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ORIGINAL ARTICLE

Production of a complete set of wheat–barley group-7 chromosome recombinants with increased grain β-glucan content

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Abstract

Key message **Wheat–barley group-7 recombinant chromosomes were selected using molecular cytogenetics and SNP markers; increased grain β-glucan content was observed in wheat plants with two and four copies of *HvCslF6*.**

Abstract The soluble dietary fiber (1–3)(1–4) mixed linked β-D-glucan from cereal grains is a valuable component of a healthy diet, which reduces risks of coronary disease and diabetes. Although wheat is an important cereal crop providing a substantial portion of daily calories and protein intake in the human diet, it has a low level of β-glucan. Owing to the plasticity of the polyploid wheat genome, agronomically important traits absent in the wheat primary gene pool can be introgressed from distant relatives. Barley (*Hordeum vulgare* L.) has a high grain β-glucan content. Earlier, we introgressed this trait into wheat in the form of whole arm compensating Robertsonian translocations (RobT) involving group-7 chromosomes of barley and all three sub-genomes of hexaploid wheat (*Triticum aestivum* L). In the presented research, we shortened the barley 7HL arms in these RobTs to small pericentromeric segments, using induced wheat–barley homoeologous recombination. The recombinants were selected using SNP markers and molecular cytogenetics. Plants, comprising barley cellulose synthase-like F6 gene (*HvCslF6*), responsible for β-glucan synthesis, had a higher grain β-glucan content than the wheat control. Three wheat–barley group-7 recombinant chromosomes involving the A, B and D sub-genomes laid the basis for a multiple-copy gene introgression to hexaploid wheat. It is hypothesized that further increases in the β-glucan content in wheat grain can be obtained by increasing the number of *HvCslF6* copies through combining several recombinant chromosomes in one line. The wheat lines with four copies of *HvCslF6* exceeded the β-glucan content of the lines with two copies.

Introduction

With increasing interest in healthy eating, development of products with health benefits becomes more important. Dietary fiber from the plant cell wall is an essential component of healthy foods. Higher intake of dietary fiber decreases the risk of cardiovascular diseases, type 2 diabetes, and colorectal and breast cancer (Reynolds et al. 2019). In human diets, cereal fiber is the largest contributor to total dietary fiber consumption. A soluble fiber (1–3)(1–4) mixed linked β-D-glucan from cereal grains such as oat and barley has been shown to reduce the risk of coronary disease and diabetes through cholesterol-lowering effects and attenuation of glycaemic and insulin responses (Brown et al. 1999; Cui et al. 2013; Sibakov et al. 2013).

Wheat is one of the world's most important cereal crops, accounting for a substantial portion of total production and in daily calories and protein intake (FAO 2018), but wheat grain has a low level of soluble β-glucans (Collins et al. 2010; Havrlentova and Kraic 2006). Although the β-glucan content ranges between 3–20% in oats and 3–7% in barley on a dry weight basis in whole meal, in wheat it is less than 1% (Beresford and Stone 1983; Collins et al. 2010; Havrlentova and Kraic 2006). Furthermore, no germplasm resources with high grain β-glucan have been found in hexaploid wheat (*Triticum aestivum* L. $2n=6x=42$, genome AABBDD) or its closest relatives from the primary and secondary (Friebe

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et al. 1996) gene pools. A survey of more than 500 germplasm accessions of diverse origin including primitive, synthetic and elite lines of tetraploid and hexaploid wheats and diploid relatives indicated insufficient genetic diversity to initiate a breeding program for high β-glucan (Marcotuli et al. 2019; Pritchard et al. 2011). Wheat grain quality can be changed through introgressing of genes of distantly related wild and cultivated relatives from the tertiary gene pool via interspecific hybridization and chromosome engineering (Cseh et al. 2011, 2013; Danilova et al. 2018a; Rakszegi et al. 2017; Turkosi et al. 2018). High β-glucan content (up to 7%) was found in some *Aegilops* species from the tertiary gene pool (diploid *Ae. umbellulata* Zhuk., *Ae. markrafii* (Greuter) Hammer and tetraploid species containing U and C genomes) (Marcotuli et al. 2019; Rakszegi et al. 2017). Among species from the wheat tertiary gene pool successfully used in interspecific hybridization, barley has the highest grain β-glucan content.

Barley grain β-glucan is a quantitative trait controlled by several genes with different impacts. Genes from the *Cellulose synthase-like* (*Csl) F* gene family *HvCslF4*, *HvCslF6* and *HvCslF9* mapped on chromosome arms 2HS, 7HL and 1HS significantly influence grain β-glucan content in barley. The transcripts of *HvCslF6* and *HvCslF9* predominate in the endosperm during grain development with higher expression of *HvCslF6* (Burton et al. 2008; Doblin et al. 2009; Schreiber et al. 2014). A mutation in the *HvCslF6* locus caused complete lack of β-glucan in grain (Tonooka et al. 2009). Overexpression of *HvCslF4* and *HvCslF6* in transgenic barley with extra copies of these genes resulted in more than 50% and 80% increase in grain β-glucan content, respectively (Burton et al. 2011). Additional candidate genes influencing synthesis of grain β-glucan are *HvGlb1* and *HvGlb2*, which code for the (1,3;1,4)- β-D-glucan endohydrolase isoenzymes EI and EII and co-localize with QTLs on 1HL (Han et al. 1995) and 7HL (Li et al. 2008; Kim et al. 2011; Houston et al. 2014). An increase of *HvGlb1* transcripts at the beginning of endosperm development was observed in some barley cultivars (Burton et al. 2008).

Several barley genes were introgressed to bread wheat, expressed in a wheat background and resulted in an increase of grain β-glucan. The expression of *HvCslF9* and *HvCslF6* in wheat 1HS ditelosomic and 7H disomic addition lines was detected in grain (Cseh et al. 2013). *HvCslF6* introgressed in the form of compensating and non-compensating Robertsonoan translocations (RobT) resulted in 1.5–2-fold increase of β-glucan in wheat grain, depending on the wheat or barley genotype (Cseh et al. 2011, 2013; Danilova et al. 2018a; Turkosi et al. 2018). It is not known how wheat and barley genes, controlling synthesis of β-glucan and other grain cell wall components interact in introgression lines. In hexaploid wheat, ten sets of *TaCslF* sequences similar to ten barley *HvCslF* genes were found in all three wheat genomes and were located on the same homoeologous chromosomes as in barley. *HvCslF6* was mapped by FISH at the pericentromeric region of 7HL (Danilova et al. 2018a). Three sequences, similar to *HvCslF6*, were located in the pericentromeric region on the long arms of all wheat group-7 chromosomes (Danilova et al. 2018a; Schreiber et al. 2014). Nemeth et al. (2010) showed the influence of *TaCslF6* genes on β-glucan content in grain of hexaploid wheat by RNA interference suppression, which resulted in 30–52% decrease in β-glucan. In tetraploid durum wheat (*Triticum turgidum* subsp. *durum*, $2n=4x=28$, genome AABB), Marcotuli et al. (2018) found a positive correlation between expression of *CslF6* and β-glucan content in grain. Neither Marcotuli et al. (2018) nor Nemeth et al. (2010) investigated the expression and impact of A-, B- or D-genome *CslF6* homoeologues on β-glucan synthesis in wheat grain.

The hexaploid nature of the bread wheat genome gives an opportunity to double and triple doses of introgressed barley gene *HvCslF6* in wheat by chromosome engineering and possibly significantly increase the grain β-glucan content. Earlier, we reported the construction of a complete set of wheat–barley group-7 RobT chromosomes T7AS·7HL, T7BS·7HL and T7DS·7HL containing *HvCslF6* (Danilova et al. 2018a). If combined, these RobTs would not be transmitted stably to the progenies because of meiotic abnormalities and low fertility due to paring between the shared 7H arms on different wheat homoeologues. Interstitial recombinants for the wheat–barley group-7 homoeologues, consisting of a 7HL segment at the proximal region of the long arm and a wheat segment at the distal end (T7WS·7HL-7WL), can be combined in one wheat line resulting in four or six doses of *HvCslF6*. Here, we report construction of a complete set of these recombinant chromosomes and increased grain β-glucan content in wheat lines with two or four copies of *HvCslF6*.

