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ORIGINAL ARTICLE

Genetic modification of spikelet arrangement in wheat increases grain number without significantly affecting grain weight

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Abstract

Crop yield is determined by the acquisition and allocation of photoassimilates in sink organs. Therefore, genetic modification of sink size is essential for understanding the complex signaling network regulating sink strength and source activities. Sink size in wheat depends on the number of spikelets per spike, floret/grain number per spikelet as well as the grain weight or dry matter accumulation. Hence, increasing spikelet number and improving sink size are targets for wheat breeding. The main objective of the present work was to genetically modify the wheat spike architecture, i.e., the sink size by introgressing the 'Miracle wheat' or the *bh*^{t}*-A1* allele into an elite durum wheat cv. Floradur. After four generations of backcrossing to the recurrent parent, Floradur (FL), we have successfully developed Near Isogenic Lines (NILs) with a modified spikelet arrangement thereby increasing spikelet and grain number per spike. Genotyping of *bh*^{t}*-A1* NILs using the Genotyping-By-Sequencing approach revealed that the size of the introgressed donor segments carrying *bh*^{t}*-A1* ranged from 2.3 to 38 cM. The size of the shortest donor segment introgressed into *bh*^{t}*-A1* NILs was estimated to be 9.8 mega base pairs (Mbp). Phenotypic analysis showed that FL-*bh*^{t}*-A1*-NILs (BC3F2 and BC3F3) carry up to seven additional spikelets per spike, leading to up to 29% increase in spike dry weight at harvest (SDW_h). The increased SDW_h was accompanied by up to 23% more grains per spike. More interestingly, thousand kernel weight (TKW) did not show significant differences between FL-*bh*^{t}*-A1*-NILs and Floradur, suggesting that besides increasing spikelet number, *bh*^{t}*-A1* could also be targeted for increasing grain yield in wheat. Our study suggests that the genetic modification of spikelet number in wheat can be an entry point for improving grain yield, most interestingly and also unexpectedly without the trade-off effects on TKW. Hence, FL-*bh*^{t}*-A1-NILs* are not only essential for increasing grain number, but also for understanding the molecular and genetic mechanism of the source–sink interaction for a clearer picture of the complex signaling network regulating sink strength and source activities.

Keywords Wheat · Spike · Spikelet · Genetic modification · Spikelet arrangement · Grain number · Near Isogenic Lines · Source–sink · Sink size · Thousand kernel weight

Introduction

Grain yield increase of wheat in the past were mainly based on linear increase in the number of grains per meter square (Serrago et al. 2013; Ferrante et al. 2017), while spikelet number, grain weight and biomass were largely unchanged (Royo et al. 2007; Alvaro et al. 2008; Sanchez-Garcia et al. 2013). Even the tremendous increase in wheat grain yield after the introduction of the semi-dwarfing varieties has

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been mainly due to an increase in grains per spikelet without change in spikelet number (Youssefian et al. 1992a, b; Thomas 2017). Interestingly, unlike other cereal crops like barley, maize, rice, and sorghum, each wheat spikelet bears more than one grain, making the wheat spikelet the most essential grain yield component. Since the number and arrangement of each spikelet are under strong genetic, hormonal, and environmental control (McSteen 2009; Boden et al. 2015; Poursarebani et al. 2015; Youssef et al. 2017; Dixon et al. 2018), the isolation and characterization of genes that regulate inflorescence architecture is important for a clearer picture of grain yield barriers in wheat. With the fact that grain growth in wheat is also reported to be sink limited (Borras et al. 2004; Miralles and Slafer 2007; Zhang et al. 2010; Serrago et al. 2013), modifying the sink size, i.e., spikelet number is key to further understand the physiological and genetic basis of the source–sink relationship in wheat.

So far, several approaches were suggested for increasing wheat's yield potential (Fischer 2007; Reynolds et al. 2009; Foulkes et al. 2011; Gonzalez et al. 2011; Guo et al. 2017), including increasing the spike fertility index. The spike fertility index, also termed as fruiting efficiency (Slafer et al. 2015; Alonso et al. 2018), is the number of grains set per unit spike dry weight at anthesis (Ferrante et al. 2012). Because improving harvest index by directly increasing floret fertility has been very tough (Austin et al. 1980; Guo and Schnurbusch 2015; Guo et al. 2016), an alternative approach, such as enhancing spikelet number per spike should be critically considered.

The wheat inflorescence is characterized by an unbranched spike, whereupon sessile spikelets are distichously attached to the inflorescence's central axis (i.e., the rachis). In wheat, the number of spikelets per spike is fixed after the initiation of the terminal spikelet (Bonnet 1967; McMaster 1997). However, spike length and spikelet number are also known to be influenced by environmental factors, such as temperature and day length (Friend 1965; Rawson 1971; Rawson and Richards 1993). Generally, a longer spike development phase combined with optimum temperature and light promotes the initiation of more spikelets in wheat (Friend 1965; Rawson 1970, 1971; Pinthus and Millet 1978; Fischer 1985; Rawson and Richards 1993; Shaw et al. 2013). In this regard, the reproductive success of the wheat plant is predominantly controlled by three sets of genes namely vernalization, photoperiod, and the earliness per se genes (Yan et al. 2003, 2004, 2006; Beales et al. 2007; Lewis et al. 2008; Gawroński et al. 2014). The earliness per se genes are classes of genes regulating flowering time independently of vernalization and photoperiod and are important for the wide adaptation of wheat to different environments (Cockram et al. 2007; Lewis et al. 2008; Faricelli et al. 2010; Zikhali et al. 2016). By accelerating flowering time, i.e., shortening of the vegetative phases, *Photoperiod-1* (*Ppd1*) is implicated in the reduction of spikelet and tiller number in wheat (Worland et al. 1998; Shaw et al. 2013; Guo et al. 2018). Furthermore, with the rising global temperature, several crops flower earlier in warmer temperature (Craufurd and Wheeler 2009; Ellwood et al. 2013).

