

Genome-Wide Analysis of HAESA/HAESA-Like Kinase Family in Rice

Chengliang Wang¹, Dong Li¹, Pei Wang¹, Defu Chen^{2*}, Xiwen Chen^{1#}

¹Department of Biochemistry and Molecular Biology, College of Life Sciences, Nankai University, Tianjin, China

²Department of Genetics and Cell Biology College of Life Sciences, Nankai University, Tianjin, China

Email: *chendefu@nankai.edu.cn, #xiwenchen@nankai.edu.cn

How to cite this paper: Wang, C.L., Li, D., Wang, P., Chen, D.F. and Chen, X.W. (2020) Genome-Wide Analysis of HAESA/HAESA-Like Kinase Family in Rice. *American Journal of Plant Sciences*, 11, 1254-1269. <https://doi.org/10.4236/ajps.2020.118089>

Received: June 24, 2020

Accepted: August 15, 2020

Published: August 18, 2020

Copyright © 2020 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

In leucine-rich repeat (LRR) receptor-like kinase XI subfamily, *Arabidopsis* HAESA (AtHAE) and two closely related HAESA-LIKE (AtHSL1 and AtHSL2) constitute a small branch. Several reports have described the function and the involved signaling pathway that AtHAE and AtHSLs are involved in. However, the family members and functions of HAE and HSL in rice have not been reported. Here, we performed a genome-wide analysis of the HAE/HSL kinase family in rice. A total of 17 OsHSLs were identified in the genome. Of these, only *Os11g11890* was annotated as HSL2; all the other members were annotated as HSL1. Phylogenetic analysis revealed that OsHSLs diverged into three groups, with three *Arabidopsis* members constituting a subgroup of group I. Domain analysis revealed that all the homologues had 9-19 LRR repeats and a typical kinase domain at the C-terminus, except that four members lost or evolved their kinase domains. Expression analysis revealed that OsHSLs were co-expressed with genes involved in biotic and abiotic stresses. Microarray data revealed that most OsHSLs were highly expressed in the vegetative tissues and only two members were highly expressed in the reproductive tissues. Most OsHSLs changed their expression profiles when subjected to drought, and salt stress treatments. Our results provide an overview of OsHSL gene family in rice, and suggest that OsHSLs possibly function under biotic and abiotic stresses, thus would help for elucidating the function of OsHSLs gene family *in vivo*.

Keywords

HAESA and HAESA-Like, Gene Family, Rice (*Oryza sativa* L.), *Arabidopsis thaliana*, Receptor-Like Kinase

1. Introduction

Physiological responses of plants are stimulated by a great deal of signals from

cell-cell and cell-environment interactions, thus leading to a series of signal transductions. These signal transductions are usually accomplished by reversible phosphorylation of protein kinases (PKs). As the substrates and functions of most PKs in plants have not been identified, plant PKs were temporarily named receptor-like kinases (RLKs) [1]. RLKs can be divided into several families according to their extracellular domains. The leucine-rich repeat (LRR) RLKs constitute the largest family of RLKs. Approximately 225 LRR-RLK genes, belonging to 23 subfamilies, have been identified in *Arabidopsis*. Sixty of them have been described the function [2]. The LRR domain is generally 20 - 30 amino acids long, and forms α/β helix. These structural characteristics allow LRR-RLKs to participate in protein-protein interactions, whereby modulating cellular functions such as signal transductions, stress response, and immunity [1].

In the LRR-RLK XI subfamily, *Arabidopsis thaliana* (*At*) HAESA (AtHAE) and two closely related HAESA-LIKE proteins—AtHSL1 and AtHSL2—constitute a small branch [3]. These three homologous proteins have a similar extracellular LRR domain and cytoplasmic Ser/Thr protein kinase domain [3]. A great number of reports have described the function and signaling pathways that AtHAE and AtHSL2 are involved in [3] [4] [5]. AtHAE is specifically expressed in abscission zones of the floral organs as well as at the base of the pedicels and petioles. Disruption of HAE results in delayed abscission of floral organs in *Arabidopsis* [4]. Single *hae* or *hsl2* mutant appears phenotypically normal, while the double mutant *hae/hsl2* has a complete loss of floral organ abscission [6]. *INFLORESCENCE-DEFICIENT IN ABSCISSION* (*IDA*) encodes a small secreted peptide that acts as the upstream ligand of HAE and HSL2. Plants with mutations in *IDA* present with similar phenotypes to *hae/hsl2* mutant [6]. *AGAMOUS-like 15* (*AGL15*), a MADS-domain transcription factor, positioned downstream of HAE, creates a positive feedback loop in transcriptional regulation of *HAE* via a mitogen-activated protein kinase (MAPK) MKK4/MKK5 manner [7]. Subtilisin-like proteinase (SBT) has also been reported to be involved in the IDA-HAE/HSL2 floral organ abscission signaling [8]. Furthermore, IDA-HAE/HSL2 pathway not only functions in developmentally regulated floral organ abscission, but also in drought-induced abscission of cauline leaves [5]. The IDA-HAE/HSL2 signaling is part of a genetic network regulated by auxin and regulates expression of the genes involved in cell wall remodeling during lateral root emergence [9]. Recently, HSL1 has been reported to act as a cognate receptor for CLAVATA3/ESR-RELATED 9/10 (CLE9/10) to regulate stomatal lineage cell division [10]. Genes encoding abscission signaling components of IDA or IDA-LIKE (IDL) peptides and its receptor HSL proteins were also identified in representative Angiosperm orders from an evolutionary prospective [11]. However, HAE and HSL homologues, and their functions in rice have not systematically been validated.

To further understand the functions of HAE and HSL kinases and the regulating networks, we performed a comprehensive genome-wide *in silico* analysis of the gene family in rice. Our results provide an overview of *HAE/HSL* family

in rice, facilitating further functional studies about this gene family.

