Possibilities of gene transfer from related species for the increase of β-glucan content of wheat

PD OTKA 105594

Interspecific and intergeneric hybridisation provides an opportunity to transfer agronomically useful traits from related species into hexaploid wheat (*Triticum aestivum* L. 2n=6x=42, AABBDD). One relative species that has been considered is barley (*Hordeum vulgare* L., 2n=2x=14, HH) as it carries genes for abiotic stress tolerance and has good nutritional parameters. Wheat/alien chromosome addition lines are excellent resources to study the effect of alien chromatin in the genetic background of wheat and they can be used as a starting point for gene transfer from an alien chromosome. Moreover, these disomic and ditelosomic additions facilitate the definition of the physical position of genes and DNA markers within particular chromosomal regions. When using alien genes from relative species in wheat improvement, the alien target chromosome arm needs to be in the form of a cytologically stable, compensating wheat-alien Robertsonian translocation. The production of wheat-barley compensating translocation lines is an important intermediate step in wheat breeding programmes.

Barley chromosome 7H carries a number of important genes. For example, it has been shown that drought tolerance in barley is controlled by QTLs located on 7H and a QTL for salinity tolerance during germination was mapped to 7H. Salt stress analysis of the Asakaze komugi/Manas 7HL ditelosomic addition line suggest that the 7HL chromosome arm is a good candidate for improving salinity tolerance of wheat during germination. The 7HL disomic addition line exhibited higher salt tolerance during germination and in early developmental stages than the wheat parent, which may be due to the elevated osmotic adjustment capacity of these addition lines, similar to that found for barley cv. Manas (Darko et al. 2014).

The dietary fibre (1,3;1,4)-β-D-glucan (ß-glucan), is a major quality parameter of cereals. Grain ß-glucan content is the most important factor from the aspect of human health maintenance. The grain of barley (*Hordeum vulgare* L.) is one of the most important ß-glucan sources having a ß-glucan content ten times higher than that of wheat (*Triticum aestivum* L.). Barley β-glucans are beneficial to human health, as they are a major source of soluble dietary fibres and have been recognised both as potential cholesterol-lowering polysaccharides (Kerckhoffs et al., 2003; Shelat et al., 2011) and as non-specific immune-activators (Allendorf et al., 2005). Dietary fibres such as β-glucan can play an important role in preventing diseases such as type2 diabetes and cardiovascular disorders (Brownlee, 2011).

Cellulose synthase-like (*CSL*) genes are candidates to encode the enzymes that synthesise the backbone of various non-cellulosic linked cell wall polysaccharides (Doblin et al. 2009).

Aims of the project were

I.) *Expression analysis of HvCSLF9 and HvCSLF6 genes by quantitative RT-PCR*

HvCSLF9 and *HvCSLF6* genes were found to have the highest expression detected in developing barley endosperms (Burton et al. 2008). The *HvCSLF9* and *HvCSLF6* genes were mapped to loci near the centromeres of chromosomes 1H and 7H, respectively, which were close to QTLs for barley grain β-glucan content. For this reason the present study aimed to analyse the expression of these genes in wheat genetic background.

In the progenies of the Mv9/Igri 1HS isochromosomic and 7H disomic addition lines 10-10 plants, carrying a complete set of wheat chromosomes and two 1HS chromosome arms or two 7H chromosomes, respectively, were selected using GISH (Fig 1.).

