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Identification of Brown Planthopper (*Nilaparvata lugens* Stål.) Stress Response Genes in Rice using RNASeq Data

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ABSTRACT: Brown planthopper (*Nilaparvata lugens* Stål) is one of the major destructive pests in Asia and South East Asia region. Application of chemical pesticides is hazardous and produces resurgence in BPH biotypes. Therefore, identification of resistant genes and development of new genotypes can reduce the crop loss. Here, we have performed *in silico* analysis of RNASeq data from leaf and sheath samples of a resistant genotype, Qingliu in control and infested condition. This study discovered total 1050 significant differentially expressed genes (DEGs) in leaf and 244 DEGs in sheath samples with 1.5 fold change and adjusted p-value < 0.05. Further, these significant DEGs were undergone for building protein- protein network and GO enrichment analysis using STRING algorithm. This study reveals, genes belong to cellulose synthase family and genes related to signal protein like calmodulin-binding domain protein, Nitric oxide synthase and amino acid encoding are found to be up-regulated in leaf samples. Further, genes in leaf region involve in carbon metabolism, photosynthetic carbon fixation, biosynthesis of amino acid, nitrogen compound metabolic process are observed to be down-regulated which signified the shutdown of key metabolic process in stress condition. On the other hand, genes involves in hormonal pathways such as Salicyclic acid, Jasmonic acid, Abscisic acid and MAPK are found to be up-regulated in BPH infested sheath region. This study will help to understand the molecular mechanism involves in rice BPH interaction and may be used in future breeding programme to develop improved genotypes against BPH which can help to boost production and productivity.

Keywords: Brown planthopper, RNASeq analysis, Differential expression of genes, Transcriptomic study.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple crops, feeding almost half of the world population. Rice production is associated with massive limiting factors such as genetic constitution, climate, soil properties, biotic and abiotic stresses of the area where it is grown. Losses due to biotic stresses are mainly caused by insect, micro-organisms (viruses, bacteria and fungi) and nematodes The major biotic stresses such as bacterial leaf blight (BLB), sheath blight, blast, brown spot (BS), false smut (FS), brown planthopper (BPH), yellow stem borer (YSB), and gall midge (GM) yield and quality of rice grains contributing to about 25% loss (Savary *et al*., 2000). Particularly, in Asia insect pests such as Brown planthopper (BPH) and White Brown planthopper (WBPH) are the major insect pests. These insects destroy rice plants by sucking the phloem sap which in turn causes significant loss in grain yield (Muduli *et al*., 2021). Conventionally insects are being managed by the application of high doses of environmentally harmful synthetic insecticides (Heong and Hardy 2009). To overcome the harmful effect of these insecticides several studies have been carried out to identify inherent resistance against BPH in rice germplasms including the wild and weedy relatives. With the advent of rice reference genome and gene annotation data, the genetics and genomics of rice varieties with respect to BPH resistance is now better understood. Several genes are involved in BPH resistance. Since 1960s several studies have been performed to identify, characterize and utilize rice land races that exhibit resistance to planthoppers (Pathak *et al*., 1969; IRRI, 1979; Heinrichs *et al*., 1985). So far 40 major genes and 72 QTLs conferring resistance to BPH attack have been reported by using suitable mapping populations. Majority of these identified genes and QTLs have been localized in few rice chromosomes such as Chr1, Chr3, Chr4, Chr6, Chr11 and Chr12 (Muduli *et al*., 2021).

The Next-Generation Sequencing (NGS) platform and high-throughput sequencing pipelines have been utilized successfully to analyze and characterize the mapped genes& QTLs. Such analysis provide better insight into identification of various candidate genes, their expression patterns and signaling pathways that are involved in providing resistance to BPH. In addition to these, Differential Gene Expression (DGE) analysis

provides better understanding of resistance mechanism of rice plant against BPH. The RNA-Seq analysis allows researchers to detect both known and novel features in a single assay, enabling the identification of transcript isoforms, gene fusions, single nucleotide variants, and other features without the need of prior knowledge. Under BPH attack, different biochemical pathways governing resistance mechanism is triggered by plant's innate immunity. These biochemical changes are associated with the up-regulation and down regulation of different genes during infestation of period. Of the various biochemical changes, hormonal signaling pathways and transcriptional factors are the critical mediators of plant defense mechanism in response to BPH attack. Hence information on transcription factors in BPH-susceptible rice following to BPH infestation has been generated through various micro array and RNA-seq experiments (Wang *et al.*, 2012; Lv *et al.*, 2014). Transcriptomics study of BPH6 transgenic line and wild type Nipponbare after BPH infestation identified the significant DEGs that are involved in important metabolic processes, molecular function and cellular component of rice crop (Tan *et al*., 2020). Similarly cloning and characterization of major BPH resistant genes (BPH6, BPH9, BPH14, & bph29) and its alleles have provided information about their role in regulating hormonal signaling pathways like Salicyclic acid (SA), Jasmonic Acid (JA)& Ethylene (ET) and mediating the resistance pattern during pest attack (Du *et al*., 2020).