2. Methods

2.1. Identification, Chromosomal Location, and Phylogenetic Tree Construction of HAE/HSL Kinase Gene Families

Homologous OsHSL proteins and gene sequences in rice were explored using *Arabidopsis HAE*, *HSL1* and *HSL2* with the BLAST suite program in three databases—GenBank at NCBI (<https://www.ncbi.nlm.nih.gov/gene>), Rice Data (<http://www.ricedata.cn/gene>), and Ensemble Plants (<http://plants.ensembl.org/index.html>) [12]—with an inclusion threshold of $E < 1e^{-30}$. Meanwhile, a phylogenetic tree of the rice LRR-RLK XI family was constructed, and the members that included in the HAEs or HSLs subgroup were also categorized as the homologous OsHSL proteins. The annotations and alternative splicing patterns of the corresponding genes were determined in Rice Data and Ensemble Plants. The exon-intron structures were analyzed using the Gene Structure Display Server. The exact chromosomal locations were determined by BLASTN search of the obtained sequences against Rice Data database and TAIR (The *Arabidopsis* Information Resource) database (<https://www.arabidopsis.org/>) and displayed using Map Chartver. 2.2 [13]. An unrooted phylogenetic tree of *AtHAE*, *AtHSLs*, and *OsHSLs* was built by the neighbor joining tree (NJ-tree) facility of this program with bootstrapping (1000 iterations) after manual adjustment of the alignment using the Genedoc program [14].

2.2. Domain Architecture and Protein Structure of HAE and HSL Kinase Family

The transmembrane helices of the proteins were predicted at TMHMM Server ver. 2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>). LRR domain was predicted using LRRfinder2.0 as described in Offord *et al.* [15]. Recommended values for upper and lower boundaries are 95% and 80%, respectively. The additional domains (outlier homologues, homologues of known structure), was identified by SMART (<http://smart.embl-heidelberg.de/>), which contains Swiss-Prot, SP-TrEMBL and stable Ensemble proteomes in Normal SMART. Signal peptides were identified by SIGNALP-5.0. To exhibit the structural divergence of *AtHAE*, *AtHSLs*, and *OsHSLs*, the conserved motifs in the encoded proteins were analyzed with the MEME online program ver. 4.9.0, and visualized with WebLogo [16].

2.3. Co-Expression and Expression Profile Analyses of HAE and HSL Kinases in Rice

The co-expression analysis data was accessed from RiceXPro (<http://ricefrend.dna.affrc.go.jp/>). Then RiceFREND was used to identify functionally related genes in various biological pathways to construct the correlated

gene network [17]. We selected two guide genes, then set “Hierarchy”, and finally clicked “Draw” to generate the network.

The spatio-temporal gene expression profile of rice during its entire development and growth stages, the gene expression profile in the leaves and seeds under various abiotic stress were derived from the microarray data integrated in RiceXPro and GEO databases.

3. Results

3.1. Identification of HAE/HSL Kinase Gene Family in Rice

To identify *HAE* and *HSL* kinase family in rice, the amino acid sequences of *Arabidopsis* HAE, HSL1 and HSL2 were used to perform independent TBLASTN searches through the above-mentioned databases. Thirteen possible *HSL1* and *HSL2* genes were identified in the rice genome. Next, we clustered the protein kinases in the genome into eleven groups. Altogether 17 possible *HSL* kinase homologues, including the above-mentioned thirteen sequences, were deemed as putative *OsHSLs* (Table 1). Of them, only *Os11g11890* had been annotated as *HSL2*, while all the other sequences had been annotated as *HSL1* (Table 1).

Most (14/17) of the rice sequences have one splicing pattern; while two sequences (*Os01g53920* and *Os01g65650*) have two patterns, and one (*Os06g36270*) has five. All three *Arabidopsis* sequences have only one splicing pattern (Table 1). The rice sequences have different number of introns in different alternative splicing isoforms. Twelve isoforms have one intron, three isoforms have two introns, one (*Os11g11890-HSL2*) has eleven introns, and one sequence (*Os01g53920*) does not have any intron (Table 1). Conversely, all three sequences in the *Arabidopsis* genome have only one intron (Table 1). The three *Arabidopsis* genes were distributed on chromosomes 1, 4 and 5 (Figure S1(A)). The 17 sequences localized to all the chromosomes in the rice genome, except for chromosomes 3, 4, 7 and 10, with chromosomes 2 and 11 containing most of the sequences (Figure S1(B)).

3.2. Phylogenetic Analysis of HAE/HSL Kinase Gene Family in Rice and *Arabidopsis*

To illustrate the evolutionary and functional relatedness of HAE/HSL gene families in rice and *Arabidopsis*, an unrooted phylogenetic tree was constructed with 17 rice and 3 *Arabidopsis* cDNA sequences. These sequences were found to diverge into three groups (Figure 1). Group I and II contain most of the rice sequences. Group III contains only one rice gene (*Os11g11890*), the only sequence that was annotated as *HSL2*. Interestingly, three *Arabidopsis* sequences (*AtHSL1*, *AtHSL2* and *AtHAE*) constitute a subgroup of group I, which also includes three rice genes. This observation indicates that *OsHSLs*, *AtHAE* and *AtHSLs* probably originated from common ancestral genes [18], and have some functional similarity.

3.3. Domain Architecture of HAE and HSL Kinase Family in Rice and Arabidopsis

Plant RLKs commonly consist of an extracellular LRR domain, transmembrane motif, and intracellular kinase domain. We firstly predicted the transmembrane helices of the HAE and HSL kinases in rice and *Arabidopsis*. Most OsHSLs have one transmembrane helix except that *Os02g13420*, *Os02g13460* and *Os11g12560* do not have any. Although one or more transmembrane helices were also identified at the N-terminal portion of some proteins, such as *Os09g15700* and *Os12g43660* (Figure S2), these helices are not valid as they are derived from signal peptides [19]. Next, the domain architectures of HAE and HSL kinases of rice and *Arabidopsis* were analyzed (Figure 2). Almost all the proteins have typical LRR-PK domain arrangement, with LRR domain residing at the N-terminus,

Table 1. Summary of the identified HAESA and HAESA-like kinase family in rice and *Arabidopsis*.

