Expression analysis of cellulose synthase-like genes in durum wheat

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Cellulose synthase-like \textit{CslF} and \textit{CslH} genes have been implicated in the biosynthesis of \(\beta\)-glucans, a major cell wall constituents in grasses and cereals. The low \(\beta\)-glucan content of durum wheat and lack of information of the biosynthesis pathway make the expression analysis in different developmental stages of grain endosperm an interesting tool for the crop genetic improvement. Specific genome sequences of wheat \textit{CslF6} and \textit{CslH} were isolated and the genomic sequence and structure were analysed in the cv. Svevo. In starchy endosperm at five developmental stages (6, 12, 21, 28 and 40 days after pollination) \textit{CslF6} and \textit{CslH} transcripts were differentially expressed. A peak of \textit{CslF6} transcription occurred at 21 dap, while \textit{CslH} was abundant at 28 dap. Significant variations were detected for both the genes in the genotypes. Significant and positive correlation were detected between \(\beta\)-glucan content and \textit{CslF6} gene expression at 21 dap and 40 dap, while no significant correlation was observed for \textit{CslH} gene. On the overall, our correlation analysis reflected data from previous studies on other species highlighting how the abundance of transcripts encoding for \textit{CslF6} and \textit{CslH} enzymes were not necessarily a good indicator of enzyme activity and/or \(\beta\)-glucan deposition in cell wall.

Wheat is one of the most important cereal in the world, providing nutrients for humans and animals. Non-starch polysaccharides (NSP) are important components of wheat kernels and many studies have shown that they have health benefits, including immunomodulatory activity, cholesterol lower activity, faecal bulking effect, enhanced absorption of certain mineral, prebiotic effects, and reducing type II diabetes\textsuperscript{1}. Recently, the attention on food quality over quantity is growing in many areas of the world and dietary fibre are gaining much interest. The principal components of the NSP are \(\beta\)-glucans and arabinoxylans. Even though \(\beta\)-glucans are a minor component of wheat cell walls, they are one of the most important portion of the soluble fibre fraction with beneficial effects for human health.

As for many plant polysaccharides, how and where \(\beta\)-glucans are synthesized, which genes are involved and specific functions, interactions and activities of each enzyme are not totally available\textsuperscript{2}. A superfamily of genes are involved in the synthesis of these polysaccharides, which include \textit{cellulose synthase} (\textit{Ces})\textsuperscript{3-5} and \textit{cellulose-synthase-like} (\textit{Csl}) families\textsuperscript{6}. It has been demonstrated that the superfamily \textit{Csl} is responsible for the synthesis of several plant cell wall polysaccharides and includes subfamilies from A to H, each of which consists of multiple genes\textsuperscript{6}. For example, in rice (\textit{Oryza sativa} L.) there are 37 \textit{Csl} genes in total, while in \textit{Arabidopsis} there are 30\textsuperscript{6}. Not all \textit{Csl} subfamilies are represented in higher plant groups. Thus, the \textit{CslB} and \textit{CslG} subfamilies are found only in dicotyledons and gymnosperms, whereas the \textit{CslF} and \textit{CslH} groups are found only in monocotyledons\textsuperscript{6}, which regulate directly or indirectly the abundance and the fine structure of \(\beta\)-glucans in both grain and the other part of the plant\textsuperscript{11}.

Burton \textit{et al}..\textsuperscript{11} reported that the over-expression of a \textit{CslF} gene, under the control of an endosperm-specific promoter, resulted in the increase of \(\beta\)-glucan content and a dramatic decrease of starch in the transgenic grain. \textit{Brachypodium distachyon}, which has a \(\beta\)-glucan content over 40% of the grain weight and the starch about 6%, provides additional support for a regulatory link between starch and \(\beta\)-glucan synthesis\textsuperscript{13-15}. Furthermore, when \textit{Arabidopsis} was transformed with the \textit{OsCslF6} gene, mixed-linkage glucan was detected in the cell wall indicated that \textit{CslF6} was capable of synthesizing beta glucan\textsuperscript{6}. Four of the corresponding \textit{CslF} genes of barley were mapped to a locus on chromosome 2H (\textit{HvCslF3}, \textit{HvCslF4}, \textit{HvCslF8}, \textit{HvCslF10}) and two other genes on