Large numbers of RNASeq datasets are generated by the use of available low cost and high throughput sequencing technology. These generated datasets are available at various public databases such as Gene Expression Omnibus (GEO) and Sequence Read Archive (SRA) of National Centre for Biotechnological Information (NCBI). Rice-BPH interaction RNASeq data available in these databases can be analyzed using suitable bioinformatics pipelines to find out the novel hub genes that are up/down regulated under infestation. The information generated will be helpful to the plant breeder to develop new BPH resistance rice varieties. Further, local rice land races that are showing resistance towards BPH phenotypically can be explored for their genomics connection by comparing with the RNASeq data available at these databases.

In the present study, we have performed *in silico* analysis of RNASeq data of Qingliu genotype (leaf and sheath) in both control and infested condition to generate knowledge on BPH resistance mechanism. Significant DEGs and their involvement in different biochemical and metabolic pathways related to BPH attack were also studied and correlated. Simultaneously, Gene Ontology (GO) enrichment analysis of these reported hub genes was also identified to support the information related to BPH stress on rice plants at molecular level. Information obtained from this study will elucidate the mechanism of BPH resistance in rice plant.

MATERIALS AND METHOD

RNASeq data collection: Illumina raw RNA reads (Accession: PRJNA689251) of Qingliu rice variety (NATIONAL TAIWAN UNIVERSITY) under control and infested (24h) condition available publicly at NCBI-SRA (https://www.ncbi.nlm.nih.gov/sra) repository were collected for analysis. Twenty RNASeq data sets (10 each for leaf and sheath parts) with unique accessions i.e. leaf control (SRR13356498, SRR13356499, SRR13356510, SRR13356491 & SRR13356492), leaf infested (SRR13356505, SRR13356506, SRR13356507, SRR13356508 & SRR13356509), sheath control (SRR13356500, SRR13356501, SRR13356502, SRR13356503 & SRR13356504) and sheath infested (SRR13356493, SRR13356494, SRR13356495, SRR13356496 & SRR13356497) were retrieved and converted into FASTQ format for further analysis using SRA-Toolkit (https://hpc.nih.gov/apps/sratoolkit.html).

Data processing and DEG computation. The raw RNASeq reads (20 samples) in fastq format were grouped into leaf (10nos) and sheath (10nos) with each containing 5 control and 5 infested data sets. FastQC tool (Simons, 2010) was used to check the quality of the raw reads generated from high throughput sequencing pipelines considering Phred ($Q = -10\log_{10}P$) score parameter. Further, good quality reads obtained from 20 samples were subjected to adapter trimming and quality trimming (Phred +33) using fastx_clipper and fastq_quality_trimmer algorithm of fastx toolkit (Hannon 2010). Rice reference genome and gene structural information (in .gtf file format) were downloaded from Rice Annotation Project database (RAP db) (https://rapdb.dna.affrc.go.jp/) followed by indexing of reference rice genome using HISAT-2 algorithm. HISAT-2 (a rapid and efficient aligner) was used to align the processed RNASeq reads (high quality, filtered or cleaned) for 20 samples against the reference rice genome (Kim *et al*., 2015). The sam files generated from mapped pair end reads were further converted into corresponding bam (binary) files using Samtools (Li *et al*., 2009). Furthermore, the assembly of mapped reads was performed using StringTie2, a reference-guided, accurate and faster transcriptome assembler suitable for both short and long reads (Kovaka *et al.*, 2019). The assembled reads for each group i.e. 10 leaves and 10 sheath samples were collected separately and differential expressed gene (DEG) counts were calculated using Feature Counts tool (Liao *et al.*, 2014).

Discovery of significant DEG. Analysis and visualization of identified DEG were performed using BiocManager and R Studio 4.2.2 packages. Initially, data normalization was carried out using DESeq2 (Love *et al.*, 2014) of package of R. Significant DEGs were computed considering $\log_2$ fold change > 0.585 (up-regulated) and < 0.585 (down-regulated) and with corrected p-value i.e. -log 10 (p-value) < 0.05. Further, removal of false positive DEGs from the data sets were carried out with adjusted p-value < 0.05. The up regulated and down regulated genes were separated from significant DEGs of leaf and sheath data sets and subjected for downstream analysis.

Protein-protein network and Gene Ontology analysis. Significant LOCs were converted to official

Ensembl gene ids using DAVID (https://david.ncifcrf.gov/tools.jsp) tool. Further, protein-protein interaction, gene ontology and pathway analysis of probable BPH response genes was performed using STRING web server which generates network on the basis of physical and functional association between proteins. Gene ontology and pathway analysis were performed for statistically significant enriched nodes.

RESULTS

Data Processing and discovery of differentially expressed Locus. Processed and good quality (Phred +33) reads from 10 leaf (5 control and 5 infested) and 10 sheath (5 control and 5 infested) data samples were mapped separately (Table 1) against reference genome of *Oryza sativa Japonica* group (https://rapdb.dna.affrc.go.jp/) in order to discover key BPH response locus (LOC) counts. HISAT2 aligner algorithm computed overall 92.05% and 91.66% aligned reads to reference genome in leaf and sheath samples respectively (Table 1), indicating good alignment. Again, from these aligned reads, total 33,681 and 10,953 LOCs were obtained in case of leaf (Fig. 1A) and sheath (Fig. 1B) samples respectively. The ambiguous data (sum of counts <=50) were discarded from the list which resulting in 21,374 and 8,278 counts for leaf and sheath group respectively.

