JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

GENOME-WIDE IDENTIFICATION AND ANALYSIS OF THE *CslF* GENE FAMILY IN BARLEY (*Hordeum vulgare* L.)

Mutthanthirige Don Lalith Chandana Nishantha[1,2], Diddugodage Chamila Jeewani[1,3], Guangwei Xing[1], Xiaojun Nie[1] and Song Weining[1]*

Address(es):
[1]State Key Laboratory of Crop Stress Biology in Arid Areas, College of Agronomy, Northwest A&F University, Yangling, Shaanxi, 712100, China.
[2]Directorate of Agriculture and Livestock, Army Cantonment, Panagoda, Homagama, 10200, Sri Lanka.
[3]Grain Legumes and Oil Crops Research and Development Centre, Department of Agriculture, Angunakolapelessa, 82220, Sri Lanka.

*Corresponding author: mdlcnishantha@gmail.com

doi: 10.15414/jmbfs.2020.10.1.122-126

ARTICLE INFO

Received 27. 6. 2019
Revised 11. 5. 2020
Accepted 22. 5. 2020
Published 1. 8. 2020

Regular article

OPEN ACCESS

ABSTRACT

The Cellulose synthase-like (*Csl*) F family has been considered as one of the most crucial genes regulating β-glucan synthesis. It is a cereal cell wall component, holding advantages for human nutrition, but disadvantages in animal nutrition, malting and brewing industries. Based on a genome-wide search method, present study identified barley *CslF* gene family members by considering the importance of (1,3;1,4)-β-D-glucan and newly completed barley genome. A sum of Eighteen *CslF* genes were recognized in the barley genome. Then, phylogenetic analyses classified them into 3 groups, that shared conserved motif compositions. A new motif, D,D,WQxxD was also found, which was responsible for the cellulose synthase. Furthermore, using RNA-seq data, the *HvCslFs* expression profiles were systematically examined in different tissues and tissue-specific candidates were found. Lastly, interaction network analysis identified 11 *CslF* genes involved in the interaction network. All together, prsent task provides valuable evidence about the genomic organization and evolutionary relationship of the *CslF* gene family in barley, and facilitate the functional surveys of *CslF* genes in barley and beyond.

Keywords: Barley; β-glucan; *CslF*; Phylogenetic analysis; Expression profile

INTRODUCTION

Barley (*Hordeum vulgare*), the fourth main cereal cultivated worldwide is an ancient crop, which is used as feed, food, malt and brewing industries **(Arngren *et al.*, 2011)**. It is also a highly adapted crop species, which can be grown in both desert and fertile lands **(Newman *et al.*, 2006)**. Barley grain endosperm cell wall contains (1, 3; 1, 4)-β-D-glucan (hereafter mentioned as β-glucan) **(Fincher *et al.*, 2004)** and considering the rich β-glucan availability, researches on barley are increased during last decades **(Bhatty, 1999; Bilgi *et al.*, 2004)**. β- glucan is a partially water-soluble linear polysaccharides molecule, which contains glucose **(Johansson *et al.*, 2004)** linked by both β-(1→3) and β-(1→4)-linkages **(Rimsten *et al.*, 2003)**. Barley β-glucan is beneficial for human health, which may reduce the risks of cardiovascular diseases mainly coronary heart disease, high serum cholesterol, colorectal cancer, non-insulin-dependent diabetes, obesity and hypertension **(Li *et al.*, 2003; Keogh *et al.*, 2003; Brennan *et al.*, 2005)**. Also, it has immune modulating properties, increasing vitamin and mineral bio-availability, and important in gut physiology while influencing spatial memory performance of children **(Klopfenstein, 1988; Thorburn *et al.*, 1993; Murphy *et al.*, 2004; http://www.nutraingredients.com)**. At the same time, β-glucan has adverse impact on processing applications of cereals mainly on brewing and malting while anti-nutritive for mono-gastric animals feed formulations. In feed formulations, it affects growth and feed conversion efficiency, nutritional intake of animals and stickiness of droppings. Further, when used for brewing, it reduces haze formation and the rate of wort filtration in beer, and negatively affect the malt extraction recovery **(Hesselman *et al.*, 1982; Wang *et al.*, 1992; Brennan *et al.*, 2005)**. Glycosyltransferases are in charge of the production of major wall polysaccharides ie.; mannans, xyloglucans and β-glucans **(Scheller *et al.*, 2010)**. The backbones of wall polysaccharides synthases are encrypted by an enormous multigene family which is named as the Cellulose synthase/Cellulose synthase-like (CesA/Csl) superfamily **(Richmond *et al.*, 2000)**. It includes several sub families such as cellulose synthase sub-family (CesA) and cellulose synthase-like (Csl) sub-families, CslA to CslJ, and all of them consist of multiple genes **(Schwerdt *et al.*, 2015)**. β-glucan synthesis is done by the *CslF* gene family **(Richmond *et al.*, 2000; Burton *et al.*, 2006; Schreiber *et al.*, 2014)**.