Materials and methods

Plant material

The material used in this study included the wheat cv. Chinese Spring (CS) TA3008, the *ph1b* mutant stock in CS background TA3809, the set of three wheat lines with RobTs T7AS·7HL, T7BS·7HL and T7DS·7HL containing *HvCslF6* gene from barley cv. Betzes, accessions TA5790, TA5792 and TA9795L1 (Danilova et al. 2018a) and the translocation line with an inactivated barley centromere T7AS·7ALdel·7HL containing both *HvCslF6* and a wheat *TaCslF6* orthologue in a 7AL pericentromeric segment (Danilova et al. 2018b). All lines are maintained by the Wheat Genetics Resource Center at Kansas State University. The lines, homozygous for RobTs T7AS·7HL,

T7BS·7HL and T7DS·7HL, were crossed twice with the *ph1b* stock. From these crosses, plants homozygous for *ph1b* and heterozygous for a RobT were selected and self-pollinated. Their progenies were screened by molecular markers to find putative wheat–barley recombinant chromosomes and verified cytologically.

Cytogenetic analyses

Chromosome preparations, probe labeling and the fluorescent in situ hybridization (FISH) were performed as described previously (Kato et al. 2004, 2006) with minor modifications (Danilova et al. 2012). FISH with HvCslF6 and barley specific *copia*-like retroelement BARE-1 (Manninen and Schulman 1993; Waugh et al. 1997; Wicker et al. 2009) probes was used to confirm the presence of gene *HvCslF6* on recombinant chromosomes. Preparation of BARE probe was described earlier (Danilova et al. 2018a). The HvCslF6 probe (cDNA clone AK365097 from the Genebank of the National Institute of Agrobiological Sciences, Tsukuba, Japan) was labeled with Texas red-5-dCTP and the BARE probe was labeled using Fluorescein-12-dUTP (PerkinElmer, Waltham, MA, USA).

Genomic in situ hybridization with the addition of FISH probes (FGISH) oligonucleotide Cy5-$(GAA)_9$ and TEX-pAs1 (Danilova et al. 2012), labeling microsatellite $(GAA)_n$ and D-genome-specific Afa-family tandem repeat (Nagaki et al. 1995) was used to identify chromosomes and confirm recombination events. The FGISH probe mixture (10 µl/slide) in 2 × SSC − 1 × TE buffer contained 30 ng of barley gDNA probe labeled with Fluorescein-12-dUTP, 6 ng of Cy5-$(GAA)_9$, 35 ng of TEX-pAs1 (synthesized by Integrated DNA Technologies, Inc., Coralville, IA, USA) and 750 ng of autoclaved salmon sperm DNA. The mixture was added to a slide and covered with a 22 × 22 plastic cover slip. Slides were placed in metal tray floating in boiling water bath for 5 min to denature the probe and chromosomal DNA as described by Kato et al. (2004, 2006) and transferred to 55 °C oven for overnight hybridization. Slides were washed in 2 × SSC buffer for 5 min at room temperature and 20 min at 55 °C. Chromosome preparations were mounted and counterstained with 4′,6- diamidino-2-phenylindole (DAPI) in Vectashield (Vector Laboratories, Burlingame, CA, USA).

Images were captured using a Zeiss Axioplan 2 microscope with a cooled charge-coupled device camera CoolSNAP HQ2 (Photometrics, Tucson, AZ, USA) and AxioVision 4.8 software (Carl Zeiss, Thornwood, NY, USA) and processed using the Adobe Photoshop software (Adobe Systems Incorporated, San Jose, CA, USA).

Molecular markers analysis

Genomic DNA was isolated from leaves of 2-week-old seedlings using a BioSprint 96 workstation according to the Qiagen BioSprint DNA Plant Handbook protocol (QIAGEN Inc., Valencia, CA, USA). We used Single Nucleotide Polymorphism (SNP) to distinguish barley and wheat alleles. The 8 µl PCR Allelic Competitive Extension (PACE) reaction mixture contained 4 µl of DNA sample with a concentration of 10–20 ng/µl, 0.11 µl of 72x primer mix and 4 µl of a 2x PACE Genotyping Mastermix, standard ROX − 150 nM (3CR Bioscience Ltd, Welwyn Garden City, UK). The 72x SNP-specific primer mix contained two allele-specific forward primers and one common reverse primer. PACE PCR was performed in a PTC-221 MJ Research thermal cycler under the following conditions: initial denaturation 15 min at 94 °C, then 45 cycles of 94 °C − 20 s, 56 °C − 1 min and held at 10 °C. The PCR results were read using a Bio-Rad CFX96 machine (Bio-Rad Laboratories, Inc., Hercules, CA, USA) after reading cycle: 37 °C for 1 min, with Bio-Rad CFX Manager 3.1 software. PACE PCR primer sequences are listed in Table 1. Primers were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA, USA). For developing PACE markers, sequences with determined physical positions were selected on the wheat cytogenetic map (Danilova et al. 2014) or barley physical map (Mayer et al. 2012; Kersey et al. 2016).

Measuring β-glucan content

Seeds used for β-glucan measurements were collected from plants grown in CMP6050 chambers (Conviron, Canada) with a 14 h light: 10 h dark photoperiod and temperature settings of 20 °C day, 17 °C night until heading (45 days), then temperatures were set to 30 °C day and 20 °C night until maturity. The β-glucan content on a dry weight basis was measured in whole-grain flour in four replicates using a β-Glucan Assay Kit for mixed linkage 1,3:1,4-β-D-glucan in cereal grains (Megazyme, Ireland) according to the manufacturer's instructions (AACC Method 32-23.01). Grain samples were milled using a coffee grinder. The size of the flour particles was verified using 0.5 mm sieve to confirm that flour passes the 0.5 mm mesh. For the test, grain from 3–5 plants of each genotype was combined in equal ratios into 15 g samples, which were used for grain moisture measurement, calculation of 1000 kernel weight (TKW) and grinding. Moisture was measured in the grain using the near infrared light spectroscopy with a DA 7250 NIR Analyzer (Perten, Sweden) in four replicates. The average moisture value was used for all calculations. Statistical data analysis, calculation of average values and confidence at level equal 95% were performed using Microsoft Excel functions and ANOVA analysis tools.

Table 1 PCR primers

Marker	Forward primer 5′–3′	Reverse primer 5′–3′	PCR product, bp	Sequence	Position on chromosome
46ALcent	HEX-TTACTACTAGTTGCA GTTAGGGGA	CCCTTGGTAAATTCGATTCA	63	tplb0013b07[a]	7WL 0.1-0.2
46HLcent	FAM-TCCTTTACTACTAGT TGCAGTTAGGT		65		
43ALdist	HEX-CATGTTCAGTGACAG AAGGTT	TGAATGATGGACCAAATA CTGC	64	tplb0007o14	7WL 0.9
43HLdist	FAM-TACATGTTCAGTGTC TGGGGA		68		
30BLcent	HEX-TTGTGTATGTGATCC TGCCATC	TCGACAAGTACAGGTAAC CATCTT	56	tplb0013b07	7WL 0.1-0.2
30HLcent	FAM-TTGTGTATGTGATCC TGCCAAT				
25BLdist	HEX-AGTACATATTTAGCG ATGATTTTTTG	CAAGCAGTGACTAGTAAT TAAGCC	55	EST BE490073	7BL7-0.63-0.78, 7DL3-0.82-1.00
25HLdist	FAM-AGTAGATCTTTAGCC ATGATATTTTTC			7H phys. map	653 532 254: 653 580 997
35DLcent	HEX-GTGTCCAATAGAGGG AGAAAGGTAG	ATGCTACCAGCTCCTTCA CAAATAC	80	tplb0013b07	7WL 0.1-0.2
35HLcent	FAM-GTCCAATAGAGtGAG AAAGGTACCC		78		
40DLdist	HEX-GAGCCATCCTTCCCA TCACTTCCA	GGCAAAGATAATGGGGAG AGCAAC	65	tplb0007o14	7WL 0.9
40HLdist	FAM-AGAGCCATCCTTGCC ATCACTTCT		66		

[a]tplb0013b07 wheat cDNA; position on 7H physical map is 397,635,373 bp

Hordeum vulgare CslF6 mRNA (EU267181) position on 7H physical map is 378,385,072 bp

http://plants.ensembl.org/Hordeum_vulgare/Tools/Blast/Results

Results

Development of barley and wheat homoeologue specific shifted SNP markers

Previously, we produced a set of wheat lines with RobT chromosomes consisting of wheat group-7 short arms and 7HL of barley (Danilova et al. 2018a). Recombination events between 7HL and wheat group-7 long arms can be detected by the disassociation of molecular markers located in the proximal and distal regions of a targeted chromosome arm. Pairs of co-dominant SNP markers were developed for barley 7HL and each of the wheat homoeologues 7AL, 7BL and 7DL using genic sequences corresponding to physically mapped wheat cDNAs (Danilova et al. 2014) or sequences selected from the barley physical (Mayer et al. 2012; Kersey et al. 2016) and wheat deletion maps (Qi et al. 2004).