Adispersion plot (Fig. 2) was generated using mean of DESeq2 normalized counts (leaf: 20,079, sheath: 8,240) to trace out highly BPH regulated LOCs in both leaf and sheath samples. Resulted dispersion plot showed significant gene counts lying within fitted threshold curve.

Table 1: Sample features and alignment rate to the reference genome.

Sr. No.	Sample ID	Plant part	Type	Size (Gb)	Overall alignment (%)
1.	SRR13356491	Leaf	Control	2.09	92.07
2.	SRR13356492	Leaf	Control	2.25	91.62
3.	SRR13356498	Leaf	Control	2.35	92.79
4.	SRR13356499	Leaf	Control	2.11	93.49
5.	SRR13356510	Leaf	Control	2.34	92.24
6.	SRR13356505	Leaf	Infested	1.93	93.11
7.	SRR13356506	Leaf	Infested	1.96	92.60
8.	SRR13356507	Leaf	Infested	1.87	92.16
9.	SRR13356508	Leaf	Infested	2.15	93.40
10.	SRR13356509	Leaf	Infested	2.10	93.08
11.	SRR13356493	Sheath	Control	2.05	91.53
12.	SRR13356494	Sheath	Control	2.29	92.56
13.	SRR13356495	Sheath	Control	2.05	90.52
14.	SRR13356496	Sheath	Control	2.07	91.46
15.	SRR13356497	Sheath	Control	2.36	91.53
16.	SRR13356500	Sheath	Infested	2.40	92.05
17.	SRR13356501	Sheath	Infested	2.16	90.64
18.	SRR13356502	Sheath	Infested	2.15	91.12
19.	SRR13356503	Sheath	Infested	2.17	93.08
20.	SRR13356504	Sheath	Infested	1.84	92.20

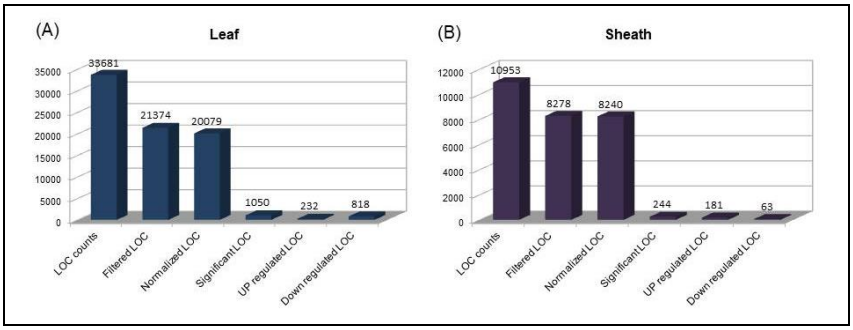


Fig. 1. Diffentially expressed gene Locus (LOC) counts observed before and after analysis.

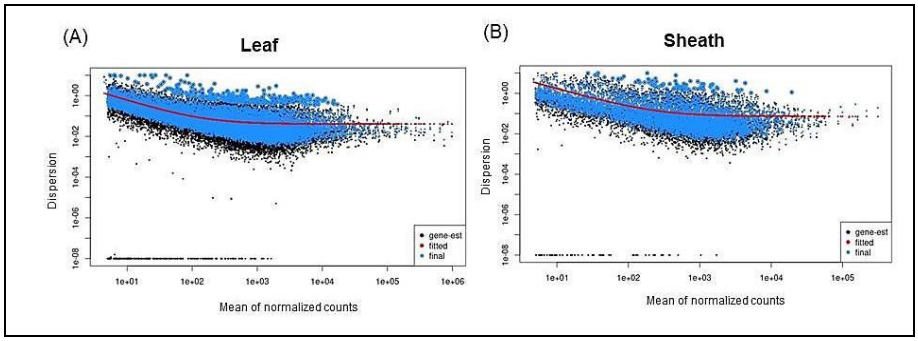
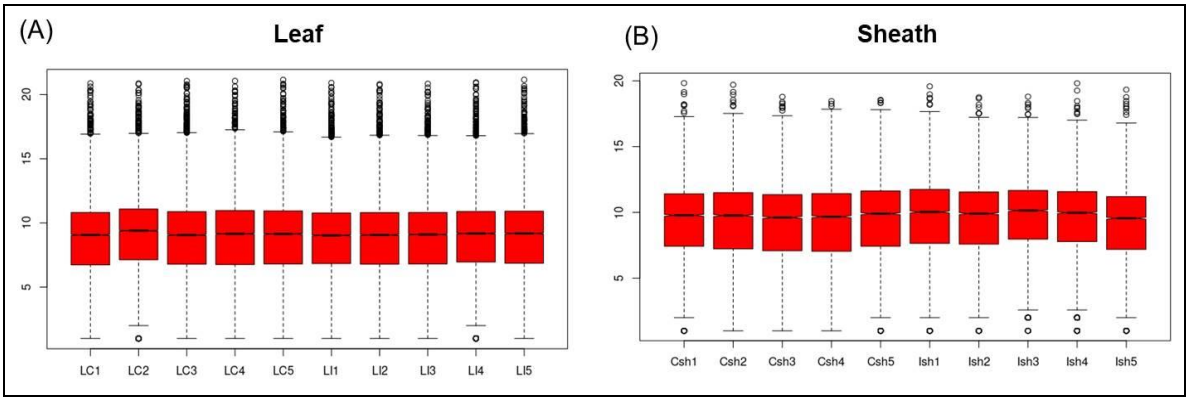


