

Genome-wide characterization of Rice Black Streaked Dwarf Virus-responsive genes in rice

DOI: 10.25177/JFST.5.2.RA.615

Research

Received Date: 25th Nov 2019Accepted Date: 15th Feb 2020Published Date: 28th Feb 2020

Copy rights: © This is an Open access article distributed under the terms of International License.

Jiaowen Pan¹, Pengfei Wang³, Guilin Zhang¹, Lianqun Yang¹, Fangyin Yao^{1,2}¹ Biotechnology Research Center, Shandong Academy of Agricultural Sciences / Key Laboratory of Genetic Improvement, Ecology and Physiology of Crops, Jinan 250100, Shandong, China² College of Life Sciences, Shandong Normal University, Jinan 250100, Shandong, China³ Shandong Academy of Grape; Shandong engineering research center for Grape cultivation and deep-processing, Jinan 250100, Shandong, China

CITATION

Jiaowen Pan, Genome-wide characterization of Rice Black Streaked Dwarf Virus-responsive genes in rice (2020) Journal of Food Science & Technology 5(2) pp:66-82

ABSTRACT

Background: Rice black streaked dwarf virus (RBSDV) is an important pathogen disease in rice and gramineous planting regions all over the world. The major phenotypes of RBSDV infected rice were dwarf with dark green leaves, which generally resulted in seriously loss of yield. The RBSDV known so far is preoperatively transmitted to rice in a persistent manner by small brown planthopper (SBPH), instead of transmitting to offspring through ovary.

Results: To identify RBSDV responsive genes and to explore and clarify the molecular mechanisms involved in plant-virus interaction, digital gene expression profile (DGE) analysis was performed by high-throughput sequencing using wild-type (WT) and RBSDV infected rice leaves (IRL) as materials. A total of 165,975 and 165,940 unique tags were obtained in IRL and WT libraries, respectively. In comparison with the control, 896 differentially expressed genes (DEGs) were obtained, of which 500 DEGs were up regulated and 396 DEGs were down regulated. Functional analysis showed that DEGs mainly classified into 10 groups, including metabolism, stress pathogen and defense, signal transduction, Transporter, transcription and post-transcription, cell structure and division etc. To further validate reliability and authenticity of the data, 10 DEGs were randomly picked and Real-time RT-PCR was carried out, and the expression trends of 7 genes were in line with the RNA-seq results. By searching the RBSDV related miRNA database of rice, 10 targeted genes of 6 significantly changed miRNAs were also identified in these DEGs.

Conclusions: The data derived from RNA-seq were valid and credible. Through this research, a series of candidate RBSDV-responsive genes were obtained, and special signal transduction and metabolism pathways were built and pulled out in rice. This study provided further insight into the molecular mechanisms during compatible and incompatible interactions between viruses and their host plants.

1. INTRODUCTION

Rice black streaked dwarf virus (RBSDV), which belongs to the genus *Fijivirus* within the family *Reoviridae*, could be propagated and infect rice, barley, wheat, sorghum, maize and other host plants by planthopper *laodelphax striatellus* in a persistent manner. The harmful influence of corresponding disease is reported to cause extensive and devastating crop losses in grain and forage yield, which ultimately leading serious economic loss in Asian countries such as China and Japan [1]. The infection of rice by RBSDV usually cause distant disease symptoms and growth abnormalities, such as severe dwarfing, more ineffective tillerings, poorly developed roots, rigidity leaf with almost no heading [2].

The whole genome of RBSDV consists of 10 double-stranded RNA segments (S1–S10) [3,4]. The complete nucleotide sequences of these segments have been determined and the functions of encoding proteins also have been preliminary researched. The mechanism by which many proteins are involved in plant virus interaction has also been studied. For instance, S7 contains two ORFs which encoding p7-1 and p7-2 proteins. Therein P7-1 may be disruption of the lignin biosynthesis and H₂O₂-dependent polymerization pathways, and P7-2 might involve in the -virus interaction [5,6]. S8 encodes core capsid protein, which may act as a nuclear transcriptional inhibitory factor in plant cells that participated in host cell gene expression regulation to make it suitable for virus propagation[1]. Although the biological functions of viral genome becoming increasingly clear, there were still no effective strategies to monitoring and control this disease as the insufficient data of the interactions between RBSDV and its host plants.

Microarray investigation revealed that various disease resistance genes, cell wall-related genes and development-related genes were altered in RBSDV infected maize[7]. Proteomic investigation also revealed that the dramatic changes of the basal metabolism processes in infected maize. Moreover, the demands for antioxidant enzymes, G-proteins, lipoxygenases and UDP-glucosyltransferase BX9 all increased in the virus infected plants, which indicated that they may participate in disease resistant response[2]. Using

comparative proteomic analysis, 72 differently expressed proteins which belonged to 11 groups were detected in RBSDV permanently infected rice[8]. Using deep sequencing approach, the expression of 14 miRNAs in leave and 16 miRNAs in root were found significantly changed in RBSDV infected rice [9]. Genetic analysis also showed that the resistance of rice variety Tetep to RBSDV was controlled by quantitative trait locis (QTLs), and three new QTLs were also identified [10].

High-throughput sequencing technology has been used to researches of various virus infected plant species, such as *Arabidopsis thaliana* [11,12], *Nicotiana* [13], *Zea mays* [14], potato[15] and rice[16]. As a model plant, the multiple genome and transcriptome databases and bioinformatical resources of rice are already available, which make it practicable to carry out high-throughput sequencing.

In this research, using WT and the RBSDV infected rice leaves (IRL) as materials, the genes expression profiles were investigated by RNA-seq, and the physiological and biochemical indexes were detected. Finally, about 896 DEGs were identified, and partial expression patterns were further verified. The expression patterns of several disease-related miRNAs targeted genes were also detected. The expression profiles or transcriptomes of the genes have provided effective clues for plants disease resistance breeding and germplasm innovation.

2. MATERIALS AND METHODS

2.1 Plant materials

Epidemiological investigation of RBSDV was carried out in Jining, Shandong. As the production and quality of RBSDV susceptible rice variety “Zixiangnuo 2315” were heavily infected by RBSDV infection, ten RBSDV infected rice plants with symptoms were picked while the healthy strains were harvested as control locally (Figure 1). All of the rice samples were stored in -80°C for further analysis. To verify whether the dwarf symptoms were caused by RBSDV infection, two pairs of specific PCR primers (supplement table 2) of published virus S9 and S10 were designed.



Fig1. The phenotypes of rice at tillering stage. Left: Wild type; Right: Rice infected with RBSDV

2.2. RNA extraction and cDNA library construction

Total RNA of WT and IRL were extracted using Trizol reagent, and the crude extracts were treated with RNase-free DNase I (TaKaRa, Dalian, China). In order to reduce the individual error, the RNA of 3 independent strains of RBSDV infected or wild type plants were mixed as one sample. The total RNA concentration was examined with a spectrophotometer (Nanodrop ND-2000, ThermoFisher Scientific, Wilmington, DE, USA), and the RNA sample integrity was verified by a Bio-Analyzer 2100 (Agilent Technologies, Santa.Clara, USA). The cDNA libraries construction and Sequencing at BGI Tech Solutions Co., Ltd. (BGI Tech, Shenzhen, China).

2.3. Identification of differential expressed genes (DEGs)

Obtaining and screening the unambiguous clean tags, the number of unambiguous clean tags for each gene were calculated and then normalized to TPM (number of transcripts per million clean tags).

The DEGs between two samples were identified by a rigorous algorithm method. The "FDR (False Discovery Rate) ≤ 0.001 and the absolute value of $\log_2 \text{Ratio} \geq 1$ " were used as the threshold to judge the significance of gene expression difference. The biological replication was analysis using Pearson correlation. According to the correlation results, DEGs were selected based on the NOISeq method of $Q \geq 0.6$ [17].

2.4. Gene Ontology (GO) and pathway enrichment analysis of DEGs

GO and pathway annotation and enrichment analysis were based on the Gene Ontology Database (<http://www.geneontology.org/>) and KEGG pathway Database (<http://genome.jp/kegg/>).

