few SNS from (1) and the last 30 lines from (2). MP and FP were thus used for BSA.

In a given pool, ten grains of each line were germinated. All leaves were sampled from each line, and then, leaves from all of the lines in each pool were mixed for DNA extraction by Beijing CapitalBio Technology Co., Ltd. (https://www.capitalbiotech.com/). Genotyping of the two pools was carried out using the 660 K SNP array (Beijing CapitalBio Technology. Co., Ltd.) as described in our previous study (Qu et al. 2022). PolyHighResolution (PHR) SNPs, regarded as the high-quality genotyping data, were retained by setting the following thresholds: DQC (Dish QC) > 0.82 and CR (Call-Rate) > 94. Further, SNPs with a heterozygous genotype (e.g., A/T and C/G) and missing information were eliminated and only the homozygous SNPs (e.g., A/A, T/T, C/C and G/G) were retained for subsequent analyses. The chromosomal interval related to SNS loci was determined according to the following steps. (1) The polymorphic SNPs were obtained by comparing SNPs in two pools with extreme phenotype values. (2) The number of polymorphic SNPs on each chromosome and the proportion of polymorphic SNPs relative to the total SNPs on each chromosome were calculated. Then, the chromosome with the highest proportion of candidate SNS loci was identified. (3) The number of polymorphic SNPs within a 40 Mb step on the candidate chromosome was calculated. Then, the physical interval with the most SNPs carrying possible SNS loci was obtained.

### KASP marker development and genetic map construction

Polymorphic SNPs on the candidate chromosomes identified from the 660 K SNP array analysis were transformed into kompetitive allele-specific PCR (KASP) markers. Primer sequences were designed using the online primer design pipeline PolyMarker (http://polymarker.tgac.ac.uk/). The polymorphic markers were used to genotype 214 lines of SSY after testing the specificity of KASP markers for the two parents. The KASP amplification reaction system included 5 µL of SsoFast EvaGreen mixture (Bio-Rad, Hercules, CA, USA): 2.85 µL of deionized water, 1.4 µL of mixed primers and 0.75 of µL DNA. The real-time PCR (Bio-Rad, CFX-96) system was used for this process. The cycling parameters were as follows: 94 °C for 15 min, 10 cycles of 20 s at 94 °C and 60 s at 61–55 °C (drop 0.6 °C, per cycle). Then, 26 cycles were performed at 94 °C for 20 s and 55 °C for 1 min. After the PCR procedure, fluorescence data was collected at 37 °C for 1 min. The sequences of the designed KASP markers are displayed in Table S2.

JoinMap 4.0 (Jansen et al. 1995) was used to construct a genetic linkage map based on the genotypes obtained from KASP markers in the SSY population. The genetic linkage map was drawn using MapChart V2.3 (Voorrips 2002).

### Identification and validation of major QTL

The Biparental Populations (BIP) module of IciMapping was used for QTL detection. The critical logarithm of odds (LOD) score for a QTL was set at 2.5 (Lin et al. 1996).The QTL mapping for multi-environmental trials (MET) module of IciMapping was used to perform a QTL × environment (QE) interaction analysis of SNS. Among the detected QTL, those repeatedly detected in more than three environments and explained greater than 10% of phenotypic variation were considered to be major and stable loci (Ma et al. 2019; You et al. 2021). QTL were named in accordance with the International Rules of Genetic Nomenclature (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm).

The flanking marker of the major QTL was used to verify its effect in SCN and S83 populations. According to the genotyping results, each population was grouped into three classes: (1) lines with homozygous alleles for increased SNS derived from parent S849-8, (2) lines carrying homozygous alleles of SY95-71 and (3) lines with heterozygous alleles (excluding analysis). The Student's *t* test ($p < 0.05$) was used to evaluate the differences between (1) and (2).

### Comparison with previously reported QTL and prediction of candidate genes

The physical locations of flanking markers and predicted genes in the major and stable QTL were obtained by blasting against the CS reference genome (IWGSC RefSeq v2.1) and the wild emmer genome (*Triticum turgidum* ssp *dicoccoides*, WEWSeq v2.0) (Zhu et al. 2021). Using the same method, physical locations of previously reported genes or QTL related to SNS on 7A were obtained. We analyzed the annotations and functions of these genes using UniProt (https://www.uniprot.org/). Furthermore, the expression patterns of the predicted genes were obtained from the wheat expression database of Chinese Spring Development (pair) in WheatOmics (Ma et al. 2021).

## Results

### Phenotypic evaluation

The SNS of S849-8 was significantly higher than that of SY95-71 in six environmental datasets ($p < 0.05$, Table 1, Fig. 1a). The SNS of the SSY population ranged from 15.88 to 32.50, and the standard deviation (SD) was 2.09 to 3.16.