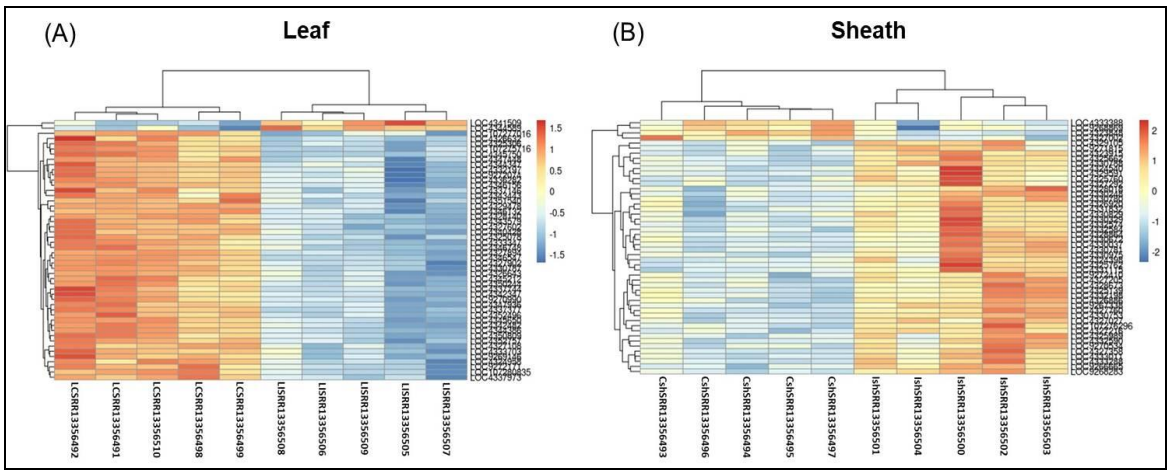
Fig. 2. Significant Locus counts plotted after normalization using dispersion plot.

The sample wise normalized counts of both leaf and sheath samples were plotted using box plot (Fig. 3). Computed median value in each sample of leaf (Fig. 3A) and sheath (Fig. 3B) groups showed minimal deviation in both control and infested (24h) stages. A total of 1050 (leaf) and 244 (sheath) significant LOCs (Fig. 1) were obtained with fold change > 1.5 (up regulation) and fold change < 1.5 (down regulation) and adjusted p-value < 0.05. It was observed that in case of the infested leaf samples higher number (818 counts) of genes were down regulated while in sheath samples greater numbers of genes (181) were upregulated. The same observation was also noticed from the heat maps plotted for top fifty significant LOCs (Fig.4). The heat map clearly indicated that fifty significant LOCs of leaf samples were highly up regulated in control whereas down regulated in infested samples (Fig. 4A). Similarly, fifty significant LOCs were down regulated in control and up regulated in infested sheath samples. In addition, all of these 50 LOCs were found to be highly down regulated in two particular infested samples (SRR13356505, SRR13356507) of leaf (Fig. 4A). Similarly, high up regulation of fifty significant LOCs were observed in two particular sheath infested samples (SRR13356500, SRR13356502) in comparison to others (Fig. 4B). All the significant LOCs that are observed in leaf and sheath groups were mapped in DAVID web tool in order to identify official ENSEMBL gene ids for carrying out further downstream analysis.



Five replicates of leaf control (LC1, LC2, LC3, LC4, LC5), Five replicates of leaf samples with BPH infested (LI1, LI2, LI3, LI4, LI5), Five replicates of sheath control (Csh1, Csh2, Csh3, Csh4, Csh5), Five replicates of sheath samples with BPH infested (Ish1, Ish2, Ish3, Ish4, Ish5)

Fig. 3. Box plot showed median distribution of significant DEGs among leaf and sheath samples.



(5 control: LCSRR13356491, LCSRR13356492, LCSRR13356498, LCSRR13356499, LCSRR13356510; 5 infested: LISRR13356505, LISRR13356506, LISRR13356507, ISRR13356508, LISRR13356509) and 10 sheath (5 control: CshSRR13356493, CshSRR13356494, CshSRR13356495, CshSRR13356496, CshSRR13356497 ; 5 infested: IshSRR13356500, IshSRR13356501, IshSRR13356502, IshSRR13356503, IshSRR13356504) samples were plotted using heat map. Red colour indicates highly upregulated genes and blue colour indicates highly down regulated genes.

Fig. 4. Significant DEGs in 10 leaf.

Protein-protein Network and Enrichment analysis of hub genes. Total 201 (up-regulated), 715 (down-regulated) genes of leaf samples and 160 (up-regulated), 52 (down-regulated) genes of sheath samples were successfully converted to official ENSEMBL gene ids. The significant DEGs identified in leaf and sheath samples were used for building protein- protein network using STRING algorithm. It has been observed that there are seven clusters among 19 up-regulated genes of leaf samples (Fig. 5A) with high confidence (0.700) parameter. Similarly, physical and functional network between 31 down-regulated genes was established in leaf samples (Fig. 6A) with highest (0.900) string confidence. In addition, 8

numbers of up-regulated genes were clustered into two groups within sheath samples (Fig. 5B) with high confidence (0.700) parameter. Similarly, connection between only two down-regulated genes was observed with medium confidence (0.400) parameter (Fig. 6B) from sheath samples whereas 35 disconnected nodes (proteins) were also identified within the network.