Considering the significance of β-glucan for human health, brewing and malting industries, it's important to aware that *CslF* gene family which is direct for β-glucan synthesis. In this study we examined the *CslF* gene family in barley based on a bioinformatics search using latest genomic information. The phylogenetic tree, interaction network, conserved motifs and gene expression pattern of *CslF* were further systematically analyzed. Present study provides the basic genomic organization information of *CslF* genes in barley, and it will help for further functionalstudies.

MATERIAL AND METHODS

CslF genes in barley

Using the method given by Wang *et al*., 2016 all possible members of barley *CslF* gene family were recognized. To create a local protein database, all existing protein sequences for *Hordeum vulgare* L. were retreived from the Ensemble database (http://plants.ensembl.org/index.html) **(Bolser *et al.*, 2016)**. The available *CslF* genes of *Arundo donax*, *Avena sativa*, *Brachypodium distachyon*, *Zea mays*, *Sorghum bicolor*, *Triticum aestivum*, *Oryza sativa* and *Triticum urartu* depositing in National Center for Biotechnology Information (NCBI) database and hmm-build tool embedded in HMMER 3.0 were utilized to build a hidden Markov model (HMM) profile. Then the HMM profile and the Hmmsearch tool embedded in HMMER 3.0 were applied to search barley proteins **(Wheeler *et al.*, 2013)**. Conserved domains of barley *CslF* members were further confirmed by InterProScan database **(Zdobnov *et al.*, 2001)** and PFAM **(Finn *et al.*, 2016).** Lastly, sequence verification was done by a BLASTN (Nucleotide BLAST) similarity search compared to barley expressed sequence tags (ESTs) deposited in the NCBI database. The Mw (molecular weight) of candidate and theoretical pI (isoelectric point) value of genes were calculated by online compute pI/Mw tool **(Gasteiger *et al.*, 2003)**. Using the CELLO v2.5 web server, subcellular localization of those genes were predicted **(Yu *et al.*, 2006)**.

Phylogenetic analysis and multiple alignments

ClustalW tool was used to perform multiple sequence alignments **(Larkin *et al.*, 2007)**. Then the phylogenetic tree was created by combining neighbor-joining

method and bootstrap test method with thousand replications in MEGA 6.0 software **(Tamura *et al*., 2013)**. The conserved motifs of *CslF* were predicted by the MEME program **(Bailey *et al*., 2009)**.

CslF RNA-seq datasets expression profiles

RNA-seq datasets obtained from NCBI Sequence Read Archive (SRA) was utilized to learn the expression profile of *HvCslF* genes in various tissues. Sample information and the data used were depicted in Table S1. TopHat and Cufflinks software were used to analyze gene expressions **(Trapnell *et al*., 2012)**. For each gene, the FPKM value was taken. To generate the heat map, log10-transform (FPKM +1) values of *HvCslF* genes were used.

Analysis of Co-expression network

To investigate the gene function and regulatory pathway, the most commonly used method is study of co-expression networks. Barley *CslF* genes co-expression network was created with the help of WGCNAR_1.49 package by analyzing RNA-seq data using weighted correlation network analysis **(Langfelder *et al*., 2008)**.

RESULTS AND DISCUSSION

Genome-wide identification of the *CslF* gene family in barley

To find out the members of *CslF* family in barley, we performed a HMM search using the latest updated genome resource and totally 18 non-redundant *CslF* genes have been recognized in barley genome (Tab 1). Based on the chromosome location, the predicted barley *CslF* genes were then designated as *HvCslF1* to *HvCslF18*. *CslF* genes count in barley (18) was greater than maize (7) and rice (8) **(Schwerdt *et al*., 2015; Penning *et al*., 2009)**. *HvCslF* cascade genes locations were not random along barley chromosomes. Four *HvCslF* genes per chromosome (total 12) were located on 2, 5 and 7 chromosomes, while 3 genes were located in chromosome 1. One gene per each chromosome was on chromosome 3 and 6. There were no *HvCslF* genes found on chromosome 4.

According to the literature, previous studies have discovered only 10 *CslF* genes in the *CslF* subfamily in barley **(Schreiber *et al*., 2014; Burton *et al*., 2011)**. Present experiment, identified 18 *CslF* genes in barley using newly completed barley genome through a genome-wide search. According to **Burton *et al*., (2008)**, the 7 *CslF* genes found from barley were divided into two groups and 4 *HvCslF* genes were mapped to 2H chromosome. Rest of the *HvCslF* genes were mapped to chromosomes 7H, 5H and 1H. In 2014, **Schreiber *et al*., (2014)** mapped 10 *HvCslF* genes on barley chromosomes, where 5 *HvCslF* genes in chromosome 2H and the rest of *HvCslF* genes have been mapped to chromosomes 7H, 5H and 1H.

The putative *HvCslF* proteins' length was starting from 606 to 1207 amino acids, with theoretical pI extending from 6.04 to 8. 83 and putative molecular weights (Mw) starting from 68500.08 up to 132914.06 Da. According to the Subcellular localization analysis, most of the *HvCslF* genes were localized in Inner Membrane (Table 1). BLASTN search against UniGene database and barley EST using the *HvCslF* genes as queries was done to find out the actual presence of above putative genes. Results showed that all *HvCslF* genes had EST support, suggesting they are truly found in barley genome.

