



# A breeding strategy targeting the secondary gene pool of bread wheat: introgression from a synthetic hexaploid wheat

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## Abstract

**Key message** Introgressing one-eighth of synthetic hexaploid wheat genome through a double top-cross plus a two-phase selection is an effective strategy to develop high-yielding wheat varieties.

**Abstract** The continued expansion of the world population and the likely onset of climate change combine to form a major crop breeding challenge. Genetic advances in most crop species to date have largely relied on recombination and reassortment within a relatively narrow gene pool. Here, we demonstrate an efficient wheat breeding strategy for improving yield potentials by introgression of multiple genomic regions of de novo synthesized wheat. The method relies on an initial double top-cross (DTC), in which one parent is synthetic hexaploid wheat (SHW), followed by a two-phase selection procedure. A genotypic analysis of three varieties (Shumai 580, Shumai 969 and Shumai 830) released from this program showed that each harbors a unique set of genomic regions inherited from the SHW parent. The first two varieties were generated from very small populations, whereas the third used a more conventional scale of selection since one of bread wheat parents was a pre-breeding material. The three varieties had remarkably enhanced yield potential compared to those developed by conventional breeding. A widely accepted consensus among crop breeders holds that introducing unadapted germplasm, such as landraces, as parents into a breeding program is a risky proposition, since the size of the breeding population required to overcome linkage drag becomes too daunting. However, the success of the proposed DTC strategy has demonstrated that novel variation harbored by SHWs can be accessed in a straightforward, effective manner. The strategy is in principle generalizable to any allopolyploid crop species where the identity of the progenitor species is known.

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Ming Hao and Lianquan Zhang have contributed equally to this work.

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## Introduction

Bread wheat (*Triticum aestivum*) arose from an allopoloidization event that occurred around 10,000 years ago and involved the tetraploid species *T. turgidum* and the diploid species *Aegilops tauschii* (Kihara 1944; McFadden and Sears 1944). Since that time, the widespread dispersal from the site of origin in the Fertile Crescent, in conjunction with intensive selection, first by early farmers and later through deliberate breeding, resulted in adaptation to a wider range of environments than occupied by any other crop species (Dubcovsky and Dvorak 2007). While breeding over the past century has delivered a notable increase in yield potential, a yield plateau now seems to have been reached, leading to concerns that the expanding global demand for wheat will not be easily met without breeding innovations (Hawkesford et al. 2013). The consensus is that it will be necessary to

expand the germplasm base to meet this challenge (Tester and Langridge 2010; Moore 2015).

It has been recognized for some time that use of the bread wheat secondary gene pool, represented by the progenitor species, has the potential to introduce a substantial amount of novel genetic variation for exploitation by wheat breeders. Given that the species is derived from a relatively small number of founder amphiploids, recreating these hybrids by crossing *T. turgidum* with *Ae. tauschii* to produce so-called synthetic hexaploid wheats (SHWs) should offer a major opportunity to widen the genetic base of the crop (McFadden and Sears 1944; Mujeeb-Kazi et al. 1996). Efforts in this direction have confirmed that use of SHWs as breeding parents can enhance yields across a diverse range of environments (Hoisington et al. 1999; Coghlan 2006; Warburton et al. 2006; Dreisigacker et al. 2008; Trethowan and Mujeeb-Kazi 2008). The major disadvantage of SHWs is that they inevitably harbor genes that negatively impact key domestication traits (Zhang et al. 2013). As a result, the contribution of SHWs to elite commercial varieties remains relatively minor (Yang et al. 2009; Ogonnaya et al. 2013; Börner et al. 2015). Here, we present a simple, reproducible breeding method that is designed to enhance yield based on the idea of selection of genomic segments inherited from an SHW parent.

## Materials and methods

### Plant materials

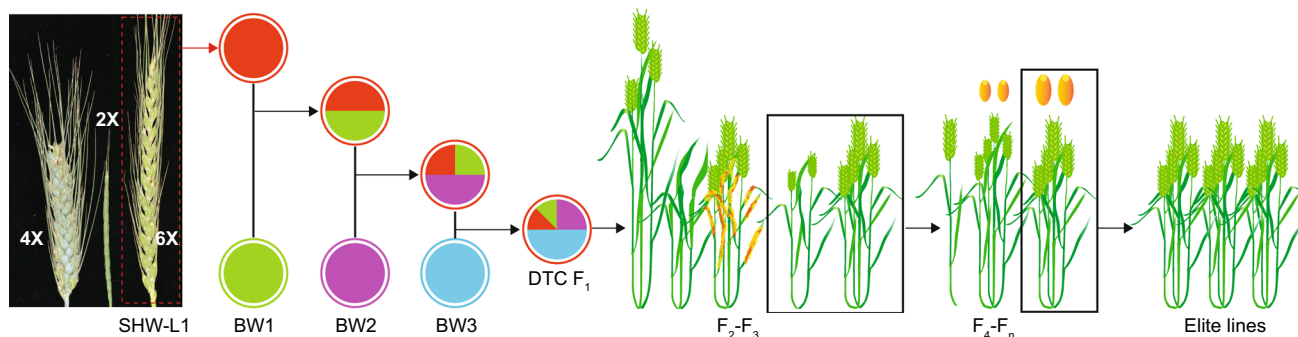
SHW-L1 was bred from a chromosome-doubled amphiploid between Chinese *T. turgidum* ssp. *turgidum* landrace AS2255 as female parent and Iranian *Ae. tauschii* ssp. *tauschii* accession AS60 as the male parent (Zhang et al. 2004). AS60 is quite distinct from bread wheat D genome, as