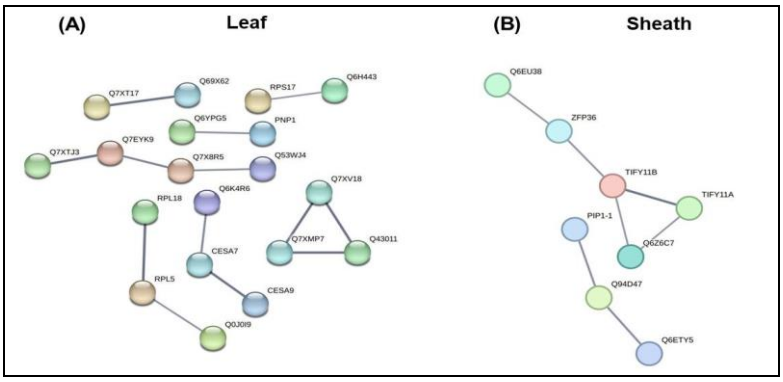


Fig. 5. Protein-protein network established between highly up-regulated genes in leaf and sheath samples.

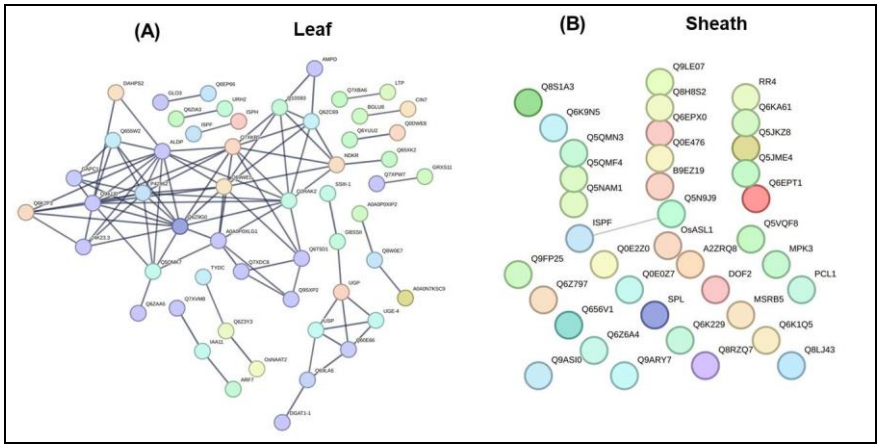


Fig. 6. Protein-protein network established between highly down-regulated genes in leaf and sheath samples.

Afterwards, GO enrichment of these genes was inspected from the string results. Up-regulated genes that are observed in leaf sample were found to be involved in carbon-nitrogen ligase activity (GO: 0016884) with glutamine as an amino-n-donor and in a single metabolic pathway (KEGG: dosa01100). Again, several metabolic processes such as metabolic process (GO: 0008152), cellular process (GO: 0009987), organic substance metabolic process (GO: 0071704), primary metabolic process (GO: 0044238), cellular metabolic process (GO: 0044237), nitrogen compound metabolic process (GO: 0006807), and organic substance biosynthesis process (GO: 1901576) were highly up-regulated in BPH infested leaf samples (Fig. 7).

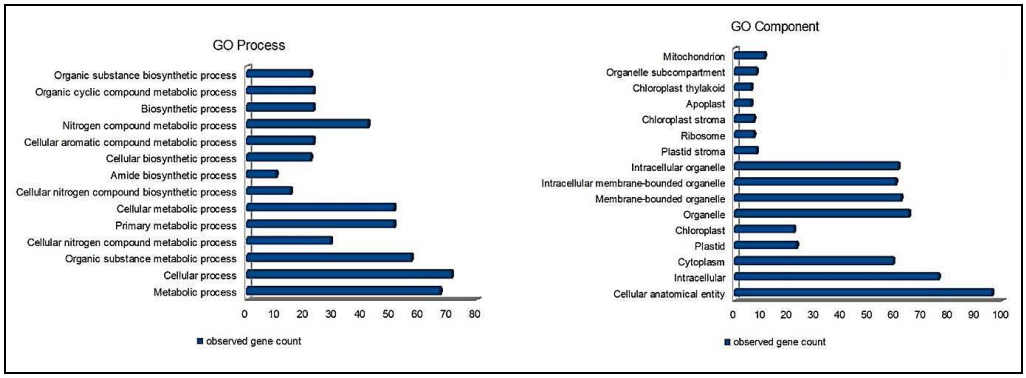


Fig. 7. Gene Ontology (GO) enrichment analysis of highly up-regulated genes in leaf samples.

The down-regulated genes in leaf sample involvement in different GO process, GO function, cellular compartments, KEGG pathways and STRING clusters are depicted in Fig. 8. However, many significant down-regulated genes in BPH infested leaf samples were observed to participate in different important pathways such as biosynthesis of secondary metabolites (54) (KEGG: dosa01110), carbon metabolism (22) (KEGG: dosa01200), biosynthesis of amino acid (17) (KEGG: dosa01230), carbon fixation in photosynthetic organisms (10) (KEGG: dosa00710), and purine metabolism (10) (KEGG: dosa00230) of crop plant (Fig. 8).