2.5 Quantitative Real-time PCR (qRT-PCR) Analysis

The gene-specific primers were designed using primer3.0 plus (supplement table 2). The qRT-PCR was performed using FastStart Universal SYBR Green Master (Roch, Shanghai, China) according to the manufacturer's instructions. Each reaction was performed refer to Liu Wei report [18]. The rice actin gene (LOC_Os03g50885) was used as internal control. Quantification of the relative changes in gene expressions were performed using the $2^{-\Delta\Delta CT}$ method as described by Livak and Schmittgen [19].

2.6. MicroRNA expression analysis

Referred to the data of RBSDV responsive miRNA-targeted genes [9], the putative target miRNAs of DEGs in this research were obtained.

3. RESULTS

3.1. Verification of virus infected rice strains

The RBSDV susceptible rice that sampling from Jining were preliminary screened according to Li Shuo(2015) report [20,21]. After been screened by specific primers of virus segments of S8 and S9 by RT-PCR, eight RBSDV infected rice strains with notable stunting symptoms and nine healthy strains were obtained (Supplement figure 1).

Considering RBSDV and rice stripe virus (RSV) all can be transmitted by planthopper *laodelphax striatrlus* simultaneously, the RSV specific primers (Supplement table 2) were also used to distinguish single virus infection or co-infection. The results showed that only one strain was detected to be infected with both RBSDV and RSV, other strains were all RBSDV single virus infection.

Table 1. Distribution of tags in two DEG libraries

Category	Parameter	IRL	WT
Raw Data	Total	4069983	4854614
	Distinct Tag	467951	416253
Clean Tag	Total number	3662063	4559710
	Distinct Tag number	165975	165940
	Total number	3014868	3961765
	Distinct Tag % of clean tag	82.33%	86.89%
All Tag Mapping to Gene	Distinct Tag number	91293	104584
	Distinct Tag % of clean tag	55.00%	63.03%
	Total number	1808480	2304034
	Total % of clean tag	49.38%	50.53%
Unambiguous Tag Mapping to Gene	Distinct Tag number	57703	65457
	Distinct Tag % of clean tag	34.77%	39.45%
	number	39198	39706
	% of ref genes	59.09%	59.85%
All Tag-mapped Genes	number	17424	17785
	% of ref genes	26.27%	26.81%
	Total number	349285	415276
	Total % of clean tag	9.54%	9.11%
Mapping to Genome	Distinct Tag number	40048	41422
	Distinct Tag % of clean tag	24.13%	24.96%
	Total number	297910	182669
	Total % of clean tag	8.14%	4.01%
Unknown Tag	Distinct Tag number	34634	19934
	Distinct Tag % of clean tag	20.87%	12.01%

3.2 Tags identification and quantification

After library construction and RNA-seq analysis, total of 4,069,983 and 4,854,614 tags were obtained separately in IRL and WT. There about 3,662,063 and 4,559,710 clean tags were left after per-filter. Then, only tags with less than one copy remain in the library and were used for further analysis. Finally, 165,975 and 165,940 unique tags for the IRL and WT were obtained, respectively. All clean tags were mapped to the reference genes and genome of rice. There were 65,457 (39.45% of clean tags) unique tags in WT library and 57,703 (34.77% of clean tags) unique tags in IRL library, and the unique tags were mapped to 17,785 (26.81%) and 17424 (26.27%) of the reference genes, separately (Table 1). Meanwhile, 41,422 (24.96%) and 40,048 (24.13%) unique tags were mapped to reference genome (Table 1).

3.3. Gene expression analysis between WT and IRL libraries

About 896 DEGs were identified between WT and IRL libraries, among which 500 genes were up regulated and 396 genes were down regulated. The DEGs were mainly divided into 10 groups, among which more than 50% of DEGs were involved in stress and

defense response and metabolic processes. Other DEGs were classified into hormone biosynthesis and signal transduction, Protein biosynthesis, cell structure and division (Figure 3).

Considering the screening of the disease resistance genes and breeding of disease resistance rice varieties, the categories of photosynthesis-related DEGs, hormone biosynthesis and signal transduction related DEGs, pathogenesis-related DEGs, and other remarkable DEGs were analyzed emphatically.

3.3.1 Photosynthesis-related DEGs

Here totally 23 photosynthesis-related DEGs were down-regulated in RBSDV-infected rice. Among which 18 DEGs belong to photosystem II reaction center protein, chlorophyll A-B binding protein, photosystem I reaction center protein, photosynthetic electron transport and F-type ATPase (Table 2). There were 5 DEGs that function in the Calvin-Benson cycle, including two fructose-1,6-bisphosphatase, one phosphoribulokinase/Uridine kinase family protein, one ribulose bisphosphate carboxylase small chain, and one phosphoglycerate kinase protein (Table 2 and Table 3).

Table 2. Photosynthesis-related genes

	Locus ¹	Annotation	Log ₂ Ratio ² (IRL/WT)	Q value ³
Photosystem II (PSII)	LOC_Os04g16770.1	photosynthetic reaction center protein, putative, expressed	-1.81	0.74
	LOC_Os08g25900.1	PsbP, putative, expressed	-1.21	0.66
	LOC_Os08g02630.1	photosystem II core complex proteins psbY, chloroplast precursor, putative, expressed	-1.38	0.74
	LOC_Os03g21560.1	photosystem II 11 kD protein, putative, expressed	-1.82	0.73
Light harvesting complexes of PSII	LOC_Os09g17740.1	chlorophyll A-B binding protein, putative, expressed	-1.44	0.75
	LOC_Os01g52240.1	chlorophyll A-B binding protein, putative, expressed	-1.43	0.75
	LOC_Os07g37550.1	chlorophyll A-B binding protein, putative, expressed	-1.76	0.70
	LOC_Os04g38410.1	chlorophyll A-B binding protein, putative, expressed	-1.66	0.76
	LOC_Os09g17740.1	chlorophyll A-B binding protein, putative, expressed	-1.44	0.75
Photosystem I (PSI)	LOC_Os08g44680.1	photosystem I reaction center subunit II, chloroplast precursor, putative, expressed	-1.35	0.74
	LOC_Os09g30340.1	photosystem I reaction center subunit, chloroplast precursor, putative, expressed	-1.25	0.72
	LOC_Os12g08770.1	photosystem I reaction center subunit N, chloroplast precursor, putative, expressed	-1.12	0.70
	LOC_Os04g33830.1	membrane protein, putative, expressed	-1.80	0.78
Photosynthetic Electron transport	LOC_Os06g01210.1	plastocyanin, chloroplast precursor, putative, expressed	-1.54	0.77
	LOC_Os06g01850.1	ferredoxin--NADP reductase, chloroplast precursor, putative, expressed	-1.52	0.73
F-type ATPase	LOC_Os07g32880.1	ankyrin repeat domain containing protein, expressed	-1.28	0.71
	LOC_Os03g17070.1	cysteine-rich repeat secretory protein 55 precursor, putative, expressed	-1.23	0.71

1 based on Rice Genome Annotation Project database

2 Log₂-based differential expression ratio

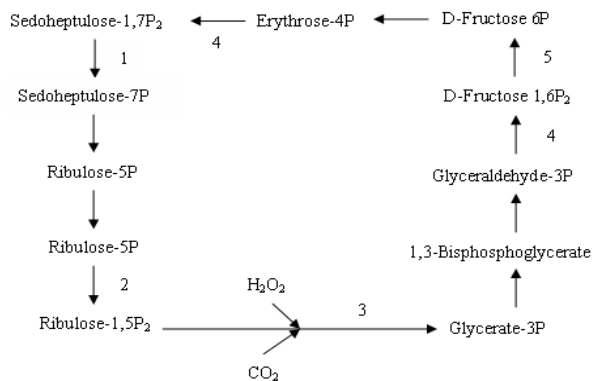
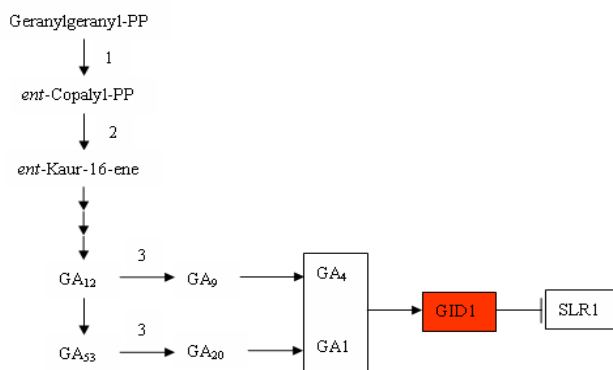


