Development of a new 7BS.7HL winter wheat-winter barley Robertsonian translocation line conferring increased salt tolerance and (1,3;1,4)-β-D-glucan content

Edina Türkösi1, Eva Darko2, Marianna Rakszegi3, István Molnár4, Márta Molnár-Láng1*, András Cseh5*  

1 Department of Plant Genetic Resources, Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary, 2 Department of Plant Physiology, Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary, 3 Cereal Breeding Department, Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary, 4 Maize Breeding Department, Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary, 5 Molecular Breeding Department, Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary  

* cseh.andras@agrar.mta.hu (AC); langne.molnar.marta@gmail.com (MML)

Abstract

Interspecific hybridization between bread wheat (Triticum aestivum, 2n = 42) and related species allows the transfer of agronomic and quality traits, whereby subsequent generations comprise an improved genetic background and can be directly applied in wheat breeding programmes. While wild relatives are frequently used as sources of agronomically favourable traits, cultivated species can also improve wheat quality and stress resistance. A salt-tolerant ‘Asakaze’/’Manas’ 7H disomic addition line (2n = 44) with elevated β-glucan content, but with low fertility and an unstable genetic background was developed in an earlier wheat-barley prebreeding programme. The aim of the present study was to take this hybridization programme further and transfer the favourable barley traits into a more stable genetic background. Taking advantage of the breakage-fusion mechanism of univalent chromosomes, the ‘Rannaya’ winter wheat 7B monosomic line was used as female partner to the 7H addition line male, leading to the development of a compensating wheat/barley Robertsonian translocation line (7BS.7HL centric fusion, 2n = 42) exhibiting higher salt tolerance and elevated grain β-glucan content. Throughout the crossing programme, comprising the F1-F4 generations, genomic in situ hybridization, fluorescence in situ hybridization and chromosome-specific molecular markers were used to trace and identify the wheat and barley chromatin. Investigations on salt tolerance during germination and on the (1,3;1,4)-β-D-glucan (mixed-linkage glucan [MLG]) content of the seeds confirmed the salt tolerance and elevated grain MLG content of the translocation line, which can be directly applied in current wheat breeding programmes.
Introduction

The exponential increase in the world population and the recent challenges imposed by the changing climate make it essential for crop breeding programmes to put the emphasis on developing higher-yielding cultivars with better nutritional parameters. Bread wheat (*Triticum aestivum*, 2n = 6x = 42), one of the most important crop species, is significantly affected by environmental stresses that seriously reduce its productivity [1,2]. Among the environmental factors, soil salinization, which has been worsened by climate change, is one of the most constraining abiotic stresses threatening wheat yields. The breeding of salinity-tolerant, high-yielding varieties is therefore a valuable solution to ensure food security. This can be achieved via prebreeding programmes that transfer genes responsible for salt tolerance from related species into wheat. Barley (*Hordeum vulgare*, 2n = 2x = 14) is one of the most salt-tolerant crops, with significant variation among genotypes [3–5]. It can be cultivated on saline soil as it maintains growth despite accumulating Na⁺ in the leaves. Salt stress causes a complete metabolic rearrangement in the cells, affecting the sugar, amino acid and polyamine metabolism, and ion and redox homeostasis [6–8]. Similarly to other plant species, salt tolerance in barley is inherited as a polygenic trait with numerous quantitative trait loci (QTLs) mapped on several chromosomes including 7H [9,10].

Barley has a place in healthy diet, as the grain has a high content of (1,3;1,4)-β-D-glucan (MLG), a polysaccharide that helps to lower blood cholesterol and regulates blood sugar levels in humans [11,12]. The cellulose synthase-like F6 (*HvCslF6*) gene that encodes the putative MLG synthase has been mapped to barley chromosome 7H [13,14]. Located within the centromeric region of the long arm (7HL), the *HvCslF6* gene has been proved to play a key role in controlling β-glucan biosynthesis, and its down-regulation results in decreased MLG within the endosperm [15,16].

Barley belongs to the tertiary gene pool of wheat, so abiotic stress resistance and advantageous composition parameters can be transferred into wheat via wide hybridization. Crossing wheat with barley is challenging to achieve and the F₁ hybrids can only be raised in embryo culture, due to the lack of the endosperm [17].

The Ukrainian six-rowed winter barley ‘Manas’ may carry new allelic variation compared to the two-rowed spring cultivar ‘Betzes’ (generally used in wheat-barley crosses) as it has better agronomic traits (e.g. abiotic stress tolerance and yield ability), and it is well adapted to Central European conditions [18]. A hybrid previously developed between the winter wheat ‘Asakaze’ and the barley cultivar ‘Manas’ was subsequently screened to select a series of disomic and ditelosomic addition lines carrying the individual chromosomes/chromosome arms from barley in the wheat background [18,19]. Addition lines make it possible to study the effect of a specific barley chromosome added to wheat. However, as aneuploids are genetically unstable, this requires continuous cytogenetic checks. Wheat/barley translocation lines carrying 42 chromosomes are mostly stable and are directly applicable in prebreeding programmes [20,21]. Furthermore, compensating translocations are particularly valuable, as the alien homoeologous chromosomes are better able to compensate for the loss of a wheat chromosome segment [22]. In a previous study the ‘Asakaze’/‘Manas’ 7H disomic addition (2n = 44) and 7HL ditelosomic addition lines (2n = 42+2 telocentrics) were characterized in detail for abiotic stress resistance and nutritional parameters. Germination tests performed under salt conditions and salt tolerance experiments in early development stages revealed higher salinity tolerance for both lines [19,23]. The addition of the barley chromosome 7H, carrying the gene responsible for MLG synthesis (*HvCslF6* gene), to the hexaploid chromosome set of wheat has been found to increase the MLG content of the grain [24].

