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Genome-wide identification and characterization of Xylanase Inhibitor Protein (XIP) genes in cereals

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Abstract

Xylanase inhibitor proteins (XIPs) have been reported to be involved in plant defense mechanisms, more predominantly against fungal pathogens. XIPs have mostly been well characterized in model plant species, and very little is known about their distribution, organization and evolution in cereals. In the present study, we have identified XIPs in four cereal plant species, including three major crops sorghum, maize, rice and a model species *Brachypodium*. The genome-wide analysis identified 10, 20, 13 and 31 *XIP* genes respectively for *Brachypodium*, sorghum, maize and rice. The number of identified genes is in well accordance with the genome size except for maize. Interestingly, most of the *XIP* genes were observed to be intron-less, and clustered together on the chromosomes. The *XIP* genes organization is much similar to the lower eukaryotes and fungi suggesting the possibility of horizontal gene transfer from the pathogen. The phylogenetic analysis of *XIP* revealed two major groups, and minor subgroups mostly representing gene clusters. Gene expression evaluation using publically available data suggested LOC_Os11g47500 and LOC_Os11g47510are candidate genes for resistance against *Magnaporthe grisea* pathogen in rice. The information provided here would be helpful for the identification of candidate resistance genes in other cereals.

Key words: Xylanase inhibitor proteins, resistance genes, genome-wide analysis, gene family, gene clusters, fungal diseases

Introduction

Xylanase inhibitors involved in the fundamental biological processes are novel class of proteins (Flatman et al. 2002; Bellincampi et al. 2004; Moscetti et al. 2015). In plants, xylanase inhibitor proteins (XIPs) limit fungal infection by inhibiting the xylanases secreted by fungus during the early stage of infection. Thus, the identification of genes encoding XIP has great importance to develop resistance against plant fungal pathogens. In wheat, a *XIP-I* gene has been identified that encode protein, which inhibits xylanases belonging to GH10 and GH11 families of glycoside hydrolases (Flatman et al. 2002). Sequence and structural similarities indicate that XIP-I is related to chitinases of family GH18, regardless of its lack of glycoside hydrolases activity. The multi-functionality of GH18 members makes it more interesting for genomic annotations and prediction of molecular function.

Previously, structural features essential for xylanase inhibitor have been predicted by the high-resolution crystal structures of XIP-I in complex with GH10 (from *Aspergillus nidulans*) and GH11 (from *Penicillium funiculosum*) xylanases (Payan et al. 2004). The study revealed the novel inhibition mechanism of XIP-Ishowing that XIP-1 protein has two independent enzyme-binding sites, allowing simultaneous binding of two glycoside hydrolases with different folds. XIP-I is the member of GH18 family that includes chitinases from various species, like bacteria, fungi, nematodes, insects, plants, and mammals. In cereals, the presence of xylanase inhibitor is not surprising as they have hemicellulose in their cell wall, which is mainly composed of arabinoxylan. Xylanase and their inhibitors are, particularly, required for the proliferation, degradation of cell wall. The ability to degrade xylan represents an important attribute for a fungal pathogen

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to infect plant tissues. Indeed, secretion of xylanases by rice pathogens was reported for *Magnaporthe grisea*, the fungal pathogen that causes rice blast disease (Wu et al. 1995), and *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial leaf blight, a serious disease in rice (Wu et al. 1995; Ryeand Withers 2000). Xylanases secreted by rice pathogens are found to be associated with the virulence that suggests the potential role of XIPs in plant defense (Bellincampi et al. 2004). XIPs could have evolved from chitinases as a part of the plant defense pathway to act both on the xylanases secreted by pathogens and on the pathogen itself. The Plant chitinases exhibit rapid evolution by acting as prime targets for the co-evolution of plant–pathogen interactions. In both cases, the function is oriented towards a general role in plant defense and the production of inhibitors prevents the plant to undergo unnecessary metabolic costs. Therefore, genome-wide study of XIP genes is necessary to understand the evolution of genes, their organization and distribution.

Recent development in DNA sequencing technology has made several genomes available in public domain (www.phytozome.net). The complete genome sequence has been explored in many ways to understand genome organization and evolution in plants (Sonah et al. 2011a). Sequenced genome served as a basis for the development of molecular markers, identification of candidate genes, and map based cloning of several agronomical important genes (Singh et al. 2010; Sharma et al. 2011; Sonah et al. 2011b). Many studies have demonstrated identification of novel genes in cereals through comparative genomic approaches (Deshmukh et al. 2010, Kadam et al. 2012). For instance, a gene sequence of silicon transporter (Lsi1) identified in rice has been used to identify its homologous in wheat, maize, sorghum, barley and soybean (Deshmukh et al. 2013). Likewise, there are many examples of utilization of well-characterized gene information for the identification of its novel homologs in related species (Kulkarni et al. 2012). Similarly, the wheat XIP-1, well characterized for structure and function can be used to identify its homologs in other related grass family members. Presently, cereal genomes like rice, sorghum and maize are available with the well annotated data for structure and organization of genes. Moreover, availability of genome sequence of *Brachypodium,* which is a closer relative of wheat than that of rice (Vogel 2010), has provided a model system to study large genome like wheat, barley, and oat.

In present investigation, extensive analysis of *XIP-1* like genes identified in fully sequenced genome of rice, maize, sorghum and *Brachypodium* was performed to understand their distribution and organization in these genomes.

Materials and methods

Identification of XIP-1 like genes

DNA as well as protein sequence of predicted genes of rice from TIGR (http://rice.plantbiology.msu.edu/), and maize, sorghum and *Brachypodium* from Phytozome (http://www.phytozome.net/) database were retrieved in batches. These sequences were used to create local database 'cereal-proteome' for proteins and 'cereal-genes' for DNA sequence of predicted gene models. The *XIP-1* like genes were searched in rice, maize, sorghum and *Brachypodium* genome using xylanase inhibitor protein I (XIP-1, GI:284178233, ADB81849.1) gene cloned from wheat.

Protein sequences of candidate genes similar to *XIP-1* gene were identified based on a BLASTP search (Altschul et al. 1990) at a thresholde-value of 10^{-20} using *XIP-1* gene of wheat as a query. The *XIP-1* like genes identified in cereals genomes were located on respective chromosomes using MapChart 2.2 software tool (Voorrips 2002).

Identification of conserved domains

Conserved domains were identified in XIP-1 like candidate genes using BLAST search at NCBI-conserved domain database (http://www.ncbi.nlm.nih.gov/). Motifs conserved among the XIPs from four cereal species were identified using MEME (Multiple EM for Motif Elicitation) software suite (http://meme-suite.org). The setting with minimum width 6 and maximum width 50 amino acid motifs were used for prediction. The final output of MEME was manually evaluated. To construct 3D protein structure, all the identified XIP sequences were submitted to the Phyre2 protein-modeling server (www.sbg.bio.ic.ac.uk/~phyre2). Quality of predicted model was assessed based on the z-score calculated using the Prosa server (https://prosa.services.came.sbg.ac.at/prosa.php).

