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Identification of Rice *RRM1* Gene Family and Its Resistance to Rice Blast

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Abstract: In order to enhance understanding of RNA-binding proteins in rice, a comprehensive investigation was conducted on the *RRM1* gene family of rice, encompassing genome-wide identification and exploration of its role in rice blast resistance. Physical and chemical properties of the *OsRRM1* gene family in rice was analyzed, including conserved domain, motif, location information, gene structure, phylogenetic tree, collinearity analysis, cis-acting elements, GO, and KEGG. Furthermore, the expression patterns of the *OsRRM1* gene were examined at different time intervals following rice blast treatment. Furthermore, the alterations in expression patterns of selected *OsRRM1* genes were assessed using quantitative real-time PCR(qRT-PCR). A total of 212 members of the *OsRRM1* gene family were identified, which were dispersed across 12 chromosomes. Many of these genes exhibit multiple exons and introns, all of which encompass the conserved RRM1 domain and share analogous motifs. This observation suggests a high degree of conservation within the encoded sequence domain of these genes. Phylogenetic analysis revealed the existence of five subfamilies within the *OsRRM1* gene family. Furthermore, the investigation of the promoter region identified homeopathic elements that are involved in nucleic acid binding and interaction with multiple transcription factors. By employing GO and KEGG analysis, four *RRM1* genes were tentatively identified as crucial contributors to plant immunity, while the *RRM1* gene family was also found to have a significant involvement in the complex of alternative splicing. Additionally, gene expression analysis indicated that the majority of *OsRRM1* genes exhibited constitutive expression. The results of the qRT-PCR analysis revealed distinct temporal changes in the expression pattern of the *OsRRM1* gene following rice blast treatment. These findings contribute to the existing knowledge of the *OsRRM1* gene family, establish a foundation for further investigation into the role of the *OsRRM1* gene in response to rice blast infection, and hold theoretical significance for future studies on the functionality of the *OsRRM1* gene.

Keywords: rice; gene family; rice blast; bioinformatics

1. Introduction

Gene expression must abide by strict laws, and each step needs to be strictly regulated, which is often regulated at the transcription level through DNA cis-acting elements and transcription factor binding[1,2]. Studies have shown that post-transcriptional regulation plays an important role in regulating gene expression of plant. Post-transcriptional regulation involves multiple processes, namely alternative splicing, RNA editing, RNA transport from the nucleus to the cytoplasm, RNA stabilization, and translation, which require the help of RNA binding proteins (RBPs) [1,3]. In order to achieve sequence-specific recognition of regulation in different levels and regulatory targets, there are several RNA binding domains with conserved characteristics in RBPs, such as RRM (RNA Recognition motif) domains[4,5].

The RNA recognition motif (RRM), also known as the RNA binding domain (RBD) or ribonucleoprotein domain (RNP), is one of the most abundant protein domains in eukaryotes and was first identified in the late 1980s[6–9]. The RNA recognition motif (RRM) domain is an important player in the regulation of development, signaling, gene expression, and cell

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differentiation[10–13]. RRM is a structurally conserved region consisting of about 80-90 amino acids, consisting of two short consensus sequences: RNP1 (hexapeptide) and RNP2 (octapeptide) [14]. It folds into a $\alpha\beta$ sandwich with a typical $\beta_1\alpha_1\beta_2\beta_3\alpha_2\beta_4$ topology that forms a four-stranded antiparallel β-sheet packed against two α-helices[15]. The specificity of RNA binding is determined by multiple exposures to surrounding amino acids[14,16]. In some cases, a third helix is present during RNA binding[17]. The largest single-stranded RNA-binding proteome is the eukaryotic RNA recognition motif (RRM) family, which contains eight amino acid RRM1 consensus sequences[8,18]. RRM proteins have a variety of RNA-binding preferences and functions, including heteroribonucleo proteins (hnRNPs), proteins associated with alternative splicing regulation (SR, U2AF, Sxl), protein components of small ribonucleoproteins (U1 and U2 snRNPs), and proteins that regulate RNA stability and translation (PABP) [18–20]. The RRM in the heterodimer splicing factor U2 snRNP cofactor (U2AF) appears to have two RRM-like domains with special features for protein recognition[21]. This motif also appears in some single-stranded DNA-binding proteins[16].

Rice (*Oryza sativa Japonica*) is one of the main food crops in the world, which plays an irreplaceable role in China's food security and is also an important model crop selected by biological research. However, there are few reports on *OsRRM1* gene family. Previously unknown *RRM1* transcription factors have been identified that interact directly with *NLR* to activate plant defense, establishing a direct link between transcriptional activation of immune responses and *NRL*-mediated pathogen perception[22]. Although the rice genome encodes a large number of OsRRM1 proteins, the exact number and function of these gene families in rice remains unclear.

Magnaporthe Oryza is one of the most widespread and harmful worldwide fungal diseases caused by rice blast fungus. It may infect rice at all stages of growth and development, seriously affecting the yield and quality of rice, and thus threatening the global food security. Although the traditional chemical control means can quickly and effectively control diseases and pests, long-term use of pesticide will not only bring severe environmental problems, but also increase economic costs, which is not conducive to the sustainable development of agriculture[23]. The resistance of germplasm resources has a wide range of genetic variation, and thus the host plant's own resistance is the most effective, economical and environmentally friendly method to against *Magnaporthe Oryza* [24]. Many of studies have shown that the adaptability of rice blast fungus to the host changes frequently, and the resistance of rice varieties can only be maintained for 3 to 5 years[23,25,26]. Plant genomes express a large number of RRM-containing proteins, but only a few RRM proteins have been elucidated for their roles in plants, including immunity in plants, possibly through RNA processing[27–30]. Some researchers have identified possible members of the *RRM* transcription factor family, but have not predicted the role of all *RRM* genes in transcriptional activation in rice and other plants[31]. Therefore, it is necessary to further study the regulation of gene network during rice blast occurrence and to explore and identify new blast resistance genes, which has important theoretical and practical significance for the breeding of new varieties resistant to rice blast.

In this study, bioinformatics was used to identify and characterize the whole genome of *RRM1* gene family in rice. The gene structure, physical and chemical properties, domain and phylogenetic characteristics of *RRM1* gene family in rice were studied. In addition, RNA-seq was used to analyze the expression patterns of *RRM1* gene family in different time periods after rice blast fungus treatment. At the same time, the expression changes of *RRM1* family genes in response to stress resistance were analyzed by quantitative real-time PCR. This study increased the understanding of *OsRRM1* gene family, and provided a basis for further investigation of the function of *OsRRM1* gene under infection of rice blast fungus, and played a certain theoretical role for the subsequent study of the function of *OsRRM1* gene family.

2. Materials and Methods

2.1. Identification and physicochemical properties of RRM1 gene family members in rice

Rice(*Oryza sativa Japonica*) genome sequence, annotation files, protein sequences, and gene structure file were downloaded from the Ensembl Plants database

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(http://plants.ensembl.org/index.html). Download the HMM (Hidden Marov Model) PF00076.24 (RRM1 domain) of the RRM1 gene family from the Pfam database (http://pfam.xfam.org/). Using HMM SEARCH tool sequence in HMMER3.2 software to search and analyze, RRM1 gene family in rice was predicted, and the E-value was less than 1×10^{-5}. Domain analysis of identified RRM1 candidate sequences was performed using conserved RRM1 domain sequences in Pfam database (PF00076.24) and SMART online analysis software (http://smart.embl.de/). Using ExPASy (https://www.expasy.org/protparam/) online tools to predict protein isoelectric point, molecular size, length of protein sequences of amino acids. Use the Cell-PLoc 2.0 (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/) online tools for protein subcellular localization prediction analysis.

2.2. Chromosome location of OsRRM1 gene family and construction of phylogenetic tree

The position of *RRM1* gene on chromosome was analyzed through the rice gene sequence file downloaded from the Ensembl Plants database, and the chromosome location map was drawn by TBtools software. The NJ (neighbor-joining method) phylogenetic tree of *RRM1* protein was constructed using MEGA11.0(Molecular Evolutionary genetics Analysis11.0) with the Bootstrap value of 1000 and use default for other parameters, and then the online software Itol(https://itol.embl.de/) was used to beautify the tree.

2.3. Analysis of conserved domain, gene structure and motif of OsRRM1 gene family

Conserved domains of the identified gene families were analyzed using the online tool Pfam (https://pfam.xfam.org/), and visualized by TBtools[32].

Through the plant genome database Ensembl plant rice gene structure annotation files downloaded (http://plants.ensembl.org/), the structure information of the members of the *RRM1* gene family identified were analyzed using TBtools software for drawing genetic structure.

The conserved motif location of the identified *RRM1* gene family was predicted using online MEME (https://meme-suite.org/meme/doc/meme). The parameter was set to 10 motifs and the other parameters were default. The prediction results were plotted using TBtools software.

2.4. Interspecies collinearity analysis of OsRRM1 gene family

The collinearity analysis and prediction of *RRM1* gene in rice and *Arabidopsis Thaliana* were carried out, and the collinearity map was drawn by TBtools software.

2.5. GO and KEGG analysis of OsRRM1 gene family

GO and KEGG analysis of the *OsRRM1* gene family was performed using PlantRegMap(http://plantregmap.gao-lab.org/) and Kobas(http://kobas.cbi.pku.edu.cn/kobas3/), respectively. All analysis results were calculated with $q<0.05$. Prism8.0 was used to plot the path name as the ordinate and $-\log_{10}$(q-value) as the abscissa.

2.6. Analysis of presumptive cis-regulatory elements in the promoter region of OsRRM1 gene

Use TBtools software to predict cis acting elements in the 2000bp upstream gene promoter region of *OsRRM1* in the PlantCARE Database (https://bioinformatics.psb.ugent.be/webtools/plantcare/html).

2.7. Expression pattern analysis of RRM1 gene in rice treated with blast fungus

TBtools software was used to map the expression patterns of *OsRRM1* gene family members identified under rice blast fungus treatment.

2.8. Plant materials and rice blast stress treatment

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The experimental material is rice(*O.Sativa L.spp.japonica,var nippobare*). The mature seeds were placed in petri dishes, sterilized with 2%NaClO, soaked at 28 ° C for 48 hours, and then placed in perforated PCR plates. PCR plates were isolated by placing 24 seeds as three biological replicates. The treatment group and control group were repeated with two plates. All seedlings were placed in a growth chamber with a photoperiod of 14 h (day) /10 h (night) and a temperature cycle of 28/24 ℃.

