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Analysis of GDSL lipase (GLIP) family genes in rice (*Oryza sativa*)

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Abstract

Lipase is one of the lipid hydrolyzing enzymes, distributed broadly throughout plants, animal and microorganisms. GDSL-lipases is one of the lipases that exhibit a GDSL motif GxSxxxxG, in which the active site Serine is located near the N-terminus and display a Gly-Asp-Ser-(Leu) [GDS(L)] motif in conserved block I. However, the knowledge about their roles in developmental processes and response to various stimuli are still very limited in rice. A systematic analysis revealed the presence of at least 113 GDSL lipase (GLIP) genes in the rice genome. The tandem gene duplications have contributed a major role in expansion of this gene family. Phylogenetic analysis classed proteins into three groups; OsGLIP group B contained 56 genes, 50 in group A and only 2 genes in group C. The organization of putative motifs indicated potential diverse functions of GLIP gene family members in rice. Microarray data analysis revealed tissue and developmental stage-specific expression patterns of several OsGLIP genes. 38 OsGLIP genes were especially expressed in stigma and seed germination, several genes expressed exclusively in root and 17 OsGLIP genes were induced by any of three stresses. Our analysis also suggests differential accumulation of cluster genes during these processes. Our analyses indicated OsGLIP genes may have potential roles in rice development and abiotic stresses.

Keywords: GLIP, *Oryza sativa*, chromosomal localization, phylogenetic analysis, expression patterns, microarray.
Abbreviations: MeJA - methyl jasmonic acid SA -salicylic acid ABA-abscisic acid

Introduction

Lipase is one of the lipid hydrolyzing enzymes, distributed broadly throughout plants, animals, and microorganisms. Three highly conserved amino acid residues (Ser, Asp, and His), which form the catalytic triad, are present in lipase family members (Arpigny and Jaeger, 1999; Hong et al., 2008). A pentapeptide consensus motif (Gly-X-Ser-X-Gly) is found in members of the lipolytic enzyme family, which includes lipases and esterases. Lipases preferentially hydrolyze long-chain acylglycerols (10 carbon atoms at least), whereas esterases are specific for short-chain acylglycerols (10 carbon atoms at most) (Ling et al., 2006). GDSL lipases represent a subfamily of lipolytic enzymes, which like other members of the lipase and esterase families, possess a conserved catalytic triad (Akoh et al., 2004; Lee and Cho, 2003). Most lipases contain a GxSxG motif, but GDSL lipases exhibit a GDSL motif GxSxxxxG, in which the active site Ser is located near the N-terminus and displays a Gly-Asp-Ser-(Leu) [GDS(L)] motif in conserved block I (Brick et al., 1995; Lee et al., 2009; Updegraff et al., 2009; Upton and Buckley, 1995).

All of GDSL enzymes have a consensus sequence that is divided into five conserved sequence blocks (I–V). The GDSL family is further classified based on the strict conservation of the catalytic residues S, G, N, and H within conserved blocks I, II, III, and V, respectively (Dalrymple et al., 1997; Ling et al., 2006; Mølgaard et al., 2000; Lo et al., 2003). GDSL-lipases are involved principally in the regulation of plant development, morphogenesis, *Enod8* is a member of the GDSL lipase/esterase family that is isolated from *Medicago sativa* root nodules (Pringle and Dickstein, 2004). *BnLIP2* (*Brassica napus*.L) encodes a GDSL lipase which is induced during germination and maintained in mature seedlings (Ling et al., 2006). GDSL lipases also perform critical roles in the biotic and abiotic stress responses of plants. *Br-sil1* (*Brassica rapa* salicylate-induced lipase-like 1) is induced by salicylic acid and pathogens (Lee and Cho, 2003). *GLIP1* is an Arabidopsis GDSL lipase that possesses anti-microbial activity and regulates pathogen resistance to *Alternaria brassicicola* in association with ethylene signaling (Hong et al., 2008; Oh et al., 2005), *GLIP2* playes a role in resistance to *Erwinia carotovora* via negative regulation of auxin signaling (Lee et al., 2009; Yu et al., 2010). In hot pepper, *CaGLIP1* confers resistance to *Xanthomonas campestris pv. vesicatoria* that has been shown to modulate pathogen and wound stress resistance, and is induced in by MeJA (Hong et al., 2008; Kim et al., 2008; Park et al., 2001).

Over-expression of *AtLTL1* is induced by SA treatment increased salt tolerance and other environmental stresses in yeast and Arabidopsis (Hong et al., 2008; Naranjo et al., 2006; Riemann et al., 2007). In addition, GDSL-lipases are indicated as participating in hormonal pathways related to growth processes (Kiba et al., 2005; Naranjo et al., 2006). The researches of *GER1* (rice), *RGE1* (Arabidopsis) and *EXL4* (Arabidopsis) also evidenced a correlation between the expression of GDSL-lipases and other developmental processes (Kondou et al., 2008; Riemann et al., 2007; Updegraff et al., 2009). Partial GDSL-lipases involve in the metabolism of cutin and wax (Broun et al., 2004; Kim et al., 2008; Kondou et al., 2008; Panikashvili et al., 2010; Riemann et al., 2007; Takahashi et al., 2010). An unrooted phylogenetic tree is constructed from the amino acid sequences of 604 GDSL lipases from seven species (*Arabidopsis thaliana*, *Populus trichocarpa*, *Vitis vinifera*, *Orysa sativa*, *Sorghum bicolor*, *Picea sitchensis*,

Table 1. Putative motifs predicted in OsGILP proteins.

	Group A	Group B
Block I	FGDSxVDxGNNN	FxFGDSxxDTGN
Block II	TGRFSNGxxxxD	PTGRxSDGRLxxDF
Block III	SLFxxxxGxxND	SLFxxGEIGGNDY
Block IV	GARxxxxxxxxPxGCxP	GARxxVVPGxxPxGCxP
Block V	FWDxxHPTEAAN	SWDGxHxTEAAY

Significant motifs (e-value <e-100) of more than 10 amino acid length present in at least 20 GILP proteins were predicted by MEME research. The consensus sequence GILP proteins containing are given.