Phylogenetic analysis of XIP-1 like sequences

The protein sequences were aligned using ClustalW with default options, and the alignment was manually corrected using the alignment editor Jalview (Waterhouse et al. 2009). Aligned sequences were trimmed at both ends to eliminate regions of poor

alignment. A phylogenetic tree was constructed using the Bootstrap neighbor-joining (NJ) method in MEGA4 (Tamura et al. 2007). The stability of tree nodes was tested by bootstrap analysis with 1,000 replicates.

Gene expression

Massively Parallel Signature Sequencing (MPSS) data for the rice XIP was downloaded from the website of Rice MPSS database (mpss.udel.edu). The sum of abundances of 17bp-tag signatures listed for each library was compared among the leaf tissue samples collected 24 hours after inoculation with *Magnaporthe grisea* from the resistant Nipponbare-Pi9 and susceptible Nipponbare cultivars (Nakano et al. 2006).

Results and discussion

Distribution of XIP-1 like genes in cereals

A total of 74 XIP-1 genes were identified in four fully sequenced cereal genomes with BLASTp searched using wheat XIP-1 (GI:284178233) as query. These consisted of 10, 20, 13, and 31 genes respectively for *Brachypodium*, sorghum, maize and rice genomes. The distribution of *XIP-1* genes was not uniform in the four plant genomes analyzed. Most of the genes similar to *XIP-1* (71.6%) were found in clusters (Table 1, Fig. 1). For instance, in *Brachypodium* 7 genes out of 10 were present in clusters including a cluster of three genes on chromosome 2 and two clusters each with two genes on chromosome 4. Likewise, sorghum has five clusters of XIP-1 genes distributed on three different chromosomes representing 16 out of 20 XIP-1 genes. In maize, three clusters of XIP-1 genes are present that includes a cluster on chromosome 3 and two clusters on chromosome 7. Similarly, in rice genome, 5 clusters are present on 5 different chromosomes. Largest cluster of genes having 11 tandem repeated genes are present on rice chromosome 11. Several theories have been speculated for the occurrence of such gene clusters in eukaryotes for instance horizontal gene transfer mostly from prokaryotes (bacterial operons), uneven crossing-over followed by tandem duplications, and co-evolution of metabolically related genes having similar selection pressure (Song et al. 2010; Dean 2007). Compared to plants and higher animals, fungi genomes have higher frequency of gene clusters (Dean 2007). Horizontal gene transfer from the fungi could be one of the reasons for *XIP-1* like gene clustersin plants. The genes, which are not in cluster, may arise later with mechanism like copy-paste or cut-paste mostly associated with transposons, chromosomal translocations and shuffling of the genomes.

Table 1. Details of xylanase inhibitor protein (XIP) gene clusters identified in the four plant genome

Species	Cluster	Genes	Gene_ID
Brachypodium*	Bd-02	3	Bd2g55610 (1), Bd2g55620 (1), Bd2g55630
	Bd-04a	2	Bd4g09420 (1), Bd4g09430 (1)
	Bd-04b	2	Bd4g40110, Bd4g40120
Sorghum*	Sb-02a	2	Sb02g004650, Sb02g004660,
	Sb-02b	4	Sb02g009360, Sb02g009370, Sb02g009380, Sb02g009390
	Sb-03	5	Sb03g040560 (1), Sb03g040570 (1), Sb03g040580 (1), Sb03g040590 (1), Sb03g040600,
	Sb-05a	2	Sb05g008530, Sb05g008550,
	Sb-05b	3	Sb05g023690 (1), Sb05g023700, Sb05g023710,
Maize*	Gm-03	3	GM2G430936, GM2G430942, GM2G023650 (2)
	Gm-07a	2	GM2G328171, GM2G162359
	Gm-07b	3	GM2G130686, GM2G160265, GM2G080547 (1),
Rice[#]	Os-01	2	Os01g64100 (1), Os01g64110
	Os-05	4	Os05g15770, Os05g15850, Os05g15880, Os05g15920
	Os-06	2	Os06g24990 (1), Os06g25010
	Os-08	3	Os08g40680, Os08g40690, Os08g40740 (1)
	Os-11	11	Os11g47500, Os11g47510, Os11g47520, Os11g47530, Os11g47550, Os11g47560, Os11g47570, Os11g47580, Os11g47590, Os11g47600, Os11g47610
Total	16	53	Intronless 39, with intron 14

*Phytozome locus Id, [#]TIGR locus Id, prefix are shortened. Numbers given in brackets are number of introns present in the gene

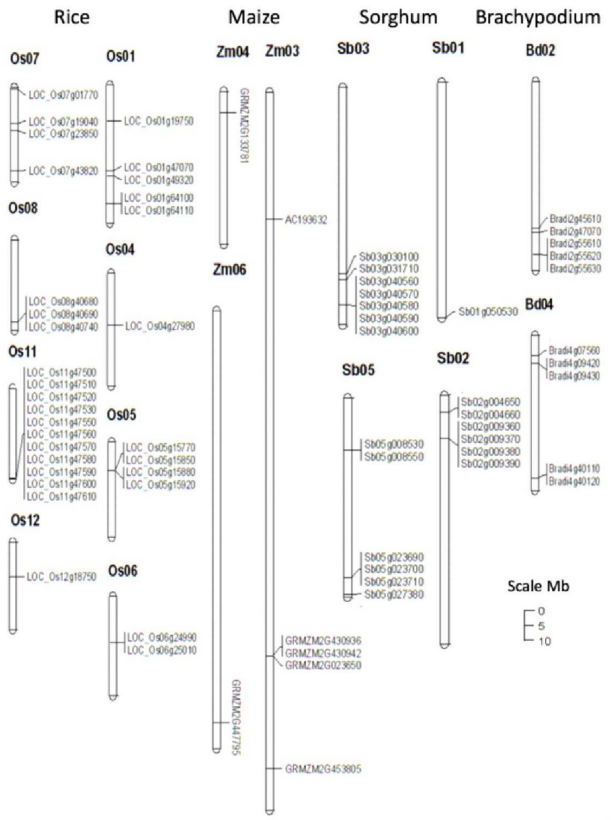

Fig. 1. Genomic distribution of xylanase inhibitor proteins (XIP) genes in rice, maize, sorghum and *Brachypodium* genomes

Availability of fully sequenced genome of *Brachypodium*, sorghum, maize and rice has made it possible for the first time to identify all the XIP-1like genes in these genomes. The numbers of genes in these species are well agreeing with assumption made on the basis of genome size. However, this is only true with the Brachypodium, Sorghum and rice. Maize genome is very large and having more duplicated region (Hughes et al. 2014). Even though, the XIP like genes identified in maize is less than the Sorghum and rice. Besides having gaint genome, maize has three times lesser gene density compared to the rice and most of the other cereal genomes (Schnable et al. 2009). Most of the maize genome is covered by repetitive DNA sequences, transposons and retrotransposons (Schnable et al. 2009). Considering the facts it is obvious to have relatively lesser XIPs frequency in the maize genome.

Gene structure and organization

The XIP genes generally have very few or no introns. About 75% of total XIP-1 genes identified in four plant genomes are without intron. Out of 74 genes, 15 genes have single, and 4 genes have more than one intron. Moreover, genes in cluster mostly have same number of introns and have more similarity among them. For instance, in *Brachypodium*, cluster Bd-04a all genes are with single intron whereas cluster Bd-04b has intron-less genes (Table 1). In addition, cluster Bd-02 includes two genes with single intron and one intron-less. Likewise, in sorghum, maize and rice, most of the gene clusters have genes with similar structure (Table 1). Most interestingly the largest gene cluster observed on chromosome 11 of rice has all the 11 genes with intron-less structure. This cluster of tandem repeated gene has been interrupted with the presence of one LOC_Os011g47540 gene. This gene might be inserted in the cluster later on or might be the cluster itself duplicated, as all the 11 genes are highly similar.