For rice blast stress, rice seedlings were cultivated in artificial growth chamber until the three-leaf stage. Finally, suspensions of blast fungus with a concentration of $1*10^5$ times were used as stress treatment. The rice blast strain was *guy11*. At 0, 12, 24, 36 and 48h after infecting *Magnaporthe Oryza* (*guy11*), the young rice leaves were immediately frozen in liquid nitrogen and stored at -80℃ for later use.

2.9. Analysis of OsRRM1 gene expression by qRT-PCR

RNA was extracted from treated plant leaves. Total RNA was extracted using the RNA Total RNA Extraction Kit (Takara). The first strand cDNA was synthesized using PrimeScript First strand cDNA Synthesis Kit (Takara) with a reaction volume of 20 μl, consisting of 1 μg total RNA, 4 μl 5xPrime Script RT premix and RNA-free enzyme ddH_2O. The PCR procedure is as follows: 95℃ for 2 minutes, then 35 cycles, 95 ℃ for 5 seconds, 60℃ for 30 seconds.

Quantitative real-time PCR is performed on the ABI 7500 quantitative real-time fluorescent quantitative PCR system according to the manufacturer's instructions. Primer Premier 5 was used to design specific primers targeting the *OsRRM1* gene (Table 1). *Actin* is used as a reference gene. The qRT-PCR was performed in a final volume of 20 μl and consisted of 2 μl cDNA, 10 μl 2*SYBR green premix (Takara), and 1 μl forward and reverse primers. The amplification procedure is as follows: initial denaturation at 95 ℃ for 5 minutes; 35 cycles, denatured at 95 ℃ for 10 seconds, annealed at 60 ℃ for 20 seconds; And finally extended for 20 seconds at 72 ℃. Three biological replicates and three technical replicates were performed for each cDNA sample. Relative expression values are calculated by the $2^{-\Delta\Delta CT}$ method.

Table 1. Specific primers for *OsRRM1* gene.

Gene	F-primer	R-primer
RRM1-15	GGATGTGACTGAAGCTCGGGTGATC	GGATGTGACTGAAGCTCGGGTGATC
RRM1-61	GGAGGTCTTGGAAGCCAAGGTCATC	CCATCCATGTCAGCGCCATCAAG
RRM1-76	CACTGAAGCAAAGGTGGTTTTTGAC	GAGCTTTATCGACAGTGATCGCC
RRM1-207	CTTGGATGGAAAGGATCTCGATGG	CATAGCCACCGCCTCCATAG
Actin	CCAATCGTGAGAAGATGACCCA	CCATCAGGAAGCTCGTAGCTCT

3. Results

3.1. Screening and identification of RRM1 gene family members in rice

In this study, domains (Pfam: PF00076.24) predicted that 212 *RRM1* genes (all with E values less than 1×10^{-5}) were identified in the whole genome of rice (*Oryza sativa Japonica*), and their conserved domain was analyzed by Pfam (Figure 1). The results showed that all 212 *OsRRM1* genes contained *RRM1,* but the location in the gene was different. These genes are named *OsRRM1-1-OsRRM1-212* based on their physical location on the chromosome (Table 2). Use Expasy (https://web.expasy.org/protparam/) article analyzed *212 OsRRM1* gene molecular weight, length, isoelectric point, amino acids, et al. The results showed that the length of amino acids encoding the 212 rice *RRM1* genes ranged from 53aa to 1160aa, the molecular weight ranged from 5837Da to 127816Da, and the theoretical isoelectric point distribution ranged from 3.97 to 12.37, which made the study of the *OsRRM1* gene family more difficult. Subcellular localization prediction showed that *OsRRM1* was mainly located in the nucleus, followed by the extracellular matrix, mitochondria, chloroplast, cell membrane, and intracytoplasmic matrix. This suggests that these proteins function

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were mainly in the nucleus. In addition, functional reports of several previously studied genes are listed.

Table 2. Basic information of *OsRRM1* gene identified in rice.

Gene	RAP	Number of Amino Acid	Molecular Weight	pI	subcellular localization
RRM1-1	*Os01g0101600*	978	106323.33	9.56	nucleus
RRM1-2	*Os01g0155600*	324	36893.16	11.27	nucleus
RRM1-3	*Os01g0209400*	308	33656.38	8.94	nucleus
RRM1-4	*Os01g0265800*	490	49279.96	5.09	nucleus
RRM1-5	*Os01g0316600*	124	13965.3	9.91	chloroplast
RRM1-6	*Os01g0367300*	698	79763.67	10.57	nucleus
RRM1-7	*Os01g0502800*	53	5837.75	10.27	chloroplast
RRM1-8	*Os01g0614500*	447	44269.69	8.43	nucleus
RRM1-9	*Os01g0619000*	163	18016.54	5.76	extracellular space
RRM1-10[33]	*Os01g0636700*	469	52108.23	8.63	nucleus
RRM1-11	*Os01g0867800*	439	49316.8	6.46	nucleus
RRM1-12	*Os01g0876500*	100	11385	7.72	chloroplast
RRM1-13	*Os01g0876800*	300	31951.79	8.91	extracellular space
RRM1-14[34–36]	*Os01g0907900*	683	71779.19	6.37	nucleus
RRM1-15	*Os01g0916600*	150	15546.9	8.01	chloroplast thylakoid lumen
RRM1-16	*Os01g0938200*	460	48888.22	8.72	nucleus
RRM1-17	*Os01g0945800*	363	40073.63	6.61	nucleus
RRM1-18	*Os01g0956600*	608	68184.84	7.8	nucleus
RRM1-19	*Os01g0958500*	310	31802.78	8.34	nucleus
RRM1-20	*Os01g0959000*	432	48111.49	12.37	chloroplast thylakoid lumen
RRM1-21	*Os01g0974701*	116	12459.19	9.74	mitochondrion
RRM1-22	*Os02g0122800*	249	28953.55	10	nucleus
RRM1-23	*Os02g0131700*	448	49261.45	5.02	nucleus
RRM1-24	*Os02g0167500*	957	105767.07	7.88	extracellular space
RRM1-25	*Os02g0179900*	240	28105.95	8.85	nucleus
RRM1-26	*Os02g0221500*	397	40265.08	5.63	nucleus
RRM1-27	*Os02g0244600*	359	38737.53	5.62	nucleus
RRM1-28	*Os02g0252100*	265	30466.57	11.09	nucleus
RRM1-29	*Os02g0319100*	811	90295.23	6.27	nucleus
RRM1-30	*Os02g0497700*	480	50879.51	5.01	nucleus
RRM1-31[37]	*Os02g0517531*	1001	110368.83	6.39	nucleus
RRM1-32	*Os02g0536400*	656	74812.42	9.43	nucleus
RRM1-33	*Os02g0567900*	259	28284.5	9.18	nucleus
RRM1-34	*Os02g0602600*	386	41584.82	7.67	nucleus
RRM1-35	*Os02g0610400*	467	51689.83	5.56	nucleus
RRM1-36	*Os02g0610600*	200	22797.31	11.33	nucleus
RRM1-37[38,39]	*Os02g0612300*	243	28573.22	5.44	chloroplast
RRM1-38	*Os02g0714000*	287	30609.94	9.32	nucleus
RRM1-39	*Os02g0719800*	428	47331.12	5.57	nucleus
RRM1-40	*Os02g0730800*	399	43547.8	6.15	extracellular space
RRM1-41	*Os02g0755400*	176	18512.61	9.99	mitochondrion
RRM1-42	*Os02g0757900*	212	24083.82	5.07	nucleus
RRM1-43	*Os02g0788300*	295	32235.18	7.72	nucleus

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RRM1-44	*Os02g0788400*	289	32009.09	8.66	nucleus
RRM1-45	*Os02g0789400*	185	21023.33	11.24	nucleus
RRM1-46	*Os02g0815200*	316	34612.01	5.17	chloroplast thylakoid lumen
RRM1-47	*Os03g0123200*	252	28108.69	7.64	nucleus
RRM1-48	*Os03g0136800*	296	32305.94	9.02	nucleus
RRM1-49	*Os03g0174100*	416	46056.33	5.35	nucleus
RRM1-50	*Os03g0265600*	125	13993.55	7.86	chloroplast
RRM1-51	*Os03g0278300*	238	24720.42	9.83	chloroplast
RRM1-52	*Os03g0278500*	647	72627.76	8.43	nucleus
RRM1-53	*Os03g0278800*	173	18433.86	9.3	chloroplast outer membrane
RRM1-54	*Os03g0285900*	330	37042.2	11	nucleus
RRM1-55	*Os03g0286500*	310	32704.09	9	extracellular space
RRM1-56	*Os03g0298800*	232	26100.86	9.44	chloroplast
RRM1-57	*Os03g0326600*	467	51073.78	9.06	nucleus
RRM1-58	*Os03g0344100*	264	29782.1	10.08	nucleus
RRM1-59	*Os03g0363800*	243	27781.69	10.83	nucleus
RRM1-60	*Os03g0374575*	217	25589.48	11.17	nucleus
RRM1-61	*Os03g0376600*	265	28556.57	4.5	chloroplast outer membrane
RRM1-62	*Os03g0376900*	464	49564.37	6.39	nucleus
RRM1-63	*Os03g0388000*	205	24739.51	10.27	nucleus
RRM1-64	*Os03g0418800*	523	56761.18	8.75	chloroplast
RRM1-65	*Os03g0566500*	429	46194.37	9.62	chloroplast
RRM1-66	*Os03g0569900*	402	43945.82	5.34	extracellular space
RRM1-67	*Os03g0670700*	196	20375.4	6.73	nucleus
RRM1-68	*Os03g0681900*	308	34036.6	9.05	nucleus
RRM1-69[40]	*Os03g0713600*	284	30904.71	5.06	nucleus
RRM1-70	*Os03g0748900*	278	29986.94	9.23	nucleus
RRM1-71	*Os03g0801800*	959	105396.52	9.48	nucleus
RRM1-72	*Os03g0809900*	197	21969.34	5.2	nucleus
RRM1-73	*Os03g0811700*	130	14710.82	9.49	chloroplast
RRM1-74	*Os03g0824300*	523	58186.08	7.22	nucleus
RRM1-75	*Os03g0826400*	312	36258.57	9.25	nucleus
RRM1-76	*Os03g0836200*	205	21823.38	8.29	nucleus
RRM1-77	*Os03g0854300*	441	48288.94	10.11	nucleus
RRM1-78	*Os04g0118900*	245	28783.89	9.94	nucleus
RRM1-79	*Os04g0306800*	649	72026.14	9.09	nucleus
RRM1-80	*Os04g0372800*	486	51446	5.1	nucleus
RRM1-81	*Os04g0394300*	903	97243.83	8.7	nucleus
RRM1-82	*Os04g0414300*	137	15074.25	9.93	chloroplast
RRM1-83	*Os04g0449900*	387	41807.64	8.68	extracellular space
RRM1-84	*Os04g0467300*	484	51314.72	7.33	nucleus
RRM1-85	*Os04g0496400*	476	53576.63	4.69	nucleus
RRM1-86	*Os04g0497600*	435	48295.81	5.49	nucleus
RRM1-87	*Os04g0504800*	659	71231.24	8.95	extracellular space
RRM1-88	*Os04g0510500*	462	51785.72	5.01	nucleus
RRM1-89	*Os04g0543200*	774	86649.43	5.64	nucleus
RRM1-90	*Os04g0591000*	291	31672.86	6.05	mitochondrion
RRM1-91	*Os04g0611500*	536	60240.64	9.16	nucleus
RRM1-92	*Os04g0620700*	707	75253.44	4.85	nucleus