**Table 1** Phenotype of the parents and RILs in this study

| Environment | Parents | | S849-8 × SY95-71(SSY) | | | |
|---|---|---|---|---|---|---|
| | S849-8 | SY95-71 | Min–max | Mean | SD | $H^2$ |
| 2021WJ | 28.88** | 19.83 | 15.88–32.50 | 25.20 | 2.69 | |
| 2021CZ | 25.40** | 20.75 | 16.20–28.75 | 23.73 | 2.09 | |
| 2021YA | 27.17* | 23.33 | 18.00–31.67 | 24.56 | 2.85 | |
| 2022WJ | 25.63** | 19.25 | 16.00–29.63 | 23.07 | 2.72 | |
| 2022CZ | 27.40** | 20.40 | 16.00–32.00 | 24.84 | 3.16 | |
| 2022YA | 27.43** | 21.71 | 18.50–31.80 | 26.01 | 2.94 | |
| BLUP | 26.85** | 21.08 | 17.59–30.68 | 24.56 | 2.33 | 0.62 |

*RILs* recombinant inbred lines, *SD* standard deviation, $H^2$ the broad-sense heritability, *BLUP* best linear unbiased prediction, *WJ* Wenjiang, *CZ* Chongzhou, *YA* Ya'an

*Difference is significant at the 0.05 level. **Difference is significant at the 0.01 level

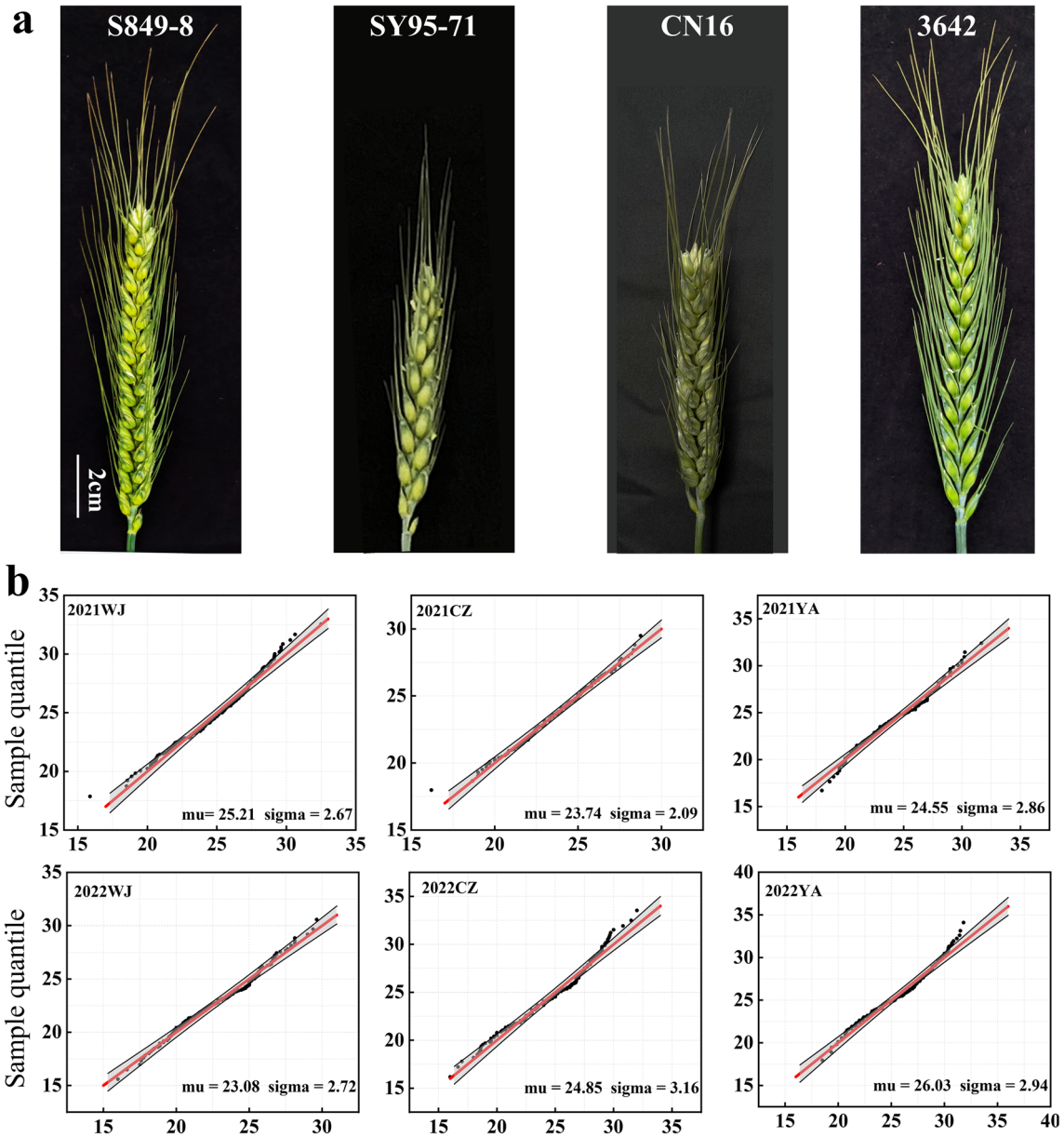


**Fig. 1** Phenotypic characteristics and frequency distribution of SNS. **a** Spikes of the parent 'S849-8,' 'SY95-71,' 'CN16,' and '3642'; the white line represents the scale bar = 2 cm. **b** Quantile–quantile (Q–Q) plots of the distribution of SNS in six different environments

**Table 2** Correlation coefficients for SNS in different environments

| Environments | 2021WJ | 2021CZ | 2021YA | 2022WJ | 2022CZ | 2022YA |
|---|---|---|---|---|---|---|
| 2021WJ | 1 | | | | | |
| 2021CZ | 0.77** | 1 | | | | |
| 2021YA | 0.63** | 0.66** | 1 | | | |
| 2022WJ | 0.88** | 0.77** | 0.62** | 1 | | |
| 2022CZ | 0.84** | 0.76** | 0.60** | 0.89** | 1 | |
| 2022YA | 0.83** | 0.72** | 0.61** | 0.89** | 0.87** | 1 |