Different studies have also reported that late flowering wheat cultivars are vulnerable to post-anthesis stress that lead to a substantial reduction in yield and grain quality (Liu et al. 2014; Myers et al. 2014; Asseng et al. 2015). Therefore, to offset the trade-off between accelerated flowering and spikelet number, other mechanisms of increasing spikelet number need to be considered.

Genes controlling wheat spikelet arrangement have already been discovered (Boden et al. 2015; Dobrovolskaya et al. 2015; Poursarebani et al. 2015; Dixon et al. 2018). One of these genes is the wheat spikelet meristem identity gene, *TtBH-A1* (Poursarebani et al. 2015). A non-synonymous mutation that has occurred in the highly conserved AP2/ERF DNA binding domain of *TtBH-A1* altered the function of *TtBH-A1*, resulting in the so-called 'Miracle wheat' or spike-branching wheat phenotype. 'Miracle wheat' mutants deviate from the canonical spike form by developing mini-spike-like branches and/or secondary or supernumerary spikelets (SS) along the spike, but similarly have often lost terminal spikelet formation with increased spikelet and grain number per spike (Poursarebani et al. 2015).

Aiming to further characterize the 'Miracle wheat' allele, especially from the perspectives of increasing spikelet and grain number, we introgressed the *bh*^{t}*-A1* allele into an elite durum wheat cv. Floradur. Unlike the 'Miracle wheat' (TRI 19165, which is the donor parent), Floradur-*bh*^{t}*-A1*-NILs are typically characterized by the formation of supernumerary spikelets (SS) instead of developing mini-spike-like structures. Nevertheless, Floradur-*bh*^{t}*-A1*-NILs showed increased spike dry weight at harvest that was accompanied by significant increase in grain number per spike, suggesting that Floradur-*bh*^{t}*-A1*-NILs are interesting genetic materials for the genetic manipulation of grain yield in wheat. This is also because, Floradur-*bh*^{t}*-A1*-NILs are not only carrying the 'Miracle wheat' allele, but also incorporated the semi-dwarfing allele, *Rht-B1*, which make them useful wheat germplasm that have combined two plant architectural genes in a single elite genetic background to further assess new opportunities for increasing grain yield production.

Materials and methods

Plant materials

A German elite durum spring wheat cultivar, Floradur (FL), was used as a recurrent parent. 'Miracle wheat' accession

TRI 19165 was used as a donor parent for the 'Miracle wheat' or the *bh*^{t}*-A1* allele. TRI 19165 is a spike-branching mutant landrace from the gene bank collection of the Leibniz Institute for Plant Genetics and Crop Plant Research (IPK, Germany).

Backcrossing schemes

Floradur and TRI 19165 were grown side by side in a greenhouse for crossing. TRI 19165 was used as a pollen parent to generate F1 plants. Emasculation of the female parent was initiated when half of the spike emerged from the flag leaf sheath. All young florets were surgically removed from each spikelet except the basal two florets. The terminal and very young spikelets were completely removed. After removing the anthers, the whole spike was covered with bags to avoid any pollen cross-contamination. After pollination, the spike was covered again to protect any pollen cross-contamination. The complete scheme is shown in Fig. 1. Accordingly, four times backcrossing was made to the recurrent parent Floradur.

One genome-specific CAPS marker, which was developed based on the SNP in the *TtBH-A1* gene (T287C) which gave rise to the 'Miracle wheat' or *bh*^{t}*-A1* allele (Poursarebani et al. 2015), was used for the foreground (FG) selection in all generations. Heterozygosity of the F1 plants was checked using two microsatellite markers, *xgwm155* (chr.3A) and *xgwm95* (chr.2A). PCR conditions and fragment analysis were conducted as described by Röder et al. (1998). All true-bred heterozygous F1 plants were backcrossed with Floradur following the same procedure described earlier (see Fig. 1).

Embryo rescue (ER)

To shorten the generation time, embryos 21 days after pollination were rescued from the first two generations, i.e., BC1F1 and BC2F1 plants (Fig. 1). This saved the time from ripening to harvest. First, the immature grains were removed from all BC1F1 and BC2F1 plants. Then, the grains were rinsed in 70% alcohol for about 1 min. After rinsing the grains with sterilized water, the samples were washed with a washing buffer containing 20% hypochlorite and tween20. After 7 min of shaking, samples were repeatedly washed with sterilized water. The embryos from the grains were surgically removed in a laminar hood and carefully placed on B5 medium in a Petri dish (Duchefa biochemie, Haarlem, The Netherlands) for about 72 h at 24 °C under a dark condition in a growth chamber. The calli were then transferred to fresh new media in a magenta box under long day conditions (16 h light and 8 h dark) for about 10–12 days at 24 °C. Seedlings were then transplanted under greenhouse conditions in a pot size of 2 L filled with substrate 2 (Klasmann-Deilmann GmbH, 49744 Geeste, Germany), compost and sand with a proportion of 2:2:1, respectively. Plants received all the standard treatments for wheat including fertilizers and pesticides. DNA was extracted from these plants for the background (BG) and FG selections. All heterozygous plants