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RRM1-93	*Os04g0624800*	376	40858.93	5.59	nucleus
RRM1-94	*Os04g0625800*	425	46195.8	5.99	extracellular space
RRM1-95[41]	*Os04g0636900*	515	52204.84	5.79	nucleus
RRM1-96	*Os04g0641400*	144	16026.58	4.61	nucleus
RRM1-97	*Os04g0682400*	1008	110200.99	6.17	nucleus
RRM1-98[42]	*Os04g0684500*	901	101135.53	6.65	chloroplast inner membrane
RRM1-99	*Os05g0102800*	955	104522	6.01	nucleus
RRM1-100	*Os05g0105900*	380	42434.11	12.18	nucleus
RRM1-101	*Os05g0114500*	290	32890.31	6.85	nucleus
RRM1-102	*Os05g0120100*	323	36222.41	10.83	nucleus
RRM1-103	*Os05g0140500*	204	22104.33	5.18	nucleus
RRM1-104	*Os05g0154800*	253	28203.66	9.2	cytoplasm
RRM1-105	*Os05g0162600*	338	39019.1	9.83	nucleus
RRM1-106	*Os05g0223200*	104	11486.44	8.03	nucleus
RRM1-107	*Os05g0223300*	102	11702.99	5.06	nucleus
RRM1-108	*Os05g0303700*	254	29800.11	8.77	nucleus
RRM1-109	*Os05g0364600*	319	36105.16	11.2	nucleus
RRM1-110	*Os05g0373400*	466	50213.29	8.1	nucleus
RRM1-111	*Os05g0376000*	209	23394.61	9.14	nucleus
RRM1-112	*Os05g0437300*	444	49754.23	6.41	nucleus
RRM1-113[40]	*Os06g0112400*	261	27763.35	6.23	nucleus
RRM1-114	*Os06g0127500*	265	28209.55	7.14	nucleus
RRM1-115	*Os06g0151200*	300	32650.85	5	nucleus
RRM1-116	*Os06g0170500*	482	54009.89	8.12	nucleus
RRM1-117	*Os06g0187900*	185	21183.36	11.29	nucleus
RRM1-118	*Os06g0219600*	204	23178.94	5.19	nucleus
RRM1-119	*Os06g0220600*	343	36170.91	9.63	chloroplast outer membrane
RRM1-120	*Os06g0248200*	164	17952.57	5.98	nucleus
RRM1-121	*Os06g0256200*	294	31817.7	10.97	nucleus
RRM1-122	*Os06g0566100*	292	29810.49	9.33	nucleus
RRM1-123	*Os06g0589700*	399	43823.12	9.17	nucleus
RRM1-124	*Os06g0622900*	275	29594.2	8.39	nucleus
RRM1-125	*Os06g0670400*	469	53864.27	5.38	nucleus
RRM1-126	*Os06g0670500*	564	64975.32	5.63	nucleus
RRM1-127	*Os06g0687500*	219	23922.07	9.52	endomembrane system
RRM1-128	*Os06g0698400*	123	13222.7	5	nucleus
RRM1-129	*Os06g0724600*	164	18503.88	10.31	nucleus
RRM1-130	*Os07g0102500*	438	47703.93	9.53	nucleus
RRM1-131	*Os07g0124600*	377	41006.31	6.68	nucleus
RRM1-132	*Os07g0158300*	364	39084.91	4.61	mitochondrion
RRM1-133	*Os07g0180800*	411	46253.74	9.65	nucleus
RRM1-134	*Os07g0237100*	340	36144.67	10.27	chloroplast
RRM1-135	*Os07g0281000*	486	54334.99	6.72	nucleus
RRM1-136	*Os07g0296200*	394	43291.14	8.3	nucleus
RRM1-137	*Os07g0516900*	251	27613.79	6.3	extracellular space
RRM1-138	*Os07g0549800*	133	14421.25	9.41	chloroplast outer membrane
RRM1-139	*Os07g0583500*	474	54197.46	6.55	extracellular space
RRM1-140	*Os07g0584500*	472	50477.44	5.94	nucleus
RRM1-141	*Os07g0602600*	238	23564.23	8.54	mitochondrion

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RRM1-142	*Os07g0603100*	569	62175.74	6.15	nucleus
RRM1-143	*Os07g0615400*	427	46723.56	7.19	nucleus
RRM1-144	*Os07g0623300*	275	32242.91	11.35	nucleus
RRM1-145[43]	*Os07g0631900*	264	28099.31	4.75	chloroplast thylakoid lumen
RRM1-146	*Os07g0633200*	213	24820.57	10.68	nucleus
RRM1-147	*Os07g0663300*	427	46493.89	9.17	nucleus
RRM1-148	*Os07g0673500*	296	33141.48	10.64	nucleus
RRM1-149	*Os08g0113200*	838	95016.62	5.47	endomembrane system
RRM1-150	*Os08g0116400*	302	32739.26	6.4	nucleus
RRM1-151	*Os08g0117100*	319	35941.88	6.02	chloroplast outer membrane
RRM1-152	*Os08g0139000*	111	11938.8	9.55	chloroplast outer membrane
RRM1-153	*Os08g0190200*	442	47809.63	5.86	extracellular space
RRM1-154	*Os08g0192900*	572	60393.68	4.98	nucleus
RRM1-155	*Os08g0314800*	660	71558.46	7.55	nucleus
RRM1-156	*Os08g0320100*	350	36738.65	9.22	nucleus
RRM1-157	*Os08g0385900*	279	32947.48	11.88	nucleus
RRM1-158	*Os08g0412200*	214	25104.07	10.05	chloroplast
RRM1-159	*Os08g0416400*	503	54742.2	7.66	nucleus
RRM1-160	*Os08g0427900*	286	30491.17	11.05	nucleus
RRM1-161	*Os08g0436000*	461	49888.14	6.46	nucleus
RRM1-162	*Os08g0483200*	269	29132.14	9.39	mitochondrion
RRM1-163	*Os08g0486200*	289	33541.09	11.8	nucleus
RRM1-164	*Os08g0490300*	603	64733.85	6.09	nucleus
RRM1-165	*Os08g0492100*	362	38125.83	9.22	nucleus
RRM1-166	*Os08g0504600*	684	75299.38	6.19	nucleus
RRM1-167	*Os08g0520300*	447	48765.28	6.86	nucleus
RRM1-168	*Os08g0547000*	294	31708.05	7.08	nucleus
RRM1-169	*Os08g0557100*	194	21388.83	4.95	chloroplast
RRM1-170	*Os08g0567200*	235	26254.56	9.77	nucleus
RRM1-171	*Os09g0115400*	662	71630.27	6.45	mitochondrion
RRM1-172[44]	*Os09g0123200*	738	79658.29	9.09	nucleus
RRM1-173	*Os09g0279500*	245	26681.14	8.53	chloroplast thylakoid lumen
RRM1-174[45]	*Os09g0298700*	1005	110844.59	6.79	nucleus
RRM1-175	*Os09g0299500*	160	17315.17	5.76	extracellular space
RRM1-176	*Os09g0314500*	353	38868.39	5.96	nucleus
RRM1-177	*Os09g0462700*	441	46949.78	8.52	chloroplast
RRM1-178	*Os09g0476100*	604	64263.07	6.3	nucleus
RRM1-179	*Os09g0491756*	290	34087.52	8.92	nucleus
RRM1-180	*Os09g0513700*	375	43193.42	9.74	nucleus
RRM1-181[46]	*Os09g0516300*	900	97198.57	6.85	nucleus
RRM1-182	*Os09g0527100*	149	16616.66	8.8	nucleus
RRM1-183	*Os09g0527500*	235	25960.25	8.81	nucleus
RRM1-184[47]	*Os09g0549500*	276	29500.33	9.18	nucleus
RRM1-185	*Os09g0565200*	322	35425.05	4.41	mitochondrion
RRM1-186	*Os10g0115600*	463	55113.96	9.1	nucleus
RRM1-187	*Os10g0151800*	438	47821.62	4.98	nucleus
RRM1-188	*Os10g0167500*	374	40267.56	3.97	nucleus

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RRM1-189	*Os10g0321700*	317	32244.11	4.59	chloroplast thylakoid lumen
RRM1-190	*Os10g0439600*	330	34829.59	4.96	nucleus
RRM1-191	*Os10g0457000*	355	38849.39	8.55	nucleus
RRM1-192	*Os10g0470900*	464	45620.47	6.24	nucleus
RRM1-193	*Os10g0569200*	719	83181.8	4.98	nucleus
RRM1-194	*Os11g0100200*	219	24033.05	9.87	nucleus
RRM1-195	*Os11g0133600*	298	32998.39	7.65	nucleus
RRM1-196	*Os11g0139500*	189	21471.25	4.13	extracellular space
RRM1-197	*Os11g0176100*	495	52955.01	6.43	extracellular space
RRM1-198[48]	*Os11g0250000*	441	48446.94	5.68	nucleus
RRM1-199	*Os11g0549537*	242	26479.77	6.08	chloroplast
RRM1-200	*Os11g0620100*	441	47561.26	6.86	nucleus
RRM1-201	*Os11g0636900*	550	61141.76	7.78	nucleus
RRM1-202	*Os11g0637700*	467	49048.64	8.44	nucleus
RRM1-203	*Os11g0704700*	511	57960.23	10.14	chloroplast
RRM1-204[49]	*Os12g0100100*	228	24809.9	9.87	nucleus
RRM1-205	*Os12g0131000*	300	33277.87	8.81	chloroplast
RRM1-206	*Os12g0136200*	502	55072.87	5.03	nucleus
RRM1-207	*Os12g0502200*	258	25044.52	4.74	mitochondrion
RRM1-208	*Os12g0572400*	263	30186.19	10.9	nucleus
RRM1-209[50–52]	*Os12g0572800*	1160	127816.97	8.61	plasma membrane
RRM1-210	*Os12g0577100*	414	47380.57	9.1	nucleus
RRM1-211	*Os12g0587100*	947	106893.09	9.14	nucleus
RRM1-212[53]	*Os12g0632000*	162	16083.1	6.31	nucleus

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Figure 1. The conserved domain of *OsRRM1* gene family.

