Funct Integr Genomics (2016) 16:153–169
DOI 10.1007/s10142-016-0474-3

ORIGINAL ARTICLE

Analysis of SSH library of rice variety Aganni reveals candidate gall midge resistance genes

Dhanasekar Divya[1,4] · **Y. Tunginba Singh**[2,3] · **Suresh Nair**[2] · **J. S. Bentur**[1,4]

Received: 24 September 2015 / Revised: 19 December 2015 / Accepted: 7 January 2016 / Published online: 22 January 2016

Abstract The Asian rice gall midge, *Orseolia oryzae*, is a serious insect pest causing extensive yield loss. Interaction between the gall midge and rice genotypes is known to be on a gene-for-gene basis. Here, we report molecular basis of HR− (hypersensitive reaction—negative) type of resistance in Aganni (an *indica* rice variety possessing gall midge resistance gene *Gm8*) through the construction and analysis of a suppressive subtraction hybridization (SSH) cDNA library. In all, 2,800 positive clones were sequenced and analyzed. The high-quality ESTs were assembled into 448 non-redundant gene sequences. Homology search with the NCBI databases, using BlastX and BlastN, revealed that 73% of the clones showed homology to genes with known function and majority of ESTs belonged to the gene ontology category 'biological process'. Validation of 27 putative candidate gall midge resistance genes through real-time PCR, following gall midge infestation, in contrasting parents and their derived pre-NILs (near isogenic lines) revealed induction of specific genes related to defense and metabolism. Interestingly, four genes, belonging to families of leucine-rich repeat (LRR), heat shock protein (HSP), pathogenesis related protein (PR), and NAC domain-containing protein, implicated in conferring HR+ type of resistance, were found to be up-regulated in Aganni. Two of the reactive oxygen intermediates (ROI)–scavenging-enzyme-coding genes Cytosolic Ascorbate Peroxidase1, 2 (*OsAPx1* and *OsAPx2*) were found up-regulated in Aganni in incompatible interaction possibly suppressing HR. We suggest that Aganni has a deviant form of inducible, salicylic acid (SA)-mediated resistance but without HR.

Keywords HR− type of resistance · ESTs · RT-PCR · Induced resistance · Biotype

Dhanasekar Divya and Y. Tunginba Singh contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s10142-016-0474-3) contains supplementary material, which is available to authorized users.

✉ Suresh Nair
suresh@icgeb.res.in

✉ J. S. Bentur
jbentur@yahoo.com

[1] Indian Institute of Rice Research, Rajendranagar, Hyderabad 500030, India

[2] International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110067, India

[3] Present address: Department of Botany, Mizoram University, Tanhril, Aizawl, Mizoram 796004, India

[4] Present address: Agri Biotech Foundation, Rajendranagar, Hyderabad 500030, India

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops for more than one half of the world's population. Productivity of the crop is affected by damage caused by insect pests/pathogens. The Asian rice gall midge, *Orseolia oryzae* (Wood Mason), is one of the important rice pests causing an annual yield loss to the tune of about US$80 million in India (Bentur et al. 2003). Development of rice cultivars with resistance to the pest is the desirable approach to mitigate this loss. However, adaptation of pest populations and evolution of virulent biotypes is the cause of concern. Hence, identification and characterization of resistance genes in rice are essential to the development of durable resistance against the pest.

Plants posses an array of defense arsenal against pathogens and insect pests. While plant defense against pathogens generally involves salicylic acid (SA)-mediated response resulting in expression of hypersensitive reaction (HR), defense against insects involves jasmonic acid (JA)-mediated response (Thaler et al. 2012). Often these two pathways act antagonistically. Genotype-specific variations in defense in the same plant species against specific race/biotype of the pest are also reported. Such diversity is the result of co-evolution and negate the counter-defense strategies of the invading organisms (Xu et al. 2013).

The rice gall midge interaction is genetically well-characterized. Until now, 11 R genes have been identified from different rice varieties and mapped on to different chromosomes (Yasala et al. 2012). None of these genes is effective against all the seven gall midge biotypes reported in India (Vijaya Lakshmi et al. 2006). Incompatibility between the host and the gall midge, i.e., plant resistance is manifested in two distinct ways, i.e., with or without the expression of hypersensitive reaction (HR+ or HR− types), wherein the HR+ type results in tissue necrosis at the feeding site, while HR− type response is without tissue necrosis (Bentur and Kalode 1996). Conversely, compatible interaction or plant susceptibility occurs when virulent maggots successfully establish in the plant to complete the life cycle to emerge as adults. Of the known R genes, only *Gm1* and *Gm8* confer HR− type resistance (Bentur et al. 2003). The indica rice cultivar Aganni, carrying gall midge resistance gene *Gm8*, has a wide range of resistance across gall midge biotypes. This gene has been tagged and mapped within 0.43-Mb region on chromosome 8 of rice between the flanking SSR markers RM22685 and RM22709 (Sama et al. 2012).

In our earlier studies on the molecular basis of rice-gall midge interactions, we have analyzed differentially expressed genes in the rice variety Suraksha (possessing gall midge resistance gene *Gm11*) expressing HR+ type of resistance, using suppressive subtraction hybridization (SSH) cDNA library (Rawat et al. 2013). Defense mechanism in this cultivar showed similarity with that observed in rice against pathogens. In contrast, microarray analysis of resistance mechanism in the rice variety Kavya carrying *Gm1* gene (with HR− type of resistance) revealed the resistance to be a novel type (Rawat et al. 2012a). Using both SSH and microarray approaches compatible interactions were also studied in gall midge-susceptible rice TN1 (with no R gene) and also in Kavya (with *Gm1* gene) and Suraksha (with *Gm11* gene) challenged with the virulent biotype of the gall midge (Rawat et al. 2012b). These studies revealed up-regulation of genes involved in primary metabolism, nutrient relocation, cell organization, DNA synthesis, and down-regulation of genes involved in defense, secondary metabolism, and signaling. This study also identified few putative 'susceptibility genes'. In this paper we have examined the molecular basis of resistance in another rice variety Aganni, carrying *Gm8* – a gene conferring HR− type of resistance—and show that pathways involved here are not similar to those described earlier.

Materials and methods

Plant material for suppressive subtraction hybridization (SSH) cDNA library construction

Rice variety Aganni carrying gall midge resistance gene *Gm8* with HR− type of resistance was used for SSH cDNA library construction. Aganni seeds were germinated and grown in plastic trays (60 × 30 × 30 cm) with fertilizer-enriched rice soil under standard conditions. Each tray had 12 lines of 20 plants each. When plants were 15-day-old, one of the trays was exposed to gall midge biotype 4 (GMB4) adults (50 females and 10 males). Another tray was used as the control where the plants were not exposed to the insects. Two days after insect release, both the trays were transferred to a high humidity chamber (>90 % RH) for two days (for egg incubation and maggot establishment in infested plants). Plants were sampled at five different time points 24, 48, 72, 96, and 120 hours after infestation (hai). Plants from the uninfested trays were also sampled at the same time point.

Plant material for gene validation

Two sets of plants – test lines with contrasting phenotypes, − were used for validation of shortlisted genes for real-time PCR. The two contrasting parents—gall midge-susceptible TN1 and gall midge-resistant Aganni formed the first set. Two pre-NILs (near isogenic lines) designated as susceptible 34S and resistant 74R selected from the 426 F_{10} recurrent inbred lines (RILs) from the cross TN1 X Aganni with over 93 % genetic similarity as assessed with 320 Simple Sequence Repeats (SSR) markers formed the second set of plants (data not shown). Seeds of the test lines were germinated and grown in plastic trays (60 × 30 × 30 cm) with fertilizer-enriched rice soil under standard conditions. Plants were grown, infested with GMB1, and sampled as mentioned earlier (Rawat et al. 2013). Plants from the uninfested trays were also sampled at the same time point.

RNA extraction

The sampled plants were cut at the base, dissected under stereo binocular microscope to remove live and dead maggots from the infested plants. Uninfested plants were also similarly dissected out despite the fact there would be no maggots in these tissues. The meristematic tissue (region ~1 cm from the base) was cut and preserved in RNALater (Qiagen, Germany). Later, the tissues were crushed in liquid N_2 and total RNA was

isolated using TRIzol (Invitrogen, USA) according to the manufacturer's protocol. Equal amounts of total RNA were pooled from all the time intervals, for both the sets of plants, separately. Total RNA 700 μg from uninfested and 350 μg from infested plants was used for mRNA isolation by magnetic separation after annealing to 5′-biotinylated oligodT primer and subsequently immobilized on streptavidin-linked paramagnetic beads, as described earlier (Mishra et al. 2005).

Driver preparation

First-strand cDNA was synthesized, using Superscript III reverse transcriptase (Invitrogen, USA) along with 5′-biotinylated anchored oligodT [T(18) VN] primer, from 5 μg of mRNA isolated from the control plants, according to the manufacturer's protocol. Subsequent purification of the cDNA was performed by hydrolyzing the mRNA by alkaline treatment (30 min at 55 °C in 0.1 N NaOH) followed by removal of excess oligodT primers according to Mishra et al. (2005).

Suppressive subtraction hybridization (SSH)

Five-fold excess of 5′-biotinylated first-strand cDNA populations, from the control plants (driver) were mixed with one microgram of mRNA (tester) from the infested Aganni variety. The samples were kept for hybridization for 4 h at 68 °C in 50 μl RNase-free hybridization buffer (10 mM Tris–HCl pH 7.5, 0.5 M KCl, and 5 mM EDTA) in a silanized eppendorf tube. SSH library was prepared as per Rawat et al. (2012b). Double-strand cDNA was synthesized using the sequence abundant, normalized, and enriched, differentially up-regulated, subtracted mRNA. The double-stranded cDNA was unidirectionally ligated to lambda-ZAP vector, in vitro packaged and allowed to infect XL1-Blue MRF *E. coli* cells according to the manufacturer's instructions using a UNI-ZAP XR cDNA library construction kit (Stratagene, USA).

Screening for recombinant clones

The recombinant clones were stored temporarily at −20 °C after transferring into 96-well format storage plates containing 200 μl of SM buffer (100 mM NaCl, 8 mM $MgSO_4.7H_2O$, 50 mM Tris–HCl pH 7, 0.04 % gelatin) supplemented with 5 % DMSO and 0.5 % chloroform. The cDNA clones were PCR amplified with 100 ng of universal M13 forward and reverse primers and recombinant phages having insert sizes of ≥200 bp were selected and re-arrayed into new storage plates. PCR was performed using 1 μl of phage suspension as template in 100 μl of PCR reaction volume (1X PCR buffer, 200 μM dNTPs and 5U *Taq* polymerase) for 30 cycles with following PCR conditions, 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min. The PCR products were purified using StrataPrep 96 PCR purification kit (Stratagene, USA) to remove un-incorporated primers and nucleotides. Next, the purified PCR products were dried and sent for sequencing (Macrogen, South Korea).

Data processing

The obtained sequence data for each clone were processed using MacVector (MacVector Inc, USA, version 11.1.2) suite of programs to trim out vector sequences. The inbuilt Phred and Phrap program of MacVector was used to assemble the sequences into contigs.

Data analysis

Homology search was performed using BlastN and BlastX (http://www.ncbi.nlm.nih.gov/BLAST/ version 2.2.29) to search for homologous sequences in the non-redundant nucleotide and protein databases, respectively. Selected clones were manually annotated using the information from the highest scoring matches returned by both the analyses. Functional classification of the ESTs was performed using Gene Ontology (http://www.ricearray.org/analysis/GO/). Further ESTs were assigned to the main metabolic pathways using Kyoto Encylopedia of Genes and Genomes (http://www.ricearray.org/analysis/KO/). All the processed sequences have been submitted to the GenBank.