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Wheat is not recognized as a significant source of β-glucan because of its low β-glucan content. In contrast, barley grains are recognized as a major source of dietary fiber. The biosynthetic pathway of β-glucan has been deeply studied in barley, but limited data are reported in wheat. Therefore, we report the A and B genome sequences of the two main genes, CslF6 and CslH, involved in the β-glucan biosynthesis in durum wheat, and the expression study carried out in the endosperm at different developmental stages to clarify the transcription pattern of those two genes and the linkage with fiber accumulation.

Isolation of genomic sequences of Csl genes in durum wheat. The sequences corresponding to CslF6 and CslH genes from Oryza sativa (Os08g06380 and Os04g35030, respectively) and Hordeum vulgare (MLOC_57200 and MLOC_53007, respectively) were used as starting points to isolate the durum wheat sequences. As reported in Supplementary File S1, using the cv. Svevo, the A and B homoeologous sequences of the Csl durum genes were isolated with the corresponding cDNAs. Due to the availability of SNP data in bread and durum wheat, the current analysis allowed to detect some SNP markers inside both genes. In particular, two (IWB6109 and IWB23981) and four (IWB4561, IWB4622, IWB14446, IWB66376) SNP markers, corresponding to CslF6 and CslH gene sequences, respectively, were identified. The SNPs within CslF6 gene mapped on chromosomes 7, whereas those of the other gene localized on chromosome group 2 (Table 1).

Characterization of wheat Csl gene sequences. As our main interest was the study of the possible relationships between Csl genes and β-glucan accumulation in the wheat grain, we analyzed the genomic sequence and structure of CslF6 and CslH genes in the cv. Svevo (Fig. 1a,b). Relying on our bioinformatics analysis regarding the CslF6-7A gene, the genomic sequence was 5,879 bp, including an mRNA of 2,838 bp and a protein of 945 amino acids. For CslF6-7B, the genomic sequence length was 5,628 bp, allowing to obtain a cDNA of 2,826 bp coding a protein of 941 amino acids.

Figeneshewa et al. gene prediction was used to define the intron/exons structure predicting a similarity between both the wheat CslF6 genes composed by 3 exons and 2 introns sharing an identity of 97% among the homoeologues. Differences were underlined for the first exon between the A and B genome, while exons two and three displayed 100% of identity (Fig. 1a). BLAST analysis using Phytozome v.7 software (http://www.phytozome.net) with O. sativa and H. vulgare genomes allowed the comparison of wheat CslF6 genes with the orthologues genes located on chromosome 8 of rice (locus name: Os08g06380) and chromosome 7 of barley (locus name: MLOC_57200). The rice CslF6 had a sequence of 5,438 bp with a CDS of 2,859 bp, whereas the barley gene had a sequence of 5,188 bp with a CDS of 2,724 nucleotides. Considering the wheat CslF6-7A with the rice and barley genomic sequences, the identities detected were 88% and 96%, respectively, and 96% between wheat CDS and the other two CDS considered. A similar intron/exons structure was observed between CslF6-7A from wheat and CslF6 from rice and barley.
composed by 3 exons and 2 introns (Fig. 1a). An identity of 88% were detected between wheat and rice *CslF6* genomic sequence and 87% between the two cDNA sequences. Whereas identity of 96% was found aligning both the *CslF6-7B* genomic sequence and mRNA with the barley *CslF6* ones.

Comparison between both the wheat *CslF6* protein sequences (A and B genome) and rice and barley enzymes showed identity of 85% and 98%, respectively.

