粳稻云引稻瘟病抗性近等基因系与免疫应答相关的LRR-RLKs差异基因分析

林悦龙^{1,2,3}, 王昱澎^{1,2,3}, 朱永生^{2,3}, 郑燕梅^{2,3}, 蔡秋华^{2,3}, 何炜^{2,3}, 陈丽萍^{2,3}, 谢鸿光^{2,3}, 魏毅东^{2,3}, 谢华安^{1,2,3}, 张建福^{1,2,3*}

1. 福建农林大学农学院, 福州 350002;

2. 福建省农业科学院水稻研究所, 福州 350019;

3. 闽台作物有害生物生态防控国家重点实验室/水稻国家工程实验室/杂交水稻国家重点实验室华南研究基地/福建省作物种质创新与分子育种省部共建国家重点实验室培育基地/国家水稻改良中心福州分中心/农业农村部华南杂交水稻种质创新与分子育种重点实验室/福建省作物分子育种工程实验室/福建省水稻分子育种重点实验室, 福州 350003

* 联系人, E-mail: jianfzhang@163.com

2021-03-05 收稿, 2021-04-23 修回, 2021-04-25 接受, 2021-04-26 网络版发表

国家重点研发计划(2017FD0100100)、福建省省属公益类科研专项(2020R1023008)和福建省科技重大专项(2020NZ08016)资助

摘要 在广谱抗稻瘟病品种云引与普感稻瘟病品种丽江新团黑谷(Lijiangxintuanheigu, LTH)构建的近等基因系抗、感病子代基因组重测序的差异基因中, 从与免疫应答(immune response)相关的分类单元里得到8个基因序列有差异的基因。它们都为编码富含亮氨酸重复序列的类受体蛋白激酶基因, 并聚集于11号染色体的邻近位置, 且与已知的白叶枯病抗性基因*Xa21*具有一定同源性。为进一步探究这些类受体蛋白激酶类基因在稻瘟病抗性中可能发挥的功能, 我们分析了这些基因的结构、进化及抗感子代间的序列差异类型, 同时还分别采用离体和喷雾接菌的方式对云引和LTH进行稻瘟病接种并取样。通过实时荧光定量PCR(quantitative real time polymerase chain reaction, qRT-PCR)发现, 部分基因能受到稻瘟病菌的诱导, 推测它们可能在水稻对抗稻瘟病菌中发挥一定作用, 为进一步挖掘稻瘟病相关抗性基因奠定基础。

关键词 近等基因系, 免疫应答, 富含亮氨酸重复序列的类受体蛋白激酶, 稻瘟病

水稻(*Oryza sativa*)是世界主要的粮食作物之一, 具有悠久的栽培与驯化历史, 同时也是植物科学研究中重要的单子叶模式植物。然而, 在水稻的生产过程中经常会遇到许多病害, 其中, 被称为水稻“癌症”的稻瘟病, 田间发病严重时可致减产50%, 甚至绝收^[1,2]。因此, 不断培育抗稻瘟病水稻品种、发掘稻瘟病抗性相关基因以及研究水稻在稻瘟病致病过程中的免疫应答过程和机理对于水稻育种与农业生产具有重要意义。

在过去的几十年间, 通过图位克隆的方法已经从

水稻中克隆了多个稻瘟病抗性基因^[3,4]。近年来, 不断兴起的高通量测序技术以及生物信息学分析工具, 也让研究者能够通过基因组测序、集群分离分析法(bulked segregant analysis, BSA)^[5]、基因芯片表达谱数据^[6-8]以及全基因组关联分析(genome wide association study, GWAS)^[9,10]等方式对水稻相关抗病基因进行深入发掘。而构建好相关农艺性状的遗传群体对于高通量的组学分析具有重要基础作用。例如, 近等基因系, 是指将带有目标性状的供体亲本与受体亲本(又称轮

引用格式: 林悦龙, 王昱澎, 朱永生, 等. 粳稻云引稻瘟病抗性近等基因系与免疫应答相关的LRR-RLKs差异基因分析. 科学通报, 2021, 66: 3873-3885

Lin Y L, Wang Y P, Zhu Y S, et al. Analysis of immune response-related LRR-RLKs differential genes in the rice blast resistance near-isogenic lines of Japonica Variety Yunyin (in Chinese). Chin Sci Bull, 2021, 66: 3873-3885, doi: [10.1360/TB-2021-0242](https://doi.org/10.1360/TB-2021-0242)

回亲本)进行杂交后再进行多轮回交,再自交分离从而得到稳定的带有目标性状且遗传背景与受体亲本相近的品系.这个品系与轮回亲本即称为一对近等基因系,近等基因系对于特定效应基因的精确分析、定位或具有重要功能^[11].

为了进一步挖掘水稻中抗稻瘟病基因以及参与稻瘟病免疫应答过程的相关基因,本研究从对稻瘟病抗谱较广的云引与高感稻瘟病的丽江新团黑谷(Lijiang-xintuanheigu, LTH)构建的近等基因系^[12,13]抗感病子代基因组重测序的差异基因中,从GO(Gene Ontology)注释与免疫应答(immune response)相关的分类单元里得到了8个在基因序列上有差异的基因.研究发现,它们均为编码富含亮氨酸重复序列的类受体蛋白激酶(Leucine-rich repeat receptor-like protein kinase, LRR-RLK),并处于同一染色体邻近位点.通过分析这些基因的结构类型、进化,并采用离体和喷雾接菌方式对云引和LTH进行稻瘟病菌接种和取样,结合实时荧光定量PCR(quantitative real time polymerase chain reaction, qRT-PCR)进行表达分析.结果发现,部分基因能受到稻瘟病菌侵染的诱导,推测它们可能在水稻对抗稻瘟病菌的过程中发挥一定作用.

1 材料与方法

1.1 供试材料

水稻品种云引、LTH由福建省农业科学院水稻国家工程实验室保存提供;稻瘟病菌生理小种四川-43、RB17分别由中国农业科学院作物科学研究所雷财林研究员、福建农林大学功能基因组学研究中心王宗华教授实验室提供.

1.2 试验方法

(i) 差异基因相关信息的获取.通过RiceData网站的“基因&QTL”栏目(<https://www.ricedata.cn/gene/>)检索测序获得的差异基因座号,进一步通过RAP-DB(<https://rapdb.dna.affrc.go.jp/index.html>)和RGAP(<http://rice.plantbiology.msu.edu/index.shtml>)链接网站获得以日本晴基因组为参考的相关基因信息(包括基因长度、编码区、氨基酸序列等).

(ii) 差异基因的结构、比对与进化分析.基因的保守结构域通过HMMER网站(<https://www.ebi.ac.uk/Tools/hmmer/>)查询获得;氨基酸比对相似性矩阵由EBI

网站提供的Clustal Omega(<https://www.ebi.ac.uk/Tools/msa/clustalo/>)在线比对程序获得;系统进化树由MEGA7.0软件构建,采用Neighbor joining(NJ)邻位归并法计算,bootstrap replications参数为1000,模型为Poisson model, Gaps/Missing Data Treatment选Partial deletion,程度约为50%,用于进化树构建的水稻和拟南芥LRR-RLK相关基因序列分别由RGAP、NCBI(<https://www.ncbi.nlm.nih.gov/>)和Phytozome(<https://phytozome.jgi.doe.gov/pz/portal.html>)网站获得.

