Genome-Wide Exploration of Thaumatin-like Protein (TLP) Gene Family in Cereals

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Research article

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Abstract

Background: TLP genes are the members of a conserved pathogenesis-related protein 5 (PR-5) gene family. They play role in abiotic stress response, hormone signaling, cell death, cold tolerance, enzyme inactivation, fruit maturation and seed germination. In this study, we characterized the TLP gene family in barley with specific emphasis on germination and malting. Results: We identified 19 TLP genes from the reference genome of *Hordeum vulgare* L. cv. Morex and 37, 28 and 35 TLP genes from the *Oryza sativa*, *Brachypodium distachyon* and *Sorghum bicolor* genome respectively. Comparative phylogenetic analysis and thaumatin domain organization of TLPs using the conserved region classified the TLP family into nine groups. Data revealed that localized gene duplications contributed to the expansion of the TLP gene family in cereals with diverse exon/intron structures. In the barley genome, most HvTLPs were localized on chromosome 5H. The differential spatiotemporal expression pattern of HvTLP genes in barley indicate that TLPs have been expressed predominantly in the embryo, developing grains, root and shoot tissues. Additionally, transcript abundance of HvTLP genes was measured between 16 hrs. to 96 hrs. of grain germination. Differential expression of HvTLP14, HvTLP17 and HvTLP18 in the malting variety (Morex), as compared to the feed variety (Steptoe) at different stages of seed germination indicates their possible role in malting. Conclusion: Barley genome contains higher number (19) of TLP genes as previously thought (8). This study provides a description of the TLP gene family in barley and their differential expression between 16-96 hrs. of germination. The results indicate their possible involvement in the malting process. Keywords: Cereals, Barley, Thaumatin like-proteins, Phylogenetics, Expression analysis

Background

Thaumatin-like proteins (TLPs), are part of a large PR (pathogenesis-related) gene family, involved in a broad range of defense and developmental processes in plants, fungi, and animals (Brandazza et al., 2004). In Plants, TLPs are members of the PR-5 gene family that includes permatin, osmotin, osmotin-like proteins (OLPs), synthesis of which is mainly triggered in response to biotic and abiotic stress. However, it is also developmentally regulated during seed germination [1] and fruit ripening [2], which perform defense and development related functions 93 [3].

TLPs have high sequence similarity with sweet tasting disulfide thaumatin protein, initially identified in the West African shrub *Thaumatococcus daniellie* [4]. TLPs are highly conserved 24-34 kDa proteins with polypeptides comprising of 225-319 amino acid residues [5]. Further, they have 5 to 8 disulfide linkages depending upon the number of cysteine residues, ranging from 10-16. These disulfide structures provide stability and resistance to proteins against pH, high temperature induced denaturation, and protease degradation [6]. TLPs having 10 conserved cysteines are designated as small TLPs and have been identified in various monocotyledonous and coniferous plant species [5, 7, 8].

TLPs are involved in the plant defense against numerous biotic and abiotic stresses [5]. Overexpression of TLPs resulted in induced resistance against downy mildew, *Macrophomia phaseolina*, *Phytophthora infestans* and salinity stress [9-11]. TLPs have roles in plant development and physiology, including

tolerance to freezing stress [12], antifungal activity [11], fruit ripening [13] and seed germination [14]. TLPs have been identified in various plants including Moss (*Physcomitrella*), *Arabidopsis*, Rice, Maize, Poplar and Barley [1, 5, 15].

Barley grain is typically used for malting purpose, and recently the role of *TLPs* has been shown in the germinating grains during the malting process [1]. Barley grain endosperm is composed of 75% non-starch polysaccharides, such as (1, 3, 1, 4)- β -D glucan “(hereafter, β -glucan)” and arabinoxylans [16]. Lower β -glucan content has been considered as a desirable trait to determine malting quality [17]. A barley TLP (HvTLP8), having a carbohydrate binding motif (CQTGDCGG), has been implicated in the redox- regulated interaction with the β -glucan [1]. In addition, barley TLPs have been found to be involved in antifungal activity [18], antimicrobial activity [19] and binding with carbohydrates [20].

Considering the importance of TLP genes that are associated to various defense, development and physiological responses, and diversity of TLP gene members in different plant species, it is of our great interest to investigate the global status and evolution of the TLP gene family in barley and of other cereals involved in brewing industry, especially sorghum (a cereal with gluten free beer). There is lack of information about the status of TLP gene family members in cereal grains. We are specifically interested in TLPs which possess carbohydrate binding domain that may interact with different polysaccharides moieties during the germination and malting process. Our data provide the global status of TLP gene family and its expansion in rice, Brachypodium, sorghum, and barley. The availability of updated genome sequence databases of several plant species facilitated us to explore the TLP gene family status in cereals.