*WJ* Wenjiang, *CZ* Chongzhou, *YA* Ya'an

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level

**Table 3** Correlation between SNS and other agronomic traits in the SSY population

| Trait | SNS |
|---|---|
| PH | 0.36** |
| SL | 0.53** |
| AD | 0.70** |
| PTN | 0.06 |
| TKW | −0.09 |

*SNS* spikelet number per spike, *PH* plant height, *SL* spike length, *AD* anthesis date, *PTN* productive tiller number, *TKW* thousand-kernel weight

*Significance level at $p < 0.05$. **Significance level at $p < 0.01$

The $H^2$ for SNS was 0.62, indicating that the trait was mainly determined by genetic factors (Table 1). The frequency distribution of SNS was close to a normal distribution in all environments indicating multi-genic inheritance (Fig. 1b). Significant and positive correlations for SNS were detected among six environments with Pearson's correlation coefficients ranging from 0.60 to 0.89 (Table 2).

## Correlation analysis

We further analyzed the correlations between SNS and other agronomic traits (PH, SL, AD, PTN and TKW) based on BLUP datasets. Pearson correlation coefficients ranged from −0.09 to 0.70 (Table 3). There was a significant and positive correlation between SNS and PH, SL, and AD ($p < 0.01$). SNS had no significant correlations with PTN and TKW. ANOVA indicated that environment (E), genotype (G) and genotype × environment (G × E) interactions had significant effects on SNS (Table S3).

## BSA and wheat 660 K analysis

In the BSA-660 K analysis, 905 polymorphic SNPs were detected after removing the unreliable SNPs with heterozygous genotypes (e.g., A/T and C/G) and sites with missing information. The number of polymorphic SNPs was highest on chromosome 7A (i.e., 696). The proportion of polymorphic SNPs was also highest on chromosome 7A (Fig. 2a). These results indicated that chromosome 7A may contain a major locus for SNS. SNPs were enriched in the physical

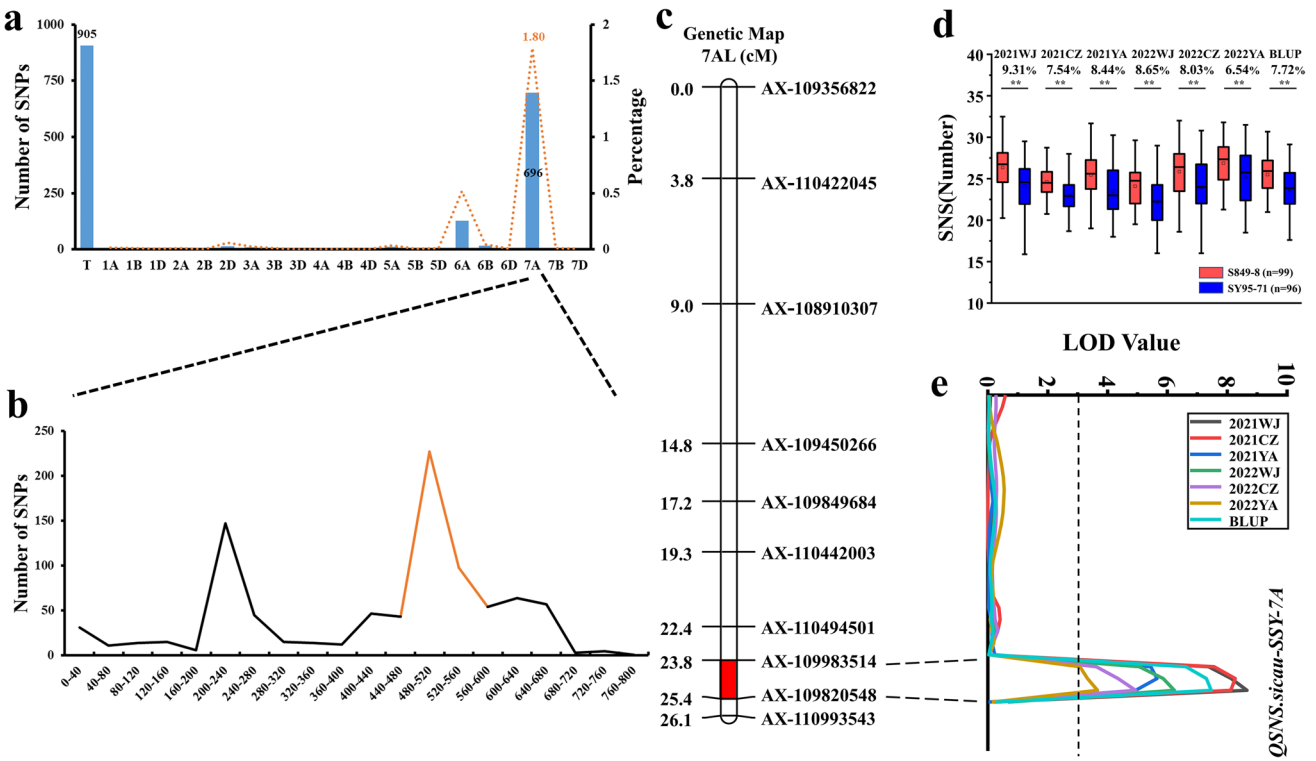


**Fig. 2** BAS-660 K SNP analysis and construction of genetic map. **a** Overview of PolyHighResolution SNP analyses by the 660 K SNP array; 'Number of SNPs' represents the number of polymorphic SNPs, T: total polymorphic SNPs; 'Percentage' represents the ratio of the number of polymorphic SNPs to the total ones. **b** Distribution of different SNPs in different segments of chromosome 7A; the horizontal axis is the chromosome position, and the vertical axis is the number of SNPs. **c** Genetic map of the QTL for SNS identified in SSY population. **d** SSY population were divided into two haplotype groups based on the genotype of flanking markers, and the SNS differences caused by the corresponding quantitative trait loci (QTL) were represented. **e** Log of odds (LOD) value of QTL for SNS of chromosome 7A