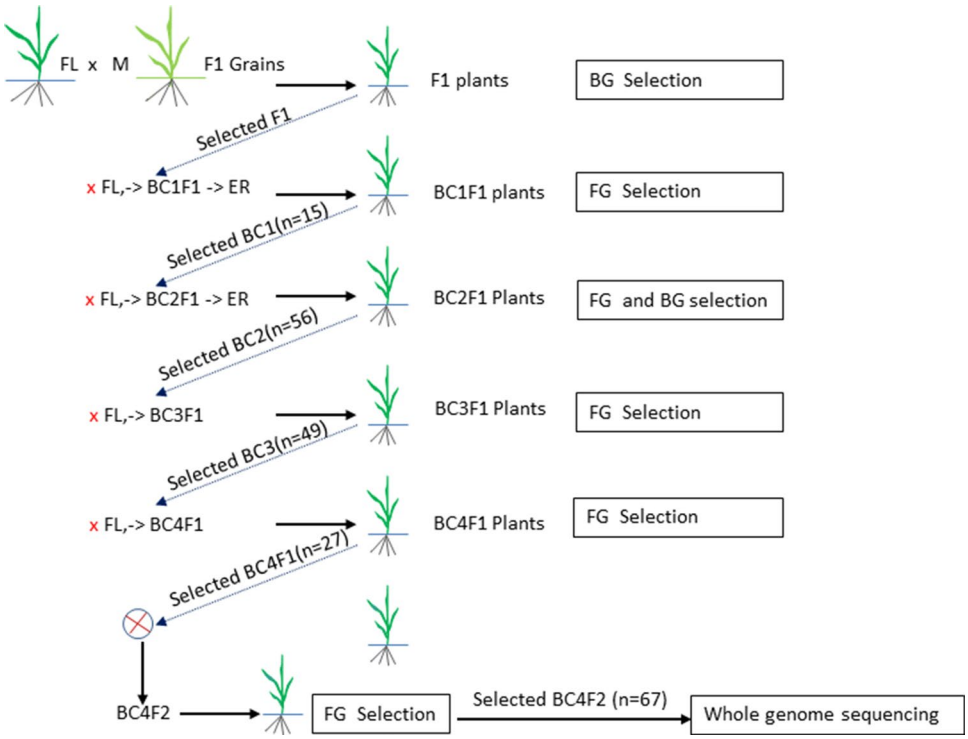

Fig. 1 Scheme for the development of Floradur-*bh*^{t}*-A1*-NILs. *FL* Floradur, *M* mutant (TRI 19165), *F1* the first filial generation, *BG* background selection, *FG* foreground selection, *BC1F1* the first backcross generation of F1, *ER* embryo rescue, *BC2F1* the second backcross generation of F1, *BC3F1* the third backcross generation of F1, *BC4F1* the fourth backcross generation of F1; circle with a cross show selfing; *BC4F2* the fourth backcross generation obtained by selfing BC4F1 and GBS, Genotyping-By-Sequencing

were selected and backcrossed with Floradur following the same procedure described earlier (Fig. 1).

Background selection

Forty-seven polymorphic wheat microsatellite markers were selected for the background selection (Supplementary Table 1). The selection was made in such a way that the polymorphism can be easily detected on a 3% standard agarose gel. PCR conditions for each of these markers were followed as described earlier by Röder et al. (1998). Then, the background selection was applied to 56 heterozygous BC2F1 plants. The recurrent parent genome recovery (RpGR) was calculated as follows

$$\text{RpGR} = \frac{\text{HO} + (\text{HE}/2)}{\text{Total number of markers}} \times 100$$

where RpGR is the recurrent parent genome recovery, HO is the number of homozygous markers (Floradur alleles); HE is the number of heterozygous markers (alleles).

Genotyping

After checking the zygosity of 90 BC4F2 plants using the *bh*^{t}*-A1* CAPS marker, all *bh*^{t}*-A1* homozygous ($n = 23$) and heterozygous ($n = 44$) plants were genotyped following the novel two-enzyme Genotyping-By-Sequencing (GBS) approach for the whole genome (Poland et al. 2012). Adapters were trimmed from reads with cutadapt version 1.8.dev0 (Martin 2011). Trimmed reads were mapped to the chromosome-shotgun assemblies of bread wheat cultivar Chinese Spring (The International Wheat Genome Sequencing Consortium (IWGSC) 2014) with BWA mem version 0.7.12 (Li 2013), converted to BAM format with SAMtools (Li et al. 2009) and sorted with Novosort (Novocraft Technologies Sdn Bhd, Malaysia, http://www.novocraft.com/). Multi-sample variant calling was performed with SAMtools version 0.1.19 (Li 2011). The command "mpileup" was used with the parameters "-C50 –DV". The resultant VCF file was filtered with an AWK script provided as Text S3 by Mascher et al. (2013). Only bi-allelic SNPs were used. Homozygous genotype calls were set to missing if their coverage was below 1 or their genotype quality was below 3. Heterozygous genotype calls were set to missing if their coverage below 4 or their genotype quality was below 10. An SNP was discarded when (1) its quality score was below 40, (2) its heterozygosity was above 20%, (3) its minor allele frequency was below 10%, or (4) had more than 66% missing data. Genotype calls were filtered and converted into genotype matrix with an AWK script available as Text S3 of Mascher et al. (2013). Chromosomal locations and genetic positions were taken from population sequence (POPSEQ) data (Chapman et al. 2015). Raw sequence data are available from the European Nucleotide Archive under accession PRJEB24999.

Phenotyping of homozygous BC3F2 and BC3F3 plants

Grains from selfed BC3F1 plants were randomly picked and grown as BC3F2 plants on a 96-well tray under greenhouse conditions. After 4 weeks of vernalization (at 4 °C) and hardening for 1 week (at 15 °C), seedlings were directly transplanted into the field characterized by a silty loam soil. The distance between rows and plants was 20 and 10 cm, respectively. DNA was extracted from each of these plants for the foreground selection, i.e., homozygous *bh*^{t}*-A1* plants, for phenotyping. Following the field evaluation, homozygous progenies from BC3F2 plants, i.e., BC3F3 plants were also phenotyped under greenhouse conditions. In both environments, the recurrent parent Floradur was used for comparison. In total, about 118, i.e., 27 plants at BC3F2 and 91 plants at BC3F3 generation were evaluated for this study. Crop husbandry (fertilization, watering, and pesticides) was applied uniformly to all plants in the field and the greenhouse following the recommended rate for wheat cultivation as required. For phenotypic data analysis, we used unpaired two-tailed Student's *t* test to check for trait differences among progenies.

Results

Marker-assisted foreground and background selections

At all generations, the *bh*^{t}*-A1* CAPS marker was used as a diagnostic marker for the selection of the *bh*^{t}*-A1* allele (Fig. 2a). BG selection was made once at BC2F1 using 47 polymorphic wheat microsatellite markers (Supplementary Table 1). Calculated RpGR based on 56 heterozygous BC2F1 plants is shown in Fig. 2b. Based on RpGR, five plants namely P-69, P-103, P-114, P-126, and P-170 were selected to establish five families of the NILs. These plants were then backcrossed to Floradur to generate BC3F1 plant families. Heterozygous progenies from BC3F1 were further backcrossed to Floradur to generate BC4F1 grains for all the five NILs families (Fig. 1).