Table 1 Features of putative barley cellulose synthase-like (*HvCslF*) genes

Gene	Ensemble Barley Gene ID	Chromosome Number	Amino Acid Length	pI	Mw (Da)	EST Count	Subcellular Location
HvCslF1	HORVU0Hr1G038120	Unknown	1144	8.26	128803.08	19	Cytoplasmic
HvCslF2	HORVU1Hr1G022900	1	802	8.18	90216.61	9	InnerMembrane
HvCslF3	HORVU1Hr1G026320	1	920	6.5	100340.87	18	Cytoplasmic
HvCslF4	HORVU1Hr1G039250	1	966	8.39	109510.29	21	InnerMembrane
HvCslF5	HORVU2Hr1G042240	2	821	6.18	92464.71	9	InnerMembrane
HvCslF6	HORVU2Hr1G042250	2	897	7.89	99823.28	9	InnerMembrane
HvCslF7	HORVU2Hr1G042350	2	869	7.92	97507.51	9	InnerMembrane
HvCslF8	HORVU2Hr1G042370	2	900	7.01	101803.96	7	InnerMembrane
HvCslF9	HORVU3Hr1G071770	3	1041	6.04	116685.80	20	Cytoplasmic
HvCslF10	HORVU5Hr1G023640	5	1207	8.32	132914.06	5	InnerMembrane
HvCslF11	HORVU5Hr1G064230	5	686	8.72	77120.31	23	InnerMembrane
HvCslF12	HORVU5Hr1G110000	5	1023	8.22	114840.73	17	InnerMembrane
HvCslF13	HORVU5Hr1G118270	5	1141	8.83	129402.91	16	InnerMembrane
HvCslF14	HORVU6Hr1G050750	6	838	8.72	94826.85	21	InnerMembrane
HvCslF15	HORVU7Hr1G005270	7	1188	7.07	131851.52	26	InnerMembrane
HvCslF16	HORVU7Hr1G070010	7	947	8.54	103629.28	20	InnerMembrane
HvCslF17	HORVU7Hr1G081850	7	606	8.51	68500.08	5	InnerMembrane
HvCslF18	HORVU7Hr1G121040	7	834	6.59	93563.78	9	InnerMembrane

Legend: Molecular weight (Mw), Expressed sequence tags (EST) and Isoelectric point (pI),

Analysis of multiple alignments, phylogenety and conserved motif of *HvCslF*

Using ClustalW software, the full-length protein sequences of the 18 *HvCslF* were aligned to evaluate the phylogenetic relationships of the *HvCslF* genes **(Larkin *et al*., 2007)**. Further, the MEGA 6.0 with neighbor joining (NJ) method has been used to phylogenetic tree construction **(Tamura *et al*., 2013)**. We observed highly conserved motif regions in the sequences of the *HvCslF* genes (Figure 1A). Considering the phylogenetic analysis *HvCslF*s were divided into three groups (Figure 1B), which included 6 (Group I), 4 (Group II), and 8 (Group III) members, respectively. The results showed some evolutionary changes found in barley *CslF* genes, which was consistent with those in *Oryza sativa* **(Burton *et al*., 2008)**. Furthermore, each *CslF* cluster recognized by phylogenetic analysis, has same composition of conserved motifs. Totally 15 motifs have been recognized in *HvCslF* proteins. All of the *CslF* gene products contain a D,D,D,QxxRW motif, which has been called as the nucleotide sugar-binding domain as well as the catalytic site of enzymes **(Richmond *et al*., 2000; Schwerdt *et al*., 2015; Doblin *et al*., 2009)**. Motif 3 (IPR00150) is an important motif in the *HvCslF* family, being responsible for the cellulose synthase, which was found by InterProScan analysis. Other most important motifs in the *HvCslF* family are Motifs 1 and 2, which containing D,D,WQxxD. Conserved domains analysis and identification may assist to identify gene's functional units as well as elaborate their tasks in growth and development of a plant. The Motif D, D,D,QxxRW is available in other cereals like rice, maize, *and Brachypodium distachyon* **(Schwerdt *et al*., 2015)**.