To identify barley specific SNPs, conservative among different cultivars, two sequences from barley cvs. Morex, Barke or Bowman (Mayer et al. 2012) were aligned with orthologous sequences of the wheat A-, B- and D-genomes from the flow-sorted wheat CS sequence database (Mayer et al. 2014) using NCBI BLAST functions (Altschul et al. 1990). To ensure selection of wheat SNPs that are not CS specific, but conservative among different wheat cultivars and distinctive for a particular wheat sub-genome, we added into alignments orthologous sequences of closest diploid wheat relatives (*Triticum urartu* Tumanian ex Gandilyan (Ling et al. 2018), *Aegilops speltoides* Tausch (Alaux et al. 2018) and *Ae. tauschii* Coss. (Luo et al. 2017) for A-, B- and D-genome alignments, respectively). Only SNPs present in both a wheat sub-genome and a corresponding diploid genome and in both barley cultivars were selected for designing primers for the PACE genotyping array. In alignments analyzed, we found very few three-genome SNPs (that differentiated barley and one out of three wheat sub-genomes) present in one site and suitable for primer design. Two of them were used to develop markers 25BHLdist and 30BHLcent. In contrast, two SNPs, specific for barley and one out of three wheat sub-genomes located several nucleotides apart were quite common (Fig. 1). We used these closely located SNP pairs to develop PACE assays consisting of two "shifted" forward primers specific for barley and one of wheat sub-genomes and one common reverse primer.

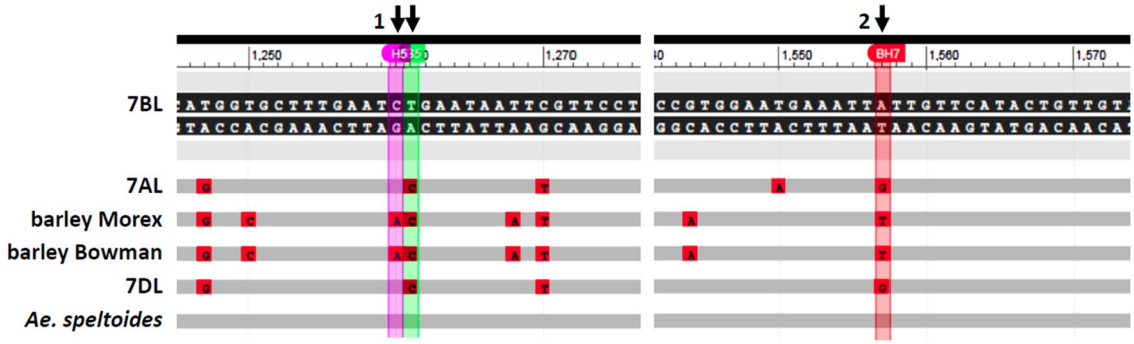

Fig. 1 Development of barley and wheat homoeologue-specific shifted SNP markers. Alignment of wheat, barley, and *Ae. speltoides* sequences (tplb0013b07). **1.** Barley and B/S-genome specific SNPs are located 1 bp apart (shifted). **2.** Three SNPs specific for genomes B/S, H and A/D are located at the same site

The shifted co-dominant barley–wheat homoeologue specific SNP primers were developed for all three wheat sub-genomes and barley at proximal and distal positions of the long arm of group-7 chromosomes (Table 1, Fig. 2) and used in PACE genotyping assays for further screenings for putative recombinants in the *ph1b* background. With these SNP assays, we run two PCRs for each plant. We demonstrated the approach with shifted primer sets to be effective for differentiating closely related wheat sub-genomes and barley.

Screening for wheat–barley recombinant chromosomes

The progenies of three sets of plants, homozygous for *ph1b* and heterozygous for each RobT, were screened using PACE genotyping assays. Putative recombinant plants with a disassociation of distal and proximal 7L markers or with undetermined results were verified by FGISH. In total, 2525 seedlings from three populations were screened (Table 2).

At the beginning of the experiment, we screened the first population for a 7AL-7HL recombination with a set of four dominant SNP markers: two 7HL markers (distal and proximal) and two 7AL markers (not shown). Genotyping from four PACE reactions accumulated a high portion of undetermined results, which explains the high percentage of plants from population-1, checked by FGISH (32.6% Table 2). We therefore switched to pairs of co-dominant SNP markers, and two PACE reactions had fewer undetermined results, which reduced the amount of FGISH screenings almost by three times. Even without taking into consideration undetermined results, the percentage of plants with marker disassociation was higher than the percentage of targeted 7WL-7HL recombinations confirmed by FGISH. Cytological analysis showed that a recombination between wheat 7L homoeologues resulted in the 'false' marker disassociation.

For each wheat genome, we found targeted wheat–barley recombinant chromosomes containing proximal barley segment and additional recombinants containing distal barley segments of different lengths (Fig. 3, Table 2). Previously, we located the *HvCslF6* gene on 7HL very close to the centromere (relative distance ~ 0.13) using FISH (Danilova et al. 2018a). According to the BLAST results, the sequence of *HvCslF6* gene (EU267181) is more proximal than the barley orthologue of wheat cDNA tplb0013b07 used for designing pericentromeric molecular markers (Table 1); their positions on the 7H physical map are 378,385,072 bp and 397,635,373 bp, respectively (Ensembl Plants release 42—Dec 2018 © EMBL-EBI; Kersey et al. 2018). Provided the correct ordering of the barley physical map, the recombinant barley translocation segments, detected by tplb0013b07-based markers should contain the *HvCslF6* gene. In the recombinant chromosomes T7AS·7HL-7AL and T7BS·7HL-7BL barley segments were very short, and we additionally verified the presence of *HvCslF6* gene by multicolor FISH. We found that in both recombinant chromosomes the HvCslF6 probe signals were located within the barley translocation (Fig. 3). A sizable barley segment in recombinant chromosome T7DS·7HL-7DL was long enough (~ 75% of 7DL) to contain *HvCslF6* (Fig. 3).

The selected plants with recombinant chromosomes were self-pollinated to produce homozygous introgression lines for preliminary β-glucan test. They also were crossed with CS to eliminate the *ph1b* mutant allele. Plants with recombinant chromosome T7AS·7HL-7AL were crossed with RobTs T7BS·7HL and T7DS·7HL, and their progenies were self-pollinated. Homozygous progenies were selected by PACE genotyping assays and confirmed by FGISH (Fig. S1). The spikes of these plants are shown in Fig. 4. We observed karyotype abnormalities in some plants homozygous for *ph1b* and translocation chromosomes T7AS·7HL-7AL and T7DS·7HL-7DL (Fig. S1), which did not involve the barley chromatin. Meiosis irregularity caused by the absence of the major homologous pairing locus *Ph1* can explain these