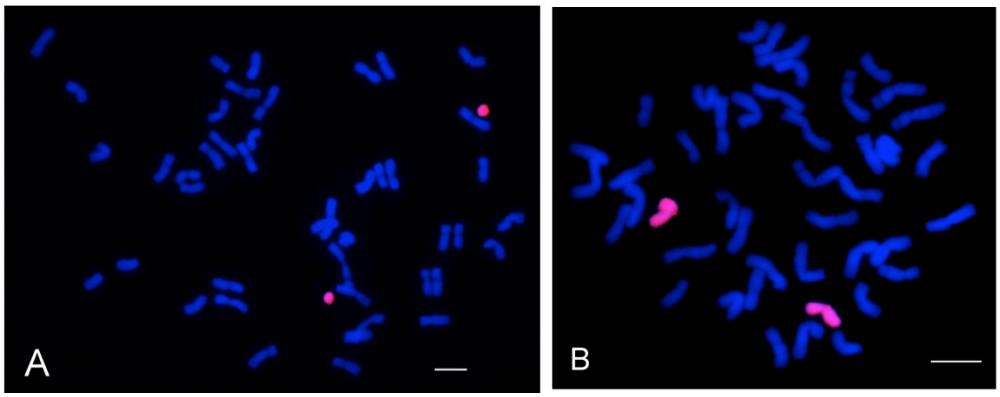

Figure 1.

A. Genomic *in situ* hybridisation (GISH) on mitotic chromosomes of the 'Mv9kr1'/'Igri' 1HS ditelosomic addition line. Labelled genomic DNA of barley was used as probe. The 1HS barley chromosome arm is highlighted in red. The chromosomes were counterstained with DAPI (blue). Scale bar represents 10 μm.

B. Genomic *in situ* hybridisation (GISH) on mitotic chromosomes of the 'Mv9kr1'/'Igri' 7H disomic addition line. Labelled genomic DNA of barley was used as probe. The 7H barley chromosome is highlighted in red. The chromosomes were counterstained with DAPI (blue). Scale bar represents 10 μm.

The expression of the *HvCSLF9* and *HvCSLF6* genes in the genetic background of wheat was determined by quantitative RT-PCR. The expression pattern of the *HvCSLF9* gene transcript showed a gradual increase throughout grain development (6, 10, 14, 18, 22, 26 days after pollination), while the *HvCSLF6* gene was normally transcribed at relatively high levels (Fig 2.). In leaves, the transcript of the *HvCSLF9* gene could not be detected at the end of tillering, while the *HvCSLF6* gene was still strongly expressed at this time in the 7H addition line (Fig 2). The present study mapped the *HvCslF9* gene to the short arm of the 1H chromosome.

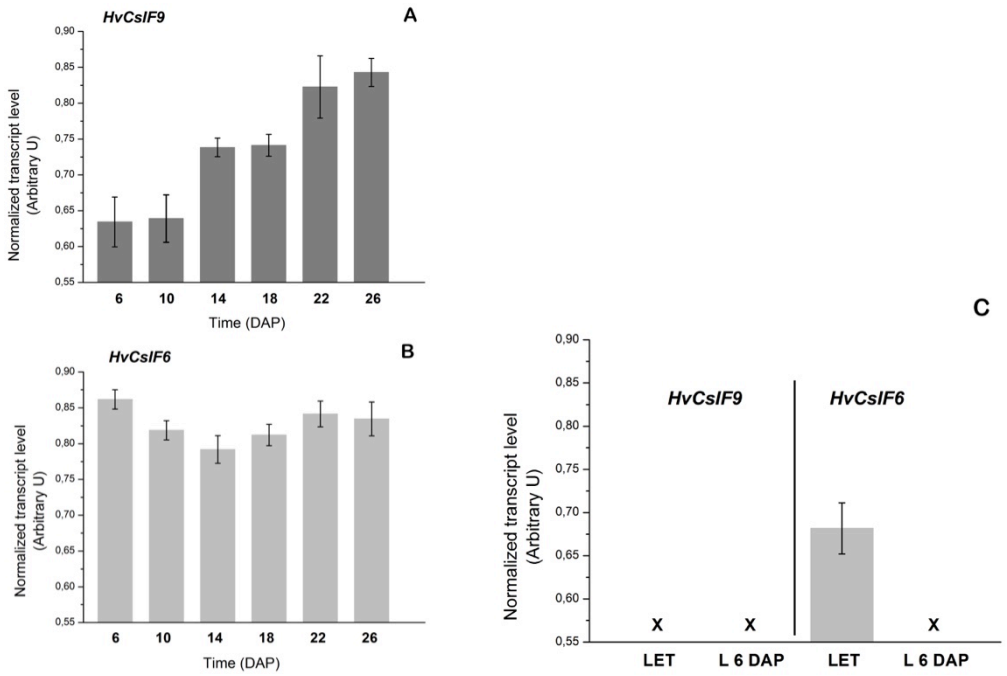

Figure 2.