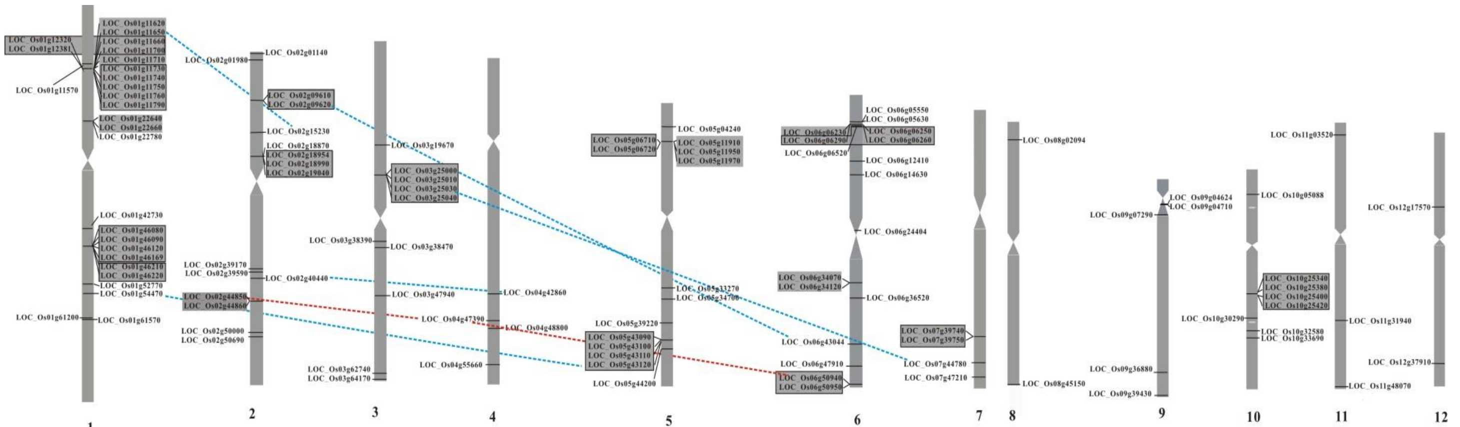

Fig 1. Chromosomal distribution of OsGILP genes. Graphical represent of the location of different GILP genes IDs on rice chromosomes. The genes labeled on left side are oriented from bottom to top and those on right side are oriented from top to bottom on rice chromosomes. The genes localized on duplicated chromosomal segments are connected by blue and red dashed lines. Fifteen clusters (three on chromosome 1, 2, 5 and 6, one on chromosome 3, 7 and 10) of tandemly arranged OsGILP genes are indicated in gray rectangles and duplicated-pairs in boxes. The chromosome number is indicated at the bottom of each chromosome.

Physcomitrella patens). The topology of the tree depicts two major and one minor subfamily. This division is also supported by the unique gene structure of each subfamily (Volokita et al., 2011). However the roles of OsGLIP genes in development and abiotic stresses are rarely reported.

Results and Discussion

Identification of genes encoding GILP proteins in rice and tandem duplications

From TAIR and GRAMNENE database, one hundred and thirteen GILP genes were obtained (latest data). The comparison with Volokita et al. (2011) research shows that 108 genes were identical, while others had discrepancy. To investigate the contribution of gene duplication in the expansion of GILP gene family, the chromosomal location of each GILP gene was determined based on the information provided by RGAP. These GILP genes were located in all 12 chromosomes (Fig. 1). The position of gene impacts the function, most GLIP genes were in cluster at arms of chromosomes, a few at the telomeric ends and near centromere. The rice genomes have undergone several rounds of genome-wide duplication events, including polyploidy, which have great impact on the expansion of a gene family in the genome. The chromosomal localization of most members of this family indicated a non-random distribution. As these genes were located within ≤5 genes in between them, possibility for tandem duplication was analyzed using Vista Tools for Comparative Genomics (Frazer et al., 2004). A total of fifteen clusters of tandem arranged genes were observed on different chromosomes. Chromosome 1 and 5 harbored three clusters of GILP genes (29 members of group B), respectively. Chromosome 2 contained three clusters of 7 GILP genes (all members of group A). Chromosome 6 has three clusters of 8 GILP genes, first and second cluster of 6 members of group B, third cluster of 2 members of group A. Chromosome 3, 7 and 10 have one cluster of GILP genes, all members were belonging to group B. This data suggested the major contribution of tandem duplications (60 of 113, 53.1%) to the GILP family expansion. On the other hand, we found 13 members of GILP family located on the segment-duplicated regions. Since the number of GILP genes located on segment-duplicated regions was less than those present in tandem, tandem duplications appear to have a major role in expansion of this gene family.

OsGLIP genes phylogenetic analyses

All of 113 GDSL-lipases homologous proteins were retrieved for phylogenetic analyses using their full-length protein sequences. Based on the protein sequence alignments and evolutionary relationships, the largest number of OsGLIP genes (56) were included in group B followed by 50 genes in group A, and one minor group C with just 2 members. This result was in agreement with Volokita et al. (2011) (Fig. 2). The additional putative conserved motifs in GLIP proteins were investigated using MEME program. The results show that there were five consensus sequences in Block I-V (Table 1). The GDSL motif was found to be close to the N-terminus. The sequences FGDSxxDxG are more conservative in rice, Ser in Block I, Asp in Block III or Asp and His Block V, putatively constituting the catalytic triad SDH. The exact location of active residue Asp has not been exactly confirmed yet (Ling et al., 2006; Upton and Buckley, 1995; Volokita et al., 2011). Most notably the numbers of Block V was larger than Block III in rice. There were no specific to the members of a particular class, the same blocks contained different conservative ammo acid residues within groups (Table 1). These putative conserved sequences in same motif might provide diversity in functions of the OsGLIP proteins.