In addition, *CslF6* gene sequences from bread and durum wheat were compared (Fig. 1a) showing for both the A and B genome an identity of 99%. In details, Chinese Spring *CslF6-7A* had a genomic sequence of 5,879 bp

| Gene | Enzyme | SNP name | SNP id | Allele | Scaffold |
|------|--------|----------|--------|--------|----------|
| *CslF6* | Cellulose synthase-like protein F6 | BS00009773_51 | IWB6109 | T/G | TGACv1_scaffold_592076_7BS: 95,657-95,721 |
|  |  | | | | TGACv1_scaffold_555973_7AL: 251,795-251,905 |
|  |  | | | | TGACv1_scaffold_577473_7BL: 37,700-37,810 |
|  |  | | | | TGACv1_scaffold_607937_7DL: 4,686-4,796 |
| *CslH* | Cellulose synthase-like protein H | BobWhite_c90_1565 | IWB4561 | A/G | TGACv1_scaffold_158387_2DL: 95,998-96,108 |
|  |  | | | | TGACv1_scaffold_094351_2AL: 6,791-6,898 |
|  |  | | | | TGACv1_scaffold_129372_2BL: 297,426-297,698 |
|  |  | | | | TGACv1_scaffold_129372_2BL: 298,012-298,119 |
|  |  | | | | TGACv1_scaffold_129372_2BL: 298,012-298,119 |
|  |  | | | | TGACv1_scaffold_158387_2DL: 94,798-94,905 |
|  |  | | | | TGACv1_scaffold_094351_2AL: 6,791-6,898 |
|  |  | | | | TGACv1_scaffold_094351_2AL: 6,791-6,898 |

Table 1. List of β-glucan genes in wheat with corresponding SNP markers, allele change and scaffold localization.

Figure 1. Comparison of *CslF6* (a) and *CslH* (b) gene structures in rice, barley, durum wheat (A and B genome) and bread wheat (A, B and D genome) is shown based on coloured boxes highlighting conserved exons. Intron and exon sizes are shown as well as the whole gene (in brackets the total length). Rice, barley and both wheat *CslF6* share the same structure with three exons of conserved sizes and two introns. Differences were detected in *CslH* structure among the three species. Black dashed line indicates the absence of intron sequence.
and the same structure and exons length of durum wheat, while differences were detected into the introns length. Interesting results were observed for the bread B genome, which showed a gene of 5,843 bp with same exon number and length, different introns amino acid number and a transposome of 212 bp into the first intron, which could be one of the reason of a different and higher final β-glucan content in bread wheat. In addition, the transposome gene located in the first intron of the B gene is responsible of the synthesis of four different transcripts of 941 aa, 827 aa, 806 aa and 643 aa, respectively. Interesting results were detected for the CslH genomic sequence and structure. In details, the CslH-2A gene sequence was 3,089 bp, counting a cDNA of 2,259 bp and a protein of 726 amino acids, while the CslH-2B had a gene sequence length of 3,156 bp, transcribing a mRNA of 2,277 bp and a final protein size of 595 amino acids. Different intron/exons structure was detected between the two CslH homeologues: 9 exons and 8 introns were reported for the A genome gene and 8 exons and 7 introns for the B genome with 95% of identity. The difference among the intron/exon numbers is due to the merging of the exons 3 and 4 of the A genome into exon 3 of the B genome (Fig. 2b).

Again the CslH gene sequences from Chinese Spring and Svevo were compared highlighting same gene length of 3,089 bp and intron/exon structures for A genome. Three different transcripts were found for CslH-2A counting of 752 aa, 748 aa and 737 aa.

Differences were detected examining sequences from bread and durum B genomes. The Chinese Spring CslH, in fact, showed a gene of 3,295 bp, with a cDNA of 2,400 bp and a transcript of 799 with a gene structure similar to the A genome with 9 exons and 8 introns.