Materials And Methods

Sequence Retrieval and Identification of Thaumatin like-proteins in Cereals TLP domain sequence was retrieved from conserved domain database (CDD) (<http://www.ncbi.nlm.nih.gov/guide/domains-structures/>) and used as a query sequence to do protein blast (BLASTp) (<https://blast.ncbi.nlm.nih.gov/Blast>) in the NCBI (www.ncbi.nlm.nih.gov) database. BLASTp was performed in non-redundant protein sequences database by using e value cut off of e^{-10} to retrieve TLPs protein sequences for *Hordeum vulgare*, *Oryza sativa*, *Sorghum bicolor* and *Brachypodium distachyon*. In terms of selecting gene models, only the longest gene models were selected for further analysis. In addition, identified barley HvTLP gene sequences were also verified using IPK barley (<http://webblast.ipk136.gatersleben.de/barley/>) and Ensembl Plants database (http://plants.ensembl.org/Hordeum_vulgare/Info/Index) to retrieve their gene IDs. Furthermore, screening was performed, and only those genes having the thaumatin family signature were selected for further analysis. The pipeline for the bioinformatic analysis is shown in (Fig. 1B). Identified genes were also confirmed as TLPs from SMART (<http://smart.embl-heidelberg.de>) and Pfam (<https://pfam.xfam.org>) database.

Protein Sequence, Motif Identification 142 and Phylogenetic Analysis Multiple sequence alignment of amino acid sequences of TLPs from rice, sorghum, barley and *Brachypodium* (Additional file 1: Table S1) was used to construct the phylogenetic tree. Multiple sequence alignments were performed using MUSCLE (<http://www.ebi.ac.uk/Tools/msa/muscle/>) alignment tool with default settings [21]. An unrooted Neighbor-Joining phylogenetic tree was generated using MEGA v7 program (1000 bootstrap replicates)[22-25].

Chromosomal Location, Exon/Intron Structure and Alternative Splice Variants Analysis of Barley TLP

Genes Chromosomal location, intron/exon structures, and predicted alternative splice variants of the HvTLPs were determined using the *H. vulgare* ensembl database (http://plants.ensembl.org/Hordeum_vulgare/Info/Index). Initially, 32 TLP proteins with the thaumatin signature were identified in Genbank using BLASTp as described in a previous section; upon blasting these sequences against the ensemble database, we determined that these 32 amino acid sequences aligned to only 19 genetic loci. In all subsequent analyses, only the first gene isoform, normally denoted with a “.1”, was used are provided in (Additional file2: Table S2). Exon/intron structures of the barley HvTLP genes were illustrated by using online Gene Structure Display Server (GSDS; <http://gsds.cbi.pku.edu.cn>) which displays the gene length and exon position [26, 27].

Gene Expression Analysis of Novel Barley TLPs in Different Tissues of a Barley Variety, Morex We used publicly available Morex RNA-seq database (International Barley Genome Sequencing 163 2012) to study the expression patterns of 19 novel barley HvTLP genes in eight different developmental stages, tissues, and inflorescence treatments. CAR5 expression data was collected from developing grains, bracts removed (5 DPA), ROO was collected from roots from the seedlings (10 cm shoot stage), LEA was taken from Shoots from the seedlings (10 cm shoot stage), EMB was collected from 4-day embryos dissected from germinating grains, CAR15 was collected from developing grain, bracts removed (15 DPA), INF1 was taken from young developing inflorescences (5mm), INF2 was taken from developing inflorescences (1-1.5 cm), and NOD was taken from developing tillers at 6-leaf stage, third internode. The heat map of HvTLP transcript abundance was generated by using the online Mev tool (<http://www.tm4.org/mev.html>) with the average hierarchical clustering method.

Plant Material and Growth Conditions Two barley varieties (Malt: Morex and Feed: Steptoe) were used as experimental material. Mature barley seeds were surface sterilized with 20% NaOCl and 70% ethanol. They were germinated in the dark on wet filter paper in sterile petri dishes at 21°C. Germinating seeds were collected at 16, 48, and 96 h and were flash frozen in liquid nitrogen and stored in -80°C until further processing.