shown by previous phylogenetic (Wang et al. 2013) and gene expression analyses (Hao et al. 2017; Ramírez-González et al. 2018). An  $F_7$  generation recombinant inbred line (RIL) population from the cross SHW-L1  $\times$  Chuanmai 32 was previously described by Yu et al. (2014).

### The double top-cross and two-phase selection strategy

Double top-cross (DTC) was used to establish  $F_1$  populations that retain only theoretical 12.5% of the nucleus genome of the synthetic wheat SHW-L1 (Fig. 1). Hybrid seeds for each of the first top-cross combinations were planted in one 2 m row, and one to three hybrid plants resistant to stripe rust were randomly chosen for the second top-cross. The bulked hybrid seeds formed DTC  $F_1$  populations; those grown in seasonal nurseries were selected for stripe rust resistance but there was no selection in off-season nurseries. DTC  $F_2$  bulks were harvested from all retained  $F_1$ s.

Two-phase selection (2PS) was used to reduce population size. The first phase was used in  $F_2$ – $F_3$  generations to eliminate serious defects of synthetic wheat, while the second phase was used in  $F_4$ – $F_n$  to improve yields (Fig. 1). The full selection process is outlined in Table S1. All  $F_2$  plants were planted in a seasonal nursery, with selection aimed at eliminating four SHW-L1 traits: tough glumes, late maturity, tall stature and stripe rust susceptibility. 100–200  $F_2$  individuals were typically screened, with larger populations for crosses involving less established varieties.  $F_3$  bulks were planted in either seasonal or off-season nurseries, with selection repeated against the same SHW-L1 traits in seasonal nurseries. In addition, plants with low fertility, non-uniform tiller length and short upper internodes were discarded, and all retained plants bulked. From  $F_4$  onward, planting was restricted to seasonal nurseries. Individual plant selections



**Fig. 1** Double top-cross (DTC) strategy used to introgress genes from the SHW selection SHW-L1. The expected outcome of the crosses (DTC  $F_1$ ) is a genetic background in which 87.5% of the sequence derives from three bread wheat parents indicated by BW1–BW3. Selection in the early generations ( $F_2$ – $F_3$ ) focused on short stature,

early maturity, resistance to stripe rust and soft glumes. In the later generations ( $F_4$ – $F_n$ ), selection was imposed on yield and its components. Elite selections were used in yield tests. The spikes were taken from SHW-L1 (6x) and its *T. turgidum* (4x) and *Ae. tauschii* (2x) parents

focused on yield components including spikes per plant, grains per spike and 1000 grain weight (TGW).

Seasonal plantings were sown at the Dujiangyan Experimental Station (31°01'N, 103°32'W) and at the Wenjiang Experimental Station (30°36'N, 103°41'W) in 2 m rows containing 20 plants (Table S1), with each row separated from its neighbor by 30 cm. The stripe rust susceptible variety SY95-71 was planted either side of each experimental row and inoculated with a mixture of locally predominant races. Off-season (summer) plantings of the F<sub>1</sub> and F<sub>3</sub> materials were conducted at Maerkang (31°92'N, 102°13'W, ~2600 m a.s.l): There, ~50 plants were sown in each 2.0 m row, with an inter-row separation of 30 cm.

### Yield testing

Elite selections were tested at Wenjiang Experimental Station, with management according to local methods. A small number of lines were entered into official uniformity variety trials, including regional variety tests (RVTs) run over two cropping seasons and province productivity trials (PTs) over one cropping season. The PT trial required a plot size ten times the size of the RVT trial and was intended to replicate typical farmer conditions. The official experiments involving Shumai 580 were conducted in Yunnan province in 2013–2015, while the experiments of Shumai 969 and Shumai 830 were carried out in Sichuan province in 2010–2013 and 2014–2017, respectively.