3.2. Chromosome localization and phylogenetic tree analysis of OsRRM1 gene family

The positions of 212 *OsRRM1* genes on chromosomes were mapped using TBtools software (Figure 2). There were 212 *OsRRM1* genes distributed on all 12 chromosomes, among which 31 *OsRRM1* genes were the most distributed on chromosome 3, and only 8 OsRRM1 genes were the least distributed on chromosome 10. Distinct gene clusters were formed on chromosomes 1, 2, and 3.

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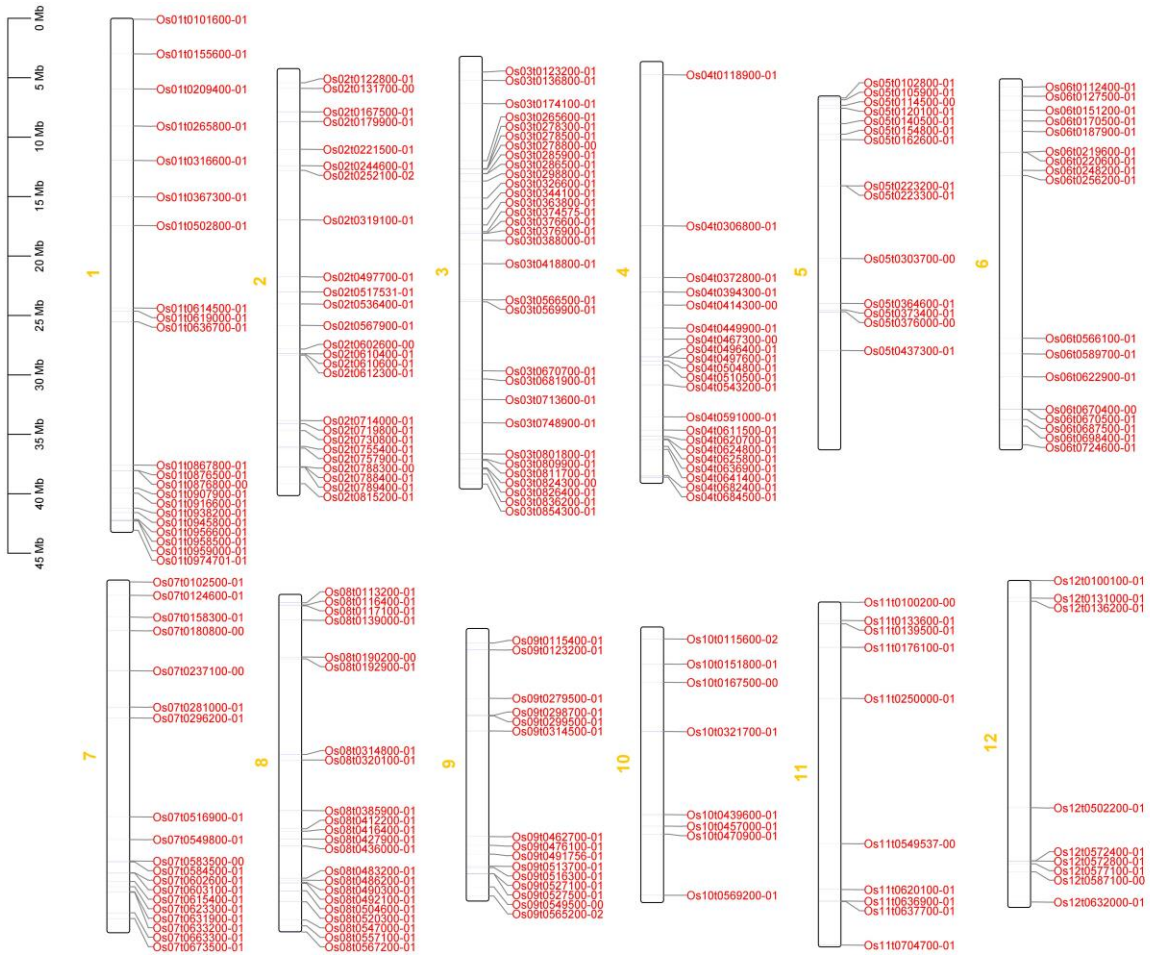

Figure 2. Chromosome mapping of *OsRRM1* gene family.

In order to study the phylogenetic relationship of *OsRRM1* protein, a phylogenetic tree was constructed for 21 OsRRM1 protein sequences in rice (Figure 3). According to the topological structure of the evolutionary tree, 212 OsRRM1 proteins can be divided into 5 groups. The fifth group (Branch marks green) contained the highest amount of OsRRM1 protein and 61 proteins in total; The third group (Branch marks dark green) contained 58 RRM1 proteins, the second group (Branch marks red) and the fourth group (Branch marks blue) contained 33 RRM1 proteins and 53 RRM1 proteins respectively. The first group (Branch marks orange) had the lowest number of RRM1 proteins, seven in total.

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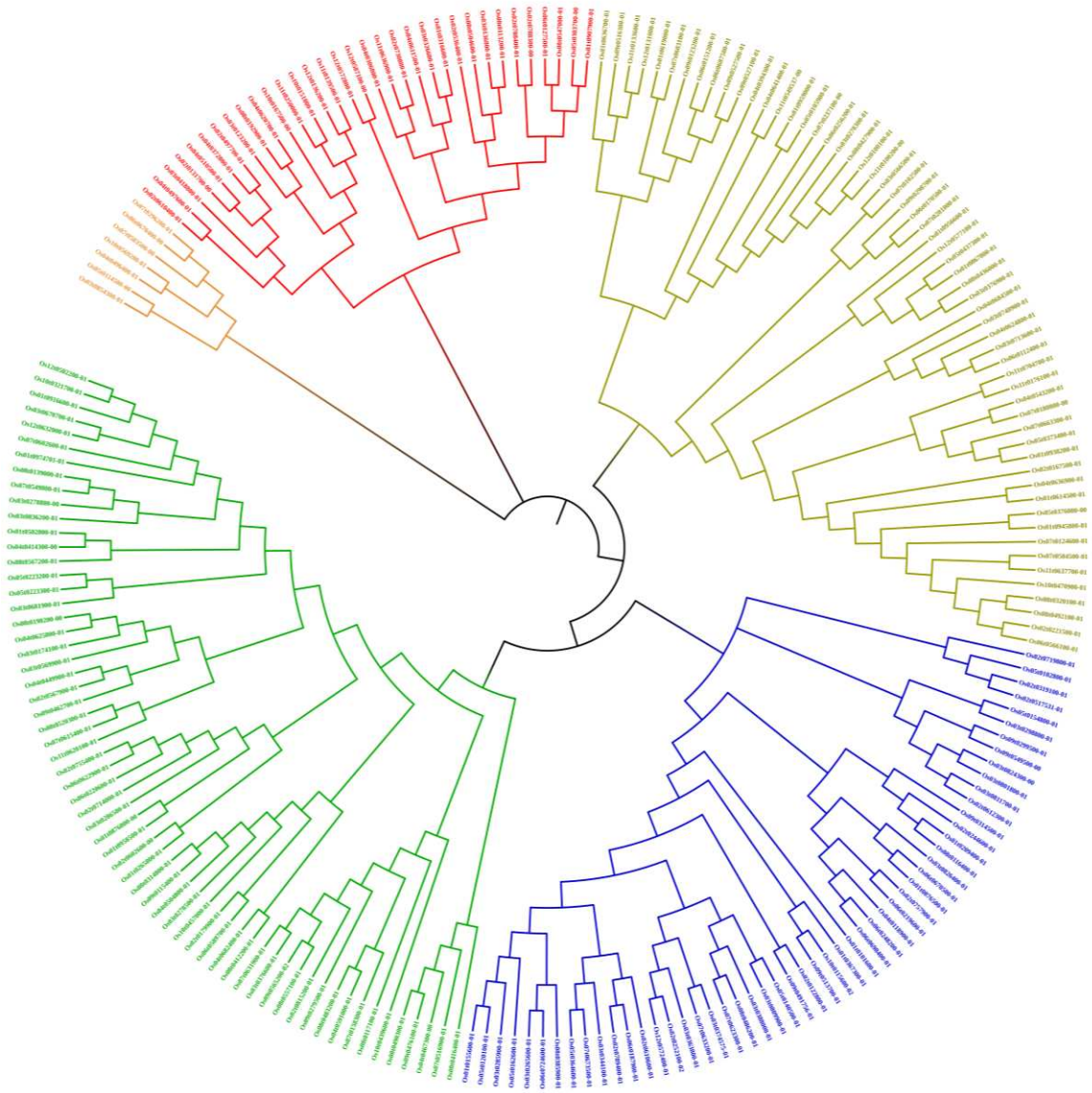

Figure 3. Phylogenetic tree of *OsRRM1* gene family.

3.3. Motif analysis and gene structure analysis of OsRRM1 gene family

The evolution of a family is mainly manifested by the diversity of gene structure and the change of conserved motifs. In order to better understand the structure of *OsRRM1* gene, the exon intron structure of *OsRRM1* gene was analyzed using annotated information from the rice reference genome (Figure 4). The results showed that 212 *OsRRM1* genes had large differences in sequence length and exons and introns, but the same as the clustering results of evolutionary tree, genes in the same group usually had similar structure, but their intron lengths were different. It was also found that exon-intron patterns in the same phylogenetic taxa showed great similarity. This may be the result of replication of these sequences, which may also prove that the classification results are reliable.

Then, the online prediction tool MEME was used to identify the conserved motifs of OsRRM1 protein. Multiple motifs exist in 212 OsRRM1 protein sequences (Figure 4), and the types and numbers of motifs are highly overlapping. In addition, gene families within the same subfamily in the evolutionary tree are composed similarly on the motif.

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Figure 4. Motif analysis and prediction of *OsRRM1* gene family(left) and Schematic diagram of gene structure of *OsRRM1* gene family(right).