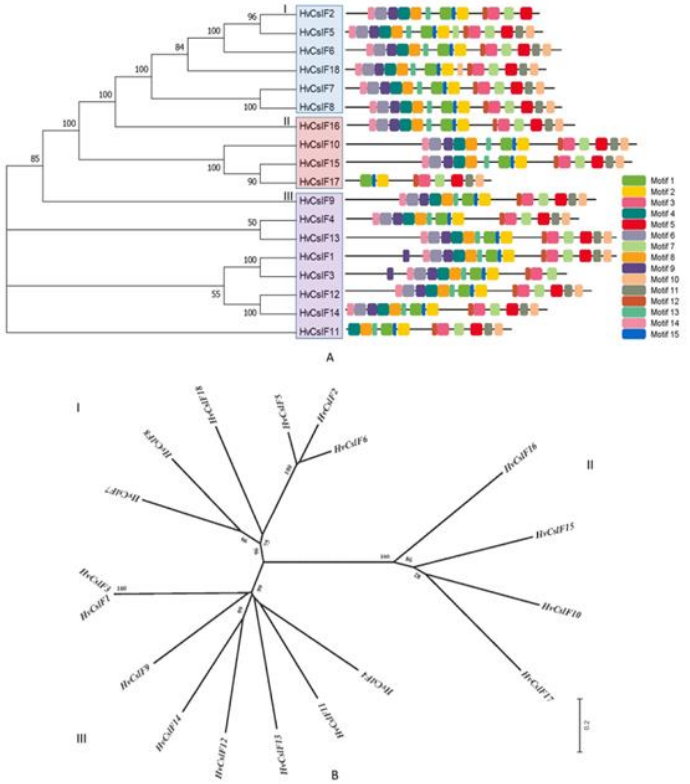

Figure 1 Conserved motifs composition and phylogenetic relationship of the barley *CslF* genes (A). Conserved domain composition (right) and Phylogenetic analysis (left) of *CslF* genes. (B) Radial representation of the Phylogenetic analysis.

HvCslF genes expression pattern

By using RNA-seq data from NCBI database (https://www.ncbi.nlm.nih.gov/), the expression patterns of *HvCslF*s in seven barley tissues was studied (Table S1). The heat map (Figure 2) indicated that, only 11 genes detected in several barley tissues and, their expression levels were highly variable. Most of the tissues showed higher expression of *HvCslF1, 3, 4, 9* and *15* genes. Among these tissues, the highest number of genes (5) were expressed in the grain. In the grain, *HvCsIF9* shows the highest expression and *HvCsIF14* was only expressed in the grains. Hence, these two genes could be suggested as the main candidate genes for β-glucan synthesis in grain. Some *HvCslF*s were highly expressed in specific tissues such as, *HvCslF1, 9* and *15* were highly expressed in palea, grain and lodicule, respectively, and *HvCslF3* was also relatively highly expressed in palea and lodicule tissues, proposing that those genes may play crucial roles in these tissues. The other 5 genes, *HvCslF2, 6, 8, 12,* and *13* were not expressed in the studied tissues. It is worth mentioning that, the *CslF* genes specificity has been stated in rice and maize **(Wang *et al*., 2010; Penning *et al*., 2009)**.

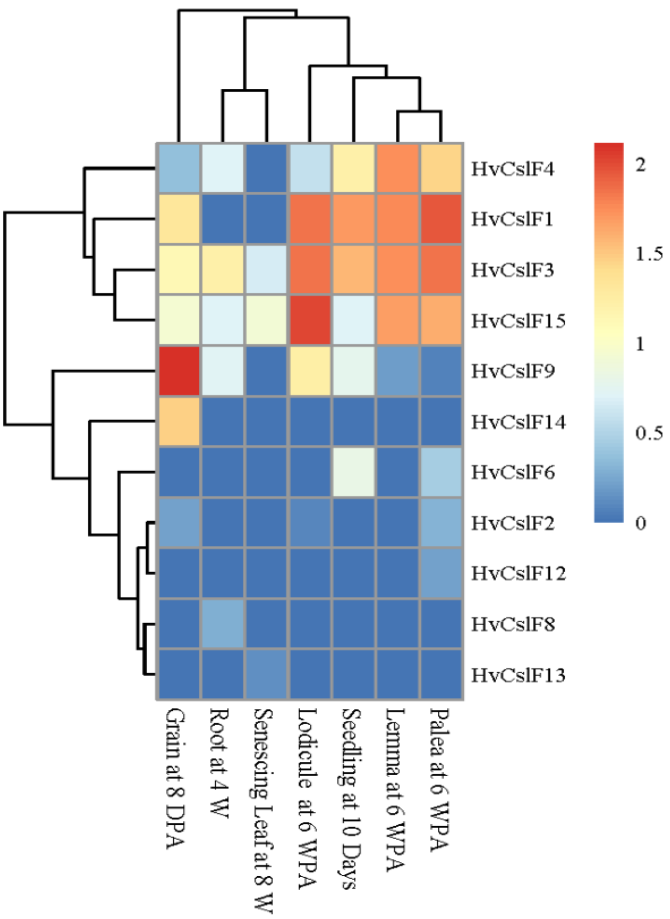

Figure 2 *HvCslF* genes expression pattern in various tissues of barley. Expression levels indicate by Blue (decreased) and Red (increased) colors. Weeks (W), Weeks after post anthesis (WPA), Days after post anthesis (DPA).

Several studies showed that, the *CslF* genes have a key role in β-glucan production **(Burton *et al*., 2011)**. For example, *CslF* genes in *Oryza sativa* **(Hazen *et al*., 2002)**, *Triticum aestivum* **(Jobling, 2015)** and *Zea mays* **(Alexandrov *et al*., 2009)** were reported to regulate β-glucan synthesis. Also, expression of barley *CslF* genes are highly variable under the abiotic stresses such as water stress. Quantity of barley β-glucan is affected by the quantity of water supply during the maturity. Moisture level in soil and β-glucan in the barley kernel has a negative corelation **(Guler, 2003)**. Barley β-glucan content is highly affected by dry conditions prevailing at grain maturation period (Hang et al., 2007). β-glucan levels in barley grains change dramatically during its growth and development due to numerous expression patterns of *HvCslF* genes in various tissues at different times. **Gibeaut *et al*., (2005)** observed, increased β-glucan level in barley coleoptiles walls at the elongation phase, followed by cessation of growth at about 5 days, β-glucan content rapidly decreases. The transient nature of β-glucan in maize coleoptiles is described by **Mccann *et al*., 2007**.

