第30卷 第1期 华 中 农 业 大 学 学 报 Vol. 30 No. 1
2011年 2月 Journal of Huazhong Agricultural University Feb. 2011,121～123

水稻抗病相关基因的分离克隆和功能鉴定*

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水稻白叶枯病和稻瘟病分别是由白叶枯病菌(*Xanthomonas oryzae* pv. *oryzae*,Xoo)和稻瘟病菌(*Magnaporthe grisea*)引起,是世界水稻生产中的两大重要病害,造成的损失巨大。通过改良水稻自身防御体系来控制病害,是一种既经济又绿色的方法。鉴定水稻抗病相关基因,研究水稻抗病机理对改良水稻有着重要的理论意义和应用前景。

植物激素生长素诱导的信号通常被认为能调节植物的生长和发育。本研究报道的水稻基因*GH*3-8是1个生长素反应基因,在依赖于生长素的发育中发挥功能,同时也调节不依赖于水杨酸和茉莉酸的信号路径的抗病反应。白叶枯病菌诱导水稻至少在被侵染部位合成生长素,而生长素继而诱导水稻大量合成松弛细胞壁的蛋白质——伸展蛋白(α-和β-expansins),破坏细胞壁对病原菌的先天屏障作用,以利病原菌在水稻中生长繁殖。在携带有抗病基因*Xa*21或*Xa*26抗病水稻品种中,病原菌引起的水稻感染部位生长素的合成可诱导水稻快速合成IAA酰胺合成酶GH3-8。GH3-8通过催化IAA-氨基酸的合成抑制生长素的作用,从而阻止细胞壁的松弛,增强植物对病原菌的自身免疫功能。超量表达*GH*3-8基因增强水稻对白叶枯菌的抗性,同时也延缓了植株的生长和发育,至少部分抑制了生长素信号,从而抑制了α-和β-expansins的合成。本研究结果揭示了病原菌利用生长素作为毒性因子侵染水稻的机理以及水稻应对这一毒性因子的调控途径,同时也从一个方面解释了植物在抗病反应中通常要付出生长被抑制的代价的原因。

超量表达*GH*3-8导致植株不育。通过正反交试验显示*GH*3-8超量表达植株是雄性和雌性都不育。通过形态学观察发现,*GH*3-8超量表达植株的雌蕊柱头发育不正常;通过激光共聚焦显微镜对雌蕊胚囊观察发现,*GH*3-8超量表达植株的成熟胚囊发育不正常,这可能是*GH*3-8超量表达植株雌性不育的原因。通过形态学观察发现,*GH*3-8超量表达植株的雄蕊和野生型无异,但花粉碘染显示,*GH*3-8超量表达植株大部分花粉都败育,这可能是*GH*3-8超量表达植株雄性不育的原因。通过分析*GH*3-8基因表达模式,显示*GH*3-8基因特异在雄蕊高表达,并随着花的发育表达强弱也不断变化,而在雌蕊基本检测不出表达。组织和时间的特异表达也印证了*GH*3-8在调控花的发育中起作用。利用酵母单杂交技术,筛选得到和*GH*3-8基因启动子互作的几个生长素反应因子。其中*OsARF*8超量表达激活*GH*3-8基因的表达,证明*OsARF*8是调控*GH*3-8基因表达的转录因子。通过分析*OsARF*8基因表达模式,显示*OsARF*8基因特异地在雌蕊高表达,而在雄蕊表达很低。*OsARF*8基因超量表达植株和野生型植株相比育性下降。花粉碘染显示*OsARF*8基因超量表达植株大部分花粉败育;检测雌蕊没有发现和野生型有显著差异。花粉的育性下降可能是*OsARF*8超量表达植株育性下降的原因。生长素信号路径中的基因(*OsARF*8和*GH*3-8)的不正常表达影响了水稻育性,说明生长素信号可能在调控水稻育性中有重要作用。检测水稻穗发育中生长素分布,也显示生长素和穗的发育密切相关。