Table 3. Calvin-Benson cycle related genes

Designation	Locus ¹	Annotation	Log ₂ Ratio ² (IRL/WT)	Q value
1	LOC_Os04g16680.1	fructose-1,6-bisphosphatase, putative, expressed	-1.38	0.73
2	LOC_Os02g47020.1	phosphoribulokinase/Uridine kinase family protein, expressed	-1.12	0.69
3	LOC_Os12g19381.1	ribulose biphosphate carboxylase small chain, chloroplast precursor, putative, expressed	-1.41	0.75
4	LOC_Os12g07210.1	serine esterase family protein, putative, expressed	-1.08	0.65
5	LOC_Os03g16050.1	fructose-1,6-bisphosphatase, putative, expressed	-1.88	0.67

1 based on Rice Genome Annotation Project database

2 Log₂-based differential expression ratio



3.3.2. Synthesis, metabolism and signal transduction related DEGs of plant hormones

Considering the dwarf and stunted phenotypes of RBSDV infected rice, multiple clues all indicated that RBSDV disease is closely related to the synthesis and metabolism of plant hormones[22-24]. The genes involved in corresponded processes and signal transduction pathways were focused.

Genes related to Gibberellins biosynthesis and signaling

Here three significantly DEGs of GA biosynthesis were identified. The expression decreased genes mainly included one *ent-copalyl* diphosphate synthase (CPS) and two gibberellins 20 oxidase 2 (GA20ox 2). Meanwhile, the expression levels of four genes encoding GID1 were increased (Table 4), which known to be involved in GA signaling pathway.

Genes related to brassinolide biosynthesis and signaling

Two DEGs encoding cytochrome P450 were induced to

express by RBSDV which identified to be involved in BRs biosynthesis. In the BRs signal transduction pathway, the BRASSINOSTEROID -INSENSITIVE 1 (BRI1), BRI1 ASSOCIATED RECEPTOR KINASE1 (BAK1) and BSU1 were all found to be up-regulated under disease infected conditions (Table 5).

In addition, there were two DEGs which participate in BR biosynthesis regulation. One gene named *OsCYP51G3* which may encode obtusifoliol 14 α -demethylase was up-regulated. Previous investigation showed that decreased *OsCYP51G3* expression could reduce phytosterol and BR concentrations, and cause typical phenotypic changes related to phytosterol and BR deficiency, including dwarf plants, erect leaves, semi-sterile pollen grains, and shorter cells[25]. Other gene *OsGSR1* was down-regulated, which could activate BR synthesis by directly regulating a BR biosynthetic enzyme at the post-translational level and regulate GA signaling as a positive regulator [26].

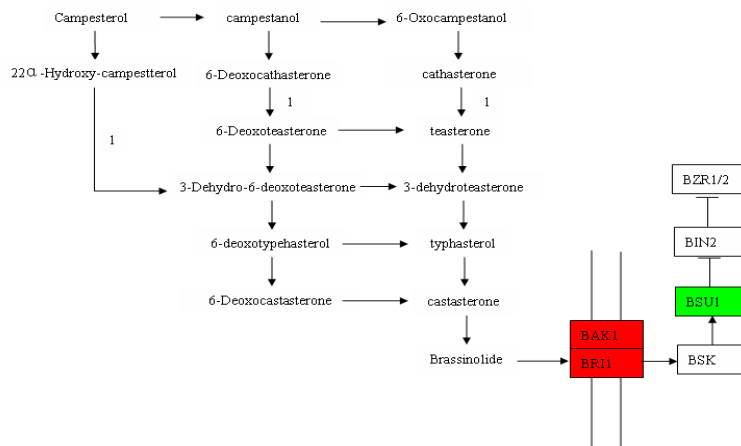
Table 4. Genes related to Gibberellins biosynthesis and signaling

Designation	Locus ¹	Annotation	Log ₂ Ratio ² (IRL/WT)	Q value
1	LOC_Os09g15050.1	ent-kaurene synthase A, chloroplast precursor, putative, expressed	-2.34	0.69
2	LOC_Os04g10060.1	ent-kaurene synthase, chloroplast precursor, putative, expressed	8.38	0.69
	LOC_Os02g36140.2	terpene synthase, putative, expressed	3.39	0.79
3	LOC_Os05g34854.1	gibberellin 20 oxidase 2, putative, expressed	-1.38	0.73
	LOC_Os05g34854.2	gibberellin 20 oxidase 2, putative, expressed	-7.87	0.62
GID1	LOC_Os03g57640.1	gibberellin receptor GID1L2, putative, expressed	1.62	0.74
	LOC_Os03g15270.1	gibberellin receptor GID1L2, putative, expressed	1.10	0.69
	LOC_Os11g04350.1	cell death associated protein, putative, expressed	9.56	0.83
	LOC_Os07g34280.1	CXE carboxylesterase, putative, expressed	2.38	0.78

¹ based on Rice Genome Annotation Project database

² Log₂-based differential expression ratios

Boxes of red indicate that the corresponding genes are predominantly induced.

**Table 5.** Genes related to brassinolide biosynthesis and signaling

Designation	Locus ¹	Annotation	Log ₂ Ratio ² (IRL/WT)	Q value
1	LOC_Os01g43710.1	WRKY11, expressed	1.14	0.70
	LOC_Os04g48200.1	cytochrome P450, putative, expressed	8.28	0.67
BRI1	LOC_Os11g39420.1	jacalin-like lectin domain containing protein, expressed	4.12	0.73
	LOC_Os04g42620.1	uncharacterized protein At4g06744 precursor, putative, expressed	-1.03	0.65
BAK1	LOC_Os01g04580.1	Ser/Thr protein kinase, putative, expressed	1.60	0.66
	LOC_Os02g48080.1	cysteine-rich receptor-like protein kinase 7 precursor, putative, expressed	1.18	0.60
	LOC_Os01g12430.1	receptor-like protein kinase, putative, expressed	1.40	0.60
	LOC_Os01g12420.1	expressed protein	1.92	0.61
	LOC_Os07g43570.1	TKL_IRAK_DUF26-lc.25 - DUF26 kinases have homology to DUF26 containing loci, expressed	-1.31	0.68
BSU1	LOC_Os10g30530.1	lectin-like receptor kinase, putative, expressed	1.39	0.60
	LOC_Os09g07460.1	fasciclin-like arabinogalactan protein 8 precursor, putative, expressed	1.09	0.61

¹ based on Rice Genome Annotation Project database

² Log₂-based differential expression ratios

Boxes of red indicate that the corresponding genes are predominantly induced.

Boxes of green indicate that the corresponding genes are predominantly suppressed.

3.3.3 Pathogenesis-related DEGs.

The results showed that 75 pathogenesis-related DEGs were identified, among them 61 genes up-regulated and 14 genes were down-regulated after infection. Specifically, there were 7 SCP-like extracellular protein encoded genes, 8 chitinase genes, 6 thaumatin genes, 12 glycosyl hydrolase genes, 11 peroxidase genes, 10 proteinase inhibitor genes, 7 Cupin domain containing protein genes and 14 other PR-like genes (Table 6).