**Table 4** Quantitative trait loci (QTL) for SNS detected in the 'S849-8' × 'SY95-71' population

| QTL | Environment | Chromosome | Interval (cM) | Left marker | Right marker | LOD | PVE (%) | Add |
|---|---|---|---|---|---|---|---|---|
| *QSNS.sicau-SSY-7A* | 2021WJ | 7AL | 23.8–25.4 | *AX-109983514* | *AX-109820548* | 8.66 | 15.54 | 1.06 |
| | 2021CZ | 7AL | 23.8–25.4 | *AX-109983514* | *AX-109820548* | 8.27 | 15.72 | 0.85 |
| | 2021YA | 7AL | 23.8–25.4 | *AX-109983514* | *AX-109820548* | 5.65 | 11.93 | 1.02 |
| | 2022WJ | 7AL | 23.8–25.4 | *AX-109983514* | *AX-109820548* | 6.23 | 12.31 | 0.89 |
| | 2022CZ | 7AL | 23.8–25.4 | *AX-109983514* | *AX-109820548* | 4.93 | 8.32 | 0.89 |
| | 2022YA | 7AL | 23.8–25.4 | *AX-109983514* | *AX-109820548* | 3.66 | 6.86 | 0.75 |
| | BLUP | 7AL | 23.8–25.4 | *AX-109983514* | *AX-109820548* | 7.47 | 14.12 | 0.85 |

*LOD* logarithm of odds, *PVE* phenotype variance explained, *Add* additive effect of a QTL, *WJ* Wenjiang, *CZ* Chongzhou, *YA* Ya'an, *BLUP* best linear unbiased prediction

interval of 440–560 Mbp, indicating that the putative locus for SNS is likely located in this interval (Fig. 2b).

## Linkage map construction and QTL mapping

KASP markers were developed based on polymorphic SNPs on chromosome 7A. Then, we obtained 10 pairs of markers that were polymorphic between two parents and used these to genotype the SSY population. Finally, a genetic map with a length of 26.1 cM was constructed by combining the genotyping results of different markers (Fig. 2c). *QSNS.sicau-SSY-7A* was mapped between *AX-109983514* and *AX-109820548* with an interval of 1.6 cM in six environments and the BLUP dataset (Table 4). This locus explained 6.86–15.72% of the phenotypic variation. The lines carrying positive alleles from S849-8 showed significantly increased SNS ($p < 0.01$, by 6.54–9.31%) in six environments and the BLUP dataset (Fig. 2c). *QSNS.sicau-SSY-7A* had significant effects ($p < 0.05$) on KL, TKW and SL, while it was not related to PH, AD or PTN (Fig. S2). *QSNS.sicau-SSY-7A* could be detected in multiple environments by using QE interaction effects, further indicating that it is a major and stably expressed locus (Table S4).

## Validation of the major QTL for SNS

To verify the effect of *QSNS.sicau-SSY-7A*, a KASP marker (*KASP-AX-109983514*) tightly linked to this locus was used for genotyping the SCN and S83 populations (Fig. 3). Each population was divided into two groups according to the genotyping results (excluding heterozygotes). As expected, groups with positive alleles at *QSNS.sicau-SSY-7A* showed significantly higher SNS (by 4.64% and 6.26% in SCN and S83 populations, respectively) than those in groups with negative alleles (Fig. 3).

## Effects of *QSNS.sicau-SSY-7A* and *WAPO1* on SNS and other yield-related traits in the SSY population

Previous studies have identified *WAPO1* as a candidate gene for increasing SNS on chromosome 7AL (Kuzay et al. 2019). We genotyped the SSY population using functional markers of *WAPO1* (Ding et al. 2022b) and reconstructed the map according to the genotyping results. *WAPO1* was far from *QSNS.sicau-SSY-7A* (Fig. S2). *QSNS.sicau-SSY-7A* was still mapped between *AX-109983514* and *AX-109820548* based on the reconstructed map (Table S5). These results indicate that *QSNS.sicau-SSY-7A* and *WAPO1* are not likely allelic. The effects of *QSNS.sicau-SSY-7A* and *WAPO1* on SNS were further analyzed. Compared with SNS in lines without positive alleles for SNS, those with the combination of *QSNS.sicau-SSY-7A* and *WAPO1* showed significantly higher SNS by up to 10.55%, and those with *QSNS.sicau-SSY-7A* or *WAPO1* showed significantly higher SNS, by 6.52% and 6.66%, respectively. Lines with the combination of *QSNS.sicau-SSY-7A* and *WAPO1* exhibited significantly higher SNS than those of lines with *QSNS.sicau-SSY-7A* (4.31%) or *WAPO1* (4.17%). In addition, there was no significant difference between the two lines with only *QSNS.sicau-SSY-7A* or *WAPO1* (Fig. 4a).