The aim of the present study was to develop a stable, fully fertile compensating winter wheat/winter barley translocation line (2n = 42) directly amenable to wheat breeding
programmes, which ultimately enhances salt tolerance and the MLG content of the grain. The breakage-fusion mechanism of univalent chromosomes was exploited to produce a new 7BS.7HL Robertsonian translocation line (RobT) and the new line was characterized by means of molecular genetic and cytogenetic methods (genomic in situ hybridization: GISH and fluorescence in situ hybridization: FISH), which confirmed the high salinity tolerance during germination and the higher MLG content of the grain, proving that 7BS.7HL RobT is a valuable genetic material readily applicable in wheat improvement.

**Material and methods**

**Plant material**

A crossing programme was initiated in order to develop a compensating Robertsonian translocation in a wheat background, including the long arm of barley chromosome 7H, in order to obtain a stable, fully fertile genetic material carrying salt tolerance and elevated MLG content. A ‘Rannaya’ 7B winter wheat monosomic stock was pollinated with the previously generated ‘Asakaze’/Manas’ 7H disomic addition line. Double monosomics for wheat 7B and barley 7H chromosomes (carrying 42 chromosomes) were selected in the F1 generation using 7H-specific molecular microsatellite and STS markers and Feulgen staining. F1 plants were self-pollinated and descendants in the F2 generation were screened with specific markers for the 7HS and 7HL arms to detect individuals carrying the long arm of 7H. Plants with a monosomic Robertsonian translocation were identified in the F3 generation using GISH, and disomic (homozygous) wheat-barley centric fusions were selected in the F4 generation (S1 Fig). Fertility (number of seeds/spike) was assigned based on the main spikes of plants grown in phytotron growth cabinets and in the Martonvásár nursery in 2014–2015 and 2015–2016, respectively.

In phytotron experiments vernalisation was carried out at 4°C for 6 weeks, after which the vernalised plants were grown in 2 L pots filled with a 2:1:1 mixture of garden soil, humus and sand. The plants were grown until tillering under an initial regime of 15°C day: 10°C night temperature, 12 h light: 12 h dark photoperiod. The temperature was raised by increments of 2°C after tillering (day length 14 h), stem elongation (16 h illumination), flowering and 2 weeks after fertilization.

**Genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH)**

Mitotic chromosome spreads were obtained from germinating root tips as described by Lukaszewski [25]. GISH technique has been used to distinguish alien chromosomes from wheat chromosomes and wheat-alien translocated chromosomes in a wheat background [26, 27]. The GISH experiment was carried out using total genomic barley DNA labelled with a dig-nick-translation mix (Roche Diagnostics, Mannheim, Germany). Unlabelled wheat genomic DNA was used as a blocking agent at a ratio of 35:1. Probe detection was carried out with anti-digoxigenin-Rhodamine (Roche). Slides were mounted in Vectashield antifade solution (Vector Laboratories, Burlingame, USA) containing 2 μg mL−1 4′-6-diamidino-2-phenylindole (DAPI). FISH was performed after rinsing the GISH hybridization signals off in 4× saline-sodium citrate (4X SSC) Tween 20 (Sigma Aldrich, Darmstadt, Germany) at room temperature. Wheat-specific DNA repetitive probes (pSc 119.2, Afa family and pTa 71) [28–30] were used to identify the wheat chromosome arm involved in the Robertsonian translocation and to confirm the presence of the entire wheat genome (except the 7BL chromosome arm). The Afa family and pSc119.2 repetitive probes were amplified and labelled with PCR using digoxigenin-11-dUTP and biotin-16-dUTP, respectively, while the 45S rDNA clone pTa71 was
labelled with 50% biotin-16-dUTP and 50% digoxigenin-11-dUTP by nick translation. Digoxi-
genin and biotin signals were detected with anti-digoxigenin-Rhodamine Fab fragments and streptavidin-FITC (Roche), respectively. The fluorescence signals were visualized using a Zeiss Axioskop-2 fluorescence microscope (Zeiss, Oberkochen, Germany) equipped with filter sets appropriate for DAPI, FITC, Rhodamine and the simultaneous detection of FITC and Rhodamine (double filter set). Images were captured with a Spot CCD camera (Diagnostic Instruments, Sterling Heights, USA) and processed with Image Pro Plus software (Media Cybernetics, Silver Spring, USA).

SSR and STS marker analysis

Genomic DNA was extracted from fresh young leaves of wheat cultivar 'Asakaze', barley culti-

var 'Manas' and the wheat-barley RobT using Quick Gene-Mini80 (FujiFilm, Japan) with a Quick-Gene DNA tissue kit (FujiFilm, Japan) according to the manufacturer’s instructions. barley chromosome arm-specific SSR markers (Bmac0031-7HS, HvID-7HL) [31] and the gene-specific HvCslF6 STS marker [32] were used to reveal the presence of the 7HS and 7HL barley chromosome arms. The 7BL chromosome arm-specific SSR marker (Wmc311-7BL) [33] was used to reveal the absence of the 7BL wheat chromosome arm. The primer pairs were tested on DNA templates containing genomic DNA from wheat cultivar ‘Rannaya’, the ‘Asa-

kaze’/’Manas’ 7H disomic addition line (7H), and the ‘Asakaze’/’Manas’ RobT. PCR amplifica-
tion was carried out under the conditions described by Cseh et al. [34]. PCR products were separated with a Fragment Analyzer Automated CE System equipped with a 96-Capillary Array Cartridge (Advanced Analytical Technologies, USA). The results were interpreted using PROsize v2.0 software (Advanced Analytical Technologies, USA).

Morphological characterization of the 7BS.7HL RobT

The number of seeds per main spike was determined from 2 × 10 plants for each genotype ('Rannaya' wheat cultivar, 7H addition line and 7BS.7HL RobT) in experiments carried out in the Martonvásár phytotron and nursery.