Table 6. Disease resistance related genes

PR family	Locus ¹	Annotation	Log ₂ ratio ² (IRL/WT)	Q value
PR1	LOC_Os07g03580.1	SCP-like extracellular protein, expressed	6.64	0.93
	LOC_Os07g03319.1	SCP-like extracellular protein, expressed	6.27	0.87
	LOC_Os10g11500.1	SCP-like extracellular protein, expressed	5.40	0.82
	LOC_Os07g03600.1	SCP-like extracellular protein, expressed	4.99	0.70
	LOC_Os07g03710.1	SCP-like extracellular protein, expressed	4.06	0.83
	LOC_Os07g03590.1	SCP-like extracellular protein, expressed	3.84	0.78
	LOC_Os01g28450.1	SCP-like extracellular protein, expressed	3.14	0.83
PR2	LOC_Os01g71340.1	glycosyl hydrolases family 17, putative, expressed	2.67	0.82
	LOC_Os01g71820.1	glycosyl hydrolases family 17, putative, expressed	2.15	0.78
	LOC_Os01g71670.1	glycosyl hydrolases family 17, putative, expressed	1.52	0.77
PR3	LOC_Os05g33130.1	CHIT17 - Chitinase family protein precursor, expressed	2.56	0.82
	LOC_Os05g33140.1	CHIT17 - Chitinase family protein precursor, expressed	1.02	0.67
	LOC_Os03g30470.1	CHIT4 - Chitinase family protein precursor, expressed	2.64	0.81
	LOC_Os03g31690.1	CHIT4 - Chitinase family protein precursor, expressed	-1.14	0.62
	LOC_Os06g51050.1	CHIT7 - Chitinase family protein precursor, expressed	2.67	0.82
	LOC_Os06g51060.1	CHIT7 - Chitinase family protein precursor, expressed	1.16	0.71
PR4	LOC_Os11g37950.1	WIP3 - Wound-induced protein precursor, expressed	1.39	0.75
	LOC_Os11g37960.1	WIP4 - Wound-induced protein precursor, expressed	3.08	0.831
	LOC_Os11g37970.1	WIP5 - Wound-induced protein precursor, expressed	1.52	0.68
PR5	LOC_Os12g38120.1	thaumatin family domain containing protein, expressed	2.00	0.77
	LOC_Os12g43450.1	thaumatin family domain containing protein, expressed	1.47	0.76
	LOC_Os03g45960.1	thaumatin, putative, expressed	3.07	0.81
	LOC_Os12g43490.1	thaumatin, putative, expressed	3.04	0.83
	LOC_Os12g43510.1	thaumatin, putative, expressed	1.49	0.61
	LOC_Os03g46070.1	thaumatin, putative, expressed	1.33	0.74
	LOC_Os01g62260.1	thaumatin, putative, expressed	-1.04	0.68
PR6	LOC_Os01g03340.1	BBTI4 - Bowman-Birk type bran trypsin inhibitor precursor, expressed	1.41	0.75
	LOC_Os01g03360.1	BBTI5 - Bowman-Birk type bran trypsin inhibitor precursor, expressed	2.55	0.82
	LOC_Os01g03390.1	BBTI7 - Bowman-Birk type bran trypsin inhibitor precursor, expressed	1.21	0.72

PR8	LOC_Os11g18730.1	glycosyl hydrolase family 3 protein, putative, expressed	-1.67	0.76
	LOC_Os11g47530.1	glycosyl hydrolase, putative, expressed	8.3	0.68
	LOC_Os11g47560.1	glycosyl hydrolase, putative, expressed	3.88	0.69
	LOC_Os05g15880.1	glycosyl hydrolase, putative, expressed	3.76	0.79
	LOC_Os11g47550.1	glycosyl hydrolase, putative, expressed	3.70	0.61
	LOC_Os11g47600.1	glycosyl hydrolase, putative, expressed	2.35	0.78
	LOC_Os11g47510.1	glycosyl hydrolase, putative, expressed	1.94	0.69
	LOC_Os10g28120.1	glycosyl hydrolase, putative, expressed	1.94	0.80
	LOC_Os11g47570.1	glycosyl hydrolase, putative, expressed	1.85	0.62
	LOC_Os11g47580.1	glycosyl hydrolase, putative, expressed	1.79	0.68
	LOC_Os01g47070.1	glycosyl hydrolase, putative, expressed	1.61	0.77
	LOC_Os10g28080.1	glycosyl hydrolase, putative, expressed	1.43	0.75
	LOC_Os05g15770.1	glycosyl hydrolase, putative, expressed	1.37	0.74
PR9	LOC_Os05g04490.1	peroxidase precursor, putative, expressed	2.62	0.76
	LOC_Os03g13180.1	peroxidase precursor, putative, expressed	1.98	0.71
	LOC_Os01g22352.1	peroxidase precursor, putative, expressed	1.83	0.77
	LOC_Os05g06970.1	peroxidase precursor, putative, expressed	1.80	0.72
	LOC_Os08g01120.1	peroxidase precursor, putative, expressed	1.63	0.63
	LOC_Os04g59150.1	peroxidase precursor, putative, expressed	1.05	0.62
	LOC_Os09g29490.1	peroxidase precursor, putative, expressed	-1.41	0.60
	LOC_Os07g48010.1	peroxidase precursor, putative, expressed	-1.74	0.73
	LOC_Os01g10850.1	peroxidase precursor, putative, expressed	-1.86	0.67
PR10	LOC_Os08g28670.1	pathogenesis-related Bet v I family protein, putative, expressed	2.31	0.71
	LOC_Os12g36850.1	pathogenesis-related Bet v I family protein, putative, expressed	1.83	0.75
	LOC_Os12g36830.1	pathogenesis-related Bet v I family protein, putative, expressed	1.75	0.75
	LOC_Os01g14590.1	pathogen-related protein, putative, expressed	1.41	0.70
PR13	LOC_Os12g26960.1	THION34 - Plant thionin family protein precursor, expressed	2.39	0.72
PR14	LOC_Os05g06780.1	LTPL104 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	-1.54	0.75
	LOC_Os03g14654.1	LTPL108 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	1.21	0.69
	LOC_Os02g44320.1	LTPL113 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	-1.38	0.74
	LOC_Os04g55159.1	LTPL125 - Protease inhibitor/seed storage/LTP family protein precursor, putative, expressed	-1.30	0.73
	LOC_Os10g40420.1	LTPL138 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	-1.50	0.72
	LOC_Os06g49190.1	LTPL154 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	1.61	0.72
	LOC_Os10g36100.1	LTPL157 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	3.61	0.83
	LOC_Os01g59900.1	LTPL65 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	-1.06	0.67
	LOC_Os07g07790.1	LTPL75 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	2.30	0.66

PR15	LOC_Os08g35760.1	Cupin domain containing protein, expressed	-1.99	0.80
PR16	LOC_Os08g08960.1	Cupin domain containing protein, expressed	4.04	0.73
	LOC_Os08g09000.1	Cupin domain containing protein, expressed	3.83	0.76
	LOC_Os08g08980.1	Cupin domain containing protein, expressed	2.17	0.75
	LOC_Os08g09010.1	cupin domain containing protein, expressed	1.76	0.75
	LOC_Os08g09060.1	Cupin domain containing protein, expressed	1.08	0.68

1 based on Rice Genome Annotation Project database
2 Log₂-based differential expression ratio

3.3.4 Other remarkable DEGs

Except the DGEs that classified into specific processes above, there still have 23 DEGs that present significant high expression levels, and 10 DEGs that showed decreased expression levels in RBSDV infected rice ($\log_2\text{Ratio IRL/WT}>5$) (Supplement Table 1). Among which, there were 17 up-regulated genes and 7 down-regulated genes that have annotations in rice.

In the data, eight DGEs were defense-and stress-related genes, including MYB transcription factor, OsSub13 [27] putative subtilisin, terpene synthase [28] and DUF26 [29,30]. Two DGEs related to hormone metabolic, including GDSL-like lipase/acylhydrolase regulated of ethylene signal and flavin monooxygenase involved in auxin biosynthesis[31,32].

OsCML26, which reported to play important roles in the signal transduction and transcriptional regulation networks of Ca²⁺ signaling in response to different abiotic stress, was induced to express significantly ($\log_2\text{Ratio}=8.64$) after RBSDV infected [33,34].

In the data, we also found two cell wall-related genes, one was POEI5-Pollen Ole e I allergen and extensin family protein precursor (LOC_Os10g05820.1), which is a key enzyme in the biosynthesis of lignin cell wall precursors, the expression level of this gene was down-regulated after RBSDV infected ($\log_2\text{Ratio}=-8.03$). The other was pectinesterase, which is a cell-wall-associated enzyme that facilitates plant cell wall modification

and subsequent breakdown. The expression level of this gene was up-regulated in infected rice ($\log_2\text{Ratio}=7.80$).