The effects of *QSNS.sicau-SSY-7A* and *WAPO1* on other agronomic traits were further analyzed (Figs. 4b, S3). When excluding the effect of *WAPO1*, lines with *QSNS.sicau-SSY-7A* significantly increased TKW, KL and SL by up to 4.15%, 1.87% and 4.35%, respectively, compared with values in lines without *QSNS.sicau-SSY-7A* or *WAPO1* (Fig. 4b, Fig. S3). The lines possessing the positive allele at *QSNS.sicau-SSY-7A* showed significantly higher TKW, KL and SL values (by 5.27%, 2.56% and 6.77%, respectively), than those in lines possessing the positive allele at *WAPO1* (Figs. 4b, S3). *WAPO1* had no significant effect on TKW, KL or

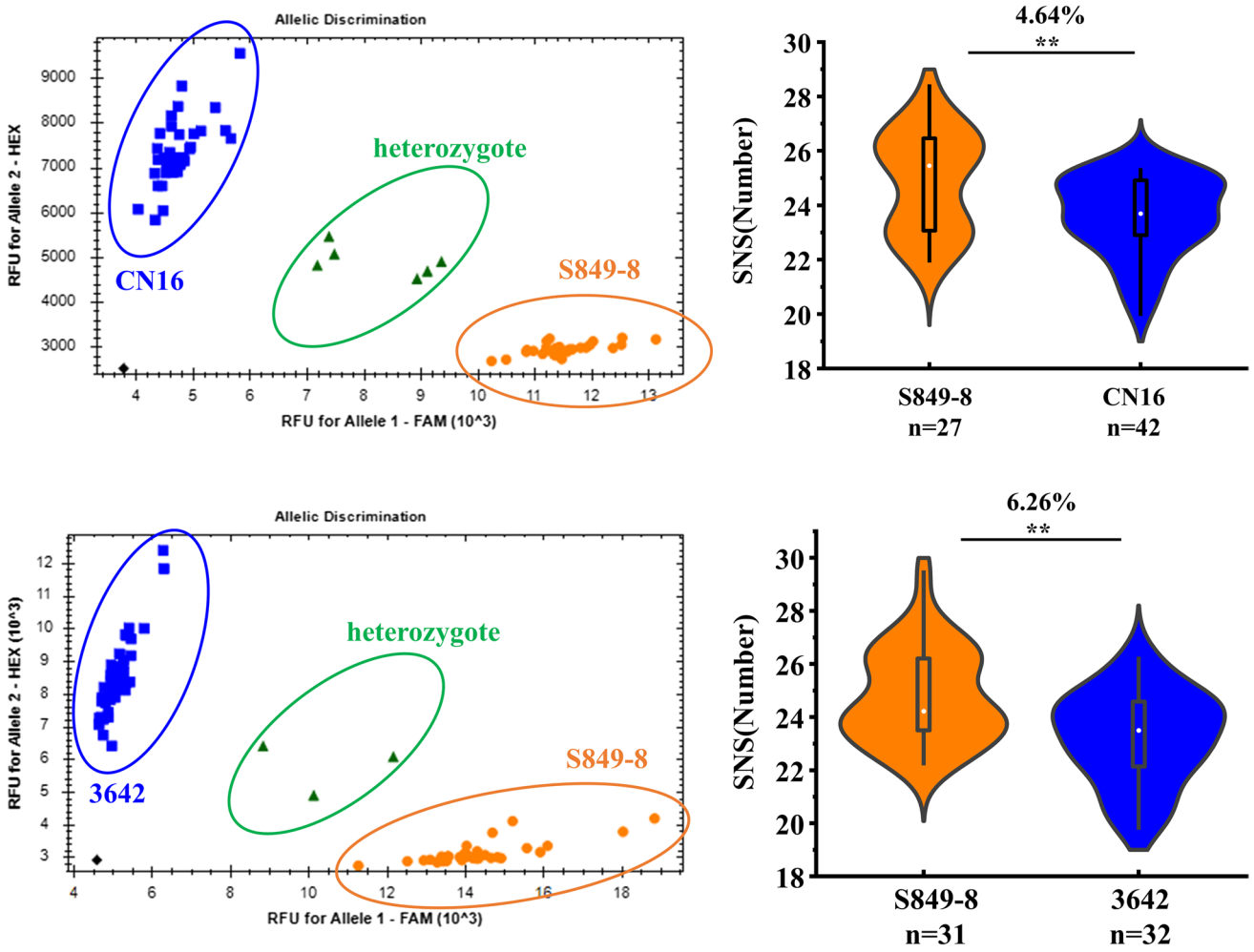


**Fig. 3** Validation of major QTL in different genetic backgrounds. Effects of *QSNS.sicau-SSY-7A* in SCN (S849-8×CN16) and S83(S849-8×3642) populations. 'Blue' and 'red' represent haplotype group with and without the positive allele of the corresponding QTL based on the genotype of flanking markers, respectively. **Significance at the 0.01 probability level; *significance at the 0.05 probability level

SL; however, it had a significant negative effect ($p < 0.05$, −4.76%) on PTN (Figs. 4b, S3).