Presently, several gene clusters have been discovered in fungi, plant and animal genomes (Andersen et al. 2013; Patil et al. 2015; Noordermeer et al. 2011). In lower organisms such gene clusters are common due to the typical gene regulation and compact genome. Unlike, plants genomes are larger and more complex, even though, several genes are present in cluster on the chromosome. This might be because of the structural role of chromatin in gene expression or may be because of horizontal gene transfer from the microbes. The XIP-1 like genes has much more similarity with the fungal *chitinase* genes (Flatman et al. 2002; Channamallikarjuna et al. 2010). This suggests the possibility of horizontal transfer of gene and subsequent evolution.

Domain structure, functionality and phylogenetic relationship among XIP-1 genes

Similarity search against NCBI-conserved domain database showed significant match for all the genes with the domain belongs to GH18_chitinase-like superfamily and more specific to GH18_hevamine_ XipI_class_III (Table S1 (Supplemntary Table 1 available online at http://www.isgpb.co.in: vi-vii)). Moreover, SignalP-4.0 search performed for XIP-1 genes revealed the presence of signal peptide for 72 out of 74 genes (Table S2 (Supplemntary Table 2 available online at http://www.isgpb.co.in: i-v)). No signal peptide was detected for the two rice genes namely LOC_Os01g19750 and LOC_Os07g23850. Both of the genes are not present in cluster and might be formed with artifacts during evolution. Furthermore, to search conserved motif MEME search was performed for all the 74 genes. A total of three

conserved motifs were detected in XIP-1 genes (Fig. 2). However, none of these are present in signal peptide region (Fig. 2a). Homology modeling of XIP genes showed 3D structure witheight barrels foldssimilar to glycoside hydrolase family 18. A representative 3D structure of rice XIP is shown in Fig. 2c.

The amino acid sequences of *XIP-1* like genes were aligned in order to find similarity among genes and to construct the neighbor-joining (NJ) phylogenetic tree. Phylogenetic analysis of *XIP-1* genes revealed that genes in cluster are more similar than genes in different clusters. The bootstrap values obtained for most of the nodes were very high. This suggests the groups obtained are statistically more significant. On the basis of phylogenetic tree, *XIP-1* like genes in cereals can be divided into two major classes (Fig. 3). However, we have not observed any functional relevance of the classification. The *XIP-1* like genes from different plant species identified here exhibits high level of similarity. This is likely to be more helpful for the cloning of *XIP-1* homologous in related cereal species.

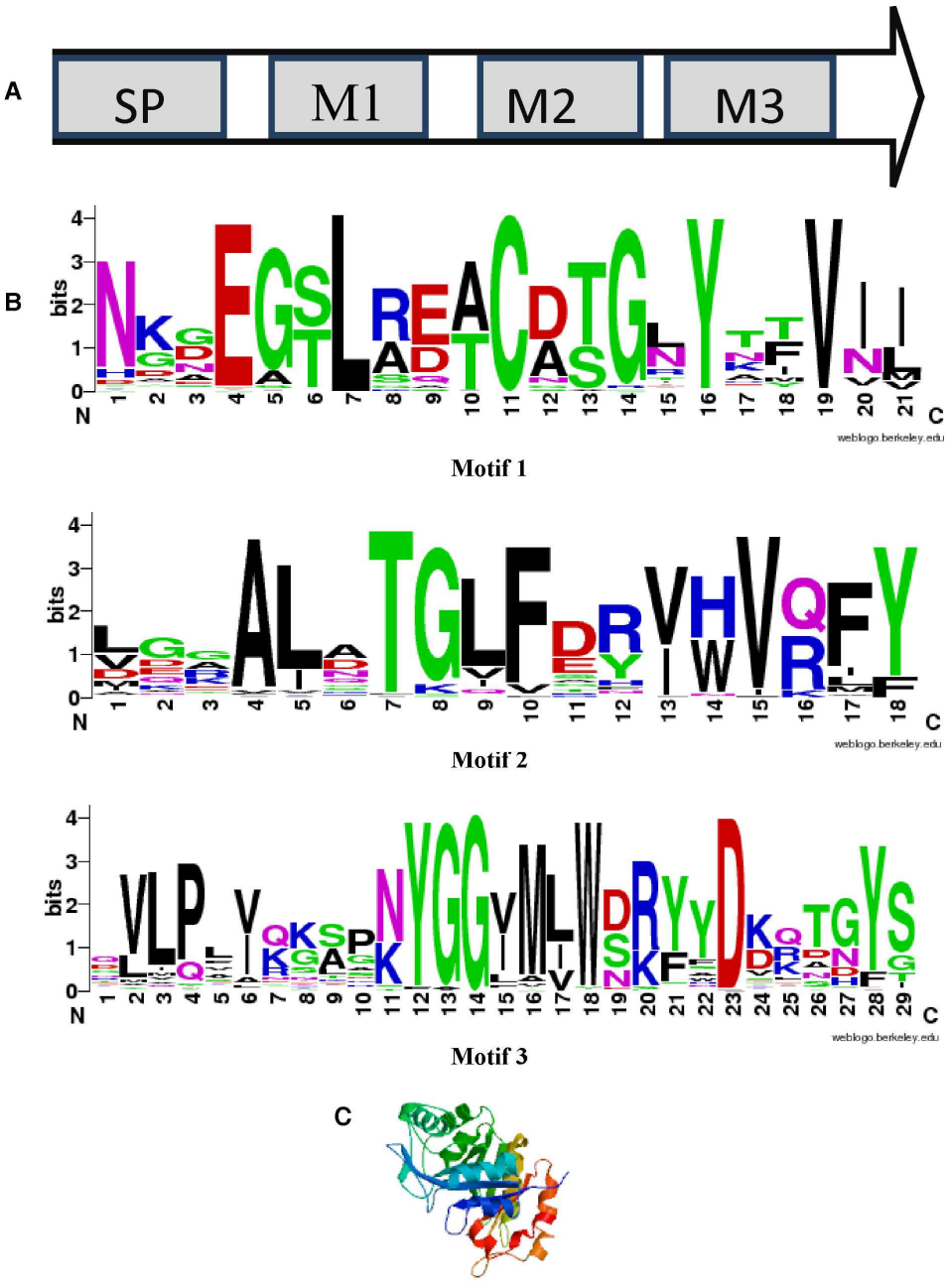

Fig. 2. Conserved motif detected among the 74 xylanase inhibitor proteins (XIPs) identified in Brachypodium, sorghum, maize and rice genome by using MEME software tool. (a) location of the signal peptide and motifs in the gene and (b) conserved amino acids in motif with the bit score; (c) Representative protein 3D structure of XIP (LOC_Os11g47590) constructed with Swissmodel tool (http://swissmodel.expasy.org)