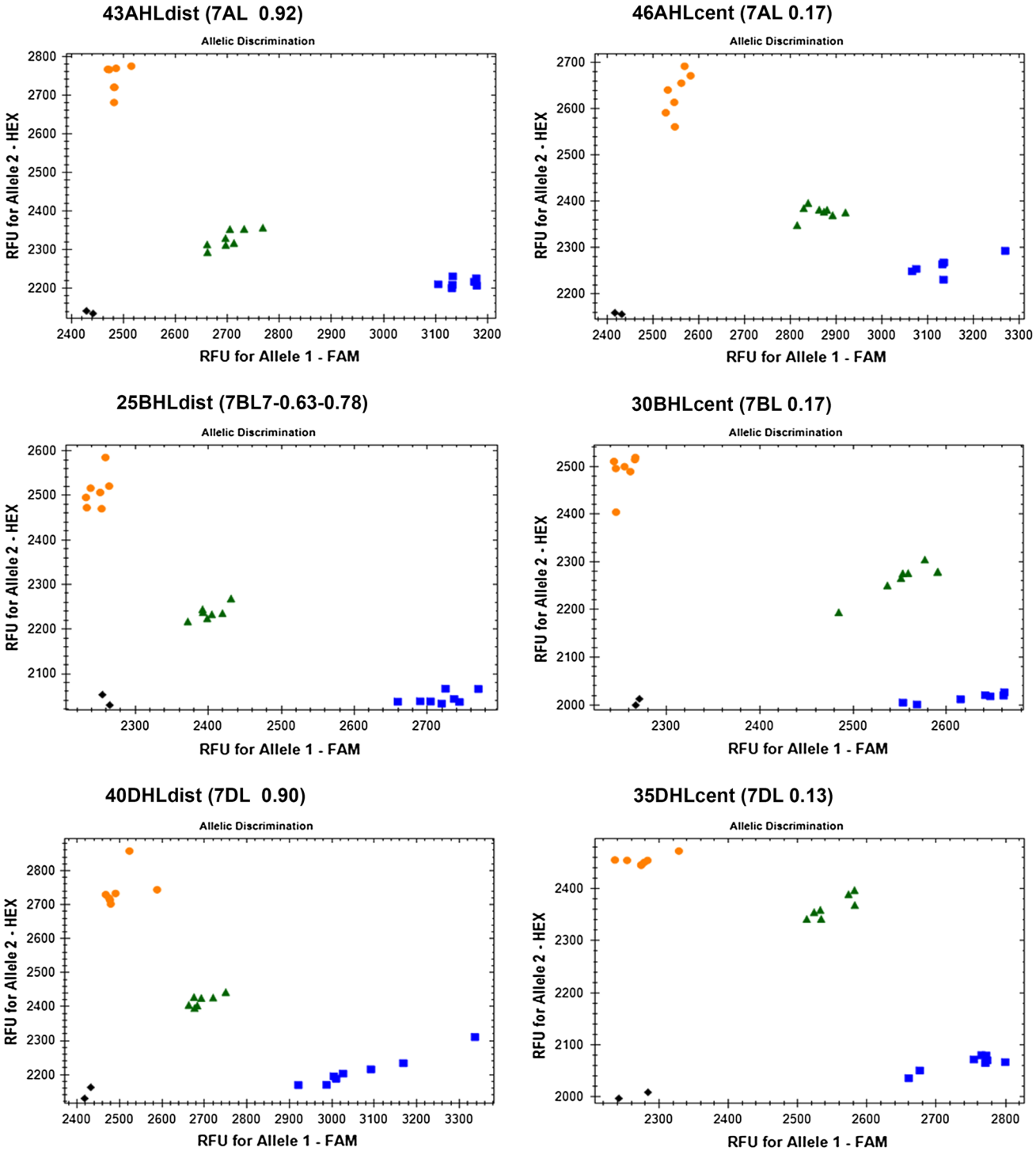

Fig. 2 PACE genotyping assays. Co-dominant barley–wheat subgenome specific SNP markers with known physical positions (designations correspond to Table 1). Left column—distal markers, right column—pericentromeric markers. Physical position of each sequence on group-7 wheat chromosomes is shown as a relative distance from the centromere (Danilova et al. 2014); 25BHLdist—EST BE490073 position is shown (Qi et al. 2004). Plants homozygous on barley alleles-blue squares, heterozygous on barley alleles-green triangles and homozygous on wheat alleles-yellow circles

Table 2 Screening for wheat–barley recombinant chromosomes

RobT	Total plants screened	Plants screened by FGISH	Number of 7WL-7HL recombinants found	% of recombinants
7AS·7HL	620	202 (32.6%)	3	0.48
7BS·7HL	1183	160 (13.5%)	2	0.17
7DS·7HL	722	72 (10.0%)	1	0.14

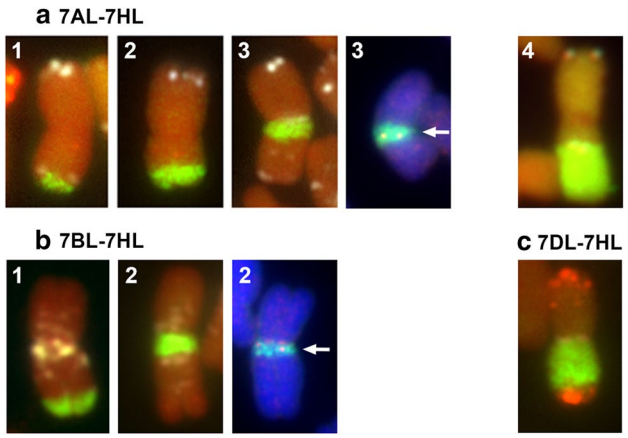

Fig. 3 Wheat–barley recombinant and translocation chromosomes. Summary. **a** Three 7AL-7HL recombinant chromosomes were found (1-3); translocation chromosome T7AS·7ALdel·7HL is also shown (4); **b, c.** Two 7BL-7HL and one 7DL-7HL recombinant chromosomes were found (bottom panel). The *CslF6* gene (arrows) was detected within barley pericentromeric chromatin on recombinant chromosomes T7AS·7HL-7AL and T7BS·7HL-7BL. FGISH images **a** (1-3, 4), **b** (1, 2) and **c**: barley chromatin is green, (GAA)n repeat is white, pAs1 repeat is red, chromosomes counterstained with DAPI are shown in orange. FISH images **a** 3, **b** 2: barley chromatin is green, *CslF6* gene is labeled in red, chromosomes counterstained with DAPI are blue

abnormalities. As a result, plants with T7DS·7HL-7DL had a low seed set (Fig. 4).

β-Glucan content in grain of wheat–barley translocation lines

We measured the β-glucan content in grain from three sets of plants homozygous for the wheat–barley recombinant chromosome and containing two copies of the *HvCslF6* gene from barley, substituting wheat orthologues from each of A, B or D wheat genomes; in two sets of plants containing four copies of *HvCslF6* substituting four wheat orthologues and in plants homozygous for translocation chromosomes with an inactivated barley centromere T7AS·7ALdel·7HL, containing two copies of *HvCslF6* in addition to wheat *CslF6* A-genome orthologues.

Plants were grown under high-temperature conditions, because previous experiments showed a higher β-glucan content under higher temperatures during grain filling (Swanston et al. 1997; Molina-Cano et al. 2007; Danilova et al. 2018a). Lines homozygous for the RobT translocations containing 7HL as well as the lines carrying *HvCslF6* in recombinant chromosomes showed statistically significant 16–20% increase in β-glucan content above the wheat control (Table 3). We did not observe any significant difference between plants homozygous for RobTs T7AS·7HL and T7BS·7HL and plants with the corresponding recombinant chromosomes, indicating that the *HvCslF6* gene carried in the proximal recombinant translocations is indeed conferring the increased β-glucan content. Grain from plants with RobT T7DS·7HL contained less β-glucan than plants with the corresponding recombinant chromosome. Grain from the line with T7AS·7ALdel·7HL contained slightly more β-glucan than the lines with *HvCslF6* substituting wheat 7A-orthologous gene, but it had TKW lower than T7AS·7HL-7AL. Considering the negative correlation between TKW and β-glucan content (Danilova et al. 2018a), the low TKW of T7AS·7ALdel·7HL can explain the difference in β-glucan. Thus, transfer of *HvCslF6* in the interstitial recombinants is largely or completely responsible for the observed increased grain β-glucan.

Two lines with four copies of *HvCslF6* (homozygous for T7AS·7HL-7AL, RobTs T7BS·7HL and T7AS·7HL-7AL, T7DS·7HL) significantly exceeded the β-glucan content of the CS wheat control and the corresponding lines with two copies of *HvCslF6* by 70–90% and 25–57%, respectively. Considering the highest TKW of the line, homozygous for T7AS·7HL-7AL and RobTs T7BS·7HL (Table 3), the highest β-glucan content in this line can be attributed to the presence of four *HvCslF6* copies.