Primer designing and real-time PCR validation of differentially expressed genes

Primer pairs were designed using Primer3 software tool (http://frodo.wi.mit.edu/) or Primer Express Software (Applied Biosystems) from the genes shortlisted, from those that were sequenced, for validation of expression using quantitative real-time PCR (qPCR). The primers were designed so as to amplify a 60–150-bp product (Table 1).

In all, 27 candidate genes were validated via real-time reverse transcription PCR with four sets of samples derived from the test lines (TN1, Aganni, 74R line, and 34S line). Real-time PCR was conducted using cDNA prepared from RNA sampled from independent infestations at two time points viz., 24 and 120 hours after infestation (hai). Three micrograms of RNA was used for first-strand cDNA synthesis using the SuperScript III RT (Invitrogen, USA) following the manufacturer's guidelines. Real-time-PCR was performed using Applied Biosystems 7500 Real Time PCR System with the SYBR green chemistry (Applied Biosystems, USA) according to the manufacturer's instructions. Rice ubiquitin gene, *OsUbq* (GenBank accession no. AK059694), was used as the endogenous control. Real-time PCR reaction volume of 20 μl contained 10 μl SYBR green PCR master mix (Applied Biosystems, USA), 500 nM each of forward and reverse primers and 20 ng of the cDNA. To calculate mean

Table 1 Sequence information of primers used for the real-time PCR of the shortlisted ESTs from the SSH cDNA library of Aganni

S. no	Gene name	Locus ID	Primer sequences	Product size (bp)	Tm (°C)
1	Peptidyl-prolyl *cis-trans* isomerase	LOC_Os08g44520	F: ACGACAACGTCATCTTCCAT R: GTGCTTCAGTTCTGGCCTAA	134	58
2	Auxin-responsive Aux/IAA gene family member	LOC_Os01g13030	F: AGACAAGGATGGTGACTGGA R: AAGTCCAATTGCATCTGAGC	140	60
3	Leucine-rich repeat family protein	LOC_Os11g42660	F: ATCTGCAACAGGGTATTCCA R: TTCTATGTTGGTTCCCCTCA	126	58
4	Heat shock protein	LOC_Os12g32986	F: GTGCCTGCTACAAAGAAGGA R: GAGTCAACAACACCCCTCAC	143	58
5	Pathogenesis-related Bet v I family protein	LOC_Os08g28670	F: GGACCTGCACTTCATCACTT R: AGGTGACATGGTAGGGATCA	130	57
6	Chitin-inducible gibberellin-responsive protein	LOC_Os01g65900	F: ACACTTCCAAGGGAAAGTCC R: ATCTCTCCACACGATCAGGA	137	60
7	NAC domain-containing protein 67	LOC_Os03g60080	F: GGGGTCAAGACTGATTGGAT R: TCTCCCACTCGTTCTTCTTG	139	58
8	Serine hydroxymethyltransferase,	LOC_Os11g26860	F: TCGAGAAGGACTTTGAGCAG R: CAGCTTGAGGTTCTCGATGT	143	58
9	WW domain-containing protein	LOC_Os06g19470	F: TCCTCCATCAGAGCAAGTTC R: GAGGAGCTGGTTCATTCTCA	143	58
10	COP9 signalosome complex subunit 5b	LOC_Os04g56070	F: GGTTGGCTGGTATCACTCAC R: ACCAGCAGAAACAGTCCTTG	140	59
11	bZIP transcription factor domain-containing protein	LOC_Os05g34050	F: TGTTAGCCTTCTTGGTTTGC R: TGACACTCAACACCCTAGCA	113	58
12	Acyl-CoA synthetase protein	LOC_Os05g25310	F: GGTCTTCCCAAGGGTGTAAT R: CAGATAAACATCCCCTGTGC	101	57
13	Plasminogen activator inhibitor 1 RNA-binding protein	LOC_Os05g45660	F:GGAGGAGAAGAGGAAAGCAC R: CCTTGTCAGATCCCAGTTTG	128	58
14	Aspartic proteinase oryzasin-1 precursor	LOC_Os05g49200	F: TACTGGGGTAGTCAGCCAAG R:ACAGACCAACCTGAGAGCAG	68	58
15	Erythronate-4-phosphatedehydrogenase	LOC_Os11g26850	F: ACACTGGCATCATTGTCCTT R: CTTCTCCTTCCACAGCTCAA	131	58
16	Amino acid transporter	LOC_Os01g66010	F: GTACGTGGTGCAGAAGAAGG R: AACCTTGAGATCCGACATGA	136	58
17	Ketol-acid reductoisomerase, chloroplast precursor	LOC_Os01g46380	F: ACCAGGATGTTGATGGAAGA R: TACAGCCCCAAGCAAAATAC	136	57
18	Abscisic stress-ripening, putative	LOC_Os11g06720	F: CCTTCGCCCTGTATGAGAA R: TCCTTCTTCTTCTCGTGGTG	136	58
19	Monodehydroascorbate reductase	LOC_Os09g39380	F: GTGTTTCCTGAACCTTGGTG R: CATCACCATTAGCATCAGCA	142	58
20	OsAPx1 - Cytosolic Ascorbate Peroxidase encoding gene 1-8	LOC_Os03g17690	F: AGGGACCTTGGACAAGAAAC R: CAGGGCTTTGTCACTAGGAA	107	58
21	OsAPx2—Cytosolic Ascorbate Peroxidase encoding gene 4,5,6,8	LOC_Os07g49400	F: ACGTCCAACCCTTTGATCTT R: GCAGCATATTTCTCCACCAG	140	58
22	CAMK_CAMK_like.7—CAMK includes calcium/calmodulin dependent	LOC_Os07g42940	F: ATCTTCTGTCCCGGATTTTC R: TGTTGATGTCGTTCATCTGG	144	58
23	Phox domain-containing protein	LOC_Os01g61150	F:GAGGAGGAAACTGATGCAAA R: CCCAGTTGACCTGTAGTTGC	140	58
24	Glutamine synthetase, catalytic domain-containing protein	LOC_Os02g50240	F: GAGAAGATCATCGCCGAGTA R: AGCCATCGTAGTTCCACTTG	125	57
25	Enoyl-CoA hydratase/isomerase family protein	LOC_Os01g54860	F: TCTCAGCATCGACAAAGACA R: GAATCGTCAGATGGGACTTG	132	58
26	Aminocyclopropane-1-carboxylate oxidase 1	LOC_Os09g27750	F:GTGGAGAAGATGACCAAGGA R: GAAGAAGGTGCTCTCCCAGT	105	58
27	Cysteine synthase, putative	LOC_Os03g53650	F: CGGAACAGGAAAGTACCTCA R: TTTTGTGTGGACCAGGTCTT	137	58

relative expression levels, cDNAs from three independent biological samples in two technical replications each were used. PCR was initiated with a pre-incubation at 50 °C for 2 min and denaturation at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 15 s and annealing and extension at 60 °C for 1 min. A melt curve analysis was done to determine the specificity of the reaction. After normalization, quantity of each mRNA was calculated from the threshold points located in the log-linear range. The data from different PCR runs or cDNA samples were compared by using mean CT

values of the biological replicates that was normalized to the mean of CT values of the endogenous control gene. The relative standard curve method was used for the quantification of mRNA levels and displayed as relative expression values (REV). Expression ratios were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). The data were analyzed using the 7500 Sequence Detection Software (Applied Biosystems, USA) with default baseline and threshold. Mean values were compared pairwise between the time points within treatments with Tukey's HSD test and $P<0.05$ was considered as level of significance (SAS Institute Inc., version 4.3, Cary, USA). Data was presented as Relative Expression Values.

Results

Structure of SSH cDNA library

In all, 3000 clones from the Aganni library were picked and PCR amplified using M13 forward and reverse primers and sequenced. Of these, 2,800 ESTs were further considered for sequence assembly after removing low-quality sequences or those with very small inserts (<50 bp) or those clones without any inserts. The high-quality ESTs were assembled into contigs using MacVector program. These high-quality sequences had an average read length of 450 bp. Of these, 258 sequences were singletons whereas rest of the sequences assembled into 190 contigs (Table 2 and Electronic supplementary material (ESM) Table S1). All the 1,405 unique ESTs have been submitted to the EST database of GenBank (http://www.ncbi.nlm.nih.gov/dbEST) (GenBank ID: JZ546410–JZ547814). This library was used to elucidate transcriptional changes and identify transcripts that showed differential responses in rice variety Aganni triggered by the infestation with the rice gall midge biotype 4 (GMB4).

Sequencing and data analysis

Based on BlastX and BlastN homology searches of the 387 non-redundant sequences of the Aganni library, 282 (73 %) clones were homologous to genes with known function, 60 (15 %) clones were homologous to genes with unknown function and named as hypothetical proteins. Of the rest, 15 (4 %) clones did not match any known sequences in the rice database, but had matched sequences reported from other crops such as wheat, maize, mustard, and also from other species such as green algae and bacteria whereas 30 (8 %) clones did not show any hit with sequences in the NCBI database (Fig. 1, Table 2).

Table 2 A comparative analysis of SSH cDNA libraries developed form three rice varieties to highlight differential gene expression following gall midge infestation

S. no.	Data analysis	Number in		
		Aganni	Suraksha[a]	TN1[b]
1	Clones	3,000	1,930	1,450
2	Positive clones	2,800	841	552
3	Singletons	258	584	309
4	Contigs	190	257	243
5	Known function	282	615	370
6	Unknown function	60	86	48
7	No hits in rice	15	27	23
8	No hits in NCBI data base	30	12	0

[a] Rawat et al. (2013)

[b] Rawat et al. (2012b)

Gene ontology-based functional annotation

Based on gene ontology (GO) annotation, EST sequences were grouped into three categories, viz., biological process, molecular function, and cellular component. However, some ESTs were annotated in two or all the three categories. Among the ESTs under biological process, large number of ESTs pertained to biosynthetic process and translation and protein turnover (Fig. 2a). Under molecular function category, ESTs related to enzyme activity, ion binding followed by DNA binding and transferase activity were abundant (Fig. 2b). In the case of cellular component, high number of ESTs was found in membrane and intracellular group (Fig. 2c). A comparative view of the data with respect to earlier reports on Suraksha (Rawat et al. 2013) and TN1 (Rawat et al. 2012b) EST libraries is presented in supplementary figures (ESM Fig. 1A, B, C). It is evident from the data that the Aganni library had unique features, i.e., nine sub-categories, under biological process 13 sub-categories under molecular function and 13 sub-categories under cellular component that were uniquely represented in the Aganni library.

ESTs classified based on function

To obtain better understanding of the putative functions of the ESTs, these were further classified into 12 different groups, within each gene ontology categories. Four groups of ESTs namely transport, translation and protein turnover, metabolic process, and biosynthetic process were found to be in a majority in the Aganni library (Table 3). In all, 57 ESTs were present in transport group that code for monodehydroascorbatereductase, amino acid transporter, and related transporter family proteins (ESM Table S2). In translation and protein turnover group, 70 ESTs coding for

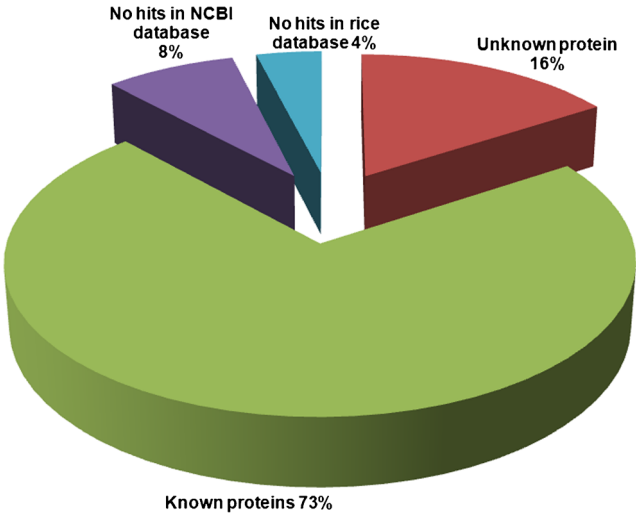


Fig. 1 Distribution of ESTs (based on sequence homology) in the SSH cDNA library prepared for rice variety Aganni challenged with GMB4

peptidylprolylcis/trans isomerase, calcium/calmodulin-dependent protein kinases, and heat shock protein were present (ESM Table S3). In the metabolic process group, 42 ESTs coding for serine hydroxymethyltransferase, acyl-CoA, erythronate-4-phosphate dehydrogenase, glutamine synthetase, cysteine synthase, and others were high in number (ESM Table S4). In the biosynthetic process group, 31 ESTs coding for ketol-acid reductoisomerase, conserved peptide U ORF-containing transcript were present (ESM Table S5).