<i>RAP_Locus ID</i>	<i>MSU ID</i>	<i>Chr</i>	<i>Intron Exon no.</i>	<i>Splice no.</i>	<i>UniProt Entry</i>	<i>Other Designations</i>
AT1G28440		1	1/2	1	Q9SGP2	HAESA-like 1
AT4G28490		4	1/2	1	P47735	HAESA, RECEPTOR-LIKE PROTEIN KINASE 5, RLK5
AT5G65710		5	1/2	1	C0LGX3	HAESA-like 2
Os01g0239700	LOC_Os01g13800.1	1	1/2	1	Q9ARQ7	receptor-like protein kinase HSL1; putative LRR receptor-like kinase
Os01g0742400	LOC_Os01g53920.1	1	1/2 or 0/1	2		receptor-like protein kinase HSL1; putative LRK1 protein
Os01g0878300	LOC_Os01g65650.1	1	2/3 or 1/2	2	Q8L3T4	receptor-like protein kinase HSL1; putative LRR receptor-like kinase
None	LOC_Os02g12910.1	2	1/2	1		receptor-like protein kinase HSL1; putative CLAVATA1 receptor kinase
Os02g0227600	LOC_Os02g13420.1	2	1/2	1		receptor-like protein kinase HSL1
Os02g0227700	LOC_Os02g13430.1	2	1/2	1	Q6H5Y7	receptor-like protein kinase HSL1; putative LRR receptor-like kinase
Os02g0228000	LOC_Os02g13460.1	2	5/6	1		receptor protein kinase CLAVATA1-like
Os02g0228300	LOC_Os02g13510.1	2	1/2	1	Q6H5Y1	receptor-like protein kinase 5
Os05g0522600	LOC_Os05g44770.1	5	1/2	1	Q5W675	receptor-like protein kinase HSL1
Os06g0557100	LOC_Os06g36270.1	6	2/3, 3/4 or 1/2	5	Q5Z7H5	receptor-like protein kinase 5
Os06g0557700	LOC_Os06g36320.1	6	2/3	1	A3BCQ7	receptor-like protein kinase 5
Os08g0376300	LOC_Os08g28870.1	8	1/2	1	Q8GVW0	receptor-like protein kinase HSL1; putative LRR receptor-like kinase receptor-like protein kinase
Os09g0326100	LOC_Os09g15700.1	9	1/2	1	Q6K3W2	receptor-like protein kinase HSL1; CLV1 receptor kinase-like
Os11g0226201	LOC_Os11g11890.1	11	11/12	1	A0A0N7KSN2	LRR receptor-like serine/threonine-protein kinase HSL2
Os11g0232100	LOC_Os11g12530.1	11	2/3	1	Q53MD2	receptor-like protein kinase HSL1
Os11g0233000	LOC_Os11g12560.1	11	2/3	1	A0A0N7KSN8	receptor-like protein kinase HSL1
Os12g0632900	LOC_Os12g43660.1	12	1/2	1	Q2QLQ5	receptor-like protein kinase HSL1

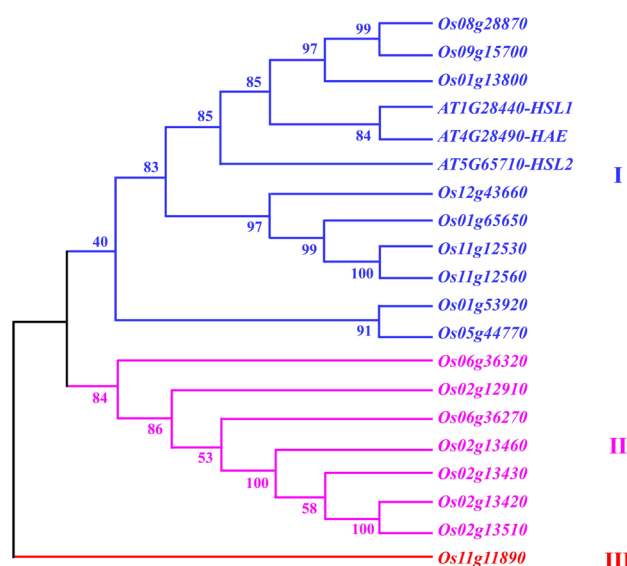


Figure 1. Unrooted neighbor-joining phylogenetic tree of *OsHSL*, *AtHAE* and *AtHSL* genes. The phylogenetic tree was generated by MEGA 6 software. Rice MSU IDs and *Arabidopsis* TAIR IDs are shown on the right side. Groups I, II and III are in blue, pink and red, respectively.

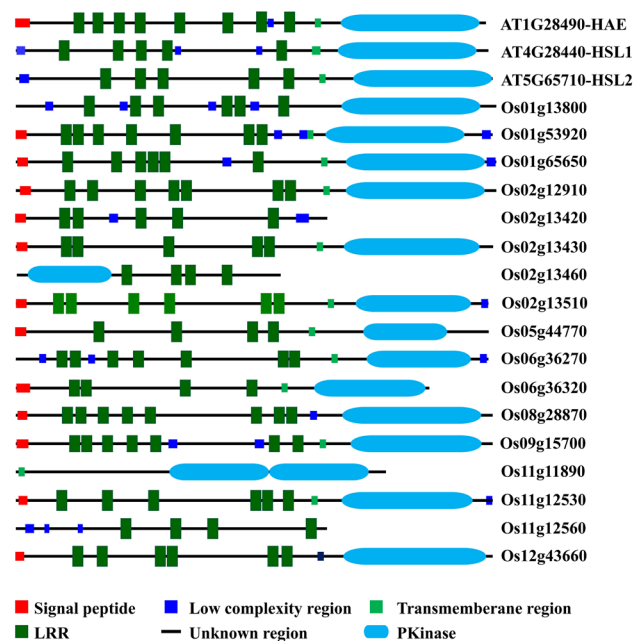


Figure 2. Diagram of the domain architectures of HAE and HSL kinases identified in rice and *Arabidopsis*. Red rectangle: signal peptide; Light green rectangle: transmembrane region; Dark green rectangle: LRR; Blue Oval: kinase domain. *Arabidopsis* TAIR IDs and Rice MSU IDs are shown on the right side of each diagram.

and the kinase domain at the C-terminus. Furthermore, while *Os02g13420* and *Os11g12560* seem to have lost their kinase domains during evolution, *Os02g13460*'s kinase domain is located at the N-terminus. Additionally, *Os11g11890* (HSL2) does not have the LRR domain. All the other HAE and HSL kinases have 9-19 LRR repeats, with most members containing 17 repeats. As for AtHAE and

AtHSLs, they show all the typical structural properties, such as domain type and arrangement.

3.4. Co-Expression Network of OsHSL Kinase Genes

To assess the function of OsHSL kinases, we performed co-expression analysis of 17 *OsHSL* genes. Ten genes were found to co-express with other genes (Figure 3). To assess the possible functions of OsHSL kinases, we examined the genes that have been reported to be co-expressed.

Os01g52480 is co-expressed with *Os12g43660*, whose homologous gene—*At4g35985*—encodes a senescence/dehydration-associated protein, and is responsive to various abiotic stresses [20]. *Os03g27390*, co-expressed with *Os08g28870*, encodes a helix-loop-helix DNA-binding protein, and is likely involved in the regulation of *OsChia4a* expression in a JA-dependent manner [21]. *Os04g45070*, co-expressed with *Os11g12530*, encodes a Remorin (originally named as pp34) and act as signaling scaffolds during development or cellular defense [22].

