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水稻抗稻瘟病机制的研究进展

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摘要 稻瘟病严重危害水稻产量与品质. 水稻抗病遗传基础及分子机制的解析, 可为水稻抗病育种提供有效抗性遗传资源和重要的理论指导. 本文从水稻稻瘟病抗病基因的克隆、抗性机制解析、产量与稻瘟病抗性的协同调控, 以及稻瘟病抗病育种等方面, 综述了近年来水稻稻瘟病抗病研究领域的主要进展, 并对目前稻瘟病抗病研究中存在的问题进行了讨论与展望.

关键词 水稻, 稻瘟病菌, 稻瘟病抗病机制, 抗病育种

水稻(*Oryza sativa*)是最重要的粮食作物之一, 养活了世界一半以上的人口, 它的安全生产对于保障社会稳定、经济发展至关重要. 稻瘟病是对水稻危害最为严重的真菌性病害, 常年发生在水稻的整个生长期, 可导致水稻减产10%~35%[1]. 培育抗病品种作为防治稻瘟病害最为经济有效和环保的途径, 在保障粮食安全方面发挥着重要作用.

在过去的20年中, 随着寄主水稻及稻瘟病菌基因组序列的测定和基因注释的完成[2~5], 水稻与稻瘟病菌的互作模式逐渐发展成为研究植物免疫系统的重要模型. 但是由于稻瘟病菌生理小种遗传的高度复杂性和变异性, 不同稻区的稻瘟病菌群体构成存在巨大差异, 使水稻稻瘟病抗病品种的推广受到区域性和时效性的限制. 本文从宿主水稻入手, 系统地梳理了近年来水稻抗稻瘟病机制的研究进展, 并对水稻稻瘟病抗病育种进行了讨论.

1 水稻稻瘟病*R*基因的克隆

1905年, 英国遗传学家Biffen[6]率先在小麦中进行抗病遗传研究, 正式拉开了植物免疫学的序幕, 并为作物抗病品种的培育指明了方向. 20世纪50年代, 美国学者Flor[7]提出基因对基因假说, 促进了寄主与病原物相互作用分子机制的深入研究. 随后, 日本学者以该假说为基础, 建立了一套稻瘟病抗病基因鉴别体系, 利用这套体系, 他们成功鉴定得到了8个水稻稻瘟病抗病(*resistance*, *R*)位点上的14个基因[8]. 迄今, 水稻中已经鉴定出超过100个稻瘟病相关的*R*基因或者位点, 它们成簇地分布在除第3号染色体外的所有染色体上(表1)[9,10].

1.1 水稻稻瘟病典型*R*基因的克隆

目前克隆的大多数*R*基因都编码含有核苷酸结合域和富含亮氨酸重复序列(nucleotide-binding/leucine-

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表 1 已克隆的*R*基因和位点

Table 1 Cloned blast resistance (*R*) genes/alleles in rice

染色体	基因名称	编码蛋白类型	供体	参考文献
1	*Pi37*	NLR	St. No. 1	[11,12]
1	*Pit*	NLR	K59	[13,14]
1	*Pish*	NLR	Nipponbare	[15,16]
1	*Pi35*	NLR	Hokkai 188	[17,18]
1	*Pi64*	NLR	Yangmaogu	[19]
2	*Pi-b*	NLR	Tohoku IL9	[20]
4	*pi21*	富含脯氨酸的蛋白	Owarihatamochi	[21,22]
4	*Pi63*/*Pikahei-1(t)*	NLR	Kahei	[23]
6	*Pi9*	NLR	*O. minuta*	[24,25]
6	*Pi2*	NLR	5173	[26,27]
6	*Piz-t*	NLR	Toride 1	[27]
6	*Pi-d2*	B-lectin类跨膜受体蛋白	Digu	[28]
6	*Pi-d3*	NLR	Digu	[29]
6	*Pi25*	NLR	Gumei2	[30]
6	*Pid3-A4*	NLR	A4 (*Oryza rufipogon*)	[31]
6	*Pi50*	NLR	Er-Ba-zhan (EBZ)	[32]
6	*Pigm*	NLR	Gumei4	[33,34]
6	*Pid4*	NLR	Digu	[35]
8	*Pi36*	NLR	Kasalath	[36,37]
9	*Pi5*/*Pi3* /*Pii*	NLR	Tetep	[38,39]
9	*Pi56*	NLR	Sanhuangzhan No. 2	[40]
11	*Pi54*	NLR	Tetep	[41,42]
11	*Pikm*	NLR	Tsuyuake	[43]
11	*Pb1*	NLR	Modan	[44]
11	*Pik*	NLR	Kusabue	[45]
11	*Pik-p*	NLR	K60	[46,47]
11	*Pia*	NLR	Sasanishiki	[48,49]
11	*Pi1*	NLR	LAC23	[50]
11	*Pi54rh*	NLR	*Oryza rhizomatis*	[51]
11	*Pi-CO39*	NLR	CO39	[49,52]
11	*Pi54of*	NLR	*Oryza officinalis* (nrcpb004)	[41]
11	*Pi-kh*	NLR	Tetep	[53]
11	*Pike*	NLR	Xiangzao143	[54]
12	*Pi-ta*	NLR	Yashiro-mochi	[55]
12	*Ptr*	含Armadillo结构域蛋白	Katy	[56]

rich-repeat, NLR)的蛋白. NLR类型的蛋白一般包含三段保守结构域: 可变的N端结构域、核苷酸结合寡聚化结构域(nucleotide-binding oligomerization domain, NOD)、C端的亮氨酸重复序列(leucine-rich repeats, LRR)[57]. NLR蛋白作为胞内免疫受体, 常常被认为是植物免疫系统的开关, 在直接或间接识别效应蛋白后,

启动效应因子激发的免疫反应(effectors triggered immunity, ETI), 引起组成性的细胞程序化死亡[58].