(iii) 稻瘟病菌的活化、培养与产孢.剪取小块实验室保存的稻瘟病菌滤纸片接种于活化培养基(酵母粉3 g、可溶性淀粉10 g、琼脂10 g、pH 6.5/1 L,高压灭菌),在25℃倒置暗培养,活化5 d左右.将活化菌丝的边缘切成小块并转接于米糠培养基(米糠30 g、酵母粉3 g、琼脂粉20 g、pH 6.5/1 L,高压灭菌),原条件继续培养7 d左右.用消毒的载玻片将生长的菌丝体刮除干净,不密封,在25℃置于持续光照下产孢5 d左右.取适量无菌水于培养皿中,用细毛刷刷洗表层的孢子,双层纱布过滤后即可镜检,并将接种孢子浓度调整至约 5×10^4 个/mL(10×10 倍显微镜下,每个视野约50个孢子).

(iv) 云引叶片离体接菌.在人工植物培养室内,将云引播种并土培45 d左右.选择长势相近且健康的植株,剪取叶龄相近的长10 cm左右的叶片用打孔器进行打孔接种,打孔时不穿透叶片,每片叶片打2~3孔.然后用消毒镊子将叶片置于离体接种培养基(琼脂5 g、25 mL抽滤除菌浓度 $C=100$ mg/L的6-BA母液/1 L,6-BA待高压冷却至50℃左右后加入)中,再吸取10 μ L混匀的孢子悬浮液(四川-43, RB17等量混匀)点在创口上,将培养基密封后置于培养箱内进行培养(温度为28℃,16 h弱光照/8 h黑暗).对照以无菌水代替,在接种后的0、12、18、24、36、48、60、72 h八个时间点分别取样,每次取3片叶片,液氮速冻后于-80℃保存.

(v) 云引、LTH的喷雾接菌.用水稻木村B营养液将云引和LTH水稻幼苗培养至3~4叶期,添加吐温-20至制备好的孢子悬浮液(四川-43),终浓度为0.02%(v/v).使用KY-3B型微型空气压缩机进行喷雾接种,摇匀后将孢子悬浮液均匀地喷洒于叶片表面,然后置于光照培养箱内(温度为28℃,湿度为90%,16 h光照/8 h黑暗)进行培养.选择在黑暗周期前接种,期间经常为叶片喷洒无菌水保湿以利于发病,对照实验组用含吐温-20的无菌水作喷雾处理,其余条件与实验组一致.在接种后的0、12、18、24、36、48、72、96 h八个时间点分别

取样, 每次取样剪取3~4株水稻幼苗上叶龄与状态相近的叶片, 液氮速冻后于-80℃保存。

(vi) RNA的提取及qRT-PCR分析. 从文献中获取或根据RGAP网站提供的参考编码序列(coding DNA sequence, CDS)序列, 使用Beacon Designer 8软件设计相关差异基因的qRT-PCR引物序列如表1。

采用购自全式金生物公司的TransZol Up Plus RNA Kit试剂盒提取叶片总RNA, 约1 μg的总RNA用TOYOBO公司提供的ReverTra Ace qPCR RT Master Mix with gDNA Remover反转录试剂进行反转录反应(20 μL体系), 具体步骤详见说明书. 将得到的cDNA稀释10倍后用于qRT-PCR反应, 反应酶液使用Roche公司提供的FastStart Universal SYBR Green Master (Rox). 反应体系为2×Rox 10 μL, 正向、反向引物各0.6 μL, 稀释的cDNA模板4 μL, ddH₂O为4.8 μL. 采用两步法进行扩增, 反应程序为: 第1步, 95℃预变性10 min; 第2步, 95℃变性15 s, 60℃退火与延伸1 min, 读取荧光信号, 40个循环; 第3步, 溶解曲线按程序软件默认设置进行. 定量PCR数据采集与结果处理由ABI QuantStudio 6 Flex荧光定量PCR仪及配套软件进行, 采用 $2^{-\Delta\Delta C_T}$ 法计算差异表达水平, 误差线在95%置信区间下计算得到。

2 结果与分析

2.1 近等基因系抗感子代重测序

将经过选育的近等基因系子代种子在37℃培养箱内浸种催芽过夜, 用营养液在植物培养室内培养至3~4叶期, 剪取抗病株系和感病株系的叶片各3个重复样本, 每个样本包含5个单株的等量叶片. 后送往公司进行基因组重测序, 在得到的所有差异基因列表中, 从与immune response相关的分类条目(GO:0045087、GO:0006955、GO:0050776)里得到8个可能与稻瘟病抗性相关的候选差异基因. 通过检索RAP-DB和RGAP网

站获得差异基因的相关注释及日本晴参考基因信息, 并分别将它们编号为IR_DEG1-8(表2)。

2.2 差异基因的结构、序列分析与相关功能报道

通过查询RGAP和HMMER网站数据库, 获得了这8个基因的结构及蛋白保守结构域(图1). 除了IR_DEG8编码蛋白长度较短, 且C端缺少保守的激酶结构域外, 其余基因都包含有类型与数量不同的LRR结构域和激酶结构域, 为典型的具有亮氨酸重复保守结构域的类型蛋白激酶基因。

在水稻日本晴参考基因组序列中, 这些基因编码氨基酸长度从534~1133不等, 通过Clustal Omega进行多重序列比对, 得到两两基因之间编码区与蛋白质序列比对的相似性矩阵(表3). 这些基因在氨基酸水平上的同源性为55%~80%之间, 具有较高同源性, 且都位于11号染色体的同一邻近位点, 推测该位点可能是一个参与水稻免疫反应过程相关的基因簇位点. 基因簇是染色体上许多基因家族成员的聚集位点. 例如抗病R(resistance)基因簇, 通常重复间隔或串联排列着大量的同源R基因. 它们之间可以通过重组和不等交换来产生更多新的R基因类型, 是R基因产生与进化的重要方式^[14~17].