Phenotypic analysis of homozygous BC3F2 and BC3F3 plants

Unlike the 'mini-spike'-like structures known to develop during genuine spike-branching in TRI 19165, spike-branching in *bh*^{t}*-A1*-NILs was reduced to the formation of secondary spikelets sharing the same rachis node with that

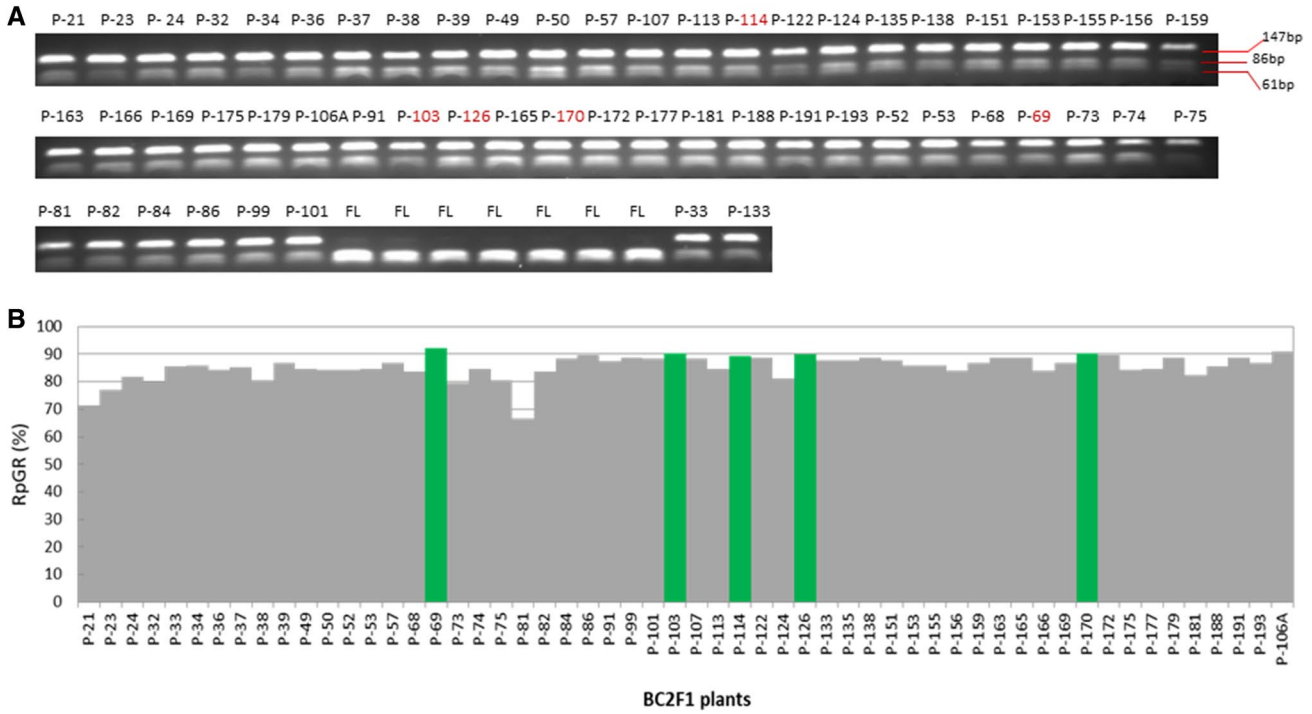

Fig. 2 Marker-assisted foreground selection (**a**) and background selection of BC2F1 based on calculated recurrent parent genome recovery (**b**). FG and BG were based on *bh*^{t}*-A1* CAPS marker and wheat microsatellite markers, respectively. Individual plant that constituted the five families of NILs is shown in green. *RpGR* recurrent parent genome recovery, *FL* Floradur

of the primary spikelets forming the so-called supernumerary spikelets (Fig. 3b). This clearly revealed the successful introgression of *bh*^{t}*-A1* into the Floradur genome. Thus, homozygous *bh*^{t}*-A1*-NILs were found to carry up to seven SS per spike (Fig. 3c). Such reduced phenotypic penetrance and expressivity is most likely due, partly, to other branch suppressing loci from Floradur located elsewhere in the genome and/or environmental effects. The summary of other