在水稻品种明恢63中抑制1个维生素B1合成基因*OsDR*8的表达,显著提高了转基因植株对白叶枯病和稻瘟病的敏感。外源应用维生素B1可以互补抑制*OsDR*8基因对水稻植株抗病的影响。几个防御反应基因包括防御信号路径的早期功能基因*OsPOX*和*OsPAL*基因以及路径下游基因*OsPR*1*a*、*OsPR*1*b*、*OsPR*4、*OsPR*5和*OsPR*10的表达

收稿日期:2010-12-03
* 2010年度全国百篇优秀博士学位论文(指导老师:王石平)
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在 *OsDR*8 抑制表达的植株中下降。这些结果说明 *OsDR*8 影响植株的抗性可能是通过影响防御反应基因的表达，*OsDR*8 的功能在信号路径的上游。另外，维生素 B_1 的积累可能是水稻植株对白叶枯病和稻瘟病的抗性所必需的。

通过筛选水稻 T-DNA 插入突变体库，发现 1 个类病斑突变体。侧翼序列分析显示 T-DNA 插在 1 个基因（命名为 *OsDR*9）的开放读码框。预测 *OsDR*9 基因编码由 180 个氨基酸组成的功能未知蛋白。*OsDR*9 基因在茎和幼穗中表达很低，而在幼苗、剑叶、叶鞘和愈伤表达较高，在根中没有检测到表达。另外 *OsDR*9 基因在老叶中比新叶表达更高。突变体对稻瘟病和胡麻叶斑病表现高抗。对有类病斑的叶片进行组织化学检测和 DNA 断裂分析显示细胞死亡具有凋亡特征。病程相关蛋白 PR4 和 PR8 以及稻瘟病相关基因 *AOS*2 在突变体中上升表达。突变体植株也积累了自发荧光物质、SA、JA 和植保素（momilactone A 和 sakuranetin）。突变体提高了超氧化物和 H_2O_2 的水平。将 1 个来源于水稻品种日本晴的包含有 *OsDR*9 基因的 10.5 kb 片段转入突变体 02Z15AM37，转基因植株类病斑表型消失，说明由于 T-DNA 插入导致的 *OsDR*9 突变是引起类病斑表型的原因。这些结果说明，*OsDR*9 是水稻抗病和细胞凋亡的 1 个负调节子。

关键词 水稻；白叶枯病；稻瘟病；抗病相关基因；生长素；水杨酸；茉莉酸；基础抗性；伸展蛋白；GH3-8；维生系 B1；类病斑突变体；凋亡

Isolation and functional characterization of pathogen-induced defense-responsive genes of rice

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Rice bacterial blight disease caused by *Xanthomonas oryza* pv. *oryza* and fungal blast disease caused by *Magnaporthe grisea*, are two of the most devastating diseases of rice worldwide. These two diseases can lead to tremendous yield loss every year. Efficient control of disease through improving rice defense system is economic and environment friendly. Characterizing rice disease resistance related genes and elucidating the mechanism of rice disease resistance are important both in scientific theory and in rice improvement.

The signaling induced by the plant growth hormoneauxin is generally recognized to regulate plant growth and development. Here we report rice *GH*3-8, an auxin-responsive gene functioned in auxin-dependent development, activates disease resistance in a salicylic acid - and jasmonic acid - signaling-independent pathway. Bacteria induce accumulation of indole-3-acetic acid (IAA), the major type of auxin in rice. IAA induces the expression of α- and β-expansins, the proteins that are known to loose cell wall, the native barrier of biotic intruder, to facilitate the growth of cells. In rice resistance variety carrying *Xa*21 or *Xa*26, the infection of bacteria induce rice to synthesize GH3-8 in infection site of rice. *GH*3-8 encodes an IAA-amino synthetase that prevents free IAA accumulation and looseness of cell wall. Overexpression of *GH*3-8 enhanced disease resistance and delayed growth and development, which is partly due to inhibiting the expression of α- and β-expansins via suppressing auxin signaling. Here we show the mechanism of bacteria hijacks auxin as virulence factor to infect rice, and the regulating pathway of rice to the virulence factor; in addition to, explain the cause that plants growth was restrained in disease resistance.