Total RNA Isolation and cDNA Synthesis Total RNA was extracted by using Spectrum Plant Total RNA kit from the germinating seed tissue according to the manufacturer' instructions (Sigma-Aldrich). cDNA was synthesized by using AffinityScript QPCR cDNA synthesis kit from 500 ng of DNaseI-treated total RNA according to the manufacturer's instructions (Agilent Technologies, USA).

RT-PCR analysis Primers were designed for five HvTLPs using IDT primer quest tool (<https://www.idtdna.com/PrimerQuest/Home/Index>) (Additional file 3: Table S3). Transcript abundance was determined using RT-PCR analysis using GoTaq® G2 Green Master Mix. The amplification conditions were 95°C for 2 minutes, followed 30 cycles at 95°C for 30 seconds, (annealing temperature was adjusted according to the primers provided in Additional file 2: Table S2) for 30 seconds, 72°C for 30 seconds and final extension at 72°C for 5 minutes. β -ACTIN was used as an expression control [28].

Results

Identification of TLP Gene Family in Cereals A total of 19, 28, 35 and 37 TLP genes were identified in barley, Brachypodium, sorghum and rice, respectively (Fig.1A). TLP domain was retrieved from CDD and was used as query to perform BLASTp in NCBI to identify TLP genes in barley, rice, sorghum and Brachypodium. Sequences of TLP candidate genes were confirmed by Pfam (Domain number: PF00314) and SMART for the presence of thaumatin family signature G-x-[GF]-x-C-x-T-[GA]-D-C-x-(1,2)-[GQ]-x-(2,3)-C. Using this approach, 32 TLP genes were identified for barley; by using the Ensembl barley blast database, it was determined that these 32 genes corresponded to only 19 genetic loci. The primary amino acid sequence associated with each loci was used for further analysis. Out of the 19 genes in barley, 8 has been previously reported (HvTLP1-8) [1]. Eleven new coding sequences of HvTLP genes ranged from 522 to 1080 base pairs. They were found to be localized on chromosome 1H (HvTLP11, HvTLP16), 3H (HvTLP13, HvTLP14 and HvTLP17), 4H (HvTLP8, HvTLP15 and HvTLP19), 5H (HvTLP3-7, HvTLP10, HvTLP12, and HvTLP18), and 7H (HvTLP1, HvTLP2 and HvTLP9). Protein length varied from 173 to 359 amino acid residues accompanied with cysteine residues ranged from 10 (HvTLP1 and HvTLP2) to 24 (HvTLP17) (Table 1). Only two new genes HvTLP14 and HvTLP17 contained the carbohydrate binding CQTGDCGG motif in their amino acid residues.

Phylogenetic Relationship of TLP Genes in Barley, Rice, Brachypodium and Sorghum A phylogenetic tree was constructed by using Neighbor-Joining method based on 119 TLP protein sequences, from Sorghum bicolor, O. sativa, H. vulgare and B. distachyon (Fig. 2A). These protein sequences were arranged in nine groups. Barley contained only 19 TLPs, a smaller number of TLPs as compared to the other species. However, this is in contrast to previous studies [1], where only 8 TLPs have been reported in the Hordeum vulgare. The number of TLPs in rice are twice of that in barley (Fig. 2B). Maximum number of (34) TLPs from the four-plant species were clustered in group 2, whereas, only six TLPs were found in both group 5 and group 7. Interestingly, seven (HvTLP5, 6, 7, 8, 10, 14 and 17) out of 19 TLPs were found in group 2. Our data suggest that orthologues for HvTLP13 and HvTLP4 genes are missing in sorghum and Brachypodium, respectively. Barley TLPs in group 2 have 35 additional amino acids residues in the N-terminal end of TLP domain. HvTLPs in group 3 possess single exon except HvTLP1, which has 2 exons. In general, barley TLPs showed closer relationship with Brachypodium followed by rice and sorghum. Interestingly, some HvTLPs located adjacent to each other on chromosome 5H were found to be clustered in the same group (group 2), i.e., HvTLP 5, 6,7 and HvTLP 12, 18 (Fig 2 and Fig 6). Similarly, phylogenetic analysis revealed that some members of rice and sorghum cluster together especially in group 3.

Gene Structure Analysis and Identification of Thaumatin Signature in Barley TLPs Classification of 19 TLPs from barley depending on the number of exons resulted in the formation of four (I-IV) groups (Fig. 3). Number of exons ranged in HvTLP genes from 1 to 4. Eleven TLPs (HvTLP2, 3, 4, 6, 8, 10, 11, 12, 13, 14) having one exon were classified in Group I and Group II includes six HvTLPs (HvTLP1, 5, 6, 9, 16, 17, 18). However, Group III (HvTLP18) and Group IV (HvTLP19) had one TLP gene member each. Except group I, all the members of other groups contained the thaumatin family signature G-x-[GF]-x-C-x-T-[GA]-D-C-x-(1,2)-[GQ]-x-(2,3)-C in their second exon. Thaumatin family signature was searched manually in the protein sequences of 37 genes but only 19 unique genes having the complete thaumatin signature were selected.