3.4. Evolutionary analysis of OsRRM1 gene family and collinearity analysis of RRM1 gene family between rice and Arabidopsis Thaliana

Phylogenetic tree was constructed by comparing 212 *OsRRM1* and 230 *AtRRM1* sequences, with a total of 442 members (Figure A1). According to the topological structure of the evolutionary tree, RRM1 proteins of the two species can be divided into five groups. Most of the RRM1 protein members of rice and *Arabidopsis* do not cluster into their own clades. Each subfamily contains members of the *RRM1* family of *Arabidopsis* and rice, and the members of each subfamily may have similar functions and domains. According to the phylogenetic relationship of protein sequences, the function of OsRRM1 protein can be predicted by the function of plant RRM1 protein with known function.

In order to further explore the evolutionary relationship of *OsRRM1* gene family, collinearity analysis was conducted between rice and *Arabidopsis Thaliana*. The results showed (Figure 5) that 20 pairs of *RRM1* genes in the two species were collinear, and no collinearity was found on chromosomes 8, 9, 10, 11 and 12 of rice and chromosome 4 of *Arabidopsis thaliana*.

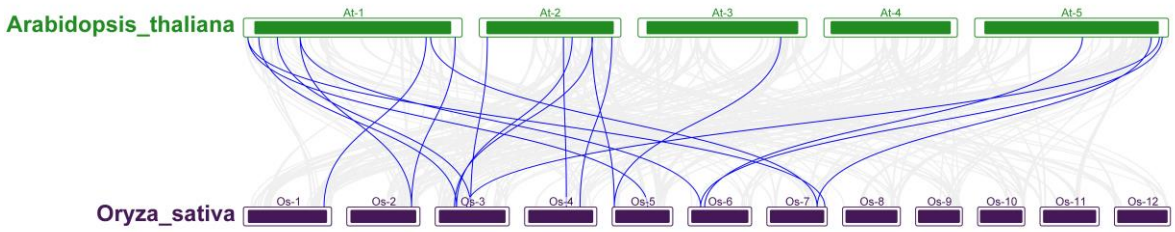

Figure 5. The synteny analysis *RRM1* gene family in *Arabidopsis thaliana* and rice.

3.5. GO and KEGG analysis of OsRRM1 gene family

GO annotation results showed (Figure 6) that the *OsRRM1* gene family plays an important role in biological processes such as innate immune response, immune response, stimulus response, defense response of biological processes, regulatory transcription negative regulation, DNA template negative regulation of gene expression, epigenetic immune system processes, and alternative splicing. These results further confirm the reported functions of *RRM1* gene in these aspects. KEGG analysis showed (Figure 6) that *OsRRM1* gene family plays an important role in alternative splicing, messenger RNA surveillance pathway, RNA transport, and RNA degradation.

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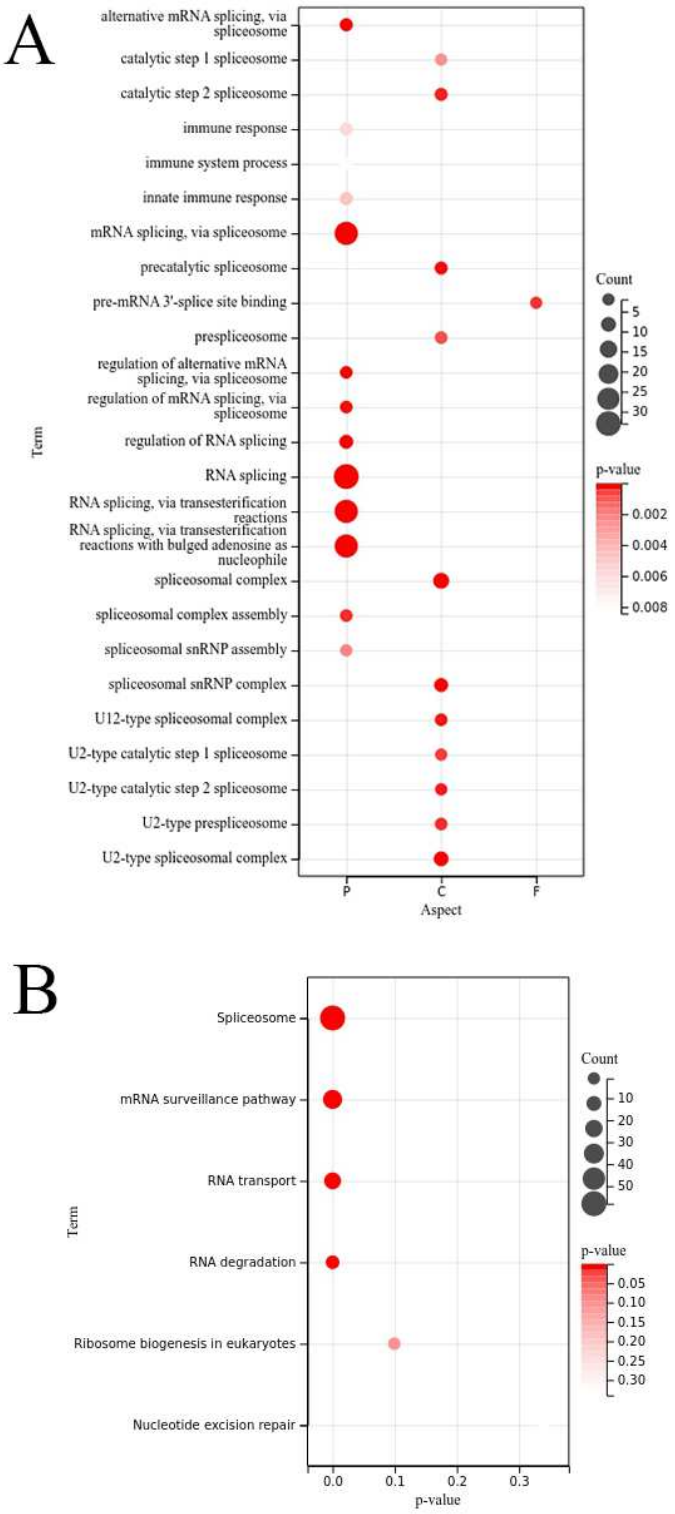

Figure 6. Geng function enrichment analysis. (A) Analysis of GO of *OsRRM1* gene family; (B) KEGG annotation of *OsRRM1* gene family.

3.6. Characterization of presumptive cis-regulatory elements in the promoter region of OsRRM1 gene

The cis-regulatory elements in the promoter region play an important role in plant response to stress. Using the PlantCARE database, we identified five stress response cy-regulatory elements in

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the upstream 2000bp of these *OsRRM1* genes, including TGACG motif (involved in JA response), CGTCA motif (involved in MeJA response), ABRE motif (involved in abscisic acid stress), TCA element (involved in salicylic acid reactivity), TGACG motif (involved in JA response), and CGTCA motif (involved in salicylic acid reactivity). WUN motif (wound response element). In the *OsRRM1* gene family, the element associated with the largest number of stress response elements was ABRE (Figure 7), and ABA was synthesized mainly in response to blast stress. These results indicate that the *OsRRM1* gene and stress-related response elements are relatively intact, but the types and amounts of stress-related elements contained in the promoter of each *OsRRM1* gene are different, suggesting that members of the *OsRRM1* gene family respond differently to rice blast stress.

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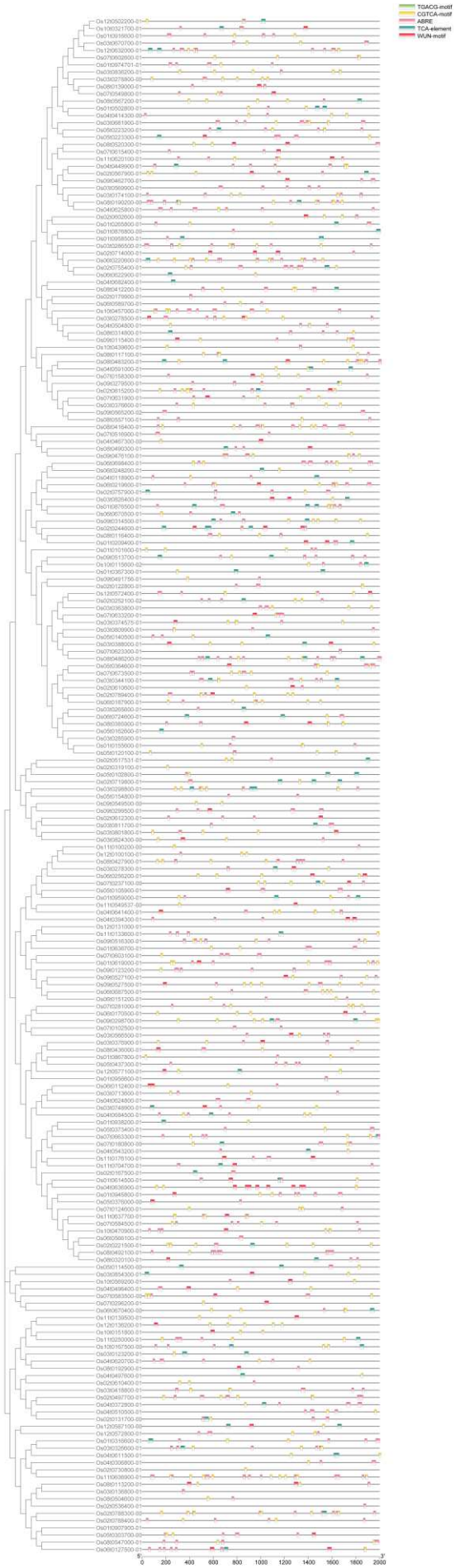

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Figure 7. Predictive cis-regulatory elements in the promoter of *OsRRM1* gene family.

3.7. Expression pattern of RRM1 gene family in rice after treatment with blast fungus

Using RNA-seq data, heat maps of 212 *OsRRM1* genes represented by log_2foldchange values were constructed at different time periods after infecting *Magnaporthe Oryza*(Figure 8). All *OsRRM1* genes were expressed, and three major clusters of expression patterns were distinguished according to the expression specificity at different time periods after treatment. The *RRM1* gene in two clusters showed an obvious up-regulation trend.