进一步比对发现, 该位点与已克隆的水稻白叶枯抗性基因Xa21^[18,19]处于11号染色体的邻近位点. Song等人^[20]于1997年从水稻品种IRBB21中克隆分析了7个Xa21的家族成员基因, 其命名分别为A1、A2、B、C、D、E、F, 并将它们分为Xa21(B、D、F)和A2(A1、A2、C、E)两类家族成员. 其中, member B成员被指派为Xa21, member D、E分别为被反转座子、转座子所截短的蛋白, 剩余成员除了A1基因编码序列正常外, member A2、C、F基因都因编码区序列突变导致蛋白质翻译提前终止而成为假基因. 这些基因与Xa21家族具有一定的序列相似性与亲缘关系. 水稻

表 1 qRT-PCR所用引物序列

Table 1 Primer sequences used for real-time PCR

基因编号	正向引物(5'→3')	反向引物(5'→3')	产物长(bp)
IR_DEG1	AACAGCCTATCGGGTGAGA	GATGGAACCAGACAGCGTATT	165
IR_DEG2	TGCCTGAAGAGATTGGTAA	CAACGATGACGGAAGAATT	86
IR_DEG3	CTGGAGAAGTAGCATTCATC	GAGAAGAAGGAGGAGGAG	90
IR_DEG6	GGCTATGCAGCACCAGAGTAT	GGCCCAGTTCAACGTACTGA	160
Ubiquitin(LOC_Os03g13170)	CAAGATGATCTGCCGCAAATGC	TTTAACCAAGTCCATGAACCCG	148

表 2 免疫应答相关分类条目差异基因列表及功能注释

Table 2 The function annotation of differential genes within the immune response term

编号	RAP/MSU基因座号	RAPDB注释	RGAP注释	参考编码区长度(bp)	氨基酸长度(aa)
IR_DEG1	Os11g0559200/ LOC_Os11g35500	蛋白激酶, 含有核心结构域的蛋白	假定的受体样蛋白激酶5前体, 表达的	2997	998
IR_DEG2	Os11g0568800/ LOC_Os11g36090	富含亮氨酸重复, 含有典型亚结构域的蛋白	假定的受体蛋白激酶, 表达的	3402	1133
IR_DEG3	Os11g0569300/ LOC_Os11g36140	含有丝氨酸/苏氨酸蛋白激酶结构域的蛋白	假定的受体样蛋白激酶2前体, 表达的	3216	1071
IR_DEG4	Os11g0569500/ LOC_Os11g36150	与类受体蛋白激酶相似	假定的受体样蛋白激酶2前体, 表达的	3108	1035
IR_DEG5	Os11g0569600/ LOC_Os11g36160	与类受体蛋白激酶相似	假定的受体样蛋白激酶2前体, 表达的	3003	1100
IR_DEG6	Os11g0569733/ LOC_Os11g36180	类受体蛋白激酶, 细菌性白叶枯病抗性	假定的受体蛋白激酶, 表达的	3114	1037
IR_DEG7	Os11g0569800/ LOC_Os11g36190	蛋白激酶, 含有催化结构域的蛋白	假定的受体蛋白激酶, 表达的	2376	791
IR_DEG8	Os11g0570000/ LOC_Os11g36200	与类受体蛋白激酶相似	假定的受体样蛋白激酶2前体, 表达的	1605	534

图 1 (网络版彩色) 差异基因的相关序列信息. (a) 基因结构; (b) 蛋白保守结构域

Figure 1 (Color online) The gene and protein information of differential genes. (a) Gene structure; (b) conserved protein domain

表 3 差异基因的编码区与氨基酸序列相似性矩阵

Table 3 Percent identity matrix of the coding and amino acid sequences among differential genes

氨基酸	编码区	Os11g35500	Os11g36090	Os11g36140	Os11g36150	Os11g36160	Os11g36180	Os11g36190	Os11g36200
Os11g35500			63	66	67	81	73	70	63
Os11g36090	61			65	68	62	64	70	66
Os11g36140	58	63			70	64	67	71	64
Os11g36150	60	65	62			65	69	76	68
Os11g36160	80	61	59	60			73	70	59
Os11g36180	67	60	57	60	66			69	64
Os11g36190	62	71	65	70	63	63			69
Os11g36200	55	59	57	60	56	58	59		

*Xa21*基因编码典型的LRR-RLK,通过监视并识别白叶枯病菌分泌的AvrXa21蛋白来触发植物体的免疫防御反应^[21,22]。LRR-RLKs基因通常由一个胞外的LRR识别结构域、一段跨膜结构域和一个胞内的激酶结构域所组成,它们的结构与哺乳动物中的促性腺激素受体以及果蝇中的Toll受体相似^[23]。在植物中,细胞主要通过细胞膜表面的模式识别受体(pattern recognition receptors, PRR)和胞内的含有核苷酸结合结构域和富含亮氨酸重复序列类免疫受体(nucleotide-binding leucine-rich repeat, NLR)来感知和识别病原菌的侵染。PRR蛋白有很多是受体蛋白激酶^[24],它们在植物体生长发育的信号转导、免疫识别以及感受环境变化中起着重要作用^[25,26]。

2.3 差异基因序列的SNP、Indel分析

单核苷酸多态性(single nucleotide polymorphisms, SNP)及插入缺失标记(insertion-deletion, Indel)的产生是基因进化或功能分化的重要标志。例如,已克隆的稻瘟病抗性基因*Pi-ta*,当该基因产物第918位氨基酸由丙氨酸突变为丝氨酸后,则会导致其抗病性的消失^[27]。而日本晴中的*Pik-h*等位基因,其RNA剪切位点的突变及产生内含子和部分Indel也可能是导致其功能丧失的原因^[28]。Thakur等人^[29]研究发现,不同水稻品种中的*Pik-h/Pi54*较广泛的SNP变化赋予了该抗性基因位点更广的抗谱。事实上,不少非功能性等位*R*基因的形成往往与基因区序列产生的SNP或Indel有关^[30-33]。

在抗感病子代重测序的基因组中,这些基因在编码区或非编码区存在不同数量的SNP和Indel(表4)。其

中,IR_DEG4基因在抗感子代中差异最大,仅在编码区中就存在27个SNP和6个Indel。其中有6个Indel均被注释为移码(FRAME_SHIFT)。对其编码区翻译氨基酸后发现,该基因编码区在感病子代中发生了提前终止。而IR_DEG8基因编码区的一个SNP被注释为终止密码子获得(STOP_GAINED),翻译氨基酸后发现,该基因编码区在抗病子代中发生了提前终止。另外,IR_DEG3和IR_DEG7在翻译的氨基酸序列上不存在差异,仅有同义突变或在基因的非编码区、上下游区域被注释存在SNP。

2.4 差异基因的进化分析

在已被报道克隆的稻瘟病抗病基因中,大部分都为具有富含核苷酸结合位点-亮氨酸重复序列结构域(nucleotide-binding site-leucine-rich repeat, NBS-LRR)的基因^[4],但也有典型的LRR-RLK蛋白激酶类基因参与了水稻对稻瘟病的抗性。Peng等人^[34]于2009年发现一个LRR-RLK基因*OsBRR1*能正调控水稻对稻瘟病的抗性,过表达该基因能提高水稻对部分稻瘟病菌株的抗性,且过表达或干扰该基因对植株正常的生长发育没有显著影响。Hu等人^[35]于2005年报道了一个水稻体细胞胚胎发生相关类受体蛋白激酶(somatic embryo-genesis receptor-like kinase, SERK)基因不仅影响胚性愈伤芽分化的形成,同时也参与了水稻的稻瘟病抗性,组成型过表达该基因能提高转化水稻的抗病性。随后,Chen等人^[36]发现,该基因*OsSERK2*也在水稻白叶枯抗性基因*Xa21*和*Xa3*介导的免疫应答中发挥着重要作用,在含有*Xa21*或*Xa3*抗性基因的背景材料中干扰*Os-*