We previously reported a major QTL for TKW *QTKW.sicau-SSY-2D* in the SSY population that was not correlated with SNS (Qu et al. 2022). The effects of *QSNS.sicau-SSY-7A* and *WAPO1* on TKW were further evaluated using QTL pyramiding in the SSY population after excluding the effect of *QTKW.sicau-SSY-2D*. Our results showed that the lines carrying the positive allele at *QSNS.sicau-SSY-7A* only significantly increased TKW by 5.53% and 7.78% over values for lines without any positive alleles and lines possessing *WAPO1* only. No significant difference was detected between those with *WAPO1* only and lines without any positive allele (Fig. 4c). Additionally, pyramiding showed that the TKW of lines carrying positive alleles at both *QSNS.sicau-SSY-7A* and *QTKW.sicau-SSY-2D* was significantly higher ($p < 0.05$, 5.36%) than that of lines containing both *WAPO1* and the positive allele at *QTKW.sicau-SSY-2D* (Fig. 4d).

## Discussion

### *QSNS.sicau-SSY-7A* is a novel and stable QTL

Wheat chromosome 7A likely contributes to the development of SNS, since it includes numerous loci associated with this trait (Xu et al. 2014; Zhai et al. 2016; Kuzay et al. 2019; Muqaddasi et al. 2019) (Table S7). *QTsn.cau-7A.1* (670.80–675.30 Mb) and *QTsn.cau-7A.2* (675.50–683.50 Mb) identified on chromosome arm 7AL are associated with SNS and AD (Chen et al. 2022c). *QSns.sau-QZ-7A* was located between *wPt-5949*

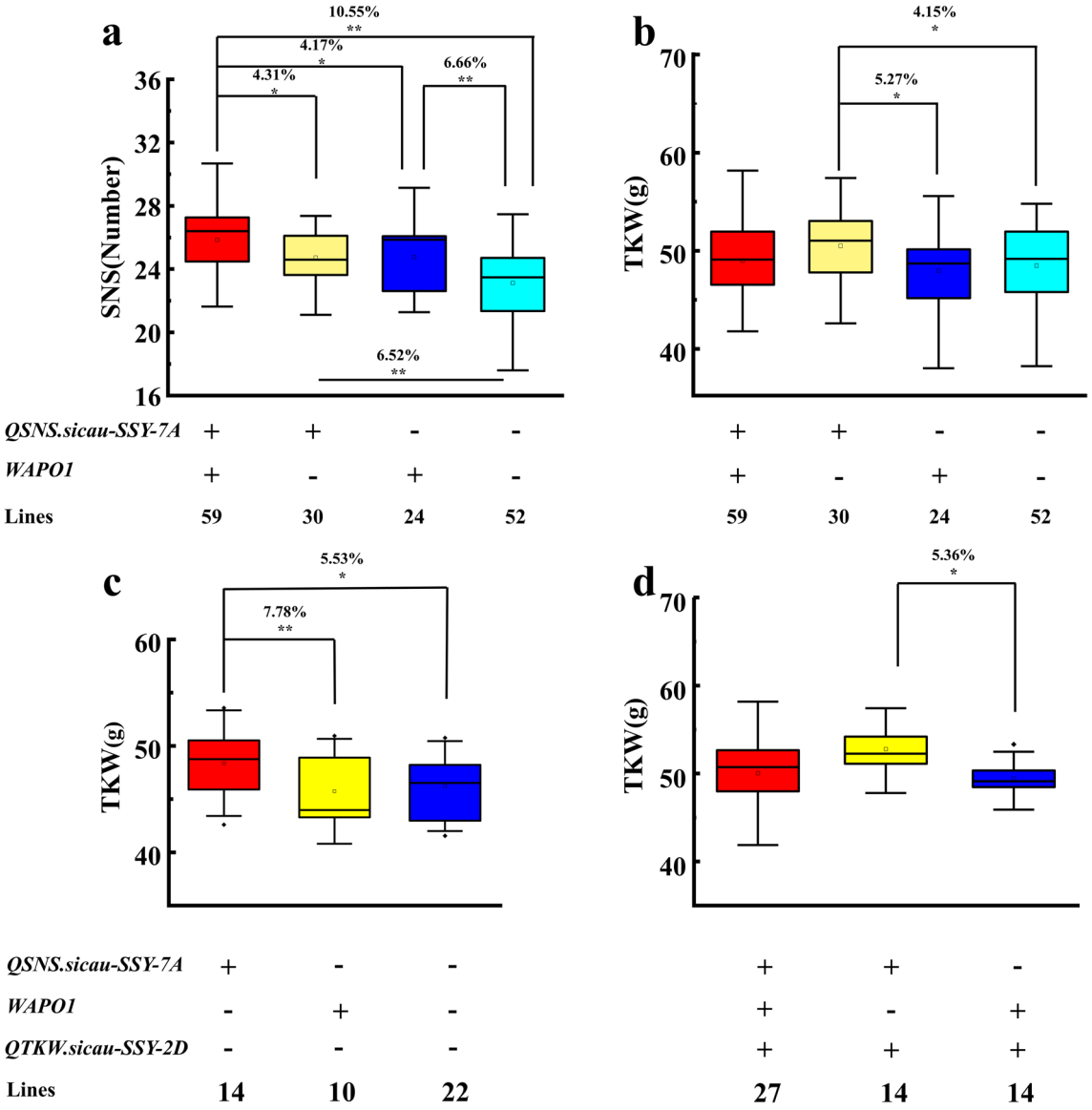


**Fig. 4** Pyramid analysis of *QSNS.sicau-SSY-7A*, *WAPO1* and *QTKW.sicau-SSY-2D*. Pyramid analysis of the effects of *QSNS.sicau-SSY-7A* and *WAPO1* on SNS (**a**) and TKW (**b**). (**c**) Genetic effects *QSNS.sicau-SSY-7A* and *WAPO1* on TKW. (**d**) Effects of *QSNS.sicau-SSY-7A* and *WAPO1* on TKW were analyzed when they were pyramided with *QTKW.sicau-SSY-2D*, respectively. '+' and '−' represent haplotype group with and without the positive allele of the corresponding QTL or gene based on the genotype of flanking markers, respectively