Table 2. Putative regulatory cis-element sequences in 5'-upstream regions of 11 OsGILP genes.

Name	Description
LTR	cis-acting element involved in low-temperature responsiveness
CCAAT-box	MYBHv1 binding site
CGTCA-motif	cis-acting regulatory element involved in the MeJA-responsiveness
TC-rich repeats	cis-acting element involved in defense and stress responsiveness
ABRE	cis-acting element involved in the abscisic acid responsiveness
ARE	cis-acting regulatory element essential for the anaerobic induction
O2-site	cis-acting regulatory element involved in zein metabolism regulation
TCA-element	cis-acting element involved in salicylic acid responsiveness
TGA-element	auxin-responsive element
TGACG-motif	cis-acting regulatory element involved in the MeJA-responsiveness
motif IIb	abscisic acid responsive element
ERE	ethylene-responsive element
HSE	cis-acting element involved in heat stress responsiveness
MBS	MYB Binding Site/MYB binding site involved in drought-inducibility
CE1	cis-acting element associated to ABRE, involved in ABA responsiveness
P-box	gibberellin-responsive element
GARE-motif	gibberellin-responsive element
TATC-box	cis-acting element involved in gibberellin-responsiveness
TATCCAT/C-motif	cis-acting regulatory element; associated with G-box like motif; involved in sugar repression responsiveness
GC-motif	enhancer-like element involved in anoxic specific inducibility
motif IIb	abscisic acid responsive element
as-2-box	involved in shoot-specific expression and light responsiveness
GCN4_motif	cis-regulatory element involved in endosperm expression
Skn-1_motif	cis-acting regulatory element required for endosperm expression

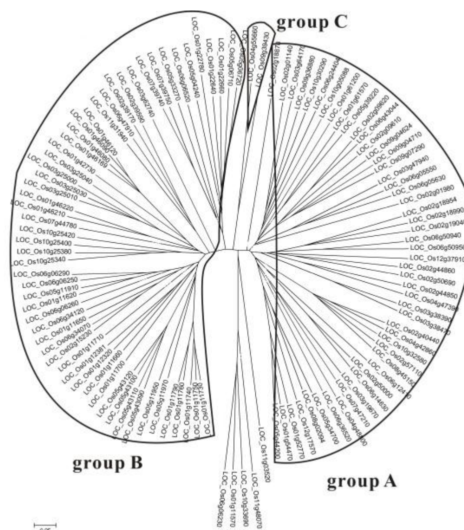

Fig 2. Phylogenetic OsGILP proteins and their classification. The unrooted tree was constructed based on multiple sequence alignment of full-length protein sequences using ClustalX program by Neighbor-joining method with 1000 bootstrap replicates. All the OsGILP proteins are classed into three groups. The bar indicates 0.05 substitutions per site.

Expression of GLIP genes during development and under abiotic stress conditions in rice

Although the roles of AtGLIP genes have been explored in various stress responses, the evidences for their role in rice growth and development were very limited (Hong et al., 2008; Kim et al., 2008; Kondou et al., 2008; Lee et al., 2009; Panikashvili et al., 2010; Updegraff et al., 2009; Volokita et al., 2011). The study of gene expression patterns of all the members of a gene family provided insight into their functional diversification. The clustering of expression profiles of 112 OsGLIP encoding genes were collected from a microarray based transcriptome profiling of 23 stages of vegetative and reproductive development (Fig. 3), three stress conditions at 7-day old shooting stage (cold, salt and desiccation) (Fig. 4) and different reproductive development under three stresses (cold, high temperature and desiccation) (Fig. 5). Lipases/esterases were active in hydrolysis and synthesis of abundant ester compounds. Extracellular lipases (*EXL1-6*) were isolated from *A. thaliana* pollen coat, anther-specific proline-rich protein genes (*APGs*) were from *A. thaliana* and *B. napus*, *BnLIP2* (*B. napus* L.) was a tissue-specific expressing gene during reproductive growth and strongly expressed during seed germination (Clauß et al., 2008; Clauß et al., 2011; Hong et al., 2008). 38 OsGLIP genes were highly expressed in stigma indicating that these genes might be expressed in stigma specifically. The expression of many OsGLIP genes was restricted to a particular tissue or developmental stage, whereas

a few OsGLIP genes exhibited expression in wide developmental stages. For example, the expression of one gene (LOC_Os02g01980) was restricted to panicle development stages (P3-P6). Eleven genes were expressed preferentially in shoot apical meristem tissue and panicle development stages (P1-P5), 27 genes expressed exclusively in root (Fig. 3). These genes may perform specific roles in these tissues or developmental stages. To reveal the functional redundancy or diversification of duplicated OsGLIP genes, their expression patterns were analyzed. The expression patterns of duplicated genes indicated their evolutionary fates. Among the fifteen gene pairs localized on duplicated chromosomal segments, four pairs of genes LOC_Os01g46080/090, LOC_Os01g46120/169, LOC_Os05g11970/950 and LOC_Os05g43100/120 exhibit similar expression patterns, whereas other pairs showed divergent expression patterns. The results suggest the evolution of GLIP gene family have occurred by gene duplication followed by retention due to sub- or neo-functionalization of the duplicated genes. Many GDSL-lipases genes have been implicated in various abiotic stress responses in plant species (Claußet al., 2008; Claußet al., 2011; Lee et al., 2009; Takahashi et al., 2010).