表 4 差异基因的SNP及Indel统计

Table 4 The statistics of SNP and Indel in differential genes

基因座号座号L	编码区			非编码区					
	SNP		Indel	SNP			Indel		
	同义突变	非同义突变		5'非编码区	3'非编码区	内含子	5'非编码区	3'非编码区	内含子
LOC_Os11g35500	0	1	0	0	0	0	0	0	0
LOC_Os11g36090	6	3	0	0	0	0	0	0	0
LOC_Os11g36140	2	0	0	0	0	0	0	0	0
LOC_Os11g36150	10	17	6	3	0	0	1	0	5
LOC_Os11g36160	10	7	0	0	0	3	0	2	0
LOC_Os11g36180	1	4	0	0	0	1	0	0	2
LOC_Os11g36190	0	0	0	0	0	1	0	0	1
LOC_Os11g36200	2	2	0	0	7	1	0	0	0

*SERK2*基因的表达后,会使*R*基因介导的抗病反应受损。此外,有研究表明,*OsSERK2*的同源基因*OsSERK1*也能正调控水稻对白叶枯病的抗性^[37],并可能在稻瘟病抗性中发挥一定作用^[38],它们都属于典型的LRR-RLK类受体蛋白激酶类基因。

将得到差异基因的参考蛋白质序列在拟南芥蛋白质数据库中比对后发现,这些基因与拟南芥EFR(EF-Tu receptor)基因具有一定同源性。*AtEFR*基因作为一个典型的PRR模式识别受体,能识别细菌的延伸因子(elongation factor Tu)蛋白,并触发植物体的免疫应答反应^[39,40]。除了*AtEFR*外,不少拟南芥的LRR-RLKs也被报道参与了植物的基础免疫应答过程,包括*AtFLS2*^[41]、*AtBAK1*^[42]、*AtPEPR1*和*AtPEPR2*^[43]等。将这些已知的LRR-RLKs抗病基因和水稻已知的白叶枯

抗病基因*Xa3/Xa26*、*Xa21*及其家族成员与差异基因的系统发育树分析后发现,这些基因与已知的*OsSERK1*、*OsSERK2*、*OsBRR1*进化距离较远,而与拟南芥的EFR基因及白叶枯抗病基因*Xa21*家族在进化关系上较近(图2)。其中,IR_DEG1和*OsXa21E*进化距离最近,但*OsXa21E*在水稻品种IRBB21中存在转座子的插入截短,将转座子序列移除后,二者在编码区与氨基酸序列的相似性高达99.6%和98.9%。据此推测,IR_DEG1可能是*OsXa21E*的完整功能性等位基因。而IR_DEG6则与*Xa21*进化距离最近,进一步序列比对发现,该基因与*Xa21*家族成员中的member F在序列上最接近,它与*Xa21*基因在编码区碱基序列的相似性为95.5%,而与*Xa21F*的一致性则在98%以上,且不存在假基因化。这些与Tan等人^[44]的研究结果相一致。另外,

图2 差异基因与相关同源基因的系统进化树

Figure 2 The phylogenetic tree of differential genes and homologous genes

IR_DEG2-4与IR_DEG7基因则独自形成一个亚簇与*Xa21*家族基因存在一定的分化. 在已知克隆的7个*Xa21*家族成员中, 除了*Xa21*和*Xa21D*分别具有完整和部分的白叶枯病抗性以外, 其余成员的功能暂未被报道^[18,45].

2.5 差异基因受稻瘟病菌诱导的表达谱分析

为进一步探究这些基因是否受到稻瘟病菌的诱导, 并在水稻对抗稻瘟病菌的免疫反应中发挥一定功能, 我们使用喷雾接种的方式对云引、LTH材料进行稻瘟病菌接种并取样, 同时也用包含致病菌的混合菌株对云引进行离体打孔接种与取样. 在正常发病后(图3), 进一步提取RNA并结合qRT-PCR分析这些基因受稻瘟病菌诱导的情况.

在喷雾接种稻瘟病菌的诱导表达实验中, 相比于对照组, 有4个基因表现出一定差异表达(图4). 在云引中, IR_DEG1与IR_DEG6二者都在接菌后的36~72 h呈现出表达量不断升高的过程, 并在接种后的72 h达到表达峰值. 但IR_DEG1在感病的LTH材料中没有受到类似的诱导作用, 而IR_DEG6在LTH中仍然表现出明显的表达上调趋势, 并也在72 h达到表达的峰值. 此外, IR_DEG2与IR_DEG3基因则在云引中表现为受到一定的抑制表达趋势, 但在LTH中, 二者的表达趋势整体上与对照组差异较不明显. 另外, 在离体接种的实验中, IR_DEG1在接种后的24 h内表达量上调幅度较对照组高, 而IR_DEG2、IR_DEG3则分别仅在12、18和24、36、72 h取样点表达量有一定幅度上调, 其余基因的表达变化相比于对照组未有明显诱导作用.

图3 稻瘟病菌接种7 d后的发病情况. (a) 云引叶片的打孔接种; (b) 云引与LTH叶片的喷雾接种

Figure 3 The disease symptoms of leaves after 7 d inoculation. (a) Punch inoculation of YY; (b) spray inoculation of YY and LTH

3 讨论

水稻对稻瘟病菌的免疫反应过程是复杂且多层次的, 涉及大量基因的协同参与调控^[46]. 在稻瘟病菌孢子附着侵染的初期, 主要由植物细胞壁成分负责感知与抵抗孢子的萌发侵染. 当病原菌突破细胞壁侵入细胞后, 还能向胞间及胞内释放一些效应因子, 包括众多的酶类基因与效应蛋白来对抗或抑制植物体自身的免疫反应并支持病原菌自身的生长^[47,48]. 某些效应子如果直接或间接地被水稻中特定的*R*基因所识别, 就会触发强烈的防御反应, 激活许多抗病信号相关通路并使植物体产生系统性抗性, 包括局部大量积累过氧化氢、产生植保素、降低营养生殖生长水平从而将更多能量提供于抗病反应, 这个免疫过程也称之为效应因子触发的免疫反应(effector-triggered immunity, ETI)^[49]. 植物体对病原菌的免疫反应在没有特定*R*基因的水稻品种中一样存在, 这样的基础防御反应通常首先由植物细胞膜表面的模式识别受体通过识别病原微生物相关的分子模式所触发. 这种抗谱较广但抗性相对较弱的先天免疫应答也称之为病原或微生物相关分子模式成分触发的免疫反应(pathogen or microbial-associated molecular pattern(PAMP or MAMP)-triggered immunity, PTI or MTI)^[50-53], 这与动物中的固有免疫与特异性免疫应答有些相似^[54]. PTI与ETI在下游信号通路上存在一定的共性. 例如, 水稻抗病调控因子*OsRAR1*、*OsSGT1*以及*OsRac1*小分子GTP酶、热激蛋白*OsHSP90*等^[55,56]在抗性基因介导或固有免疫反应中都发挥着重要作用. 此外, 它们还激活了相似的MAPK级联信号通路、转录因子和激素调控以及相关病程基因的表达^[49].