Expression of XIP-1 genes in rice

In rice, MPSS database provide extensive transcriptome data for most of the genes. The MPSS is a valuable resource that provides level of gene expression in various tissues and under different conditions (http://mpss.udel.edu). Previously, the use of MPSS for the identification of gene expression has been demonstrated in several studies (Channamallikarjuna et al. 2010; Nakano et al. 2006). Here, analysis for rice *XIP-1* genes using the 17 nucleotides long signature sequences library for tissue-specific, and biotic

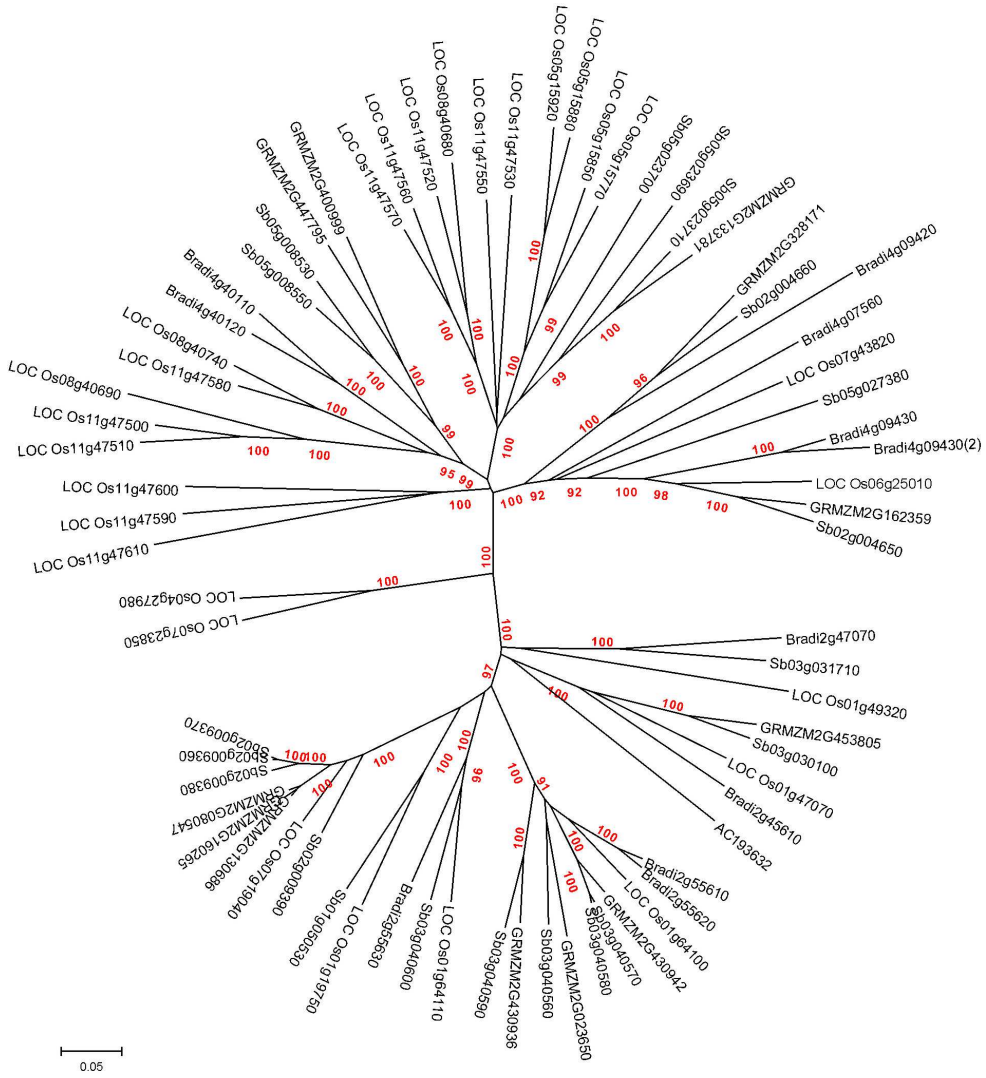

Fig. 3. Phylogenetic trees of rice xylanase inhibitor proteins (XIP) genes constructed using p-distance model of the MEGA 5.0 software tool

and abiotic stress-related traits was performed. The analysis revealed that mostly the genes in clusters showed similar expression pattern under similar conditions and tissues. Predominantly, rice *XIP-1* genes were expressed in root tissue and early stages of growth. Moreover, most of the genes were expressed against biotic stress like *Xanthomonas oryzae* and *Magnaporthe grisea*, and interestingly both of these organisms are known to secrete xylanase during early infection stage (Wu et al. 1995; Rye and Withers 2000; Bucheli et al. 1990). Further LOC_Os11g47500 and LOC_Os11g47510 genes present in cluster on rice chromosome 11 showed differential response in resistant and susceptible cultivars against *M. grisea* infection (Fig. 4). These genes were highly expressed

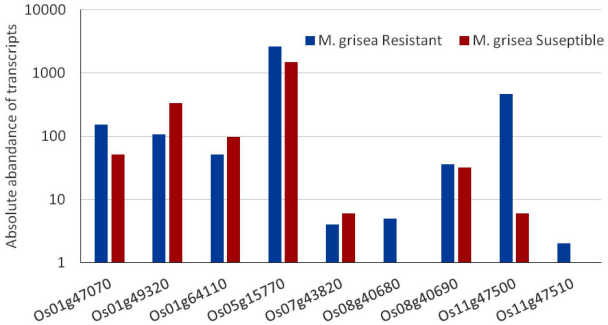

Fig. 4. Transcript abundance of rice xylanase inhibitor proteins (XIP) observed in MPSS libraries constructed using the tissue 24 hours after inoculation with *Magnaporthe grisea* to 3 week old leaves of resistant Nipponbare-Pi9 and susceptible Nipponbare cultivars. Data was obtained from Nakano et al. (2006)

24 hours after the inoculation with *M. grisea* in resistant cultivar as compared to susceptible cultivar. Recently a QTL *sbq11.1* identified for the sheath blight resistance in rice has been fine mapped (Channamallikarjuna et al. 2010). The QTL *sbq11.1* is collocated with the *XIP-1* gene cluster on chromosome 11. This makes LOC_Os11g47500 and LOC_ Os11g47510 genes more promising candidate responsible for the sheath blight resistance in rice (Channamallikarjuna et al. 2010).

In conclusion, XIP genes in plants are mostly clustered and possibly evolved through horizontal gene transfer from the fungal pathogen. The XIP genes shares high level of homology among

cereal species, and the conserved motifs observed here would be helpful for identification and annotations of novel XIPs. Integrated information of distribution, organization and gene expression will be helpful for the identification of candidate resistance genes in crop species.