Alternative Splicing in HvTLPs Alternative transcripts produce protein isoforms having amino acid sequence differences with modified cellular properties to loss of protein functions. Barley genome from Ensembl database was mined to identify if there is any alternative splicing event for HvTLP genes. Predicted alternative splice variants of 15 HvTLPs were identified and they contribute 83% towards total HvTLP splice variants. Maximum number of splice variant for a gene were 14 (HvTLP18) and three genes (HvTLP3, HvTLP10 and HvTLP13) had no splice variant product (Additional file 4: Table S4).

Spatiotemporal Expression Pattern of HvTLPs RNA-sequencing (RNA-seq) data provides important information for predicting the function of the genes. To understand the developmental role of previously known and identified novel barley TLP genes we performed comprehensive expression analysis. We did so by using MLOC ID for the TLPs (Additional file 5: Table S5) from Morex Genes-Barley RNA-seq database (<https://ics.hutton.ac.uk/morexGenes>) to investigate their spatio-temporal expression pattern. Based on the data shown in the extracted heatmap (Fig 4.), HvTLPs exhibited differential expression in developing grains (CAR5 and CAR15), root (ROO), shoot (LEA), embryo (EMB), young inflorescence (INF1), developing inflorescence (INF2) and tillers (NOD). However, HvTLP13 and 18 were found to be expressing more in CAR5 and CAR15, indicating the possibility of these genes in the development of these tissues. Only HvTLP 9, 5 and 19 showed higher expression profiles in roots and tillers, suggesting they might be involved in the development of these tissues, but they did not express in INF1& INF2, except HVTLP9. HvTLP1,2,4,5,6,7,8,9,18 and 19 were found to have elevated expression levels in EMB, suggesting their possible role in embryo development of the barley grain.

Transcript Abundance of HvTLPs During Different Stages of Barley Seed Germination The transcript abundance of 16 TLPs was measured through RT-PCR using two housekeeping genes, β -ACTIN and GAPDH, as controls (Fig. 5). HvTLP4 and HvTLP7 exhibited higher expression at all time intervals with no difference between malt and feed varieties. Similarly, HvTLP13 expressed constantly higher in all stages of seed germination. However, its expression was reduced in Steptoe (feed variety) at 96 hrs of germinating seeds. Transcripts of HvTLP5 and HvTLP6 accumulated continuously as germination proceeded between 16-96 hrs in Morex (malt variety). No or low expression of both HvTLP5 and HvTLP6 was observed in Steptoe (feed variety). Whereas, transcript abundance of HvTLP1 and HvTL2 was higher in Steptoe for similar stage of germination. Transcript of HvTLP9 was plentiful in Morex at 16 hrs after imbibition but reduced dramatically after the 16 hrs stage. Higher transcript abundance of HvTLP14,

HvTLP17 and HvTLP18 was observed in Morex than Steptoe at all time points of germination. No transcript was observed in HvTLP3, HvTLP10, HvTLP12 and HvTLP15 in both Steptoe and Morex at any stage of germination between 16-96 hrs of imbibition.

Discussion

Availability of whole genome sequences of rice, sorghum, Brachypodium and barley allowed us to perform the genome-wide exploration of TLP gene family among these crops (Fig. 1B). Here we report the global status of TLP genes in rice, sorghum, Brachypodium and barley having 37, 35, 28 and 19 genes, respectively (Fig. 1B). It is worth noting that in barley, only 8 TLP genes have been reported previously[1]. Our major focus of study was the genome-wide exploration of TLPs in barley especially during germination as our previous work established that a TLP, HvTLP8, differentially expressed during germination in malt and feed varieties and play an important role in sequestering β -glucan during malting process [1]. Barley grain contains β -glucan as a non-starch polysaccharide and its higher quantity in the grain affects the brewing process [29]. HvTLP8 possesses carbohydrate binding domain and the binding motif CQTGDCGG, which allowed its binding to β -glucan in a redox-dependent manner. [1]. In our data, we have identified two other barley TLP genes that also contain the binding motif, which is indicative of their interaction with carbohydrate moieties, which needs further investigation. Previously, 44 TLP genes were reported in rice [15]. However, our careful analysis indicates that only 19 genes are true TLPs due to the presence of the thaumatin family signature.