Our analysis showed that 42 members were with high signal value (control group signal value > 100) for rice seedlings treated with different abiotic stresses (desiccation, salt and cold) as compared to mock-treated control (7-day-old seedlings) (Fig. 4), 8 were found to be up-regulated by more than 2-fold and 15 down-regulated at desiccation stress (Fig. 6 A, B). In 7-day-old rice shooting, genes were more sensitive to desiccation stress, 14 down- and 7 up-regulated significantly. Meantime, 7 genes were down-regulated and 2 induced in salt test. However, just 3 genes were down-regulated and none up-regulated under cold condition (Fig. 6A, B). In Fig. 5, a total of 43 genes with high signal value (control group signal value > 100), 11 were found to be up-regulated and 10 down-regulated at all stresses by more than 2-fold (Fig. 6C, D). Six genes were specifically down-regulated in leaves in cold stress at booting stage and high temperature at heading/flowering stage, in which 4 of OsGILP were observed to be down-regulated in shooting leaves during cold stress; 3 in booting leaves and spike in high temperature stress; 2 in booting leaves at high temperature and desiccation and in heading/flowering spikes at cold; and none in heading/flowering spikes at desiccation and high temperature stresses. All of these genes showed high level expression in stigma and low at SAM, P2 and S3-S5 stages (Fig. 4). Microarray analysis performed on seedling, booting, heading and flowering stages subjected to cold, dehydration and high temperature stress revealed eleven 2-fold up-regulated genes in at least one of the stress conditions. In seedling leaf only one gene (LOC_Os01g11570) was induced expression by cold that was rather expressed at high level, on the other hand two genes (LOC_Os01g11650 and LOC_Os06g34120) were expressed at low level suppressed in young leaf. At booting stage two genes under dehydration stress, 6 genes were induced.

At heading and flowering stage, 4 genes were induced by high temperature in spike. The expression pattern of cluster genes was not completely identified in response to abiotic stresses. For example, the fist cluster (LOC_Os01g11710/730) genes in Fig. 6A were down-regulated under desiccation stress, while only LOC_Os01g11730 was changed under salt and cold condition more than 2 fold. The second gene cluster (LOC_Os01g46090/120/169) was also down-regulated under desiccation stress, but only one gene (LOC_Os01g46120) suppressed by salt treatment. All 3 clusters showed no change under cold stress. In the third cluster genes (LOC_Os06g34070/120) the expression responses to

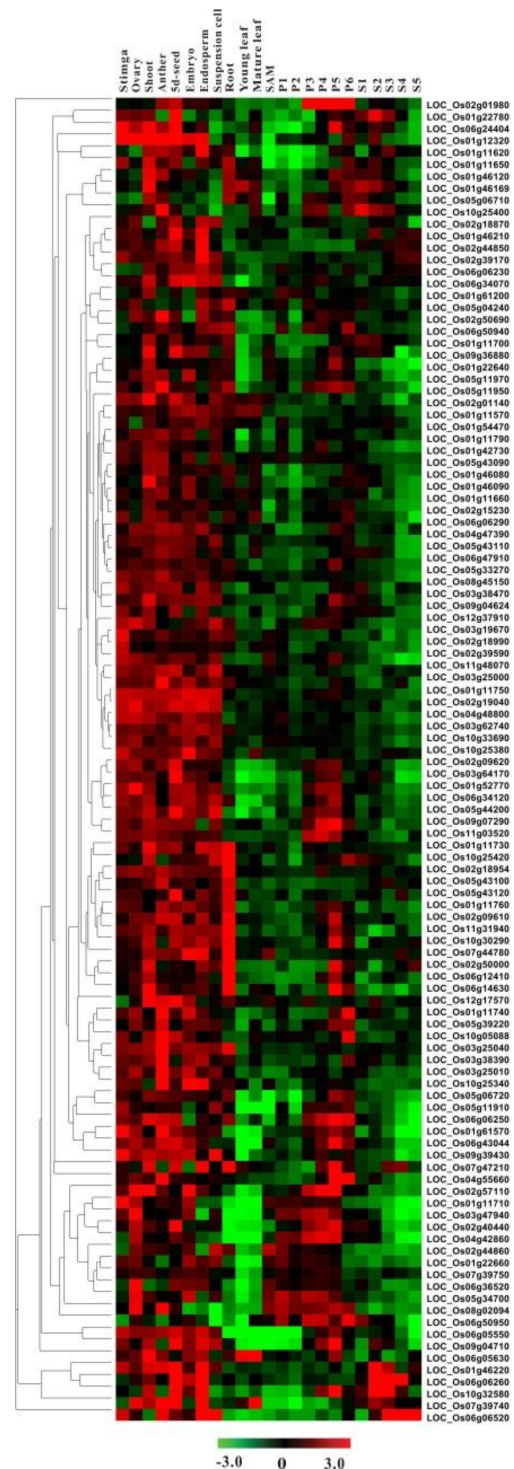

Fig 3. Differential expressions of GLIP gene family members in various tissues and developmental stages. Hierarchical clustering analysis of 112 OsGILP genes represented on Affymetrix Rice Genome Array is shown. For clustering we used average $\log_2$ signal values for three biological replicates of each sample after normalization of the raw data. The color scale for log signal values is shown at the bottom. SAM, shoot apical meristem; P1-P6, stages of panicle development; S1-S5, stages of seed development. The series GSE6893 includes microarray data from 45 hybridizations representing three biological replicates each of 15 different tissues/organs and developmental stages, three biological replicates were available only for stigma and ovary in the series GSE7951 dataset.