Ngou等人^[57]和Yuan等人^[58]的研究表明, 在植物免疫系统中, PTI与ETI是协调工作相互补充的, 单独的PTI或ETI免疫反应不足以有效对抗致病菌. PTI缺陷的植物体会导致其ETI介导的抗性失效, ETI正常抗性的实现需要PTI系统中的PRR蛋白及其相关共受体, 其主要是通过增强原先PTI反应中的免疫信号通路来产生第二层次更持久有效的防御反应以阻碍病原菌的生长. 例如, ETI能产生更大量的RBOHD蛋白但却需要PTI反应中的免疫组分来增强其磷酸化使之发挥正常功能, 从而产生更多活性氧(reactive oxygen species, ROS). 同时, PTI也能通过MAPK激酶途径增强ETI免疫中的超敏反应(hypersensitive response, HR)并产生细胞坏死.

图 4 (网络版彩色) 稻瘟病菌诱导下的差异基因表达模式
Figure 4 (Color online) The expression pattern of the differential genes after *M. grisea* inoculation

ETI可以阻止病原菌效应因子所激活的感病性(effector-triggered susceptibility, ETS), 并强化PTI反应中所产生的胼胝质沉积以及相关防御基因的表达, 二者间的相辅相成是植物体有效抵御致病菌侵染所不可或缺的。

本研究从对稻瘟病抗谱较广的云引与普通感稻瘟病的LTH的近等基因系子代基因组重测序的差异基因中, 得到了8个可能参与到稻瘟病抗性反应的LRR-RLKs基因, 且位于同一染色体区域的邻近位点, 并与水稻已知的白叶枯抗性基因 $Xa21$ 家族具有一定的同源性, 推测它们聚集的区域可能是一个参与到水稻抗病调控的基因簇位点。LRR-RLK基因是植物进化中保留的一类庞大家族基因, 仅在模式植物拟南芥中就有14个亚家族

超过200个成员的LRR-RLKs基因被识别^[59,60], 而在水稻基因组中则有超过900个LRR-RLKs基因^[61]。作为广泛存在且重要的植物细胞膜表面受体, 它们在植物体感知外界环境变化、生长发育以及逆境胁迫反应相关信号的识别与传递中起着关键的作用^[62~64]。例如, FLAGELLIN SENSITIVE 2(*FLS2*)基因负责感知病原菌的鞭毛蛋白来察觉病原菌的侵染^[41], 白叶枯抗病基因 $Xa21$ 则通过识别病原菌分泌的无毒基因 $AX21$ 的产物来触发免疫反应。还有一些则参与到了植物激素信号的转导, 如油菜素内酯受体基因 $BRI1$ 。研究表明, 这些类受体蛋白激酶类常常拥有一些共受体蛋白, 它们之间能发生互作形成异源二聚体并协同行使和强化信

号转导的功能^[65,66]。如拟南芥BAK1蛋白不仅能与FLS2蛋白形成聚合物起始植物细胞的免疫反应,同时也能与BRI1形成二聚体,并在它的内吞作用中发挥一定作用^[67]。此外,BAK1还能与RLP23、SOBIR1共同形成三源复合物来触发免疫信号通路^[68],这与稻瘟病抗性位点Pia、Pikm完整功能的实现需要分别同时存在*SasR-GA4*、*SasRGA5*以及*Pikm1-TS*、*Pikm2-TS*两个基因的原理可能相类似^[69,70]。

在合适的条件下,当稻瘟病菌的分生孢子附着到较易感病的水稻叶片表面后,12 h之内便能快速地完成孢子萌发、芽管伸长、附着胞的形成等过程。在接种后24 h左右,附着胞内已形成侵入钉并产生侵入菌丝侵入到表皮细胞中。随后,初生感染菌丝在首先被感染的细胞内不断生长与分化,在30 h左右便可延伸抵达邻近细胞处^[71~74]。而在接种的36 h左右,菌丝体的次生菌丝已经侵入到了周围的细胞中,并在之后的时间里不断向外扩张。在侵染过程的72 h左右,较先被感染的细胞群开始出现细胞死亡,在接种的4~5 d后即可逐渐产生明显可见的病斑。但对于抗病的水稻品种,该侵染过程可被抑制延迟或中断^[75~77]。在稻瘟病菌喷雾接种的早期阶段(前24 h),仅有IR_DEG6在云引和LTH中的表达都较明显地发生上调,说明该基因可能响应于稻瘟病孢子初始的侵入过程。而在接种后侵入菌丝的扩张阶段(36~72 h),IR_DEG1和IR_DEG6在云引中的表达量都不断增强。由于云引对川43菌株表现为高抗,其侵染过程可能还仅限在早期阶段,但在感病的LTH中,IR_DEG1未有相似诱导的趋势则可能是由于病原菌的免疫抑制作用或基因序列及遗传背景的差异造成。而

IR_DEG6虽然在48 h时表达量有小幅回落,但仍在72 h时达到峰值,说明二者在侵入菌丝的扩展阶段可能发挥一定功能。例如,对细胞或细胞间免疫信号的转导具有一定贡献,并起始相关防御反应来阻止病原菌的侵染扩张等。另外,IR_DEG2和IR_DEG3基因的表达在云引中与对照组相比,则整体呈现为下调趋势,但在LTH中较不明显,推测二者可能对植物的防御反应具有一定的负调控作用,对照组的几个表达峰值点则可能是由于受到其他因素如生理周期和光照、湿度等影响所造成。

而在云引叶片的离体接菌实验中,由于人工造成的叶片创伤有助于孢子的快速侵染与扩张过程,同时还存在致病菌因素。因此可能造成IR_DEG1在接种后的24 h内表达量即发生较明显上调,包括IR_DEG6在内的其余LRR-RLKs基因的表达在叶片离体后都发生明显的下调表达趋势,且与对照组较相似。可能是由于创伤及离体本身就是一种特殊的免疫或凋亡等信号,这些基因的表达受这些信号的影响要远大于来自稻瘟病菌的影响。研究表明,水稻白叶枯抗性基因*Xa21*、*Xa1*不仅能受到接种病菌的诱导,也会受到创伤的诱导^[78,79],而稻瘟病抗性基因*Pib*则会受到环境因素如暗处理的诱导^[80],但大多数的稻瘟病R基因在水稻中都呈组成型表达,起着免疫监视的作用,且不受稻瘟病菌侵染诱导表达^[30~32,81]。进一步更多的研究包括,分析基因序列上的差异可能造成对基因功能的影响等,将解析这些LRR-RLKs基因功能,以及探索它们是否参与到水稻的免疫过程并对抵抗稻瘟病菌有贡献提供更多证据。