As described above, we have identified 11 new TLPs in barley. Previously, eight known barley TLPs were classified into two groups (group 1 and 2) based on the number of cysteine residues (10 and 16) and that they were localized on chromosome 4H, 5H and 7H only [1]. However, our data emphasize that genes for TLPs could also be assigned to chromosome 1H and 3H (Fig. 6). Generally, TLPs have been considered cysteine rich proteins and maximum number of 16 cysteines have been reported in TLPs. However, our data revealed higher number of cysteine residues in some TLP. For example, HvTLP17 contains 24 cysteine residues (Table 1). It is well documented that cysteine residues result in the formation of disulphide linkages, which provides stability to proteins, especially when exposed to extremes of pH, temperature and protease degradation etc. [6]. Plant TLPs are documented as proteins having 21-26 kDa size. However, molecular weight of new TLPs identified in the present study has been calculated up to 41 kDa. Moreover, TLP genes have been identified in a wide range of plants from mosses to wheat, which the complex hexaploid genome [5, 30], suggesting that this gene family has expanded in different plant species during the process of evolution. To better understand the diversification of this gene family in small grain cereals, we performed phylogenetic analysis of predicted TLP protein sequences from rice, sorghum, Brachypodium and barley (Fig. 2A). A total of 119 TLP proteins from four different plant species were classified into nine groups. The maximum number (34) of TLP proteins were clustered in group nine (Fig. 2B). Previously, 44 TLP genes were reported in rice [15]. The way of identification was based on keywords searches in rice genome, some of which did not even have the thaumatin family signature. However, by using our approach of gene identification, as explained in the materials and method section, we found that there are only 37 true TLP genes having the thaumatin family signature in

rice. Based on our analysis, *Oryza sativa* genome has nearly two times higher number of genes (37 TLPs) as compared to Barley's 19 TLPs. This could be due to recent segmental and whole genome duplication events in rice [31, 32]. However, it appears from our data that the expansion in rice, barley, *Brachypodium* and sorghum is probably due to localized gene duplications, since many small TLP groups, which are located in close proximity on the same chromosome, have high sequence similarity. This situation can be clearly observed for the TLPs located on chromosome 5H (Fig 6). So, localized gene duplication events may be the main reason for TLP gene family expansion in barley.

Intron structures are also very important in determining the complexity of genetic structure of eukaryotic organisms [33]. Notably, 10 HvTLP genes were found without introns, whereas the 11 remaining HvTLP genes contained at least one intron (Fig. 3). This result suggests that variation in the number of intron/exons among HvTLP genes might play important role controlling their function during growth and development of barley.

Ambient temperature causes alternative splicing that functions as a molecular thermometer in plants. Recently, alternative splicing in SPL genes in barley [34] was identified and shown to have differential level of accumulation during vegetative to reproductive phases. Alternative splicing is also involved in the process of seed germination in barley [35]. Further, alternative splicing in FT genes was also identified that regulate flowering in *Brachypodium distachyon* [36]. ARF8.4 a splice variant of AUXIN RESPONSE FACTOR 8 is involved in the stamen development in *Arabidopsis* [37]. Likewise, we also investigated alternative splicing events in HvTLP genes and found that about 83% of HvTLP genes produce splice variants, implying their possible diverse role in barley growth & development (Additional file 4: Table S4).

Gene expression gives a clue for the possible functions of genes in the absence of mutant. Therefore, we examined the spatio-temporal expression patterns of HvTLPs in eight different tissues of barley (Fig. 4). The heatmap based transcript profiles of HvTLPs showed their expression was differential in different tissues however, (HvTLP1, 2, 4, 5, 6, 7, 8, 9, 18 and 19) were found to have higher expression in EMBs (Fig. 4), which shows they might have a possible role in embryo development.

Seed germination is a key step in the process of malting. In our experiments, HvTLP 4 and 7 had elevated levels of expression in all stages irrespective of malt and feed varieties, suggesting their possible role during seed germination. However, expression of HvTLP5 and HvTLP6 was found to be higher in 48hr of germination only in malting variety, which indicates that these genes may have specific role only to influence the malt biochemistry of the seed (Fig. 5) and could be considered as new gene candidates for breeding of barley varieties useful for malting and brewing. Previously, we have identified important genes which have been involved in the germination, dormancy [38] and malting process [1]. In publicly available Morex RNA-seq database, currently no data is available for germinating grains. So, we have performed the validation of HvTLP gene expression by measuring their transcript abundance in germinating barley grains of malt and feed varieties at different stages. Our motivation to conduct these experiments include our recent data, where differential expression of a HvTLP8 has been associated with the level of β -glucan in the germinating barley grains [1]. Reduction of β -glucan is an important breeding

objective in barley for breeding of the malt varieties. The association of TLPs with β -352 glucan could also lead to the development of high value and high fiber cereals by knocking out their expression using new CRISPR- based approaches.