Discussion

Shortening barley segments through induced homoeologous recombination

We used directed chromosome engineering to reduce the size of the introgressed barley chromatin by *ph1b*-induced homoeologous chromosome recombination (reviewed in Sears 1981; Friebe et al. 1996; Qi et al. 2007; Molnár-Láng and Linc 2015) in set of three wheat–barley compensating RobTs (Danilova et al. 2018a). Detection of wheat–alien homoeologous recombinants requires screening of large populations and is the most time and labor consuming step of chromosome engineering (Lukaszewski 2000; Lukaszewski et al. 2004; Qi et al. 2007). Application of PACE genotyping assays with co-dominant SNP markers proved to be

Fig. 4 Spike morphology. Plants homozygous on wheat–barley recombinant and translocation chromosomes involving 7HL and their combinations are shown. **1.** T7AS·7ALdel·7HL, **2.** T7AS·7HL-7AL, **3.** T7BS·7HL-7BL, **4.** T7DS·7HL-7DL, **CS** – cv Chinese Spring, **5.** RobT7AS·7HL, **6.** RobT7BS·7HL-7BL, **7.** RobT7DS·7HL, **8.** T7AS·7HL-7AL + RobT7BS·7HL, **9.** T7AS·7HL-7AL + RobT7DS·7HL. Bar = 1 cm

Table 3 Average β-glucan content and 1000 kernel weight (TKW) on a dry weight basis

Chromosome constitution[a], accession	β-glucan, %[c]	TKW, g
T7AS·7HL-7AL[b]	0.82 ± 0.05[d]	20.8
T7BS·7HL-7BL[b]	0.79 ± 0.05[d]	18.4
T7DS·7HL-7DL[b]	0.81 ± 0.01[d]	16.0
T7AS·7ALdel·7HL	0.97 ± 0.04[d]	17.4
T7AS·7HL-7AL, RobT 7BS·7HL	1.29 ± 0.07[d]	21.1
T7AS·7HL-7AL, RobT 7DS·7HL	1.15 ± 0.06[d]	14.4
RobT 7AS·7HL, TA5790	0.92 ± 0.05[d]	17.8
RobT 7BS·7HL, TA5792	0.80 ± 0.03[d]	16.7
RobT 7DS·7HL, TA5795 L1	0.72 ± 0.04	16.8
Chinese Spring (wheat)	0.68 ± 0.03	18.4
Betzes (barley)	5.56 ± 0.13[d]	33.6

[a]All lines were homozygous on the listed translocation chromosomes

[b]Lines were homozygous for the *ph1b* mutation

[c]Confidence intervals are shown

[d]Difference from the wheat control is statistically significant (confidence = 95%)

highly accurate, effective and significantly decreased time and effort, including cytological screening.

Chromosome rearrangements resulting in loss of colinearity between homoeologues may hinder meiotic pairing (Lukaszewski et al. 2004; Nasuda et al. 1998). Rey et al. (2018) found the 36 Mbp deletion at the terminal region of chromosome arm 7AL in CS addition line with 7H from barley Betzes produced by Islam et al. (1981) and used in our work. This rearrangement could prevent meiotic pairing and recombination between 7AL and 7HL. Crossing plants homozygous for RobT T7AS·7HL with the *ph1b* CS mutant stock allowed us to avoid this problem. The targeted wheat–barley recombinant chromosomes containing proximal barley segment and comprising the *HvCslF6* gene were obtained for all three wheat sub-genomes. The presence of *HvCslF6* was confirmed by marker positions, cytological analysis and β-glucan tests.

Rate and position of homologous recombination are affected by many factors: they have genetic control and are influenced by chromosome structure, genome sequence features and growing conditions (Darrier et al. 2017; Higgins et al. 2012; Jordan et al. 2018; Morgan et al. 2017; Phillips et al. 2015; Saintenac et al. 2011). The rate of *ph1b*-induced homoeologous recombination between wheat and alien chromosomes, originating from distantly related species of tertiary gene pool, such as barley, is even more unpredictable. It can be chromosome arm and genome specific and can vary significantly between the different genomes and chromosomes of wheat and alien introgressions. For example, in a chromosome engineering experiment involved a member of tertiary gene pool *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey ($2n = 6x = 42$, JJJ^{s}J^{s}SS), we observed a 0.06% yield of *ph1b*-induced homoeologous recombinants (per plant basis) between chromosome arms 7BL and 7S#3L (Danilova et al. 2017b). The yield of *ph1b*-induced recombinants between arms 4DS and 4J^{s}S from *Th. intermedium* was 2% (Friebe et al. 2009). Twofold differences were observed in the recombination frequencies between rye (*Secale cereale* L.) chromosome arm 1RS and wheat 1BS or 1DS in RobTs T1RS·1BL and T1RS·1DL, as well as a substantial seasonal variation (Lukaszewski 2000).

In our experiment, the recombination frequency between group-7 arms from the T7WS·7HL RobTs, estimated as the yield of plants with wheat–barley

recombinant chromosomes (per plant basis), was relatively low for the B and D genomes (0.17 and 0.14%) and higher for A genome (0.48%). In spite of the low recombination rate between 7HL and its wheat homoeologues, two out of six recombination events occurred in the pericentromeric region, although recombination at the distal ends was observed as well. This was surprising, because even crossovers between homologues preferentially happen at the distal ends during both wheat and barley meiosis (Darrier et al. 2017; Gutierrez-Gonzalez et al. 2019; Higgins et al. 2012; Jordan et al. 2018). Genetic factors influence recombination in wheat and barley (reviewed in Morgan et al. 2017; Phillips et al. 2015). Several QTLs influencing recombination rate and position were detected in long arms of wheat group-7 chromosomes, including QTLs influencing either total recombination rate, or recombination at pericentromeric regions (Jordan et al. 2018). Possibly, orthologous genes influencing position of recombination are present in barley 7HL and may affect pericentromeric crossovers. On the other hand, pairing among homoeologues may not follow the recombination patters described for homologues. Lukaszewski et al. (2004) observed a significant difference in the frequency and location of *ph1b*-induced recombination between rye chromosome arms 2RS and 2RL from RobTs T2RS·2BL, T2BS·2RL, respectively, and wheat chromosome 2B. The recombination between arms 2RL-2BL was shifted toward the terminal 25% of the arm and had frequency 14.65% (per plant basis). Unlike 2RL, rye chromosome arm 2RS lost colinearity to wheat homoeologues because of rearrangements present at the distal segment, namely terminal translocations from 6S and 7S (Devos et al. 1993; Martis et al. 2013). Thus, 2RS-2BS pairing should be reduced or absent. However, some 2RS-2BS recombination (0.48% per plant basis) was observed but was absent at the terminal region and shifted to the proximal 65%-segment of the arm. Lukaszewski et al. (2004) assumed that the fact of 2RS-2BS recombination and its shift to the interstitial and pericentromeric regions can result from a disruption of normal terminal pairing initiation between 2RS and 2BS. Possibly, under specific conditions, including a *ph1b*-induced homoeologous pairing or pairing of truncated homologues (Curtis et al. 1991; Jones et al. 2002; Qi et al. 2002), it may be initiated at random points along the arm or progress from the opposite arm of a RobT.

The influence of growing conditions on recombination rate makes the success of chromosome engineering even more unpredictable (Lukaszewski 2000). Temperature affects both rate and chromosome position of a crossover (reviewed in Morgan et al. 2017). In barley, a moderate temperature increase during meiosis results in significant shift in chiasmata distribution from distal to interstitial/proximal positions and influences different chromosome arms unequally (Higgins et al. 2012; Phillips et al. 2015).

Finally, sequence features, including the presence of particular recombination-associated repetitive motifs or genic sequences, can influence position and frequency of recombination events in wheat and barley (Darrier et al. 2017; Higgins et al. 2012; Saintenac et al. 2011).

β-Glucan content in grain

The Healthy U.S. Style Eating Pattern proposes about 100 g of whole grain daily intake for adults (U.S. Department of Health and Human Services and U.S. Department of Agriculture 2015). To obtain a significant decrease in total and LDL cholesterol, at least 2 g per day of soluble fiber should be consumed (Brown et al. 1999). If wheat whole-meal flour is a main source of soluble fiber, the target level of β-glucan in wheat grain should be 2% per dry weight. Wheat grain dietary fiber includes mostly arabinoxylan, whereas the content of soluble β-glucan is lower than 1% (Beresford and Stone 1983; Havrlentova and Kraic 2006; reviewed in Collins et al. 2010). Wheat germplasm resources do not contain genetic variation for a breeding program to generate elite wheat varieties with β-glucan content of 2% per dry weight or higher (Pritchard et al. 2011). In this situation, broadening of wheat germplasm diversity through alien gene introgression from the tertiary gene pool can be helpful.