Valuation of wheat CslH genes with the orthologous genes located on rice chromosome 4 (locus name: Os04g35030.1) and barley chromosome 2 (locus name: MLOC_53007) was carried out. The rice and barley CslH had a sequence of 5,392 bp and 3020 bp, with a CDS of 1,218 bp and 2,256 nucleotides, respectively. Analysis of identity between wheat CslH-2A and CslH-2B and rice genes showed a same score for genomic and CDS sequences (84% and 79% respectively), while 66% and 63% for the proteins. Wheat CslH-2A and CslH-2B and barley genomic sequences showed identities of 90% and 87%, respectively, with 93% for both the considered CDS and 89% and 84% for the proteins.

A comparative analysis of the durum CslH-2A gene and the correspondent bread wheat one was not carried out due to the lack of bread gene portions, while identity of 100% was detected between the two wheat CslH-2B genes.

Figure 2. Gene expression study of CslF6 (a) and CslH (b) in durum wheat endosperm at different developmental stages (6, 12, 21, 28 and 40 days after pollination). Different letters on the bars indicate datasets significantly different according to ANOVA followed by Duncan’s test (P < 0.05).
Different intron/exons structure was observed between wheat CslH-2A and rice CslH composed by 9 exons and 8 introns and 8 exons and 7 introns, respectively, due to the splitting of the rice first exon into two wheat exons (Fig. 1b). barley CslH structure showed a merge of exon 7 and 8 of durum CslH-2A into barley CslH exon 7. The CslH-2B showed the same intron/exon numbers of rice and barley CslH with 8 exons and 7 introns, but in this case rice exon 1 corresponded to wheat exons 1 and 2 and rice exons 2 and 3 merged into wheat exon 3; however barley exon 3 and 4 combined in wheat exon 3, and barley exon 7 split in wheat exons 6 and 7 (Fig. 1b).

Table 2. β-glucan content of ten durum wheat cultivar grown at Valenzano (Metropolitan City of Bari, Italy) for three years (2012, 2013 and 2016). The reported value are the mean of three biological replicates. LSD and coefficient of variation were calculated in GenStat 14.

| Source of variation | Degrees of freedom | Mean square |
|---------------------|--------------------|-------------|
| Blocks              | 2                  | 0.002       |
| Year                | 2                  | 0.017       |
| Genotype            | 9                  | 0.081***    |
| G × Y               | 17                 | 0.003       |
| Error               | 28                 | 0.003       |

Table 3. Mean squares from the analysis of variance of β-glucan content in ten wheat genotypes grown at Valenzano (Bari, Italy) in three years. ***Significant differences $P \leq 0.001$.

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| Error               | 28                 | 0.003       |

β-glucan content and expression profile of CslF6 and CslH genes. The CslF6 and CslH gene expression levels were determined in eight durum wheat genotypes (Avonlea, Canyon, Cappelli, Ciccio, Duilio, Latino, Simeto, Svevo) and in two ssp. *dicoccoides* accessions (MG4328/61, MG4413).

Total β-glucan amount for each line in three different years with LDS and coefficient of variation was reported in Table 2. The ANOVA analysis revealed highly significant variation ($P \leq 0.001$) among genotypes (Table 3). Avonlea showed the highest β-glucan content (0.70%) followed by Canyon (0.62%), while MG4413 had the lowest amount (0.28%). Our results were in line with what previously reported in barley. In fact, wheat is not recognized as a significant source of β-glucan because of it has low content in the grain, usually <1% on a dry weight basis. Although values up to 2.3% have been reported in bread wheat. The significant concentration of β-glucan in wheat grain is in the sub-aleurone layer, where low amount was found in the rest of the endosperm. Because the wheat endosperm is ground into flour and provides nutrition in the form of starch and proteins, kernels with higher β-glucan content would make durum wheat a good and complete source of nutrients for human diet.