Evaluation of the salinity tolerance of the 7BS.7HL RobT

The salt stress response of the 'Rannaya' wheat genotype, the 'Asakaze'/’Manas’ 7H disomic addition line and 7BS.7HL RobT was evaluated after germination at the seedling stage. To determine the salt stress tolerance, 3×20 seeds from each genotype were surface-sterilized in 10% sodium hypochlorite for 15 minutes, rinsed twice in distilled water and germinated on wet filter paper in Petri dishes containing 0, 100, 150, 200 or 250 mM NaCl for 8 days (3 days in the dark and 5 days in the light) at a temperature of 25˚C. The percentage of germinated seeds was determined after 5 days and the length of roots and coleoptiles was measured on the 8th day after germination.

Evaluation of (1,3;1,4)-β-D-glucan content of the seeds of 7BS.7HL RobT

Four biological replicates of mature seeds (5 plants per replicate) were collected from the fol-

lowing genotypes: 'Rannaya' wheat cultivar, 'Manas' barley cultivar, 7H disomic addition line and 7BS.7HL RobT, and milled in a Retch M400 ball laboratory mill to produce wholemeal. Samples of finely ground grains of the plants (100 mg) were used for MLG analysis in four par-

allels according to AACC Method 32–23 [35] using a Megazyme kit (Megazyme, Bray, Ire-

land). The samples were suspended and hydrated in a buffer solution (pH 6.5) and then incubated with purified lichenase enzyme and filtered. An aliquot of the filtrate was then
hydrolysed to completion with purified β-glucosidase. The quantity of produced D-glucose was determined using a glucose oxidase/peroxidase reagent.

Statistical analysis
The fertility of 7BS.7HL RobT, the wheat cultivar 'Rannaya' and the 7H disomic addition line was compared pair-wise and differences in fertility were evaluated by means of Tukey's post hoc test (SPSS 16.0) at the 0.05 significance level.

The data from the salinity tolerance experiment were also analysed using Tukey's post hoc test (SPSS 16.0) at the 0.05 significance level.

Chemical data from the MLG experiment were evaluated using single-factor analysis in the Microsoft Excel program and were also analysed using Tukey's post hoc test (SPSS 16.0) Values of P<0.05 were considered to be statistically significant.

Results
Development of the wheat-barley 7BS.7HL RobT
Earlier results revealed higher salt tolerance and MLG content in wheat-barley introgression lines carrying the 7H chromosome or 7HL arm [23,24]. In the present study the 'Rannaya' monosomic wheat line was crossed as female partner to the 'Asakaze'/Manas' 7H disomic addition line in order to induce rearrangements and develop a stable wheat/barley RobT carrying the favourable traits. Ten of the plants raised from 25 germinated F1 seeds were found to be monosomic for the 7B and 7H chromosomes when tested 7H with specific molecular markers and Feulgen staining. The F1 plants produced a total of 68 seeds with an average yield of below 10 seeds/plant, except for two individuals which yielded 21 and 22 seeds, respectively.

From the 68 F2 seeds 66 germinated and these plants were screened for the presence of the 7HS or 7HL chromosome arms with the 7HS-specific Bmac0031 SSR marker and the 7HL-specific HvCSL6 STS marker. The analysis identified two plants carrying only the 7HS barley chromosome arm and three plants containing solely the 7HL arm. Plants carrying either 7HS or 7HL were analysed in F3 with GISH in order to select progenies with wheat-barley Robertsonian translocations. Seventy-two F3 seeds were screened, of which 7 plants (9.72%) had the wheat-barley translocation (7BS.7HL). Ten other plants retained telocentrics either individually or in pairs, while three plants carried one barley isochromosome (7HS), suggesting the fusion of a telocentric pair. The barley chromatin was entirely eliminated from 52 of the F3 plants analysed. Individuals carrying the Robertsonian translocations were selfed and 50 F4 plants were screened by GISH (S1 Fig) of which three were found to be disomic lines (6%), 22 (44%) monosomic lines, 21 (42%) nullisomic lines and 4 (8%) plants carried a barley telocentric chromosomes. During the examination of disomic RobT lines the GISH results confirmed the presence of 40 wheat chromosomes and the recombinant chromosome pair (7BS.7HL) (Fig 1A). Following random selection 49 F3 seeds originated from disomic plants were germinated and analysed by GISH and these were 100% disomic 7BS.7HL translocations.

The barley chromosome arms were identified by molecular marker analysis using the above specific molecular markers (Fig 2). The whole 7HL arm was detected by means of an STS marker located in the centromeric region of 7HL (HvCSL6) and an SSR marker mapped to the telomeric region of the 7HL chromosome arm (HvID). After washing the GISH signals off, FISH was performed in order to verify the identity of the wheat chromosome arm taking part in the Robertsonian translocation. The translocated wheat chromosome arm exhibited only two subterminal pSc119.2 signals and was thus identified as 7BS (Fig 1B, Fig 3). The parental genotypes ('Rannaya' wheat, 'Manas' barley and the 7H disomic addition line used as control)
Fig 1. Genomic in situ hybridization and Fluorescence in situ hybridization on mitotic chromosomes of the 7BS.7HL wheat-barley Robertsonian translocation line. (a) Genomic in situ hybridization (GISH) on mitotic chromosomes of the
7BS.7HL Robertsonian translocation line (RobT). Labelled total genomic DNA of barley was used as probe and barley chromosome arm 7HL is highlighted in magenta (arrows). The chromosomes were counterstained with DAPI (blue).

(b) Fluorescence in situ hybridization on mitotic chromosomes of 7BS.7HL RobT. DNA repetitive probes: Afa family (red), pSc119.2 (green), pTa71 (orange). Unidentified A genomic chromosomes are labelled by an ‘A’ letter. Scale bar = 10 μm.

https://doi.org/10.1371/journal.pone.0206248.g001

and descendants of the disomic plants were maintained and multiplied in phytotron growth cabinets (F5) and in the Martonvásár nursery (F6–F7 generations).