NLR基因或位点常常重复串联在一起, 极大程度地增加了*R*基因的拷贝数, 提高了基因重组产生新型*R*基因的概率. 以第6号染色体为例, 在它的短臂近着丝粒附近聚集了*Pi2*, *Pi9*, *Pi22*, *Pi25*, *Pi26*, *Pi40*, *Pi42*, *Pigm*, *Piz*, *Piz-t*等多个*R*基因组成的基因簇[10]. 这些*R*基因或者紧密连锁或者互为等位基因, 在序列上具有高度的相似性. *Pi2*与*Piz-t*编码的R蛋白仅在3个LRR结构域上有8个氨基酸的差异[27]. *Pigm*位点含有13个NLR基因(*R1*~*R13*), 其中*R4*, *R6*, *R8*编码完整的NLR蛋白. R4和R6蛋白氨基酸序列高度一致, 仅在LRR结构域上存在4个氨基酸的差异. 遗传学实验证实, 真正识别效应蛋白启动ETI免疫反应的是*R6*编码的产物PigmR蛋白[34]. 由此可见, LRR结构域的多态性是决定NLR蛋白识别不同稻瘟病生理小种的关键.

1.2　水稻稻瘟病非典型*R*基因的克隆

虽然大多数典型的稻瘟病*R*基因编码NLR类型的蛋白, 但也有一些*R*基因编码其他类型的蛋白, 比如*pi21*, *Pid2*和*Ptr*等.

*pi21*是从栽培抗病品种“Owarihatamochi”中克隆的一个隐性非小种专化性抗病基因. 它定位在4号染色体, 只特异地提高水稻稻瘟病抗性, 对其他真菌或细菌类病害没有影响[21,22]. 野生型的*Pi21*编码一个富含脯氨酸的蛋白, 含有重金属结合结构域和蛋白互作结构域[21,22]. 在一些粳稻抗病品种中, *Pi21*编码脯氨酸的区域发生不同形式的缺失, 形成新的非典型*R*基因位点*pi21*, 导致被抑制的水稻免疫反应激活, 阻止稻瘟病菌丝在水稻细胞中的生长[22]. 像*pi21*这样的隐性抗病基因, 更容易通过基因编辑技术创制, 在水稻抗病分子育种中具有较大的应用空间.

*Pid2*是从籼稻广谱持久抗病品种地谷中克隆的一个主效抗稻瘟病基因. 它编码一个长度为825个氨基酸的B-lectin类跨膜受体蛋白激酶. Pid2氨基端含有一段疏水性信号肽、B-lectin结构域、PAN域和TM域, 羧基端含有一个典型的丝氨酸/苏氨酸激酶结构域[28]. 在水稻进化过程中, *Pid2*在不同水稻品种中产生了多种等位变异[59]. 与感病品种相比, 地谷中的*Pid2*存在一个单碱基突变, 使其编码蛋白的第441位氨基酸由甲硫氨酸突变为异亮氨酸[28]. 作为一个有活性的磷酸激酶, Pid2通过磷酸化修饰激活泛素连接酶OsPUB15, 进而启动下游免疫应答[59].

*Ptr*是从美国粳稻抗病品种Katy中克隆得到的一个非NLR类稻瘟病广谱抗病基因[56]. 它位于12号染色体的*Pi-ta*基因簇中[60,61]. *Pi-ta2*与*Ptr*序列一致[62], 编码一个包含4个Armadillo重复结构域的非典型性E3泛素连接酶, 但并不具备E3泛素连接酶活性[56]. 研究发现, *Ptr*的第4个外显子是其识别稻瘟病菌的关键区域. 在3000份水稻重组测序数据中, 仅发现48份水稻资源含有*Ptr*抗性基因[56]. *Ptr*作为一个新的稻瘟病广谱抗病基因, 抗谱比*Pi-ta*更广泛, 在未来水稻抗病育种中有较大的应用潜力.

面对抗病品种的选择压力, 稻瘟病菌群体遗传结构发生变化, 产生新的优势无毒基因型, 最终使得水稻品种抗性“丧失”. 因此, 克隆新的*R*基因, 不仅是水稻-稻瘟病互作模式研究的基础, 也是培育稻瘟病抗病新品种的关键.

2　水稻稻瘟病抗性机制解析

根据寄主与病原菌的互作方式, 稻瘟病抗性可以分为完全抗性和部分抗性两种(完全抗性又称为质量抗性或垂直抗性, 部分抗性又称为数量抗性或水平抗性). 完全抗性是指寄主与病原菌以不亲和的方式互作, 从而阻断病原菌在寄主上的繁殖, 通常由单个或多个主效*R*基因控制, 属于质量性状遗传, 具有小种专化性; 部分抗性是指寄主与病原菌以亲和的方式互作, 但病原菌的侵染在一定程度上受到抑制, 通常是由非*R*基因激活的免疫防御机制调控[63~65].

2.1　*R*基因介导的稻瘟病完全抗性机制

不同的*R*基因表现出的稻瘟病抗性特点各不相同. 有些*R*基因具有稻瘟病广谱抗性, 如*Pi9*, 它赋予水稻稻瘟病广谱抗性, 对来源于13个国家的至少43个稻瘟病菌生理小种具有抗性[25]; 有些*R*基因抗谱相对较窄, 对某些生理小种具有专化性, 如*Pid4*, 它是籼稻广谱持久抗病品种地谷的一个主效抗稻瘟病基因, 具有一定的小种专化性, 在测试的15个稻瘟病生理小种中, 仅有5个能特异激活*Pid4*介导的稻瘟病抗性[35]; 有些*R*基因在籼粳稻分化过程中产生了特异性, 如*Pid3*, 它是克隆自地谷的另一个抗稻瘟病主效基因, 它的蛋白翻译

在大部分粳稻品种中提前终止，导致粳稻中Pid3蛋白缺少完整的LRR结构域, *pid3*成为假基因[29].