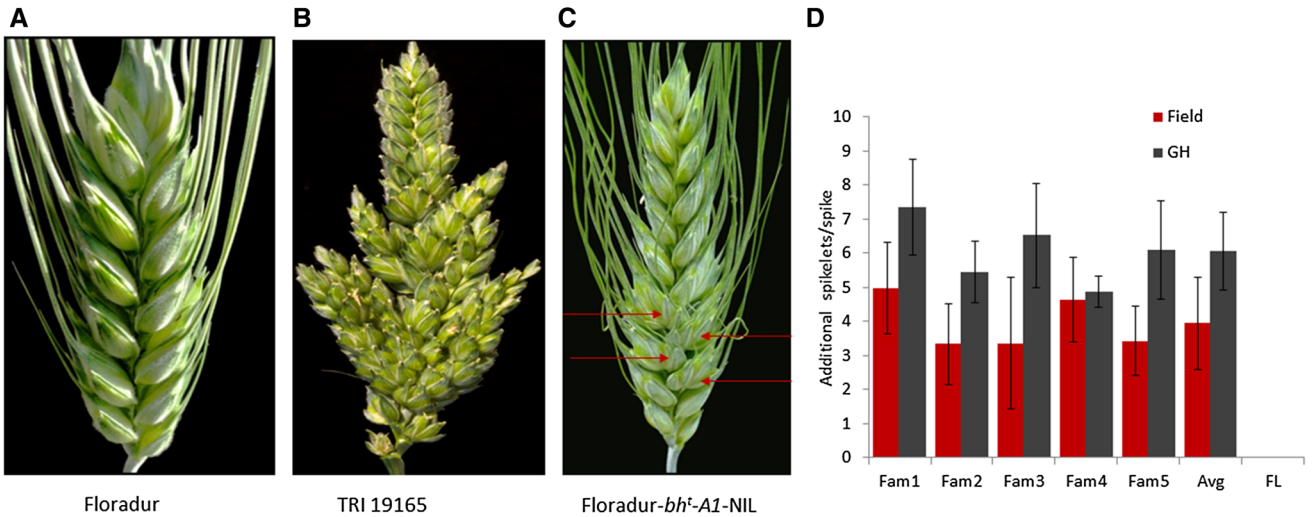

Fig. 3 Spike morphology of the Floradur (**a**), TRI 19165 (**b**), Floradur-*bh*^{t}*-A1*-NIL (**c**). Arrow indicates secondary spikelets. For clarity, awns were trimmed from TRI 19165. (**d**) Additional or secondary spikelets per spike from Floradur-*bh*^{t}*-A1*-NILs grown in the field (red bars) and greenhouse (black bars) at BC3F2 and BC3F3 generations, respectively. Error bars indicate mean ± standard deviation. *GH* greenhouse, *Fam* family, *FL* Floradur, *Avg* average

spike traits from the five plant families of *bh*^{t}*-A1*-NILs is shown in Table 1. Compared to Floradur, the *bh*^{t}*-A1*-NILs had on average more grain-bearing spikelets resulting in up to 29% of increased SDW_h, thereby increasing the grain number per spike (Table 1). Unlike the known trade-off between grain number and TKW (Pinthus and Millet 1978; Slafer and Miralles 1993; Gambín and Borrás 2010; Griffiths et al. 2015), especially for the spike-branching wheat accessions (Poursarebani et al. 2015), no significant difference in TKW between Floradur-*bh*^{t}*-A1*-NILs and Floradur was found, clearly suggesting that the *bh*^{t}*-A1* allele is useful for the manipulation of spikelet number per spike and potentially for increasing grain yield in wheat. As sink size is thought to be one of the yield-limiting factors in wheat (Borras et al. 2004; Miralles and Slafer 2007; Zhang et al. 2010), the Floradur-*bh*^{t}*-A1*-NILs are similarly an ideal resource for further assessing the source–sink interaction. Interestingly, tiller number (TN) analysis between Floradur-*bh*^{t}*-A1*-NILs and Floradur was non-significant, suggesting that *bh*^{t}*-A1* is not involved in shoot branching (Table 1). Thus, the *bh*^{t}*-A1* allele can be utilized in durum wheat breeding.

Genotyping and estimation of introgressed donor segment

GBS of 67 BC4F2 plants yielded about 9400 SNP markers spanning the whole genome. After removing all redundant markers, i.e., markers with the same genetic positions, about 799 polymorphic markers were selected to estimate the introgressed donor segment carrying the *bh*^{t}*-A1* allele. The genetic position of the markers was adopted from the POPSEQ linkage map (Chapman et al. 2015). Distribution of the markers across the linkage groups is presented in the Supplementary Table 2. Best 20 NILs with higher RpGR are shown in Table 2. Based on the graphical genotypes of the NILs (Table 3), the size of the introgressed donor segment ranged from 2.28 to 38 cM. P-69-4-4-121 and P-69-4-4-122 from Fam1 plants carry the smallest donor fragment (~2.28 cM). According to the genome sequence assembly of the Chinese Spring, Refseq V1.0 (Alaux et al. 2018), markers flanking the introgressed fragment, i.e., 2AS_5296611:2363 and 2AS_5272493:9637 were mapped at position 52,425,262 bp and 62,264,948 bp, respectively (Fig. 4). Therefore, the size of the shortest donor segment introgressed into *bh*^{t}*-A1* NILs was estimated to be 9,839,686 base pairs. Graphical representation of chromosome group 2 for the best plant from Fam 1, P-69-4-4-122 is shown in Fig. 4. Graphical representation for the remaining chromosomes for P-69-4-4-122 is shown in Supplementary Figure 1. As expected, average whole genome RpGR, i.e., 96.23%, was similar to the expected RpGR of 96.88% (Table 2). On average, RpGR for chromosomes 2A and 2B was 93.41 and 96.27%, respectively. In general, RpGR for chromosomes 2A ranged from 82 to 99%. The lowered RpGR for some of these plants

Table 1 Summary of spike phenotype of Floradur-*bh*^{t}*-A1*-NILs grown in the field and greenhouse

Environment	Trait	Family					Fam mean	Floradur (FL)
		Fam1	Fam2	Fam3	Fam4	Fam5		
Field (BC3F2)	PH	74 ± 6.3	75 ± 5.9	77 ± 5.9	82 ± 3.5	78 ± 3.3	76 ± 5.4	76 ± 3.7^{ns}
	SL	7.40 ± 0.4	7.12 ± 0.2	7.17 ± 0.2	7.15 ± 0.1	7.17 ± 0.2	7.19 ± 0.2	6.9 ± 0.2*
	SDW_h	3.02 ± 0.4	3.07 ± 0.4	3.02 ± 0.7	3.23 ± 0.1	2.85 ± 0.2	3.04 ± 0.4	2.54 ± 0.2**
	totSPS	21.8 ± 1.7	18.9 ± 1.1	18.8 ± 3.5	20.4 ± 1.8	19.3 ± 1.4	19.9 ± 1.9	14.5 ± 0.6**
	GNS	45.4 ± 6.4	46.7 ± 4.9	47.3 ± 10.9	40.7 ± 7.2	39.7 ± 5.7	43.9 ± 7	39.6 ± 2.9*
	TKW	49.6 ± 3.0	47.9 ± 2	48 ± 1.6	53.2 ± 7.2	53.8 ± 3.0	49.8 ± 2.4	48.8 ± 2.9^{ns}
	TN	14 ± 4.5	8 ± 1.4	10 ± 1.8	10 ± 2.8	12 ± 3.5	11 ± 4.0	10 ± 4.0^{ns}
GH (BC3F3)	PH	62 ± 3.4	65 ± 2.3	66 ± 2.3	67 ± 3.0	62 ± 2.0	64 ± 2.6	63 ± 3.5^{ns}
	SL	7.49 ± 0.6	6.71 ± 0.17	6.52 ± 0.3	6.46 ± 0.4	6.96 ± 0.1	6.83 ± 0.3	6.78 ± 0.2^{ns}
	SDW_h	2.46 ± 0.2	2.74 ± 0.3	2.95 ± 0.4	2.8 ± 0.3	2.63 ± 0.2	2.72 ± 0.3	2.1 ± 0.3***
	totSPS	27.6 ± 3.5	22 ± 1.1	23.6 ± 2.4	22.1 ± 1.1	23.8 ± 2.3	23.8 ± 2.1	13.3 ± 1.9***
	GNS	47.1 ± 3.8	47.7 ± 4.6	49.2 ± 5.9	46.1 ± 5.5	44.1 ± 4.1	46.9 ± 4.8	37.9 ± 3.7***
	TKW	47.1 ± 1.6	42.8 ± 1.3	42.5 ± 2.5	45.8 ± 0.8	43.9 ± 3.2	44.4 ± 1.9	42.6 ± 3.0^{ns}
	TN	4.6 ± 0.4	4.1 ± 0.8	4.3 ± 0.6	4.0 ± 0.4	4.5 ± 0.8	4.3 ± 0.6	4.0 ± 0.4^{ns}