To better understand the wheat β-glucan biosynthesis and accumulation, we analysed the expression pattern of the two main genes, CslF6 and CslH, associated with β-glucan concentration in barley. Starting from ten genotypes, our aim was to define the expression pattern of the two genes and highlight a possible correlation with final β-glucan kernel content. mRNA extracted from endosperm of the ten lines at different developing endosperm stages (6, 12, 21, 28 and 40 days after pollination) was used to determine the transcription levels of CslF6 and CslH. Considering the mean values of the ten genotypes, we observed that the expression pattern of the two genes was different. Levels of CslF6 mRNA were generally low in the first two developmental stages (6 and 12 dap) and relatively abundant in endosperm at 21 and 28 dap (Fig. 2a), while CslH transcription levels, less expressed compared to CslF6 transcripts, were low at 6 and 12 dap, moderately high at 21 dap and high at 28 and 40 dap (Fig. 2b). Our data are supported by previously studies in barley, which reported variation on the transcription levels of HvCslF6 throughout endosperm development with increases in the abundance of expression from 12 to 20 dap. Different results were reported in barley for HvCslH transcripts. Maximum transcript levels, in fact, were reached at 4 dap and slightly decreases for HvCslH were detected at 24 dap. The different data of HvCslH transcripts between our report and previously studies in barley could be due to two different reasons: the endosperm developmental stages used for the experiments (we analysed the expression pattern until 40 dap,
while in barley from 4 to 24 dap) and the role of this gene in β-glucan synthesis during secondary wall development in the two different species (wheat and barley)

The abundance of transcript of CslF6 in each genotypes was monitored independently during the development of wheat endosperm. As shown in Fig. 3 the genotypes showed different gene expression amount in the developmental stages analysed. The varieties, with higher transcript variation, resulted, for both the genes, Avonlea, Canyon, Latino, Duilio and MG4413. Transcripts of the CslF6 gene appeared statistically significant (P ≤ 0.01; P ≤ 0.05) in Avonlea, Duilio, Cappelli and MG4413 at 21 dap; Canyon, Latino, Duilio, Svevo and MG4413 at 28 dap; MG4328/61 and Latino at 40 dap (Fig. 3a). Previous studies on differential expression of cellulose synthase-like genes CslF6 in grain developmental stages confirmed our data on wheat kernel, detecting differences between barley lines during the endosperm developmental stages.

Again, differences between the ten varieties were detected for the CslH expression amount (Fig. 3b) as reported in literature on barley varieties. In detail, MG4413 showed statistical significant difference (P ≤ 0.05) at 21 dap; Latino, Duilio, Svevo and MG4413 at 28 dap ((P ≤ 0.05); and MG4413 at 40 dap (P ≤ 0.01).

The second aim of our work was to correlate the gene expression with the final β-glucan content in grains. Correlation analysis between CslF6 and CslH transcripts and β-glucan at each developmental stage were implemented in GenStat (Table 4). Significant and positive relationships were observed among β-glucan content and CslF6 expression at 21 dap and 40 dap (P ≤ 0.01 and P ≤ 0.05, respectively). The data observed were in line with what reported by Burton et al. in two barley varieties, the elite malting variety and the hulless barley.

Our experiment did not allowed us to find any significant correlations between β-glucan content and the expression of the CslH gene during the endosperm maturation. Even other results reported in literature on barley grain highlighted how levels of HvCslH transcripts were relatively low throughout the starchy endosperm during development compared to other genes involved into β-glucan biosynthesis, confirming the complexity of this trait and the necessity of further investigation at the enzymatic level and localization of gene expression.

On the overall, our correlation analysis reflected data from previous studies on other species highlighting how the abundance of transcripts encoding for CslF6 and CslH enzymes were not necessarily be a good indicator of enzyme activity and/or β-glucan deposition in cell wall. In addition, the molecular mechanisms of β-glucan biosynthesis is complex and different enzymes are crucial for their deposition and accumulation.