Os05g45410, co-expressed with *Os05g44770*, encodes for heat stress transcription factor sp17, whose disruption causes a lesion-mimic phenotype [23]. *Os01g14440* is also co-expressed with *Os05g44770*, and encodes a WRKY transcription factor, and functions in the activation of a defense response against the rice sheath blight fungus and blast fungi [24]. *Os07g23710*, another gene that co-expressed with *Os05g44770*, encodes for a cytochrome p450, which is required for anther cutin biosynthesis, and pollen exine formation in rice [25]. Three genes (*Os01g23380*, *Os12g33160*, and *Os01g15580*) are co-expressed with *Os01g13800*, and they all are NB-ARC domain containing genes, and function in resistance against phytopathogenic fungi and bacteria [26].

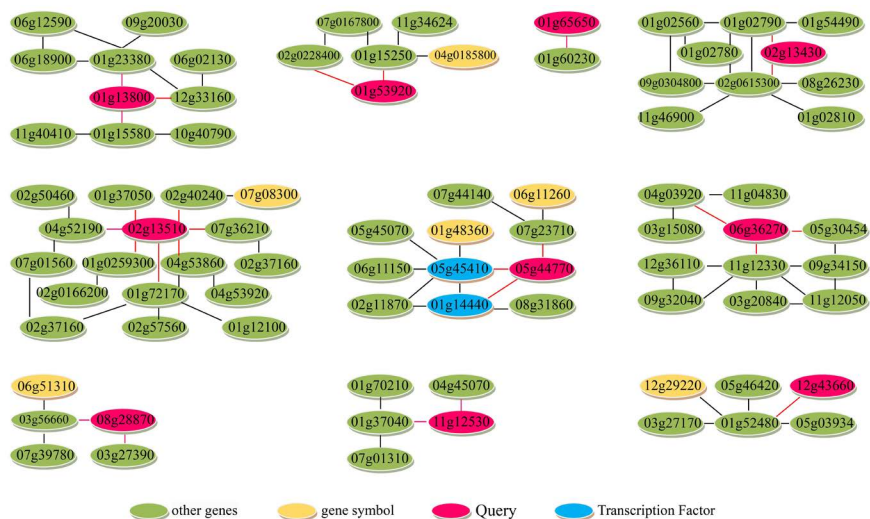


Figure 3. Co-expressed gene networks of *OsHSL* genes. Peach red, canary yellow, blue, and green circle represent the queried *OsHSL* genes, gene symbols, transcription factors, and other genes, respectively. Red line segments indicate interactions of the target protein with other proteins, and black line segments indicate possible interactions with other proteins, which may be linked to the target protein. Because the MSU IDs of several genes could not be found, they are shown only by their RAP IDs.

Seven genes (*Os01g72170*, *Os01g37050*, *Os02g40240*, *Os04g52190*, *Os01g0259500*, *Os04g53860*, *Os07g36210*) are co-expressed with *Os02g13510*, suggesting that *Os02g13510* may be involved in multiple cellular responses. Among them, *Os01g72170* is a tau class glutathione *S*-transferase and confer plants an increased tolerance to salinity and oxidative stresses [27]. *Os01g37050* is a ribonuclease III domain protein, which is involved in protein synthesis, RNA interference [28]. Two genes are co-expressed with *Os06g36270*, such as *Os11g12330* encodes a nucleotide-binding site leucine-rich repeat (NBS-LRR) protein, which recognizes specific pathogen-derived products and activates disease resistance signaling pathways [29]. And *Os04g03920* encodes a thioredoxin-like domain containing protein. These results indicate that most *OsHSLs* may function in biotic and abiotic stresses in rice.

3.5. Expression Patterns of HAE and HSL Genes in Rice

The temporal and spatial expression profiles of *HAE* and *HSL* genes in rice were investigated (Figure 4). Most *OsHSLs* showed high expression in the leaf blade, leaf sheath, and root throughout the developmental stages, with *Os02g13510* showing the highest expression. *Os08g28870* was mainly expressed in the stem, inflorescence, pistil, lemma, and palea, with the anther (1.6 - 2.0 mm) expressing the highest. *Os09g15700* also showed high expression in the reproductive tissues, such as ovary at five days after flowering (DAF) and in the embryo stage at seven and ten DAF, with anther (0.7 - 1.0 mm) expressing the highest. Conversely, *Os01g65650*, *Os01g53920*, and *Os02g13430* showed low expression in almost all the tissues.

To assess if *OsHSL* kinase genes are related to abiotic stresses, we investigated the induction patterns of *OsHSLs* in leaves and seeds under various stresses (Figure 5(A)). Nine genes (*Os01g13800*, *Os01g53920*, *Os02g12910*, *Os02g13430*, *Os05g44770*, *Os06g36270*, *Os06g36320*, *Os11g12530* and *Os12g43660*) were upregulated and three genes (*Os02g13510*, *Os08g28870*, and *Os09g15700*) were downregulated when subjected to salt stress. When under cold stress, most of the genes were downregulated or showed low expression. In the seed, most *OsHSLs* were downregulated by cold at 4 DAF, with *Os09g15700* showing the most downregulation. Six genes (*Os01g13800*, *Os02g13430*, *Os06g36270*, *Os06g36320*, *Os08g28870*, and *Os11g12530*) were upregulated under cold stress at 10 DAF (Figure 5(B)).

4. Discussion

With the completion of the rice genome sequencing project, we have already begun to pay close attention to sequence data mining and interpretation. In this study, we performed a comprehensive genome-wide analysis of *HAE/HSL* kinase family in rice. Seventeen *OsHSL* genes were identified in the rice genome. Interestingly, only one of them (*Os11g11890*) was annotated as *HSL2*; all the other sequences were annotated as *HSL1*. None of the sequences was annotated