Field data were collected from homozygous BC3F2 plants ($n = 27$) of five families along with Floradur ($n = 10$). Progenies from homozygous BC3F2 plants (i.e., BC3F3 plants, $n = 91$) of the five families were grown and phenotyped in the greenhouse

PH plant height, *SL* spike length, SDW_h spike dry weight at harvest, *totSPS* the total number of spikelets per spike, *GNS* grain number per spike, *TKW* thousand kernel weight, *TN* tiller number at harvest i.e. fertile and non-fertile combined, *Fam* family

The data represent mean ± SD (standard deviation).The significance level was calculated based on the unpaired two-tailed Student's *t* test between the family averages with Floradur. *$P_{0.05}$, **$P_{0.01}$, and ***$P_{0.001}$

Table 2 Selected Floradur-*bh*^{t}-*A1*-NILs based on calculated RpGR

Plant ID	Family	Expected at BC4	Calculated RpGR (%)			Zygosity (*TtBH-A1*)
			Chr 2A	Chr. 2B	Whole genome	
P-69-4-4-127	Fam1	96.88	90.43	97.86	97.74	+/−
P-69-4-4-126	Fam1	96.88	91.88	95.67	97.10	+/−
P-69-4-4-121	Fam1	96.88	89.92	95.94	96.38	+/−
P-69-4-4-122	Fam1	96.88	82.05	97.28	96.37	+/+
P-103-16-34-31	Fam2	96.88	95.28	98.08	97.77	+/+
P-114-27-73-69	Fam3	96.88	96.47	99.47	96.92	+/−
P-114-27-68-178	Fam3	96.88	96.22	99.25	96.51	+/−
P-114-27-68-176	Fam3	96.88	92.70	99.41	96.42	+/−
P-114-27-73-181	Fam3	96.88	96.03	99.47	96.32	+/−
P-114-27-73-192	Fam3	96.88	96.22	98.82	96.31	+/−
P-114-27-73-189	Fam3	96.88	95.21	97.33	95.58	+/−
P-114-27-73-71	Fam3	96.88	95.47	97.76	95.21	+/−
P-114-27-73-61	Fam3	96.88	99.12	98.99	95.19	+/−
P-114-27-68-53	Fam3	96.88	95.47	98.40	95.02	+/−
P-114-27-73-62	Fam3	96.88	93.39	96.63	94.56	+/−
P-126-45-103-81	Fam4	96.88	96.35	99.57	95.27	+/−
P-170-50-121-107	Fam5	96.88	89.92	86.81	97.34	+/−
P-170-50-121-103	Fam5	96.88	89.92	89.05	97.24	+/+
P-170-51-130-115	Fam5	96.88	98.55	99.15	96.14	+/−
P-170-50-121-99	Fam5	96.88	87.53	80.40	95.11	+/+
	Average	96.88	93.41	96.27	96.23	n/a

Zygosity was determined based on *bh*^{t}*-A1* CAPS marker

+/+, homozygous mutant; +/−, heterozygous; n/a, not applicable

was due to the drag-over-effect attributed to the suppressed recombination and marker-assisted selection. Taking all together, these results demonstrated that the expected RpGR at BC4 generation and one or two more rounds of backcrossing might even remove the drag-over-effects from the donor parent.

Discussion

Spikelets are the building blocks of the wheat inflorescence, also known as the spike. The number and arrangements of individual spikelets on the spike dictate the architecture or morphology of the spike and critically determines the grain yield in wheat. Thus, understanding the genetic basis of spike development is key to understand the genetic basis of gain yield formation. Due to limited knowledge of genes controlling wheat spikelet arrangement, genetic modification of spikelet arrangement was not possible until recently. In the last few years, genes altering spikelet arrangement have been discovered paving ways for the genetic modification of the spike architecture in wheat (Boden et al. 2015; Dobrovolskaya et al. 2015; Poursarebani et al. 2015; Debernardi et al. 2017; Greenwood et al. 2017).

The current study aimed to introgress the 'Miracle wheat' allele *bh*^{t}*-A1* into an elite durum wheat variety, Floradur, to better understand the effect of altered spikelet arrangement in connection with the source–sink balance and grain yield formation.

Although spike-branching increases the sink size in wheat (Dobrovolskaya et al. 2015; Poursarebani et al. 2015), the extremely low spikelet fertility hindered the direct utilization of spike-branching wheat accessions for increasing the grain yield (USDA 1916; Rawson and Ruwali 1972; Pennell and Halloran 1984; Hucl and Fowler 1992). Furthermore, the intensity of spike-branching is affected by the environment; especially by the photoperiod and temperature (Percival 1921; Sharman 1944; Pennell and Halloran 1984a, b). Interestingly, unlike the donor parent, TRI 19165, Floradur-*bh*^{t}*-A1*-NILs often form supernumerary spikelets thereby increasing spikelet and grain number per spike. Such lowered expression of spike-branching in the NILs significantly reduced the trade-off between spike-branching and spikelet fertility/TKW usually seen in the rather extreme 'Miracle wheat' phenotype (Poursarebani et al. 2015). This clearly suggests that a more attenuated phenotypic expression of *bh*^{t}*-A1* can be used to genetically modify spikelet number and spike dry weight in wheat without affecting TKW.