**Table 4. Correlation analysis between gene expression and β-glucan content at different developmental stages.**

| Genes | 6 dap | 12 dap | 21 dap | 28 dap | 40 dap |
|-------|-------|--------|--------|--------|--------|
| CslF6 | BG    | -0.062 | -0.099 | 0.835** | 0.385  | 0.670* |
| CslH  | BG    | -0.035 | 0.171  | 0.628  | 0.143  | 0.192  |

**Significant differences P ≤ 0.01. *Significant differences P ≤ 0.05.**
Δ and analyses were performed using CFX Manager 2.1 software (Bio-Rad Laboratories) using the qRT-PCR data for both genes were derived from the mean values of three independent amplification reactions of the PCR products and by sequencing of the amplified fragments (3500 Genetic Analyzer, Applied Biosystems). A specific primer pair for CslH we were interested in understanding the expression profile of the two genes during the developmental stages and A and B genomes for both genes, therefore no genome specific primer were designed. In addition, at this stage the content was determined by the Mixed-Linkage β-glucan Assay Kit (Megazyme International Ireland Ltd, Wicklow, Ireland) based on the accepted method by McCleary and Codd and included the industrial standard for barley (4.1% of β-glucan).

Material and Methods

Plant material. A set of ten genotypes, including eight cultivars of durum wheat, Triticum turgidum subsp. durum (Avonlea, Canyon, Cappelli, Cicco, Duiillo, Latino, Simeto, Svevo) and two accessions of the ssp. dicocoides (MG4328 and MG4413), were sub-chosen from a collection of 230 tetraploid wheat (Triticum turgidum L.) genotypes described by Marcotuli et al. and characterized by different total β-glucan content. A randomized complete block design with three replications and plots consisting of 1-m rows, 30 cm apart, with 80 germinating seeds per plot, was used in the field experiments. During the growing season, 10 g of nitrogen per m² was applied at the beginning of planting and standard cultivation practices were adopted. Plants were hand harvested at maturity and grain was stored at 4 °C. Using the 1093 Cycloptic Sample Mill (Tecator Foss, 119 Hillerød, Denmark), the grain was ground and passed across a 1 micron sieve. Endosperm from each genotype was collected in five developmental stages (6, 12, 21, 28 and 40 days after pollination) and stored at –80 °C for subsequent RNA extraction.

Analysis of variance, LSD and coefficient of variation were carried out for each trait using GenStat14 (version 18, VSN International Ltd, Hemel Hempstead, UK). Correlation analysis was conducted between CslF6 and CslH genes expression and β-glucan content at different developmental stages.

Cellulose synthase genes (CslF6 and CslH) isolation and characterization. The sequences of cellulose synthase-like genes from Oryza sativa and Hordeum vulgare were downloaded from the Rice Genome Annotation Project database (http://rice.plantbiology.msu.edu/) and EnsemblePlants (http://plantsensembl.org/Hordeum_vulgare/Info/Index), respectively, and used as initial query probe in the Durum Wheat Genome Database cv. Svevo (http://d-data.interomics.eu/node/1) for the wheat Csl sequences. Subsequently, the genomic and cDNA sequences were isolated and characterized amplifying target DNA from cv. Svevo. Structure of both CslF6 and CslH genes was reported by all genome specie browsers and confirmed by FGENESH program (http://www.softberry.com/berry.pl?topic=fgenes&group=programs&subgroup=gfinder).

The two CslF6 and CslH whole sequences were blasted against the available dataset of SNP marker sequences reported by Wang et al. and SNPs with ≥80% identity were considered within the Csl genes.

RNA extraction and cDNA synthesis. Annotated cDNA sequences from the two wheat genes CslF6 (KP260638.1) and CslH (AK332242.1) were used for the primer pairs design. No differences were detected among A and B genomes for both genes, therefore no genome specific primer were designed. In addition, at this stage we were interested in understanding the expression profile of the two genes during the developmental stages and among different genotypes. A specific primer pair for CslF6 was designed on exon 3 starting at 1.533 bp and with a sequence length of 260 bp, while CslH gene primers were picked at 420 bp of the last exon and counting of 195 bp sequence (Table 5).