**Morphological characterization of the 7BS.7HL RobT**

The 7H addition line showed low fertility, especially in the apical part of the spike, under both phytotron and field conditions. On the other hand, the fertility of the RobT was similar to that

![Capillary gel electrophoresis pattern](https://doi.org/10.1371/journal.pone.0206248.g002)
of the 'Rannaya' winter wheat cultivar, demonstrating that the crossing strategy had successfully restored the fertility of the 7H addition (S2 Fig). The significant improvement in fertility was reflected by the fact that although the spikes of the RobT were shorter (Fig 4) the number of seeds/spike was twice as high for the RobT than for the 7H addition line.

Salt stress response of 7BS.7HL RobT

The germination % and the root and shoot growth of the 'Rannaya' wheat cultivar, the 7H addition line and the 7BS.7HL RobT were determined after germination in NaCl solutions with various concentrations (0, 100, 150, 200 and 250 mM). These results are presented in Table 1.

All three genotypes germinated well without salt treatment and the germination percentage decreased slightly (86%) in 'Rannaya' under the mild salt stress induced by 100 mM NaCl. Above this NaCl concentration, the germination percentage decreased, especially in 'Rannaya'. When treated with 150, 200 mM and 250 mM NaCl, the germination capacity of the wheat genotype dropped considerably (to 73%, 46% and 30%, respectively). In contrast, both the 7H addition line and the RobT maintained a very high germination rate when subjected to 150, 200 and 250 mM NaCl (85%, 78% and 70% for the 7H addition line and 90%, 80% and 75% for RobT, respectively).

The salt tolerance of the 'Rannaya' wheat cultivar, the 'Asakaze'/Manas’ disomic addition line and 7BS.7HL RobT was also investigated by measuring the root and shoot lengths on the 8th day after germination. Germinating the seeds in 100, 150, 200 or 250 mM NaCl solution resulted in a decrease of root and shoot coleoptile length as a % of the control (Fig 5). The growth reduction was more pronounced in 'Rannaya' than in the addition and the RobT. For instance, the roots of the 7BS.7HL RobT were twice as long as those of the wheat parental line when treated with 200 mM NaCl (Fig 5). The differences between the genotypes were more pronounced for the roots than for the shoots. The differences in shoot lengths were not as significant due to the higher standard deviation (Fig 5). Salt stress reduced the growth vigour of
all the genotypes during the first 4 days, compared to that of the control plants (without NaCl treatment), but the introgression lines showed better growth during the subsequent 4 days and exhibited better growth vigour, resulting in greater differences between the genotypes (Fig 5).

Table 1. Germination percentage of the genotypes analysed.

|                     | 0 Mm NaCl | 100 Mm NaCl | 150 Mm NaCl | 200 Mm NaCl | 250 Mm NaCl |
|---------------------|-----------|-------------|-------------|-------------|-------------|
| Wheat cv. ‘Rannaya’ | 100<sup>a</sup> | 86<sup>b,c</sup> | 75<sup>d</sup> | 46<sup>e</sup> | 30<sup>f</sup> |
| 7H addition line    | 100<sup>a</sup> | 100<sup>a</sup> | 85<sup>b,c</sup> | 78<sup>d</sup> | 70<sup>e</sup> |
| 7BS.7HL RobT        | 100<sup>a</sup> | 100<sup>a</sup> | 90<sup>b</sup> | 80<sup>d</sup> | 75<sup>f</sup> |

Values are means ±SD of 3 x 20 replicates per genotype. Different letters indicate significant differences at P < 0.05 using Tukey’s post hoc test.

Fig 4. Phenotype of spikes of the parental genotypes and the 7BS.7HL centric fusion line. Phenotype of spikes of the parental wheat genotype ‘Rannaya’ (1), the 7H disomic addition (2) and the 7BS.7HL RobT line (3). Plants were grown in phytotron climate chambers.

https://doi.org/10.1371/journal.pone.0206248.g004
Analysis of the MLG content of 7BS.7HL RobT

The mean MLG levels in four biological replicates of the studied genotypes are presented in Fig 6, together with the LSD 5% values. The mean MLG level (mg/g dry matter) of the 'Rannaya' wheat cultivar was 7.04 mg (0.7%), while that of the barley cultivar 'Manas' was seven times as high (48.89 mg/g, 4.89%). The $\beta$-glucan content of the seeds was 8.93 mg/g (0.9%) for 7BS.7HL RobT and 10.93 mg/g (1.1%) for the 7H addition line, so the MLG level of the 7BS.7HL RobT, carrying the $HvCslF6$ gene, exceeded the $\beta$-glucan content of wheat by 26.85%.

Discussion

The 'Asakaze'/Manas' winter wheat-winter barley 7BS.7HL compensating RobT was developed with the aim of transferring agronomically favourable genes from cultivated barley to bread wheat. The present work demonstrated that a stably inherited Robertsonian translocation comprising the 7HL barley chromosome arm in a wheat background could effectively enhance the salinity tolerance of the wheat plant and increase the MLG content of the grain.

Wheat-alien amphiploids and addition lines cannot be directly used in wheat breeding programmes because of their genetic instability. On the other hand, stably inherited translocation lines carry a subchromosomal segment of an alien chromosome integrated into the wheat genome and usually comprise 42 chromosomes. A number of wheat-ancestral Robertsonian translocations were reported involving wild relatives of wheat, such as Dasypirum villosum.
Haynaldia villosa, Thinopyrum bessarabicum, Thinopyrum intermedium, Aegilops searsii and Aegilops speltoides. For instance, the stripe rust resistance gene Sr59 was introgressed into hexaploid wheat from rye through a compensating 2DS.2RL Robertsonian translocation [42]. One of the most well-known and widely applied ancestral introgressions was the transfer of the 1RS rye chromosome arm from the Russian cultivars 'Aurora' and 'Kavkaz' into the wheat background as a compensating 1BL.1RS translocation [43, 44]. Consequently, 1BL.1RS is present in more than 1000 wheat cultivars worldwide and contributed to improving the yield potential, adaptability and biotic stress resistance of wheat [45,46].