A. Normalized transcript levels (arbitrary units) of the barley *HvCSLF9* gene in developing grain of the 'Mv9kr1'/'Igri' 1HS ditelosomic addition line at 6, 10, 14, 18, 22 and 26 days after pollination (DAP). Bars on all Q-PCR plots indicate standard deviations.

B. Normalized levels of *HvCSLF6* gene transcripts (arbitrary units) in developing grain of the 'Mv9kr1'/'Igri' 7H disomic addition line at various times after pollination. Bars indicate standard deviations.

C. Normalized levels of *HvCSLF9* and *HvCSLF6* gene transcripts (arbitrary units) in leaves using leaflet samples (L) of the 'Mv9kr1'/Igri' 1HS ditelosomic addition line and the 'Mv9kr1'/'Igri' 7H disomic addition line (7H) at the end of tillering (ET) and six days after pollination (6 DAP). Bars indicate standard deviations.

Winter wheat/winter barley 'Mv9kr1'/'Igri' 1HS ditelosomic and 'Mv9kr1'/'Igri' 7H disomic addition lines carrying the *HvCSLF9* and *HvCSLF6* barley genes, respectively, were used to investigate the additive effect of barley cellulose synthase-like genes on the wheat ß-glucan content. A significantly higher ß-glucan level was detected in the leaves and grains of the wheat/barley 1HS and 7H addition lines compared to the control wheat line (Fig 3. and Fig. 4.).

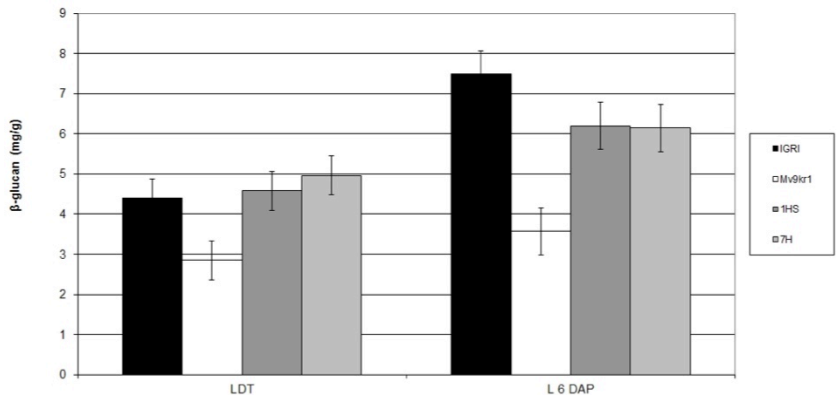

Figure 3.

Figure 3.

Histogram of the (1,3;1,4)-b-D-glucan content in the leaves (L) of barley ('Igri'), wheat ('Mv9kr1'), the 'Mv9kr1'/'Igri' 1HS ditelosomic addition line (1HS) and the 'Mv9kr1'/'Igri' 7H disomic addition line (7H) at the end of tillering (ET) and six days after pollination (6 DAP). The LSD5% values at ET and 6 DAP, respectivly, were 0.48 and 0.58.

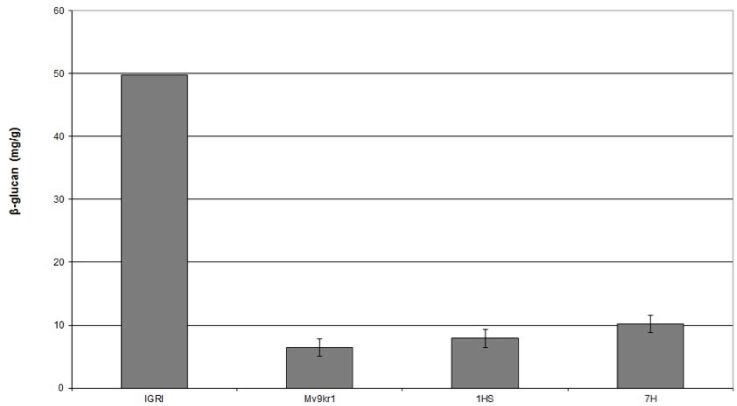

Figure 4.