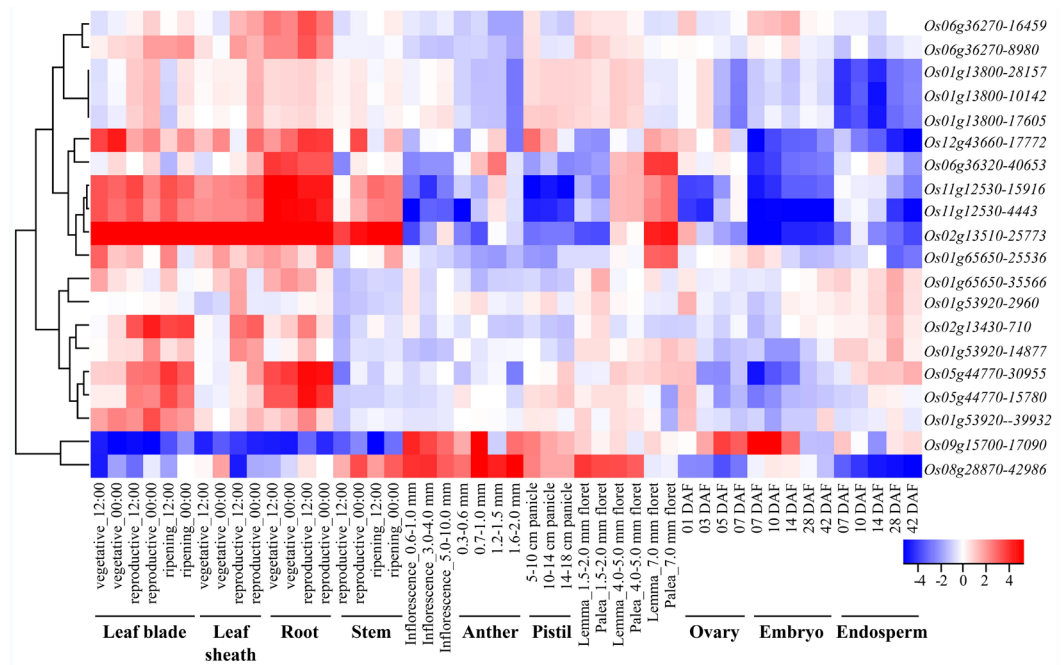


Figure 4. Tissue-specific expression patterns of *OsHSLs* genes. The expression profiles of *OsHSL* genes in various rice tissues throughout entire growth stages in the field. The numbers that follow the NSU IDs represent the feature numbers, which have been targeted by the corresponding probe. DAF: days after flowering. Data were obtained from the microarray analyses integrated in RiceXPro and normalized based on the mean expression value of each gene in all tissues analyzed. Expression values were again normalized by logarithm with the base of 2 using the R software. Red and blue boxes indicate high and low expression levels for each gene, respectively.

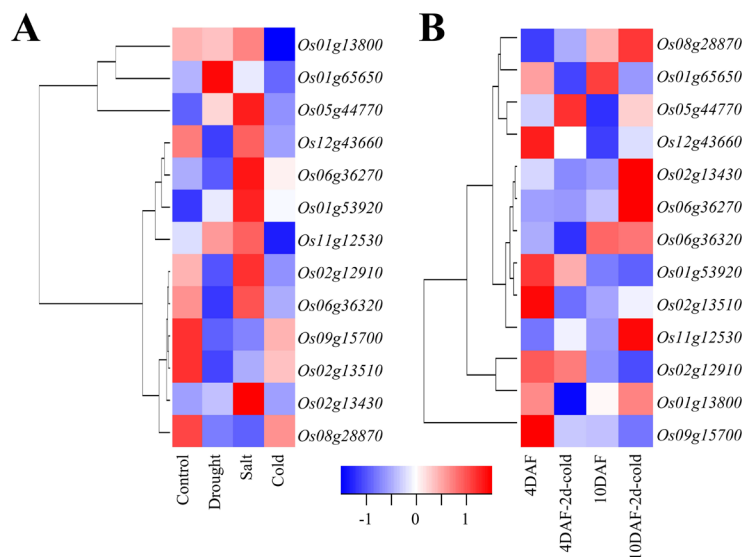


Figure 5. The expression profiles of *OsHSLs* in response to abiotic stress. Relative expression profiles of *OsHSL* genes in leaf (A) and seed (B) in response to drought, salt, and cold are shown as heatmaps. Data were obtained from the microarray analyses integrated in RiceXPro and normalized based on the mean expression value of each gene in all tissues analyzed. Expression values were again normalized by logarithm with the base of two using the R software. Red and blue boxes indicate high and low expression levels for each gene, respectively.

as *HAE* (**Table 1**). Phylogenetic analysis showed that *OsHSLs*, *AtHAE* and *AtHSLs* diverged into three groups, with three *Arabidopsis* sequences constituting a subgroup of group I alongside three rice sequences. Therefore, these homologous genes most likely originated from common ancestral genes and have some functional similarity. Interestingly, several rice sequences that are located on the same chromosomes are clustered together in the phylogenetic tree (*Os02g12910*, *Os02g13460*, *Os02g13430*, *Os02g13420*, *Os02g13510*, and *Os06g36320*, *Os06g36270*), suggesting that these paralogues were generated via intra-chromosomal duplication events during evolution [30].

Protein sequences and motifs are directly related to protein function. The LRR domain is specifically responsible for binding to signaling molecules to execute signal transduction [31], and promotes receptor-co-receptor complex formation [32], while the kinase domain phosphorylates the OH group of serine or threonine residues of the target protein. Analysis of the conservation and variation in these two domains may suggest functional similarity and divergence in rice and *Arabidopsis* *HAE/HSL* families. In this study, we found that *Os11g11890* (*HSL2*) lost the LRR domain during evolution (**Figure 1**), which may function as cytoplasmic kinase [33]. However, all the other rice HSL kinases have 8 - 18 LRR repeats, with most members containing 17 repeats. These observations indicate the degree of conservation and divergence with respect to ligand perception in the kinase family. Interesting, the LRR repeats of *AtHAESA* (21) predicted by LRRfinder is less than that observed by crystal structure analysis [32]. The discrepancy between prediction and crystal structure in Mammalian TLR2 was also reported previously [16]. Similarly, *Os02g13420* and *Os11g12560* lost their kinase domains, while *Os02g13460*'s kinase domain shifted to the N-terminus, and *Os11g11890*'s kinase domain duplicated, indicating the degree of functional diversification in these kinases.

Co-expression networks of genes usually reflect the correlation between the expression profiles of different genes and can be suggestive in predicting the gene functions. In this study, we first performed co-expression analysis to predict the function of *OsHSL* kinases. The results indicate that a large number of the identified genes that are co-expressed participate in multiple biochemical processes, such as biotic and abiotic stresses (**Figure 3**), suggesting a function of *OsHSL* gene family in stress responses. Furthermore, the temporal and spatial expression analysis revealed that most *OsHSLs* exhibited higher expression in the vegetative tissues (such as leaf blade, leaf sheath, and root) than the reproductive tissues, suggesting they function in the growth of rice plant. In contrast, *Os08g28870* and *Os09g15700* showed higher expression in the reproductive tissues (**Figure 4**), suggesting they function during the development. Importantly, most *OsHSLs* changed their expression profiles when subjected to drought and salt stresses (**Figure 5(A)**, **Figure 5(B)**). As gene expression profiles are somewhat suggestive of physiological processes governed by the genes, these data again suggest that *OsHSLs* possibly function in biotic stress responses. Until

now, the involvement of *AtHAE* and *AtHSL2* in abscission of floral organs has been reported. In addition, *AtHAE* and *AtHSL2* were also involved in mediating stress induced cauline leaf abscission in *Arabidopsis* under drought stress [5] [34]. No report about the function of *OsHSLs* exists. For instance, the functions of *OsHSLs* in abiotic stresses have not been reported previously. Thus our data provide one of the first clues about the functions of the *OsHSL* gene family.