参考文献

- 1 Ballini E, Morel J B, Droc G, et al. A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. *Mol Plant Microbe Interact*, 2008, 21: 859–868
- 2 Chen L, Yang S J. A large area of crop failure of Yuan Longping Super Rice, the controversy in Super Rice (in Chinese). *Ctry Agric Farmer*, 2015, (4): 19–22 [陈露, 杨思佳. 袁隆平超级稻减产大面积绝收, 争议超级稻. 农村·农业·农民, 2015, (4): 19–22]
- 3 Liu H T, Xu Q, He W, et al. Recent progress on the variation of blast resistance and cloning of the resistance genes in rice (in Chinese). *Fujian J Agric Sci*, 2016, 31: 545–552 [刘海涛, 徐倩, 何伟, 等. 水稻稻瘟病抗性变化及抗性基因克隆的研究进展. 福建农业学报, 2016, 31: 545–552]
- 4 Yang D W, Wang M, Han L B, et al. Progress of cloning and breeding application of blast resistance genes in rice and avirulence genes in blast fungi (in Chinese). *Chin Bull Bot*, 2019, 54: 265–276 [杨德卫, 王莫, 韩利波, 等. 水稻稻瘟病抗性基因的克隆、育种利用及稻瘟菌无毒基因研究进展. 植物学报, 2019, 54: 265–276]
- 5 Liang T, Chi W, Huang L, et al. Bulk segregant analysis coupled with whole-genome sequencing (BSA-Seq) mapping identifies a novel *pi21* haplotype conferring basal resistance to rice blast disease. *Int J Mol Sci*, 2020, 21: 2162
- 6 Jiang Z Y, Ren J P, Liu X M, et al. Comparative analysis of gene expression profiles of rice responding to different races of *Magnaporthe grisea* (in Chinese). *Sci Agric Sin*, 2013, 46: 5123–5131 [姜兆远, 任金平, 刘晓梅, 等. 水稻与稻瘟病菌不同小种互作的基因差异表达谱分析. 中国农业

科学, 2013, 46: 5123–5131]

- 7 Gao X Q. Identification and functional analysis of rice blast resistance-related genes (in Chinese). Master Thesis. Hangzhou: Zhejiang Normal University, 2015 [高晓庆. 水稻稻瘟病抗性相关基因的筛选和功能鉴定. 硕士学位论文. 杭州: 浙江师范大学, 2015]
- 8 Yu S S, Luo X, Lian L, et al. Identification of *CIPK* family in rice and qRT-PCR analysis on *OsCIPK5* induced by *Magnaporthe oryzae* (in Chinese). *Fujian J Agric Sci*, 2019, 34: 1237–1245 [喻丝丝, 罗曦, 连玲, 等. 水稻CIPK基因家族的鉴定及OsCIPK5受稻瘟病菌诱导的qRT-PCR分析. 福建农业学报, 2019, 34: 1237–1245]
- 9 Liang T M, Chen Z J, Chen S B. Research and progress of the application of genome wide analysis strategy in gene identification of rice blast resistance (in Chinese). *Mol Plant Breed*, 2019, 17: 1525–1530 [梁廷敏, 陈在杰, 陈松彪. 全基因组分析策略应用于水稻抗稻瘟病基因鉴定的研究和进展. 分子植物育种, 2019, 17: 1525–1530]
- 10 Li W, Zhu Z, Chern M, et al. A natural allele of a transcription factor in rice confers broad-spectrum blast resistance. *Cell*, 2017, 170: 114–126.e15
- 11 Ma Y Y, Zuo S M, Zhang Z J, et al. Construction and application of near-isogenic line in rice (in Chinese). *J Anhui Agric Sci*, 2008, 36: 7167–7168 [马玉银, 左示敏, 张在金, 等. 水稻近等基因系构建及其应用. 安徽农业科学, 2008, 36: 7167–7168]
- 12 Zhu Y S, Cai Q H, Xue W M, et al. Preliminary genotype analysis of broad-spectrum rice blast resistance variety Yunyin and its near-isogenic lines construction (in Chinese). *Fujian J Agric Sci*, 2012, 27: 1–6 [朱永生, 蔡秋华, 薛伟民, 等. 云引稻瘟病抗性基因型的初步分析及其近等基因系构建. 福建农业学报, 2012, 27: 1–6]
- 13 Zhu Y S, He W, Lian L, et al. Construction and genetic background recovery of blast-resistant near-isogenic lines from *japonica* variety, Yunyin (in Chinese). *Fujian J Agric Sci*, 2018, 33: 957–962 [朱永生, 何炜, 连玲, 等. 粳稻云引稻瘟病抗性近等基因系构建及遗传背景恢复分析. 福建农业学报, 2018, 33: 957–962]
- 14 Young N D. The genetic architecture of resistance. *Curr Opin Plant Biol*, 2000, 3: 285–290
- 15 McDowell J M, Simon S A. Recent insights into *R* gene evolution. *Mol Plant Pathol*, 2006, 7: 437–448
- 16 Bergelson J, Kreitman M, Stahl E A, et al. Evolutionary dynamics of plant *R*-genes. *Science*, 2001, 292: 2281–2285
- 17 Friedman A R, Baker B J. The evolution of resistance genes in multi-protein plant resistance systems. *Curr Opin Genet Dev*, 2007, 17: 493–499
- 18 Song W Y, Wang G L, Chen L L, et al. A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science*, 1995, 270: 1804–1806
- 19 Peng H, Chen Z, Fang Z, et al. Rice *Xa21* primed genes and pathways that are critical for combating bacterial blight infection. *Sci Rep*, 2015, 5: 12165
- 20 Song W Y, Pi L Y, Wang G L, et al. Evolution of the rice *Xa21* disease resistance gene family. *Plant Cell*, 1997, 9: 1279–1287
- 21 Pruitt R N, Schwessinger B, Joe A, et al. The rice immune receptor XA21 recognizes a tyrosine-sulfated protein from a Gram-negative bacterium. *Sci Adv*, 2015, 1: e1500245
- 22 Lee S W, Han S W, Bartley L E, et al. Unique characteristics of *Xanthomonas oryzae* pv. *oryzae* AvrXa21 and implications for plant innate immunity. *Proc Natl Acad Sci USA*, 2006, 103: 18395–18400
- 23 Ronald P C, Beutler B. Plant and animal sensors of conserved microbial signatures. *Science*, 2010, 330: 1061–1064
- 24 Boller T, Felix G. A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu Rev Plant Biol*, 2009, 60: 379–406
- 25 Smakowska-Luzan E, Mott G A, Parys K, et al. An extracellular network of *Arabidopsis* leucine-rich repeat receptor kinases. *Nature*, 2018, 553: 342–346
- 26 Iten M, Hoffmann T, Grill E. Receptors and signalling components of plant hormones. *J Recept Signal Transduct*, 1999, 19: 41–58
- 27 Bryan G T, Wu K S, Farrall L, et al. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. *Plant Cell*, 2000, 12: 2033–2046
- 28 Sharma T R, Madhav M S, Singh B K, et al. High-resolution mapping, cloning and molecular characterization of the *Pi-k^h* gene of rice, which confers resistance to *Magnaporthe grisea*. *Mol Genet Genom*, 2005, 274: 569–578
- 29 Thakur S, Singh P K, Das A, et al. Extensive sequence variation in rice blast resistance gene *Pi54* makes it broad spectrum in nature. *Front Plant Sci*, 2015, 6: 345
- 30 Shang J, Tao Y, Chen X, et al. Identification of a new rice blast resistance gene, *Pid3*, by genomewide comparison of paired nucleotide-binding site—Leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics*, 2009, 182: 1303–1311
- 31 Chen Z, Zhao W, Zhu X, et al. Identification and characterization of rice blast resistance gene *Pid4* by a combination of transcriptomic profiling and genome analysis. *J Genet Genomics*, 2018, 45: 663–672
- 32 Liu X, Lin F, Wang L, et al. The *in Silico* map-based cloning of *Pi36*, a rice coiled-coil—Nucleotide-binding site—Leucine-rich repeat gene that confers race-specific resistance to the blast fungus. *Genetics*, 2007, 176: 2541–2549
- 33 Chen X, Shang J, Chen D, et al. A B-lectin receptor kinase gene conferring rice blast resistance. *Plant J*, 2006, 46: 794–804
- 34 Peng H, Zhang Q, Li Y, et al. A putative leucine-rich repeat receptor kinase, OsBRR1, is involved in rice blast resistance. *Planta*, 2009, 230: 377–