High β-glucan content was detected in grain of some *Aegilops* species from the tertiary gene pool, *Ae. umbellulata*, *Ae. markrafii* and tetraploid species containing U, M and C genomes (Marcotuli et al. 2019; Rakszegi et al. 2017). Wheat disomic addition lines containing chromosomes 5U, 7U and 7M from *Ae. geniculata* Roth and *Ae. biuncialis* Vis. exceeded β-glucan content of parental wheat line (Rakszegi et al. 2017, 2019). Introgression of this trait to wheat by induced homoeologous recombination can be challenging for some *Aegilops* species because C and U genomes are known to be rearranged, i.e., not colinear to wheat and the structure of other genomes still need to be established (Danilova et al. 2017a; Molnar et al. 2016; Zhang et al. 1998). Barley chromosomes preserved colinearity and can recombine with wheat homoeologues. Barley was intensively studied as a donor of high grain β-glucan for wheat. Wheat–barley addition, substitution and RobT lines showed increased β-glucan content (Cseh et al. 2011, 2013; Danilova et al. 2018a; Turkosi et al. 2018).

Earlier, we introgressed barley *HvCslF6* to wheat in the form of three compensating RobTs T7AS·7HL, T7BS·7HL and T7DS·7HL and increased the grain β-glucan content to 0.8–1%. Although these values statistically exceeded the wheat control, they were still much lower than the 5.8% of the barley parent (Danilova et al. 2018a) or a target of 2%. Burton et al. (2011) showed that addition of *HvCslF6*

copies in transgenic barley results in a more than 80% increase in grain β-glucan content. We therefore hypothesized that increasing the number of *HvCslF6* copies in hexaploid wheat by combining several engineered wheat homoeologous chromosomes with barley introgressions could significantly increase the total β-glucan content. However, because meiotic pairing in wheat relies on homology at the telomeric chromosome ends, two or three pairs of RobTs with 7HL present in one plant, would cause meiotic abnormalities (formation of multivalents) and lead to low fertility. We had produced a few plants with four copies of *HvCslF6* by combining RobTs T7AS·7HL and T7DS·7HL and selecting homozygous lines, but they have karyotype abnormalities and their fertility was so low, that we were not able to produce enough seeds for β-glucan test (data not shown). In contrast, interstitial recombinant chromosomes can be combined in one wheat line and have regular bivalent pairing, because the respective wheat distal ends have been restored. Furthermore, large translocations can have negative impact on yield. Lines with barley RobTs showed a decrease in grain yield, which may be caused by incomplete compensation of 7H for the substituted wheat chromatin (Danilova et al. 2018a). Reduction of the barley chromatin might solve this problem. We have produced wheat–barley recombinant chromosomes consisting of wheat goup-7 short arms, the proximal part of the 7HL arm harboring the *HvCslF6* gene and the distal part derived from a wheat group-7 long arm (7AS·7HL-7AL, 7BS·7HL-7BL and 7DS·7HL-7DL). These lines exceeded the wheat control for β-glucan content and were similar to lines with wheat–barley RobTs. We did not estimate the productivity of the recombinant lines, because they were homozygous for *ph1b* and had some karyotype abnormalities that resulted in lower fertility. In the preliminary experiment, we combined two different RobTs with recombinant chromosome 7AS·7HL-7AL to analyze β-glucan content in plants with four copies of *HvCslF6* and normal fertility. These two lines had significantly higher grain β-glucan content than plants with two copies of *HvCslF6*.

It has been reported that the wheat genes orthologous to barley *HvCslF6* located on the long arm of all group-7 chromosomes (Schreiber et al. 2014, Danilova et al. 2018a), expressed in grain and influenced β-glucan content (Marcotuli et al. 2018; Nemeth et al. 2010). It is not known which of the wheat *CslF* A-, B- or D-genome orthologues are functional and more important for β-glucan synthesis in grain and how wheat and barley genes, controlling synthesis of grain cell wall components, would interact in introgression lines. To verify whether *HvCslF6*, present together with all three wheat orthologous genes, shows a higher β-glucan content over lines where *HvCslF6* substituted for one of wheat orthologues, we included to the analysis a line homozygous for T7AS·7ALdel·7HL and containing both 7A *TaCslF6* and barley *HvCslF6*. This line had β-glucan content similar to the RobT T7AS·7HL and statistically higher than three lines homozygous for recombinant chromosomes. However, this exceeding may be explained by a variation in grain size, as β-glucan content has been shown negatively correlating with TKW (Danilova et al. 2018a). Both T7AS·7ALdel·7HL and RobT 7AS·7HL had a lower TKW than recombinant 7AS·7HL-7AL. Thus, most likely, the substitution of wheat 7A-*CslF6* by barley orthologue *HvCslF6* does not influence negatively the β-glucan content in wheat grain. In our current and previous experiment (Danilova et al. 2018a), the line with RobT 7DS·7HL showed lower β-glucan than two other RobTs. Among 2 lines with four copies of *HvCslF6*, the line with barley segment present in 7AL and 7DL showed lower β-glucan than the line with *HvCslF6* substituting wheat orthologues in 7AL and 7BL. According to Marcotuli et al. (2019), grain β-glucan of hexaploid wheat (genome AABBDD) exceeded that of tetraploid wheats (genome AABB) by almost twofolds. Similary grain β-glucan of *Ae. tauschii* (genome DD) exceeded A-genome diploids, *T. urartu* Tumanian ex Gandilyan and *T. monococcum* L. almost 4 times. Based on these data, we may assume that the D-genome *CslF6* gene contributes more to grain β-glucan synthesis in hexaploid wheat than the two other orthologues.

Here, we have shown that replacement of the proximal region of the long arm of wheat group-7 chromosomes with the homoeologous barley region carring the *HvCslF6* gene gives a marked increase in β-glucan. The replacement of the wheat *TaCslF6* genes in several sub-genomes of wheat opens possibilities for stacking multiple copies of *HvCslF6*. We demonstrated that combining four copies of the allele gives a further marked increase in β-glucan. Our results open up prospects for a further increase in β-glucan in lines with multiple copies of *HvCslF6* and production of heart healthy whole-wheat products. Work is now in progress for transferring and combining wheat–barley recombinant chromosomes in elite wheat lines.

Author contribution statement BF and JP conceived the project; TVD performed the experiments, analyzed data and wrote the manuscript; all authors contributed to the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Alaux M, Rogers J, Letellier T, Flores R, Alfama F, Pommier C, Mohellibi N, Durand S, Kimmel E, Michotey C et al (2018) Linking the International Wheat Genome Sequencing Consortium bread wheat reference genome sequence to wheat genetic and phenomic data. Genome Biol 19:111

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Beresford G, Stone BA (1983) (1-]3), (1-]4)-Beta-d-glucan content of triticum grains. J Cereal Sci 1:111–114

Brown L, Rosner B, Willett WW, Sacks FM (1999) Cholesterol-lowering effects of dietary fiber: a meta-analysis. Am J Clin Nutr 69:30–42

Burton RA, Jobling SA, Harvey AJ, Shirley NJ, Mather DE, Bacic A, Fincher GB (2008) The genetics and transcriptional profiles of the cellulose synthase-like HvCslF gene family in barley. Plant Physiol 146:1821–1833

Burton RA, Collins HM, Kibble NAJ, Smith JA, Shirley NJ, Jobling SA, Henderson M, Singh RR, Pettolino F, Wilson SM, Bird AR, Topping DL, Bacic A, Fincher GB (2011) Over-expression of specific HvCslF cellulose synthase-like genes in transgenic barley increases the levels of cell wall (1,3;1,4)-beta-D-glucans and alters their fine structure. Plant Biotechnol J 9:117–135

Collins HM, Burton RA, Topping DL, Liao ML, Bacic A, Fincher GB (2010) Variability in fine structures of noncellulosic cell wall polysaccharides from cereal grains: potential importance in human health and nutrition. Cereal Chem 87:272–282