5. Conclusion

In this study, we performed a comprehensive genome-wide analysis of HAE/HSL kinase families in rice. Seventeen *OsHSLs* were identified in the rice genome. Of them, only *Os11g11890* had been annotated as *HSL2*, all the other sequences had been annotated as *HSL1*. Phylogenetic analysis suggested that *OsHSLs*, *AtHAE*, and *AtHSLs* originated from common ancestral genes have some functional similarity. Domain analysis showed that four members lost or evolved their kinase domains, suggesting functional divergence occurred in these kinases. Co-expression analyses and gene expression patterns suggested a possible function of *OsHSLs* and *AtHSL1* in biotic stresses. These results provide a wealth of information to investigators for characterization of the physiological functions and signal transduction pathways governed by *OsHSLs*.

Acknowledgements

This work was supported by the funding of the Key Program of the Natural Science Foundation of Tianjin (No. 18JCZDJC33700). This work was also supported by the funding of National Natural Science Foundation of China (No. 31870304) and the funding of the Project for Tianjin Rice Industry Technology System Innovation Team (ITTRRS2018005).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Sun, J., Li, L., Wang, P., *et al.* (2017) Genome-Wide Characterization, Evolution, and Expression Analysis of the Leucine-Rich Repeat Receptor-Like Protein Kinase (LRR-RLK) Gene Family in Rosaceae Genomes. *BMC Genomics*, **18**, 763. <https://doi.org/10.1186/s12864-017-4155-y>
- [2] Shi, T., Huang, H., Sanderson, M.J., *et al.* (2014) Evolutionary Dynamics of Leucine-Rich Repeat Receptor-Like Kinases and Related Genes in Plants: A Phylogenomic Approach. *Journal of Integrative Plant Biology*, **56**, 648-662. <https://doi.org/10.1111/jipb.12188>
- [3] Shiu, S.H. and Bleecker, A.B. (2001) Receptor-Like Kinases from *Arabidopsis* Form a Monophyletic Gene Family Related to Animal Receptor Kinases. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 10763-10768. <https://doi.org/10.1073/pnas.181141598>
- [4] Jinn, T.L., Stone, J.M. and Walker, J.C. (2000) *HAESA*, an *Arabidopsis* Leu-

- cine-Rich Repeat Receptor Kinase, Controls Floral Organ Abscission. *Genes & Development*, **14**, 108-117.
- [5] Patharkar, O.R. and Walker, J.C. (2016) Core Mechanisms Regulating Developmentally Timed and Environmentally Triggered Abscission. *Plant Physiologist*, **172**, 510-520. <https://doi.org/10.1104/pp.16.01004>
- [6] Cho, S.K., Larue, C.T., Chevalier, D., *et al.* (2008) Regulation of Floral Organ Abscission in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 15629-15634. <https://doi.org/10.1073/pnas.0805539105>
- [7] Patharkar, O.R. and Walker, J.C. (2015) Floral Organ Abscission Is Regulated by a Positive Feedback Loop. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 2906-2911. <https://doi.org/10.1073/pnas.1423595112>
- [8] Schardon, K., Hohl, M., Graff, L., *et al.* (2016) Precursor Processing for Plant Peptide Hormone Maturation by Subtilisin-Like Serine Proteinases. *Science*, **354**, 1594-1597. <https://doi.org/10.1126/science.aai8550>
- [9] Kumpf, R.P., Shi, C.L., Larrieu, A., *et al.* (2013) Floral Organ Abscission Peptide IDA and Its HAE/HSL2 Receptors Control Cell Separation during Lateral Root Emergence. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 5235-5240. <https://doi.org/10.1073/pnas.1210835110>
- [10] Qian, P., Song, W., Yokoo, T., *et al.* (2018) The CLE9/10 Secretory Peptide Regulates Stomatal and Vascular Development through Distinct Receptors. *Nature Plants*, **4**, 1071-1081. <https://doi.org/10.1038/s41477-018-0317-4>
- [11] Stø, I.M., Orr, R.J.S., Kim, F., *et al.* (2015) Conservation of the Abscission Signaling Peptide IDA during Angiosperm Evolution: Withstanding Genome Duplications and Gain and Loss of the Receptors HAE/HSL2. *Frontiers in Plant Science*, **6**, 931. <https://doi.org/10.3389/fpls.2015.00931>
- [12] Doxey, A.C., Yaish, M.W., Moffatt, B.A., *et al.* (2007) Functional Divergence in the *Arabidopsis* β -1,3-glucanase Gene Family Inferred by Phylogenetic Reconstruction of Expression States. *Molecular Biology and Evolution*, **24**, 1045-1055. <https://doi.org/10.1093/molbev/msm024>
- [13] Kavas, M., Baloğlu, M.C., Atabay, E.S., *et al.* (2016) Genome-Wide Characterization and Expression Analysis of Common Bean bHLH Transcription Factors in Response to Excess Salt Concentration. *Molecular Genetics and Genomics*, **291**, 129-143. <https://doi.org/10.1007/s00438-015-1095-6>
- [14] Saitou, N. and Nei, M. (1987) The Neighbor-Joining Method: A New Method for Reconstructing Phylogenetic Trees. *Molecular Biology & Evolution*, **4**, 406-425.
- [15] Offord, V. and Werling, D. (2013) LRRfinder2.0: A Webserver for the Prediction of Leucine-Rich Repeats. *Innate Immunity*, **19**, 398-402. <https://doi.org/10.1177/1753425912465661>
- [16] Wang, X.S., Zhu, H.B., Jin, G.L., *et al.* (2007) Genome-Scale Identification and Analysis of LEA Genes in Rice (*Oryza sativa* L.). *Plant Science*, **172**, 414-420. <https://doi.org/10.1016/j.plantsci.2006.10.004>
- [17] Fenart, S., Ndong, Y.P., Duarte, J., *et al.* (2010) Development and Validation of a Flax (*Linum usitatissimum* L.) Gene Expression Oligo Microarray. *BMC Genomics*, **11**, e592. <https://doi.org/10.1186/1471-2164-11-592>
- [18] Jain, M., Nijhawan, A., Arora, R., *et al.* (2007) F-Box Proteins in Rice. Genome-Wide Analysis, Classification, Temporal and Spatial Gene Expression during Panicle and Seed Development, and Regulation by Light and Abiotic Stress. *Plant Physiologist*, **143**, 1467-1483. <https://doi.org/10.1104/pp.106.091900>