Only a few wheat-barley compensating translocation lines are so far available for wheat improvement. Koba et al. [47] reported the development of a 5HS.5BL RobT in addition to the 42 wheat chromosomes from a cross between the 'Schinchunaga' wheat and 'Nyugoruden'
barley cultivars. Danilova et al. [48] produced a complete set of homoeologous group 7 compensating translocations introducing chromosome arms from the ‘Betzes’ spring barley variety into the ‘Chinese Spring’ wheat background. The ‘Manas’ barley used in the present study is a six-rowed winter variety having good agronomic traits (high yield potential, winter hardiness, salt tolerance, high MLG content) [18]. Similarly, the ‘Rannaya’ wheat used as female parent in the cross is a cultivated winter wheat with high yielding potential, making it more suitable for breeding purposes.

Depending on the wheat and alien chromosomes involved and the environmental conditions, the desired compensating wheat-alien Robertsonian translocations can be recovered at variable frequencies, ranging from low (1.77%) to fairly high (20%) [36,48–50]. In this study the efficiency of the breakage-fusion mechanism was 7.6% in the F₂ generation. Besides the crossing procedure and the detailed genetic investigation, this study focused on the phenotyping of the newly developed plant material. Wheat is not tolerant to high level of soil salt content. King et al.[51] reported the possibility of transferring salt tolerance genes from a wild relative (Thinopyrum bessarabicum) into bread wheat. In a previous study, germination tests performed under salt conditions and salt tolerance tests during early development stages confirmed the higher salinity tolerance of lines carrying the 7H barley chromosome or the 7HL arm compared to wheat and other wheat-barley addition lines (2H, 3H, 4H, 6H and 7HS) [19,23]. Detailed physiological, biochemical and molecular genetic investigations related to typical salt tolerance mechanisms revealed that Na⁺ uptake and transport are not responsible for the elevated salt tolerance of the 7H and 7HL addition lines. The higher salt tolerance was mainly due to the improved osmotic adjustment capacity of these lines [52]. Osmotic adjustment is one of the mechanisms contributing to the tolerance of salt-stressed plants [53]. QTLs associated with genes responsible for the relative water content (RWC) and water-soluble carbohydrate concentration (WSC) of plants were identified and mapped using composite interval mapping to barley chromosome arm 7HL [54]. The bSS1B gene is associated with a QTL for RWC encodes barley sucrose synthase, which is a key enzyme in the carbohydrate metabolism, catalysing fructose and UDP-glucose synthesis [55,56], while the Acl3 gene encodes a cofactor protein associated with a QTL for RWC on the 7HL arm, having a role in membrane protection during stress [54].

Earlier experiments revealed that the improved salt tolerance of the 7H addition line was due to osmotic adjustment achieved through proline accumulation and enhanced soluble carbohydrate metabolism [52]. The similar experimental design used in the present experiments showed that both the ‘Asakaze’/‘Manas’ 7H disomic addition line and the 7BS.7HL RobT performed better under salt stress conditions than the wheat parental cultivar. barley cv. ‘Manas’ and the 7HL ditelosomic addition thus gave results similar to those observed earlier for the 7H addition line, suggesting that the enhanced salt tolerance observed for the 7BS.7HL translocation line also operates via osmotic adjustment [52]. The slow root and shoot growth during the first 4 days of salt treatment and the subsequent acceleration further support this assumption, as the reprogramming of the carbohydrate metabolism and the accumulation of large pools of soluble sugars require several days. However, further investigations are needed to confirm the role of sugars in the salt tolerance mechanisms operating in the 7BS.7HL RobT line.

The seed MLG content of the ‘Manas’ barley cultivar is seven times as high as that of ‘Rannaya’ wheat. The presence of the 7HL barley chromosome arm increased the MLG content of the seeds by 26.83%, demonstrating that the HvCslF6 gene responsible for its synthesis is functional in the wheat genetic background. Recent QTL and genome-wide association studies suggested that other proteins may also play a role in (1,3;1,4)-β-D-glucan biosynthesis, implying that the introduction of other genes from barley would result in higher dietary fibre content in wheat. Candidate genes were mapped to the 1HS, 2HL, 3HS, 3HL, 4HL, 5HS, 6HS and
7HS chromosome arms [57–59]. The current model for (1,3;1,4)-β-D-glucan biosynthesis proposes that the HvCSLF6 enzyme is only responsible for creating the β-(1,4)-glucosidic linkages, which are then joined together to produce the mixed-linkage glucan through a β-(1,3)-glucosidic linkage catalysed by an as yet unidentified protein [60]. The higher MLG content of the 7H addition line compared to the RobT thus suggests that the QTLs mapped on 7HS and 7HL may have an additive effect and that the presence of both groups of loci results in a higher MLG level (Fig 6).

Besides the traits characterized in this study the 7HL chromosome arm carries other genes of breeding interest. For instance, a locus (Rps6) conferring stripe rust (Puccinia striiformis) resistance was likewise mapped to the distal region of 7HL in the barley cultivar ‘Tamalpais’ and the barley line ‘Y12’ [61]. The wheat-barley RobT produced in the present work thus has the potential to improve stripe rust resistance in wheat.

The ‘Ranaya’/Manas’ 7BS.7HL compensating RobT carries 42 chromosomes, and genomic in situ hybridization revealed a high degree of chromosome stability, implying that the introgressed agronomic characters are stably inherited. Moreover, the fertility of the 7BS.7HL RobT was similar to that of wheat, indicating that the infertility of the parental 7H addition line had been successfully overcome. The good yield potential and fertility of the RobT makes it possible to use this line directly in wheat breeding programmes to improve biotic and abiotic stress resistance and to confer better nutritional parameters on cultivated bread wheat.