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Supplementary Table 1. Similarity of 74 xylanase inhibitor proteins (XIPs) identified in four cereal genomes with the known conserved motifs available at NCBI's conserved domain database (http://www.ncbi.nlm.nih.gov/)

ID	Specification	From	To	E-Value	Accession	Short name
AC193632	specific	28	302	2.62E-132	cd02877	GH18_hevamine_XipI_class_III
AC193632	superfamily	28	302	2.62E-132	cl10447	GH18_chitinase-like
AC193632	superfamily	379	659	2.53E-81	cl21453	PKc_like
AC193632	multi-dom	374	658	1.20E-41	smart00221	STYKc
AC193632	multi-dom	28	293	3.81E-27	pfam00704	Glyco_hydro_18
Bradi2g45610	specific	26	299	1.82E-115	cd02877	GH18_hevamine_XipI_class_III
Bradi2g45610	superfamily	26	299	1.82E-115	cl10447	GH18_chitinase-like
Bradi2g45610	multi-dom	26	288	7.98E-18	pfam00704	Glyco_hydro_18
Bradi2g47070	specific	33	311	2.27E-120	cd02877	GH18_hevamine_XipI_class_III
Bradi2g47070	superfamily	33	311	2.27E-120	cl10447	GH18_chitinase-like
Bradi2g47070	superfamily	405	675	1.61E-67	cl21453	PKc_like
Bradi2g47070	multi-dom	33	302	5.11E-24	pfam00704	Glyco_hydro_18
Bradi2g47070	multi-dom	404	650	4.16E-21	smart00220	S_TKc
Bradi2g55610	specific	26	295	1.11E-129	cd02877	GH18_hevamine_XipI_class_III
Bradi2g55610	superfamily	26	295	1.11E-129	cl10447	GH18_chitinase-like
Bradi2g55610	multi-dom	26	284	6.71E-29	pfam00704	Glyco_hydro_18
Bradi2g55620	specific	26	295	3.97E-128	cd02877	GH18_hevamine_XipI_class_III
Bradi2g55620	superfamily	26	295	3.97E-128	cl10447	GH18_chitinase-like
Bradi2g55620	multi-dom	26	284	1.64E-28	pfam00704	Glyco_hydro_18
Bradi2g55630	specific	31	294	8.28E-134	cd02877	GH18_hevamine_XipI_class_III
Bradi2g55630	superfamily	31	294	8.28E-134	cl10447	GH18_chitinase-like
Bradi2g55630	multi-dom	31	283	9.64E-29	pfam00704	Glyco_hydro_18
Bradi4g07560	superfamily	33	304	1.89E-83	cl10447	GH18_chitinase-like
Bradi4g07560	multi-dom	39	293	1.79E-09	pfam00704	Glyco_hydro_18
Bradi4g09420	superfamily	35	166	6.13E-41	cl10447	GH18_chitinase-like
Bradi4g09430	specific	37	305	3.82E-107	cd02877	GH18_hevamine_XipI_class_III
Bradi4g09430	superfamily	37	305	3.82E-107	cl10447	GH18_chitinase-like
Bradi4g09430	multi-dom	37	294	6.70E-09	pfam00704	Glyco_hydro_18
Bradi4g09430	superfamily	37	240	2.90E-82	cl10447	GH18_chitinase-like
Bradi4g09430	multi-dom	37	195	5.44E-10	pfam00704	Glyco_hydro_18
Bradi4g40110	specific	27	295	1.44E-110	cd02877	GH18_hevamine_XipI_class_III
Bradi4g40110	superfamily	27	295	1.44E-110	cl10447	GH18_chitinase-like
Bradi4g40110	multi-dom	33	244	1.61E-12	pfam00704	Glyco_hydro_18
Bradi4g40120	specific	29	296	2.43E-119	cd02877	GH18_hevamine_XipI_class_III
Bradi4g40120	superfamily	29	296	2.43E-119	cl10447	GH18_chitinase-like
Bradi4g40120	multi-dom	34	285	2.26E-13	pfam00704	Glyco_hydro_18
GRMZM2G023650	specific	35	305	1.55E-135	cd02877	GH18_hevamine_XipI_class_III
GRMZM2G023650	superfamily	35	305	1.55E-135	cl10447	GH18_chitinase-like
GRMZM2G023650	multi-dom	35	294	5.77E-33	pfam00704	Glyco_hydro_18
GRMZM2G080547	specific	26	295	1.15E-135	cd02877	GH18_hevamine_XipI_class_III
GRMZM2G080547	superfamily	26	295	1.15E-135	cl10447	GH18_chitinase-like
GRMZM2G080547	multi-dom	26	278	6.82E-34	pfam00704	Glyco_hydro_18
GRMZM2G130686	specific	26	295	1.40E-135	cd02877	GH18_hevamine_XipI_class_III

GRMZM2G130686 superfamily	26	295	1.40E-135	cl10447	GH18_chitinase-like
GRMZM2G130686 multi-dom	26	278	7.10E-33	pfam00704	Glyco_hydro_18
GRMZM2G133781 specific	53	322	1.29E-103	cd02877	GH18_hevamine_XipI_class_III
GRMZM2G133781 superfamily	53	322	1.29E-103	cl10447	GH18_chitinase-like
GRMZM2G133781 multi-dom	55	314	1.31E-09	pfam00704	Glyco_hydro_18
GRMZM2G160265 specific	26	295	3.95E-133	cd02877	GH18_hevamine_XipI_class_III
GRMZM2G160265 superfamily	26	295	3.95E-133	cl10447	GH18_chitinase-like
GRMZM2G160265 multi-dom	26	278	3.62E-33	pfam00704	Glyco_hydro_18
GRMZM2G162359 specific	44	310	1.47E-102	cd02877	GH18_hevamine_XipI_class_III
GRMZM2G162359 superfamily	44	310	1.47E-102	cl10447	GH18_chitinase-like
GRMZM2G162359 multi-dom	44	299	3.20E-09	pfam00704	Glyco_hydro_18
GRMZM2G328171 specific	38	304	3.57E-97	cd02877	GH18_hevamine_XipI_class_III
GRMZM2G328171 superfamily	38	304	3.57E-97	cl10447	GH18_chitinase-like
GRMZM2G328171 multi-dom	38	303	1.87E-09	pfam00704	Glyco_hydro_18
GRMZM2G400999 superfamily	30	305	4.19E-91	cl10447	GH18_chitinase-like
GRMZM2G400999 multi-dom	36	294	0.000119919	pfam00704	Glyco_hydro_18
GRMZM2G430936 specific	27	297	1.03E-132	cd02877	GH18_hevamine_XipI_class_III
GRMZM2G430936 superfamily	27	297	1.03E-132	cl10447	GH18_chitinase-like
GRMZM2G430936 multi-dom	27	286	3.37E-27	pfam00704	Glyco_hydro_18
GRMZM2G430942 specific	26	292	1.44E-126	cd02877	GH18_hevamine_XipI_class_III
GRMZM2G430942 superfamily	26	292	1.44E-126	cl10447	GH18_chitinase-like
GRMZM2G430942 multi-dom	26	287	2.99E-31	pfam00704	Glyco_hydro_18
GRMZM2G447795 specific	33	312	1.41E-106	cd02877	GH18_hevamine_XipI_class_III
GRMZM2G447795 superfamily	33	312	1.41E-106	cl10447	GH18_chitinase-like
GRMZM2G447795 multi-dom	39	170	3.86E-07	pfam00704	Glyco_hydro_18
GRMZM2G453805 specific	27	293	6.45E-137	cd02877	GH18_hevamine_XipI_class_III
GRMZM2G453805 superfamily	27	293	6.45E-137	cl10447	GH18_chitinase-like
GRMZM2G453805 multi-dom	27	295	6.25E-30	pfam00704	Glyco_hydro_18
LOC_Os01g19750 specific	51	333	3.43E-124	cd02877	GH18_hevamine_XipI_class_III
LOC_Os01g19750 superfamily	51	333	3.43E-124	cl10447	GH18_chitinase-like
LOC_Os01g19750 multi-dom	51	322	1.28E-28	pfam00704	Glyco_hydro_18
LOC_Os01g47070 specific	27	299	9.91E-140	cd02877	GH18_hevamine_XipI_class_III
LOC_Os01g47070 superfamily	27	299	9.91E-140	cl10447	GH18_chitinase-like
LOC_Os01g47070 multi-dom	29	297	1.78E-30	pfam00704	Glyco_hydro_18
LOC_Os01g49320 specific	28	300	8.72E-126	cd02877	GH18_hevamine_XipI_class_III
LOC_Os01g49320 superfamily	28	300	8.72E-126	cl10447	GH18_chitinase-like
LOC_Os01g49320 multi-dom	28	289	5.84E-30	pfam00704	Glyco_hydro_18
LOC_Os01g64100 specific	26	295	5.63E-131	cd02877	GH18_hevamine_XipI_class_III
LOC_Os01g64100 superfamily	26	295	5.63E-131	cl10447	GH18_chitinase-like
LOC_Os01g64100 multi-dom	26	287	4.87E-29	pfam00704	Glyco_hydro_18
LOC_Os01g64110 specific	31	303	3.75E-140	cd02877	GH18_hevamine_XipI_class_III
LOC_Os01g64110 superfamily	31	303	3.75E-140	cl10447	GH18_chitinase-like
LOC_Os01g64110 multi-dom	32	292	1.26E-33	pfam00704	Glyco_hydro_18
LOC_Os04g27980 specific	33	298	7.63E-135	cd02877	GH18_hevamine_XipI_class_III
LOC_Os04g27980 superfamily	33	298	7.63E-135	cl10447	GH18_chitinase-like
LOC_Os04g27980 multi-dom	33	288	4.65E-21	pfam00704	Glyco_hydro_18
LOC_Os05g15770 specific	25	281	1.01E-119	cd02877	GH18_hevamine_XipI_class_III