Conclusion

Our results provide novel information about the status of TLP gene family in cereals as knowledge about this gene family is scarce. Due to the availability of sequencing data and new tools, we were able to identify new TLPs which were previously unknown. Interestingly, some of these TLPs possess higher number of cysteine residues than previously thought. One of the cysteine rich TLP, HvTLP8, has been found to be associated with β -glucan and the interaction was found to be dependent on the redox status, so the role of newly identified cysteine rich TLPs demand further elucidation, especially for their activity and stability. It is worth noting that some TLPs express during germination and their polymorphism in malt and feed varieties could help in understanding their role in malting and for the development of novel molecular markers for breeding of the future generation of malting barley varieties. New information about TLP gene family in cereals can also be helpful to further investigate their possible functional response towards developmental, physiological, and defense related stimuli in cereals.

Abbreviations

TLP: Thaumatin like-protein; PR-5: Pathogenesis-related proteins; OLPs: Osmotin-like proteins, HR: Hypersensitive response; SAR: Systemic acquired resistance; SA: salicylic acid, ROS: Reactive oxygen species

Declarations

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Availability of data and materials All the data are included in the supplementary files.

Authors Contribution: Il extracted the sequences, analyzed them, and wrote the paper; RKT carried out the RNA-seq analysis, wrote parts and edited the manuscript; OW conceived the project along with JS, and wrote parts and edited the paper; JS conceived and supervised the project, and helped to write the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate N/A

Consent for publication N/A

Competing interests The authors declare that they have no competing interests.

References

1. Singh S, Tripathi RK, Lemaux PG, Buchanan BB, Singh J: Redox-dependent interaction between thaumatin-like protein and β -glucan influences malting quality of barley. *Proceedings of the National Academy of Sciences* 2017;201701824.
2. Fils-Lycaon BR, Wiersma PA, Eastwell KC, Sautiere PJPp: A cherry protein and its gene, abundantly expressed in ripening fruit, have been identified as thaumatin-like. 1996, 111(1):269-273.
3. van Loon LC, Rep M, Pieterse CM: Significance of inducible defense-related proteins in infected plants. *Annu Rev Phytopathol* 2006, 44:135-162.
4. Velazhahan R, Datta SK, Muthukrishnan S: The PR-5 family: thaumatin-like proteins. *Pathogenesis-related proteins in plants* 1999:107-129.
5. Petre B, Major I, Rouhier N, Duplessis S: Genome-wide analysis of eukaryote thaumatin-like proteins (TLPs) with an emphasis on poplar. *BMC Plant Biol* 2011, 11(1):33.
6. Ghosh R, Chakrabarti C: Crystal structure analysis of NP24-l: a thaumatin-like protein. *Planta* 2008, 228(5):883-890.
7. Fierens E, Gebruers K, Voet AR, De Maeyer M, Courtin CM, Delcour JA: Biochemical and structural characterization of TLXI, the *Triticum aestivum* L. thaumatin-like xylanase inhibitor. *J Enzyme Inhib Med Chem* 2009, 24(3):646-654.
8. Liu JJ, Sturrock R, Ekramoddoullah AK: The superfamily of thaumatin-like proteins: its origin, evolution, and expression towards biological function. *Plant Cell Rep* 2010, 29(5):419-436.
9. He R, Wu J, Zhang Y, Aguero CB, Li X, Liu S, Wang C, Walker MA, Lu J: Overexpression of a thaumatin-like protein gene from *Vitis amurensis* improves downy mildew resistance in *Vitis vinifera* grapevine. *Protoplasma* 2017, 254(4):1579-1589.
10. Subramanyam K, Arun M, Mariashibu TS, Theboral J, Rajesh M, Singh NK, Manickavasagam M, Ganapathi A: Overexpression of tobacco osmotin (Tbosm) in soybean conferred resistance to salinity stress and fungal infections. *Planta* 2012, 236(6):1909-1925.
11. Acharya K, Pal AK, Gulati A, Kumar S, Singh AK, Ahuja PS: Overexpression of *Camellia sinensis* thaumatin-like protein, CsTLP in potato confers enhanced resistance to *Macrophomina phaseolina* and *Phytophthora infestans* infection. *Mol Biotechnol* 2013, 54(2):609-622.
12. Chun J, Yu X, Griffith M: Genetic studies of antifreeze proteins and their correlation with winter survival in wheat. *Euphytica* 1998, 102(2):219-226.
13. Salzman RA, Tikhonova I, Bordelon BP, Hasegawa PM, Bressan RA: Coordinate accumulation of antifungal proteins and hexoses constitutes a developmentally controlled defense response during fruit ripening in grape. *Plant Physiol* 1998, 117(2):465-472.
14. Seo PJ, Lee A-K, Xiang F, Park C-M: Molecular and functional profiling of *Arabidopsis* pathogenesis-related genes: insights into their roles in salt response of seed germination. *Plant and Cell Physiology* 2008, 49(3):334-344.
15. Cao J, Lv Y, Hou Z, Li X, Ding L: Expansion and evolution of thaumatin-like protein (TLP) gene family in six plants. *Plant growth regulation* 2016, 79(3):299-307.
16. Houston K, Russell J, Schreiber M, Halpin C, Oakey H, Washington JM, Booth A, Shirley N, Burton RA, Fincher GB: 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.
17. Wang J, Zhang G, Chen J, Wu F: The changes of β -glucan content and β -glucanase activity in barley before and after malting and their relationships to malt qualities. *Food Chemistry* 2004, 86(2):223-228.
18. Hejgaard J, Jacobsen S, Svendsen I: Two antifungal thaumatin-like proteins from barley grain. *FEBS Lett* 1991, 291(1):127-131.
19. Gorjanović S, Beljanski MV, Gavrović-Jankulović M, Gojgić-Cvijović G, Pavlović MD, Bejosano F: Antimicrobial Activity of Malting Barley Grain Thaumatin-Like Protein Isoforms, S and R. *Journal of the Institute of Brewing* 2007, 113(2):206-212.
20. Osmond RI, Hrmova M, Fontaine F,