Overexpression of *GH*3-8 results in sterility of plants. Analysis of forward and reverse cross showed that *GH*3-8-overexpressing plants were male sterility and female sterility. We found that the stigmas of *GH*3-8-overexpressing plants are abnormal by morphological observation. We observed the mature embryo sac of *GH*3-8-overexpressing plants using laser scanning confocal microscopy. The result showed

that the mature embryo sacs of *GH*3-8-overexpressing plants were abnormal. This may be the reason of female sterility. No obvious difference was observed in stamen between *GH*3-8-overexpressing plants and wide-type plant, but the most of pollen of *GH*3-8-overexpressing plants were sterile. This may be the reason of male sterility. *GH*3-8 had high expression level in stamen, and the expression of *GH*3-8 changes as the development of flower. Tissue and time differential expression confirmed the role of *GH*3-8 in regulating flower development. We identified several auxin responsive factors (ARFs) that interacted with the promoter of *GH*3-8 by analysis of yeast one hybrid. Overexpression of *OsARF*8 in Mudanjiang 8 activated the expression of *GH*3-8. This result suggested that *OsARF*8 is the transcription factor in regulating the expression of *GH*3-8. *OsARF*8 expressed highly in pistil, but lowly in stamen. Fertility of *GH*3-8-overexpressing plants was lower than that of wide type. The most of pollen of *OsARF*8-overexpressing plants were sterile. The overexpression of auxin signaling genes (*OsARF*8 and *GH*3-8) resulted in decrease of rice fertility. This result suggested that auxin plays a critical role in regulating flower development. The detection of the auxin distribution in panicle development showed that auxin is affinitively related with panicle development.

The transgenic plants with repressed expression of *OsDR*8 showed reduced resistance or susceptibility to *Xanthomonas oryzae* pv. *oryzae* and *Magnaporthe grisea* causing bacterial blight and blast, respectively. The putative product of *OsDR*8 was highly homologous to an enzyme involved in the biosynthesis of the thiazole precursor of thiamine. Exogenous application of thiamine could complement the compromised defense of the *OsDR*8-silenced plants. The expression level of several defense-responsive genes including the earlier function genes of defense transduction pathway, *OsPOX* and *OsPAL*, and the downstream genes of the pathway, *OsPR*1*a*, *OsPR*1*b*, *OsPR*4, *OsPR*5 and *OsPR*10, was also decreased in the *OsDR*8-silenced plants. These results suggest that the influence of *OsDR*8 on disease resistance in rice may be through the regulation of expression of other defense-responsive genes and the site of OsDR8 function is on the upstream of the signal transduction pathway. In addition, the accumulation of thiamine may be essential for bacterial blight resistance and blast resistance.

A mutant with lesion mimics on the leaves was found through screening a rice T-DNA inserted pool. The T-DNA was inserted into the open reading frame (ORF) of a gene named *OsDR*9. The predicted encoding product of *OsDR*9 consists of 180 amino acids with unknown function. *OsDR*9 had very low expression level in stem and young panicle but higher level in seedling, flag leaf, sheath and callus; no *OsDR*9 expression was detected in the root. In addition, *OsDR*9 had higher expression level in old leaf than young leaf. The mutant was highly resistant to *Magnaporthe grisea* causing fungal blast disease and *Bipolaris oryzae* causing *Cochliobolus miyabeanus* disease in field. Histochemical detection and DNA fragmentation of the leaves developed lesion mimics showed that the cell death had the same features of apoptosis. In addition, the expression of pathogenesis related (PR) proteins genes *PR*4 and *PR*8 as well as a blast resistance related gene *AOS*2 was upregulated in the mutant. The mutant also accumulated autofluorescent materials, salicylic acid and phytoalexins (both momilactone A and sakuranetin). The mutant contained elevated levels of superoxide and H_2O_2. A 10.5-kb fragment harboring the *OsDR*9 gene from rice variety Nipponbare was transferred into the mutant. Lesion mimic phenotype was disappeared in the transgenic plants, indicating that knockout of *OsDR*9 by T-DNA insertion caused the lesion mimic mutant phenotype. These results suggest that OsDR9 is a negative regulator in rice disease resistance and apoptosis.

Key words　*Oryza sativa*; bacterial blight; fungal blast; disease resistance related genes; auxin; salicylic acid (SA); jasmonic acid (JA); basal resistance; development; expansin; GH3-8; thiamine; lesion mimic mutant (LMM); apoptosis

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