3.4. Gene Ontology (GO) enrichment analysis of DEGs

In order to understand the functions of these DEGs, all of these genes were mapped to GO database and classified into different groups according to GO terms comparing to the genome background.

In the category of molecular function (MF) the GO terms of oxidoreductase activity and binding which including pattern binding, carbohydrate binding and ion binding were significantly overrepresented, other terms such as chitinase activity were also dominant.

When looking into the category of biological process (BP), the terms of response to stress and response to biotic stimulus were found overrepresented in the IRL library, which were also found in other plants infected with pathogen. Other terms such as aminoglycan catabolic process, amine metabolic process and polysaccharide catabolic process were also dominant.

Meanwhile, in the category of cellular component (CC), an increased percentage of DEGs corresponding to the terms of plastid, thylakoid and cytoplasmic vesicle were mainly appeared in the RBSDV infected rice. The results above hinted that the regulation of photosynthesis efficiency and vesicle transportation may play important role during the plant-pathogen interaction process (Figure2).

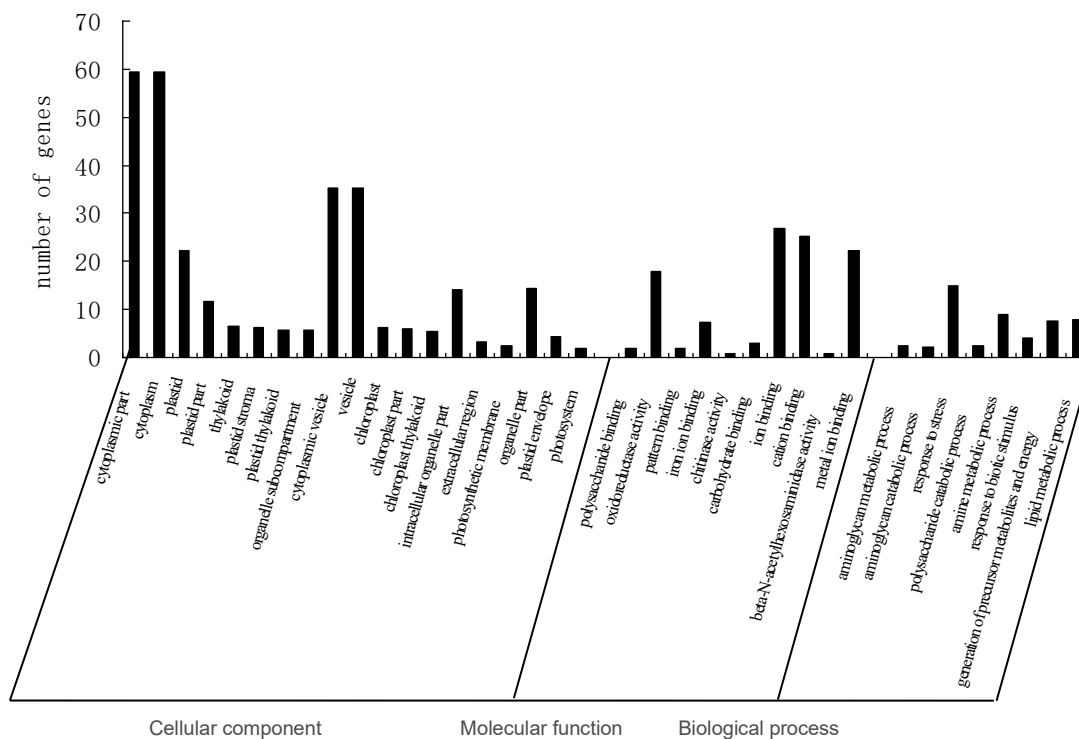


Figure 2. GO analysis of DEGs between the WT and IRL libraries.

3.5. Pathways enrichment analysis of DEGs

The DEGs identified in RBSDV infected rice were also grouped into different biological pathways by enrichment analysis. Among 896 DEGs, total of 508 DEGs were annotated in 104 pathways. Therein 19 pathways were significantly enriched (Q value ≤ 0.05). The enriched pathways were all in metabolism and biosynthetic pathways. In metabolism pathways included carbohydrate metabolism, energy metabolism, nucleotide metabolism, lipid metabolism, metabolism of terpenoids, polyketides and biosynthesis of other

secondary metabolites. The biosynthetic pathways mainly include biosynthesis of plant hormones. There were 60 DEGs belong to the biosynthesis of plant hormones, of which 44 DEGs were up-regulated and 16 DEGs were down-regulated. The biosynthesis of plant hormones pathways involved the biosynthesis of IAA, ABA, GA, JA and SA. The results suggested that RBSDV infect may cause the change of the host hormone levels, thus affect the growth and development of the host and stimulate the host disease resistance response (Figure3).

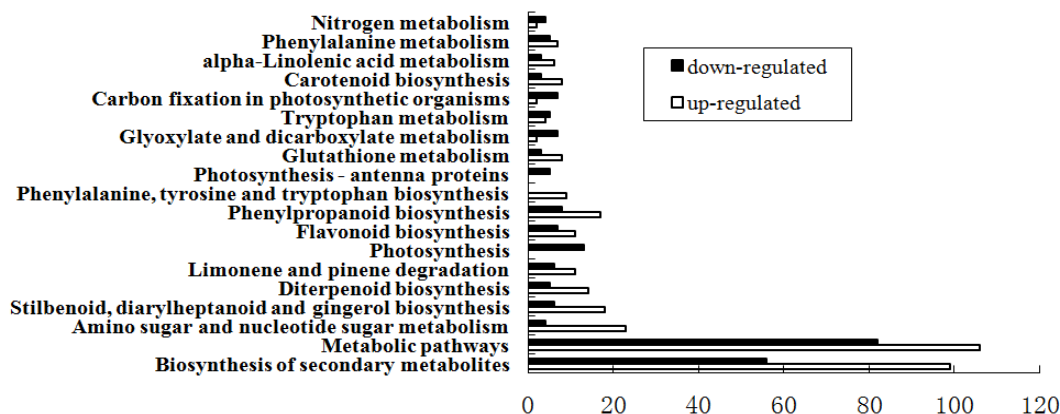


Figure 3: Pathways enrichment analysis of DEGs between the WT and IRL libraries.

Table 7. The target DEGs of miRNA

miRNA	Target gene	Log ₂ Ratio (IRL/WT)	Description
miR528	Os09g20090	2.85	ozone-responsive apoplastic protein
	Os02g48080	1.18	cysteine-rich receptor-like protein ki-
	Os01g66170	-1.35	SNARE associated Golgi protein, puta- tive, expressed
miR164	Os12g05260	1.56	phytosulfokines precursor, putative, expressed
	Os06g46270	1.19	NAC gene
	Os12g41680	0.92	No apical meristem protein, putative,
miR396	Os02g48080	1.18	cysteine-rich receptor-like protein ki- nase 7 precursor, putative, express
miR827	Os04g48390	1.70	OsSPX-MFS1
miRn11	Os10g39520	-1.12	MLO domain containing protein, puta-
miR166	Os04g48290	-0.95	MATE efflux family protein, putative, expressed

3.6 Verification of profiles of DEGs

To validate the expression profiles of DEGs, 10 DEGs were picked randomly and analyzed by qRT-PCR using rice actin as internal control. The results showed that except 3 genes, the variation trends of other 7 genes were consistent with RNA-seq results (Supplement figure 2).

3.7 MicroRNA expression analysis

There were 10 DEGs targeted by miR164, miR166, miR396, miR528, miR827, miRn11. Including NAC gene, ozone-responsive apoplastic protein, OsSPX-MFS1, and so on (Table 7). But the expression of DEGs was notable unless ozone-responsive apoplastic protein. And the expression levels of these miRNAs will be further analyzed by qRT-PCR.