ESTs in another set of four groups relating to transcription and modification, defense related, signal transduction, and DNA structure, synthesis, and cell organization were also abundant in the Aganni library. In the transcription group ESTs coding for NAC domain-containing protein, WW domain-containing protein and auxin-responsive protein were present (ESM Table S6). In the defense related group, ESTs coding for abscisic stress-ripening, pathogenesis-related Bet v I family protein and plasminogen activator inhibitor (ESM Table S7) were present. In the signal transduction group 14 ESTs were present. These ESTs code for COP9 signalosome complex subunit 5b, protease inhibitor, and histidine-containing phosphotransferase protein (ESM Table S8). In the DNA structure, synthesis, and cell organization, group ESTs coding for FACT complex subunit SPT16, ABC transporter, ATP-binding protein were present (ESM Table S9). The third set of four groups such as cell wall metabolism, cell redox, primary metabolism, and homoiothermy had relatively lower number of ESTs in Aganni. In the cell wall metabolism group, eight ESTs related to phox domain-containing protein and MLO domain-containing protein were present (ESM Table S10). In Cell redox group, 19 ESTs coding for peroxidase precursor and glyceraldehyde 3 phosphate dehydrogenase were present (ESM Table S11). In primary metabolism group, nine ESTs coding for aspartic proteinase, transporter family protein (ESM Table S12) were present. In homoiothermy group eight ESTs related to AP2 domain-containing protein and membrane associated DUF588 domain-containing protein were present (ESM Table S13). Significantly, no EST was detected under cell signaling and secondary metabolism groups.

Chromosomal distribution of ESTs

The ESTs identified as being up-regulated during the rice gall midge infestation and whose homology was determined by using BlastN against the rice annotation database (TIGR) and Gramene database were mapped (*in silico*) to the rice chromosomes. Highest number of ESTs from the Aganni library mapped to chromosome 3 followed by those mapping to chromosome 1, 5, 4, 2, and 12, each consisting of 56, 47, 32, 31, 26, and 21 ESTs, respectively. In contrast, less than 20 ESTs mapped on to the remaining chromosomes 6, 7, 9, 10, and 11; while only 15 ESTs mapped to chromosome 8.

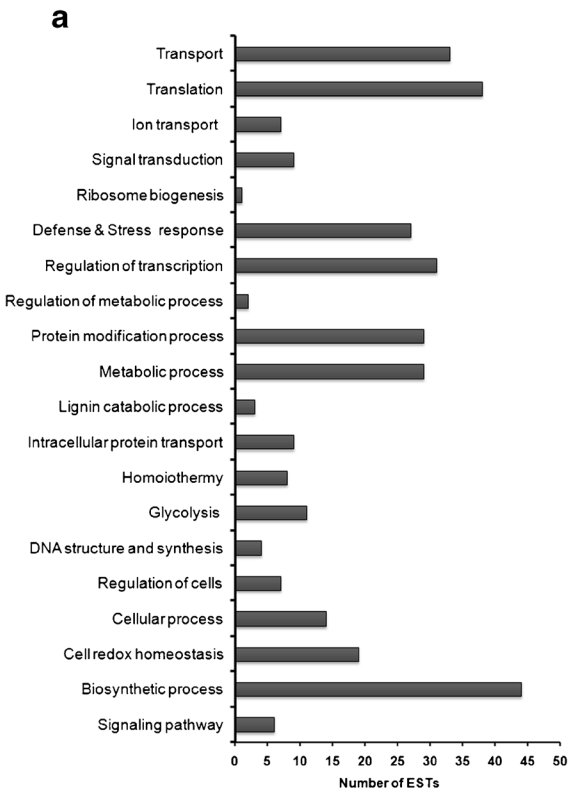
Validation of candidate gall midge resistance genes through real-time reverse transcription PCR (rtPCR)

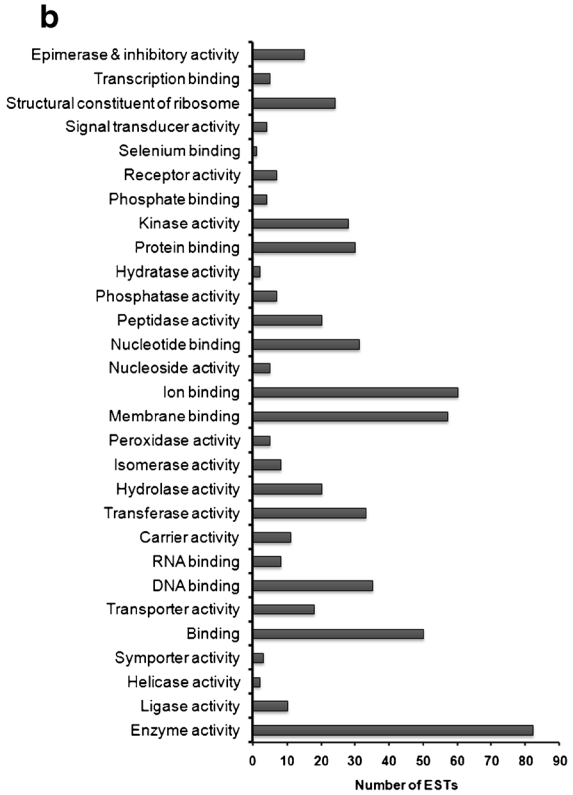
Relative expression of 27 ESTs, picked from the above library, in response to rice gall midge infestation was studied in real-time expression in TN1, Aganni, 34S, and 74R rice lines at various time points after gall midge infestation (Table 1). The basis of selecting these genes has been their reported role in plant stress management based on recent literature (ESM Table S14).

Genes induced in Aganni and 74R

Eight of the ESTs were found significantly up-regulated in both Aganni and 74R at 24 hai when compared with their respective uninfested plants (Table 4). These were (1) abscisic stress-ripening-ASR (contig 374), (2) NAC domain (contig 368), (3) peptidylprolylcis/trans isomerase- PPI (contig 425), (4) glutamine synthetase-GS (contig 478), (5) serine hydroxymethyltransferase-SHMT (contig 358), (6) WW domain-containing protein (contig 415), (7) OsAPx1—cytosolic ascorbate peroxidase encoding gene 1–8 (contig 399), (8) plasminogen activator inhibitor- PAI (contig 456). Expression of these genes decreased at 120 hai (Fig. 3a, b). Only one EST, (9) pathogenesis-related Bet v I family was induced (contig 458, Table 4) in Aganni and 74R at

Fig. 2 Distribution of ESTs from Aganni SSH cDNA library based on the functional classification, into the three principal Gene Ontology (GO) categories: **a** biological process, **b** molecular function, **c** cellular component. Values represent the number of ESTs found under each sub-category under the three main GO categories


a
Transport
Translation
Ion transport
Signal transduction
Ribosome biogenesis
Defense & Stress response
Regulation of transcription
Regulation of metabolic process
Protein modification process
Metabolic process
Lignin catabolic process
Intracellular protein transport
Homoiothermy
Glycolysis
DNA structure and synthesis
Regulation of cells
Cellular process
Cell redox homeostasis
Biosynthetic process
Signaling pathway
0 5 10 15 20 25 30 35 40 45 50
Number of ESTs


b
Epimerase & inhibitory activity
Transcription binding
Structural constituent of ribosome
Signal transducer activity
Selenium binding
Receptor activity
Phosphate binding
Kinase activity
Protein binding
Hydratase activity
Phosphatase activity
Peptidase activity
Nucleotide binding
Nucleoside activity
Ion binding
Membrane binding
Peroxidase activity
Isomerase activity
Hydrolase activity
Transferase activity
Carrier activity
RNA binding
DNA binding
Transporter activity
Binding
Symporter activity
Helicase activity
Ligase activity
Enzyme activity
0 10 20 30 40 50 60 70 80 90
Number of ESTs

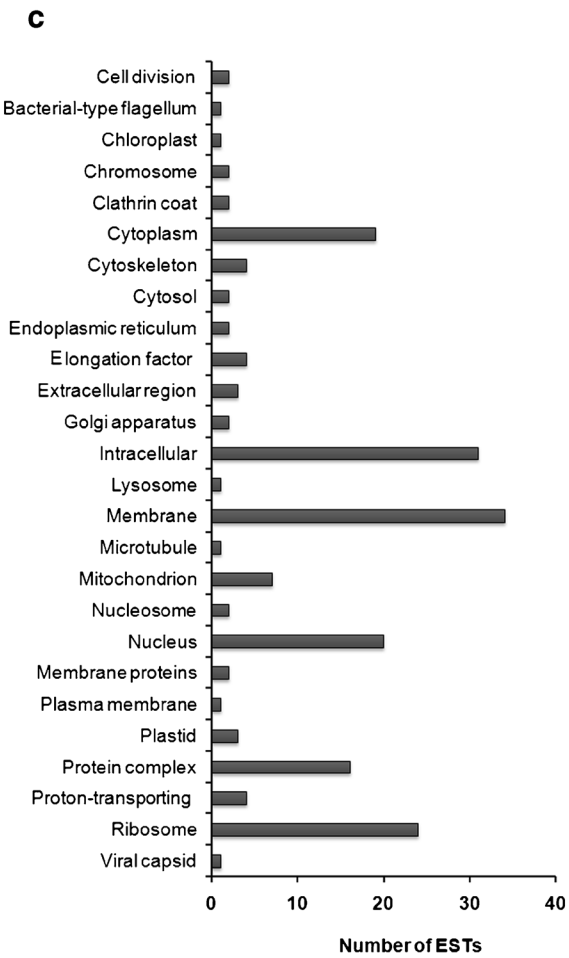

c
Cell division
Bacterial-type flagellum
Chloroplast
Chromosome
Clathrin coat
Cytoplasm
Cytoskeleton
Cytosol
Endoplasmic reticulum
Elongation factor
Extracellular region
Golgi apparatus
Intracellular
Lysosome
Membrane
Microtubule
Mitochondrion
Nucleosome
Nucleus
Membrane proteins
Plasma membrane
Plastid
Protein complex
Proton-transporting
Ribosome
Viral capsid
0 10 20 30 40
Number of ESTs

Table 3 Relative abundance of ESTs under different functional groups detected in three SSH cDNA libraries

S. no.	Group	Number of ESTs in SSH cDNA library of		
		Aganni Incompatible	Suraksha[a] Incompatible	TN1[b] Compatible
1	Transport	57	22	14
2	Translation and protein turnover	70	122	100
3	Metabolic process	42	0	0
4	Biosynthetic process	31	0	0
5	Transcription and modification	27	59	28
6	Defense related	27	33	17
7	Signal transduction	14	20	13
8	DNA structure, synthesis, and cell organization	15	44	19
9	Cell wall metabolism	8	15	13
10	Cell redox	19	4	9
11	Primary metabolism	9	39	33
12	Homoiothermy	8	0	0
13	Cell signaling	0	33	16
14	Secondary metabolism	0	9	5

[a] Rawat et al. (2013)

[b] Rawat et al. (2012b)

120 hai. However, there was no induction observed at 24 hai (Fig. 4a, b).