Imberty A, Fincher GB: Binding interactions between barley thaumatin-like proteins and (1, 3)- β -D-glucans. *The FEBS Journal* 2001, 268(15):4190-4199. 21. Dhaliwal AK, Mohan A, Gill KS: Comparative analysis of ABCB1 reveals novel structural and functional conservation between monocots and dicots. *Frontiers in plant science* 2014, 5:657. 22. Saitou N, Nei M: The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution* 1987, 4(4):406-425. 23. Kaur S, Dhugga KS, Beech R, Singh J: Genome-wide analysis of the cellulose synthase-like (Csl) gene family in bread wheat (*Triticum aestivum* L.). *BMC Plant Biol* 2017, 17(1):193. 24. Kumar S, Stecher G, Tamura K: MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol* 2016, 33(7):1870-1874. 25. Kaur S, Dhugga KS, Gill K, Singh J: Novel Structural and Functional Motifs in cellulose synthase (CesA) Genes of Bread Wheat (*Triticum aestivum*, L.). *PLOS ONE* 2016, 11(1):e0147046. 26. Hu B, Jin J, Guo AY, Zhang H, Luo J, Gao G: GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics* 2015, 31(8):1296-1297. 27. Tripathi RK, Goel R, Kumari S, Dahuja A: Genomic organization, phylogenetic comparison, and expression profiles of the SPL family genes and their regulation in soybean. *Dev Genes Evol* 2017, 227(2):101-119. 28. Kaur R, Singh K, Singh J: A root-specific wall-associated kinase gene, HvWAK1, regulates root growth and is highly divergent in barley and other cereals. *Functional & Integrative Genomics* 2013, 13(2):167-177. 29. Gupta M, Abu-Ghannam N, Gallagher E: Barley for brewing: Characteristic changes during malting, brewing and applications of its by-products. *Comprehensive reviews in food science and food safety* 2010, 9(3):318-328. 30. Fierens E, Rombouts S, Gebruers K, Goesaert H, Brijs K, Beaugrand J, Volckaert G, Van Campenhout S, Proost P, Courtin CM et al: TLXI, a novel type of xylanase inhibitor from wheat (*Triticum aestivum*) belonging to the thaumatin family. *Biochem J* 2007, 403(3):583-591. 31. Thiel T, Graner A, Waugh R, Grosse I, Close TJ, Stein N: Evidence and evolutionary analysis of ancient whole-genome duplication in barley predating the divergence from rice. *BMC Evolutionary Biology* 2009, 9(1):209. 32. Yu J, Wang J, Lin W, Li S, Li H, Zhou J, Ni P, Dong W, Hu S, Zeng C: The genomes of *Oryza sativa*: a history of duplications. *PLoS biology* 2005, 3(2):e38. 33. Koonin EV: The origin of introns and their role in eukaryogenesis: a compromise solution to the introns-early versus introns-late debate? *Biology direct* 2006, 1(1):22. 34. Tripathi RK, Bregitzer P, Singh J: Genome-wide analysis of the SPL/miR156 module and its interaction with the AP2/miR172 unit in barley. *Scientific Reports* 2018, 8(1):7085. 35. Zhang Q, Zhang X, Wang S, Tan C, Zhou G, Li C: Involvement of Alternative Splicing in Barley Seed Germination. *PLOS ONE* 2016, 11(3):e0152824. 36. Qin Z, Wu J, Geng S, Feng N, Chen F, Kong X, Song G, Chen K, Li A, Mao L: Regulation of FT splicing by an endogenous cue in temperate grasses. *Nature communications* 2017, 8:14320. 37. Napoli N, Ghelli R, Brunetti P, De Paolis A, Cecchetti V, Tsuge T, Serino G, Matsui M, Mele G, Rinaldi G et al: A Newly Identified Flower-Specific Splice Variant of AUXIN RESPONSE FACTOR8 Regulates Stamen Elongation and Endothecium Lignification in *Arabidopsis*. *The Plant Cell* 2018. 38. Singh M, Singh JJE: Seed development-related expression of ARGONAUTE4_9 class of genes in barley: possible role in seed dormancy. 2012, 188(1):123-129.