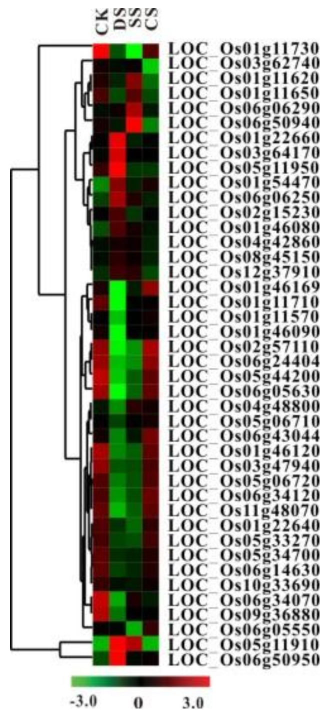

Fig 4. Differential expressions of OsGILP genes in response to three abiotic stresses. Hierarchical clustering of 42 *OsGILP* genes showing the significant differential expression under three abiotic stress conditions. DS, desiccation stress; SS, salt stress; CS, cold stress; CK, control (7-days old seedlings, signal value > 100). The color bar representing $\log_2$ signal values is shown at the bottom.

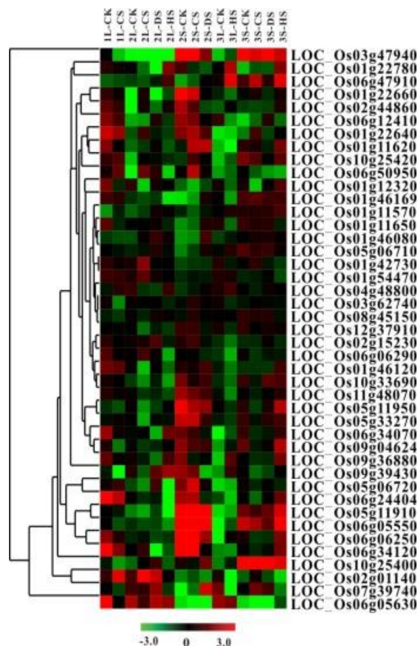

Fig 5. Differential expressions of *OsGILP* genes in response to various biotic stress conditions. Hierarchical clustering of 43 GST genes showing significant differential expression in differential developmental stages under various abiotic conditions is shown. 1, seedling stage; 2, booting stage; 3, heading and flowering stage; L, leaf; S, spike; CK, as compared with an untreated rice (signal value > 100 in any tissue); CS, cold stress; DS, dehydration stress; HS, high temperature stress. The color bar representing $\log_2$ signal values is shown at the bottom.

desiccation stress was observed while no change under salt condition detected. In this cluster only one gene were down-regulated by cold stress. Two cluster genes induced by any stress in Fig. 6B. The first pair of genes (LOC_Os05g11910/950) both induced by desiccation but not under cold stress. The second cluster genes, one (LOC_Os06g50940) induced by desiccation, another (LOC_Os06g50950) by salt stress. No gene of cluster was down-regulated under different reproductive development stages under cold, high temperature and desiccation stresses (Fig. 6C). Fig. 6D shows that one cluster genes (LOC_Os01g46080/090/169) were significantly induced by stresses, in which all of 3 genes were up-regulated in booting spikes under desiccation stress. Only LOC_Os01g46080 was induced in booting leaf at desiccation, while LOC_Os01g46169 was suppressed in leaf at high temperature and LOC_Os01g46090 in spike at heading/flowering stage under high temperature circumstances. The analysis suggested that expression of different members of the OsGILP gene cluster was regulated differentially after abiotic stresses in different tissues, which might be attributed to their tolerance or susceptibility behavior to stress.

Analysis of cis-elements in 17 abiotic stress responding genes

To investigate the probable explanation for the 17 abiotic stress up-regulated genes, we analyzed their promoter sequences, about 1.5 kb upstream from translational start site. The results of Plant-CARE database and the PLACE databases shows that a total of 21 types of CREs were identified responding to abiotic stress, which contained 3 kinds of growth regulatory elements (as-2-box, Skn-1_motif and GCN4_motif) (Table 2). The names and functions of these 24 motifs were shown in Table 2. Their locations on upstream sequences are displayed in Fig. 7.

TGACG-motif, ABRE and ARE motifs were found in 12, 11 and 10 out of 17 genes, respectively. In reverse, the motifs CE1, motif IIb and TATC-box were just found in one gene. Other motifs distributed in 3 to 9 genes, apart from GC-motif stretching in 2 genes. Totally 9 genes contained 6-8 CREs, one gene (LOC_Os01g42730) had the biggest number of 14, following 2 genes (LOC_Os01g42730 and LOC_Os06g50940). There were many phytohormone auxin responsive cis-acting elements, such as MeJA, ABA, zein, ethylene, gibberellins.

This analysis revealed that the regulatory elements were more conserved in duplicated OsGILP genes with similar expression patterns (for example, LOC_Os05g11910/950 and LOC_Os06g50940/950) as compared to the GLIP genes with divergent expression patterns (such as, LOC_Os01g46080/090/169). The difference in the regulatory elements of duplicated genes might explain their divergent expression patterns. However, the existence of some other regulatory mechanism, which is responsible for divergent expression patterns and/or non-functionalization of one of the duplicates, could not be ruled out.

Materials and methods

Plant materials and growth conditions

The viable seeds of cultivated rice Pei'ai 64S (*Oryza sativa* L. *indica*) were suspended in a sterile solution of 0.1% $HgCl_2$ for 10 min, washed 3 times using running water, immersed for 3 days under 25 ^{o}C and water changed once a day, then were germinated and grown in distilled water at 37°C for 2~3 days. They were sown in batches in pots that put in net basin at Institute of Subtropical Agriculture of Chinese Academy of