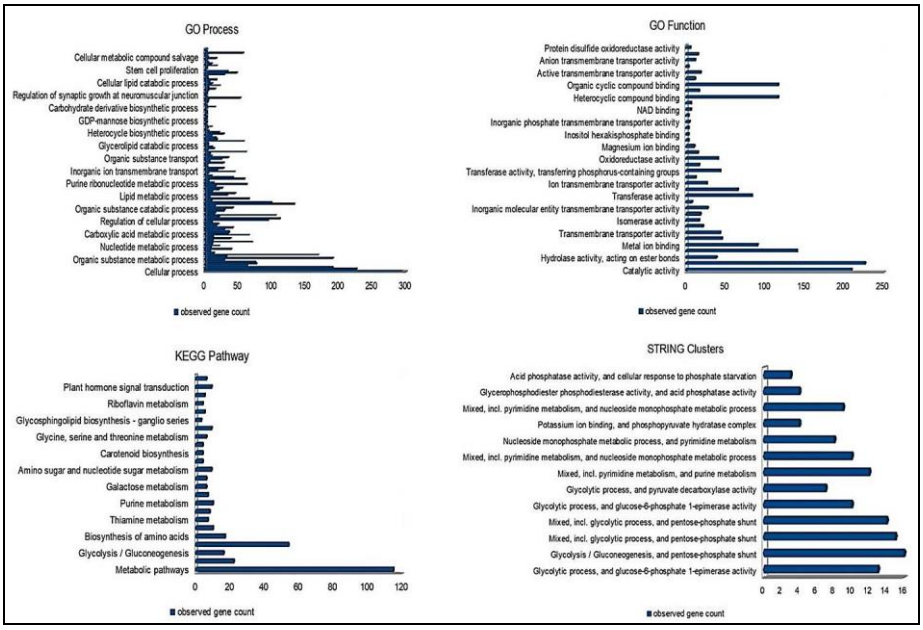


Fig. 8. Gene Ontology (GO) enrichment analysis of highly down-regulated genes in leaf samples.

Similarly, biochemical pathways such as regulation of jasmonic acid mediated signaling pathway, and MAPK signaling pathway (CL: 26429), jasmonic acid signaling pathway, and stamen filament development (CL: 26431) and plant hormone signal transduction pathway (KEGG: dosa04075) were observed to be up regulated in BPH infested sheath samples(Fig. 9). Additionally, in response to BPH attack few biological processes such as response to stimulus (GO: 0050896), response to chemicals (GO: 0042221), response to organic substance (GO: 0010033), response to hormone (GO: 0009725) and response to oxygen-containing compounds (GO: 9101700) were also noticed to be up-regulated in sheath samples (Fig. 9). Most of the down regulated genes in sheath samples were located in several GO component such as Intracellular (GO: 0005622), Organelle (GO: 0043226), Membrane-bounded organelle (GO: 0043227), Intracellular organelle (GO: 0043229), Cellular anatomical entity (GO: 0110165) (Fig. 10).

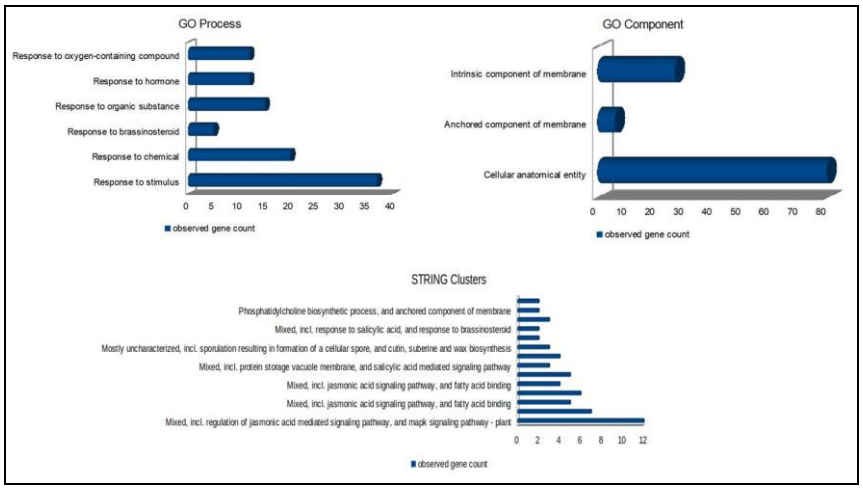


Fig. 9. Gene Ontology (GO) enrichment analysis of highly up-regulated genes in sheath samples.

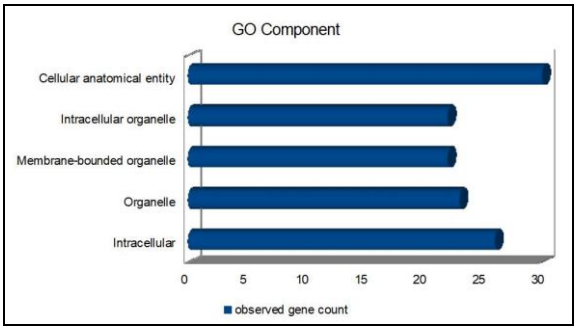


Fig. 10. Gene Ontology (GO) enrichment analysis of highly down-regulated genes in sheath samples.