Cseh A, Kruppa K, Molnar I, Rakszegi M, Dolezel J, Molnar-Lang M (2011) Characterization of a new 4BS.7HL wheat-barley translocation line using GISH, FISH, and SSR markers and its effect on the beta-glucan content of wheat. Genome 54:795–804

Cseh A, Soos V, Rakszegi M, Turkoesi E, Balazs E, Molnar-Lang M (2013) Expression of HvCslF9 and HvCslF6 barley genes in the genetic background of wheat and their influence on the wheat beta-glucan content. Ann Appl Biol 163:142–150

Cui SW, Wu Y, Ding H (2013) The range of dietary fibre ingredients and a comparison of their technical functionality. Elsevier Science, ProQuest Ebook Central

Curtis CA, Lukaszewski AJ, Chrzastek M (1991) Metaphase I pairing of deficient chromosomes and genetic mapping of deficiency breakpoints in common wheat. Genome 34:553–560

Danilova TV, Friebe B, Gill BS (2012) Single-copy gene fluorescence in situ hybridization and genome analysis: Acc-2 loci mark evolutionary chromosomal rearrangements in wheat. Chromosoma 121:597–611

Danilova TV, Friebe B, Gill BS (2014) Development of a wheat single gene FISH map for analyzing homoeologous relationship and chromosomal rearrangements within the Triticeae. Theor Appl Genet 127:715–730

Danilova TV, Akhunova AR, Akhunov ED, Friebe B, Gill BS (2017a) Major structural genomic alterations can be associated with hybrid speciation in *Aegilops markgrafii* (Triticeae). Plant J 92:317–330

Danilova TV, Zhang G, Liu W, Friebe B, Gill BS (2017b) Homoeologous recombination-based transfer and molecular cytogenetic mapping of a wheat streak mosaic virus and *Triticum* mosaic virus resistance gene *Wsm3* from *Thinopyrum intermedium* to wheat. Theor Appl Genet 130:549–556

Danilova TV, Friebe B, Gill BS, Poland J, Jackson E (2018a) Development of a complete set of wheat-barley group-7 Robertsonian translocation chromosomes conferring an increased content of beta-glucan. Theor Appl Genet 131:377–388

Danilova TV, Friebe B, Gill BS, Poland J, Jackson E (2018b) Chromosome rearrangements caused by double monosomy in wheat-barley group-7 substitution lines. Cytogenet Gen Res 154:45–55

Darrier B, Rimbert H, Balfourier F, Pingault L, Josselin AA, Servin B, Navarro J, Choulet F, Paux E, Sourdille P (2017) High-resolution mapping of crossover events in the hexaploid wheat genome suggests a universal recombination mechanism. Genetics 206:1373–1388

Devos KM, Atkinson MD, Chinoy CN, Francis HA, Harcourt RL, Koebner RMD, Liu CJ, Masojc P, Xie DX, Gale MD (1993) Chromosomal rearrangements in the rye genome relative to that of wheat. Theor Appl Genet 85:673–680

Doblin MS, Pettolino FA, Wilson SM, Campbell R, Burton RA, Fincher GB, Newbigin E, Bacic A (2009) A barley cellulose synthase-like *CSLH* gene mediates (1,3;1,4)-beta-D-glucan synthesis in transgenic *Arabidopsis*. Proc Natl Acad Sci USA 106:5996–6001

FAO (2018) FAOSTAT. Food balance sheets. Crop production. http://www.fao.org/faostat/en/#compare (verified 25 June 2018). FAO, Rome, Italy

Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: Current status. Euphytica 91:59–87

Friebe B, Qi LL, Wilson DL, Chang ZJ, Selfers DL, Martin TJ, Fritz AK, Gill BS (2009) Wheat-*Thinopyrum intermedium* recombinants resistant to wheat streak mosaic virus and *Triticum* mosaic virus. Crop Sci 49:1221–1226

Gutierrez-Gonzalez JJ, Mascher M, Poland J, Muehlbauer GJ (2019) Dense genotyping-by-sequencing linkage maps of two Synthetic W7984 × Opata reference populations provide insights into wheat structural diversity. Sci Rep-UK 9:1793

Han F, Ullrich SE, Chirat S, Menteur S, Jestin L, Sarrafi A, Hayes PM, Jones BL, Blake TK, Wesenberg DM, Kleinhofs A, Kilian A (1995) Mapping of beta-glucan content and beta-glucanase activity loci in barley grain and malt. Theor Appl Genet 91:921–927

Havrlentova M, Kraic J (2006) Content of beta-D-glucan in cereal grains. J Food Nutr Res 45:97–103

Higgins JD, Perry RM, Barakate A, Ramsay L, Waugh R, Halpin C, Armstrong SJ, Franklin FC (2012) Spatiotemporal asymmetry of the meiotic program underlies the predominantly distal distribution of meiotic crossovers in barley. Plant Cell 24:4096–4109

Houston K, Russell J, Schreiber M, Halpin C, Oakey H, Washington JM, Booth A, Shirley N, Burton RA, Fincher GB, Waugh R (2014) A genome wide association scan for (1,3;1,4)-beta-glucan content in the grain of contemporary 2-row spring and winter barleys. BMC Genom 15:907

Islam AKMR, Shepherd KW, Sparrow DHB (1981) Isolation and characterization of euplasmic wheat-barley chromosome addition lines. Heredity 46:161–174

Jones E, Rybka K, Lukaszewski J (2002) The effect of a deficiency and a deletion on recombination in chromosome 1BL in wheat. Theor Appl Genet 104:1204–1208

Jordan KW, Wang S, He F, Chao S, Lun Y, Paux E, Sourdille P, Sherman J, Akhunova A, Blake NK, Pumphrey MO, Glover K, Dubcovsky J, Talbert L, Akhunov ED (2018) The genetic architecture of genome-wide recombination rate variation in allopolyploid wheat revealed by nested association mapping. Plant J 95:1039–1054

Kato A, Lamb JC, Birchler JA (2004) Chromosome painting using repetitive DNA sequences as probes for somatic chromosome identification in maize. Proc Natl Acad Sci USA 101:13554–13559

Kato A, Albert PS, Vega JM, Birchler JA (2006) Sensitive fluorescence in situ hybridization signal detection in maize using directly labeled probes produced by high concentration DNA polymerase nick translation. Biotech Histochem 81:71–78

Kersey PJ, Allen JE, Armean I, Boddu S, Bolt BJ, Carvalho-Silva D, Christensen M, Davis P, Falin LJ, Grabmueller C et al (2016) Ensembl Genomes 2016: more genomes, more complexity. Nucl Acids Res 44:D574–D580

Kersey PJ, Allen JE, Allot A, Barba M, Boddu S, Bolt BJ, Carvalho-Silva D, Christensen M, Davis P, Grabmueller C et al (2018) Ensembl Genomes 2018: an integrated omics infrastructure for non-vertebrate species. Nucl Acids Res 46:D802–D808

Kim H-S, Park K-G, Baek S-B, Kim J-G (2011) Inheritance of (1–3)(1–4)-beta-D-glucan content in barley (*Hordeum vulgare* L.). J Crop Sci Biotechnol 14:239–245

Li JZ, Baga M, Rossnagel BG, Legge WG, Chibbar RN (2008) Identification of quantitative trait loci for beta-glucan concentration in barley grain. J Cereal Sci 48:647–655

Ling HQ, Ma B, Shi X, Liu H, Dong L, Sun H, Cao Y, Gao Q, Zheng S, Li Y et al (2018) Genome sequence of the progenitor of wheat A subgenome *Triticum urartu*. Nature 557:424–428

Lukaszewski AJ (2000) Manipulation of the 1RS.1BL translocation in wheat by induced homoeologous recombination. Crop Sci 40:216–225

Lukaszewski AJ, Rybka K, Korzun V, Malyshev SV, Lapinski B, Whitkus R (2004) Genetic and physical mapping of homoeologous recombination points involving wheat chromosome 2B and rye chromosome 2R. Genome 47:36–45

Luo MC, Gu YQ, Puiu D, Wang H, Twardziok SO, Deal KR, Huo N, Zhu T, Wang L, Wang Y et al (2017) Genome sequence of the progenitor of the wheat D genome *Aegilops tauschii*. Nature 551:498–502

Manninen I, Schulman AH (1993) Bare-1, a *Copia*-like retroelement in barley (*Hordeum vulgare* L.). Plant Mol Biol 22:829–846