Interactions between the *HvCslF* family members

Present experiment, created the interaction network of the *HvCslF* family considering the various tissues in barley. Using RNA-seq data, WGCNAR package **(Langfelder, *et al*., 2008)** provided a wide-ranging functions to perform the analysis of weighted correlation network. For example in Figure 3, 11 out of 18 HvCslF genes were found in the co-relation network analysis. Nine HvCslF genes (*HvCslF1*, *HvCslF2*, *HvCslF4*, *HvCslF9*, *HvCslF10*, *HvCslF11*, *HvCslF12*, *HvCslF13* and *HvCslF15*) were involved in a single cascade, illustrating that they have close relationship with each other, and 2 *HvCslF* genes (*HvCslF5* and *HvCslF7*) located separately, indicating that they have no close relationship with the other 9 *HvCslF* genes. Furthermore, 3 of the 9 *HvCslF* genes in Group III (Figure 1), including *HvCslF4*, *HvCslF9* and *HvCslF11*, showed a close association with each other than the other genes in the cascade.

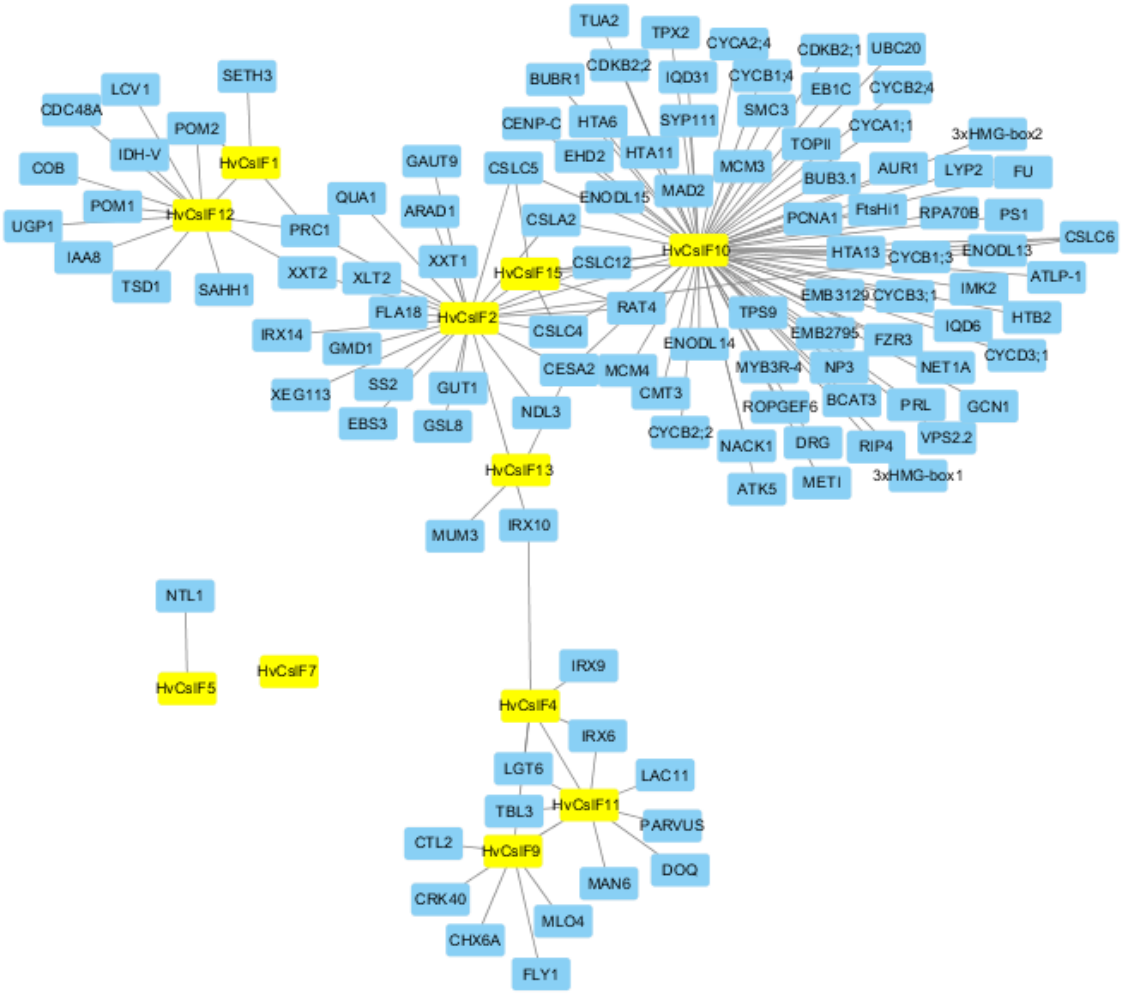

Figure 3 The interaction network of *HvCslF* genes in barley

In the network, the largest group was *HvCslF10* followed by *HvCslF2* and *HvCslF12*, respectively. This emphasizes that, these *HvCslF* genes have more relationship with other genes in the genome. For example, in the *HvCslF10* group, five Cyclin B (CYCB) genes are interacting, which are responsible for the cell cycle regulation, that regulates the gap 2 (G2) /mitosis (M) transition **(Ishida *et al.*; 2008Lin *et al.*, 2017)**. The *HvCslF2* group has interaction with GSL genes, suggested to have a crucial role in the synthesis of callose, which belongs to glucan synthase-like (GSL) family **(Shu *et al.*, 2014; Töller *et al.*, 2008)**. The *HvCslF12* group has interaction with UGP genes, which are responsible for Plant UDP-glucose (UDPG) pyrophosphorylase (UGPase) which are essential in the metabolism or production of UDPG, an essential metabolite for cell wall and sucrose synthesis **(Meng *et al.*, 2007; Meng *et al.*, 2008)**. The interaction network constructed in this study between *HvCslF*s provides information for further studies on β-glucan synthesis and other genes which have interaction with *HvCslF* genes in barley and genomes of other species.

CONCLUSION

The evolution relationship, expression profiles and genome organization of the *CslF* gene family in barley were investigated in the present study. Totally, 18 *HvCslF* genes were identified based on a bioinformatics search using latest genomic information. The gene expression pattern, phylogenetic tree, conserved motifs as well as interaction network of the *CslF* was further systematically analyzed. This is the first study to report the barley *CslF* family at the genome scale, which is providing the candidates for advance functional studies, and facilitates to expose the regulatory mechanism of the *CslF* family involving in development and growth in barley and beyond.