*R*基因与对应的*Avr*基因间遵循“基因对基因”的互作模式. 目前，约有24个稻瘟病菌的*Avr*基因位点被定位，其中12个被克隆，9个在水稻中筛选到与之对应的*R*基因[10]. 这些*Avr*基因大多编码小于200个氨基酸的分泌性蛋白，它们作为病原菌效应子被宿主的R蛋白识别. 但也有一些*Avr*基因编码的蛋白不具备分泌蛋白的特征，比如*ACE1*编码的非核糖体多聚乙酰合酶，被认为参与合成次级代谢物，进而激活宿主免疫[66]. 通常情况下, *R*基因在抗性植物中呈组成性表达[67]. 在识别病原菌分泌的AVR蛋白后, R蛋白构象发生改变, 进入激活状态，启动ETI免疫反应[68~70]. 但也有部分*R*基因的表达在稻瘟病菌侵染后发生显著变化，如*Pi5-1*, *pi21*, *Pi54rh*和*Pb1*等[22,38,44,51].

目前，水稻R蛋白与对应的稻瘟病菌AVR蛋白之间的识别机制主要有两种模式. 第一种是以Pita/AVR-Pita, Pi54/AVR-Pi54, Pik/AVR-Pik, Pia/AVR-Pia, Pi-CO39/AVR-CO39等为代表的直接互作模式[49,71~73]. Pita/AVR-Pita是最早被报道可以直接相互作用的一对R/AVR蛋白. *Avr-Pita*编码含有223个氨基酸的锌离子金属蛋白酶. 从稻瘟病菌中分泌进入水稻细胞后, Avr-Pita利用C端176个氨基酸识别并结合Pita的LRD(leucine-rich domain)结构域，进而启动Pita介导的免疫反应[67,74](图1A). Pia抗性位点由2个NLR蛋白组成(RGA4和RGA5)，其中RGA5负责识别AVR蛋白，RGA4负责启动下游免疫应答信号. 正常情况下，RGA5与RGA4形成异源二聚体, 抑制RGA4活性, 避免其激活后引起的超敏反应阻碍水稻正常生长. 当RGA5识别AVR蛋白(Avr-CO39或AVR-Pia)后，RGA4被释放，免疫反应激活[49,71,75](图1B). 第二种是以Pii/AVR-Pii和Piz-t/AVR-Piz-t等为代表的间接互作模式. AVR-Pii不能直接被Pii识别，它首先被水稻细胞中的“诱饵”蛋白Exo70捕获形成复合体，随后落入Pii介导的免疫应答“陷阱”[76](图1C). AVR-Piz-t同样不能直接被Piz-t识别，需要泛素连接酶APIP6[77]和APIP10[78,79]、转录因子APIP5[80]、Nup98同源蛋白APIP12[81]、钾离子通道蛋白OsAKT1(APIP7)[82]、胰蛋白酶抑制剂APIP4[83]等多种类型的互作蛋白作为桥梁, 激活抗病反应(图1D). 水稻R蛋白与稻瘟病菌AVR蛋白之间的识别机制极为复杂. 多数水稻R蛋白识别的稻瘟病菌Avr蛋白还未被鉴定到. 2019年，我国学者发现首个植物抗病小体激活免疫的全新机制[68]，为稻瘟病R/AVR蛋白互作研究提供了方向.

*R*基因与*AVR*基因之间是协同进化的关系. AVR-Pik的识别需要邻近的一对NLR蛋白(Pik-1和Pik-2)共同参与, 其中Pik-1负责识别AVR-Pik, 并与Pik-2形成复合体, 共同激活宿主ETI免疫反应, 属于直接识别模式. 值得注意的是，*AVR-Pik*位点有5个等位基因(*AVR-Pik-A*, *-B*, *-C*, *-D*和*-E*), 其中*AVR-Pik-D*分布最广, 是这5个等位基因进化的“祖先”. 与之对应，水稻进化产生了7个*Pik*等位基因(*Pi1*, *Pik*, *Pikm*, *Pikp*, *Piks*, *Pikh*和*Pike*), 特异地识别不同的*AVR-Pik*: *Pik*特异地识别*AVR-Pik-D*和*-E*; *Pikp*和*Piks*特异地识别*AVR-Pik-D*; *Pikh*和*Pikm*特异地识别*AVR-Pik-A*，*-D*和*-E*[84]，这充分的体现了*R*基因与*AVR*基因之间的协同进化.

2.2 转录因子参与调控的稻瘟病部分抗性机制

转录因子(transcriptional factors, TFs)在水稻稻瘟病抗病反应中发挥关键作用(图2A). 目前发现的58个TFs家族中[85]，植物所特有的WRKY型转录因子对水稻稻瘟病广谱抗性起重要作用. 很多抗性相关基因的启动子含有W-box基序(TTGACT/C), 该基序可特异地被WRKY转录因子识别. 转录组测序分析表明, 水稻中超过30个*WRKY*基因的表达水平受稻瘟病菌侵染的影响[86,87]. WRKY转录因子通过转录激活或转录抑制调控水稻抗性相关基因的表达, 进而调控稻瘟病抗性[88]. 比如，OsWRKY45起转录激活作用，它可以激活*P450*等多个抗病相关基因的表达，进而增强水稻对稻瘟病的广谱抗性[89,90]. OsWRKY42起转录抑制作用，它可以与活性氧(reactive oxygen species，ROS)清除基因*OsMT1d*的启动子结合，抑制其表达，从而提高细胞内ROS的含量, 最终增强水稻稻瘟病抗性[91].

