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小麦抗病基因类似序列*BRG1*的分离与功能分析

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Identification and Functional Analysis of a Wheat Resistance Analogous Gene *BRG1*

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摘要 利用cDNA-AFLP技术筛选到1个在抗黄矮病的小麦-中间偃麦草易位系YW642中特异表达的小麦抗病基因类似序列(BYDV resistance-related gene1, *BRG1*)的基因片段。利用cDNA末端快速扩增技术(RACE)和RT-PCR技术，从YW642中分离出*BRG1*基因全长cDNA序列，获得了一个通读的抗病同源基因cDNA序列，编码由645个氨基酸组成的蛋白质，包含1个NB-ARC保守结构域和3个LRR结构域，该蛋白属于nucleotide-site binding, leucine-rich repeats (NBS-LRR)家族。荧光定量或半定量RT-PCR表达分析表明，*BRG1*在抗病小麦易位系YW642叶片中优势表达，受BYDV的诱导，BYDV接种后48 h表达量最高，*BRG1*在感病小麦亲本中8601中表达量始终较低，随BYDV接种时间延长呈轻微的下调趋势；而且外源激素水杨酸(SA)与茉莉酸(JA)处理可上调该基因在YW642中的表达。利用病毒介导的基因沉默技术分析了*BRG1*基因的功能，结果表明该基因沉默的抗病小麦易位系YW642中BYDV相对含量增加，但未造成抗病性表型显著改变，说明该基因参与抗黄矮病反应，但不是小麦抗黄矮病重要基因。

关键词： 小麦-中间偃麦草易位系 小麦黄矮病抗性 cDNA-AFLP 抗病基因类似序列 病毒介导的基因沉默

Abstract: Barley yellow dwarf virus (BYDV) can cause wheat yellow dwarf. In this study, we isolated a fragment of a wheat resistance analogous gene, tentatively named BYDV response gene1 (*BRG1*),which was expressed in the BYDV resistant wheat-*Thinopyrum intermedium* translocation line YW642 but was not expressed in the BYDV susceptible wheat Zhong 8601 by using cDNA-AFLP technique.The full-length cDNA sequence of the gene *BRG1* was obtained by rapid amplification of cDNA end (RACE) and RT-PCR methods. The gene *BRG1* encodes a NBS-LRR protein consisting of 645 amino acid residues，which possesses one typical NB-ARC domain and three leucine-rich domains. The result of expression analysis by Q-RT-PCR method indicated that the expression of *BRG1* gene was predominant in the BYDV resistant translocation line YW642 and induced by BYDV infection, reached the peak at 48 hour post inoculation with BYDV, whereas the express level of the gene in the susceptible wheat parent Zhong8601 was lower than that in the resistant wheat YW642, and showed a decline tendency with BYDV infection time. The mRNA expressionof *BRG1* gene in YW642 wasup-regulated by salicylic acid (SA) and jasmonate (JA). Virus induced gene silencing technique was used to conduct functional analysis on the gene *BRG1*. The results showed that after BYDV infection, BYDV relative content in *BRG1* knocked-down YW642 was higher than that in YW642 expressing *BRG1* gene, whereas the silenced *BRG1* gene did not obviously alter the plant phenotype to BYDV infection, suggesting that the gene *BRG1* may be involved in the host response to BYDV infection but not be an important gene.

Keywords: Wheat-Thinopyrum intermedium translocation line Resistance to barley yellow dwarf virus (BYDV) cDNA-AFLP Virus-induced gene silencing

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