### Fluorescence in situ hybridization (FISH) karyotyping

FISH was used to identify gross chromosomal variants and to investigate the transmission of certain specific chromosomal segments. The FISH methodology used followed Komuro et al. (2013) and Zhao et al. (2018), utilizing as probes FAM- or TAMRA-labeled (CTT)<sub>10</sub>, pSc119.2, pTa-535, pTa71 and pTa-713, all synthesized by TSINGKE Biological Technology Company (Chengdu, Sichuan, China).

### Single-nucleotide polymorphism (SNP) genotyping

Genomic DNA was isolated from seedling leaves using a Plant Genomic DNA Kit (TIANGEN Biotech (Beijing) Co., Ltd.). Chip-based genotyping was performed by CapitalBio Corporation ([www.capitalbio.com](http://www.capitalbio.com)) using a wheat 55 K SNP array, following the Affymetrix Axiom 2.0 Assay Manual Workflow protocol. The flanking sequences for each SNP allowed the location of most of the assays to be mapped onto the bread wheat reference sequence ([https://urgi.versailles.inra.fr/download/iwgsc/IWGSC\\_RefSeq\\_Assemblies/v1.0/](https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Assemblies/v1.0/)), by imposing a BLASTN E-value threshold of 10<sup>-10</sup> and allowing a maximum mismatch of one base. The SNP

genotyping was used in phylogenetic analysis following Suzuki and Shimodaira (2006). The distribution of missing markers on each chromosome was analyzed. The ratios of missing markers within neighboring 10 Mb intervals along individual chromosomes were calculated.

### Analysis of introgressed segments

SNP markers that were heterozygous in either the parents or the final varieties were removed, and the remaining markers used to calculate parental contributions to the three varieties and to characterize SHW-L1 introgressions. Introgressions were estimated by computing the ratios of same SNP to the total SNPs scored between a variety and its four parents using a sliding window of 10 Mb and step length of 1 Mb and considered to run from the first window to the start of the last consecutive window that had the highest ratio of SHW-L1 SNPs. Graphical representations were constructed using the R package ggplot2 (v.2.2.1) (Wickham 2016). The gene sequences of known major genes determining yield-related traits (Nadolska-Orczyk et al. 2017) and aspects of flour quality were downloaded from GenBank and located on the whole-genome reference sequence. A subset was PCR-amplified from genomic DNA and amplicons sequenced by the TSINGKE Biological Technology Company; primer information is in Table S2. SDS-PAGE was used to identify the composition of high molecular weight glutenin subunits (HWM-GSs), as described by Wan et al. (2005).

### Quantitative trait loci (QTL) analysis

The RIL population created from the cross SHW-L1 × Chuanmai 32 was used in a QTL analysis. The relevant phenotypic data were gathered from experiments carried out over the period 2008–2011 (Yu et al. 2014) and were supplemented by fresh data collected in the 2014–2017 at Wenjiang and 2017 at Beijing. QTL were identified using QTL IciMapping v4.1 software (Li et al. 2007) ([www.isbreeding.net](http://www.isbreeding.net)). Threshold values were calculated using 1000 permutations, assuming a 0.05 type I error. QTL lying within a 50 Mb region defined by flanking markers were used to estimate possible QTL introgressions in the new varieties. A introgression was considered if the QTL region overlapped with an introgressed SHW-L1 region detected by sliding window analysis.