(1,3;1,4)-b-D-glucan content in the grains of barley ('Igri'), wheat ('Mv9kr1'), the 'Mv9kr1'/'Igri' 1HS ditelosomic addition line (1HS) and the 'Mv9kr1'/'Igri' 7H disomic addition line (7H). There was a statistically significant increase ($P < 0.05$) in (1,3;1,4)-β-D-glucan content in 1HS and 7H addition lines. The bars show the LSD5% value: 1.4.

The results provide new insights into the expression and regulation of the *HvCSLF* genes in the genetic background of wheat and indicate that cisgenesis can be used to increase the leaf and grain ß-glucan content in wheat.

II.) *Production of new high ß-glucan content Rannaja/Manas Robertsonian translocations*

Plants carrying 40 or 41 chromosomes were selected from the progenies of the monosomic stocks of Rannaja 7A, 7B, 7D using Feulgen staining (Figure 5.).

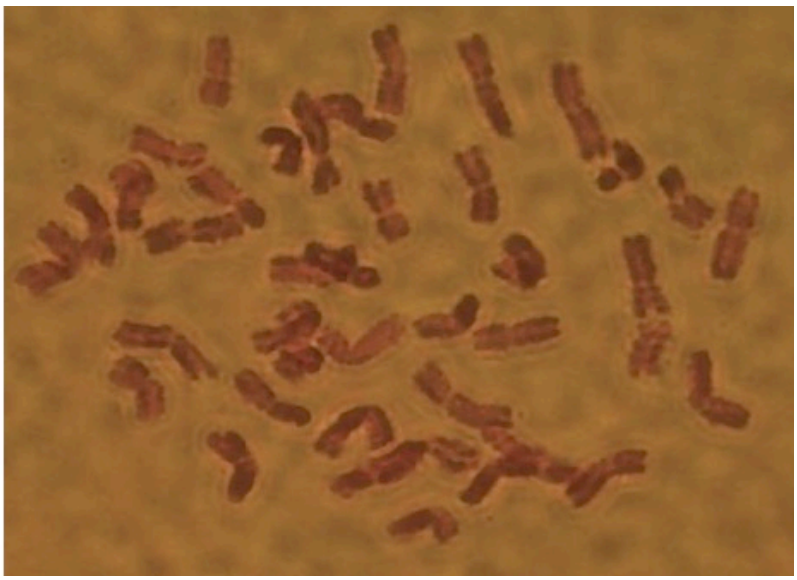

Figure 5.

Feulgen's-stained chromosome preparation of Rannaja 7B monosomic line.

Female plants of the Rannaja 7A, 7B, 7D monosomes were crossed with the disomic wheat/barley (Asakaze komugi/Manas) 7H addition line. In the progenies of the F1 hybrids, plants carrying 42 chromosomes (20" + 7A', 7B', 7D' + 7H') were selected by GISH. Nine 20"+7A'+7H', eleven 20"+7B'+7H' and eight 20"+7D'+7H' double monosomic plants were identified and self-pollinated to produce new Rannaja/Manas 7AS.7HL and/or 7BS.7HL and/or 7DS.7HL translocation lines. The 161 F_2 plants were screened with the help of 7HS (Bmac0031) and 7HL (HvCSLF6, HvID) specific molecular markers. In the 7A group only one plant carried the 7HS chromosome arm while two plants were detected to carry the 7HL arm. Two plants with 7HS arm and three plants with the 7HL arm were identified from the 7B group by molecular markers. The 7D group included five plants with the 7HL chromosome arm. All the F2 plants were selfed and the new translocation lines were selected from the 104 F_3 plants using the GISH technique. In the F3 generation of the 7B group six plants were selected carrying a monosomic 7BS.7HL centric fusion (Figure 6.). In the F3 progenies of the 7D and 7A group only telosomics and isochromosomes were detected. .