Genes induced only in 74R

Five ESTs were induced only in 74R at 24 hai. These were (1) erythronate-4-phosphate dehydrogenase-EDPP (contig 401, 3.05-fold), (2) cysteine synthase-CS (GenBank ID: JZ546692, 2.96-fold), (3) acyl-CoA synthetase (GenBank ID: JZ547652, 2.76-fold), (4) aspartic proteinase (AP) oryzasin-1 (contig 451, 2.55-fold) and (5) OsAPx2 - Cytosolic Ascorbate Peroxidase encoding gene 4,5,6,8 (contig 404, 2-fold) (Fig. 5a, b). Another five ESTs viz., (1) leucine-rich repeat (LRR) family protein (GenBank ID: JZ546538, 3.58-fold), (2) amino acid transporter (contig 497, 3.38-fold), (3) Phox domain-containing protein (contig 461, 2.30-fold), (4) heat shock protein-HSP (GenBank ID: JZ547665, 2.28-fold) and (5) COP9 signalosome complex subunit 5b (GenBank ID: JZ546688, 2.10-fold) (Fig. 6a, b) were induced at 120 hai.

Genes induced inTN1 and 34S

One EST, i.e., peptidyl prolylcis/trans isomerase (contig 425), registered 2.07- and 2.33-fold increase, respectively, in transcription levels in TN1 and 34S line at 24 hai, when compared with the respective uninfested plants (Table 5). Another three ESTs were found to be induced only in 34S line at 24 hai viz., abscisic stress-ripening (contig 374, 2.62-fold), aspartic proteinase oryzasin-1 (contig 451, 2.04-fold) and erythronate-4-phosphate dehydrogenase (contig 401, 2.03-fold) (Fig. 7a, b). None of EST was found up-regulated at 120 hai in this set of plants.

Genes with low level of induction

In addition to the 19 genes listed above, remaining eight genes registered low level of induction (≤2.0-fold) in the genotypes tested (ESM Fig. 2A, B).

Discussion

Plants have evolved diverse defense mechanisms to protect themselves from various biotic stresses. They have the ability to detect invading pathogen/insect and respond with inducible defenses by producing toxic chemicals and invoking deliberate cell death—apoptosis. Generally, defense against the biotrophic pathogens and some phloem feeding insects is associated with the SA pathway (Hua 2009) and that against the necrotrophic pathogens and leaf-feeding insects with JA pathway (Turner et al. 2002). Pathogenesis-related (PR) genes are associated with plant defense against both pathogens and insects along with initiation of signal transduction processes related to induction of systemic acquired resistance (SAR) (Van Loon 1997). Since the interactions between plant and invading pathogen/insect are dynamic and constantly subjected to coevolving selection pressures, several alternative

Table 4 Relative expression values for the selected ESTs at 24 and 120 in Aganni and 74R upon infestation by GMB1 compared with respective uninfested controls

S. no	Gene name	Locus ID	Fold values after gall midge infestation in			
			Aganni		74R	
			24 hai	120 hai	24 hai	120 hai
1	Abscisic stress-ripening	LOC_Os11g06720	4.66	0.97	9.92	0.54
2	NAC domain-containing protein	LOC_Os03g60080	3.98	0.29	4.12	0.16
3	Peptidyl-prolyl *cis*-trans isomerase	LOC_Os08g44520	3.87	1.46	3.82	0.19
4	Glutamine synthetase, catalytic domain-containing protein	LOC_Os02g50240	2.51	0.4	3.23	0.82
5	Serine hydroxymethyltransferase,	LOC_Os11g26860	2.21	0.73	2.37[a]	1.75[a]
6	WW domain-containing protein	LOC_Os06g19470	2.17	0.31	3.53	0.32
7	OsAPx1—cytosolic ascorbate peroxidase	LOC_Os03g17690	2.05[a]	1.3[a]	2.44	0.6
8	Plasminogen activator inhibitor 1	LOC_Os05g45660	2.09	1.37	2.02	1.06
9	Pathogenesis-related Bet v I family protein	LOC_Os08g28670	0.85	5.37	0.44	2.5
10	Erythronate-4-phosphate dehydrogenase	LOC_Os11g26850	1.75[a]	1.69[a]	3.05	1.53
11	Cysteine synthase	LOC_Os03g53650	1.16	1.86	2.96	0.57
12	Acyl-CoA synthetase protein	LOC_Os05g25310	1.56	0.71	2.76	0.98
13	Aspartic proteinase oryzasin-1	LOC_Os05g49200	1.38[a]	0.52[a]	2.55	0.77
14	OsAPx2—cytosolic ascorbate peroxidase	LOC_Os07g49400	1.92	0.8	2	0.95
15	Leucine-rich repeat family protein	LOC_Os11g42660	0.56	1.97	0.08	3.58
16	Amino acid transporter	LOC_Os01g66010	0.53[a]	0.83[a]	0.32	3.38
17	Phox domain-containing protein	LOC_Os01g61150	0.6[a]	0.66[a]	0.29	2.3
18	Heat shock protein	LOC_Os12g32986	0.48	1.86	0.35	2.28
19	COP9 signalosome complex subunit 5b	LOC_Os04g56070	0.8	1.63	0.85[a]	2.1[a]

hai hours after infestation

[a] Not significantly different between two time points(Turkey's HSD test; $P \geq 0.05$)

defense pathways are involved in the same plant species against same or similar group of organisms. This is evident from the fact that multiple R genes, often representing multiple pathways and present in the same host (Ellis and Jones 2003), are effective against the same pathogen/insect, e.g., over 60 genes are reported in rice conferring resistance against blast (*Magnaporthe oryzae*) (Chen et al. 2005), over 38 genes against bacterial blight (*Xanthomonas oryzae* pv *oryzae*) (Natrajkumar et al. 2012), over 27 genes against the brown planthopper, *Nilaparvata lugens* (Huang et al. 2013; Fujita et al. 2013), and 11 genes against the gall midge (Yasala et al. 2012). Despite the knowledge that these gall midge resistance genes confer a varying spectrum of resistance, against the seven known biotypes of the pest, and differ in expression of HR, the commonality, or diversity in terms of pathway(s) involved in the manifestation of resistance is yet to be elucidated.

Our recent studies involving gene cloning and characterization (Sama et al. 2014) or through analysis of differential gene expression profiles of genes identified through SSH cDNA libraries (Rawat et al. 2012b; Rawat et al. 2013) or microarrays (Rawat et al. 2012a) have revealed diversity in defense pathways triggered by some of the key R genes of rice involved in conferring resistance to gall midge. A comparative analysis of SSH cDNA libraries developed for the rice variety Suraksha (with *Gm11* gene conferring HR+ type resistance), TN1 with no gall midge resistance genes and Aganni (with *Gm8*) (Table 2) revealed relatively lower percentage (73 %) of genes with known function in Aganni as compared with that in Suraksha (83 %) or TN1 (84 %). As observed in the case of Suraksha, Aganni too had a higher percentage of ESTs that mapped on to chromosome 3. But Aganni library revealed highest percentage (6.7 %) of ESTs with no homologies to sequences in the NCBI database. Gene ontology and functional annotation (Table 3) revealed highest proportion of ESTs under translation and protein turnover group in all of them. However, cell signaling and secondary metabolism related ESTs were conspicuously absent in Aganni library. Understandably, defense related ESTs were more abundant in Aganni and Suraksha as compared with those in TN1 library. Significantly, ESTs related to catabolic process and homoiothermy were present only in the Aganni library. In view of a large number of distinct ESTs under several subgroups of all the three categories of GO annotation (ESM Fig. 1A, B, C),

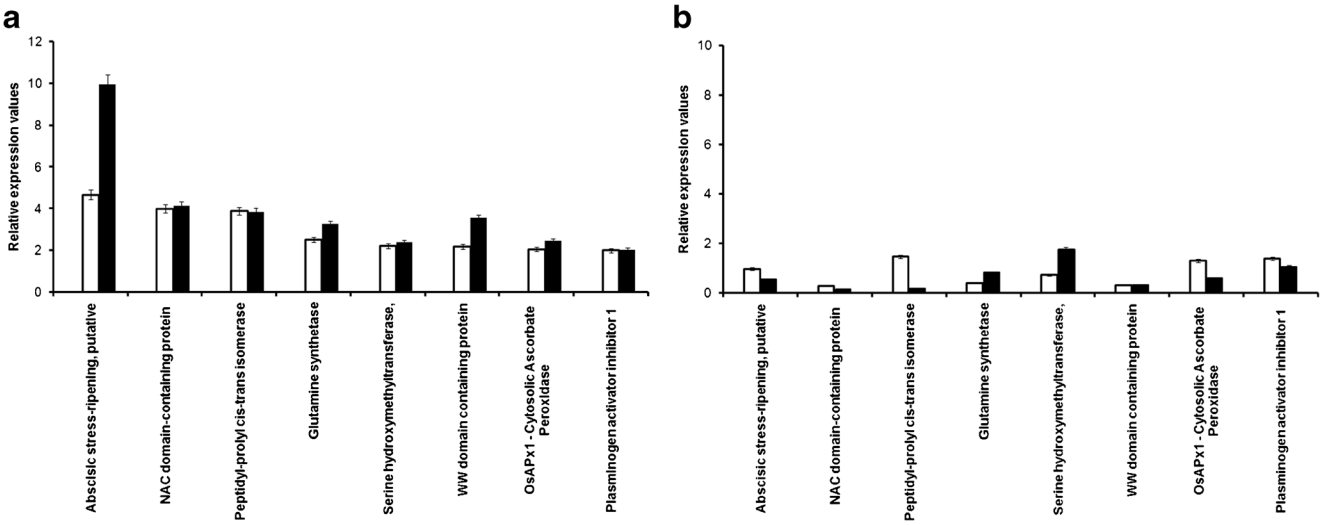


Fig. 3 Relative expression profiles of 8 ESTs induced in Aganni and 74R (≥2-fold) when compared with the respective uninfested control **a** at 24 hai and **b** at 120 hai. *Error bars* represent mean ± S.D. *Light bars* represent Aganni-GMB1 and *dark bars* 74R-GMB1 incompatible interactions, respectively

Aganni library was unique compared with other genotypes TN1 and Suraksha studied earlier (Rawat et al. 2012b; Rawat et al. 2013).