除了WRKY家族成员外, bZIP类、C2H2类、NAC类等其他类型的转录因子在水稻稻瘟病广谱抗病信号通路中发挥了重要作用. bZIP类的转录因子APIP5可以同时结合稻瘟病病菌的AVR蛋白Avr-Piz-t和水稻R蛋白Piz-t. 当水稻缺失Piz-t时，Avr-Piz-t能抑制APIP5的转录活性，增强效应子触发的坏死，帮助稻瘟病菌进入死体营养阶段. 当宿主含有Piz-t时，Piz-t可增强APIP5蛋白的稳定性，阻止细胞坏死的发生，抑制稻瘟病菌从活体营养阶段向死体营养阶段的过渡[80]. C2H2

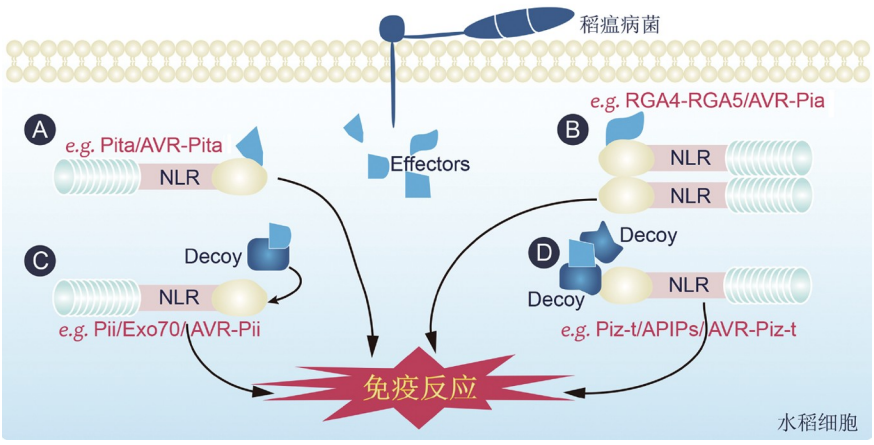

图 1　AVR蛋白与R蛋白间的互作模式. A: Pita/AVR-Pita之间的直接互作模式; B: RGA4, RGA5/AVR-Pia之间的直接互作模式; C: 由Exo70介导的Pii/AVR-Pii之间的间接互作模式; D: 由各类APIPs介导的Piz-t/AVR-Piz-t之间的间接互作模式

Figure 1　Models of molecular interaction between AVR and R proteins. A: Directly recognition pattern of Pita/AVR-Pita; B: directly recognition pattern of RGA4, RGA5/AVR-Pia; C: indirectly recognition pattern of Pii/AVR-Pii mediated by Exo70; D: indirectly recognition pattern of Piz-t/AVR-Piz-t mediated by APIPs

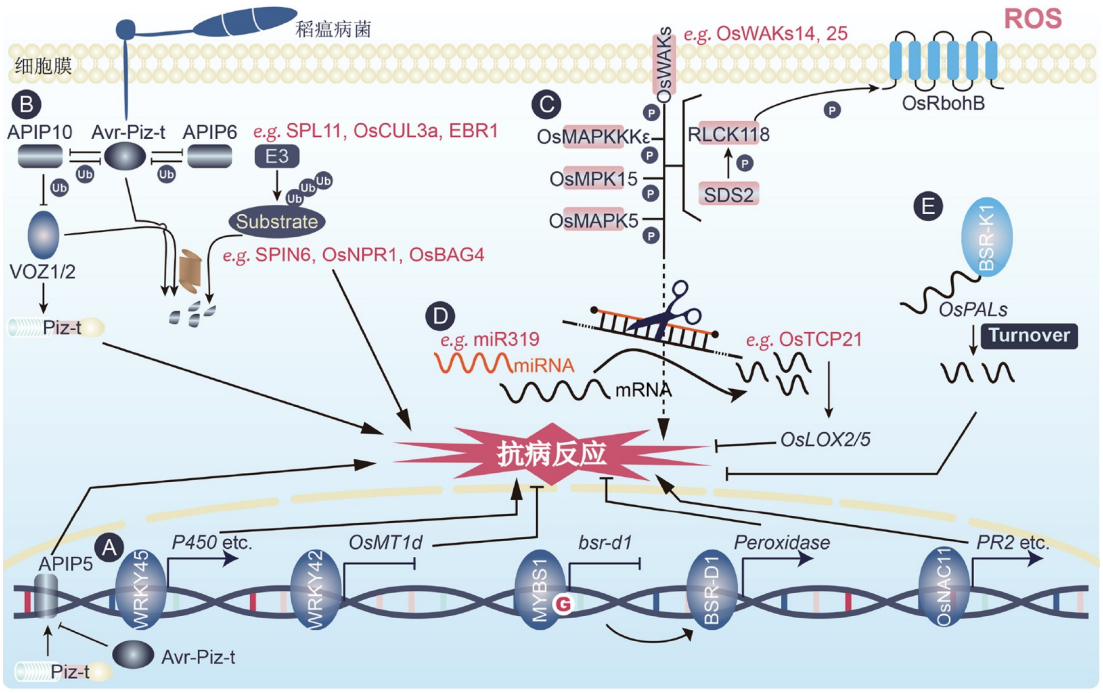

图 2　非*R*基因介导的水稻抗稻瘟病机制. A: 转录因子WRKY45和bsr-d1等介导的水稻抗稻瘟病工作模型; B: 泛素连接酶APIP6和APIP10等介导的水稻抗稻瘟病工作模型; C: 磷酸激酶OsMAPK5和RLCK118等介导的水稻抗稻瘟病工作模型; D: 小RNA miR139和miR398等介导的水稻抗稻瘟病工作模型; E: RNA结合蛋白BSR-K1介导的水稻抗稻瘟病工作模型

Figure 2　A working model for the non-R protein mediated rice blast resistance. A: Rice blast resistance mediated by transcriptional factors, such as WRKY45 and bsr-d1; B: rice blast resistance regulated by E3 ligases, such as APIP6, APIP10; C: protein kinases, such as OsMAPK5 and RLCK118, are involved in multiple signaling pathways of rice blast resistance; D: miRNAs are regulators contributing to rice blast resistance, such as miR139 and miR398; E: RNA binding protein BSR-K1 negatively regulates rice blast resistance

类转录因子bsr-d1克隆自籼稻广谱持久抗病品种地谷, 它通过调控过氧化氢(hydrogen peroxide, H_2O_2)酶基因的表达来调节H_2O_2的积累, 从而影响水稻的稻瘟病广谱抗性. *bsr-d1*提高稻瘟病广谱抗性的同时, 不影响水

稻产量和品质，在水稻抗病育种中具有广阔应用前景[92]. NAC类转录因子*OsNAC111*的表达受到稻瘟病菌侵染的诱导. 过量表达*OsNAC111*能促进抗病相关(*pathogen related*, *PR*)基因*PR2*和*PR8*的表达，诱导细胞内ROS的产生, 提高水稻对稻瘟病的抗性[93].