DISCUSSION

Several attempts have been made to increase the durability of resistance in response to the various planthopper populations with increased virulence pattern by identifying major genes and pyramiding two or more resistance genes into a single susceptible variety (Fujita *et al*., 2013; Hu *et al*., 2013). Preliminary studies on resistance mechanism suggested the up regulation of the callose synthase genes in resistant lines inducing callose deposition in sieve tube, thereby restricting the sucking of the phloem sap from the major feeding site (Hao *et al.,* 2008). Gupta *et al.* (2019) through *in silico* approach identified five differentially expressed key hub genes belonging to cellulose synthase family namely Os05g0176100, Os06g0683200, Os07g0208500,Os07g0252400 and Os07g0424400 in Bt rice and suggested these five genes are possible cause for controlling infestation.

Two up regulated genes belonging to cellulose synthase family namely Os10g0467800 (CESA7) and Os09g0422500 (CESA9) (Table 2) was observed in the leaf of resistant variety, Qingliu. This finding confirmed the presence of BPH resistant genes as it plays major role for increasing the cellulose synthesis, a major structural component in cell wall formation. More cellulose synthesis hardens the cell wall and help plants to heal the damage tissue caused by pest attack. Out of the two cellulose synthase genes, Os10g0467800 was previously reported by Gupta *et al.* (2019) whereas; Os09g0422500 is a novel discovery of this study which is for cellulose biosynthetic process and cell wall organization. This gene enhances lignocellulose synthesis and accumulation on the cell walls, thereby creating a strong barrier for piercing insect.

Table 2: Major Significant DEGs discovered in leaf samples.

RAP	Protein id	Functional characterization	UP/Down regulation
Os06g0265000	Q43011	Asparagine synthetase, Long-distance transport of asparagin	Up
Os06g0598800	Q69X62	3-ketoacyl-CoA synthase (Lipid metabolism; fatty acid biosynthesis)	Up
Os04g0530600	Q7X8R5	Thioredoxin M2, chloroplastic	Up
Os10g0467800	CESA7	Cellulose synthase	Up
Os09g0422500	CESA9	Cellulose synthase A catalytic subunit 9, Cell wall biosynthesis and plant growt	Up
Os02g0299200	Q6K4R6	calmodulin-binding region domain containing protein	Up
Os02g0104700	Q6YPG5	Putative nitric oxide synthase, produces nitric oxide (NO) which is a messenger molecule involved in hormonal signaling and defense responses in plant	Up
Os11g0171300	ALDP	Fructose-bisphosphatealdolase, Plays a key role in glycolysis and gluconeogenesis	Down
Os01g0118000	Q94JJ0	Catalytic Activity (Fructose-bisphosphatealdolase)	Down
Os09g0327400	Q6K2P3	Catalytic Activity (glucose-6-phosphate 1-epimerase)	Down
Os04g0677500	Q7XKB5	Pyruvate kinase	Down
Os11g0148500	Q2RAK2	Cytosolic pyruvate kinase	Down
Os08g0109300	Q6ZC69	Probable adenylate kinase 2, chloroplastic, Catalyzes the reversible transfer of the terminal phosphate group between ATP and AMP	Down
Os03g0130400	Q10S93	Probable adenylate kinase 1, chloroplastic, Catalyzes the reversible transfer of the terminal phosphate group between ATP and AMP	Down
Os07g0693500	AMPD	Probable AMP deaminase, AMP deaminase plays a critical role in energy metabolism	Down

In addition to that, it is also observed that resistant rice plants have the ability to sustain the BPH attack by increasing the level of primary metabolites as well as repairing the damaged tissues. As evidence from the earlier studies on comparative transcriptomic profiling showed that genes that are involved in the primary metabolism were up regulated in resistant lines (BPH15 gene introgressed line) than the susceptible line (recipient line) (Lv *et al*., 2014). This present study supported the above facts as protein-protein interaction network of significant DEGs carried out by STRING database search identified different clusters consisting of key protein which encode the enzymes of primary metabolic pathways. The enzymes such as Asparagine synthase (Q43011), 3-ketoacyl-CoA synthase (Q69X62) regulating the lipid metabolism, Glucose-6-phosphate-1-dehydrogenase (Q7EYK9) and putative nitric oxide synthase (Q6YPG5) were found to be up regulated.

Amino acid encoding gene i.e. Asparagine (Os06g0265000) (Table 2) that helps in nitrogen assimilation, distribution and remobilization within plant via phloem and also it acts as secondary signal messenger when plant is infested with sucking pest like BPH (Shigematsu *et al*., 1982; Sogawa *et al*., 1982). Besides that, other important element of signal transduction such as kinase cascades and genes related to Ca^{2+} signaling pathways send signals to downstream response genes (Wu and Baldwin 2010). In this study, calmodulin-binding domain protein (Ca^{2+}signaling related genes, Os02g0299200) was found to be up regulated in leaf suggesting the activation of signal perception after BPH feeding. Apart from this signaling protein, another gene has been identified that encodes Nitric oxide synthase (Os02g0104700) involved in Nitric oxide (NO) production found to be up regulated in leaf. This finding is also established by Li *et al.* (2019) that Nitric oxide synthase plays a major role in the plant defence. This NO acts as messenger molecule

for hormonal signaling associated with defense response in rice plants. Therefore, when rice plants sense an invasion from BPH, the calmodulin-binding domain protein and Nitric oxide synthase enzyme are up regulated involved in transmitting resistance signal to the downstream regulatory network.