4. DISCUSSION

4.1. Effects of RBSDV on photosynthetic rate and energy metabolism

Plant infected with RBSDV usually induces photosynthetic rate decrease. The long-term RBSDV accumulation in rice lead to the decrease of photosynthetic parameters, including the net photosynthetic rate (Pn), the stomatal conductance (Cond), the intercellular CO₂ concentration (Ci), and the transpiration speed (Tr). Some related proteins such as chlorophyll a/b binding proteins, Rubisco activase small isoform precursor and calvin cycle related proteins were also

changed [8]. Here, 24 DEGs that related to photosynthesis of photosystem II (PSII), photosystem I (PSI), photosynthetic electron transport, F-type ATPase and the Calvin cycle were down-regulated under virus infection.

Down regulated DEGs of photosynthetic reaction including *psbA*(LOC_Os04g16770.1), *psbP*(LOC_Os08g02630.1), *psb27*(LOC_Os03g21560.1), *psbY*(LOC_Os08g02630.1) and *psbW*(LOC_Os03g21560.1). The *psbA* encode photosynthetic reaction center protein D1, and the *psbP* encode subunit of the oxygen evolving complex (OEC). The decrease of the protein products of these two genes will affect the assembly and/or stability of the PSII-LHCII supercomplexes in the gram regions of the thylakoid membrane [35-37]. The *PsbY* protein was reported to take part in the cyclic electron flow around PSII in order to protect PSII against photoinhibition, and also could stabilize the binding of *PsbE* and *PsbF* to the heme group by providing a shelter of Cyt b₅₅₉ from oxidizing compounds[38]. The *psb27* was known to be essential for efficient recovery of the photodamaged PSII. In *Arabidopsis*, under high-light-illumination, PSII activity and the content of the PSII reaction center protein D1 decreased more significantly in the *psb27* mutant than in wild-type (WT) plant[39,40]. Taken together; the decrease of these

photosynthetic genes will affect the stable of PSII complex and the recovery of the photodamaged PSII. Five Chlorophyll A-B binding protein genes were down-regulated in RBSDV infected rice. Considering chlorophyll A-B binding protein is a component of the light-harvesting complex of photosynthesis II (LHC II), down regulation of these genes may inhibit LHC II function and further affect the photosynthetic process.

In the photosystem I, there were 4 DEGs including *psa D* (LOC_Os08g44680.1), *psaG* (LOC_Os09g30340.1), *psaN* (LOC_Os12g08770.1) and *psaO* (LOC_Os04g33830.1). In view of the functions of proteins encoded by these genes, the down regulation of these genes may affect stable and activity of PSI, and influence the energy balance between photosystems [41-43]. The reduction of plastocyanin (PC) and ferredoxin--NADP reductase(FNR) may inhibit the electron transport from PS II to PSI. RBSDV infection also inhibited the carbon fixation of plants by decreasing the expressions of 5 DEGs which encoding Calvin cycle enzyme.

Taken together, the PSI, PSII and electron transport were infected in BSDV infected rice, and thereby infected the photosynthesis efficiency and energy accumulation, which further results in abnormal growth, dwarfing and fruitless symptoms of infected plants.

4.2. The relationship between dwarfing symptoms and hormones

Dwarfism is a typical characteristic symptom of RBSDV infected plants. Considering phytohormones play multiple roles in regulation growth and development of plants, their variations in RBSDV infected rice were analyzed in depth.

Gibberellins (GA) are a group of tetracyclic diterpenoid compounds, which essential for many processes of plant growth and development [44-46]. The loss of function in GA synthesis and signaling resulted in dwarfism in rice and Arabidopsis plants, and transgenic plant expressing genes for GA degradation showed the dwarfism [47-49]. In RBSDV infected

rice the GA content reduces significantly [50]. In this research, partial DEGs in GA biosynthesis were decreased and in GA signaling pathway were increased, suggested that RBSDV infection might inhibit the accumulation of gibberellins, but activate GA signaling transduction in rice.

Brassinosteroid (BR) is other important phytohormone in plants. Brassinosteroids involve in various physiological processes including plant growth, development and immunity [51]. In rice, a low concentration of BL (10^{-9} M) could slightly enhance leaf sheath and root length, but it will inhibit leaf sheaths and roots growth when the contents were accumulated over certain levels, which resulted in decreased seeding height and root length [52]. Consistent with this, mutant or transgenic plants with excessive enhanced BR levels or BR signaling usually have reduced height to a certain degree in rice. In our data, two DEGs in BR biosynthesis were up-regulated significantly under virus infection, and this give us a hint that it may be caused by over accumulation of BR that lead to the dwarfism of RBSDV infected rice. The detailed mechanisms were still under investigation.

4.3. The roles of PRs in RBSDV infected rice

By now, many theoretical researches on the effects of plant *PR* genes have mainly focused on plant-fungi/bacteria interactions [53]. Some research found that the induced patterns of the *PRs* were quite different in virus infected rice, hinting the divergent defense mechanisms during defense response. In this research, 75 *PRs* that 61 up-regulated and 14 down-regulated were identified in the highly RBSDV susceptible rice Zixiangnuo2315. The *PR* have been classified into 17 groups (PR1-PR17) based on their amino acid sequences and biological functions [54]. In our data, These 75 *PRs* belong to eleven *PR* subfamilies, and the DEGs of PR1, PR14 and PR16 changed significantly in this research [54]. Especially seven DEGs of PR1 (including *OsPR1a* and *OsPR1b*) were dramatically induced to express about 10 to 3000 folds, which displayed vital roles in RBSDV and host interaction[55,56]. Here ten *PR* genes belong to PR14 subfamily, which further defined as

LTP genes were also changed, among which 6 genes were down-regulated about 3 to 285 folds and 4 genes were up-regulated about 3 to 20 folds[57]. As reported that LTP genes such as *OsC6* may participate in development regulation of pollen, the phenotypes of no heading or sterility of RBSDV infected rice may association with down-regulation of LTPs [58].

Supplementary Data

<https://www.siftdesk.org/articles/images/615/supplement%20data.pdf>

Funding

This work was supported by Shandong Provincial Natural Science Foundation (No. ZR2019QC003), the National Key Research and Development Program of China (2016YFD0100903-9), Agricultural scientific and technological innovation project of Shandong Academy of Agricultural Sciences (CXGC2018E13 and CXGC2016A02), and the Young Talents Training program of Shandong Academy of Agricultural Sciences (2016-2018).