### Statistical analysis

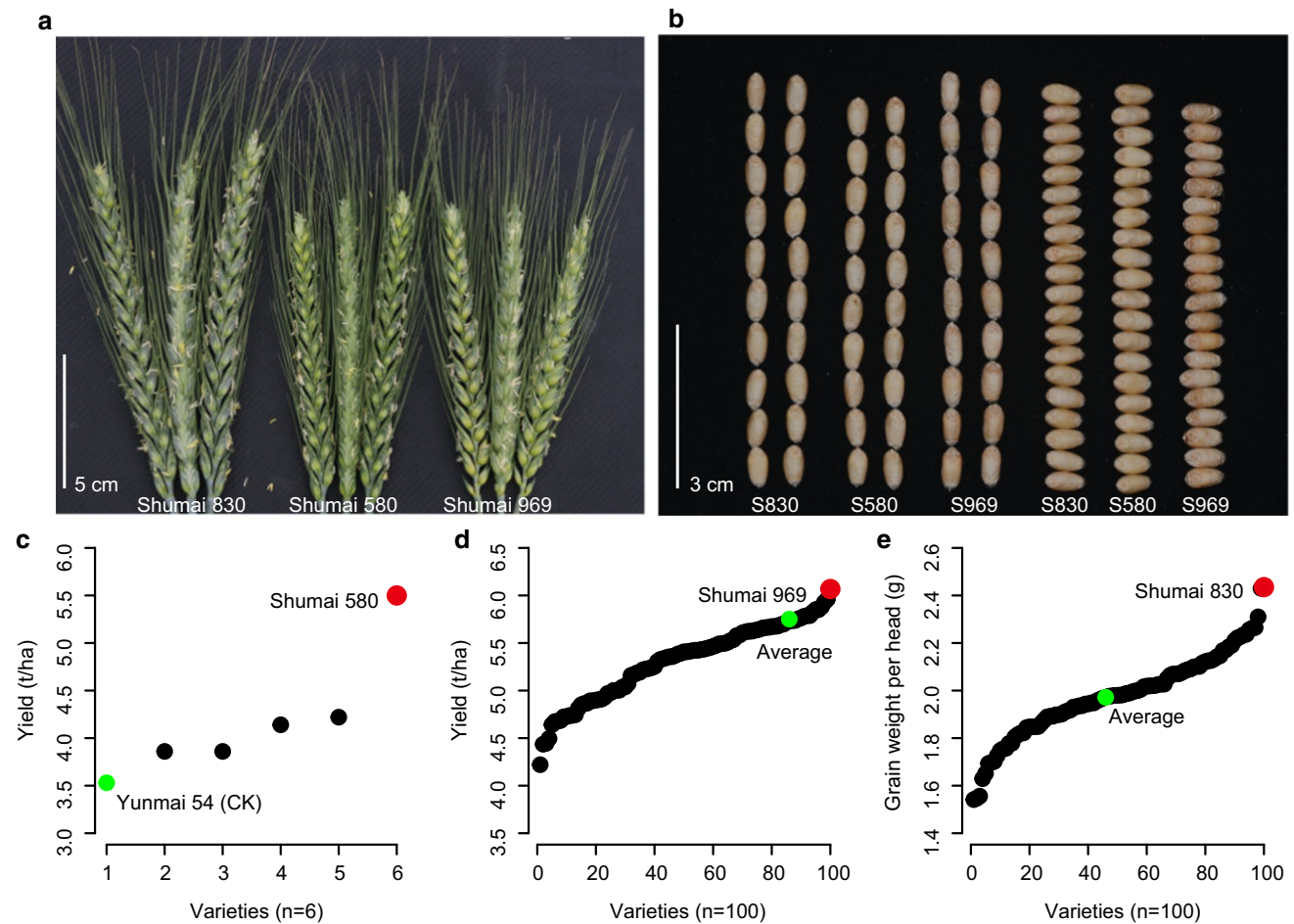
All statistical analyses were performed using programs implemented in either R (v3.4.4) software or in Microsoft Excel 2007.

## Results

### Yield performance of the new varieties

A total of 67 DTC  $F_1$  combinations, involving 51 bread wheat lines (ten in 2003–2004 and 57 in 2005–2006), were made. Three commercial varieties have been released from three combinations (Table S1): These have been named Shumai 580, Shumai 969 and Shumai 830. The first two of these were generated from relatively small populations (respectively, ca. 650 and 1150  $F_1$ – $F_4$  plants), whereas the third required a more conventional scale of selection (ca. 6360 plants). The three varieties differed with respect to spike size and their grain characteristics (Fig. 2a, b) and were superior in yield to other leading genotypes (Fig. 2c–e).

Yield superiority of Shumai 580 was particularly clear in the Yunnan province productivity trial (PT), where it outperformed five locally bred varieties by at least 30% and the commercial check cultivar Yunmai 54 by 56% (Fig. 2c). Shumai 969 was the only variety yielding over 6 t/ha in Sichuan province productivity trials conducted during 2007–2017 (Fig. 2d). The high yield potential of Shumai 830 was mainly attributed to high grain weight. The Sichuan regional variety test (RVT) data collected for 100 varieties released over the past decade showed that Shumai 830 spikes have been consistently heavier than those of other varieties (Fig. 2e). The high yield potential of the other two varieties also relates to improvements in TGW (Table S3). Shumai 969 and Shumai 830 produced grain with a relatively high TGW, even though the anthesis to maturity period was relatively short, indicating rapid grain filling.



**Fig. 2** Spikes and grain of the three Shumai varieties and their yield potential compared to current elite genotypes. **a** Shumai 830 formed long spikes. **b** Shumai 830 set large grains. **c** Shumai 580 outperformed the yield of five check varieties by at least 30% in a Yunnan province productivity trial conducted in 2015–2016, according to the official trial data provided by the Yunnan Seed Administration Sta-

tion. **d** Shumai 969 out-yielded 99 current varieties in the Sichuan provincial productivity trials conducted over the period 2007–2017, according to the official trial data provided by the Sichuan Seed Administration Station. **e** Shumai 830 produced a larger grain mass per spike compared to 99 current varieties in Sichuan provincial regional variety trials conducted over the period 2007–2017

## The genotypic composition of the three Shumai varieties

FISH karyotyping results (Fig. S1) indicate that the 1BL/1RS wheat/rye translocation present in some of the parents was not transmitted to these varieties, but that chromosome segments from SHW-L1 were present in some (2DS) or all (5DS).