Marcotuli I, Gadaleta A, Mangini G, Signorile AM, Zacheo SA, Blanco A, Simeone R, Colasuonno P (2017) Development of a high-density SNP-based linkage map and detection of QTL for beta-glucans, protein content, grain yield per spike and heading time in durum wheat. Int J Mol Sci 18:1329. https://doi.org/10.3390/ijms18061329

Marcotuli I, Colasuonno P, Blanco A, Gadaleta A (2018) Expression analysis of cellulose synthase-like genes in durum wheat. Sci Rep 8:15675

Marcotuli I, Colasuonno P, Cutillo S, Simeone R, Blanco A, Gadaleta A (2019) B-glucan content in a panel of *Triticum* and *Aegilops* genotypes. Genet Res Crop Evol 66:897–907

Martis MM, Zhou RN, Haseneyer G, Schmutzer T, Vrana J, Kubalakova M, Konig S, Kugler KG, Scholz U, Hackauf B, Korzun V, Schon CC, Dolezel J, Bauer E, Mayer KFX, Stein N (2013) Reticulate evolution of the rye genome. Plant Cell 25:3685–3698

Mayer KFX, Waugh R, Langridge P, Close TJ, Wise RP, Graner A, Matsumoto T, Sato K, Schulman A, Muehlbauer GJ et al (2012) A physical, genetic and functional sequence assembly of the barley genome. Nature 491:711–717

Mayer KFX, Rogers J, Dolezel J, Pozniak C, Eversole K, Feuillet C, Gill B, Friebe B, Lukaszewski AJ, Sourdille P et al (2014) A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science 345:286

Molina-Cano JL, Moralejo M, Elia M, Munoz P, Russell JR, Perez-Vendrell AM, Ciudad F, Swanston JS (2007) QTL analysis of a cross between European and North American malting barleys reveals a putative candidate gene for beta-glucan content on chromosome 1H. Mol Breed 19:275–284

Molnar I, Vrana J, Buresova V, Capal P, Farkas A, Darko E, Cseh A, Kubalakova M, Molnar-Lang M, Dolezel J (2016) Dissecting the U, M, S and C genomes of wild relatives of bread wheat (*Aegilops* spp.) into chromosomes and exploring their synteny with wheat. Plant J 88:452–467

Molnár-Láng M, Linc G (2015) Wheat–barley hybrids and introgression lines. In: Molnár-Láng M, Ceoloni C, Doležel J (eds) Alien introgression in wheat. Cytogenetics, molecular biology, and genomics. Springer, Cham, pp 315–346

Morgan CH, Zhang H, Bomblies K (2017) Are the effects of elevated temperature on meiotic recombination and thermotolerance linked via the axis and synaptonemal complex? Philos Trans R Soc Lond B Biol Sci. https://doi.org/10.1098/rstb.2016.0470

Nagaki K, Tsujimoto H, Isono K, Sasakuma T (1995) Molecular characterization of a tandem repeat, Afa family, and distribution among Triticeae. Genome 38:479–486

Nasuda S, Friebe B, Busch W, Kynast RG, Gill BS (1998) Structural rearrangement in chromosome 2M of *Aegilops comosa* has prevented the utilization of the Compair and related wheat-*Ae. comosa* translocations in wheat improvement. Theor Appl Genet 96:780–785

Nemeth C, Freeman J, Jones HD, Sparks C, Pellny TK, Wilkinson MD, Dunwell J, Andersson AA, Aman P, Guillon F, Saulnier L, Mitchell RA, Shewry PR (2010) Down-regulation of the *CSLF6* gene results in decreased (1,3;1,4)-beta-D-glucan in endosperm of wheat. Plant Physiol 152:1209–1218

Phillips D, Jenkins G, Macaulay M, Nibau C, Wnetrzak J, Fallding D, Colas I, Oakey H, Waugh R, Ramsay L (2015) The effect of temperature on the male and female recombination landscape of barley. New Phytol 208:421–429

Pritchard JR, Lawrence GJ, Larroque O, Li Z, Laidlaw HK, Morell MK, Rahman S (2011) A survey of beta-glucan and arabinoxylan content in wheat. J Sci Food Agric 91:1298–1303

Qi LL, Friebe B, Gill BS (2002) A strategy for enhancing recombination in proximal regions of chromosomes. Chromosome Res 10:645–654

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD, Akhunov ED, Dvorak J, Linkiewicz AM, Ratnasiri A et al (2004) A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. Genetics 168:701–712

Qi L, Friebe B, Zhang P, Gill BS (2007) Homoeologous recombination, chromosome engineering and crop improvement. Chromosome Res 15:3–19

Rakszegi M, Molnar I, Lovegrove A, Darko E, Farkas A, Lang L, Bedo Z, Dolezel J, Molnar-Lang M, Shewry P (2017) Addition of *Aegilops* U and M chromosomes affects protein and dietary fiber content of wholemeal wheat flour. Front Plant Sci 8:1529

Rakszegi M, Darko E, Lovegrove A, Molnar I, Lang L, Bedo Z, Molnar-Lang M, Shewry P (2019) Drought stress affects the protein and dietary fiber content of wholemeal wheat flour in wheat/*Aegilops* addition lines. PLoS ONE 14:e0211892

Rey E, Abrouk M, Keeble-Gagnere G, Karafiatova M, Vrana J, Balzergue S, Soubigou-Taconnat L, Brunaud V, Martin-Magniette ML, Endo TR, Bartos J, International Wheat Genome Sequencing C, Appels R, Dolezel J (2018) Transcriptome reprogramming due to the introduction of a barley telosome into bread wheat affects more barley genes than wheat. Plant Biotech J 16:1767–1777

Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L (2019) Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. Lancet 393:434–445

Saintenac C, Faure S, Remay A, Choulet F, Ravel C, Paux E, Balfourier F, Feuillet C, Sourdille P (2011) Variation in crossover rates across a 3-Mb contig of bread wheat (*Triticum aestivum*) reveals the presence of a meiotic recombination hotspot. Chromosoma 120:185–198

Schreiber M, Wright F, MacKenzie K, Hedley PE, Schwerdt JG, Little A et al (2014) The barley genome sequence assembly reveals three additional members of the *CslF* (1,3;1,4)-β-glucan synthase gene

family. PLoS ONE 9(3):e90888. https://doi.org/10.1371/journal.pone.0090888
Sears ER (1981) Transfer of alien genetic material to wheat. In: Evans IT, Peacock WJ (eds) Wheat science—today and tomorrow. Cambridge University Press, Cambridge, pp 75–89
Sibakov J, Lehtinen P, Poutanen K (2013) Cereal brans as dietary fibre ingredients. Elsevier Science, ProQuest Ebook Central, Amsterdam, pp 153–168
Swanston JS, Ellis RP, PerezVendrell A, Voltas J, MolinaCano JL (1997) Patterns of barley grain development in Spain and Scotland and their implications for malting quality. Cereal Chem 74:456–461
Tonooka T, Aoki E, Yoshioka T, Taketa S (2009) A novel mutant gene for (1-3,1-4)-beta-D-glucanless grain on barley (*Hordeum vulgare* L.) chromosome 7H. Breed Sci 59:47–54
Turkosi E, Darko E, Rakszegi M, Molnar I, Molnar-Lang M, Cseh A (2018) Development of a new 7BS.7HL winter wheat-winter barley Robertsonian translocation line conferring increased salt tolerance and (1,3;1,4)-beta-D-glucan content. PLoS One 13:0206248
U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015–2020 dietary guidelines for Americans. 8th edn. December 2015. https://health.gov/dietaryguidelines/2015/guidelines/
Waugh R, McLean K, Flavell AJ, Pearce SR, Kumar A, Thomas BBT, Powell W (1997) Genetic distribution of Bare-1-like retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphisms (S-SAP). Mol Gen Genet 253:687–694
Wicker T, Taudien S, Houben A, Keller B, Graner A, Platzer M, Stein N (2009) A whole-genome snapshot of 454 sequences exposes the composition of the barley genome and provides evidence for parallel evolution of genome size in wheat and barley. Plant J 59:712–722
Zhang H, Jia J, Gale MD, Devos KM (1998) Relationships between the chromosomes of *Aegilops umbellulata* and wheat. Theor Appl Genet 96:69–75