2.3 泛素连接酶参与调控的稻瘟病部分抗性机制

泛素化修饰是指泛素分子在泛素激活酶、泛素结合酶、泛素连接酶等一系列特殊酶的作用下，对靶蛋白进行特异性修饰的过程. 泛素化修饰在蛋白定位、稳定性和功能等方面都起着十分重要的作用，是调控水稻稻瘟病抗性的重要生化过程(图2B).

泛素连接酶可以直接与AVR蛋白互作，如APIP6, APIP10和OsPUB15等[59,77,78]. 稻瘟病菌的效应蛋白Avr-Piz-t可靶向结合水稻两个不同的泛素连接酶APIP6和APIP10. 当效应蛋白Avr-Piz-t进入水稻细胞后，会立即被APIP6和APIP10识别，引起Avr-Piz-t降解并阻止病原菌的侵入; Avr-Piz-t同时也会干扰APIP6和APIP10的功能，以此抵抗APIP6和APIP10介导的病原分子模式(pathogen associated molecular pattern, PAMP)激发的免疫反应(PAMPs triggered immunity, PTI)[77,78]. 当细胞内含有R蛋白Piz-t时，Avr-Piz-t的侵入会抑制APIP10泛素修饰功能，维持维管植物单锌指蛋白VOZ1/VOZ2的稳定性. 随后VOZ1/VOZ2与Piz-t形成复合体, 促进Piz-t富集, 激活体内ETI反应[79].

*spl11*是水稻的一个类病斑突变体，对稻瘟病和白叶枯病表现为广谱非小种专化抗性[94]. *SPL11*编码一个U-box类E3泛素连接酶，通过介导SPIN6和OsRac1蛋白的稳定性，进而调控水稻稻瘟病广谱抗性. 稻瘟病菌侵染会快速“唤醒”*SPL11*的表达，促进SPIN6的泛素化和降解，继而解除对OsRac1活性的抑制，诱发ROS和下游防卫基因的表达，抵御稻瘟病菌侵袭[95,96]. 和*spl11*类似，类病斑突变体*oscul3a*同样对稻瘟病和白叶枯病具有广谱非小种专化抗性. 作为“脚手架”蛋白，OsCUL3a首先招募含有RING结构域的锌指蛋白OsRBX1a和OsRBX1b形成CRL(cullin-RING ligase)复合体, 随后结合并促进底物OsNPR1的降解[97]. 像*spl11*和*oscul3a*这样的类病斑突变体，往往导致防御相关基因组成性表达，对不同病原菌都具有广谱非小种专化抗性, 是研究植物免疫机制的基础材料.

水稻中参与稻瘟病广谱抗性的泛素连接酶还包括EBR1和OsBBI1. EBR1特异地靶向降解OsBAG4, 稳定体内ROS含量, 避免细胞程序化死亡. 功能缺失突变体*ebr1*表现出稻瘟病广谱抗性[98]. *OsBBI1*的表达受稻瘟病菌诱导. 过量表达*OsBBI1*可以增加水稻细胞壁酚类物质的积累和蛋白交联, 促进ROS迸发, 增强水稻稻瘟病广谱抗性[99].

2.4 磷酸激酶参与调控的稻瘟病部分抗性机制

蛋白磷酸化修饰普遍存在于细胞信号调控网络中. 在水稻与稻瘟病菌的互作过程中, 关键调控蛋白的磷酸化状态是免疫信号激活的关键(图2C).

受体类激酶(receptor-like kinase, RLK)和受体类胞质激酶(receptor-like cytoplasmic kinase, RLCK)是水稻稻瘟病广谱抗病的关键组分. 过量表达受体类激酶OsWAK14, OsWAK25, OsWAK91和OsWAK92均可显著提高水稻对稻瘟病的抗性[100,101]. BSR1是一个典型的RLCK蛋白，其过量表达能提高水稻对稻瘟病菌、白叶枯菌等多种病原菌的抗性[102]. SDS2作为一个RLK蛋白，通过RLCK118将磷酸化修饰信号传递给NADPH氧化酶OsRbohB，促进植物细胞内ROS的迸发，增强水稻对稻瘟病的抗性[103]. RLK和RLCK往往形成类似的复合体，比如BAK1-BIK1[104]和OsSERK1-OsRLCK185[105]等，是植物免疫信号由胞外向胞内传递的桥梁.

丝裂原活化蛋白激酶(mitogen-activated protein kinases, MAPK)是进化保守的丝/苏氨酸蛋白激酶, 将稻瘟病抗病信号从细胞质传递到细胞核[106]. *OsMAPK5*功能缺失突变体表现为*PR*基因持续高表达、ROS迸发增强、对稻瘟病等多种病害的抗性提高[107]. *OsMPK15*负调*PR*基因的表达和ROS的积累, 影响水稻对稻瘟病等多种病原菌的广谱抗性[108]. *OsMAPKKKε*是水稻稻瘟病抗性的正调控因子. 受体激酶OsCERK1的底物OsRLCK185能直接磷酸化OsMAPKKKε，进而激活MAPKK4和MAPK3/6，建立一条线性化的水稻免疫信号通路[105]. MAPKs与RLK，RLCK的这种互作模式, 是利用蛋白质磷酸化实现免疫信号级联放大, 是植物免疫的普遍机制.

2.5 其他非*R*基因参与调控的稻瘟病部分抗性机制

与植物激素合成相关的蛋白，如水稻乙烯生物合

成酶OsACS2等，也在水稻稻瘟病广谱抗病中发挥重要作用. 提高*OsACS2*的表达量，可以增强乙烯合成，促进*PR*基因表达，提高水稻对多种稻瘟病菌生理小种的抗性，且不影响水稻农艺性状，在抗病育种中具有较好的应用潜力[109].