In all, 51,160 SNP sequences were mapped onto Chinese Spring reference sequence: 17,879 mapped to A genome sites, 18,203 to B genome sites and 15,078 to D genome sites. Except for chromosome 4D, which was marked by 1087 SNP loci, each chromosome had at least 2000 markers. On the basis of allelic status at the 51,160 SNP sites, it was clear that SHW-L1 is genetically very distant from any of the bread wheat varieties used as parents in the DTC (Fig. S2). This is particularly applied to the D genome, as the Axiom assay returned missing data for a large number of SNP loci in SHW-L1 (Fig. S3).

The number of SHW-L1 alleles present in the three varieties represent 17.2% (Shumai 580), 13.4% (Shumai 830) and 13.8% (Shumai 969) of the scored SNP loci (Table S4). These were unevenly distributed across the wheat genome, with only 69 loci, including a block of 56 on chromosome 5D, present in all three varieties (Fig. 3a, b).

The number of introgressed segments per variety varied from 57 to 83 (Fig. 3b; Table S5), most of which were shorter than 30 Mb in length. Assuming an overall genome size of 14.05 Gb (IWGSC 2018), the overall ratio of SHW-L1 DNA retained by Shumai 969 was 12.4%, and the ratios for Shumai 830 and Shumai 580 were, respectively, 13.5 and 15.0% (Table S5).

## QTL analysis on a SHW-L1/Chuanmai 32 RIL population

We detected 86 QTL influencing 12 yield-related traits from an analysis of a SHW-L1/Chuanmai 32 RIL population (Table S6). The 86 QTL mapped to 19 of the 21 chromosomes (the exceptions were chromosomes 5D and 7D) (Table S7) were particularly frequent on chromosomes 5A (17 loci) and 2D (11 loci). On the basis of a box plot analysis, the SHW-L1 *Vrn-A1* allele on 5A appeared to hasten anthesis, whereas the *Ppd-D1* allele on 2D delayed it (Fig. S4). In addition, the presence of *Vrn-B1* allele in SHW-L1 was also associated with a QTL for early heading (Table S7). One half of the QTL mapped to sites on A genome chromosomes. Interesting, 40 out of 86 favorable yield-related QTL alleles were inherited from SHW-L1.