Acknowledgments: Authors are gratitude to China government scholarship.

REFERENCES

Alexandrov, N. N., Brover, V. V., Freidin, S., Troukhan, M. E., Tatarinova, T. V., Zhang, H., Feldmann, K. A. (2008). Insights into corn genes derived from large-scale cDNA sequencing. *Plant Molecular Biology*, 69(1-2), 179–194. https://doi.org/10.1007/s11103-008-9415-4

Arngren, M., Hansen, P. W., Eriksen, B., Larsen, J., & Larsen, R. (2011). Analysis of Pregerminated Barley Using Hyperspectral Image Analysis. *Journal of Agricultural and Food Chemistry*, 59(21), 11385–11394. https://doi.org/10.1021/jf202122y

Bailey, T. L., Boden, M., Buske, F. A., Frith, M., Grant, C. E., Clementi, L. Noble, W. S. (2009). MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Research*, 37(Web Server), W202–W208. https://doi.org/10.1093/nar/gkp335

Bhatty, R. S. (1999). β-Glucan and Flour Yield of Hull-less Barley. *Cereal Chemistry Journal*, 76(2), 314–315. https://doi.org/10.1094/cchem.1999.76.2.314

Bilgi, B., & Celik, S. (2004). Solubility and emulsifying properties of barley protein concentrate. *European Food Research and Technology*, 218(5), 437–441. https://doi.org/10.1007/s00217-004-0895-4

Bolser, D., Staines, D. M., Pritchard, E., & Kersey, P. (2016). Ensembl Plants:Integrating Tools for Visualizing, Mining, and Analyzing Plant Genomics Data. *Methods in Molecular Biology*, 115–140. https://doi.org/10.1007/978-1-4939-3167-5_6

Brennan, C. S., & Cleary, L. J. (2005). The potential use of cereal (1→3,1→4)-β-d-glucans as functional food ingredients. *Journal of Cereal Science*, 42(1), 1–13. https://doi.org/10.1016/j.jcs.2005.01.002

Burton, R. A., Collins, H. M., Kibble, N. A. J., Smith, J. A., Shirley, N. J., Jobling, S. A., … Fincher, G. B. (2011). Over-expression of specific HvCslF cellulose synthase-like genes in transgenic barley increases the levels of cell wall (1,3;1,4)-β-d-glucans and alters their fine structure. *Plant Biotechnology Journal*, 9(2), 117–135. https://doi.org/10.1111/j.1467-7652.2010.00532.x

Burton, R. A., Jobling, S. A., Harvey, A. J., Shirley, N. J., Mather, D. E., Bacic, A., & Fincher, G. B. (2008). The Genetics and Transcriptional Profiles of the Cellulose Synthase-Like HvCslF Gene Family in Barley. *Plant Physiology*, 146(4), 1821–1833. https://doi.org/10.1104/pp.107.114694

Burton, R. A. (2006). Cellulose Synthase-Like CslF Genes Mediate the Synthesis of Cell Wall (1,3;1,4)- -D-Glucans. *Science*, 311(5769), 1940–1942. https://doi.org/10.1126/science.1122975

Doblin, M. S., Pettolino, F. A., Wilson, S. M., Campbell, R., Burton, R. A., Fincher, G. B Bacic, A. (2009). A barley cellulose synthase-like CSLH gene mediates (1,3;1,4)- -D-glucan synthesis in transgenic Arabidopsis. *Proceedings of the National Academy of Sciences*, 106(14), 5996–6001. https://doi.org/10.1073/pnas.0902019106