Figure 6.
Photos of the wheat/barley 7BS.7HL monosomic translocation line.

These results have been confirmed by FISH and molecular marker analysis using 7BS (Xgwm46), 7BL (Xgwm611), 7HS (Bmac0031) and 7HL (HvCSLF6) specific markers. The selected plants with the 7BS.7HL centric fusion were selfed and the F4 generation was screened to detecte disomic individuals. Three plants were identified to date with disomic 7BS.7HL translocation chromosomes (Figure 7).

The 7BS.7HL disomic Robertsonian translocation lines will be used to increase the β-glucan content and to improve salt and drought tolerance in wheat. It is planned to study the above characteristics in the translocation line.

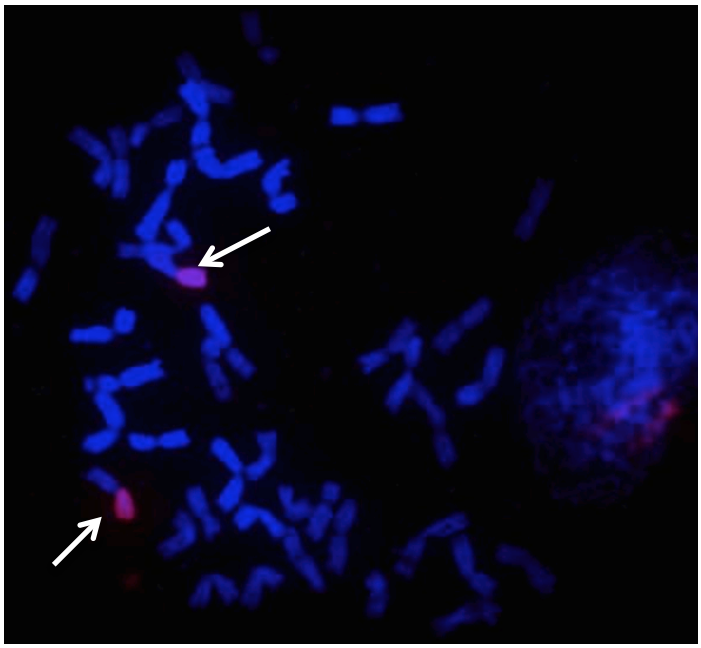

Figure 7.

Genomic *in situ* hybridisation (GISH) on mitotic chromosomes of the wheat/barley 7BS.7HL disomic translocation line. Labelled genomic DNA of barley was used as probe. The 7HL barley chromosome arm is highlighted in red. The chromosomes were counterstained with DAPI (blue).

III.) *Exploration of new Aegilops CSLF genes*

Disomic Mv9/*Ae. biuncialis* addition lines and disomic Chinese Spring/ -*Ae. geniculata,-Ae.umbellulata* and,*-Ae. comosa* addition lines were screened by GISH and FISH (Figure 8.). Progenies were multiplied together with the parental genotypes for the determination of β-glucan content.