## Major genes and QTL introgressed from SHW-L1

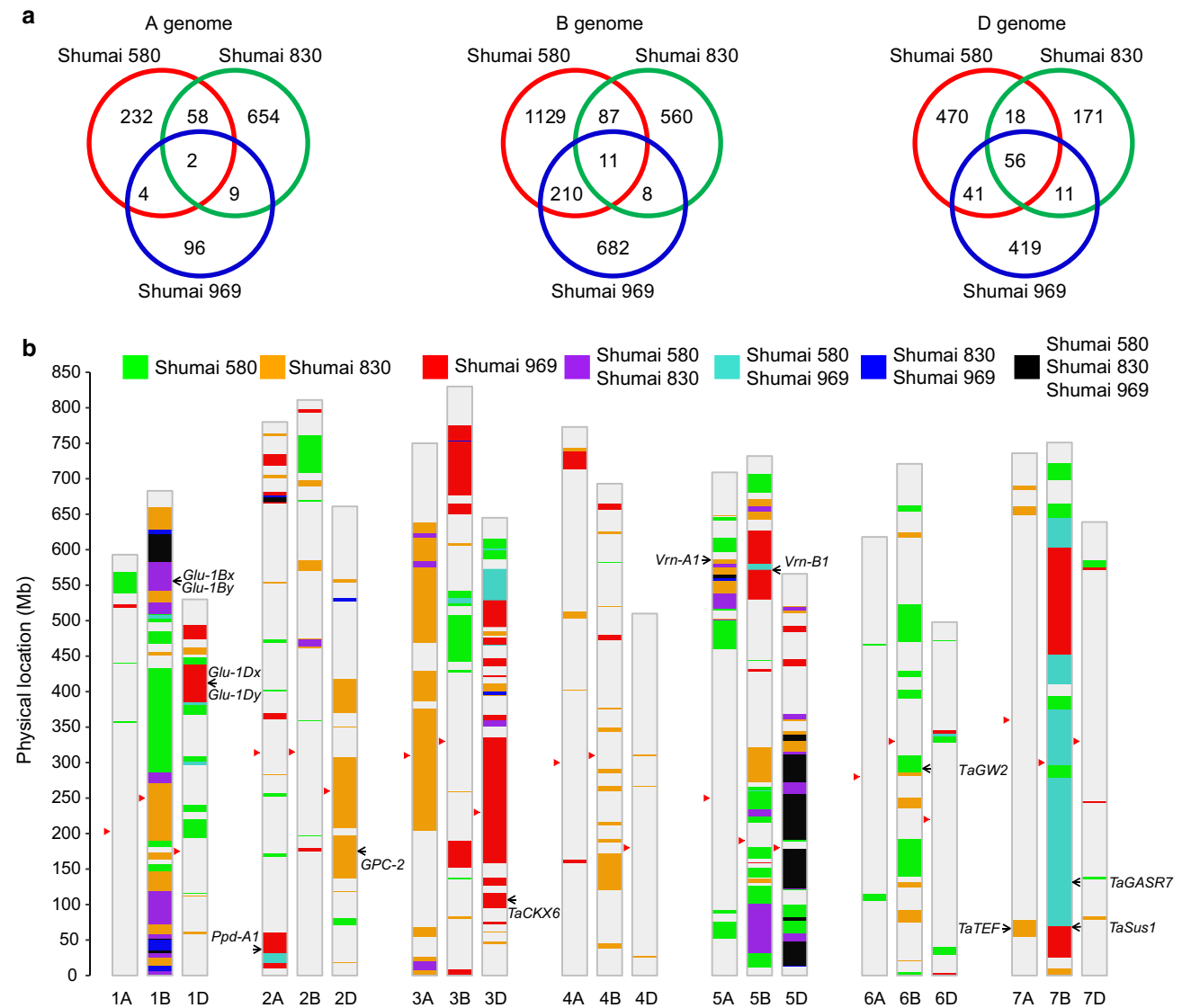
All three Shumai varieties inherited *Ppd-D1*, *Rht-B1* and *Rht-D1* alleles from a bread wheat parent (Table 1). However, Shumai 969 inherited *Ppd-A1* and *Vrn-B1* alleles from SHW-L1, whereas Shumai 830 inherited a *Vrn-A1* allele from the synthetic parent (Fig. 3b). Shumai 830 inherited *TaTEF* and *GPC-2* alleles from SHW-L1, while Shumai 969 inherited *TaGASR7*, *TaCKX6* and *TaSus1* and Shumai 580 inherited *TaGW2* and *TaGASR7* alleles from SHW-L1. In addition, Shumai 830 and Shumai 580 retained the SHW-L1 *Glu-B1* allele and Shumai 969 harbored the SHW-L1 *Glu-D1* allele, which encode endosperm proteins affecting flour quality.

The analysis was then extended to the 86 QTL detected from the SHW-L1/Chuanmai 32 RIL population (Table S7). Of these, 37 were introgressed into the three Shumai varieties. The presence of each of the *Vrn-A1* and *Vrn-B1* alleles in SHW-L1 was associated with a QTL for early heading (Table S7), and its *Glu-B1* allele was associated with a QTL for early heading. There were no favorable QTL associated with any of the other introgressed major genes referred to above (Table 1), probably caused by the interference of SHW-L1 traits such as tough glumes on QTL detection.

The numbers of SHW QTL contributing negatively to traits as predicted from the RIL analysis were nine of 20 in Shumai 580, ten of 20 in Shumai 830 and four of seven in Shumai 969 (Table S7). Three positive SHW-L1 QTL for spike length (on chromosomes 2A and 5A) and one for spikelet number on chromosome 2D were inherited by Shumai 830.

## Discussion

Unadapted germplasm including SHWs have been used as resources of new variations for wheat improvement. A few large-scale pre-breeding programs have been launched for this goal, such as the Wheat Pre-breeding Project (2011), the Wheat Improvement Strategic Programme (Moore 2015) and CIMMYT's Seeds of Discovery (Singh et al. 2018). Typically, SHWs have been crossed and then backcrossed (A/B//B) or top-crossed (A/B//C) to wheat varieties or elite lines to produce breeding populations from which new elite lines, or in a few cases varieties, have been selected (Yang et al. 2009; Ogonnaya et al. 2013). For example, this strategy has been successfully used in China, and four SHW-derived varieties were released in 2003–2005. One of these (Chuanmai 42) has proven to be both a high yielding variety and an outstanding crossing parent, from which 12 commercially released varieties (including Shumai 969) have been bred (Li et al. 2014).