LOC_Os05g15770	superfamily	25	281	1.01E-119	cl10447	GH18_chitinase-like
LOC_Os05g15770	multi-dom	27	279	5.44E-12	pfam00704	Glyco_hydro_18
LOC_Os05g15850	specific	36	287	1.97E-110	cd02877	GH18_hevamine_XipI_class_III
LOC_Os05g15850	superfamily	36	287	1.97E-110	cl10447	GH18_chitinase-like
LOC_Os05g15850	multi-dom	38	208	2.13E-07	pfam00704	Glyco_hydro_18
LOC_Os05g15880	specific	31	293	3.27E-104	cd02877	GH18_hevamine_XipI_class_III
LOC_Os05g15880	superfamily	31	293	3.27E-104	cl10447	GH18_chitinase-like
LOC_Os05g15880	multi-dom	37	215	2.88E-07	pfam00704	Glyco_hydro_18
LOC_Os05g15920	specific	32	291	4.16E-106	cd02877	GH18_hevamine_XipI_class_III
LOC_Os05g15920	superfamily	32	291	4.16E-106	cl10447	GH18_chitinase-like
LOC_Os05g15920	multi-dom	34	286	6.66E-11	pfam00704	Glyco_hydro_18
LOC_Os06g25010	superfamily	33	234	4.10E-84	cl10447	GH18_chitinase-like
LOC_Os06g25010	multi-dom	33	197	6.53E-09	pfam00704	Glyco_hydro_18
LOC_Os07g19040	specific	26	295	4.12E-136	cd02877	GH18_hevamine_XipI_class_III
LOC_Os07g19040	superfamily	26	295	4.12E-136	cl10447	GH18_chitinase-like
LOC_Os07g19040	multi-dom	26	278	5.97E-36	pfam00704	Glyco_hydro_18
LOC_Os07g23850	specific	12	267	9.20E-120	cd02877	GH18_hevamine_XipI_class_III
LOC_Os07g23850	superfamily	12	267	9.20E-120	cl10447	GH18_chitinase-like
LOC_Os07g23850	multi-dom	13	256	6.85E-20	pfam00704	Glyco_hydro_18
LOC_Os07g43820	superfamily	38	314	1.39E-95	cl10447	GH18_chitinase-like
LOC_Os07g43820	multi-dom	38	303	1.34E-06	pfam00704	Glyco_hydro_18
LOC_Os08g40680	specific	34	292	9.12E-97	cd02877	GH18_hevamine_XipI_class_III
LOC_Os08g40680	superfamily	34	292	9.12E-97	cl10447	GH18_chitinase-like
LOC_Os08g40680	multi-dom	34	214	7.65E-11	pfam00704	Glyco_hydro_18
LOC_Os08g40690	specific	41	311	3.41E-97	cd02877	GH18_hevamine_XipI_class_III
LOC_Os08g40690	superfamily	41	311	3.41E-97	cl10447	GH18_chitinase-like
LOC_Os08g40690	multi-dom	41	262	2.36E-06	pfam00704	Glyco_hydro_18
LOC_Os08g40740	specific	31	281	1.41E-105	cd02877	GH18_hevamine_XipI_class_III
LOC_Os08g40740	superfamily	31	281	1.41E-105	cl10447	GH18_chitinase-like
LOC_Os08g40740	multi-dom	31	270	4.81E-07	pfam00704	Glyco_hydro_18
LOC_Os11g47500	specific	34	302	3.60E-117	cd02877	GH18_hevamine_XipI_class_III
LOC_Os11g47500	superfamily	34	302	3.60E-117	cl10447	GH18_chitinase-like
LOC_Os11g47500	multi-dom	34	249	1.12E-07	pfam00704	Glyco_hydro_18
LOC_Os11g47510	specific	43	309	7.04E-113	cd02877	GH18_hevamine_XipI_class_III
LOC_Os11g47510	superfamily	43	309	7.04E-113	cl10447	GH18_chitinase-like
LOC_Os11g47510	multi-dom	42	270	6.87E-09	pfam00704	Glyco_hydro_18
LOC_Os11g47520	specific	30	285	9.47E-106	cd02877	GH18_hevamine_XipI_class_III
LOC_Os11g47520	superfamily	30	285	9.47E-106	cl10447	GH18_chitinase-like
LOC_Os11g47520	multi-dom	30	189	2.75E-10	pfam00704	Glyco_hydro_18
LOC_Os11g47530	specific	32	288	5.43E-123	cd02877	GH18_hevamine_XipI_class_III
LOC_Os11g47530	superfamily	32	288	5.43E-123	cl10447	GH18_chitinase-like
LOC_Os11g47530	multi-dom	32	281	1.21E-14	pfam00704	Glyco_hydro_18
LOC_Os11g47550	specific	30	284	4.67E-112	cd02877	GH18_hevamine_XipI_class_III
LOC_Os11g47550	superfamily	30	284	4.67E-112	cl10447	GH18_chitinase-like
LOC_Os11g47550	multi-dom	35	209	3.72E-13	pfam00704	Glyco_hydro_18
LOC_Os11g47560	specific	31	281	4.03E-110	cd02877	GH18_hevamine_XipI_class_III