Recent paradigm shift in gene-for-gene hypothesis of plant defense (Stuart 2015) elaborates four phases of defense. Basal immunity is primarily directed at evading host un-adapted pathogens and triggered by pathogen associated molecular patterns (PAMPs). However, the first line of defense against host-adapted pathogens is mediated by effector triggered immunity (ETI). The main class of genes in pathogen elicitor recognition is nucleotide-binding site leucine-rich repeat (NBS-LRR) (Marone et al. 2013). This immunity is race specific. The diversity of plant defense against a wide range of pathogens and their races, also insects and their biotypes, is reflected in presence of hundreds of such genes in any plant genome, as exemplified in rice above. Generally the plant LRR protein coding R genes are race specific and also associated with hypersensitive response mediated induced resistance (Belkhadir et al. 2004). There have been several recent studies elaborating on structure based specificity among such genes. The NBS-LRR proteins are large, abundant proteins involved in the plant defense through detection of diverse pathogens, including bacteria, viruses, fungi, nematodes, insects, and oomycetes (McHale et al. 2006). NBS-LRR proteins are classified into two sub groups. The first subgroup consists of proteins that have a distinct N-terminal region (TIR domain) that resembles the cytoplasmic domains of the *Drosophila* protein Toll and the mammalian interleukin-1 receptor protein. The proteins of the second subgroup lack this region but may have a leucine zipper or coiled coil-domain in the N-terminal region (Pan et al. 2000). NBS and TIR domains have a conserved signaling function but LRR domain is involved in the pathogen recognition by either direct interaction with avr protein or indirectly by binding to the protein complex between avr protein and other host proteins (Kobe and Diesenhofer 1995). Rice genome has 653 NBS-LRR genes (Marone et al. 2013). Rice blast resistance genes *Pi50*, *Pi-ta*, *Pi9*, and *Pi54* encode NBS-LRR proteins (Su et al. 2015; Bryan et al. 2000; Qu et al. 2006; Ramkumar et al. 2011). Three of the bacterial blight (BB) resistance genes, *Xa1*, *Xa21,* and *Xa26* are also members of the NBS-LRR family (Hu et al. 2015; Sun et al. 2004). Two of the brown planthopper resistance genes *Bph14* and *Bph26* encode coiled coil-nucleotide binding leucine-rich repeat (CC-NB-LRR) protein (Du et al. 2009; Tamura et al. 2014). Seven nucleotide

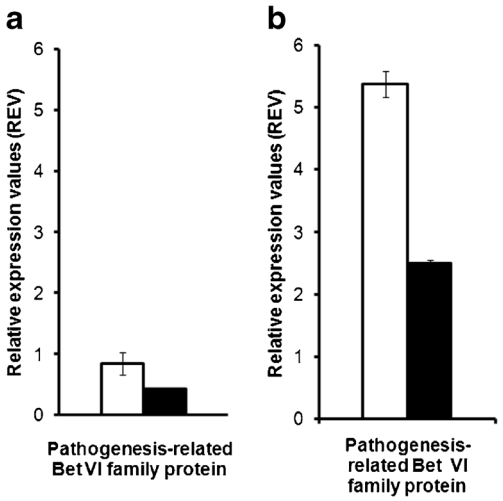


Fig. 4 Relative expression profile of one EST induced in Aganni and 74R (≥2-fold) when compared with the respective uninfested control **a** at 24 hai and **b** at 120 hai. *Error bars* represent mean ± S.D. *Light bars* represent Aganni-GMB1 and *dark bars* 74R-GMB1 incompatible interactions, respectively

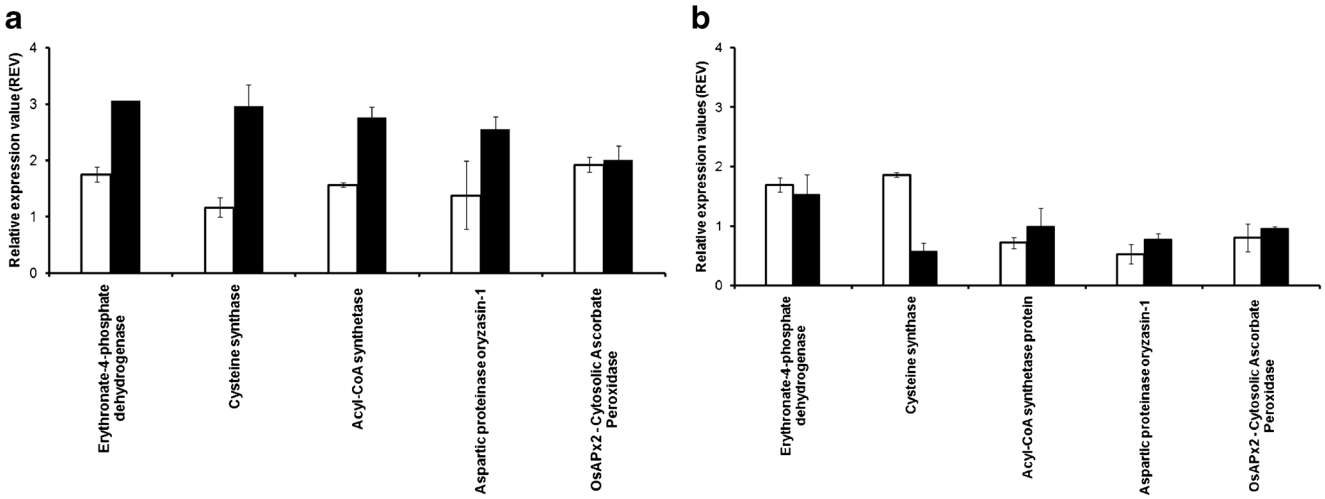


Fig. 5 Relative expression profiles of 5 ESTs induced only in 74R (≥2-fold) when compared with the respective uninfested control **a** at 24 hai and **b** at 120 hai. *Error bars* represent mean ± S.D. *Light bars* represent Aganni-GMB1 and *dark bars* 74R-GMB1 incompatible interactions, respectively

binding (NB) and/or LRR genes are reported in the rice genome segments with gall midge resistance genes *Gm2*, *gm3*, *Gm4*, *Gm6*, *Gm7*, and *Gm11* (Yasala et al. 2012) all conferring HR+ type resistance. Of these genes, NB-ARC and NBS-LRR have been functionally validated, respectively, as the candidate gene for *gm3* and *Gm4* (Sama et al. 2014; Divya et al. 2015). In the present study one of the LRR genes (LOC_Os11g42660) was found up-regulated in incompatible interaction (Fig. 6b), though it is not located on chromosome 8 to which the *Gm8* gene has been mapped (Sama et al. 2012). LRR genes require trans-partners to be effective and several heat shock proteins (HSP) act as such trans-partners (Belkhadir et al. 2004). Heat shock proteins (HSP) belong to a multigenic family and are known to function as chaperones, which assists protein folding in response to environmental stress (Sarkar et al. 2013; Mayer and Bukau 2005). HSP 70 family plays an important role in protecting the rice plants from heat stress (Jung et al. 2013). Genome wide interactions of HSPs in rice revealed Hsp70s to be involved in CHO metabolism, innate immunity, and photosystem II repair (Wang et al. 2014). Heat shock proteins play a central role in the early sensing of H_2O_2 in plants (Miller and Mittler 2006) and complement the action of NBS-LRR protein (Kadota and Shirasu 2012), e.g., in *Arabidopis* (Hubert et al. 2003) tobacco (Liu et al. 2004); and potato (Lu et al. 2003). In our earlier study, a HSP gene (LOC_Os03g16860) was noted to be up-regulated

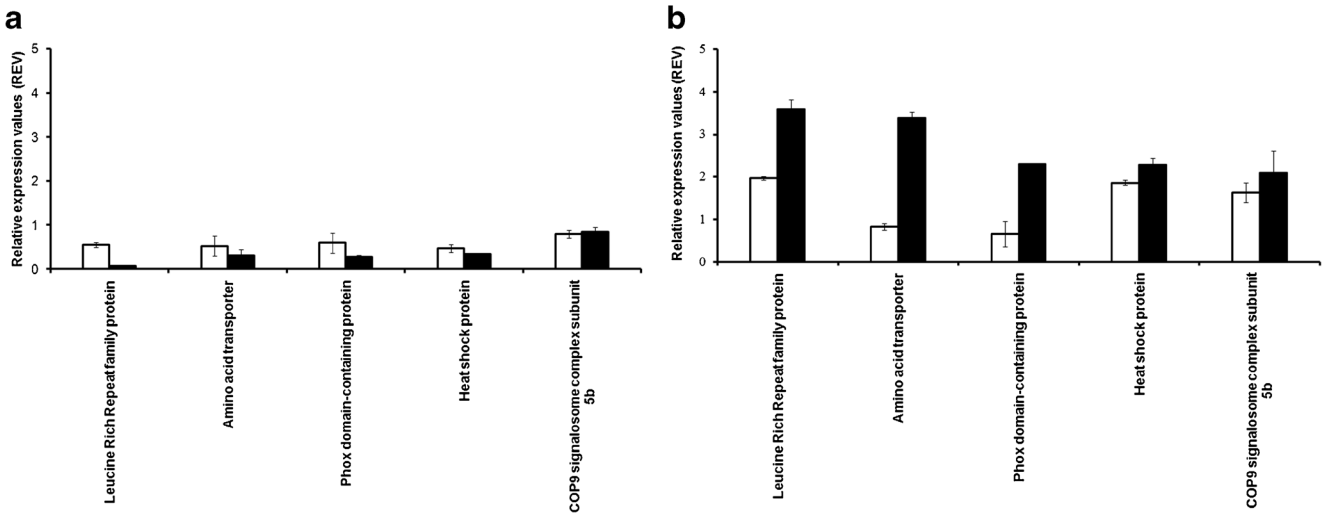


Fig. 6 Relative expression profiles of another five ESTs induced only in 74R (≥2-fold) when compared with the respective uninfested control **a** at 24 hai and **b** at 120 hai. *Error bars* represent mean ± S.D. *Light bars* represent Aganni-GMB1 and *dark bars* 74R-GMB1 incompatible interactions, respectively

Table 5 Relative expression values for the selected ESTs at 24 and 120 in TN1 and 34S upon infestation by GMB1 compared with respective uninfested controls

S. no	Gene name	Locus ID	Fold values after gall midge infestation			
			TN1		34S	
			24 hai	120 hai	24 hai	120 hai
1	Peptidyl-prolyl *cis*-trans isomerase	LOC_Os08g44520	2.07	0.4	2.33	0.08
2	Abscisic stress-ripening, putative	LOC_Os11g06720	0.74[a]	0.6[a]	2.62	0.67
3	Aspartic proteinase oryzasin-1 precursor	LOC_Os05g49200	0.74	0.2	2.04	0.47
4	Erythronate-4-phosphate dehydrogenase	LOC_Os11g26850	1.65	0.35	2.03	0.42

hai hours after infestation

[a] Not significantly different between two time points(Turkey's HSD test; $P \geq 0.05$)

in Suraksha at 120 hai (Rawat et al. 2013). At present, another HSP (LOC_ Os12g32986) gene was induced following gall midge infestation (Fig. 6b).

Recognition of invading pathogen by LRR gene is immediately followed by ion flux in the target cells triggering production of reactive oxygen species (ROS), such as hydrogen peroxide, superoxide radical, and singlet oxygen, often leading to expression of hypersensitive reaction (HR). HR results in death of host cells invaded by pathogen/insect. One of the genes, likely to be involved in calcium dependent oxidative burst, CAMK_CAMK_like.7—CAMK was not found induced in this study (ESM Fig. 2A, B). Reactive oxygen species (ROS) /oxidation reduction can be activated during the course of normal aerobic metabolism or when plant is exposed to a variety of stress conditions. A Phox domain-containing protein coding gene was involved in ROS production in *Arabidopis* (Torres et al. 2002). A gene of this family was also found up-regulated in our study (Fig. 6b). However, ascorbate peroxidases play a major role in H_2O_2 scavenging to prevent the plant from ROS damage (Zou et al. 2005). Two cytosolic APX isoforms (*OsAPX1* and *OsAPX2*) have been characterized for their role in rice plant defense. *OsAPX1/2* knockdown plants (AP1/2s) exhibited enhanced accumulation of H_2O_2 (Ribeiro et al. 2012). In the present study *OsAPx1*—Cytosolic Ascorbate Peroxidase encoding gene 1–8 and *OsAPx2*—Peroxidase encoding gene 4, 5, 6, 8 were upregulated (Fig. 5a, b) suggesting that ROI scavenging enzymes successfully prevented ROS damage in Aganni and prevented HR. Rice genotypes resistant to gall midge display diversity in HR expression. Those genotypes that express HR showed higher expression of LRR, HSP, and PR genes (Rawat et al. 2013); while in Kavya with *Gm1* gene that did not express HR, these genes were not up-regulated (Rawat et al. 2012b). Hence, it is of interest to note that Aganni with *Gm8*