MicroRNA(miRNA)是一类长度约20~24个核苷酸的非编码单链RNA，它们广泛参与转录后基因表达调控[110,111]. 稻瘟病菌侵染会引起水稻体内多个miRNA表达谱发生显著变化[112]，但这些miRNA在水稻稻瘟病抗病过程中的作用各不相同. 比如，水稻miR319是加速稻瘟病菌侵染的“助推器”. 稻瘟病菌侵染会促进miR319的积累，进而抑制下游转录因子OsTCP21的表达，削弱茉莉酸合成途径中关键基因*LOX2*和*LOX5*的表达，降低水稻体内茉莉酸含量，最终破坏寄主免疫[113](图2D). miR398是水稻抗病的“同盟军”，过量表达miR398可以增加水稻H_2O_2含量，提高稻瘟病广谱抗性[114].

RNA结合蛋白BSR-K1含有四肽重复结构域，是水稻稻瘟病抗性的负调控因子. *Bsr-k1*的缺失导致苯丙氨酸裂解酶基因*OsPALs*的表达量升高，增强水稻的基础免疫能力，进而提高水稻的稻瘟病广谱抗性[115](图2E).

*R*基因介导的稻瘟病完全抗性，在水稻育种和生产中得到了广泛应用. 但是，稻瘟病菌的快速变异和群体结构的不稳定性，可能导致抗病品种抗性“丢失”. 非*R*基因介导的稻瘟病部分抗性虽然效力相对较弱，但抗谱广、抗性持久稳定. 因此，充分解析二者的分子机制，实现*R*基因和非*R*基因的取长补短，是培育广谱持久抗病水稻品种的关键.

3　水稻产量与稻瘟病抗性的协同调控

通常认为，植物抗性与产量性状之间存在拮抗关系，犹如鱼与熊掌不可兼得. 这主要是因为植物识别病原菌后，会消耗大量能量用于激活免疫系统，导致产量等生理指标遭受不同程度损失. 为解决抗性与产量间的“矛盾”，迫切需要阐明水稻协同调控两种性状的分子机制.

3.1　水稻产量与稻瘟病抗性的拮抗关系

早在19世纪60年代，育种学家就发现，*R*基因的导入虽然可以增强作物的抗病性，但也不同幅度地降低了作物产量[116]，给作物高产抗病育种带来了障碍.

水稻的多数类病斑突变体由于细胞程序化死亡失控，细胞内H_2O_2含量增加，*PR*基因表达量提高，植株对稻瘟病等多数病害表现为广谱抗性[117]. 但这类突变体的生长发育往往存在一定缺陷. 比如*lmm5*，*lmm9150*和*spl4*等稻瘟病抗病突变体都不同程度地出现了生长矮小、早衰、产量低等生长发育受阻的表型[118~120].

一些稻瘟病抗病基因的强表达不利于水稻生长. *OsWRKY45*编码抗病相关转录因子，过量表达*OsWRKY45*可以增强水稻对稻瘟病的抗性，但在一定程度上影响了水稻的生长[89]. *NPR1*是介导植物系统获得性免疫反应的关键调控因子. 水稻中异源表达拟南芥的*NPR1*，可以提高对稻瘟病、白叶枯等多种病害的广谱抗性[121,122]. 然而*NPR1*会过度激活水稻免疫系统，引起类病斑的产生，进而延缓植株发育、降低水稻产量[122]. 为了避免这些抗病相关基因对产量的负面影响，往往需要精确的表达调控机制[123,124]，因而限制了其在育种中的广泛应用.

3.2　水稻产量与稻瘟病抗性的平衡机制

抗性和发育虽存在拮抗关系，但二者在水稻中可协调共存. 围绕产量和稻瘟病抗性的平衡机制研究，近年来取得了突破性进展[125](图3). 稻瘟病持久广谱抗病位点*Pigm*是从谷梅4号中克隆得到的一个*R*基因位点. 该位点对水稻产量没有负面影响，已在生产中得到广泛应用. *Pigm*位点含有多个NLR基因，其中只有两个基因编码功能蛋白：*PigmR*和*PigmS*. PigmR在水稻的叶、茎秆、穗等器官中组成型表达，可以自身形成同源二聚体，发挥广谱抗病功能，但PigmR降低水稻千粒重，导致产量下降. PigmS的表达受RNA介导的DNA甲基化调控，在花粉中特异高表达. 一方面，PigmS可以与PigmR形成异源二聚体，抑制PigmR功能；另一方面，PigmS可以提高水稻结实率，抵消PigmR对产量的负面影响，最终实现水稻高抗的同时不影响产量[34].

转录因子IPA1在平衡水稻稻瘟病抗性与产量之间发挥重要作用. 磷酸化修饰是IPA1调控高产抗病的关键枢纽[126]. 通常情况下，IPA1结合产量相关基因的启动子，促进其表达，调控水稻理想株型的建成与水稻产量. 稻瘟病菌侵染引起IPA1磷酸化. 磷酸化后的IPA1更

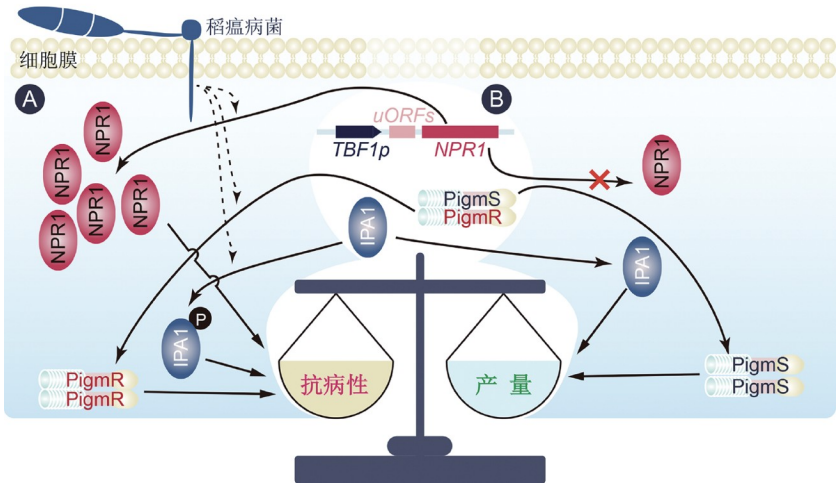

图 3 水稻稻瘟病抗性与产量的平衡. A: 稻瘟病菌侵染, 诱导*NPR1*基因表达、IPA1磷酸化、PigmR二聚体形成, 细胞内抗病反应启动; B: 当没有稻瘟病菌侵染时, *NPR1*基因低表达、IPA1处于非磷酸化状态、PigmR被抑制, 水稻产量稳定