Again STRING database search analysis on protein-protein network study of all the significant down regulated DEGs in leaf identified that genes regulating primary metabolic process such as glycolysis and gluconeogenesis (Table 3). Further GO enrichment study indicated similar results as mostly these down regulated DEGs in leaf were enriched in carbon metabolism, photosynthetic carbon fixation, biosynthesis of amino acid, nitrogen compound metabolic process, catalytic activities etc. During the host-insect interaction, there may be possible shut down of key metabolic pathways due to the down regulation of genes and adapting the tolerance mode of resistance reaction (Divya *et al*., 2021). There is also possible cause of change in expression of these genes that participate in carbon assimilation and mobilization as BPH sucks large amount of phloem-sap to get adequate sugar. The down-regulation of genes involved in the photosynthetic and primary metabolism appears to be a universal plant evolutionary adaptation to phloem-feeding insects, representing a shift in resource allocation from growth to basal defensive system (Thompson and Goggin 2006).

Mitogen activated protein kinase (MAPK) cascade is one of the major signal transduction pathways activated as earliest response by the crop plant under a biotic stress condition (Wu *et al*., 2007; Hettenhausen *et al*., 2015). Upon perceiving external stimuli from biotic attack, MAPK cascade modulates various phytohormones that are involved in resistance such as Jasmonic acid (JA), Salicyclic acid (SA), Ethylene (ET) and Abscisic acid (ABA) (Yuan *et al*., 2005; Wang *et al*., 2008). The STRING database search identified possible protein-protein interaction network on significantly up regulated DEGs of sheath region which encountered the cross-talk between major pathways and transcription factors (TF) triggered during the stress. ZFP36 (Zinc finger protein 36), one of the important probable TF involved in the cross-talk between NADPH oxidase, Hydrogen peroxide and MAPK in ABA signaling was found to be up regulated in sheath region. MAPK cascade was assumed to be involved in ABA signaling in the sheath region, conferring resistance against BPH as evidence from the earlier research.

Table 3: Major Significant DEGs discovered in sheath samples.

RAP	Protein id	Functional characterization	UP/Down regulation
Os03g0437200	ZFP36	C2H2-type zinc finger protein, Abscisic acid-induced antioxidant defence, Water stress and oxidative stress tolerance	Up
Os03g0181100	TIFY11B	Tify domain containing protein, Jasmonic acid signaling pathway. Required for the regulation of the cross-talk between NADPH oxidase, hydrogen peroxide and MAP kinase in ABA signaling	Up
Os03g0180800	TIFY11A	TIFY domain-containing transcriptional regulator, Jasmonic acid signaling pathway	Up
Os02g0666200	PIP1-1	Probable aquaporin PIP1-2, Aquaporins facilitate the transport of water and small neutral solutes across cell membranes.	Up
Os01g0585100	Q94D47	Similar to Integral membrane protein	Up
Os02g0680600	ISPF	2-C-methyl-d-erythritol 2,4-cyclodiphosphate synthase, Isoprenoid biosynthesis, Chloroplast development	Down
Os01g0832000	Q5N9J9	Cytidine diphosphate diacylglycerol synthase, Phospholipid homeostasis, Negative role in hyperosmotic stress toleranc	Down

In addition it was observed that the TFs associated with JA signaling (TIFY11B and TIFY11B) were clustered together with ZFP36 in the sheath region. Further, GO analysis of the same up-regulated DEGs of the sheath region found similar higher levels of the JA, MAPK, and SA signaling pathways (Fig. 10). Here in the sheath region, the two major hormones JA and SA are showing their synergistic behavior during infestation which is a contrast to the earlier reported classic binary model of these two hormones as they play antagonistic nature in mediating defenses responses (Du *et al*., 2020). On the other hand, the findings of Guo *et al*. (2018), who reported that the levels of both SA and JA increase rapidly following BPH infestation in plants carrying the resistant gene Bph6 compared to susceptible plants, supported our study on the expression level of these two hormones. Thus, it is obvious from the discussion presented here that sheath region contributes a major part of resistance in the variety, Qingliu by up regulating genes associated with key phytohormonal pathways.

CONCLUSIONS

Transcriptomic analysis of variety, Qingliu belongs to *Oryza sativa japonica* group infested by BPH has provided insights into the host-pest interaction followed by gene expression and related protein-protein mechanism. There are many differentially expressed genes involved in both enzymatic and biochemical mechanism of defense against BPH attack in rice. This current study revealed two DEGs (Os10g0467800 and Os09g0422500) belonging to cellulose synthase family governing the resistance behavior. Out of these two, Os10g0467800 was found to be unique in this study. Many other key DEGs involved as secondary messenger during biotic stress are Ca^{2+} signaling related genes, (Os02g0299200), Nitric oxide synthase (Os02g0104700) and amino acid encoding genes, Asparagine (Os06g0265000). Also several genes

associated with primary metabolic activities were observed to be down regulated during infestation indicating shift in resource allocation from growth to basal defensive system. Interestingly, under biotic stress, DEGs and their transcriptional factors, which control the key hormonal pathways like SA, JA, MAPK, and ABA, were highly up regulated.

FUTURE SCOPE

These findings increased the understanding on the host-insect interaction which can be used in breeding programme to transfer suitable resistant genes into a popular susceptible variety.

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