and *wPt-0961* at 669.62–700.42 Mb on chromosome 7A (Luo et al. 2016). *QSns.sau-2SY-7A* was mapped on a 4.75 cM interval and physically located between 673.87 and 677.70 Mb on chromosome arm 7AL, and *WAOP1* was deduced as a candidate gene in the region (Ding et al. 2022a). To further determine the relationship between QTLs identified in previous studies and the QTL detected in this study, we compared their physical intervals. We further verified that *QSNS.sicau-SSY-7A* and *WAPO1* were not allelic based on a reconstructed genetic map (Fig. S2, Table S7). Our results demonstrate that *QSNS.sicau-SSY-7A* may be a novel, major and stable QTL for SNS.

## Comparison of the effects of *QSNS.sicau-SSY-7A* and *WAPO1*

In this study, *QSNS.sicau-SSY-7A* was not allelic to *WAPO1*. QTL pyramiding further suggested that these loci simultaneously regulate SNS in the SSY population. Given the complex interactions between genes, pyramiding of multiple genes does not usually show a simple additive effect (Pakeerathan et al. 2019). The lines with a combination of the positive allele at *QSNS.sicau-SSY-7A* and *WAPO1* showed significantly SNS values, that is, the lines with a positive allele at either locus alone (Fig. 4). There may be

a complex genetic relationship between *QSNS.sicau-SSY-7A* and *WAPO1*, and fine mapping and map-based cloning are needed to further reveal the mechanism underlying their interaction.

Both *QSNS.sicau-SSY-7A* and *WAPO1* can increase SNS significantly. However, *QSNS.sicau-SSY-7A* exhibited better effects on other yield-related traits than those of *WAPO1*. *QSNS.sicau-SSY-7A* increased TKW, KL and SL significantly (Figs. 4, S4), while *WAPO1* had no effect on TKW and a significant negative effect on PTN (Fig. S4). Therefore, *QSNS.sicau-SSY-7A* may have greater breeding potential for improving yield.

QTLs are usually identified using linkage map analyses of genetic populations, while BSA is an elegant way to identify DNA markers closely associated with causal genes of target phenotypes (Takagi et al. 2013). The sample size and accuracy of phenotypes of an extreme mixing pool are critical to the outcome of BSA. Usually, only a few major and stably expressed QTLs for a given trait can be detected considering the limited resolution of the BSA-seq strategy (Wang et al. 2019; Zhang et al. 2021). In this study, it is possible that the lines constituting the two extreme mixing pools did not contain the homozygous allele of *WAPO1*, limiting the identification of *WAPO1* in the mapping population. Further, our results demonstrated that *QSNS.sicau-SSY-7A* and *WAPO1* are not allelic genes.

## Contributions of both *QSNS.sicau-SSY-7A* and *QTKW.sicau-SSY-2D* to yield-related traits of the wheat line S849-8

Previous studies have shown that the pyramiding of multiple excellent QTLs is an effective method to improve target traits (Fan et al. 2015; Chen et al. 2022b; Ren et al. 2022). In this study, *QTKW.sicau-SSY-2D* for TKW (independent of SNS) was used for pyramiding with *QSNS.sicau-SSY-7A* and *WAPO1* for SNS. The TKW of lines possessing the combination of positive alleles at *QSNS.sicau-SSY-7A* and *QTKW.sicau-SSY-2D* was significantly higher than those of lines with the combination of positive alleles at *WAPO1* and *QTKW.sicau-SSY-2D* (Fig. 4d). These results indicate that *QSNS.sicau-SSY-7A* and *QTKW.sicau-SSY-2D* pyramiding can increase SNS and TKW simultaneously without masking the effect of either locus. Therefore, these two favorable QTLs could be aggregated to improve yield in wheat breeding.