**Fig. 3** SHW-L1 SNP alleles and graphical genotypes of three new Shumai varieties. **a** Number of SHW-L1 SNP alleles presented in Shumai 580 (red circle), Shumai 830 (green circle) and Shumai 969 (blue) according to the A (left), B (middle), D (right) genomes. **b** SHW-L1 introgressed segments in the three varieties. The red

arrowheads indicate the centromeres. The black arrowheads indicate major genes carrying alleles inherited from the SHW-L1 parent. The colored boxes refer to introgressions from the donor SHW-L1 into Shumai varieties, for instance green for Shumai 580 and black for the shared introgression by three varieties (color figure online)

Compared to the top-cross (A/B/C), DTC (A/B/C/D) populations with one more cross have an reduction of synthetic wheat genome from theoretical 25.0 to 12.5%. However, our breeding using a small population size demonstrates that DTC is highly efficient to enhance high yielding potentials. A factor beneficial to the success is that synthetic wheat has a high frequency of favorable alleles across the genome. In our analysis on a RIL population between synthetic wheat SHW-L1 and a variety, although the expectation was that favorable yield-related QTL alleles would be less likely associated with SHW-L1, almost half (40 out of 86) of the loci behaved in this way.

Similar phenomenon was also found in synthetic wheat using the advanced backcross populations (Huang et al. 2003, 2004). Meanwhile, DTC populations are involved in three modern varieties, so that progenies have high possibility to combine different allelic variations between common wheat and synthetic wheat. Each of the released Shumai varieties harbors a unique set of genomic regions inherited from the synthetic wheat parent, highlighting the significance of synergistic recombination of yield-related alleles/traits.

The major contribution made by SHW-L1 to the high yield potential of three Shumai varieties relates to

**Table 1** Major genes assayed in the new varieties

Locus	Chromosome	Position on Chinese spring genome (bp)	Trait	Presence of SHW-L1 allele in variety <sup>a</sup>	Validation <sup>b</sup>
<i>Ppd-A1</i>	2A	36,933,684	Photoperiod response	Shumai 969	Yes
<i>Vrn-A1</i>	5A	587,411,454	Vernalization response	Shumai 830	Yes
<i>Vrn-B1</i>	5B	573,802,883	Vernalization response	Shumai 969	Yes
<i>GPC-2</i>	2D	175,331,200	Grain weight and protein content	Shumai 830	Yes
<i>TaCKX6</i>	3D	107,041,806	Grain size and weight, filling rate	Shumai 969	Yes
<i>TaTEF</i>	7A	662,277	Grain number and length	Shumai 830	Yes
<i>TaGW2</i>	6B	291,761,229	Grain size	Shumai 580	NA
<i>TaSus1</i>	7B	68,344,037	Grain size and weight	Shumai 969	NA
<i>TaGASR7</i>	7B	133,791,961	Grain length	Shumai 580, Shumai 969	NA
<i>Glu-1Bx</i>	1B	555,766,152	High molecular weight glutenin	Shumai 580, Shumai 830	Yes
<i>Glu-1By</i>	1B	555,933,492	High molecular weight glutenin	Shumai 580, Shumai 830	Yes
<i>Glu-1Dx</i>	1D	412,160,789	High molecular weight glutenin	Shumai 969	Liu et al. (2016)
<i>Glu-1Dy</i>	1D	412,217,779	High molecular weight glutenin	Shumai 969	Liu et al. (2016)
<i>Ppd-D1</i>	2D	33,952,048	Photoperiod response	None	Yes
<i>Rht-B1</i>	4B	30,861,268	Plant height	None	Yes
<i>Rht-D1</i>	4D	18,781,062	Plant height	None	NA
<i>Q</i>	5A	650,127,258	Spike morphology	None	Yes
<i>Vrn3</i>	7B	9,702,354	Vernalization response	None	Yes

NA, the gene was not used in analysis

<sup>a</sup>Based on analysis of the introgressed region

<sup>b</sup>Yes, the presence of SHW-L1 allele in variety was confirmed by gene comparison between new varieties and the parents

improvements in TGW. Additionally, some genes directly affecting TGW (Table 1) and phenology (and therefore the genes that underlie it) also have an impact. The most well-documented genes influencing growth duration are the *Vrn* (response to vernalization), *Ppd* (response to day length) and *Eps* (earliness per se, or the developmental rate) genes (Snape et al. 2001; Li et al. 2017). Growth duration genes act pleiotropically on a number of yield-related traits (Snape et al. 1985; Börner et al. 1993). The grain filling duration exhibited by SHW-L1 was relatively rapid (Yu et al. 2015). The three Shumai varieties reached maturity as early as, if not earlier, than conventional varieties, but produced heavier grains (Table S3).