In order to analyse the expression level of the two genes, total RNA was extracted from the endosperm of each genotypes using the RNeasy Plant Mini Kit (QIAGEN®) and checked on 1.5% denaturing agarose gel. All RNA samples were lead to the same concentration (1 µg) and reverse-transcribed into double stranded cDNA with QuantiTect Reverse Transciptase Kit (QIAGEN®). Data were normalized using three reference genes: Cell Division Control AAA-Superfamily of ATPases (CDC), ADP-Ribosilation Factor (ADP-RF) and RNase L Inhibitor-like protein (RLI). These genes have a stability value around 0.035 when evaluated with NormFinder software.

Table 5. Csl primer combinations used for the qPCR experiments. For each primer forward and revers sequences and product length are reported.

| Gene name | Forward sequence (5′-3′) | Reverse sequence (5′-3′) | Product length | Annealing temperature (°C) | Position |
|-----------|--------------------------|--------------------------|----------------|---------------------------|----------|
| CslF6     | AGTCGTTGACATGAGCAGGG     | GGGGCTCTTCAGAATTCCCGG    | 260            | 64                        | Exon 3   |
| CslH      | TGCTGGCTCGTGGATGCTT      | TCTGCCAGAACCGAATT       | 195            | 65                        | Exon 9   |

qPCR for cellulose synthase genes. Quantitative Real-Time PCR was carried out using Eva® GREEN in the CFX96TM Real time PCR Systems (Bio-RAD). The PCR cycle was 95 °C for 3 min followed by 40 cycles of 95 °C for 10 sec and at 60 °C for 30 sec. Amplification efficiency (98% to 100%) for the primer set was determined by amplification of cDNA with a series of six scalar dilution (1:5) per reaction. Each 10 µL PCR reaction contained 1 µL of a 1:5 dilution cDNA, 5 µL of EvaGreen Mix 10× (Bio-Rad), and 500 nM of each primer. Fluorescence signals were collected at each polymerization step. The specificity of the amplicons was confirmed by the presence of a single band of expected size for each primer pair in agarose gel (2% w/v), by a single peak melting curves of the PCR products and by sequencing of the amplified fragments (3500 Genetic Analyzer, Applied Biosystems).

Analysis of variance, LSD and coefficient of variation were used to underline significant differences between the genotypes for the two considered Csl genes.
Conclusion
Durum wheat kernel contains macronutrients such as protein, fat, and carbohydrate that are required for growth and maintenance, and also important minerals, vitamins, and other micronutrients essential for optimal health. It has been demonstrated that whole grain cereals have a key role on improving human health and lower the risk of serious, diet-related diseases. Dietary fibre is one of the most important components of whole grain cereals from this standpoint. In particular, β-glucan make up an important proportion of dietary fibre in many diets.

The identification of genes involved in the biosynthesis of the β-glucan opened the way for the genetic improvement of cereal quality parameters important in human health. Different genes are involved in β-glucan biosynthesis of different tissues/cell types, with CsILF6 and CsILH genes making the main part of the process.

The results presented here represent additional information on the gene sequences for gene involved in β-glucan pathway, biosynthesis and accumulation of β-glucan in durum wheat. The data not only are consistent with what reported in barley, but also contribute to our understanding of the genetic complexity of this important agronomic trait. It allowed us the evaluation of the expression of CsILF6 and CsILH transcript variation in wheat endosperm at different developmental stages (from 6 to 40 dap). In addition, the correlation analysis between the final β-glucan amount in wheat grains and the expression levels of these two genes open a way for further genetic studies on other genes involved in the biosynthesis and degradation of β-glucan.

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**Author Contributions**

I.M., P.C., A.B. and A.G. conceived and designed the experiments; I.M. and P.C. performed the experiments; I.M. and P.C. analysed the data; A.G. contributed reagents/materials/analysis tools; I.M., P.C., A.B. and A.G. wrote the paper.

**Additional Information**

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