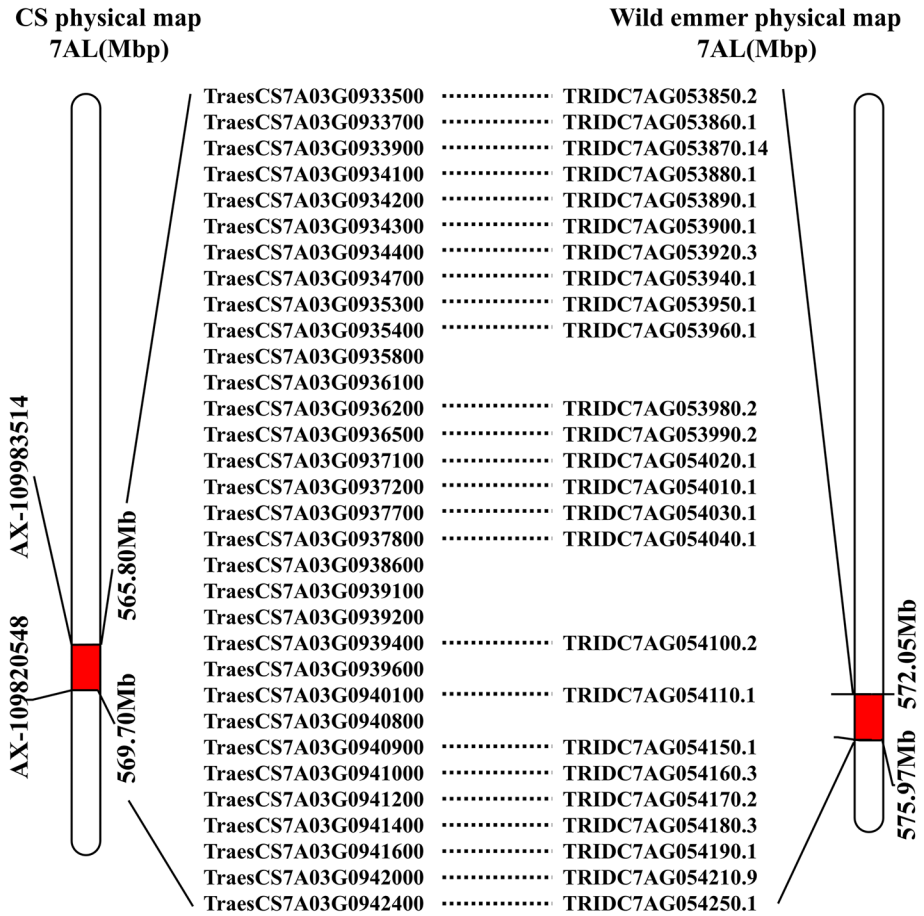


**Fig. 5** Physical interval of *QSNS.sicau-SSY-7A* and the predicted genes. Dotted line indicates the corresponding orthologs

## Great breeding potential of *QSNS.sicau-SSY-7A*

The effects of *QSNS.sicau-SSY-7A* on other yield-related traits were further analyzed. A significant correlation between SNS and SL was detected (Table 3). The lines with alternative alleles at *QSNS.sicau-SSY-7A* showed a significant difference in SL (Fig. S3). There is likely a single QTL with pleiotropic effects or a genomic region containing a set of linked genes associated with these traits (Cui et al. 2012; Kuzay et al. 2019). A locus controlling SL was not detected on chromosome 7A based on the map constructed in this study. Thus, *QSNS.sicau-SSY-7A* may have pleiotropic effects, increasing both SNS and SL simultaneously. Interestingly, lines carrying the positive allele at *QSNS.sicau-SSY-7A* showed a longer KL and higher TKW, inconsistent with observations for previously SNS QTL, which were negatively correlated with TKW (Ma et al. 2019; Ding et al. 2022a). Further analysis is needed to clarify the relationship between SNS and TKW. *QSNS.sicau-SSY-7A* showed no correlation with PH, AD or PTN (Fig. S3). Taken together, these results indicate that *QSNS.sicau-SSY-7A* may have great potential to increase wheat yield.

## Predicted genes in the interval of *QSNS.sicau-SSY-7A*

*QSNS.sicau-SSY-7A* was mapped to 565.80–569.70Mbp on 7AL of CS and 572.05–575.97 Mbp on 7AL of wild emmer. There were 32 and 28 predicted genes in this interval of CS and wild emmer, respectively, including 25 overlapping genes (Fig. 5, Table S6). Based on functional annotation and spatial–temporal expression analyses, three genes (*TraesCS7A03G0934300*, *TraesCS7A03G0939600* and *TraesCS7A03G0933900*) were highly expressed in spikes (Fig. S5), which are closely related to plant growth and development. For instance, *TraesCS7A03G0934300* encodes a replication factor C subunit (RFC), a component of the core DNA replication machinery closely associated with the cell cycle (Shultz et al. 2007). The expression levels of many cell cycle-related genes were reduced in rice mutants with reduced grain size, suggesting that these genes influence plant development by altering the cell cycle (Zhou and Xue 2020). *TraesCS7A03G0939600* encodes a Chito oligosaccharide deacetylase. Previous studies have shown that chito-oligosaccharides can improve seed germination, plant growth and development, and photosynthesis (Liu et al. 2023). *TraesCS7A03G0933900* encodes phosphatase 2C family proteins, which affect plant growth and development and stress by via the ABA signaling pathway (Jiang et al. 2022; Yu et al. 2022). In conclusion, these genes involved in plant growth and development may provide a basis for fine mapping and the identification of candidate genes QTL in future work.

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**Author contribution statement** CHZ finished the study and wrote this manuscript. JGZ participated in field work and analyzed data. CL, JNY and YLL helped with the phenotype measurement and data analysis. HPT, MD, QX, YZZ and QTJ did field work and data analysis. GYC, PFQ, YFJ and JRW collected and analyzed data. WL, ZEP, GDC and YJ helped with data analysis. ZZ, CJL and YLZ revised the manuscript. YMW discussed results and revised the manuscript. JM designed the experiments, guided the entire study, participated in data analysis, wrote and extensively revised this manuscript. All authors participated in the research and approved the final manuscript.

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**Data availability** All data generated or analyzed during this study are included in this published article and its supplementary information files or from the corresponding authors upon reasonable request.

## Declarations

**Conflict of interest** The authors have declared that no competing interests exist.

**Ethical approval** All experiments and data analyses were conducted in Sichuan. All authors contributed to the study and approved the final version for submission. The manuscript has not been submitted to any other journal.

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