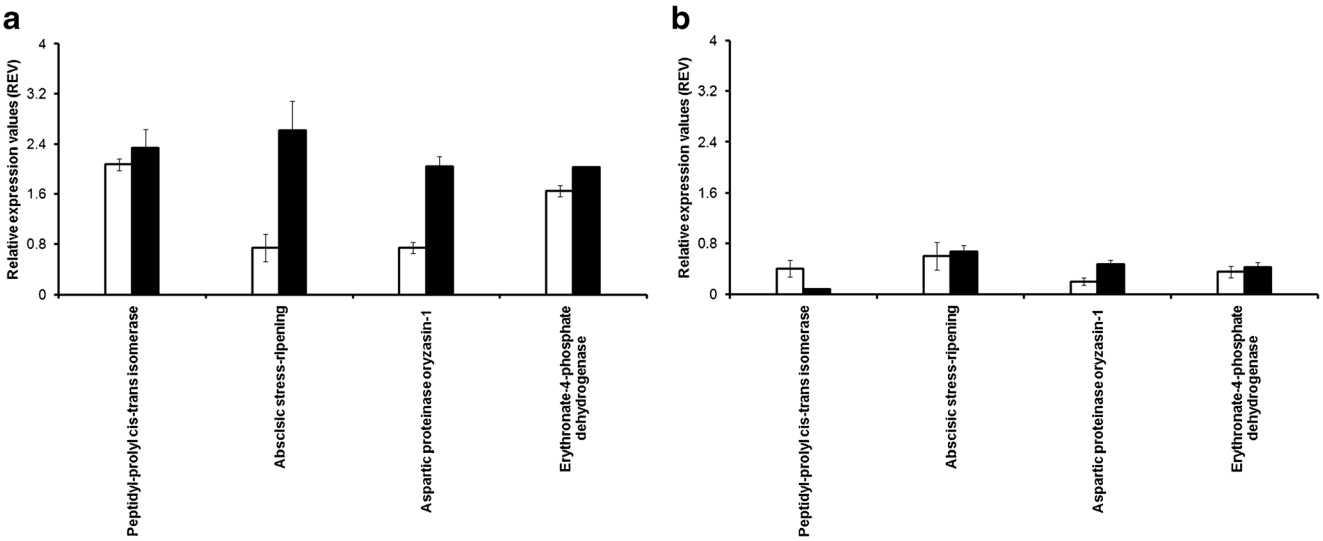


Fig. 7 Relative expression profiles of 4 ESTs induced in TN1 and in 34S (≥2-fold) when compared with the respective uninfested control **a** at 24 hai and **b** at 120 hai. *Error bars* represent mean ± S.D. *Light bars* represent TN1-GMB1 and *dark bars* 34S-GMB1 compatible interactions, respectively

gene did show enhanced expression of LRR, HSP, and PR genes but did not express HR. We associate this with enhanced activity of two of the three genes tested relating to ROS scavenging activity viz., *OsAPx1* and *OsAPx2*. However, the third gene, MDHAR (monodehyroxiascorbate reductase) did not show enhanced levels of activity (ESM Fig. 2A, B). MDHAR is a key enzyme in the oxidative stress in the plants. This enzyme involved in the ascorbate recycling in the ascorbate-glutathione cycle is important in plants against abiotic stresses (Do et al. 2014). *TaMDHAR* gene is reported to be involved in the resistance of wheat against diseases by regulating the ROS level. The knock down of this gene resulted in higher accumulation of H_2O_2 and lower APX activity (Feng et al. 2014). Yet another mechanism has been noted in rice line RP2068-18-3-5 carrying the recessive *gm3* gene and expressing HR-mediated resistance (Agarrwal et al. unpublished). Following gall midge infestation in this rice, there is enhanced expression of ascorbate peroxidase coding genes which mainly scavenge H_2O_2. However, HR in this rice is likely to be induced by singlet oxygen (Agarrwal et al. unpublished) produced by photosystem II when energy is transferred from excited chlorophyll to oxygen (Krieger-Liszkay 2005).

Once the first line of defense accomplishes the mortality of invading batch of pathogens, plant defense involves triggering of systemic acquired resistance (SAR). In systemic acquired resistance (SAR), the signal is transmitted from the infected tissue into the whole plant for induction of overall defense gene expression (Ryals et al. 1994). This step involves triggering of several transcription factors (TF) that induce genes involved in plant hormone metabolism, cell signaling, pathogenesis-related protein production and subsequently those involved in primary metabolism. At least two of the five TF genes covered in this study (NAC domain-containing protein 67, WW domain-containing protein) showed rapid induction following insect attack (Fig. 3a). In plant genomes, approximately 7 % of the coding sequences consist of transcriptional factors which proves the complexity of transcriptional regulation (Udvardi et al. 2007). NAC domain-containing proteins respond to pathogen infection (Ren et al. 2000; Selth et al. 2005; Xie et al. 1999) and to abiotic stresses (Hegedus et al. 2003; Tran et al. 2004). There are about 75 NAC domain-containing TFs in rice (Kikuchi et al. 2000). NAC19 gene in rice was strongly induced by rice blast fungus infection and after extraneous application of methyl-jasmonate and ABA (Lin et al. 2007). NAC domain-containing protein is one of the regulators in ABA pathway which leads to the up-regulation of this gene. Several classes of NAC transcriptional factors regulate the secondary cell wall formation in rice (Yoshida et al. 2013). A mutant gene CFL impairs cuticle development in rice. The wild type of this gene codes for WW domain-containing protein (Wu et al. 2011). More recently, WW domain-containing proteins were shown to regulate virus replication by acting as cell-intrinsic restriction factor (CIRF) in tomato (Barajas et al. 2015). One of the genes covered in this study, identified as TF under GO category, CIGR—chitin-inducible gibberellin-responsive protein (LOC_OS01g65900) has been identified as the candidate gene *ph1* affecting plant height and closely linked to *sd1* gene (Kovi et al. 2011). However, this TF did not get induced in the present study (ESM Fig. 2A) though it was implicated in compatible interaction earlier in TN1 (before Rawat et al. 2012b). Likewise, auxin-responsive Aux/IAA gene family member (Rawat et al. 2012a) and bZIP transcription factor domain-containing protein gene did not respond to insect infestation in Aganni. The basic leucine zipper (bZIP) domain-containing transcription factors (TFs) function as key regulators of cellular growth and differentiation in eukaryotic organisms. Till now 19 bZIP TFs have been characterized in rice. One of such TF has been associated in compatible interaction with blast fungus (Tang et al. 2015).

Genes involved in plant hormone metabolism and covered in the present studies were those coding for abscisic stress ripening (ASR) protein, COP9 signalosome complex subunit 5b (CSN5B), Aminocyclopropane-1-carboxylate oxidase 1and Enoyl-CoA hydratase/isomerase. While roles of salicylic acid and jasmonic acid in conferring plant resistance against two distinct guilds of pests are known, role of other hormones either in inducing or modulating these pathways is now being reported. ABA-related gene induces susceptibility in rice against *Xanthomonas oryzae* and *Magnaporthe oryzae* by down-regulating the SA-mediated pathway (Xu et al. 2013; Jiang et al. 2010). Rawat et al. (2012b) also reported that ABA related genes like HAV22 protein are involved in susceptibility against gall midge infestation. This ABA-related gene also triggered ROS production in cells. In *Arabidopis* two genes (*AtrbohD* and *AtrbohF*) are reported to be associated in ROS production in response to ABA and mutant of these genes showed reduced ROS production and cell death in response to pathogen infection (Kwak et al. 2003). CSN5B in *Arabidopis* is involved in regulatory role of Abscisic acid (Wang et al. 2013). Aminocyclopropane-1-carboxylate oxidase 1 gene involved in ethylene production has been implicated in cucumber for aphid resistance (Liang et al. 2015). Enoyl-CoA hydratase/isomerase family protein is reported to be involved in JA biosynthesis in coffee after leaf miner attack (Cardoso et al. 2014). However, these genes did not show increased activity in Aganni in this study.

Two genes coding PR proteins were induced following gall midge infestation. Many PR genes were up-regulated in resistant variety IR28 against false smut disease in rice (Han et al. 2015). Earlier studies had shown a dramatic 40-fold increase in transcript levels of *PR10α* in Suraksha with *Gm11* gene following infestation (Rawat et al. 2010; Rawat et al. 2013). In contrast, in Kavya with *Gm1* gene no such up-regulation of any PR gene was seen (Rawat et al. 2012a). In this study,

pathogenesis-related Bet v1 family protein, also member of PR10 family, showed up-regulation in Aganni and 74R line at late hours (Fig. 4). The plasminogen activator inhibitor (PAI) 1, another PR gene induced in Aganni at 24 hai (Fig. 3a), codes for a serine protease inhibitor and belongs to family 7 of PR proteins which are well-known in plant defense against insect pests (Lawrence and Koundal 2002). Expression of PR proteins has been considered as the marker of SAR induction.

Final step towards enhanced systemic resistance has been the modulation of host metabolism towards a possible shift in C/N ratio to favor the host. However, there are reports of C/N shift towards N in wheat during compatible interaction with the Hessian fly (Zhu et al. 2008). This shift probably favors the insect. However, in our study, focusing on an incompatible interaction, a higher number of genes involved in amino acid metabolism were found to be up-regulated (i.e., Serine hydroxymethyltransferase, Glutamine synthetase—catalytic domain-containing protein, Aspartic proteinase oryzasin-1 precursor, Cysteine synthase—putative and Amino acid transporters) were found to be upregulated (Figs. 3a and 5a) as opposed to genes encoding proteins involved in carbon metabolism (i.e., Erythronate-4-phosphatedehydrogenase and Acyl-CoA synthetase protein involved in CHO and lipid metabolism, respectively) (Fig. 5a).

Four of the genes validated in the study (Abscisic stress-ripening—putative, Erythonate-4-phosphate-dehydrogenase, Aspartic proteinase oryzasin-1 precursor, and peptidylprolylcis/trans isomerase) were up-regulated during both compatible (Fig. 7) and incompatible (Figs. 3 and 4) interaction in Aganni. The last-mentioned gene is reported to be involved in pH homeostasis in cells (Bissoli et al. 2012). It is likely that these genes respond to stress in general and prepare the plant for suitable response based on quantum and nature of stress. Likewise, ten of the genes showed >2-fold induction in 74R line but threshold level was low (≤2-fold) in the parent Aganni (Figs. 5 and 6). This could be possible if residual genome of the susceptible parent carried certain positively acting gene elements that would have been kept under check in the resistant parent. Alternatively, these genes could have been induced either early or later and missed detection with two time points (24 and 120 hai) of sampling. Such instances have also been reported in rice (Raorane et al. 2015) with reference to yield under drought related QTLs. However, more studies need to be carried out to understand the phenomenon.

Conclusions

SSH cDNA library was generated from the infested and uninfested Aganni meristematic stem tissues to identify key genes involved in the resistance pathway. Our results suggested involvement of NBS-LRR, PR, heat shock protein, and NAC domain protein coding genes, earlier observed to confer HR+ resistance in Suraksha. In addition, two genes viz., *OsAPx1* and *OsAPx2* (ROI—scavenging-enzyme) were found to be up-regulated in Aganni suggesting their possible role in the suppression of HR. Further, resistance pathway in Aganni did not share features observed in Kavya conferring HR− resistance. We suggest that Aganni has a deviant form of inducible, salicylic acid (SA)-mediated, resistance but without HR.

Acknowledgments We thank the Project Director, ICAR-IIRR, formerly, Directorate of Rice Research, Hyderabad and the Director, International Centre for Genetic Engineering and Biotechnology, New Delhi, for the facilities and encouragement. This work was partly supported by a research grant (F. no. BT/AB/FG-2 (PH-II)/2009) to JSB and (F. no. BT/AB/FG-2 (PH-II)(4B)/2009) to SN from the Department of Biotechnology (DBT), Government of India.