Figure 3 The balance between blast resistance and rice yield. Schematic of the trade-offs between plant growth and immunity mediated by NPR1, IPA1 and Pigm. Upon pathogen (such as *M. oryzae*) attack, the expression of *NPR1*, the phosphorylation of IPA1 and homodimerization of PigmR can be induced, leading to the activation of immune responses (A). However, these changes can be inhibited in the absence of pathogens, thereby promoting rice growth and yield (B)

倾向于结合抗病相关基因*WRKY45*的启动子, 促进其表达, 增强水稻稻瘟病抗性. 一旦水稻体内免疫反应启动, IPA1再通过去磷酸化修饰, 转而促进生长发育和产量相关基因表达, 保障产量, 实现抗病和产量的协同提升[126].

OsALDH2B1通过双重蛋白特性调控水稻生长和抗病. 一方面, OsALDH2B1作为线粒体乙醛脱氢酶调控花粉育性; 另一方面, OsALDH2B1发挥转录因子功能, 调控油菜素内酯、茉莉酸和水杨酸等激素介导的发育和抗病信号途径[127].

miR168-AGO1是最新报道平衡水稻稻瘟病抗性和产量的重要模块. *AGO1*是miR168的靶基因, 是RNA诱导沉默复合体的关键元件. miR168-AGO1模块通过调控miR535, miR164和miR1320等miRNA的形成, 影响这些miRNA所调控的下游抗病和生长发育相关基因的表达. 削弱miR168对*AGO1*表达的抑制, 不仅可以增强水稻对稻瘟病的抗性, 还可以促进水稻分蘖、缩短生育期、提高产量[128]. 通过操纵单个miRNA改良水稻多个重要农艺性状, 在未来作物育种中具有较大的应用空间.

利用基因工程技术控制抗性基因的表达, 同样可达到协调水稻稻瘟病抗性与产量的目的. *uORFTBF1*元件的表达特异地受病原菌诱导. 在水稻中, 将*uORFTBF1*元件与抗病基因*NPR1*融合表达, 可以提高植株对稻瘟病等多种病害的抗病性, 且不影响正常情况下植株的生长[129,130].

高产和抗病是水稻育种的两个主要目标. 因此, 研究作物高产抗病平衡机制对水稻育种至关重要. 从病原菌与宿主的互作机制、抗病基因的调控机制、病原菌与宿主的次生代谢以及营养利用等方面充分探索产量和抗病的关系, 可以为高产抗病品种的培育奠定坚实的理论基础.

4 稻瘟病抗病育种

稻瘟病抗病育种是破解稻瘟病危害的主要手段. 随着生物技术的发展, 越来越多稻瘟病抗病基因被克隆, 基因编辑等先进基因工程技术不断涌现改进, 这都为稻瘟病抗病育种的发展提供了历史契机.

4.1 稻瘟病抗病资源的挖掘与利用

由于病原真菌种群的高度变异和快速进化, 水稻稻瘟病*R*基因介导的抗性通常在3~5年失效. 生产上, 为避免*R*基因在短时期内失效, 往往需要聚合多个*R*基因, 培育广谱持久抗病品种. 非洲持久抗瘟水稻品种Moroberekan已在西非大面积种植多年, 依然具有良好

的稻瘟病广谱抗性, 其主要原因正是该品种聚合有3个主效*R*基因及10个数量性状位点(quantitative trait loci, QTLs). 广东省农业科学院水稻研究所选育的三黄占2号在生产上种植10多年, 仍具有稳定持久的稻瘟病抗性, 其抗性主要由3个主效*R*基因和5个微效基因控制[131]. 长期的生产实践证明, 挖掘稻瘟病抗性资源、克隆*R*基因, 通过分子标记辅助育种, 将多个*R*基因在栽培品种中进行聚合, 是目前控制稻瘟病害最经济有效的策略.

我国稻区分布辽阔, 抗病种质资源丰富, 主要包括病区长期自然选择保留下的高抗水稻材料、野生稻与栽培稻资源中优异的抗稻瘟病资源[132], 它们为水稻抗病基因资源的挖掘与利用提供了重要源泉. 在过去的几十年中, 我国育种学家相继筛选、鉴定、培育了谷梅4号、地谷、明恢63等多个具有广谱或持久稻瘟病抗性的种质资源, 以及一系列具有地方特色的抗病品种[34,131,133].

4.2　新技术与稻瘟病广谱抗病育种

在20世纪60年代, 袁隆平[134]首次发现了天然雄性水稻不育株, 提出了“三系”水稻杂种优势的研究设想, 开启了我国杂交水稻培育的大门. 但由于前期资源匮乏和技术瓶颈限制, 我国自主培育的第一批杂交水稻品种展现出的稻瘟病抗性较弱. 随着近年来我国科技实力的提升, 大量优异的抗病种质资源被挖掘, 为水稻抗病育种奠定了基础[135]. 利用这些资源, 研究人员克隆了大量广谱抗病基因, 解析了相关分子机制, 为稻瘟病抗病品种的定向培育提供了有利工具[136].