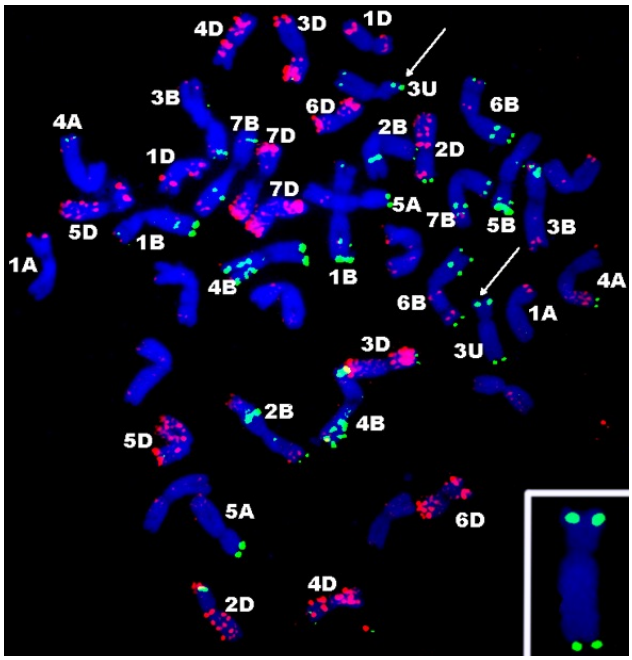

Figure 8.

Figure 8.

Fluorescence *in situ* hybridization (FISH) on mitotic chromosomes of the Mv9/*Ae. biuncialis* 3U addition line with probes for DNA repeats: Afa family (red) and pSc119.2 (green).

The verification of the disomic Mv9/*Ae. biuncialis* addition lines and disomic Chinese Spring/ *-Ae. geniculata*,*-Ae.umbellulat* and,*-Ae. comosa* addition lines using GISH and FISH were completed. The cytologically examined disomic additions were multiplied in the field to obtain sufficient amount of grains for the determination of β-glucan content. The β-glucan content of *Ae. biuncialis* and Mv9/*Ae. biuncialis* addition lines were mesured and the *Ae. biuncialis* ß-glucan level was same such as Manas barley cultivar. The Mv9/*Ae. biuncialis* 7M addition line contained a significantly higher ß-glucan level compared to the control wheat line (Figure 9.), suggesting that an orthologous gene (*CSLF*) is located on the 7M chromosome. However, the *Aegilops CSLF* gene has not been found more effective than the already identified barley *CsIF* genes.

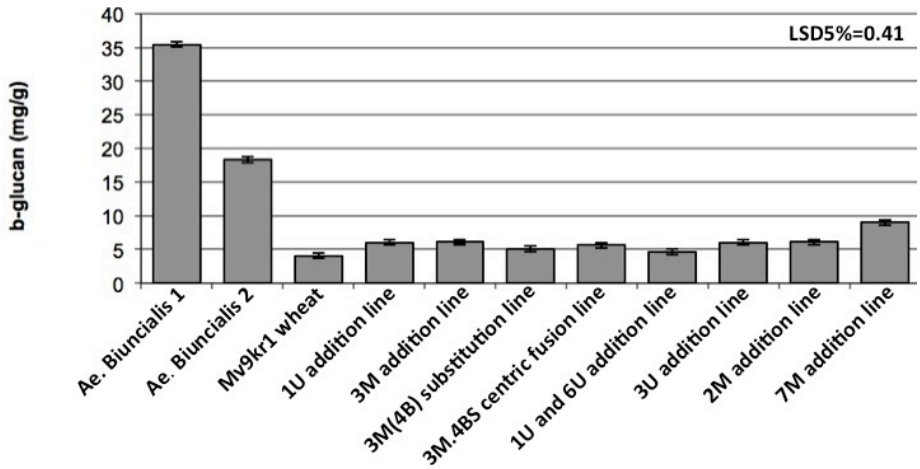

Figure 9.

(1,3;1,4)-b-D-glucan content in the grains of *Aegilops biuncialis*, wheat ('Mv9kr1'), the 'Mv9kr1'/*Ae. biuncialis* substitution line (3M(4B)), translocation line (3M.4BS) and disomic addition lines. There was a statistically significant increase ($P < 0.05$) in (1,3;1,4)-β-D-glucan content in 7M addition line. The bars show the LSD5% value: 0.41.

As the present grant proposal has been terminated 6 months before the planned deadline, results on ß-glucan level analysis of further wheat/*Aegilops* addition lines could not be included in the present report. However, these analyses are ongoing.

With the support of the present grant proposal 4 scientific papers were published in international journals. Near these papers it is planned to publish our results on the production of the 7BS.7HL translocation line.