Conclusions
The introgression of barley chromatin segments into wheat, as in the stably inherited whole arm Robertsonian translocation developed in the present study, widens the genetic diversity of cultivated bread wheat. Evidence was provided that functional genes originating from the long arm of barley chromosome 7H improved the salinity tolerance and increased the (1,3;1,4)-β-D-glucan content of the seed in the newly developed ‘Asakaze’/‘Manas’ 7BS.7HL introgression line. The genome stability and fertility of the compensating translocation line make it a suitable genetic material for direct application in wheat breeding programmes.

Supporting information
S1 Fig. Crossing strategy applied for the development of 7BS.7HL RobT.
(DOCX)

S2 Fig. Number of kernels/main spike of the 7BS.7HL RobT, ‘Rannaya’ wheat cultivar and ‘Asakaze’/‘Manas’ 7H disomic addition line. Number of kernels/main spike of ten randomly selected plants (1–10) in Martonvásár phytotron and field experiments for the 7BS.7HL RobT and for the ‘Rannaya’ wheat cultivar and ‘Asakaze’/‘Manas’ 7H disomic addition line used as control. Different letters indicate significant differences at P < 0.05 using Tukey’s post hoc test.
(DOCX)

Author Contributions
Conceptualization: András Cseh.
Data curation: András Cseh.
Formal analysis: Eva Darko, Marianna Rakszegi, András Cseh.
Funding acquisition: András Cseh.
Investigation: Edina Türkösi, Eva Darko, Marianna Rakszegi, András Cseh.

Project administration: Edina Türkösi, András Cseh.

Resources: Eva Darko, András Cseh.

Supervision: Eva Darko, István Molnár, András Cseh.

Validation: Eva Darko.

Visualization: Edina Türkösi, Marianna Rakszegi, András Cseh.

Writing – original draft: Edina Türkösi, Eva Darko, András Cseh.

Writing – review & editing: Mártá Molnár-Láng, András Cseh.

References

1. Pareek A, Sopory SK, Bohrert HJ, Govindjee. Abiotic stress adaptation in plants. Physiological, Molecular and Genomic Foundation. Dordrecht: Springer; 2010.

2. Mantri N, Patade V, Penna S, Ford R, Pang E. Abiotic stress responses in plants: present and future. In: Ahmad P, Prasad MNV, editors. Abiotic Stress Responses in Plants. Metabolism, Productivity and Sustainability. New York: Springer; 2012. pp.1–19.

3. Slavich PG, Read BJ, Cullis BR. Yield response of barley germplasm to field variation in salinity quantified using the EM-38. Aust J Exp Agr. 1990; 30:551–556.

4. Jaradat AA, Shahid M, Al-Maskri A. Genetic diversity in the Batini barley landrace from Oman: I. Spike and seed quantitative and qualitative traits. Crop Sci. 2004; 44:304–315.

5. Munns R, Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 2008; 59:651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911 PMID: 18444910

6. Widodo, Patterson JH, Newbiggin E, Tester M, Bacic A, Roessner U. Metabolic responses to salt stress of barley (Hordeum vulgare L.) cultivars, Sahara and Clipper, which differ in salinity tolerance. J Exp Bot. 2008; 60:4089–4103. https://doi.org/10.1093/jxb/erp243 PMID: 19666960

7. Wu H, Shabala L, Barry K, Zhou M, Shabala S. Ability of leaf mesophyll to retain potassium correlates with salinity tolerance in wheat and barley. Physiol Plantarum. 2013; 149:515–527.

8. Munns R, Gardner P, Tonnet ML, Rawson H. Growth and development in NaCl-treated plants. Do Na+ or Cl– concentrations in dividing or expanding tissues determine growth in barley? Aust J Plant Physiol. 1988; 15:529–540.

9. Zhou G, Johnson P, Ryan PR, Delhaize E, Zhou M. Quantitative trait loci for salinity tolerance in barley (Hordeum vulgare L). Mol Breeding. 2012; 29:427–436.

10. Ma Y, Shabala S, Li C, Lu C, Zhang W, Zhou M. Quantitative trait loci for salinity tolerance identified under drained and waterlogged conditions and their association with flowering time in barley (Hordeum vulgare L.). PLOS ONE 2018; 10(8):e0134822. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0134822

11. Kerckhoffs DA, Hornstra G, Mensink RP. Cholesterol-lowering effect of β-glucan from oat bran in mildly hypercholesterolemic subjects may decrease when β-glucan is incorporated into bread and cookies. Am J Clin Nutr. 2003; 78:221–227. https://doi.org/10.1093/ajcn/78.2.221 PMID: 12885701

12. Shelat KJ, Vilaplana F, Nicholson TM, Wong KH, Gidley MJ, Gilbert RG. Diffusion and viscosity in arabinoxylan solutions: Implications for nutrition. Carbohydr Polym. 2011; 82:46–53.

13. Molina-Cano JL, Moralejo M, Elia M, Munoz P, Russell JR, Perez-Vendrell AM et al. 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 Breeding. 2007; 19:275–284.

14. Burton RA, Wilson SM, Hrmova M, Harvey AJ, Shirley NJ, Medhurst A et al. Cellulose synthaselike CslF genes mediate the synthesis of cell wall (1,3;1,4)-β-D-glucans. Science. 2006; 311:1940–1942. https://doi.org/10.1126/science.1122975 PMID: 16574868

15. Taketa S, Yuo T, Tonooka T, Tsumuraya Y, Inagaki Y, Haruyama N et al. Functional characterization of barley betaglucanless mutants demonstrates a unique role for CslF6 in (1,3;1,4)-β-D-glucan biosynthesis. J Exp Bot. 2012; 63:381–392. https://doi.org/10.1093/jxb/erz285 PMID: 21940720

16. Nemeth C, Freeman J, Jones HD, Sparks C, Pellny TK, Wilkinson MD et al. Down-regulation of the CslF6 gene results in decreased (1,3;1,4)-β-D-glucan in endosperm of wheat. Plant Physiol. 2010; 152:1209–1218. https://doi.org/10.1104/pp.109.151712 PMID: 20089768
17. Islam AKMR, Shepherd KW. Incorporation of barley chromosomes into wheat. In: Bajaj YP, editor. Biotechnology in Agriculture and Forestry. Wheat, Vol. 13. Heidelberg: Springer; 1990. pp. 128–151.