传统的杂交育种中, 育种学家主要通过田间表型观察来挑选优异的种质资源和栽培品种, 而这需要花费较长时间和较大工作量. 现有条件下, 稻瘟病抗病品种的培育主要有两种方式, 即聚合多个*R*基因, 或利用单个广谱抗病基因. 聚合*R*基因的育种策略虽可延长品种抗性, 但育种步骤繁琐, 需耗费很长时间和花费高昂代价. 随着分子生物学技术的深入发展, 李家洋团队[137]提出了分子模块设计育种的理念, 提倡根据实际需要, 利用分子标记将多个有利基因导入同一品种, 从而在较短的时间内实现多基因的高效聚合, 培育出高产高抗优质的水稻新品种.

基因编辑技术的出现为水稻稻瘟病广谱抗病育种提供了新的技术平台. CRISPR/Cas9(clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9)系统是近年来发展迅速的基因编辑技术, 在水稻等作物育种中已展现出广阔应用前景[138]. 中国水稻研究所在长粒粳稻恢复系L1014中, 定点编辑了*Pita*, *Pi21*和*ERF922*等稻瘟病抗病基因, 提高了该恢复系的稻瘟病抗性, 丰富了水稻稻瘟病抗性育种的亲本资源[139]. 空育131是我国东北地区的常规稻主栽品种之一, 但该品种在低温和高湿的条件下易感稻瘟病, 对该品种的*Pi21*位点进行定点编辑之后, 显著增强了其稻瘟病抗性[140].

5　问题与展望

近年来, 水稻抗稻瘟病机制的研究取得了突破性进展, 但未来依然有很多亟待挖掘和解析的问题.

5.1　问题探讨

稻瘟病危害依然严重. 通过长期的努力, 稻瘟病防治得到了极大的改善, 但是稻瘟病危害仍是当前制约我国粮食稳产高产的关键因素之一. 据农业农村部的数据统计, 我国稻瘟病发生面积在2014年约为500.0万公顷, 2016年约为533.3万公顷, 2017年在此基础上又增加了约20%[141].

稻瘟病广谱抗病基因资源不足. 目前虽已克隆了多个抗病基因, 但由于我国生态环境复杂, 不同稻区的菌群结构存在较大的差异, 导致多数抗病基因在不同稻区的推广仍具有时限性和不确定性, 而且抗谱相对特异的*R*基因难以单独使用. 与此同时, 稻瘟病菌群存在易变异的特性. 如果在同一区域长期使用单一*R*基因进行抗病育种, 会对稻瘟病菌产生持续的选择压力, 致使稻瘟病菌群结构发生变化产生新的优势无毒基因型, 导致原来有效的*R*基因丧失抗性.

水稻稻瘟病抗病机制仍有许多未解之谜. 在已经克隆的*R*基因中, 一半以上都未在稻瘟病菌中找到对应的无毒基因, 相关的抗性分子机制悬而未决. 有些*R*基因虽然来源于不同的水稻品种, 命名不同, 序列存在一些差异, 但定位区间、抗谱等都极其相似, 存在异名同功的情况, 这给水稻抗稻瘟病机制的研究造成了一定的障碍. PTI和ETI作为水稻抵御外界病害的两道防线, 二者之间的抗病协同机制还有待深入系统的研究.

多数抗病基因的导入会影响水稻产量与质量. 如何“弥补”这种损失, 需要更清晰地解析抗病与代谢水平、营养利用、生物钟、光照适应等生理过程之间的交叉对话机制.

5.2 未来展望

挖掘广谱抗病资源, 以促进新的广谱抗病基因的克隆. 从世界各地广泛收集水稻种质资源, 以及不同生态区的稻瘟病生理小种, 进行系统的抗谱鉴定, 筛选出具有广谱抗性的水稻品种. 利用全基因组测序技术, 将生物信息分析与传统克隆技术结合, 有效避免抗病基因在某些水稻基因组缺失引起的定位障碍.

新老技术结合创制广谱抗病材料, 为解决稻瘟病危害构建精准的育种技术策略. 诱变育种是创制水稻新品种的传统方式之一, 包括物理诱变和化学诱变. 原丰早、浙辐802、浙辐矮等都是通过诱变育种产生的水稻品种. 利用基因编辑技术创造新的抗病资源是未来育种发展的方向之一. 一方面可以进行定向编辑产生新的抗病位点, 另一方面也可以“去掉”感病基因创制新的抗病材料. 在抗病品种创制过程中, 要充分根据不同稻区稻瘟病菌的种群分布特征与变化规律, 以及稻区的生态特点, 有的放矢地对抗病基因加以优化组合, 并通过分子技术操控抗病基因的精确表达, 从而增强水稻品种的抗病持久性.

深入解析水稻抗稻瘟病机制, 为生物防控提供重要的技术支撑. 一方面从基因功能的角度, 解析稻瘟病菌与宿主水稻之间相互识别、相互作用的分子机制, 从而为稻瘟病的防治提供理论支撑. 另一方面从代谢的角度, 解析水稻如何利用自身的次生代谢产物来抵御稻瘟病菌的侵入, 从而为生物农药的开发提供新的方向.

利用抗病机制理论引领传统技术的完善和新技术开发, 必然会加速我国水稻抗稻瘟病研究的步伐, 为国家粮食安全战略提供重要的科技支撑.

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Recent progress on rice resistance to blast disease

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Rice blast disease, caused by the fungal pathogen *Magnaporthe oryzae*, poses severe threat to rice yield and quality worldwide. It is important to identify blast resistance genes from rice germplasms and systematically study molecular mechanisms of rice resistance in order to develop effective methods for preventing rice from this disease. Here, we summarize recent advances in the study of rice blast resistance, including isolation of resistance genes, elucidation of the underlying resistance mechanisms, trade-offs between yield and resistance, and breeding of rice varieties with improved disease resistance. We further discuss the challenges in this research field and provide perspectives for future studies.

rice, rice blast, resistance mechanism, resistant breeding

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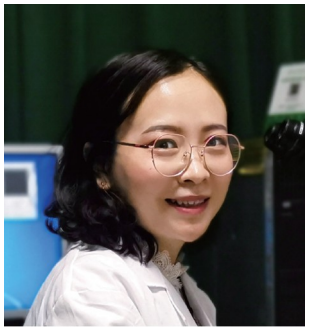

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