LOC_Os11g47560	superfamily	31	281	4.03E-110	cl10447	GH18_chitinase-like
LOC_Os11g47560	multi-dom	28	202	3.11E-14	pfam00704	Glyco_hydro_18
LOC_Os11g47570	specific	27	277	2.13E-105	cd02877	GH18_hevamine_XipI_class_III
LOC_Os11g47570	superfamily	27	277	2.13E-105	cl10447	GH18_chitinase-like
LOC_Os11g47570	multi-dom	27	197	2.81E-13	pfam00704	Glyco_hydro_18
LOC_Os11g47580	specific	34	302	1.06E-126	cd02877	GH18_hevamine_XipI_class_III
LOC_Os11g47580	superfamily	34	302	1.06E-126	cl10447	GH18_chitinase-like
LOC_Os11g47580	multi-dom	38	291	3.57E-13	pfam00704	Glyco_hydro_18
LOC_Os11g47590	specific	35	298	5.23E-120	cd02877	GH18_hevamine_XipI_class_III
LOC_Os11g47590	superfamily	35	298	5.23E-120	cl10447	GH18_chitinase-like
LOC_Os11g47590	multi-dom	41	277	7.63E-12	pfam00704	Glyco_hydro_18
LOC_Os11g47600	specific	32	299	5.32E-122	cd02877	GH18_hevamine_XipI_class_III
LOC_Os11g47600	superfamily	32	299	5.32E-122	cl10447	GH18_chitinase-like
LOC_Os11g47600	multi-dom	32	248	7.72E-12	pfam00704	Glyco_hydro_18
LOC_Os11g47610	specific	34	300	1.09E-119	cd02877	GH18_hevamine_XipI_class_III
LOC_Os11g47610	superfamily	34	300	1.09E-119	cl10447	GH18_chitinase-like
LOC_Os11g47610	multi-dom	40	289	6.75E-09	pfam00704	Glyco_hydro_18
Sb01g050530	specific	29	301	5.69E-136	cd02877	GH18_hevamine_XipI_class_III
Sb01g050530	superfamily	29	301	5.69E-136	cl10447	GH18_chitinase-like
Sb01g050530	multi-dom	29	288	3.16E-31	pfam00704	Glyco_hydro_18
Sb02g004650	specific	41	305	9.60E-105	cd02877	GH18_hevamine_XipI_class_III
Sb02g004650	superfamily	41	305	9.60E-105	cl10447	GH18_chitinase-like
Sb02g004650	multi-dom	41	294	1.39E-08	pfam00704	Glyco_hydro_18
Sb02g004660	specific	40	305	5.34E-111	cd02877	GH18_hevamine_XipI_class_III
Sb02g004660	superfamily	40	305	5.34E-111	cl10447	GH18_chitinase-like
Sb02g004660	multi-dom	40	303	6.17E-09	pfam00704	Glyco_hydro_18
Sb02g009360	specific	26	295	5.93E-139	cd02877	GH18_hevamine_XipI_class_III
Sb02g009360	superfamily	26	295	5.93E-139	cl10447	GH18_chitinase-like
Sb02g009360	multi-dom	26	278	6.11E-34	pfam00704	Glyco_hydro_18
Sb02g009370	specific	26	295	2.11E-139	cd02877	GH18_hevamine_XipI_class_III

Sb02g009370	superfamily	26	295	2.11E-139	cl10447	GH18_chitinase-like
Sb02g009370	multi-dom	26	278	5.31E-34	pfam00704	Glyco_hydro_18
Sb02g009380	specific	26	295	2.92E-138	cd02877	GH18_hevamine_XipI_class_III
Sb02g009380	superfamily	26	295	2.92E-138	cl10447	GH18_chitinase-like
Sb02g009380	multi-dom	26	278	2.63E-33	pfam00704	Glyco_hydro_18
Sb02g009390	specific	26	298	7.63E-137	cd02877	GH18_hevamine_XipI_class_III
Sb02g009390	superfamily	26	298	7.63E-137	cl10447	GH18_chitinase-like
Sb02g009390	multi-dom	26	248	6.33E-32	pfam00704	Glyco_hydro_18
Sb03g030100	specific	26	293	5.26E-138	cd02877	GH18_hevamine_XipI_class_III
Sb03g030100	superfamily	26	293	5.26E-138	cl10447	GH18_chitinase-like
Sb03g030100	multi-dom	26	295	3.71E-31	pfam00704	Glyco_hydro_18
Sb03g031710	specific	28	307	9.33E-130	cd02877	GH18_hevamine_XipI_class_III
Sb03g031710	superfamily	28	307	9.33E-130	cl10447	GH18_chitinase-like
Sb03g031710	multi-dom	27	298	2.56E-33	pfam00704	Glyco_hydro_18
Sb03g040560	specific	28	297	5.48E-132	cd02877	GH18_hevamine_XipI_class_III
Sb03g040560	superfamily	28	297	5.48E-132	cl10447	GH18_chitinase-like
Sb03g040560	multi-dom	28	287	9.39E-31	pfam00704	Glyco_hydro_18
Sb03g040570	specific	26	297	3.45E-126	cd02877	GH18_hevamine_XipI_class_III
Sb03g040570	superfamily	26	297	3.45E-126	cl10447	GH18_chitinase-like
Sb03g040570	multi-dom	26	292	1.07E-32	pfam00704	Glyco_hydro_18
Sb03g040580	specific	26	297	1.93E-125	cd02877	GH18_hevamine_XipI_class_III
Sb03g040580	superfamily	26	297	1.93E-125	cl10447	GH18_chitinase-like
Sb03g040580	multi-dom	26	292	7.42E-32	pfam00704	Glyco_hydro_18
Sb03g040590	specific	26	295	3.02E-128	cd02877	GH18_hevamine_XipI_class_III
Sb03g040590	superfamily	26	295	3.02E-128	cl10447	GH18_chitinase-like
Sb03g040590	multi-dom	26	284	1.07E-28	pfam00704	Glyco_hydro_18
Sb03g040600	specific	31	301	2.31E-133	cd02877	GH18_hevamine_XipI_class_III
Sb03g040600	superfamily	31	301	2.31E-133	cl10447	GH18_chitinase-like
Sb03g040600	multi-dom	31	290	6.37E-30	pfam00704	Glyco_hydro_18
Sb05g008530	specific	35	306	1.42E-114	cd02877	GH18_hevamine_XipI_class_III
Sb05g008530	superfamily	35	306	1.42E-114	cl10447	GH18_chitinase-like
Sb05g008530	multi-dom	36	213	1.91E-15	pfam00704	Glyco_hydro_18
Sb05g008550	specific	36	305	4.35E-115	cd02877	GH18_hevamine_XipI_class_III
Sb05g008550	superfamily	36	305	4.35E-115	cl10447	GH18_chitinase-like
Sb05g008550	multi-dom	42	217	2.55E-09	pfam00704	Glyco_hydro_18
Sb05g023690	superfamily	31	287	5.66E-93	cl10447	GH18_chitinase-like
Sb05g023690	multi-dom	33	216	1.96E-07	pfam00704	Glyco_hydro_18
Sb05g023700	specific	44	303	3.71E-102	cd02877	GH18_hevamine_XipI_class_III
Sb05g023700	superfamily	44	303	3.71E-102	cl10447	GH18_chitinase-like
Sb05g023710	specific	33	301	2.20E-99	cd02877	GH18_hevamine_XipI_class_III
Sb05g023710	superfamily	33	301	2.20E-99	cl10447	GH18_chitinase-like
Sb05g023710	multi-dom	35	293	6.74E-10	pfam00704	Glyco_hydro_18
Sb05g027380	specific	28	296	1.07E-104	cd02877	GH18_hevamine_XipI_class_III
Sb05g027380	superfamily	28	296	1.07E-104	cl10447	GH18_chitinase-like
Sb05g027380	multi-dom	28	285	1.13E-11	pfam00704	Glyco_hydro_18