References

Álvarez C, Ángeles Bermúdez M, Romero LC, Gotor C, García I (2012) Cysteine homeostasis plays an essential role in plant immunity. New Phytol 193:165–177

Ambrosone A, Batelli G, Nurcato R, Aurilia V, Punzo P, Bangarusamy DK, Ruberti I, Sassi M, Leone A, Costa A, Grillo S (2015) The *Arabidopis* RNA-binding protein AtRGGA regulates tolerance to salt and drought stress. Plant Physiol 168:292–306

Barajas D, Kovalev N, Qin J, Nagy PD (2015) Novel mechanism of regulation of tomato bushy stunt virus replication by cellular WW-domain proteins. J Virol 89:2064–2079

Belkhadir Y, Subramaniam R, Dangl JL (2004) Plant disease resistance protein signaling: NBS–LRR proteins and their partners. Curr Opin Plant Biol 7:391–399

Bentur JS, Kalode MB (1996) Hypersensitive reaction and induced resistance in rice against Asian rice gall midge, *Orseolia oryzae*. Entomol Exp Appl 78:77–81

Bentur JS, Pasalu IC, Sarma NP, Rao PU, Mishra B (2003) Gall midge resistance in rice. Directorate of Rice Research, Hyderabad

Bissoli G, Niñoles R, Fresquet S, Palombieri S, Bueso E, Rubio L, Garcia-Sanchez MJ, Fernandez JA, Mulet JM, Serrano R (2012) Peptidyl-prolyl cis-trans isomerase ROF2 modulates intracellular pH homeostasis in *Arabidopis*. Plant J 70:704–716

Bryan GT, Wu KS, Farrall L, Jia Y, Hershey HP, McAdams SA, Faulk KN, Donaldson GK, Tarchini R, Valent B (2000) A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. Plant Cell 12:2033–2046

Cardoso DC, Martinati JC, Giachetto PF, Vidal RO, Carazzolle MF, Padilha L, Filho OG, Maluf MP (2014) Large-scale analysis of differential gene expression in coffee genotypes and susceptible to leaf miner—toward the identification of candidate genes for marker assisted-selection. BMC Genomics 15:66. doi:10.1186/1471-2164-15-66

Cassone BJ, Chen Z, Chiera J, Stewart LR, Redinbaugh MG (2014) Responses of highly resistant and susceptible maize to vascular puncture inoculation with Maize dwarf mosaic virus. Physiol Mol Plant Pathol 86:19–27

Chandler JW (2015) Auxin response factors. Plant Cell Environ. doi:10.1111/pce.12662

Chen N, Goodwin PH, Hsiang T (2003) The role of ethylene during the infection of *Nicotiana tabacum* by *Colletotrichum destructivum*. J Exp Bot 54:2449–2456

Chen S, Wang L, Que ZQ, Pan RQ, Pan QH (2005) Genetic and physical mapping of *Pi37*(t), a new gene conferring resistance to rice blast in the famous cultivar St. No. 1. Theor Appl Genet 111:1563–1570

Chen H, Chen W, Zhou J, He H, Chen L, Chen H, Deng XW (2012) Basic leucine zipper transcription factor OsbZIP16 positively regulates drought resistance in rice. Plant Sci 193:8–17

Divya D, Himabindu K, Nair S, Bentur JS (2015) Cloning of a gene encoding LRR protein and its validation as candidate gall midge resistance gene, *Gm4*, in rice. Euphytica 203:185–195

Do H, Kim IS, Kim YS, Shin SY, Kim JJ, Mok JE, Park SI, Wi AR, Park H, Lee JH, Yoon HS, Kim HW (2014) Purification, characterization and preliminary X-ray crystallographic studies of monodehydroascorbate reductase from *Oryza sativa L. japonica*. Acta Crystallogr Sect F 70:1244–1248

Du B, Zhang WL, Liu BF, Hu J, Wei Z, Shi ZY, He RF, Zhu LL, Chen RZ, Han B, He G (2009) Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. Proc Natl Acad Sci U S A 106:22163–22168

Elashry A, Okumoto S, Siddique S, Koch W, Kreil DP, Bohlmann H (2013) The AAP gene family for amino acid permeases contributes to development of the cyst nematode *Heterodera schachtii* in roots of *Arabidopis*. Plant Physiol Biochem 70:379–386

Ellis JG, Jones DA (2003) Plant disease resistance genes. In: Ezekowitz RAB, Hoffmann JA (eds) Innate immunity. Humana Press Inc, Totowa, pp 27–45

Feng H, Wang X, Zhang Q, Fu Y, Feng C, Wang B, Huang L, Kang Z (2013) Monodehydroascorbatereductase gene, regulated by the wheat PN-2013 miRNA, contributes to adult wheat plant resistance to stripe rust through ROS metabolism. Biochim Biophys Acta 1839:1–12

Feng H, Liu W, Zhang Q, Wang X, Wang X, Duan X, Li F, Hunag L, Kang Z (2014) TaMDHAR4, a monodehydroascorbate reductase gene participates in the interactions between wheat and *Puccinia striiformis f. sp. tritici*. Plant Physiol Biochem 76:7–16

Fujita D, Kohli A, Horgan FG (2013) Rice resistance to planthoppers and leafhoppers. Crit Rev Plant Sci 32:162–191

Golan I, Dominguez PG, Konrad Z, Shkolnik-Inbar D, Carrari F, Bar-Zvi D (2014) Tomato abscisic acid stress ripening (ASR) gene family revisited. PLoS One 9, e107117. doi:10.1371/journal.pone.0107117

Haas FH, Heeg C, Queiroz R, Bauer A, Wirtz M, Hell R (2008) Mitochondrial serine acetyl transferase functions as a pacemaker of cysteine synthesis in plant cells. Plant Physiol 148:1055–1067

Han Y, Zhang K, Yang J, Zhang N, Fang A, Zhang Y, Liu Y, Chen Z, Hsiang T, Sun W (2015) Differential expression profiling of the early response to *Ustilaginoidea virens* between false smut resistant and susceptible rice varieties. BMC Genomics 16:955. doi:10.1186/s12864-015-2193-x

Hegedus D, Yu M, Baldwin D, Gruber M, Sharpe A, Parkin I, Whitwill S, Lydiate D (2003) Molecular characterization of *Brassica napus* NAC domain transcriptional activators in response to biotic and abiotic stress. Plant Mol Biol 53:383–397

Hind SR, Pulliam SE, Veronese P, Shantharaj D, Nazir A, Jacobs NS, Stratmann JW (2011) The COP9 signalosome controls jasmonic acid synthesis and plant responses to herbivory and pathogens. Plant J 65:480–491

Hu H, Wang J, Shi C, Yuan C, Peng C, Yin J, Li W, He M, Wang J, Ma B, Wang Y, Li S, Chen X (2015) A receptor like kinase gene with expressional responsiveness on *Xanthomonas oryzae pv. oryzae* is essential for *Xa21*-mediated disease resistance. Rice 8:1. doi:10.1186/s12284-014-0034-1

Hua LU (2009) Dissection of salicylic acid-mediated defense signaling networks. Plant Signal Behav 4:713–717

Huang D, Qiu Y, Zhang Y, Huang F, Meng J, Wei S, Li R, Chen B (2013) Fine mapping and characterization of *BPH27*, a brown planthopper resistance gene from wild rice (*Oryza rufipogon* Griff.). Theor Appl Genet 125:219–229

Hubert DA, Tornero P, Belkhadir Y, Krishna P, Takahashi A, Shirasu K, Dangl JL (2003) Cytosolic HSP90 associates with and modulates the *Arabidopis* RPM1 disease resistance protein. EMBO J 22:5679–5689

Jiang CJ, Shimono M, Sugano S, Kojima M, Yazawa K, Yoshida R, Inoue H, Hayashi N, Sakakibara H, Takatsuji H (2010) Abscisic acid interacts antagonistically with salicylic acid signaling pathway in rice-*Magnaporthe grisea* interaction. Mol Plant Microbe Interact 23:791–798

Jung KH, Gho HJ, Nguyen MX, Kim SR, An G (2013) Genome-wide expression analysis of HSP70 family genes in rice and identification of a cytosolic HSP70 gene highly induced under heat stress. Funct Integr Genomics 13:391–402

Kadota Y, Shirasu K (2012) The HSP90 complex of plants. Biochim Biophys Acta, Mol Cell Res 1823:689–697

Kikuchi K, Ueguchi-Tanaka M, Yoshida KT, Nagato Y, Matsusoka M, Hirano HY (2000) Molecular analysis of the NAC gene family in rice. Mol Gen Genet 262:1047–1051

Kobe B, Diesenhofer J (1995) A structural basis of the interactions between leucine-rich repeats and protein ligands. Nature 374:183–186

Kovi MR, Zhang Y, Yu S, Yang G, Yan W, Xing Y (2011) Candidacy of a chitin-inducible gibberellin-responsive gene for a major locus affecting plant height in rice that is closely linked to green revolution gene *sd1*. Theor Appl Genet 123:705–714

Krieger-Liszkay A (2005) Singlet oxygen production in photosynthesis. J Exp Bot 56:337–346

Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JD, Schroeder JI (2003) NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopis*. EMBO J 22:2623–2633

Lawrence PK, Koundal KR (2002) Plant protease inhibitors in control of phytophagous insects. Electron J Biotechnol 5:93–109. doi:10.2225/vol5-issue1-fulltext-3

Leung EW, Guddat LW (2009) Conformational changes in a plant ketol-acid reductoisomerase upon (Mg2+) and NADPH binding as revealed by two crystal structures. J Mol Biol 389:167–182

Liang D, Liu M, Hu Q, He M, Qi X, Xu Q, Zhou F, Chen X (2015) Identification of differentially expressed genes related to aphid resistance in cucumber (*Cucumis sativus L.*). Sci Rep 5:9645. doi:10.1038/srep09645

Lin R, Zhao W, Meng X, Wang M, Peng Y (2007) Rice gene OsNAC19 encodes a novel NAC-domain transcription factor and responds to infection by *Magnaporthe grisea*. Plant Sci 172:120–130

Liu Y, Burch-Smith T, Schiff M, Feng S, Dinesh-Kumar SP (2004) Molecular chaperone Hsp90 associates with resistance protein N and its signaling proteins SGT1 and Rar1 to modulate an innate immune response in plants. J Biol Chem 279:2101–2108

Liu S, Kandoth PK, Warren SD, Yeckel G, Heinz R, Alden J, Yang C, Jamai A, EL-Mellouki T, Juvale PS, Hill J, Baum TJ, Cianzio S, Whitham SA, Korkin D, Mitchum MG, Meksem K (2012) A soybean cyst nematode resistance gene points to a new mechanism of plant resistance to pathogens. Nature 492:256–260

Liu HC, Tian DQ, Liu JX, Ma GY, Zou QC, Zhu ZJ (2013) Cloning and functional analysis of a novel ascorbate peroxidase (APX) gene from *Anthurium andraeanum*. J Zhejiang Univ Sci B 14:1110–1120

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(−delta delta C(T)) method. Methods 25:402–408

Lu R, Malcuit T, Moffett P, Ruiz MT, Peart J, Wu AJ, Rathjen JP, Bendahmane A, Day L, Baulcombe DC (2003) High-throughput virus induced gene silencing implicates heat shock protein 90 in plant disease resistance. EMBO J 21:5690–5699

Marone D, Russo MA, Laido G, De Leonardis AM, Mastrangelo AMM (2013) Plant nucleotide binding site leucine rich repeat (NBS-LRR) genes: active guardians in host defense responses. Int J Mol Sci 14:7302–7326