18. Molnár-Láng M, Krupka K, Cseh A, Bucsi J, Linc G. Identification and phenotypic description of new wheat–six-rowed barley disomic additions. Genome. 2012; 55:302–311. https://doi.org/10.1139/G2012-013 PMID: 22439846

19. Türkösi E, Cseh A, Darko E, Molnár-Láng M. Addition of Manas barley chromosome arms to the hexaploid wheat genome. BMC Genetics. 2016; 17:87. Available from: https://bmcgenet.biomedcentral.com/articles/10.1186/s12863-016-0393-2. PMID: 27328706

20. Ivanizs L, Farkas A, Linc G, Molnár-Láng M, Molnár I. Molecular cytogenetic and morphological characterization of two wheat-barley translocation lines. PLoS ONE 2018; 13(6): e0198758. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0198758 PMID: 29889875

21. Ghazali S, Mirzaghaderi G, Majdi M. Production of a novel Robertsonian translocation from Thinopyrum bessarabicum into bread wheat. Cytol Genet. 2005; 49:378–381.

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

23. Darko E, Janda T, Majláth I, Szopko D, Dulai S, Molnár I et al. Salt stress response of wheat–barley addition lines carrying chromosomes from the winter barley “Manas”. Euphytica. 2015; 203:491–504.

24. Cseh A, Soós V, Rakszegi M, Türkösi E, Balázs E, Molnár-Láng M. Expression of HvCslF9 and HvCslF6 barley genes in the genetic background of wheat and their influence on the wheat β-glucan content. Ann Appl Biol. 2013; 163:142–150.

25. Lukaszewski AJ, Rybka K, Korzun V, Malyashev SV, Lapinski B, Whitkus R. Genetic and physical mapping of homeologous recombination points involving wheat chromosome 2B and rye chromosome 2R. Genome. 2004; 47:36–45. https://doi.org/10.1139/g03-089 PMID: 15060600

26. Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS. In situ localization of parental genomes in a wide hybrid. Ann Bot. 1989; 64:315–324.

27. Heslop-Harrison JS. The molecular cytogenetics of plants. J Cell Sci. 1991; 100:15–21.

28. Bedbrook JR, Jones J, O’Dell M, Thompson RD, Flavell RB. A molecular description of telomeric heterochromatin in secale species. Cell. 1980; 19:545–560. PMID: 6244112

29. Nagaki K, Tsujimoto H, Isono K, Sasakuma T. Molecular characterization of a tandem repeat, Afa family, and its distribution among Triticeae. Genome. 1995; 38:479–486. PMID: 7557360

30. Gerlach WL, Bedbrook JR. Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res. 1979; 7:1869–1885. PMID: 537913

31. Ramsay L, Macauly M, degli Iaissevich S, MacLean K, Cardle L, Fuller J, et al. A simple sequence repeat-based linkage map of barley. Genetics. 2000; 156:1997–2005. PMID: 11102390

32. Burton RA, Jobling SA, Harvey AJ, Shirley NJ, Mather DE, Bacic A, Fincher GB. The genetics and transcriptional profiles of the cellulose synthase-like HvCslF gene family in barley. Plant Physiol. 2008; 146:1821–1833. https://doi.org/10.1104/pp.107.114694 PMID: 18258691

33. Somers DJ, Isaac P, Edwards K. A high-density microsatellite consensus map for bread wheat (Triticum aestivum L.). Theor Appl Genet. 2004; 109:1105–1114. https://doi.org/10.1007/s00122-004-1740-7 PMID: 15490101

34. Cseh A, Krupka K, Molnár I, Rakszegi M, Doležel J, Molnár-Láng M. Characterization of a new 4BS.7HL wheat–barley translocation line using GISH, FISH, and SSR markers and its effect on the β-glucan content of wheat. Genome. 2011; 54:795–804. https://doi.org/10.1139/g11-044 PMID: 21919737

35. McCleary BV, Codd R. Measurement of (1–3),(1–4)-β-D-glucan in barley and oats: a streamlined enzymatic procedure. J Sci Food Agr. 1991; 55:303–312.

36. Zhao W, Qi L, Gao X, Zhang G, Dong J, Chen Q, Friebe B, Gill BS. Development and characterization of two new Triticum aestivum–Dasypyrum villosum Robertsonian translocation lines T1DS1V#3L and T1DL1V#3S and their effect on grain quality. Euphytica. 2010; 146:1821–1833. https://doi.org/10.1104/pp.107.114694 PMID: 18258691

37. Wilson, J. Production of wheat–Havaldia villosa Robertsonian chromosome translocations. M.Sc. Thesis, Kansas State University. 2008. Available from: http://krex.k-state.edu/dspace/bitstream/handle/2097/1085/JamieWilson2008.pdf?sequence=1.

38. Grewal S, Yang C, Hubbart Edwards S, Scholefield D, Ashling S, Burridge AJ, King IP, King J. Characterisation of Thinopyrum bessarabicum chromosomes through genome-wide introgressions into wheat. Theor Appl Genet. 2018; 131:389–406. https://doi.org/10.1007/s00122-017-3009-y PMID: 29101420

39. Lang T, La S, Li B, Yu Z, Chen Q, Li J, Yang E, Li G, Yang Z. Precise identification of wheat–Thinopyrum intermedium translocation chromosomes carrying resistance to wheat stripe rust in line Z4 and...
its derived progenies. Genome. 2018; 61:177–185. https://doi.org/10.1139/gen-2017-0229 PMID: 29470932

40. Liu W, Jin Y, Rouse M, Friebe B, Gill B, Pumphrey MO. Development and characterization of wheat-Ae. searsii Robertsonian translocations and a recombinant chromosome conferring resistance to stem rust. Theor Appl Genet. 2011; 122:1537–1545. https://doi.org/10.1007/s00122-011-1553-4 PMID: 21347655