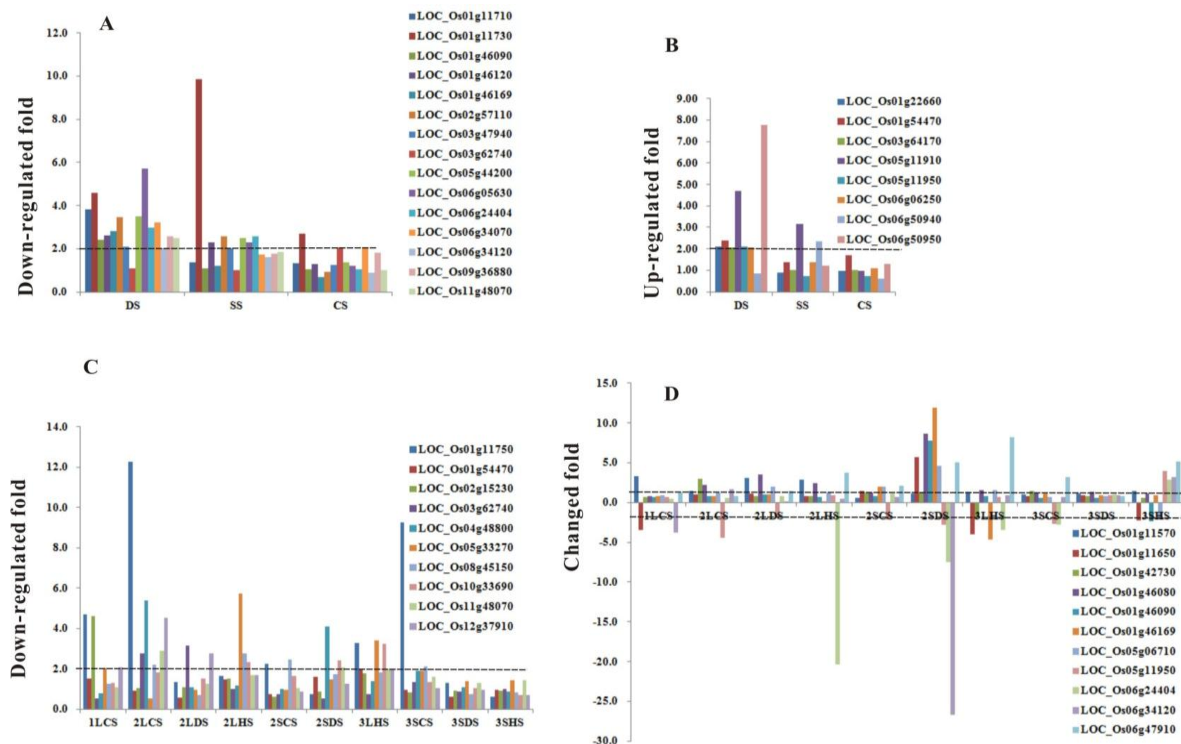

Fig 6. Genes up-regulated or down-regulated in any abiotic stresses. The genes are down- or up-regulated by more than 2-fold in response to various biotic stress conditions is compared with CK have been shown. The color rectangles represents gene. Y axis is changed fold; X axis is different tissues under various stresses. Dashed line represents expression down- or up-regulated 2 fold.

Science. Plants were divided into one control and three treatment groups. The control group was maintained under natural conditions, regular water and fertilizer management, pest and disease control. The treatment groups were exposed to drought, heat and cold stresses. At five-leaf stage, part of them was taken as the test material of the seedling stage. Other parts, as test material of booting and flowering stage are transplanted to the other pots, 5 plants from each pot.

Rice cold, heat and water-deficit treatment

For the drought tests the watering was avoided to the basin until the treatment group dried out. Meanwhile, the control group was also put in the dry shed, but with water in pots, then the leaves were harvested when they started curling after 16 h. For the heat tests, materials were put in climate incubator, PGC15.5 (Percival, USA) for 2 h under 45 ^{o}C, while the control group was put into PGC15.5 under 45 ^{o}C. For cold test, we placed the seedlings into PGC15.5 for 12h under 4 ^{o}C at booting and heading stage for 16h under 12 ^{o}C. The control group was placed into another PGC15.5, and both control and treatment group was under dark conditions.

Raw materials and sample preparation

Four or five countdown second leaves were collected from treatment and control group, and four or five leaves were harvested, which were not out of the young panicle or the middle of spiked out flower. The materials were cut into pieces, then ground into a dry powder with liquid nitrogen, and immediately divided into pre-installed 1.0 mL TRIzol extraction (Invitrogen) in 1.5 mL centrifugal tubes, about 100 mg each tube. We used low temperature marker pen to mark labels, tightly closed lid, then vigorously vortexed to make sure that samples were mixed up with TRIzol extraction and then sealed the tubes with Parafilm wrap. The samples were stored at -70 ^{o}C until required.

Total RNA isolation

Total RNA was isolated from the frozen samples using TRIzol (Invitrogen). The samples saved in -70 °C were taken out, vortexed to homogeneity, chloroform (200 μL) was added, vigorously shaken for 15 s, and then centrifuged at 12 000 × g for 15 min at 4 °C. The upper layer was carefully removed from each tube, was transferred to another centrifuge tube. Then the isopropanol (500 μL) was added, precipitated for at least 1 h at -40 °C, then centrifuged to separate the RNA. The RNA pellets were twice washed by 75% ethanol, air dried and dissolved in the appropriate volume of RNase-free water. The purity of RNA was determined by the A260/280 absorbance ratio (1.9-2.0). Isolated RNAs were stored at -70 °C, after checking the purity and integrity of 18 S, 5 S and 28 S rRNA bands on 1.5% agarose gel.

Microarray

Affymetrix expression microarray experiments was done according to manual provided by GeneTech (Biotechnology Limited Company, Shanghai, China) briefly by the following steps: (1) Total RNA extraction and purification; (2) cDNA synthesis and purification; (3) transcription cRNA synthesis and *in vitro* cRNA purification (4) cRNA fragmentation, hybridization solution preparation (5) chip hybridization (6) elution chip (7) scan chips (8) data analysis.