385

- 35 Hu H, Xiong L, Yang Y. Rice *SERK1* gene positively regulates somatic embryogenesis of cultured cell and host defense response against fungal infection. *Planta*, 2005, 222: 107–117
- 36 Chen X, Zuo S, Schwessinger B, et al. An XA21-associated kinase (OsSERK2) regulates immunity mediated by the XA21 and XA3 immune receptors. *Mol Plant*, 2014, 7: 874–892
- 37 Liao H, Xiao X, Li X, et al. OsBAK1 is involved in rice resistance to *Xanthomonas oryzae* pv. *oryzae* PXO99. *Plant Biotechnol Rep*, 2016, 10: 75–82
- 38 Song D, Li G, Song F, et al. Molecular characterization and expression analysis of *OsBISERK1*, a gene encoding a leucine-rich repeat receptor-like kinase, during disease resistance responses in rice. *Mol Biol Rep*, 2008, 35: 275–283
- 39 Kunze G, Zipfel C, Robatzek S, et al. The N terminus of bacterial elongation factor Tu elicits innate immunity in *Arabidopsis* plants. *Plant Cell*, 2004, 16: 3496–3507
- 40 Zipfel C, Kunze G, Chinchilla D, et al. Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell*, 2006, 125: 749–760
- 41 Gómez-Gómez L, Boller T. FLS2. *Mol Cell*, 2000, 5: 1003–1011
- 42 van der Burgh A M, Postma J, Robatzek S, et al. Kinase activity of SOBIR1 and BAK1 is required for immune signalling. *Mol Plant Pathol*, 2019, 20: 410–422
- 43 Krol E, Mentzel T, Chinchilla D, et al. Perception of the *Arabidopsis* danger signal peptide 1 involves the pattern recognition receptor *AtPEPR1* and its close homologue *AtPEPR2*. *J Biol Chem*, 2010, 285: 13471–13479
- 44 Tan S, Wang D, Ding J, et al. Adaptive evolution of *Xa21* homologs in Gramineae. *Genetica*, 2011, 139: 1465–1475
- 45 Wang G L, Ruan D L, Song W Y, et al. *Xa21D* encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. *Plant Cell*, 1998, 10: 765–779
- 46 Jain P, Singh P K, Kapoor R, et al. Understanding host-pathogen interactions with expression profiling of NILs carrying rice-blast resistance *Pi9* gene. *Front Plant Sci*, 2017, 8: 93
- 47 Deslandes L, Rivas S. Catch me if you can: Bacterial effectors and plant targets. *Trends Plant Sci*, 2012, 17: 644–655
- 48 Win J, Chaparro-Garcia A, Belhaj K, et al. Effector biology of plant-associated organisms: Concepts and perspectives. *Cold Spring Harbor Symp Quant Biol*, 2012, 77: 235–247
- 49 Chen X, Ronald P C. Innate immunity in rice. *Trends Plant Sci*, 2011, 16: 451–459
- 50 Teixeira P J P, Colaianni N R, Fitzpatrick C R, et al. Beyond pathogens: Microbiota interactions with the plant immune system. *Curr Opin Microbiol*, 2019, 49: 7–17
- 51 Cook D E, Mesarich C H, Thomma B P H J. Understanding plant immunity as a surveillance system to detect invasion. *Annu Rev Phytopathol*, 2015, 53: 541–563
- 52 French E, Kim B S, Iyer-Pascuzzi A S. Mechanisms of quantitative disease resistance in plants. *Semin Cell Dev Biol*, 2016, 56: 201–208
- 53 Jones J D G, Dangl J L. The plant immune system. *Nature*, 2006, 444: 323–329
- 54 Nürnberger T, Brunner F, Kemmerling B, et al. Innate immunity in plants and animals: Striking similarities and obvious differences. *Immunol Rev*, 2004, 198: 249–266
- 55 Thao N P, Chen L, Nakashima A, et al. RAR1 and HSP90 form a complex with Rac/Rop GTPase and function in innate-immune responses in rice. *Plant Cell*, 2007, 19: 4035–4045
- 56 Kawano Y, Akamatsu A, Hayashi K, et al. Activation of a Rac GTPase by the NLR family disease resistance protein pit plays a critical role in rice innate immunity. *Cell Host Microbe*, 2010, 7: 362–375
- 57 Ngou B, Ahn H K, Ding P, et al. Mutual potentiation of plant immunity by cell-surface and intracellular receptors. *Nature*, 2021, 592: 110–115
- 58 Yuan M, Jiang Z, Bi G, et al. Pattern-recognition receptors are required for NLR-mediated plant immunity. *Nature*, 2021, 592: 105–109
- 59 Xi L, Wu X N, Gilbert M, et al. Classification and interactions of LRR receptors and co-receptors within the *Arabidopsis* plasma membrane—An overview. *Front Plant Sci*, 2019, 10: 472
- 60 Wu Y, Xun Q, Guo Y, et al. Genome-wide expression pattern analyses of the *Arabidopsis* leucine-rich repeat receptor-like kinases. *Mol Plant*, 2016, 9: 289–300
- 61 Gao L L, Xue H W. Global analysis of expression profiles of rice receptor-like kinase genes. *Mol Plant*, 2012, 5: 143–153
- 62 Böhm H, Albert I, Fan L, et al. Immune receptor complexes at the plant cell surface. *Curr Opin Plant Biol*, 2014, 20: 47–54
- 63 Matsushima N, Miyashita H. Leucine-rich repeat (LRR) domains containing intervening motifs in plants. *Biomolecules*, 2012, 2: 288–311
- 64 Torii K U. Leucine-rich repeat receptor kinases in plants: Structure, function, and signal transduction pathways. *Int Rev Cytol*, 2004, 234: 1–46
- 65 Chinchilla D, Zipfel C, Robatzek S, et al. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature*, 2007, 448: 497–500

