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水稻白叶枯病隐性抗病基因*xa*13的分离与鉴定的研究*

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植物抗病基因的研究一直以来是植物分子生物学研究领域的热点之一，迄今已经从不同的宿主中分离了50多个抗病基因，其中仅有6个是隐性基因，这些隐性抗病基因的作用机理目前尚不能用广为接受的显性基因的作用机理来进行解释。水稻中已经鉴定32个主效抗白叶枯病基因，其中9个为隐性抗病基因。位于第8染色体长臂末端的*xa*13基因就是其中的1个典型，它完全隐性、特异性地抗白叶枯病菌菲律宾菌系生理小种6(PXO99)、与其他显性或隐性抗白叶枯病基因存在抗性互作，说明*xa*13基因具有特异性抗性。

为克隆*xa*13基因，采用基因组饱和杂交的原理，在9个发育时期取材构建了水稻品种明恢63全生育期均一化cDNA文库。以分子标记E6a和另一端标记S14003为起始，构建了*xa*13基因区段的物理图谱，并结合3个F_2群体和Shotgun测序结果将该基因定位于1个9.2 kb的DNA片段上。同时通过精细物理定位*xa*13基因，开发了数个与该基因紧密连锁的基于PCR技术的分子标记，为采用分子标记辅助选择*xa*13育种提供了经济、方便的分子标记。

在优化了一套适合于水稻品种IRBB13的转基因组培体系的基础上，通过农杆菌介导的遗传转化方法，将携带显性候选基因的片段转入到携带隐性抗病基因的水稻品种IRBB13中，在45株转基因植株后代中，有全长基因导入的30株的表型变为感病，并且4个单拷贝转基因的T_1代家系也符合共分离检测，从而验证了候选基因是*xa*13基因的显性基因*Xa*13，用图位克隆法成功克隆了隐性基因*xa*13和显性基因*Xa*13。同时，通过RNA干扰(RNAi)技术，分别抑制水稻品种明恢63和中花11中显性基因*Xa*13的表达，以及水稻品系IRBB13中隐性基因的表达。通过接种白叶枯病菌PXO99和定量RT-PCR分析发现，明恢63和中花11中*Xa*13基因表达受到抑制的植株表型由感病变为抗病，IRBB13中*xa*13基因表达受到抑制的植株抗性进一步加强。

有趣的是，显性基因*Xa*13和隐性基因*xa*13在花药中的表达量高于在叶片中的表达量。RNAi转基因植株中*Xa*13或*xa*13的表达受到抑制会导致植株不育或半不育，组织学观察发现这些不育单株的多数花粉的发育停留在单核花粉期或二核花粉期。原位杂交的试验证明，*Xa*13基因在花药中表达的时期与异常花粉发生的时期非常一致，*Xa*13在花粉发育的早期不表达，在单核花粉期开始高水平表达。从而说明*Xa*13基因在花粉的发育中也起着重要的作用。

进一步通过大量试验表明，显性基因*Xa*13是1个受病原调控表达的基因，由显性基因*Xa*13突变成隐性基因*xa*13的关键不是编码区内的变化，而是在启动子区的1个约18 bp的区间内的突变使基因表达受病原调控的能力丧失，且这种突变不影响其在花粉中的表达。XA13蛋白是1个未知具体生化功能的细胞膜蛋白，属于MtN3家族的成员之一。但其在白叶枯病菌PXO99侵染以及在花粉的发育中发挥着重要的功能，且可能介导新的抗病途径。

因此，本研究通过克隆隐性基因*xa*13以及它

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的显性基因 *Xa*13，不仅揭示了 1 种新的植物抗病机制：即抑制抗病基因的表达是高效抗病的基础，而且为研究植物抗病和花粉发育的关系提供了一个新的起点。

关键词　水稻；白叶枯病；黄单胞杆菌；抗病基因；cDNA 文库；图位克隆

Isolation and characterization of a recessive resistance gene, *xa*13, for bacterial blight in rice

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Studies on plant resistant genes are one of the hotspots in the field of plant molecular biology. Until now, among the over 50 isolated resistance genes, only 6 are recessive genes. The mechanisms of recessive genes can not be explained by those of dominant resistant genes, which have been universally accepted. Bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is one of the most serious diseases of rice worldwide. Thirty-two bacterial blight resistance genes (23 dominant genes and 9 recessive genes) in rice have been identified. However the *xa*13, located on the distal end of the long arm of rice chromosome 8, was a typical recessive *R* gene, which is fully recessive and specifically confers resistance to the Philippine *Xoo* race 6 (PXO99). The *xa*13 gene can interact with other dominant or recessive *R* genes, indicated that the disease resistant mechanism of *xa*13 was special.