Sequences analysis

Protein sequences of rice GDSL lipase-like (GLIP) were acquired from a query search in GRAMENE and rice genomes

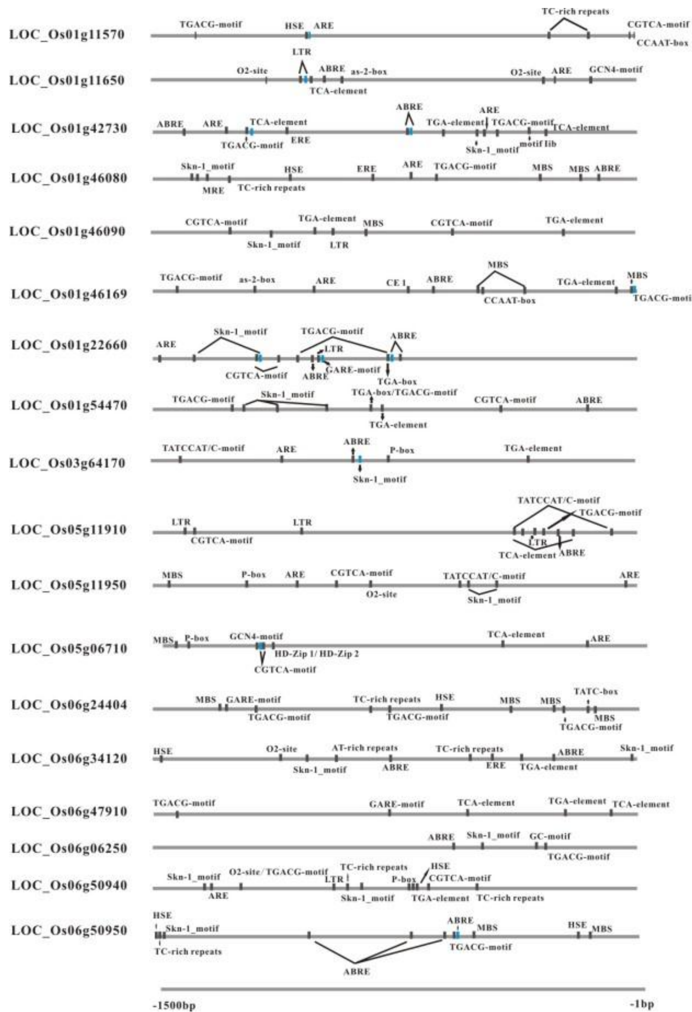

Fig 7. Distribution of cis-element sequences in 5'-upstream regions of OsGILP genes. Predicted cis-element sequences are on 5'-upstream regions of 17 up-regulated OsGILP genes. The sequence is about 1500bp from the start coden 'ATG'. Long line represents DNA sequence; little bar is cis-element.

(TIGR version 6.1) with the key word of GDSL-like lipase/acylhydrolase. GLIP motif scan was performed using InterProScan and PFAM Database searches with filter off. We identified the protein motifs of GLIP genes using MEME (http://meme.sdsc.edu/meme/meme.html) with the motif length setting at 6-20, motif for 5. GLIP genes were mapped on chromosomes by identifying their chromosomal position given in TIGR rice database. The genome organization and map location were investigated by the corresponding genome sequence with the map viewer at NCBI (http://www.ncbi.nlm.nih.gov/mapview/). The amino acid sequences of GLIPs were initially aligned using the program ClustalW (version 1.83) and computing pairwise distance. The unrooted phylogenetic tree was constructed by the Neighbor-Joining method and displayed using the same software MEGA (version 5.02). Bootstrap analysis was performed using 1000 replicates, p-distance Method, pairwise-deletion gaps data treatment. DNA sequences of rice GLIP genes and 1500 bp ahead of the translation initiation codon (ATG) were collected from the GRAMENE database. The promoters of GLIPs were analyzed by Plant-CARE database, and validated the results using the PLACE databases.

Expression analysis

We used the Gene Expression Omnibus (GEO, accession number GPL2025) database for expression data of reproductive development (GSE6893 and GSE7951) and expression data for stress treatment (GSE6901) (Jain et al., 2007) of the rice GLIP gene family. The Affymetrix CEL files of each were imported and analysed using Gene Chip Robust Multi-Array method. The IDs of probe sets present on the Affymetrix rice genome array representing the GLIP genes were identified using the Rice Multi-platform Microarray Search tool (Saeed et al., 2003). Cluster analysis on rows (using log transformation) was performed by, Average Linkage rule of Hierarchical clustering method.

Conclusion

This study provided not only an updated genomic positions, phylogenetic relationships and protein structures of the GDSL-lipase family in rice, but also the identification of several tissue- and/or developmental stage-specific and abiotic stress-responsive OsGILP genes included in various classes. Our results provided a very useful framework and starting point for revealing the function of GILP family members in rice, especially those involved in specific developmental processes and stress tolerance.

Acknowledgments

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References

Akoh CC, Lee GC, Liaw YC, Huang TH, Shaw JF (2004) GDSL family of serine esterases/lipases. Prog Lipid Res 43:534-552.

Arpigny JL, Jaeger KE (1999) Bacterial lipolytic enzymes: classification and properties. Biochem J 343:177-183.

Brick DJ, Brumlik MJ, Buckley JT, Cao JX, Davies PC, Misra S, Tranbarger TJ, Upton C (1995) A new family of lipolytic plant enzymes with members in rice, *Arabidopsis* and maize. FEBS Lett 377:475-480.

Broun P, Poindexter P, Osborne E, Jiang CZ, Riechmann JL (2004) *WIN1*, a transcriptional activator of epidermal wax accumulation in *Arabidopsis*. Natl Acad Sci 101:4706-4711.

Clauß K, Baumert A, Nimtz M, Milkowski C, Strack D (2008) Role of a GDSL lipase-like protein as sinapine esterase in *Brassicaceae*. The Plant Journal 53:802-813.

Clauß K, von Roepenack-Lahaye E, Böttcher C, Roth MR, Welti R, Erban A, Kopka J, Scheel D, Milkowski C, Strack D (2011) Overexpression of Sinapine Esterase *BnSCE3* in Oilseed Rape Seeds Triggers Global Changes in Seed Metabolism. Plant Physiol 155:1127-1145.