- [19] Krogh, A., Larsson, B., von Heijne, G., *et al.* (2001) Predicting Transmembrane Protein Topology with a Hidden Markov Model: Application to Complete Genomes. *Journal of Molecular Biology*, **305**, 567-580. <https://doi.org/10.1006/jmbi.2000.4315>
- [20] Banerjee, J., Sahoo, D.K., Dey, N., *et al.* (2013) An Intergenic Region Shared by At4g35985 and At4g35987 in *Arabidopsis thaliana* Is a Tissue Specific and Stress Inducible Bidirectional Promoter Analyzed in Transgenic Arabidopsis and Tobacco Plants. *PLoS ONE*, **8**, e79622. <https://doi.org/10.1371/journal.pone.0079622>
- [21] Miyamoto, K., Shimizu, T., Lin, F., *et al.* (2012) Identification of an E-Box Motif Responsible for the Expression of Jasmonic Acid-Induced Chitinase Gene *OsChia4a* in Rice. *Journal Plant Physiology*, **169**, 621-627. <https://doi.org/10.1016/j.jplph.2011.12.008>
- [22] Bariola, P.A., Retelska, D., Stasiak, A., *et al.* (2004) Remorins Form a Novel Family of Coiled Coil-Forming Oligomeric and Filamentous Proteins Associated with Apical, Vascular and Embryonic Tissues in Plants. *Plant Molecular Biology*, **55**, 579-594. <https://doi.org/10.1007/s11103-004-1520-4>
- [23] Yamanouchi, U., Yano, M., Lin, H., *et al.* (2002) A Rice Spotted Leaf Gene, *Spl7*, Encodes a Heat Stress Transcription Factor Protein. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 7530-7535. <https://doi.org/10.1073/pnas.112209199>
- [24] Peng, X., Hu, Y., Tang, X., *et al.* (2012) Constitutive Expression of Rice *WRKY30* Gene Increases the Endogenous Jasmonic Acid Accumulation, *PR* Gene Expression and Resistance to Fungal Pathogens in Rice. *Planta*, **236**, 1485-1498. <https://doi.org/10.1007/s00425-012-1698-7>
- [25] Li, H., Pinot, F., Sauveplane, V., Werck-Reichhart, D., *et al.* (2010) Cytochrome P450 Family Member CYP704B2 Catalyzes the ω -Hydroxylation of Fatty Acids and Is Required for Anther Cutin Biosynthesis and Pollen Exine Formation in Rice. *The Plant Cell*, **22**, 173-190. <https://doi.org/10.1105/tpc.109.070326>
- [26] Byeon, Y., Park, S., Kim, Y.S., *et al.* (2013) Microarray Analysis of Genes Differentially Expressed in Melatonin-Rich Transgenic Rice Expressing a Sheep Serotonin *N*-Acetyltransferase. *Journal of Pineal Research*, **55**, 357-363. <https://doi.org/10.1111/jpi.12077>
- [27] Sharma, R., Sahoo, A., Devendran, R., *et al.* (2014) Over-Expression of a Rice Tau Class Glutathione S-transferase Gene Improves Tolerance to Salinity and Oxidative Stresses in *Arabidopsis*. *PLoS ONE*, **9**, e92900. <https://doi.org/10.1371/journal.pone.0092900>
- [28] MacRae, I.J. and Doudna, J.A. (2007) Ribonuclease Revisited: Structural Insights into Ribonuclease III Family Enzymes. *Current Opinion in Structural Biology*, **17**, 138-145. <https://doi.org/10.1016/j.sbi.2006.12.002>
- [29] Moffett, P., Farnham, G., Peart, J., *et al.* (2002) Interaction between Domains of a Plant NBS-LRR Protein in Disease Resistance-Related Cell Death. *EMBO Journal*, **21**, 4511-4519. <https://doi.org/10.1093/emboj/cdf453>
- [30] Opassiri, R., Pomthong, B., Onkoksoong, T., *et al.* (2006) Analysis of Rice Glycosyl Hydrolase Family 1 and Expression of Os4bglu12 β -glucosidase. *BMC Plant Biology*, **6**, 33. <https://doi.org/10.1186/1471-2229-6-33>
- [31] Hunter, T. (1995) Protein Kinases and Phosphatases: The Yin and Yang of Protein Phosphorylation and Signaling. *Cell*, **80**, 225-236. [https://doi.org/10.1016/0092-8674\(95\)90405-0](https://doi.org/10.1016/0092-8674(95)90405-0)
- [32] Santiago, J., Brandt, B., Wildhagen, M., *et al.* (2016) Mechanistic Insight into a Pep-

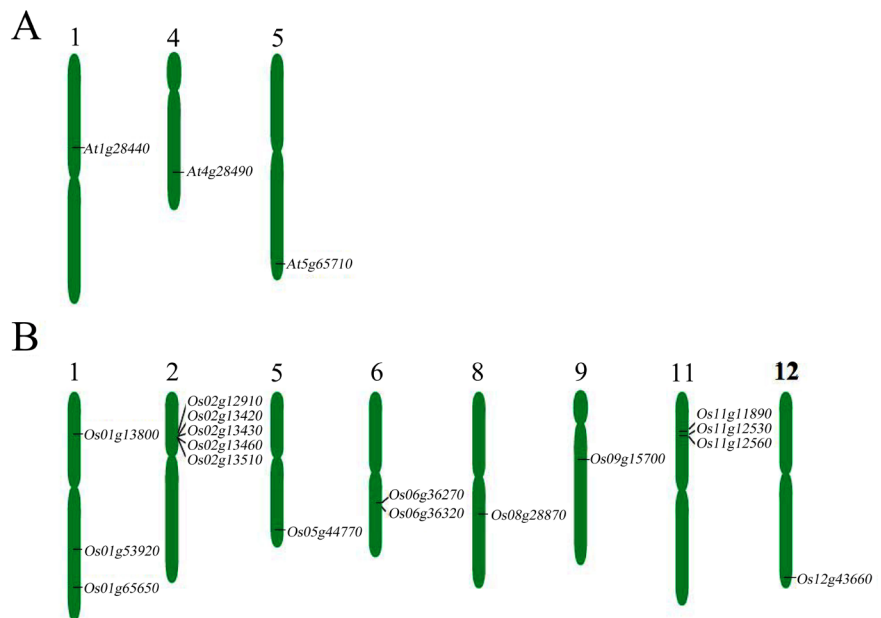
tide Hormone Signaling Complex Mediating Floral Organ Abscission. *eLife*, **5**, e1057. <https://doi.org/10.7554/eLife.15075>