- 66 Zipfel C, Oldroyd G E D. Plant signalling in symbiosis and immunity. *Nature*, 2017, 543: 328–336
- 67 Russinova E, Borst J W, Kwaaitaal M, et al. Heterodimerization and endocytosis of arabidopsis brassinosteroid receptors BRI1 and AtSERK3 (BAK1). *Plant Cell*, 2004, 16: 3216–3229
- 68 Albert I, Böhm H, Albert M, et al. An RLP23-SOBIR1-BAK1 complex mediates NLP-triggered immunity. *Nat Plants*, 2015, 1: 15140
- 69 Okuyama Y, Kanzaki H, Abe A, et al. A multifaceted genomics approach allows the isolation of the rice *Pia*-blast resistance gene consisting of two adjacent NBS-LRR protein genes. *Plant J*, 2011, 66: 467–479
- 70 Ashikawa I, Hayashi N, Yamane H, et al. Two adjacent nucleotide-binding site—Leucine-rich repeat class genes are required to confer *Pikm*-specific rice blast resistance. *Genetics*, 2008, 180: 2267–2276
- 71 Veneault-Fourrey C, Barooah M, Egan M, et al. Autophagic fungal cell death is necessary for infection by the rice blast fungus. *Science*, 2006, 312: 580–583
- 72 Samalova M, Mérida H, Vilaplana F, et al. The β -1,3-glucanotransferases (Gels) affect the structure of the rice blast fungal cell wall during appressorium-mediated plant infection. *Cell Microbiol*, 2017, 19: e12659
- 73 Ribot C, Hirsch J, Balzergue S, et al. Susceptibility of rice to the blast fungus, *Magnaporthe grisea*. *J Plant Physiol*, 2008, 165: 114–124
- 74 Giraldo M C, Dagdas Y F, Gupta Y K, et al. Two distinct secretion systems facilitate tissue invasion by the rice blast fungus *Magnaporthe oryzae*. *Nat Commun*, 2013, 4: 1996
- 75 Guo X Y, Li Y, Fan J, et al. Host-induced gene silencing of *MoAP1* confers broad-spectrum resistance to *Magnaporthe oryzae*. *Front Plant Sci*, 2019, 10: 433
- 76 Kou Y, He Y, Qiu J, et al. Mitochondrial dynamics and mitophagy are necessary for proper invasive growth in rice blast. *Mol Plant Pathol*, 2019, 20: 1147–1162
- 77 Li W, Liu Y, Wang J, et al. The durably resistant rice cultivar Digu activates defence gene expression before the full maturation of *Magnaporthe oryzae* appressorium. *Mol Plant Pathol*, 2016, 17: 354–368
- 78 Yoshimura S, Yamanouchi U, Katayose Y, et al. Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. *Proc Natl Acad Sci USA*, 1998, 95: 1663–1668
- 79 Park C J, Lee S W, Chern M, et al. Ectopic expression of rice *Xa21* overcomes developmentally controlled resistance to *Xanthomonas oryzae* pv. *oryzae*. *Plant Sci*, 2010, 179: 466–471
- 80 Wang Z X, Yano M, Yamanouchi U, et al. The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. *Plant J*, 1999, 19: 55–64
- 81 Qu S, Liu G, Zhou B, et al. The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site—Leucine-rich repeat protein and is a member of a multigene family in rice. *Genetics*, 2006, 172: 1901–1914

Summary for “粳稻云引稻瘟病抗性近等基因系与免疫应答相关的LRR-RLKs差异基因分析”

Analysis of immune response-related LRR-RLKs differential genes in the rice blast resistance near-isogenic lines of *Japonica* Variety Yunyin

Yuelong Lin^{1,2,3}, Yupeng Wang^{1,2,3}, Yongsheng Zhu^{2,3}, Yanmei Zheng^{2,3}, Qiuhua Cai^{2,3}, Wei He^{2,3}, Liping Chen^{2,3}, Hongguang Xie^{2,3}, Yidong Wei^{2,3}, Huaan Xie^{1,2,3} & Jianfu Zhang^{1,2,3*}

¹ College of Agronomy, Fujian Agriculture and Forestry University, Fuzhou 350002, China;

² Rice Research Institute, Fujian Academy of Agricultural Sciences, Fuzhou 350019, China;

³ State Key Laboratory for Ecological Control of Crop Pests between Fujian and Taiwan/National Engineering Laboratory of Rice/South China Research Base of State Key Laboratory of Hybrid Rice/Incubating Base of State Key Laboratory of Crop Germplasm Innovation and Molecular Breeding between Fujian Province and Ministry of Science and Technology/Fuzhou Branch of National Rice Improvement Center/South China Key Laboratory of Hybrid Rice Germplasm Innovation and Molecular Breeding of Ministry of Agriculture and Rural Areas/Fujian Engineering Laboratory of Crop Molecular Breeding/Fujian Key Laboratory of Rice Molecular Breeding, Fuzhou 350003, China

* Corresponding author, E-mail: jianfzhang@163.com

Rice (*Oryza sativa* L.) is a staple food in the world as well as an important monocotyledonous model plant for molecular biology, which has a long history of cultivation and domestication. However, many diseases will break out during rice production. For example, the rice blast is the most destructive disease, which can cause the heavy loss or even total crop failure. Therefore, there are constant demands to cultivate durably blast-resistant varieties and identify more broad-spectrum blast resistance genes. Besides, it's also very significant for agricultural production to elucidate the process and mechanism of immune response during the rice blast infection.

The whole-genome sequencing of the resistant and susceptible near-isogenic lines derived from the broad-spectrum blast resistance *Japonica* Variety Yunyin and the universally blast-susceptible variety Lijiangxintuanheigu (LTH) was completed. Eight differential genes were screened from the GO terms that were associated with the immune response based on the whole-genome sequence data. We analyzed their gene structure, evolution and sequence variability between the resistant and susceptible near-isogenic lines. The results indicated that the homology of these protein sequences was between 55% and 80%, and the conserved protein domain analysis showed that most of them contained different numbers and types of leucine-rich repeat domains, a transmembrane domain and a C-terminal kinase domain, which were typical leucine-rich repeat receptor-like protein kinases. Moreover, they are all located within a cluster of chromosome 11 and were homologous to the bacterial blight resistance gene *Xa21*.

The leucine-rich repeat receptor-like protein kinases (LRR-RLKs) genes are a large conserved superfamily during the plant evolution. In *Arabidopsis*, there are 14 subfamilies and more than 200 members of LRR-RLKs were identified, whereas the rice genome contained more than 900 LRR-RLKs genes. The perception and recognition of pathogen infection are mainly mediated by the plant cells surface pattern-recognition receptors (PRRs) and intracellular nucleotide-binding leucine-rich repeat (NLR) immune receptors. PRRs contain many receptor kinases including the leucine-rich repeat receptor-like protein kinases, which play an important role in the signal transduction during the plant development, immune response and sense of the environmental changes.

In order to further explore the potential function of these genes during the rice blast disease response, the rice seedlings were inoculated with *Magnaporthe oryzae* by spray and punch methods and then the expression pattern of these genes during the fungal infection were analyzed by qRT-PCR. The results showed that some genes response to the blast disease. Thus, we speculated that some of them might be involved in the rice blast disease response. This study laid a foundation for further studying genes related to the rice blast disease response.

near-isogenic lines, immune response, leucine-rich repeat receptor-like protein kinases (LRR-RLKs), rice blast

doi: [10.1360/TB-2021-0242](https://doi.org/10.1360/TB-2021-0242)