Mayer MP, Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 62:670–684

McHale L, Tan X, Koehl P, Michelmore RW (2006) Plant NBS-LRR proteins: adaptable guards. Genome Biol 7:212. doi:10.1186/gb-2006-7-4-212

Miller GAD, Mittler RON (2006) Could heat shock transcription factors function as hydrogen peroxide sensors in plants? Ann Bot 98:279–288

Mishra RN, Ramesha A, Kaul T, Nair S, Sopory SK, Reddy MK (2005) A modified cDNA subtraction to identify full-length differentially expressed genes from any given system: an alternate to DNA chip technology. Anal Biochem 345:149–157

Moon DH, Salvatierra GR, Caldas DG, de Carvalho MCG, Carneiro RT, Franceschini LM, Oda S, Labate CA (2007) Comparison of the expression profiles of susceptible and resistant *Eucalyptus grandis* exposed to *Puccinia psidii* Winter using SAGE. Funct Plant Biol 34: 1010–1018

Natrajkumar P, Sujatha K, Laha GS, Srinivasarao K, Mishra B, Viraktamath BC, Hari Y, Reddy CS, Balachandran SM, Ram T, Sheshumadhav M, Shobharani N, Neeraja CN, Ashokreddy G, Shaik H, Sundaram RM (2012) Identification and fine-mapping of *Xa33*, a novel gene for resistance to *Xanthomonas oryzae pv. Oryzae*. Phytopathology 102:222–228

Pageau K, Reisdorf-Cren M, Morot-Gaudry JF, Masclaux-Daubresse C (2006) The two senescence-related markers, GS1 and GDH, involved in nitrogen mobilization, are differentially regulated during pathogen attack and by stress hormones and reactive oxygen species in *Nicotiana tabacum L*. leaves. J Exp Bot 57:547–557

Pan Q, Wendel J, Fluhr R (2000) Divergent evolution of plant NBS-LRR resistance gene homologues in dicot and cereal genomes. J Mol Evol 50:203–213

Peng H, Yang T, Jurick WM II (2014) Calmodulin gene expression in response to mechanical wounding and *Botrytis cinerea* infection in tomato fruit. Plants 3:427–441

Qu S, Liu G, Zhou B, Bellizi M, Zeng L, Dai L, Han B, Wang G (2006) The broad-spectrum blast resistance gene *Pi9* Encodes an NBS-LRR Protein and is a member of a multigene family in rice. Genetics 172:1901–1914

Ramkumar G, Srinivasarao K, MadanMohan K, Sudershan I, Sivaranjani AKP, Gopalkrishna K, Viraktamath BC, Madhav MS (2011) Development and validation of functional marker targeting an InDel in the major rice blast disease resistance gene *Pi54* (*Pikh*). Mol Breed 27:129–135

Raorane ML, Pabuayon IM, Miro B, Kalladan R, Reza-Hajirezai M, Oane RH, Kumar A, Sreenivasulu N, Henry A, Kohli A (2015) Variation in primary metabolites in parental and near-isogenic lines of the QTL *qDTY 12.1*: altered roots and flag leaves but similar spikelets of rice under drought. Mol Breed 35:1–25

Rawat N, Sinha DK, Rajendrakumar P, Srivastava P, Neeraja CN, Sundaram RM, Nair S, Bentur JS (2010) Role of pathogenesis related genes in rice-gall midge interactions. Curr Sci 99: 1361–1368

Rawat N, Neeraja CN, Sundaram RM, Nair S, Bentur JS (2012a) A novel mechanism of gall midge resistance in the rice variety Kavya as revealed by microarray analysis. Funct Integr Genomics 12:249–264

Rawat N, Neeraja CN, Sundaram RM, Nair S, Bentur JS (2012b) Differential gene expression in gall midge susceptible rice genotypes revealed by suppressive subtraction hybridization (SSH) cDNA libraries and microarray analysis. Rice 5:8. doi:10.1186/1939-8433-5-8

Rawat N, Himabindu K, Neeraja CN, Nair S, Bentur JS (2013) Suppressive subtraction hybridization reveals that rice gall midge attack elicits plant-pathogen-like responses in rice. Plant Physiol Biochem 63:122–130

Ren T, Qu F, Morris TJ (2000) *HRT* gene function requires interaction between a NAC protein and viral capsid protein to confer resistance to turnip crinkle virus. Plant Cell 12:1917–1925

Ribeiro CW, Carvalho FEL, Rosa SB, Alves-Ferreira M, Andrade CMB, Ribeiro-Alves M, Silveira JA, Margis R, Margis-Pinheiro M (2012) Modulation of genes related to specific metabolic pathways in response to cytosolic ascorbate peroxidase knockdown in rice plants. Plant Biol 14:944–955

Ryals J, Uknes S, Ward E (1994) Systemic Acquired Resistance. Plant Physiol 104:1109–1112

Sama VSAK, Himabindu K, Naik BS, Sundaram RM, Viraktamath BC, Bentur JS (2012) Mapping and MAS breeding of an allelic gene to the *Gm8* for resistance to Asian rice gall midge. Euphytica 187:393–400

Sama VSAK, Rawat N, Sundaram RM, Himabindu K, Naik BS, Viraktamath BC, Bentur JS (2014) A putative candidate for the recessive gall midge resistance gene *gm3* in rice identified and validated. Theor Appl Genet 127:113–124

Sarkar NK, Kundnani P, Grover A (2013) Functional analysis of Hsp70 superfamily proteins of rice (*Oryza sativa*). Cell Stress Chaperones 18:427–437

Selth LA, Dogra SC, Rasheed MS, Healy H, Randles JW, Rezaian MA (2005) A NAC domain protein interacts with tomato leaf curl virus replication accessory protein and enhances viral replication. Plant Cell 17:311–325

Stuart J (2015) Insect effectors and gene-for-gene interactions with host plants. Curr Opin Insect Sci 9:1–6

Su J, Wang W, Han J, Chen S, Wang C, Zeng L, Feng A, Yang J, Zhou B, Zhu X (2015) Functional divergence of duplicated genes results in a novel blast resistance gene *Pi50* at the *Pi2/9* locus. Theor Appl Genet 128:2213–2225

Sun X, Cao Y, Yang Z, Xu C, Li X, Wang S, Zhang Q (2004) *Xa26*, a gene conferring resistance to *Xanthomonas oryzae pv oryzae* in rice, encodes a LRR receptor like kinase protein. Plant J 37:517–527

Tamura Y, Hattori M, Yoshioka H, Yoshioka M, Takahashi A, Wu J, Sentoku N, Yasui H (2014) Map-based cloning and characterization of a brown planthopper resistance gene *BPH26* from *Oryza sativa L.* ssp. *indica* cultivar ADR52. Sci Rep 4:5872. doi:10.1038/srep05872

Tang W, Ru Y, Hong L, Zhu Q, Zuo R, Guo X, Wang J, Zhang H, Zheng X, Wang P, Zhang Z (2015) System-wide characterization of bZIP transcription factor proteins involved in infection-related morphogenesis of *Magnaporthe oryzae*. Environ Microb 17:1377–1396

Thaler JS, Humphrey PT, Whiteman NK (2012) Evolution of jasmonate and salicylate signal crosstalk. Trends Plant Sci 17:260–270

Torres MA, Dangl JL, Jones JD (2002) *Arabidopis* gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc Natl Acad Sci U S A 99:517–522

Tran LS, Nakashima K, Sakuma Y, Simpson SD, Fujita Y, Maruyama K, Fujita M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2004) Isolation and functional analysis of *Arabidopis* stress-inducible NAC transcription factors that bind to a drought-responsive cis-element in the early responsive to dehydration stress 1 promoter. Plant Cell 16:2481–2498

Turner JG, Ellis C, Devoto A (2002) The jasmonate signal pathway. Plant Cell 14:153–164

Udvardi MK, Kakar K, Wandrey M, Montanari O, Murray J, Andriankaja A, Zhang JY, Benedito V, Hofer JMI, Chueng F, Town CD (2007) Legume transcription factors: global regulators of plant development and response to the environment. Plant Physiol 144:538–549

Van Loon LC (1997) Induced resistance in plants and the role of pathogenesis-releted proteins. Eur J Plant Pathol 103:753–765

Vijaya Lakshmi P, Amudhan S, Himabindu K, Cheralu C, Bentur JS (2006) A new biotype of the Asian rice gall midge *Orseolia oryzae*

(Diptera: Cecidomyiidae) characterized from the Warangal population in Andhra Pradesh, India. Int J Trop Insect Sci 26:207–211

Wang J, Zhang Z, Huang R (2013) Regulation of ascorbic acid synthesis in plants. Plant Signal Behav 8, e24536. doi:10.4161/psb.24536

Wang Y, Lin S, Song Q, Li K, Tao H, Huang J, Chen X, Que S, He H (2014) Genome-wide identification of heat shock proteins (Hsps) and Hsp interactors in rice: Hsp70s as a case study. BMC Genomics 15:344. doi:10.1186/1471-2164-15-344

Wu R, Li S, He S, Wabmann F, Yu C, Qin G, Schreiber L, Li JQ, Gu H (2011) CFL1, a WW domain protein, regulates cuticle development by modulating the function of HDG1, a Class IV homeodomain transcription factor, in rice and *Arabidopis*. Plant Cell 23:3392–3411

Wu J, Zhang Z, Zhang Q, Han X, Gu X, Lu T (2015) The molecular cloning and clarification of a photorespiratory mutant, oscdm1, using enhancer trapping. Front Genet 6:226. doi:10.3389/fgene.2015.00226

Xia Y, Yu K, Gao QM, Wilson EV, Navarre D, Kachroo P, Kachroo A (2012) Acyl CoA binding proteins are required for cuticle formation and plant responses to microbes. Front Plant Sci 3:1–18

Xie Q, Sanz-Burgos AP, Guo H, Garcia JA, Gutierrez C (1999) GRAB proteins, novel members of the NAC domain family, isolated by their interaction with a gemini virus protein. Plant Mol Biol 39:647–656

Xu J, Audenaert K, Hofte M, Vleesschauwer DD (2013) Abscisic acid promotes susceptibility to the rice leaf blight pathogen *Xanthomonas oryzae pv oryzae* by suppressing salicylic acid-mediated defenses. PLoS One 8, e67413. doi:10.1371/journal.pone.0067413

Yao X, Xiong W, Ye T, Wu Y (2012) Overexpression of the aspartic protease *ASPG1* gene confers drought avoidance in *Arabidopsis*. J Exp Bot 63:2579–2593

Yasala AK, Rawat N, Sama VSAK, Himabindu K, Sundaram RM, Bentur JS (2012) *In silico* analysis for gene content in rice genomic regions mapped for the gall midge resistance genes. Plant Omics J 5:405–413

Yoshida K, Sakamoto S, Kawai T, Kobayashi Y, Sato K, Ichinose Y, Yaoi K, Akiyoshi-Endo M, Sato H, Takamizo T, Ohme-Takagi M (2013) Engineering the *Oryza sativa* cell wall with rice NAC transcription factors regulating secondary wall formation. Front Plant Sci 4:383. doi:10.3389/fpls.2013.00383

Zhai Y, Sun Z, Zhang J, Kang K, Chen J, Zhang W (2015) Activation of the TOR signalling pathway by glutamine regulates insect fecundity. Sci Rep 5:10694. doi:10.1038/srep10694

Zhu L, Liu X, Liu X, Jeannotte R, Reese JC, Harris M, Stuart JJ, Chen M-S (2008) Hessian fly (*Mayetiola destructor*) attack causes a dramatic shift in carbon and nitrogen metabolism in wheat. Mol Plant-Microbe Interact 21:70–78

Zou LP, Li HX, Ouyang B, Zhang JH, Ye ZB (2005) Molecular cloning, expression and mapping analysis of a novel cytosolic ascorbate peroxidase gene from tomato. DNA Seq 16:456–461