Additional Files

Additional file 1: Table S1. Amino acid sequences of barley, rice, sorghum and Brachypodium TLPs used in this study. Additional file 2: Table S2. Information of unique genetic loci for barley TLPs. Additional file 3: Table S3. List of primers used for expression analysis of HvTLPs in malt and feed variety at 16 hrs, 48 hrs. and 96 hrs. of seed imbibition. Additional file 4: Table S4. Information for the HvTLPs splice variants. Additional file 5: Table S5. Gene IDs from Morex RNA-seq Assembly.

Table

Due to technical limitations, table 1 is only available as a download in the supplemental files section.

Figures

Fig. 1. (A) Number of TLP genes in Rice, Barley, Brachypodium and Sorghum. (B) Pipeline for the bioinformatics analysis.

Figure 1

(A) Number of TLP genes in Rice, Barley, Brachypodium and Sorghum. (B) Pipeline for the bioinformatics analysis.

Fig. 2. Phylogenetic analysis of TLP protein family among cereals.

Figure 2

Phylogenetic analysis of TLP protein family among cereals. The TLP domain was identified and the phylogenetic tree was constructed using neighbor-joining method with 1000 bootstraps. Terminal tree markers are indicative of species (Barley (bright green);Rice (blue); Sorghum (red) and Brachypodium (pink))

Fig. 3 Structural features of barley *TLP* genes .

Figure 3

Structural features of barley TLP genes. From left to right is 5' to 3' region containing UTR in black color, marron represents exons, blue triangle for start codon, red triangle for stop codon while black narrow line with a slit symbolizes intron and yellow triangle is representing thaumatin signature

Fig.4 Spatio-temporal expression of *HvTLP* genes in different barley tissues

Figure 4

Spatio-temporal expression of HvTLPs genes in different barley tissues. The color scale bar at the top of heat map represents FPKM normalized log2 transformed values based on “Morex” RNA-seq data and represents high and low expression respectively. EMB,LEA, INF1, INF2, ROO, NOD, CAR5 and CAR15 tissues were used for expression profiling. Details about these tissues have been earlier explained in material and methods section.

Fig.5 Transcript abundance of *HvTLP* genes at different stages of seed germination

Figure 5

Transcript abundance of *HvTLP* genes at different stages of seed germination. Gel representation of the expression profiles of *HvTLP*s (1, 2, 4, 5, 6, 7, 9, 13, 14, 16, 17 and 18) and the housekeeping genes β -actin and GAPDH at 16 hrs, 48 hrs. and 96 hrs. of germination in malting (MX= Morex) and feed (ST= Steptoe) varieties of barley using RT-PCR. 1.2 % agarose gel was used for resolving amplified bands.

Fig.6 Map locations of *HvTLP* genes in barley genome. Exact positions are shown in Table 1.

Figure 6

6 Map locations of *HvTLP* genes in barley genome. Exact positions are shown in Table 1

Supplementary Files

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