Dalrymple BP, Cybinski DH, Layton I, McSweeney CS, Xue GP, Swadling YJ, Lowry JB (1997) Three Neocallimastix patriciarum esterases associated with the degradation of complex polysaccharides are members of a new family of hydrolases. Microbiology 143:2605-2614.

Frazer KA, Pachter L, Poliakov A, Rubin EM, Dubchak I (2004) VISTA: computational tools for comparative genomics. Nucleic Acids Res 32:W273-W279. doi: 10.1093/nar/gkh458

Hong JK, Choi HW, Hwang IS, Kim DS, Kim NH, Choi DS, Kim YJ, Hwang BK (2008) Function of a novel GDSL-type pepper lipase gene, *CaGLIP1*, in disease susceptibility and

abiotic stress tolerance. Planta 227:539-558.

Jain M, Nijhawan A, Arora R, Agarwal P, Ray S, Sharma P, Kapoor S, Tyagi AK, Khurana JP (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 Physiol 143:1467-1483.

Kiba T, Naitou T, Koizumi N, Yamashino T, Sakakibara H, Mizuno T (2005) Combinatorial microarray analysis revealing *Arabidopsis* genes implicated in cytokinin responses through the His→Asp phosphorelay circuitry. Plant Cell Physiol 46:339-355.

Kim KJ, Lim JH, Kim MJ, Kim T, Chung HM, Paek KH (2008) GDSL-lipase1 (*CaGL1*) contributes to wound stress resistance by modulation of *CaPR-4* expression in hot pepper. Biochem Biophys Res Commun 374:693-698.

Kondou Y, Nakazawa M, Kawashima M, Ichikawa T, Yoshizumi T, Suzuki K, Ishikawa A, Koshi T, Matsui R, Muto S (2008) RETARDED GROWTH OF EMBRYO1, a new basic helix-loop-helix protein, expresses in endosperm to control embryo growth. Plant Physiol 147:1924-1935.

Lee DS, Kim BK, Kwon SJ, Jin HC, Park OK (2009) Arabidopsis GDSL lipase 2 plays a role in pathogen defense via negative regulation of auxin signaling. Biochem Biophys Res Commun 379:1038-1042.

Lee KA, Cho TJ (2003) Characterization of a salicylic acid-and pathogen-induced lipase-like gene in Chinese cabbage. J Biochem Mol Biol 36:433-441.

Ling H, Zhao J, Zuo K, Qiu C, Yao H, Qin J, Sun X, Tang K (2006) Isolation and expression analysis of a GDSL-like lipase gene from *Brassica napus L.* J Biochem Mol Biol 39:297-303.

Mølgaard A, Kauppinen S, Larsen S (2000) Rhamnogalacturonan acetylesterase elucidates the structure and function of a new family of hydrolases. Structure 8:373-383.

Naranjo MA, Forment J, RoldÁN M, Serrano RÓN, Vicente O (2006) Overexpression of Arabidopsis thaliana *LTL1*, a salt-induced gene encoding a GDSL-motif lipase, increases salt tolerance in yeast and transgenic plants. Plant, Cell Environ 29:1890-1900.

Oh IS, Park AR, Bae MS, Kwon SJ, Kim YS, Lee JE, Kang NY, Lee S, Cheong H, Park OK (2005) Secretome analysis reveals an Arabidopsis lipase involved in defense against Alternaria brassicicola. The Plant Cell Online 17:2832-2847.

Panikashvili D, Shi JX, Bocobza S, Franke RB, Schreiber L, Aharoni A (2010) The Arabidopsis *DSO/ABCG11* transporter affects cutin metabolism in reproductive organs and suberin in roots. Molecular plant 3:563-575.

Park CJ, Shin R, Park JM, Lee G, Yoo TH, Paek KH (2001) A hot pepper cDNA encoding a pathogenesis-related protein 4 is induced during the resistance response to tobacco mosaic virus. Mol Cells 11:122-127.

Pringle D, Dickstein R (2004) Purification of *ENOD8* proteins from Medicago sativa root nodules and their characterization as esterases. Plant physiology and biochemistry 42:73-79.

Riemann M, Gutjahr C, Korte A, Danger B, Muramatsu T, Bayer U, Waller F, Furuya M, Nick P (2007) *GER1*, a GDSL Motif-Encoding Gene from Rice is a Novel Early Light-and Jasmonate-Induced Gene. Plant Biology 9:32-40.

Saeed A, Sharov V, White J, Li J, Liang W, Bhagabati N, Braisted J, Klapa M, Currier T, Thiagarajan M (2003) TM4: a free, open-source system for microarray data management and analysis. BioTechniques 34:374-378.

Takahashi K, Shimada T, Kondo M, Tamai A, Mori M, Nishimura M, Hara-Nishimura I (2010) Ectopic expression of an esterase, which is a candidate for the unidentified plant cutinase, causes cuticular defects in Arabidopsis thaliana. Plant Cell Physiol 51:123-131.

Updegraff EP, Zhao F, Preuss D (2009) The extracellular lipase *EXL4* is required for efficient hydration of Arabidopsis pollen. Sexual plant reproduction 22:197-204.

Upton C, Buckley JT (1995) A new family of lipolytic enzymes? Trends Biochem Sci 20:178-179.

Volokita M, Rosilio-Brami T, Rivkin N, Zik M (2011) Combining Comparative Sequence and Genomic Data to Ascertain Phylogenetic Relationships and Explore the Evolution of the Large GDSL-Lipase Family in Land Plants. Mol Biol Evol 28:551-565.

Yu S, Zheng B, Zhao X, Feng Y (2010) Gene cloning and characterization of a novel thermophilic esterase from Fervidobacterium nodosum *Rt17-B1*. Acta biochimica et biophysica Sinica 42:288-295.