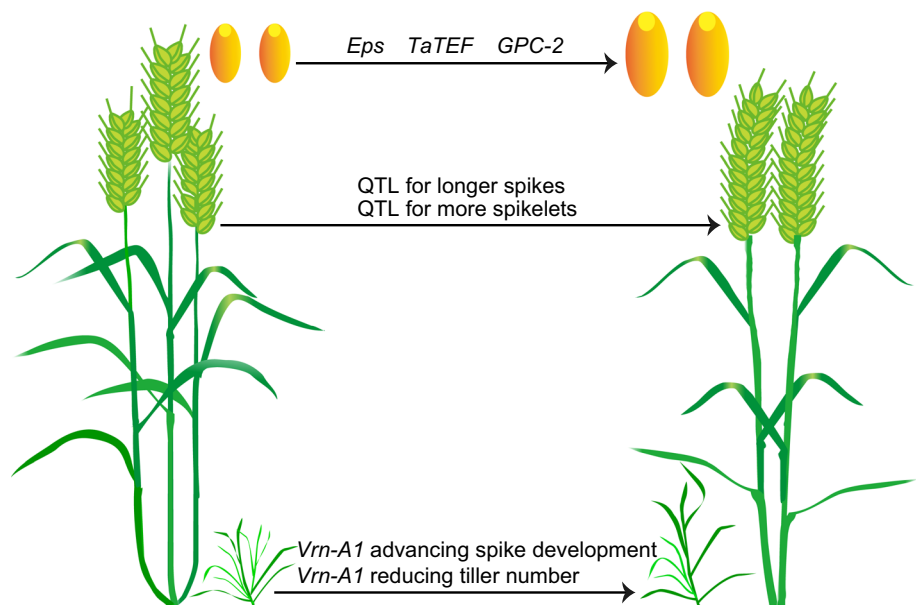
The signature trait of Shumai 830—the ability to form heavy spikes—was achieved by combining high TGW with a large number of grains per spike. This outcome suggests a pathway for producing varieties with heavy spikes, in which plant phenology is coordinated with other key traits (Fig. 4). The presence of the SHW-L1 *Vrn-A1* allele was associated with earlier flowering, the introduction of which probably reduced the requirement for vernalization, thereby resulting in a genotype producing a less than the optimal number of spikes per unit area. On the other hand, more time would then be available for the development and growth of individual spikes. Shumai 830 also harbored QTL, mapping to chromosomes 2A, 2D and 5A, which acted to increase both

the length of the spike and number of spikelets per spike. The high TGW reflected not only the presence of *TaTEF-7A* and *GPC-2* alleles from SHW-L1, but also an ability to fill the grains rapidly.

The three Shumai varieties had remarkably enhanced yield potential compared to those developed by conventional breeding. SNP-based genotyping showed that each had retained a spectrum of both beneficial and deleterious alleles from SHW-L1. Studies in tomato (Tanksley et al. 1996; Tanksley and Nelson 1996), rice (Xiao et al. 1998) and wheat (Huang et al. 2003, 2004; Pestsova et al. 2006) have confirmed that wild materials can harbor beneficial alleles, along with deleterious ones, as was also clearly the case for SHW-L1. The distinct spectrum of SHW-L1 alleles harbored by the three varieties suggests that progeny of crosses between them could enable combination of a greater number of beneficial SHW-L1 alleles, offering the prospect of further genetic advancement. The positive outcomes delivered by applying the DTC strategy imply that the secondary gene pool has great potential to contribute to wheat improvement.

Many of our leading crop species are allopolyploids and have become genetically isolated from their progenitor species; the effect of domestication and subsequent selection and breeding has been to drastically erode their genetic base (Stebbins 1950; Buckler et al. 2001). The present research has shown that a relatively simple crossing strategy can

**Fig. 4** A model devised to explain the basis of formation of heavy spikes. The high TGW achieved by Shumai 830 was associated with earliness per se (*Eps*) genes, and grain size genes *TaTEF* and *GPC-2*. A high grain number per spike was conferred by the presence of *Vrn-A1* allele from the synthetic wheat SHW-L1, along with QTL that increased the spike length and number of spikelets per spike. The presence of a *Vrn-A1* from SHW-L1 probably acted to reduce the number of spikes formed per unit area



unlock the secondary gene pool of wheat, and by inference, a similar strategy could be applied to other allopolyploid crops species (such as oat, cotton, groundnut and canola) to exploit diversity that is currently unexploited.

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**Author contribution statement** D.L., L.Q.Z., M.H. and Y.Z. designed the project; D.L., L.Q.Z., M.H., Z.W.Y., S.N., S.D., Z.H.Y., B.W., Y.Z., X.L., H.Z. and L.H. produced the new elite lines; L.B.Z., D.X., Q.L., W.C. and K.Z. performed the FISH and SDS-PAGE analyses, gene isolation and plant-type comparison. M.H., M.Y., Y.W., L.B.Z., A.L. and W.Y. performed the phenotypic and QTL analyses. M.H., D.L., J.W., M.C. and X.C. performed the SNP genotyping and statistical analyses. D.L., M.H., M.K., M.J.H. and L.M. wrote the manuscript.

### Compliance with ethical standards

**Conflict of interest** On behalf of all authors, the corresponding author declares no competing financial interests.

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