Table 3 Graphical genotypes of selected BC4F2 plants based on markers from chromosome 2A

Marker ID	Position (cM)	RP	DP	*bh*^{t}*-A1- NILs*																			
		Floradur	TRI 19165	P-69-4-4-127	P-69-4-4-126	P-69-4-4-121	P-69-4-4-122	P-103-16-34-31	P-114-27-73-69	P-114-27-68-178	P-114-27-68-176	P-114-27-73-181	P-114-27-73-192	P-114-27-73-189	P-114-27-73-71	P-114-27-73-61	P-114-27-68-53	P-114-27-73-62	P-126-45-103-81	P-170-50-121-107	P-170-50-121-103	P-170-51-130-115	P-170-50-121-99
2AS_5273839:2891	0.00	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AS_5308598:24776	1.14	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AS_5307231:8426	3.41	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AS_5165946:859	3.98	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AS_5241920:2176	4.55	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AS_5307157:4703	10.25	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AS_5218468:3257	11.39	A	B	A	B	B	B	A	A	A	A	A	A	B	A	B	B	-	A	A	B	B	B
2AS_5234045:1170	14.81	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AS_5304757:555	19.36	A	B	A	A	A	A	A	H	H	B	H	A	B	-	A	A	A	H	A	A	A	A
2AS_5210238:293	20.49	A	B	A	A	A	A	A	H	H	B	H	A	B	H	A	A	H	H	A	A	A	A
2AS_5191197:1496	29.60	A	B	A	A	A	A	A	H	H	B	H	A	H	H	A	A	-	H	A	A	A	A
2AS_5261595:7521	38.70	A	B	A	A	A	A	A	H	H	B	H	H	H	H	A	H	H	H	A	A	A	A
2AS_5211843:3921	39.84	A	B	A	A	A	A	A	H	H	B	H	H	H	H	A	H	H	H	A	A	A	A
2AS_5222631:6886	40.98	A	B	A	A	A	B	B	H	H	B	H	H	H	H	A	H	B	H	A	A	A	A
2AS_5302874:2701	41.55	A	B	A	A	A	B	B	H	H	B	H	H	H	H	A	H	H	H	A	A	A	A
2AS_5203328:4893	48.98	A	B	A	A	A	B	B	H	H	B	H	H	H	H	A	H	H	H	B	H	H	B
2AS_5296611:2363	50.11	A	B	A	A	A	A	B	H	H	B	H	H	H	H	A	H	H	H	B	H	H	B
2AS_5272493:9637	52.39	A	B	H	H	H	B	B	H	H	B	H	H	H	H	H	H	H	H	H	H	H	B
2AL_4305348:1350	58.09	A	B	H	H	A	A	A	A	H	-	A	A	-	H	A	A	A	H	A	H	-	A
2AL_6396518:3194	59.23	A	B	B	A	H	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6371388:8162	61.53	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6405678:3964	66.14	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_2738889:7610	70.81	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6362857:1852	73.18	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_902056:1293	74.32	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6434194:5615	76.02	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6436614:10508	76.59	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_39533:1795	81.15	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6360337:7083	83.42	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6363525:1891	83.99	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6436455:8936	85.13	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6376905:2965	85.70	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6340959:4778	86.83	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6436403:16131	87.97	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_996838:1288	88.54	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6330385:2031	90.24	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6395361:1966	90.81	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6403679:9419	91.95	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2AL_6403679:9485	91.95	A	B	A	A	A	-	A	A	A	A	A	B	A	A	A	B	B	A	A	A	A	B
2AL_660116:1742	92.52	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

As shown, three plants namely P-69-4-4-121, P-69-4-4-122, and P-114-27-73-61 carry the smallest donor fragment carrying *bh*^{t}*-A1*

Marker information: A, Floradur; B, TRI19165; H, Heterozygous; dash (–), missing markers

Carbon assimilation and partitioning to the developing inflorescence critically determine crop yield and productivity (Satoh-Nagasawa et al. 2006; Lawlor and Paul 2014; Sosso et al. 2015; White et al. 2016). The sink capacity of the spike, namely the number of spikelets per spike, the number of grains per spikelet (spikelet fertility) and grain weight (dry matter accumulation in each grain) strongly affect grain yield in wheat. Hence, sink strength was suggested to be a critical yield-limiting factor (Fischer 1985; Slafer and Savin 1994; Richards 1996; Borrás et al. 2004; Reynolds et al. 2007; Foulkes et al. 2011). As sink strength strongly dictates activities in the source and assimilate partitioning to the sink organs (Yu et al. 2015), it is strongly believed that increasing sink strength and activity are of major interest. Previous studies attempted to better understand to which extent source and/or sink limited wheat yields (Blade and Baker 1991; Slafer and Savin 1994; Cruz-Aguado et al. 1999; Serrago et al. 2013); however, results were rather inconclusive which might suggest that both source and sink might co-limit yield formation in wheat. The communication between the source and sink organs is regulated by a complex signaling network involving sugars, hormones, and environmental factors (Yu et al. 2015).

In this regard, a limited number of genes regulating the source–sink communication has been characterized (Aoki et al. 2002; Martinez-Barajas et al. 2011). Thus, modern molecular genetic approaches are required for a deeper understanding of the source–sink relationship in wheat. Because of the position of the secondary spikelets in relation to the primary spikelets, both are arranged laterally sharing the same rachis node, Floradur-*bh*^{t}*-A1*-NILs are important genetic resources to elucidate the mechanics of vascular tissue architecture and assimilate partitioning towards wheat spikelets.