To clone *xa*13 gene, a normalized whole-life-cycle cDNA library was firstly constructed with different tissues from 9 developmental stages using rice cultivar Minghui 63, based on the strategy of saturation hybridization with genomic DNA. Then a physical map covering the region flanked by markers E6a and S14003 was constructed by chromosome walking using Minghui 63 BAC clones. And finally *xa*13 was fine-mapped to a DNA fragment of 9.2 kb using a series of sequence-based molecular markers. Sequence analysis indicated that this region contain only one candidate gene, which was annotated by full length EST sequence that isolated from the constructed cDNA library.

Transforming IRBB13 with the DNA fragment encompassing the *Xa*13 candidate that based on the optimized tissue cultural system, including the promoter region isolated from IR64, produced 45 independent transformants. Thirty of the 45 T_0 transgenic plants were susceptible upon infection by PXO99. PCR analysis demonstrated that all of the susceptible transgenic plants had the band derived from the *Xa*13-candidate fragment, while none of the resistant plants amplified this band. T_1 families derived from four of the single-copy T_0 transgenic plants were further investigated. And the susceptibility cosegregated perfectly with the transgene in all four families, indicated that the clone of *xa*13 gene through map based cloning strategy has succeed, and the candidate gene in this fragment was indeed *Xa*13, and the allelic gene in recessive parent was *xa*13. At the same time, the expression of *Xa*13 or *xa*13 was suppressed in different rice lines using the RNA interference (RNAi) strategy. It was found that suppression of *Xa*13 expression in rice line Zhonghua 11 and MH63 significantly increased the resistance to PXO99, and the level of increased resistance was associated with the reduced accumulation of *Xa*13 transcripts. Suppressing the expression of *xa*13 in rice line IRBB13 further enhanced *xa*13-mediated resistance.

Interestingly, the expression of *Xa*13 or *xa*13 was very low in leaves but quite high in panicles and anther. And it was found that some RNAi transgenic plants with significantly reduced expression of *xa*13

or *Xa*13 had reduced spikelet fertility. The reduced fertility could be ascribed to male sterility as the *xa*13- and *Xa*13-suppressed plants had smaller anthers than its wild types and produced mostly abortive pollen. Histological analysis showed that the development of the most of microspores stopped at the unicellular pollen grain stage or bicellular pollen grain stage and gradually degenerated afterwards in those transgenic plants. Coincided with the development stage of abnormal pollen appearance, the expression of *Xa*13 in anther was identified by *in situ* hybridization. Results showed that no transcripts were detectable at early stage and *Xa*13 transcripts accumulated to high level at unicellular pollen grain stage. The coincidence in time between the accumulation of *Xa*13-transcripts in wild type plants and the appearance of pollen abortion in *Xa*13-suppressed plants during pollen development suggests an indispensable role of *Xa*13 in pollen development.

Further researches showed that the expression of *Xa*13 in leaves was modulated by pathogen inoculation. The core mutation resulted in loss-of those functions in *xa*13 genes may occur at a 18 bp region in the promoter region, rather than on gene coding region. And this mutation wouldn't influence the expression of *xa*13 in anther. Xa13 is a unknown function plasma membrane protein, belonging to the MtN3 gene family. It acts as important functions both during in the process of PXO99 aggression and anther development. Therefore it would mediate a new pathway of resistance independent from those by activated expression of pathogenesis-related genes or by increased thicken of secondary cell-wall in vascular bundle element of leaves. Above all, the cloned *xa*13 and its dominant allele *Xa*13 gene in this study, would not only display a new resistance mechanism: suppressing the expression level of resistance gene is the foundation of plant resistant to pathogen with high efficiency, but aslo provide a new jumping-off point for the study on the relationship between resistance to disease and anther development in plant.

Key words rice; bacterial blight; *Xanthomonas*; resistance gene; cDNA library; map based cloning

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