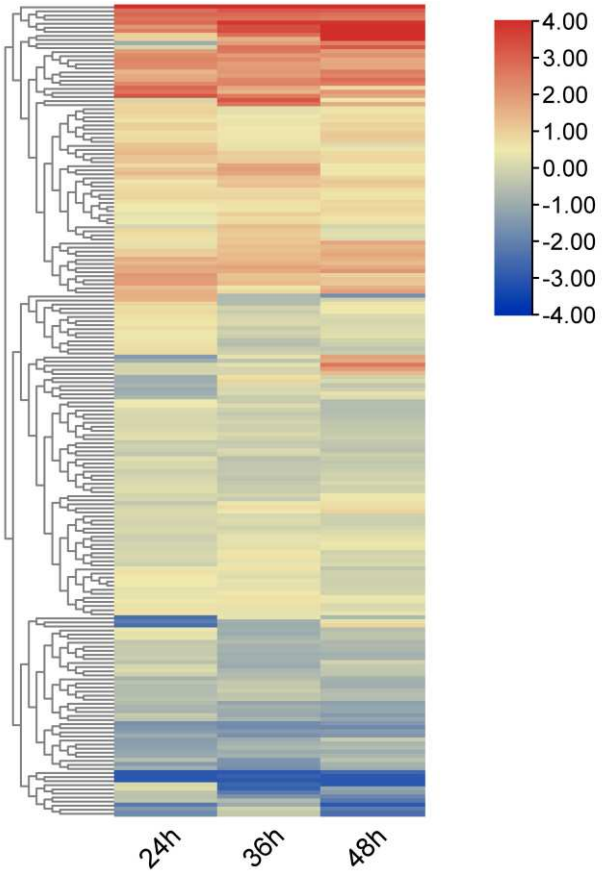

Figure 8. Expression heat map of *OsRRM1* gene family treated with *Magnaporthe oryzae*.

3.8. Expression analysis of OsRRM1 gene in response to biological stress

In order to further explore the expression changes of *OsRRM1* gene in response to biological stress, qRT-PCR was performed on 4 *OsRRM1* genes through the analysis of GO and KEGG results combined with the expression heat map to measure the transcription level of *OsRRM1* gene. There were differences in the expression levels of four *OsRRM1* genes under rice blast stress, all of which were up-regulated after treatment (Figure 9), indicating that four *OsRRM1* genes played a certain role in the development of rice blast.

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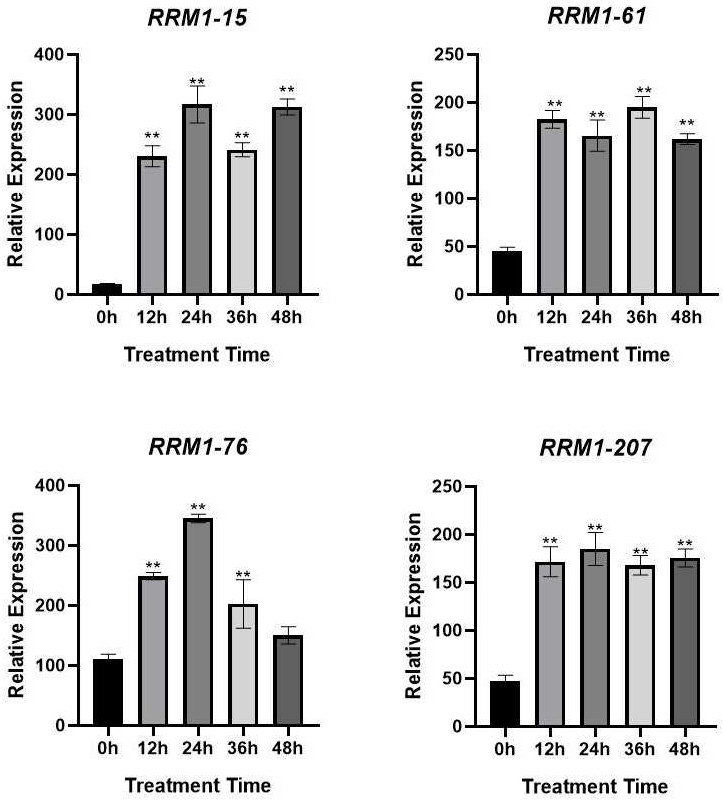

Figure 9. Expression levels of *OsRRM1* gene at different time periods under rice blast stress.

4. Discussion

Rice is one of the most important food crops and a monocotyledonous plant. In this study, bioinformatics was used to analyze the whole genome of rice *OsRRM1* gene family. The length of amino acids encoded by 212 *OsRRM1* genes ranges from 53aa to 1160aa, while Antoine Cler's study indicated that the length of RRM is 90 amino acids, which is because there are a large number of introns in the *RRM1* gene in rice, and these introns are largely discarded during transcription and translation. Therefore, the RRM1 protein encoded by the *RRM1* gene actually has only about 90 amino acids (Figure 4)[54]. It is now clear that *RRM1* is an important domain that needs to be further understood and that further biochemical and structural studies are needed to obtain a complete model of its role in cells[16]. The *RRM1* gene family is distributed in many species, 230 of which have been identified in Arabidopsis and 212 in rice. One study investigated the complete Arabidopsis genome containing proteins containing RRM and KH RNA binding domains, and the *Arabidopsis* genome encodes 196 RRM proteins[55]. The phylogenetic tree analysis of *RRM1* protein in rice and *Arabidopsis* showed that there were multiple pairs of *RRM1* homologous genes in rice and *Arabidopsis*, suggesting that these genes have similar amino acid sequences in rice and *Arabidopsis* and may have similar functions. Since rice is a monocotyledonous plant and Arabidopsis is a dicotyledonous plant, it can be inferred that the time of *RRM1* gene evolution may be earlier than the time of species differentiation. Subcellular localization prediction showed that *OsRRM1* gene was mainly located in the nucleus, followed by the extracellular matrix, mitochondria, chloroplast, cell membrane, and intracytoplasmic matrix, indicating that the above proteins mainly function in the nucleus. According to subcellular localization prediction tools, 23 Arabidopsis RRM proteins were reported to be located in chloroplasts and 10 in mitochondria[56]. This result may be due to the fact that the main site of DNA replication is in the nucleus, with a small amount of DNA replication in mitochondria and chloroplasts. In chromosome localization, 212 *RRM1* genes were found to be distributed on 12

chromosomes. In addition, there were multiple gene clusters on some chromosomes, which may be attributed to tandem duplication, resulting in gene amplification, which is of great significance in evolution. Among 212 *OsRRM1* gene sequences, CDS and introns had different numbers and large spans. However, analysis of 10 amino acid conserved motifs of 212 *OsRRM1* family proteins showed that the conserved sequences of *OsRRM1* were mostly similar, especially in homologous sequences (Figure 4).

RRM1 gene was enriched by analysis of GO and KEGG, and this family gene was mainly enriched in biological processes related to stress resistance, such as rice blast immune pathway. As previous studies have shown, the RRM protein in plant organelles is involved in various RNA processes, regulating plant development (such as flowering) and plant stress response[57]. Moreover, this gene family is highly enriched in alternative splicing and mRNA assembly processes[58]. Studies have shown that both *PSRP2* and *ORRM5* have RNA-binding activity, and it is speculated that RRM proteins increase their RNA-binding energy as RNA chaperone under stress conditions[59–61]. They are also involved in plant development and stress responses, sometimes acting as proteins or RNA-binding proteins[62,63]. In addition, several RRM proteins have been reported to be involved in plant development and stress response[59,64–66]. It can be inferred that this gene family may be involved in immunity of rice by regulating downstream gene alternative splicing. The cis-regulatory elements in the promoter region play an important role in plant response to stress. We identified five stress response cis-regulatory elements (Figure 7) in the upstream 2000bp of these *OsRRM1* genes, including TGACG motif (involved in JA response), CGTCA motif (involved in MeJA response), ABRE motif (involved in ababolic acid stress), TCA element (involved in salicylic acid reactivity), TGACG motif (involved in JA response), and TCA motif (involved in salicylic acid reactivity), WUN motif (wound response element). These results indicate that the stress-related response elements of *OsRRM1* gene are relatively complete, suggesting that members of the *OsRRM1* gene family regulate stress to a certain extent. In order to further explore the expression changes of *OsRRM1* gene in response to biological stress, qRT-PCR was performed on four *OsRRM1* gene candidates through the analysis of GO and KEGG results and combined with the expression heat map to measure the transcription level of *OsRRM1* gene. There were differences in the expression levels of four *OsRRM1* genes under rice blast stress, all of which were up-regulated after treatment (Figure 9), indicating that *OsRRM1* played a certain role in the development of rice blast, which also verified the results of S. Wang and X. Shi[67].

This study offers an initial comprehension of the *RRM1* gene family in rice, elucidating the potential roles of these genes in rice resistance, and establishing a basis for future investigations into the functions of individual members within this gene family. Subsequent steps will involve cloning, analysis of expression patterns, and functional verification of relevant genes in order to deepen our understanding and explore the significant contribution of *RRM1* genes to the growth and development of rice.

Author Contributions: Conceptualization, Xinlei Jiang, Shaochun Liu and Junying Huang; methodology, Shangwei Yu; software, Shangwei Yu; validation, Xinlei Jiang and Yuhan Huang; formal analysis, Xinlei Jiang; investigation, Junru Fu; resources, Haihui Fu and Dewei Ynag; data curation, Haihui Fu; writing—original draft preparation, Xinlei Jiang; writing—review and editing, Haihui Fu; visualization, Xinlei Jiang; supervision, Haihui Fu; project administration, Haihui Fu; funding acquisition, Haihui Fu. All authors have read and agreed to the published version of the manuscript.