REFERENCES

- [1] Liu H, Wei C, Zhong Y, Li Y (2007) Rice black-streaked dwarf virus minor core protein P8 is a nuclear dimeric protein and represses transcription in tobacco protoplasts. *FEBS Letters* 581: 2534-2540. PMID:17499245 [View Article](#) [PubMed/NCBI](#)
- [2] Li K, Xu C, Zhang J (2011) Proteome profile of maize (*Zea Mays* L.) leaf tissue at the flowering stage after long-term adjustment to rice black-streaked dwarf virus infection. *Gene* 485: 106-113. PMID:21708230 [View Article](#) [PubMed/NCBI](#)
- [3] Zhang HM, Chen JP, Adams MJ (2001) Molecular characterisation of segments 1 to 6 of Rice black-streaked dwarf virus from China provides the complete genome. *Archives of Virology* 146: 2331-2339. PMID:11811683 [View Article](#) [PubMed/NCBI](#)
- [4] Wang ZH, Fang SG, Xu JL, Sun LY, Li DW, et al. (2003) Sequence analysis of the complete genome of rice black-streaked dwarf virus isolated from maize with rough dwarf disease. *Virus Genes* 27: 163-168. PMID:14501194 [View Article](#) [PubMed/NCBI](#)
- [5] Sun F, Yuan X, Xu Q, Zhou T, Fan Y, et al. (2013) Overexpression of rice black-streaked dwarf virus p7-1 in *Arabidopsis* results in male sterility due to non-dehiscent anthers. *PLoS One* 8: e79514. PMID:24260239 [View Article](#) [PubMed/NCBI](#)
- [6] Wang Q, Tao T, Han Y, Chen X, Fan Z, et al. (2013) Nonstructural protein P7-2 encoded by Rice black-streaked dwarf virus interacts with SKP1, a core subunit of SCF ubiquitin ligase. *Virology* 10: 325. PMID:24176102 [View Article](#) [PubMed/NCBI](#)
- [7] Jia MA, Li Y, Lei L, Di D, Miao H, et al. (2012) Alteration of gene expression profile in maize infected with a double-stranded RNA fivirus associated with symptom development. *Molecular Plant Pathology* 13: 251-262. PMID:21955602 [View Article](#) [PubMed/NCBI](#)
- [8] Xu Q, Ni H, Chen Q, Sun F, Zhou T, et al. (2013) Comparative proteomic analysis reveals the cross-talk between the responses induced by H₂O₂ and by long-term rice black-streaked dwarf virus infection in rice. *PLoS One* 8: e81640. PMID:24312331 [View Article](#) [PubMed/NCBI](#)
- [9] Sun Z, He Y, Li J, Wang X, Chen J (2015) Genome-wide characterization of rice black streaked dwarf virus-responsive microRNAs in rice leaves and roots by small RNA and degradome sequencing. *Plant Cell Physiology* 56: 688-699. PMID:25535197 [View Article](#) [PubMed/NCBI](#)
- [10] Zhou T, Du L, Wang L, Wang Y, Gao C, et al. (2015) Genetic analysis and molecular mapping of QTLs for resistance to rice black-streaked dwarf disease in rice. *Scientific Reports* 5: 10509. PMID:26198760 [View Article](#) [PubMed/NCBI](#)
- [11] Gao R, Liu P, Yong Y, Wong SM (2016) Genome-wide transcriptomic analysis reveals correlation between higher WRKY61 expression and reduced symptom severity in Turnip crinkle virus infected *Arabidopsis thaliana*. *Scientific Reports* 6: 24604. PMID:27086702 [View Article](#) [PubMed/NCBI](#)
- [12] Sun F, Fang P, Li J, Du L, Lan Y, et al. (2016) RNA-seq-based digital gene expression analysis reveals modification of host defense responses by rice stripe virus during disease symptom development in *Arabidopsis*. *Virology* 13: 202. PMID:27912765 [View Article](#) [PubMed/NCBI](#)
- [13] Pesti R, Kontra L, Paul K, Vass I, Csorba T, et al. (2019) Differential gene expression and physiological changes during acute or persistent plant virus interactions may contribute to viral symptom differences. *PLoS One* 14: e0216618. PMID:31051010 [View Article](#) [PubMed/NCBI](#)

- [14]Ghorbani A, Izadpanah K, Dietzgen RG (2018) Changes in maize transcriptome in response to maize Iranian mosaic virus infection. 13: e0194592. PMID:29634778 [View Article](#) [PubMed/NCBI](#)
- [15]Goyer A, Hamlin L, Crosslin JM, Buchanan A, Chang JH (2015) RNA-Seq analysis of resistant and susceptible potato varieties during the early stages of potato virus Y infection. *BMC Genomics* 16: 472. PMID:26091899 [View Article](#) [PubMed/NCBI](#)
- [16]Zheng W, Ma L, Zhao J, Li Z, Sun F, et al. (2013) Comparative transcriptome analysis of two rice varieties in response to rice stripe virus and small brown planthoppers during early interaction. *PLoS One* 8: e82126. PMID:24358146 [View Article](#) [PubMed/NCBI](#)
- [17]Tarazona S, Garcia-Alcalde F, Dopazo J, Ferrer A, Conesa A (2011) Differential expression in RNA-seq: a matter of depth. *Genome Res* 21: 2213-2223. PMID:21903743 [View Article](#) [PubMed/NCBI](#)
- [18]Liu W, Wang PF, Li Z, Wang QG, Wang YY, et al. (2019) Genome-Wide Identification and Expression Analysis of the Cytidine Deaminase Subfamily in Rice. *Russian Journal of Plant Physiology* 66: 203-213. [View Article](#)
- [19]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. PMID:11846609 [View Article](#) [PubMed/NCBI](#)
- [20]Li S, Wang X, Xu J, Ji Y, Zhou Y (2015) A simplified method for simultaneous detection of Rice stripe virus and Rice black-streaked dwarf virus in insect vector. *J Virol Methods* 211: 32-35. PMID:25455902 [View Article](#) [PubMed/NCBI](#)
- [21]Cho SY, Jeong RD, Yoon YN, Lee SH, Shin DB, et al. (2013) One-step multiplex reverse transcription-polymerase chain reaction for the simultaneous detection of three rice viruses. *J Virol Methods* 193: 674-678. PMID:23850700 [View Article](#) [PubMed/NCBI](#)
- [22]Zhang H, Tan X, Li L, He Y, Hong G, et al. (2019) Suppression of auxin signalling promotes rice susceptibility to Rice black streaked dwarf virus infection. *Mol Plant Pathol* 20: 1093-1104. PMID:31250531 [View Article](#) [PubMed/NCBI](#)
- [23]Xie K, Li L, Zhang H, Wang R, Tan X, et al. (2018) Abscisic acid negatively modulates plant defence against rice black-streaked dwarf virus infection by suppressing the jasmonate pathway and regulating reactive oxygen species levels in rice. *Plant, Cell & Environment* 41: 2504-2514. PMID:29920686 [View Article](#) [PubMed/NCBI](#)
- [24]Tao T, Zhou CJ, Wang Q, Chen XR, Sun Q, et al. (2017) Rice black streaked dwarf virus P7-2 forms a SCF complex through binding to *Oryza sativa* SKP1-like proteins, and interacts with *GID2* involved in the gibberellin pathway. *PLoS One* 12: e0177518. PMID:28494021 [View Article](#) [PubMed/NCBI](#)
- [25]Xia K, Ou X, Tang H, Wang R, Wu P, et al. (2015) Rice microRNA osa-miR1848 targets the obtusifoliol 14alpha-demethylase gene *OsCYP51G3* and mediates the biosynthesis of phytoosterols and brassinosteroids during development and in response to stress. *New Phytol* 208: 790-802. PMID:26083975 [View Article](#) [PubMed/NCBI](#)
- [26]Wang L, Wang Z, Xu Y, Joo SH, Kim SK, et al. (2009) *OsGSR1* is involved in crosstalk between gibberellins and brassinosteroids in rice. *Plant J* 57: 498-510. PMID:18980660 [View Article](#) [PubMed/NCBI](#)
- [27]Jordá L, Vera P (2000) Local and Systemic Induction of Two Defense-Related Subtilisin-Like Protease Promoters in Transgenic Arabidopsis Plants. Luciferin Induction of *PR* Gene Expression. *Plant Physiology* 124: 1049-1058. PMID:11080282 [View Article](#) [PubMed/NCBI](#)
- [28]Yoshitomi K, Taniguchi S, Tanaka K, Uji Y, Akimitsu K, et al. (2016) Rice terpene synthase 24 (*OsTPS24*) encodes a jasmonate-responsive monoterpene synthase that produces an antibacterial gamma-terpinene against rice pathogen. *J Plant Physiol* 191: 120-126. PMID:26771167 [View Article](#) [PubMed/NCBI](#)
- [29]Ambawat S, Sharma P, Yadav NR, Yadav RC (2013) MYB transcription factor genes as regulators for plant responses: an overview. *Physiol Mol Biol Plants* 19: 307-321. PMID:24431500 [View Article](#) [PubMed/NCBI](#)
- [30]Miyakawa T, Hatano K, Miyauchi Y, Suwa Y, Sawano Y, et al. (2014) A secreted protein with plant-specific cysteine-rich motif functions as a mannose-binding lectin that exhibits antifungal activity. *Plant Physiol* 166: 766-778. PMID:25139159 [View Article](#) [PubMed/NCBI](#)
- [31]Yamamoto Y, Kamiya N, Morinaka Y, Matsuoka M, Sazuka T (2007) Auxin biosynthesis by the *YUCCA* genes in rice. *Plant Physiol* 143: 1362-1371. PMID:17220367 [View Article](#) [PubMed/NCBI](#)
- [32]Kim HG, Kwon SJ, Jang YJ, Nam MH, Chung JH, et al. (2013) *GDSL LIPASE1* modulates plant immunity through feedback regulation of ethylene signaling. *Plant Physiol* 163: 1776-1791. PMID:24170202 [View Article](#) [PubMed/NCBI](#)