Supplementary Table 2. Details of signal peptides and transmembrane domains in xylanase inhibitor proteins (XIP)

identified in four cereal species

Sequence Id	Phobos results			Signal_IP4_result			
	TM domain		Signal peptide	Signal start	Signal end	Score	Signal peptide
AC193632	1	YES	n9-20c25/26o307-330i	1	25	0.916	YES
Bradi2g45610	0	YES	n10-17c25/26o	1	25	0.854	YES
Bradi2g47070	1	YES	n8-19c24/25o331-354i	1	24	0.822	YES
Bradi2g55610	0	YES	n9-20c25/26o	1	25	0.943	YES
Bradi2g55620	0	YES	n9-20c25/26o	1	25	0.909	YES
Bradi2g55630	0	YES	n5-15c23/24o	1	20	0.821	YES
Bradi4g07560	0	YES	n7-17c25/26o	1	28	0.687	YES
Bradi4g09420	0	YES	n9-22c27/28o	1	27	0.776	YES
Bradi4g09430	0	YES	n12-27c32/33o	1	32	0.811	YES
Bradi4g09430	0	YES	n12-27c32/33o	1	32	0.811	YES
Bradi4g40110	0	YES	n8-19c23/24o	1	23	0.914	YES
Bradi4g40120	0	YES	n9-20c25/26o	1	24	0.865	YES
GRMZM2G023650	0	YES	n18-29c34/35o	1	24	0.865	YES
GRMZM2G080547	0	YES	n2-13c18/19o	1	25	0.814	YES
GRMZM2G130686	0	YES	n2-13c18/19o	1	18	0.859	YES
GRMZM2G133781	0	YES	n31-41c48/49o	1	18	0.859	YES
GRMZM2G160265	0	YES	n2-13c18/19o	1	18	0.87	YES
GRMZM2G162359	0	YES	n18-30c38/39o	1	38	0.794	YES
GRMZM2G328171	0	YES	n9-28c33/34o	1	33	0.725	YES
GRMZM2G400999	0	YES	n10-21c26/27o	1	26	0.744	YES
GRMZM2G430936	0	YES	n10-21c26/27o	1	26	0.831	YES
GRMZM2G430942	0	YES	n5-20c25/26o	1	25	0.872	YES
GRMZM2G447795	0	YES	n10-20c24/25o	1	29	0.874	YES
GRMZM2G453805	0	YES	n10-21c26/27o	1	26	0.906	YES
LOC_Os01g19750	1	0	o23-43i				No
LOC_Os01g47070	0	YES	n11-22c26/27o	1	26	0.843	YES
LOC_Os01g49320	0	YES	n11-22c27/28o	1	27	0.829	YES
LOC_Os01g64100	0	YES	n11-21c25/26o	1	25	0.84	YES
LOC_Os01g64110	0	YES	n13-24c29/30o	1	29	0.884	YES
LOC_Os04g27980	0	YES	n7-19c27/28o	1	28	0.682	YES
LOC_Os05g15770	0	YES	n6-14c19/20o	1	21	0.851	YES
LOC_Os05g15850	0	YES	n5-18c23/24o	1	23	0.884	YES
LOC_Os05g15880	0	YES	n6-16c21/22o	1	21	0.865	YES
LOC_Os05g15920	0	YES	n7-18c24/25o	1	24	0.945	YES
LOC_Os06g25010	0	YES	n8-23c28/29o	1	28	0.734	YES
LOC_Os07g19040	0	YES	n7-18c23/24o	1	25	0.738	YES
LOC_Os07g23850	0	0	i				No
LOC_Os07g43820	0	YES	n10-25c33/34o	1	33	0.885	YES
LOC_Os08g40680	0	YES	n8-19c29/30o	1	24	0.851	YES
LOC_Os08g40690	0	YES	n13-24c32/33o	1	32	0.776	YES
LOC_Os08g40740	0	YES	n8-19c27/28o	1	27	0.879	YES
LOC_Os11g47500	0	YES	n10-21c26/27o	1	26	0.925	YES
LOC_Os11g47510	0	YES	n9-27c32/33o	1	32	0.917	YES

LOC_Os11g47520	0	YES	n6-19c27/28o	1	27	0.901	YES
LOC_Os11g47530	0	YES	n8-23c28/29o	1	28	0.862	YES
LOC_Os11g47550	0	YES	n8-21c26/27o	1	26	0.859	YES
LOC_Os11g47560	0	YES	n5-16c21/22o	1	26	0.811	YES
LOC_Os11g47570	0	YES	n4-15c21/22o	1	21	0.895	YES
LOC_Os11g47580	0	YES	n13-24c29/30o	1	24	0.878	YES
LOC_Os11g47590	0	YES	n10-23c31/32o	1	31	0.888	YES
LOC_Os11g47600	0	YES	n6-21c25/26o	1	29	0.773	YES
LOC_Os11g47610	0	YES	n10-21c29/30o	1	29	0.76	YES
Sb01g050530	0	YES	n10-23c28/29o	1	28	0.899	YES
Sb02g004650	0	YES	n15-30c35/36o	1	35	0.809	YES
Sb02g004660	0	YES	n12-27c35/36o	1	35	0.764	YES
Sb02g009360	0	YES	n7-18c25/26o	1	18	0.87	YES
Sb02g009370	0	YES	n7-18c25/26o	1	18	0.894	YES
Sb02g009380	0	YES	n7-18c25/26o	1	18	0.865	YES
Sb02g009390	0	YES	n10-21c25/26o	1	25	0.833	YES
Sb03g030100	0	YES	n10-20c25/26o	1	25	0.837	YES
Sb03g031710	1	YES	n5-13c21/22o322-346i	1	22	0.76	YES
Sb03g040560	0	YES	n11-22c27/28o	1	27	0.91	YES
Sb03g040570	0	YES	n10-20c25/26o	1	25	0.909	YES
Sb03g040580	0	YES	n10-20c25/26o	1	25	0.909	YES
Sb03g040590	0	YES	n11-21c25/26o	1	25	0.908	YES
Sb03g040600	0	YES	n11-22c27/28o	1	27	0.769	YES
Sb05g008530	0	YES	n10-23c31/32o	1	31	0.774	YES
Sb05g008550	0	YES	n9-27c32/33o	1	32	0.913	YES
Sb05g023690	0	YES	n7-19c27/28o	1	25	0.865	YES
Sb05g023700	0	YES	n13-28c35/36o	1	35	0.777	YES
Sb05g023710	0	YES	n10-21c28/29o	1	28	0.868	YES
Sb05g027380	0	YES	n3-18c23/24o	1	23	0.854	YES

TM= Transmembrane