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Appendix A

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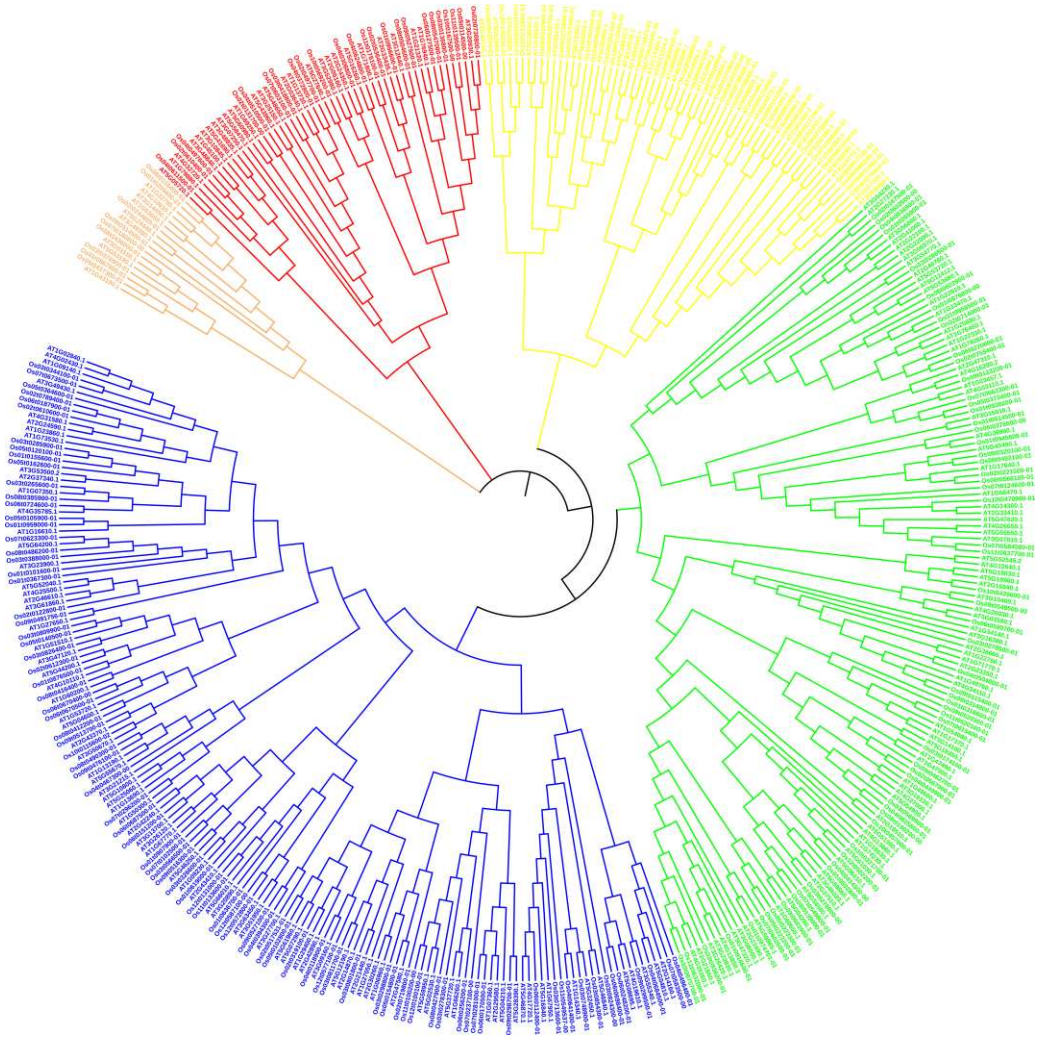

Figure A1. Phylogenetic tree of *RRM1* gene families in *Arabidopsis* and rice.

References

1. D. S. Latchman. "Transcriptional Gene Regulation in Eukaryotes." In *Els*, 2011.
2. E. L. Jeune and A. G. Ladurner. "Book Review." *Protein Science* 13, no. 7 (2004): 1950–52.
3. D. A. Jackson, A. Pombo and F. Iborra. "The Balance Sheet for Transcription: An Analysis of Nuclear Rna Metabolism in Mammalian Cells." *Faseb j* 14, no. 2 (2000): 242-54.
4. Z. J. Lorković and A. Barta. "Genome Analysis: Rna Recognition Motif (Rrm) and K Homology (Kh) Domain Rna-Binding Proteins from the Flowering Plant Arabidopsis Thaliana." *Nucleic Acids Res* 30, no. 3 (2002): 623-35.
5. C. G. Burd and G. Dreyfuss. "Conserved Structures and Diversity of Functions of Rna-Binding Proteins." *Science* 265, no. 5172 (1994): 615-21.
6. G. Dreyfuss, M. S. Swanson and S. Piñol-Roma. "Heterogeneous Nuclear Ribonucleoprotein Particles and the Pathway of Mrna Formation." *Trends in Biochemical Sciences* 13, no. 3 (1988): 86-91.
7. S. A. Adam, T. Nakagawa, M. S. Swanson, T. K. Woodruff and G. Dreyfuss. "Mrna Polyadenylate-Binding Protein: Gene Isolation and Sequencing and Identification of a Ribonucleoprotein Consensus Sequence." *Mol Cell Biol* 6, no. 8 (1986): 2932-43.
8. R. J. Bandziulis, M. S. Swanson and G. Dreyfuss. "Rna-Binding Proteins as Developmental Regulators." *Genes Dev* 3, no. 4 (1989): 431-7.
9. G. Dreyfuss, V. N. Kim and N. Kataoka. "Messenger-Rna-Binding Proteins and the Messages They Carry." *Nat Rev Mol Cell Biol* 3, no. 3 (2002): 195-205.

10. J.-E. Gomes, S. E. Encalada, K. A. Swan, C. A. Shelton, J. C. Carter and B. Bowerman. "The Maternal Gene Spn-4 Encodes a Predicted Rrm Protein Required for Mitotic Spindle Orientation and Cell Fate Patterning in Early C. Elegans Embryos." *Development* 128 21 (2001): 4301-14.
11. X. Zhan, B. Qian, F. Cao, W. Wu, L. Yang, Q. Guan, X. Gu, P. Wang, T. A. Okusolubo, S. L. Dunn, J. K. Zhu and J. Zhu. "An *Arabidopsis* Pwi and Rrm Motif-Containing Protein Is Critical for Pre-Mrna Splicing and Aba Responses." *Nat Commun* 6 (2015): 8139.
12. K. Paukku, M. Backlund, R. A. De Boer, N. Kalkkinen, K. K. Kontula and J. Y. Lehtonen. "Regulation of At1r Expression through Hur by Insulin." *Nucleic Acids Res* 40, no. 12 (2012): 5250-61.
13. M. K. O'Bryan, B. J. Clark, E. A. McLaughlin, R. J. D'Sylva, L. O'Donnell, J. A. Wilce, J. Sutherland, A. E. O'Connor, B. Whittle, C. C. Goodnow, C. J. Ormandy and D. Jamsai. "Rbm5 Is a Male Germ Cell Splicing Factor and Is Required for Spermatid Differentiation and Male Fertility." *PLoS Genet* 9, no. 7 (2013): e1003628.
14. C. Maris, C. Dominguez and F. H. Allain. "The Rna Recognition Motif, a Plastic Rna-Binding Platform to Regulate Post-Transcriptional Gene Expression." *Febs j* 272, no. 9 (2005): 2118-31.
15. K. Nagai, C. Oubridge, T. H. Jessen, J. Li and P. R. Evans. "Crystal Structure of the Rna-Binding Domain of the U1 Small Nuclear Ribonucleoprotein A." *Nature* 348, no. 6301 (1990): 515-20.
16. A. Cléry, M. Blatter and F. H. Allain. "Rna Recognition Motifs: Boring? Not Quite." *Curr Opin Struct Biol* 18, no. 3 (2008): 290-8.
17. E. Birney, S. Kumar and A. R. Krainer. "Analysis of the Rna-Recognition Motif and Rs and Rgg Domains: Conservation in Metazoan Pre-Mrna Splicing Factors." *Nucleic Acids Research* 21, no. 25 (1993): 5803-16.
18. C. C. Query, R. C. Bentley and J. D. Keene. "A Common Rna Recognition Motif Identified within a Defined U1 Rna Binding Domain of the 70k U1 Snrnp Protein." *Cell* 57, no. 1 (1989): 89-101.
19. J. C. Chambers, D. Kenan, B. J. Martin and J. D. Keene. "Genomic Structure and Amino Acid Sequence Domains of the Human La Autoantigen." *J Biol Chem* 263, no. 34 (1988): 18043-51.
20. Sachs, Davis and Kornberg. "A Single Domain of Yeast Poly(a)-Binding Protein Is Necessary and Sufficient for Rna Binding and Cell Viability." *Mol.cell.biol* (1987).
21. C. L. Kielkopf, S. Lücke and M. R. Green. "U2af Homology Motifs: Protein Recognition in the Rrm World." *Genes Dev* 18, no. 13 (2004): 1513-26.
22. K. Zhai, Y. Deng, D. Liang, J. Tang, J. Liu, B. Yan, X. Yin, H. Lin, F. Chen and D. Yang. "Rrm Transcription Factors Interact with Nlrs and Regulate Broad-Spectrum Blast Resistance in Rice." *Mol Cell* (2019).
23. J. Jeon, G. W. Lee, K. T. Kim, S. Y. Park, S. Kim, S. Kwon, A. Huh, H. Chung, D. Y. Lee, C. Y. Kim and Y. H. Lee. "Transcriptome Profiling of the Rice Blast Fungus *Magnaporthe Oryzae* and Its Host *Oryza Sativa* During Infection." *Mol Plant Microbe Interact* 33, no. 2 (2020): 141-44.
24. H. K. Manandhar, H. J. Lyngs Jørgensen, S. B. Mathur and V. Smedegaard-Petersen. "Suppression of Rice Blast by Preinoculation with Avirulent *Pyricularia Oryzae* and the Nonrice Pathogen Bipolaris Sorokiniana." *Phytopathology* 88 7 (1998): 735-9.
25. F. Nasir, L. Tian, C. Chang, X. Li, Y. Gao, L. P. Tran and C. Tian. "Current Understanding of Pattern-Triggered Immunity and Hormone-Mediated Defense in Rice (*Oryza Sativa*) in Response to *Magnaporthe Oryzae* Infection." *Semin Cell Dev Biol* 83 (2018): 95-105.
26. H. Moriyama, S. I. Urayama, T. Higashiura, T. M. Le and K. Komatsu. "Chrysoviruses in *Magnaporthe Oryzae*." *Viruses* 10, no. 12 (2018).
27. D. H. Lee, D. S. Kim and B. K. Hwang. "The Pepper Rna-Binding Protein Carbp1 Functions in Hypersensitive Cell Death and Defense Signaling in the Cytoplasm." *Plant J* 72, no. 2 (2012): 235-48.
28. Nina and F. J. C. O. i. P. Biology. "Rna-Binding Proteins in Plants: The Tip of an Iceberg?" (2002).
29. Z. J. J. T. i. P. S. Lorkovi? "Role of Plant Rna-Binding Proteins in Development, Stress Response and Genome Organization." 14, no. 4 (2009): 229-36.
30. V. Woloshen, S. Huang and X. Li. " Review Article Rna-Binding Proteins in Plant Immunity." (2011).
31. K. Zhai, Y. Deng, D. Liang, J. Tang, J. Liu, B. Yan, X. Yin, H. Lin, F. Chen, D. Yang, Z. Xie, J.-Y. Liu, Q. Li, L. Zhang and Z. He. "Rrm Transcription Factors Interact with Nlrs and Regulate Broad-Spectrum Blast Resistance in Rice." *Mol Cell* 74, no. 5 (2019): 996-1009.e7.
32. C. Chen, H. Chen, Y. Zhang, H. R. Thomas and R. Xia. "Tbtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data." *Molecular Plant* 13, no. 8 (2020).
33. Y. Hu, N. Zhu, X. Wang, Q. Yi, D. Zhu, Y. Lai and Y. Zhao. "Analysis of Rice *Snf*2 Family Proteins and Their Potential Roles in Epigenetic Regulation." *Plant Physiol Biochem* 70 (2013): 33-42.