- [33]Chinpongpanich A, Limruengroj K, Phean OPS, Limpaseni T, Buaboocha T (2012) Expression analysis of calmodulin and calmodulin-like genes from rice, *Oryza sativa* L. *BMC Res Notes* 5: 625. PMID:23134977 [View Article](#) [PubMed/NCBI](#)
- [34]Boonburapong B, Buaboocha T (2007) Genome-wide identification and analyses of the rice calmodulin and related potential calcium sensor proteins. *BMC Plant Biol* 7: 4. PMID:17263873 [View Article](#) [PubMed/NCBI](#)
- [35]Thornton LE, Ohkawa H, Roose JL, Kashino Y, Keren N, et al. (2004) Homologs of plant PsbP and PsbQ proteins are necessary for regulation of photosystem ii activity in the cyanobacterium *Synechocystis* 6803. *Plant Cell* 16: 2164-2175. PMID:15258264 [View Article](#) [PubMed/NCBI](#)
- [36]Ido K, Ifuku K, Yamamoto Y, Ishihara S, Murakami A, et al. (2009) Knockdown of the PsbP protein does not prevent assembly of the dimeric PSII core complex but impairs accumulation of photosystem II supercomplexes in tobacco. *Biochim Biophys Acta* 1787: 873-881. PMID:19285950 [View Article](#) [PubMed/NCBI](#)
- [37]Plochinger M, Schwenkert S, von Sydow L, Schroder WP, Meurer J (2016) Functional Update of the Auxiliary Proteins PsbW, PsbY, HCF136, PsbN, TerC and ALB3 in Maintenance and Assembly of PSII. *Front Plant Sci* 7: 423. PMID:27092151 [View Article](#) [PubMed/NCBI](#)
- [38]von Sydow L, Schwenkert S, Meurer J, Funk C, Mamedov F, et al. (2016) The PsbY protein of Arabidopsis Photosystem II is important for the redox control of cytochrome b559. *Biochim Biophys Acta* 1857: 1524-1533. PMID:27220875 [View Article](#) [PubMed/NCBI](#)
- [39]Chen H, Zhang D, Guo J, Wu H, Jin M, et al. (2006) A Psb27 homologue in Arabidopsis thaliana is required for efficient repair of photo-damaged photosystem II. *Plant Mol Biol* 61: 567-575. PMID:16897475 [View Article](#) [PubMed/NCBI](#)
- [40]Nowaczyk MM, Hebel R, Schlodder E, Meyer HE, Warscheid B, et al. (2006) Psb27, a cyanobacterial lipoprotein, is involved in the repair cycle of photosystem II. *Plant Cell* 18: 3121-3131. PMID:17114356 [View Article](#) [PubMed/NCBI](#)
- [41]Jensen PE, Haldrup A, Zhang S, Scheller HV (2004) The PSI-O subunit of plant photosystem I is involved in balancing the excitation pressure between the two photosystems. *J Biol Chem* 279: 24212-24217. PMID:15169790 [View Article](#) [PubMed/NCBI](#)
- [42]Chitnis VP, Ke A, Chitnis PR (1997) The PsaD subunit of photosystem I. Mutations in the basic domain reduce the level of PsaD in the membranes. *Plant Physiol* 115: 1699-1705. PMID:9414569 [View Article](#) [PubMed/NCBI](#)
- [43]Zygadlo A, Jensen PE, Leister D, Scheller HV (2005) Photosystem I lacking the PSI-G subunit has a higher affinity for plastocyanin and is sensitive to photodamage. *Biochim Biophys Acta* 1708: 154-163. PMID:15953472 [View Article](#) [PubMed/NCBI](#)
- [44]Wang Y, Zhao J, Lu W, Deng D (2017) Gibberellin in plant height control: old player, new story. *Plant Cell Rep* 36: 391-398. PMID:28160061 [View Article](#) [PubMed/NCBI](#)
- [45]Achard P, Genschik P (2009) Releasing the brakes of plant growth: how GAs shutdown DELLA proteins. *J Exp Bot* 60: 1085-1092. PMID:19043067 [View Article](#) [PubMed/NCBI](#)
- [46]Achard P, Gusti A, Cheminant S, Alioua M, Dhondt S, et al. (2009) Gibberellin signaling controls cell proliferation rate in Arabidopsis. *Curr Biol* 19: 1188-1193. PMID:19576768 [View Article](#) [PubMed/NCBI](#)
- [47]Oikawa T, Koshioka M, Kojima K, Yoshida H, Kawata M (2004) A role of OsGA20ox1, encoding an isoform of gibberellin 20-oxidase, for regulation of plant stature in rice. *Plant Mol Biol* 55: 687-700. PMID:15604710 [View Article](#) [PubMed/NCBI](#)
- [48]Ji SH, Gururani MA, Lee JW, Ahn BO, Chun SC (2014) Isolation and characterisation of a dwarf rice mutant exhibiting defective gibberellins biosynthesis. *Plant Biol (Stuttg)* 16: 428-439. PMID:23944972 [View Article](#) [PubMed/NCBI](#)
- [49]
- [50]Luo Y, Dong X, Yu T, Shi X, Li Z, et al. (2015) A Single Nucleotide Deletion in Gibberellin20-oxidase1 Causes Alpine Dwarfism in Arabidopsis. *168: 930-937*. PMID:25941313 [View Article](#) [PubMed/NCBI](#)
- [51]Huang R, Li Y, Tang G, Hui S, Yang Z, et al. (2018) Dynamic phytohormone profiling of rice upon rice black-streaked dwarf virus invasion. *J Plant Physiol* 228: 92-100. PMID:29886196 [View Article](#) [PubMed/NCBI](#)
- [52]Tang J, Han Z, Chai J (2016) Q&A: what are brassinosteroids and how do they act in plants? *BMC Biol* 14: 113. PMID:28007032 [View Article](#) [PubMed/NCBI](#)
- [53]Tong H, Xiao Y, Liu D, Gao S, Liu L, et al. (2014) Brassinosteroid regulates cell elongation by modulating gibberellin metabolism in rice.

- Plant Cell 26: 4376-4393. PMID:25371548 [View Article](#) [PubMed/NCBI](#)
- [54]Ali S, Ganai BA, Kamili AN, Bhat AA, Mir ZA, et al. (2018) Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. Microbiol Res 212-213: 29-37. PMID:29853166 [View Article](#) [PubMed/NCBI](#)
- [55]Sels J, Mathys J, De Coninck BM, Cammue BP, De Bolle MF (2008) Plant pathogenesis-related (PR) proteins: a focus on PR peptides. Plant Physiol Biochem 46: 941-950. PMID:18674922 [View Article](#) [PubMed/NCBI](#)
- [56]Agrawal GK, Jwa NS, Rakwal R (2000) A novel rice (*Oryza sativa* L.) acidic PR1 gene highly responsive to cut, phytohormones, and protein phosphatase inhibitors. Biochem Biophys Res Commun 274: 157-165. PMID:10903912 [View Article](#) [PubMed/NCBI](#)
- [57]Agrawal GK, Rakwal R, Jwa NS (2000) Rice (*Oryza sativa* L.) OsPR1b gene is phytohormonally regulated in close interaction with light signals. Biochem Biophys Res Commun 278: 290-298. PMID:11097833 [View Article](#) [PubMed/NCBI](#)
- [58]Finkina EI, Melnikova DN, Bogdanov IV, Ovchinnikova TV (2017) Plant Pathogenesis-Related Proteins PR-10 and PR-14 as Components of Innate Immunity System and Ubiquitous Allergens. Curr Med Chem 24: 1772-1787. PMID:27784212 [View Article](#) [PubMed/NCBI](#)
- [59]Zhang D, Liang W, Yin C, Zong J, Gu F, et al. (2010) OsC6, encoding a lipid transfer protein, is required for postmeiotic anther development in rice. Plant Physiol 154: 149-162. PMID:20610705 [View Article](#) [PubMed/NCBI](#)