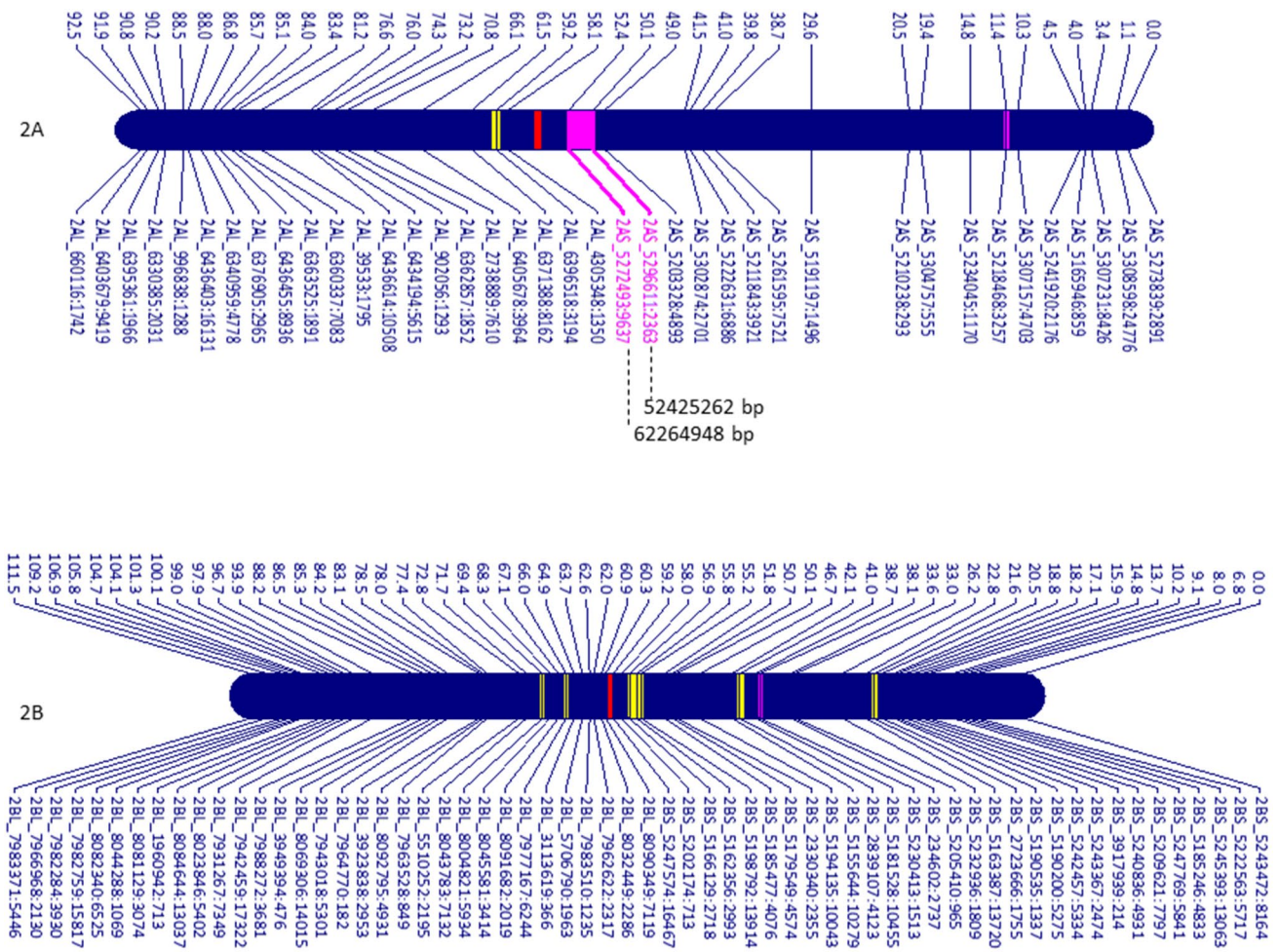

Fig. 4 Graphical representation of chromosome group 2 for the best plant P-69-4-4-122. Markers with a purple font are those markers flanking the donor segment carrying the *bh*^{t}*-A1* allele. Names and the corresponding positions (cM) of markers are indicated on the right and left sides of the chromosome, respectively. The physical genomic position of markers flanking the introgressed segment is shown below each marker. The position was according to the genome sequence assembly of the Chinese Spring, Refseq V1.0. Red bar demarcates the centromeric region. The blue portion of the chromosome indicates genome of the recurrent parent (Floradur), purple indicates batches of donor segments, and yellow indicates heterozygous region

The tremendous harvest index increase after the introduction of the semi-dwarfing allele during the green revolution was due, partly, to the diversion of more assimilates to the developing spike which otherwise would be invested in the growth and development of the culm (Flintham et al. 1997; Khush 2001). Interestingly, Floradur-*bh*^{t}*-A1*-NILs are semi-dwarf carrying the reduced plant height gene (*Rht-B1*), which makes them an excellent wheat ideotype for studying the physiological and genetic basis of source–sink interaction and yield formation in wheat. Hence, Floradur-*bh*^{t}*-A1*-NILs are important wheat genetic resources not only for increasing grain number per spike, but also, for the systematic discovery of *TtBH-A1* downstream target genes for the detailed understanding of the molecular genetics of wheat spike morphogenesis. Our study further suggests genetic modification of spikelet number as an entry point for improving yield, most interestingly and also unexpectedly without the trade-off effects on TKW. Therefore, despite the negative relationship between grain number and grain weight in wheat, this work illustrates the possibilities of increasing grain number per spike without significantly affecting the grain weight. However, whether this genetic material, or its derivatives, will have any yield advantages under real world conditions is still to be shown in multi-year and multi-location field evaluations. Nevertheless, as the sink size in Floradur-*bh*^{t}*-A1*-NILs is genetically controlled, Floradur-*bh*^{t}*-A1*-NILs are also beneficial for understanding the physiological and molecular basis of source–sink interaction for a clearer picture of the complex signaling network regulating sink strength and source activities in wheat.

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Author contributions TS conceived and supervised the project. GMW developed the diagnostic CAPS marker, NILs, and performed the phenotyping. MM assembled the GBS data and generated the genotypic matrix file. GMW and TS analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest We declare that there was no conflict of interest.

Human participants or animals This article does not contain any studies with human participants or animals performed by any of the authors.

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