41. Liu W, Koo DH, Friebe B, Gill BS. A set of Triticum aestivum-Aegilops segetoides Robertsonian translocation lines. Theor Appl Genet. 2016; 129:2359–2368. https://doi.org/10.1007/s00122-016-2774-3 PMID: 27558995

42. Rahmatov M, Rouse MN, Nirmala J, Danilova T, Friebe B, Steffenson BJ, Johansson E. A new 2DS-2RL Robertsonian translocation transfers stem rust resistance gene Sr59 into wheat. Theor Appl Genet. 2016; 129:1383–1392. https://doi.org/10.1007/s00122-016-2710-6 PMID: 27025509

43. Zeller FJ, Hsam SLK. Broadening the genetic variability of cultivated wheat by utilizing rye chromatin. In: Sakamoto S. editor. Proceedings of the 6th International Wheat Genetics Symposium. Kyoto University;1983. pp. 161–173.

44. Braun HJ, Payne TS, Morgounov AI, Ginkel MV, Rajaram S. The challenge: one billion tons of wheat by 2020. In: Slinkard AE. editor. Proceedings of the Ninth International Wheat Genetics Symposium. University of Saskatchewan: University Extension Press;1998. pp 33–40.

45. Lukaszewski AJ. Frequency of 1RS/1AL and 1RS/1BL translocations in the United States wheats. Crop Sci. 1990; 30:1151–1153.

46. Schlegel R, Korzun V. About the origin of 1RS-1BL wheat-rye chromosome translocations from Germany. Plant Breeding.1997; 116:537–540.

47. Koba T, Takumi S, Shimada T. Isolation, identification and characterization of disomic and translocated barley chromosome addition lines of common wheat. Euphytica. 1997; 96:289–296.

48. Danilova TV, Friebe B, Gill BS, Poland J, Jackson E. Development of a complete set of wheat–barley group 7 Robertsonian translocation chromosomes conferring an increased content of β-glucan. Theor Appl Genet. 2018; 131:377–388. https://doi.org/10.1007/s00122-017-3008-z PMID: 29124282

49. Lukaszewski AJ. Manipulation of the genome by chromosome breakage. In: Gill BS, Raupp WJ editors. Proceedings of the US-Japan symposium, classical and molecular cytogenetic analysis. Manhattan: 1994. pp 136–139.

50. Friebe B, Zhang P, Linc G, Gill BS. Robertsonian translocations in wheat arise by centric misdivision of univalents at anaphase I and rejoining of broken centromeres during interkinesis of meiosis II. Cyto-genet Genome Res. 2005; 109:293–297. https://doi.org/10.1159/000084212 PMID: 15753589

51. King IP, Forster BP, Law CC, Cant KA, Orford SE, Gorham J et al. Introgression of salt-tolerance genes from Thinopyrum bessarabicum into wheat. New Phytol. 1997; 137:75–81.

52. Darko E, Gierczik K, Hudák O, Forgó P, Pál M, Türkösi E et al. Differing metabolic responses to salt stress in wheat-barley addition lines containing different 7H chromosomal fragments. PLoS ONE. 2017; 123:20. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0174170.

53. Negro S, Schmöckel SM, Tester M. Evaluating physiological responses of plants to salinity stress. Ann Bot. 2017; 119:1–11. https://doi.org/10.1093/aob/mcw191 PMID: 27707746

54. Diab AA, Teulat-Merah B, This D, Ozturk NZ, Benscher D, Sorrells ME. Identification of drought-inducible genes and differentially expressed sequence tags in barley. Theor Appl Genet. 2004; 109:1417–1425. https://doi.org/10.1007/s00122-004-1755-0 PMID: 15517148

55. Sánchez de la Hoz P, Vicente-Carbajosa J, Mena M, Carbonero P. Homologous sucrose synthase genes in barley (Hordeum vulgare) are located in chromosomes 7H (syn 1) and 2H. Evidence for a gene translocation? Febs Lett. 1992; 310:46–50. PMID: 1388123

56. Kleines M, Eister RC, Rodrigo MJ, Blervačq AS, Salamini F, Bartels D. Isolation and expression analysis of two stress-responsive sucrose-synthase genes from the resurrection plant Craterostigma plantagineum (Hochst.). Planta. 2009; 209:13–24. https://doi.org/10.1007/s004250050602 PMID: 10467027

57. Islamovic E, Obert DE, Oliver RE, Harrison SA, Ibrahim A, Marshall JM et al. Genetic dissection of grain beta-glucan and amylose content in barley (Hordeum vulgare L.). Mol. Breeding. 2013; 31:15–25.

58. Shu X, Rasmussen SK. Quantification of amylose, amylopectin, and β-glucan in search for genes controlling the three major quality traits in barley by genome-wide association studies. Front. Plant Sci. 2014; 5: 197. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4030205/. https://doi.org/10.3389/fpls.2014.00197 PMID: 24860587

59. Houston K, Russell J, Schreiber M, Halpin C, Oakley H, Washington JM et al. A genome wide association scan for (1,3;1,4)-β-glucan content in the grain of contemporary 2-row spring and winter barleys. BMC Genomics. 2014; 15(1):907. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4213503.
60. Wilson SM, Ho YY, Lampugnani ER, Van de Meene AML, Bain MP, Bacic A, Doblina MS. Determining the subcellular location of synthesis and assembly of the cell wall polysaccharide (1,3;1,4)-β-D-Glucan in grasses. Plant Cell. 2015; Available from: www.plantcell.org/cgi/doi/10.1105/tpc.114.135970.

61. Li K, Hegarty J, Zhang C, Wan A, Wu J, Guédira GB et al. Fine mapping of barley locus *Rps6* conferring resistance to wheat stripe rust. Theor Appl Genet. 2016; 129:845–859. https://doi.org/10.1007/s00122-015-2663-1 PMID: 26875072