Fincher, G. B., & Stone, B. A. (2004). CEREALS | Chemistry of Nonstarch Polysaccharides. *Encyclopedia of Grain Science*, 206–223. https://doi.org/10.1016/b0-12-765490-9/00107-5

Finn, R. D., Coggill, P., Eberhardt, R. Y., Eddy, S. R., Mistry, J., Mitchell, A. L., … Bateman, A. (2015). The Pfam protein families database: towards a more sustainable future. *Nucleic Acids Research*, 44(D1), D279–D285. https://doi.org/10.1093/nar/gkv1344

Gasteiger, E. (2003). ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Research*, 31(13), 3784–3788. https://doi.org/10.1093/nar/gkg563

Getting their oats helps kids perform better. Available online: Http://www.Nutraingredients.Com /news/ng.Asp?Id=35877 (accessed on 02.04.2018).

Gibeaut, D. M., Pauly, M., Bacic, A., & Fincher, G. B. (2005). Changes in cell wall polysaccharides in developing barley (Hordeum vulgare) coleoptiles. *Planta*, 221(5), 729–738. https://doi.org/10.1007/s00425-005-1481-0

Güler, M. (2003). Barley grain β-glucan content as affected by nitrogen and irrigation. *Field Crops Research*, 84(3), 335–340. https://doi.org/10.1016/s0378-4290(03)00100-x

Hang, A., Obert, D., Gironella, A. I. N., & Burton, C. S. (2007). Barley Amylose and β-Glucan: Their Relationships to Protein, Agronomic Traits, and Environmental Factors. *Crop Science*, 47(4), 1754. https://doi.org/10.2135/cropsci2006.06.0429

Hazen, S. P., Scott-Craig, J. S., & Walton, J. D. (2002). Cellulose Synthase-Like Genes of Rice. *Plant Physiology*, 128(2), 336–340. https://doi.org/10.1104/pp.010875

Hesselman, K., Elwinger, K., & Thomke, S. (1982). Influence of increasing levels of β-glucanase on the productive value of barley diets for broiler chickens. *Animal Feed Science and Technology*, 7(4), 351–358. https://doi.org/10.1016/0377-8401(82)90004-9

Ishida, T., Kurata, T., Okada, K., & Wada, T. (2008). A Genetic Regulatory Network in the Development of Trichomes and Root Hairs. *Annual Review of Plant Biology*, 59(1), 365–386. https://doi.org/10.1146/annurev.arplant.59.032607.092949

Jobling, S. A. (2015). Membrane pore architecture of the CslF6 protein controls (1-3,1-4)-β-glucan structure. *Science Advances*, 1(5), e1500069. https://doi.org/10.1126/sciadv.1500069

Johansson, L., Tuomainen, P., Ylinen, M., Ekholm, P., & Virkki, L. (2004). Structural analysis of water-soluble and -insoluble - β glucans of whole-grain oats and barley. *Carbohydrate Polymers*, 58(3), 267–274. https://doi.org/10.1016/j.carbpol.2004.06.041

Keogh, G. F., Cooper, G. J., Mulvey, T. B., McArdle, B. H., Coles, G. D., Monro, J. A., & Poppitt, S. D. (2003). Randomized controlled crossover study of the effect of a highly β-glucan–enriched barley on cardiovascular disease risk factors in mildly hypercholesterolemic men. *TheAmerican Journal of Clinical Nutrition*, 78(4), 711–718. https://doi.org/10.1093/ajcn/78.4.711

Klopfenstein, C.F. (1988). The role of cereal beta-glucans in nutrition and health. *Cereal Foods World*, 33, 865–869.

Langfelder, P., & Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics*, 9(1). https://doi.org/10.1186/1471-2105-9-559

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, 23(21), 2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Li, J., Kaneko, T., Qin, L.-Q., Wang, J., & Wang, Y. (2003). Effects of barley intake on glucose tolerance, lipid metabolism, and bowel function in women. *Nutrition*, 19(11-12), 926–929. https://doi.org/10.1016/s0899-9007(03)00182-5

Lin, P., Shen, C., Chen, H., Yao, X. H., & Lin, J. (2016). Improving tobacco freezing tolerance by co-transfer of stress-inducible CbCBF and CbICE53 genes. *Biologia Plantarum*, 61(3), 520–528. https://doi.org/10.1007/s10535-016-0687-2

McCann, M. C., Defernez, M., Urbanowicz, B. R., Tewari, J. C., Langewisch, T., Olek, A., Carpita, N. C. (2007). Neural Network Analyses of Infrared Spectra for Classifying Cell Wall Architectures. *Plant Physiology*, 143(3), 1314–1326. https://doi.org/10.1104/pp.106.093054

Meng, M., Geisler, M., Johansson, H., Mellerowicz, E. J., Karpinski, S., & Kleczkowski, L. A. (2007). Differential tissue/organ-dependent expression of two sucrose- and cold-responsive genes for UDP-glucose pyrophosphorylase in Populus. *Gene*, 389(2), 186–195. https://doi.org/10.1016/j.gene.2006.11.006