34. M. Mimura and J. Itoh. "Genetic Interaction between Rice Plastochron Genes and the Gibberellin Pathway in Leaf Development." *Rice (N Y)* 7, no. 1 (2014): 25.
35. T. Kawakatsu, J.-I. Itoh, K. Miyoshi, N. Kurata, N. Alvarez, B. Veit and Y. Nagato. "Plastochron2 Regulates Leaf Initiation and Maturation in Rice." *The Plant Cell* 18, no. 3 (2006): 612-25.
36. G. S. Xiong, X. M. Hu, Y. Q. Jiao, Y. C. Yu, C. C. Chu, J. Y. Li, Q. Qian and Y. H. Wang. "Leafy Head2, Which Encodes a Putative Rna-Binding Protein, Regulates Shoot Development of Rice." *Cell Res* 16, no. 3 (2006): 267-76.
37. J. Lyu, D. Wang, P. Duan, Y. Liu, K. Huang, D. Zeng, L. Zhang, G. Dong, Y. Li, R. Xu, B. Zhang, X. Huang, N. Li, Y. Wang, Q. Qian and Y. Li. "Control of Grain Size and Weight by the Gsk2-Large1/Oml4 Pathway in Rice." *Plant Cell* 32, no. 6 (2020): 1905-18.
38. M. Isshiki, Y. Matsuda, A. Takasaki, H. L. Wong, H. Satoh and K. J. P. B. Shimamoto. "Du3, a Mrna Cap-Binding Protein Gene, Regulates Amylose Content in Japonica Rice Seeds." *Plant Biotechnology* (2008).
39. M. Yano, K. Okuno, H. Satoh and T. Omura. "Chromosomal Location of Genes Conditioning Low Amylose Content of Endosperm Starches in Rice, Oryza Sativa L." *Theor Appl Genet* 76, no. 2 (1988): 183-9.
40. K. Zhai, Y. Deng, D. Liang, J. Tang, J. Liu, B. Yan, X. Yin, H. Lin, F. Chen, D. Yang, Z. Xie, J. Y. Liu, Q. Li, L. Zhang and Z. He. "Rrm Transcription Factors Interact with Nlrs and Regulate Broad-Spectrum Blast Resistance in Rice." *Mol Cell* 74, no. 5 (2019): 996-1009.e7.
41. T. Liu, X. Zhang, H. Zhang, Z. Cheng, J. Liu, C. Zhou, S. Luo, W. Luo, S. Li, X. Xing, Y. Chang, C. Shi, Y. Ren, S. Zhu, C. Lei, X. Guo, J. Wang, Z. Zhao, H. Wang, H. Zhai, Q. Lin and J. Wan. "Dwarf and High Tillering1 Represses Rice Tillering through Mediating the Splicing of D14 Pre-Mrna." *Plant Cell* 34, no. 9 (2022): 3301-18.
42. X. Liu, J. Lan, Y. Huang, P. Cao, C. Zhou, Y. Ren, N. He, S. Liu, Y. Tian, T. Nguyen, L. Jiang and J. Wan. "*Wsl5*, a Pentatricopeptide Repeat Protein, Is Essential for Chloroplast Biogenesis in Rice under Cold Stress." *J Exp Bot* 69, no. 16 (2018): 3949-61.
43. Q. Lu, S. Ding, S. Reiland, A. Rödiger, B. Roschitzki, P. Xue, W. Gruissem, C. Lu and S. Baginsky. "Identification and Characterization of Chloroplast Casein Kinase Ii from Oryza Sativa (Rice)." *J Exp Bot* 66, no. 1 (2015): 175-87.
44. "Conservation and Divergence of Fca Function between *Arabidopsis* and Rice." *Plant Molecular Biology* 58, no. 6 (2005): 823-38.
45. S. Y. Chen, Z. Y. Wang and X. L. Cai. "Osrrm, a Spen-Like Rice Gene Expressed Specifically in the Endosperm." *Cell Res* 17, no. 8 (2007): 713-21.
46. S.-Y. Chen, Z.-Y. Wang and X.-L. Cai. "Osrrm, a Spen-Like Rice Gene Expressed Specifically in the Endosperm." *Cell Res* 17, no. 8 (2007): 713-21.
47. K. J. Kwak, H. J. Jung, K. H. Lee, Y. S. Kim, W. Y. Kim, S. J. Ahn and H. Kang. "The Minor Spliceosomal Protein U11/U12-31k Is an Rna Chaperone Crucial for U12 Intron Splicing and the Development of Dicot and Monocot Plants." *PLoS One* 7, no. 8 (2012): e43707.
48. Y. Cai, M. E. Vega-Sánchez, C. H. Park, M. Bellizzi, Z. Guo and G. L. Wang. "Rbs1, an Rna Binding Protein, Interacts with Spin1 and Is Involved in Flowering Time Control in Rice." *PLoS One* 9, no. 1 (2014): e87258.
49. S. Sharma, C. Kaur, S. L. Singla-Pareek and S. K. Sopory. "Ossro1a Interacts with Rna Binding Domain-Containing Protein (Osrbd1) and Functions in Abiotic Stress Tolerance in Yeast." *Front Plant Sci* 7 (2016): 62.
50. T. Zhao, L. Ren, Y. Zhao, H. You, Y. Zhou, D. Tang, G. Du, Y. Shen, Y. Li and Z. Cheng. "Reproductive Cells and Peripheral Parietal Cells Collaboratively Participate in Meiotic Fate Acquisition in Rice Anthers." *Plant J* 108, no. 3 (2021): 661-71.
51. S. Miyazaki, Y. Sato, T. Asano, Y. Nagamura and K. Nonomura. "Rice Mel2, the Rna Recognition Motif (Rrm) Protein, Binds in Vitro to Meiosis-Expressed Genes Containing U-Rich Rna Consensus Sequences in the 3'-Utr." *Plant Mol Biol* 89, no. 3 (2015): 293-307.
52. K. Nonomura, M. Eiguchi, M. Nakano, K. Takashima, N. Komeda, S. Fukuchi, S. Miyazaki, A. Miyao, H. Hirochika and N. Kurata. "A Novel Rna-Recognition-Motif Protein Is Required for Premeiotic G1/S-Phase Transition in Rice (*Oryza Sativa L.*)." *PLoS Genet* 7, no. 1 (2011): e1001265.
53. C. Sahi, M. Agarwal, A. Singh and A. Grover. "Molecular Characterization of a Novel Isoform of Rice (*Oryza Sativa L.*) Glycine Rich-Rna Binding Protein and Evidence for Its Involvement in High Temperature Stress Response." *Plant Science* 173, no. 2 (2007): 144-55.

54. A. Cléry, M. Blatter and F. H. T. Allain. "Rna Recognition Motifs: Boring? Not Quite." *Curr Opin Struct Biol* 18, no. 3 (2008): 290-98.
55. Z. J. Lorković and A. Barta. "Genome Analysis: Rna Recognition Motif (Rrm) and K Homology (Kh) Domain Rna-Binding Proteins from the Flowering Plant Arabidopsis Thaliana." *Nucleic Acids Research* 30, no. 3 (2002): 623-35.
56. X. Shi, M. R. Hanson and S. Bentolila. "Functional Diversity of *Arabidopsis* Organelle-Localized Rna-Recognition Motif-Containing Proteins." *Wiley Interdiscip Rev Rna* (2017): e1420.
57. X. Shi, M. R. Hanson and S. Bentolila. "Functional Diversity of *Arabidopsis* Organelle-Localized Rna-Recognition Motif-Containing Proteins." *Wiley Interdiscip Rev Rna* 8, no. 5 (2017).
58. X. Zhan, B. Qian, F. Cao, W. Wu, L. Yang, Q. Guan, X. Gu, P. Wang, T. A. Okusolubo, S. L. Dunn, J.-K. Zhu and J. Zhu. "An *Arabidopsis* Pwi and Rrm Motif-Containing Protein Is Critical for Pre-Mrna Splicing and Aba Responses." *Nat Commun* 6, no. 1 (2015): 8139.
59. J. Y. Kim, S. J. Park, B. Jang, C. H. Jung, S.-j. Ahn, C. H. Goh, K. Cho, O. Han and H. Kang. "Functional Characterization of a Glycine-Rich Rna-Binding Protein 2 in *Arabidopsis Thaliana* under Abiotic Stress Conditions." *The Plant journal* 50 3 (2007): 439-51.
60. T. Xu, K. Lee, L. Gu, J.-I. Kim and H. Kang. "Functional Characterization of a Plastid-Specific Ribosomal Protein Psrp2 in *Arabidopsis Thaliana* under Abiotic Stress Conditions." *Plant Physiology and Biochemistry* 73 (2013): 405-11.
61. "Cold Shock Domain Proteins and Glycine-Rich Rna-Binding Proteins from *Arabidopsis Thaliana* Can Promote the Cold Adaptation Process in Escherichia Coli." *Nucleic Acids Research* (2007).
62. S. Wang, G. Bai, S. Wang, L. Yang, F. Yang, Y. Wang, J. K. Zhu and J. Hua. "Chloroplast Rna-Binding Protein Rbd1 Promotes Chilling Tolerance through 23s Rrna Processing in *Arabidopsis*." *PLoS Genet* 12, no. 5 (2016): e1006027.
63. X. Shi, A. Germain, M. R. Hanson and S. Bentolila. "Rna Recognition Motif-Containing Protein Orrm4 Broadly Affects Mitochondrial Rna Editing and Impacts Plant Development and Flowering." *Plant Physiol* 170, no. 1 (2016): 294-309.
64. M. Vermel, B. Guermann, L. Delage, J. M. Grienenberger, L. Maréchal-Drouard and J. M. Gualberto. "A Family of Rrm-Type Rna-Binding Proteins Specific to Plant Mitochondria." *Proc Natl Acad Sci U S A* 99, no. 9 (2002): 5866-71.
65. K. J. Kwak, Y. O. Kim and H. Kang. "Characterization of Transgenic *Arabidopsis* Plants Overexpressing Gr-Rbp4 under High Salinity, Dehydration, or Cold Stress." *J Exp Bot* 56, no. 421 (2005): 3007-16.
66. Z. J. Lorković. "Role of Plant Rna-Binding Proteins in Development, Stress Response and Genome Organization." *Trends Plant Sci* 14, no. 4 (2009): 229-36.
67. Shuai, Wan, Ge, Ba, Shu, Wan, Leiyun, Yang, Fen and Y. J. P. Genetics. "Chloroplast Rna-Binding Protein Rbd1 Promotes Chilling Tolerance through 23s Rrna Processing in *Arabidopsis*." (2016).