- [33] Matsushima, N., Tanaka, T., Enkhbayar, P., *et al.* (2007) Comparative Sequence Analysis of Leucine-Rich Repeats (LRRs) within Vertebrate Toll-Like Receptors. *BMC Genomics*, **8**, 124. <https://doi.org/10.1186/1471-2164-8-124>
- [34] Patharkar, O.R., Walter, G., Walker, J.C., *et al.* (2017) Leaf Shedding as an Anti-Bacterial Defense in Arabidopsis Cauline Leaves. *PLoS Genetics*, **13**, e1007132. <https://doi.org/10.1371/journal.pgen.1007132>

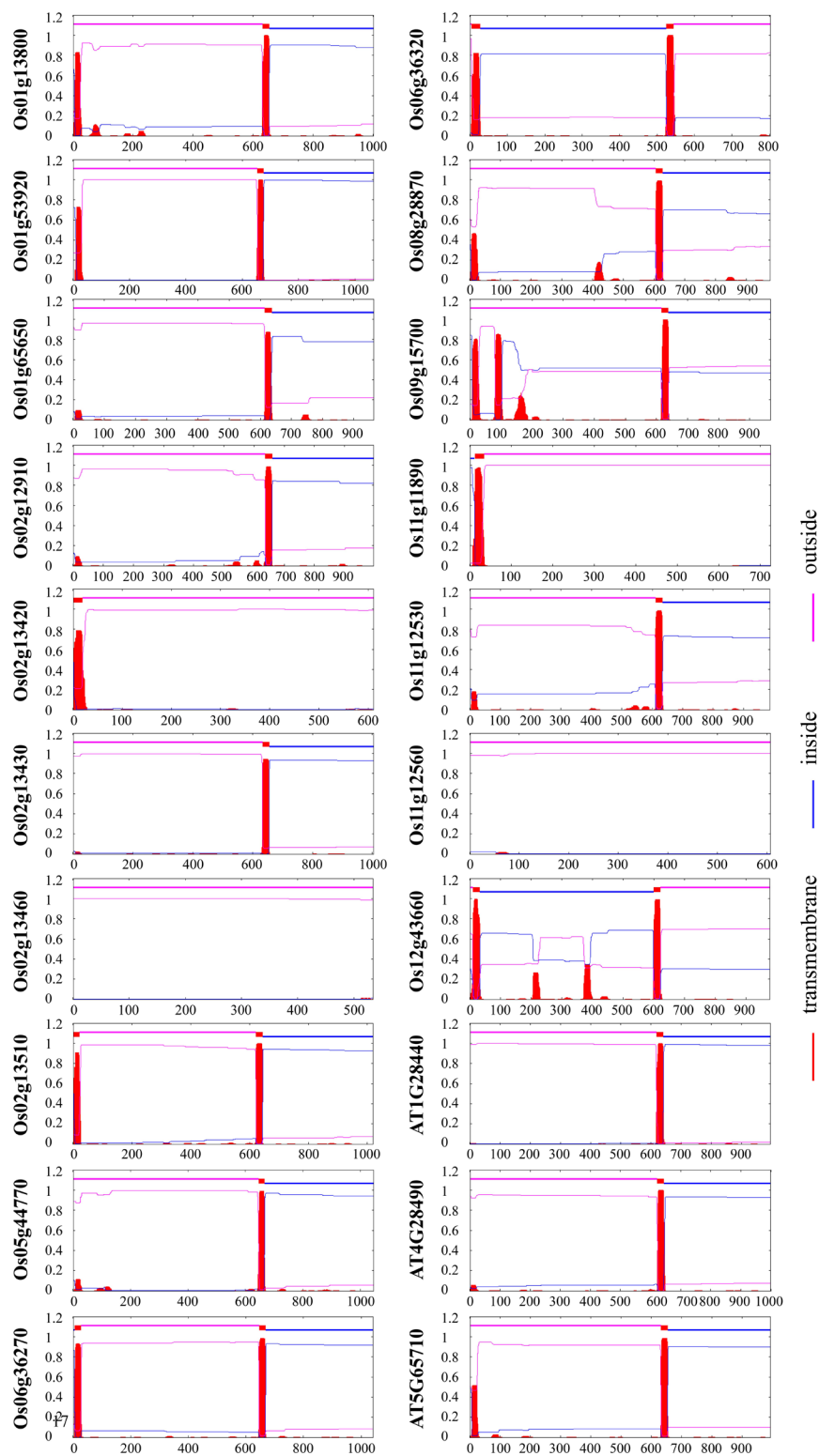
Abbreviations

AGL, AGAMOUS-like; CLE, CLAVATA3/ESR-RELATED; DAF, days after flowering; HAE, HAESA; HSL, HAESA LIKE; IDA, INFLORESCENCE-DEFICIENT IN ABCISSION; LRR, leucine-rich repeat; MAPK, mitogen-activated protein kinase; PKs, protein kinases; RLK, receptor-like kinases; SBT, subtilisin-like protease; TAIR, The *Arabidopsis* Information Resource.

Supplementary Material



Supplementary Figure S1. Distribution of OsHSL, AtHAE, and AtHSL genes on rice and Arabidopsis chromosomes. (A) *AtHAE* and *AtHSL* genes are located on 3 of the 5 chromosomes in *Arabidopsis* genome. (B) *OsHSL* genes are located on 8 of the 12 chromosomes in rice genome. Chromosomes that do not contain any of the genes are not shown. The constriction on the chromosomes (vertical bars) indicates the position of the centromere. *Arabidopsis* TAIR IDs and rice MSU IDs are shown on the chromosomes.



Supplementary Figure S2. Trans-membrane helix (THM) prediction of HAE and HSLs kinase families in rice and Arabidopsis. Amino acids corresponding to red and blue filaments indicate the location in or out of cytomembrane and ones below red grid are considered at trans-membrane region.