Meng, M., Wilczynska, M., & Kleczkowski, L. A. (2008). Molecular and kinetic characterization of two UDP-glucose pyrophosphorylases, products of distinct genes, from Arabidopsis. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 1784(6), 967–972. https://doi.org/10.1016/j.bbapap.2008.02.021

Murphy, E. A., Davis, J. M., Brown, A. S., Carmichael, M. D., Mayer, E. P., & Ghaffar, A. (2004). Effects of moderate exercise and oat β-glucan on lung tumor metastases and macrophage antitumor cytotoxicity. *Journal of Applied Physiology*, 97(3), 955–959. https://doi.org/10.1152/japplphysiol.00252.2004

Newman. (2006). A Brief History of Barley Foods. *Cereal Foods World.* https://doi.org/10.1094/cfw-51-0004

Penning, B. W., Hunter, C. T., Tayengwa, R., Eveland, A. L., Dugard, C. K., Olek, A. T., … Carpita, N. C. (2009). Genetic Resources for Maize Cell Wall Biology. *Plant Physiology*, 151(4), 1703–1728. https://doi.org/10.1104/pp.109.136804

Richmond, T. A., & Somerville, C. R. (2000). The Cellulose Synthase Superfamily. *Plant Physiology*, 124(2), 495–498. https://doi.org/10.1104/pp.124.2.495

Rimsten, L., Stenberg, T., Andersson, R., Andersson, A., & Åman, P. (2003). Determination of β-Glucan Molecular Weight Using SEC with Calcofluor Detection in Cereal Extracts. *Cereal Chemistry Journal*, 80(4), 485–490. https://doi.org/10.1094/cchem.2003.80.4.485

Scheller, H. V., & Ulvskov, P. (2010). Hemicelluloses. Annual Review of Plant Biology, 61(1), 263–289. https://doi.org/10.1146/annurev-arplant-042809-112315

Schreiber, M., Wright, F., MacKenzie, K., Hedley, P. E., Schwerdt, J. G., Little, A., Halpin, C. (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

Schwerdt, J. G., MacKenzie, K., Wright, F., Oehme, D., Wagner, J. M., Harvey, A. J., Fincher, G. B. (2015). Evolutionary Dynamics of the Cellulose Synthase Gene Superfamily in Grasses. *Plant Physiology*, 168(3), 968–983. https://doi.org/10.1104/pp.15.00140

Shu, X., & Rasmussen, Sã¸. K. (2014). Quantification of amylose, amylopectin, and Î²-glucan in search for genes controlling the three major quality traits in barley by genome-wide association studies. *Frontiers in Plant Science*, 5. https://doi.org/10.3389/fpls.2014.00197

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, 30(12), 2725–2729. https://doi.org/10.1093/molbev/mst197

Thorburn, A., Muir, J., & Proietto, J. (1993). Carbohydrate fermentation decreases hepatic glucose output in healthy subjects. *Metabolism*, 42(6), 780–785. https://doi.org/10.1016/0026-0495(93)90249-n

Töller, A., Brownfield, L., Neu, C., Twell, D., & Schulze-Lefert, P. (2008). Dual function of Arabidopsis glucan synthase-like genes GSL8 and GSL10 in male gametophyte development and plant growth. *The Plant Journal*, 54(5), 911–923. https://doi.org/10.1111/j.1365-313x.2008.03462.x

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D. R., Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nature Protocols*, 7(3), 562–578. https://doi.org/10.1038/nprot.2012.016

Wang, L., Guo, K., Li, Y., Tu, Y., Hu, H., Wang, B., Peng, L. (2010). Expression profiling and integrative analysis of the CESA/CSL superfamily in rice. *BMC Plant Biology*, 10(1), 282. https://doi.org/10.1186/1471-2229-10-282

Wang, L., Newman, R. K., Newman, C. W., & Hofer, P. J. (1992). Barley β-Glucans Alter Intestinal Viscosity and Reduce Plasma Cholesterol Concentrations in Chicks. *The Journal of Nutrition*, 122(11), 2292–2297. https://doi.org/10.1093/jn/122.11.2292

Wang, M., Yue, H., Feng, K., Deng, P., Song, W., & Nie, X. (2016). Genome-wide identification, phylogeny and expressional profiles of mitogen activated protein kinase kinase kinase (MAPKKK) gene family in bread wheat (Triticum aestivum L.). *BMC Genomics*, 17(1). https://doi.org/10.1186/s12864-016-2993-7

Wheeler, T. J., & Eddy, S. R. (2013). nhmmer: DNA homology search with profile HMMs. *Bioinformatics*, 29(19), 2487–2489. https://doi.org/10.1093/bioinformatics/btt403

Yu, C.-S., Chen, Y.-C., Lu, C.-H., & Hwang, J.-K. (2006). Prediction of protein subcellular localization. *Proteins: Structure, Function, and Bioinformatics*, 64(3), 643–651. https://doi.org/10.1002/prot.21018

Zdobnov, E. M., & Apweiler, R. (2001). InterProScan - an integration platform for the signature-recognition methods in InterPro. *Bioinformatics*, 17(9), 847–848. https://doi.org/10.1093/bioinformatics/17.9.847