作物学报 2004 30 (3): 189-195 ISSN: 0496-3490 CN: 11-1809/S

研究论文

利用中间偃麦草抗病基因同源序列分离黄矮病抗性候选基因克隆

张增艳,许景升,刘耀光,王晓萍,林志珊,辛志勇

中国农业科学院作物育种栽培研究所，农业部作物遗传育种重点实验室，北京100081

收稿日期 2003-2-26 修回日期 2003-6-26 网络版发布日期 接受日期

摘要 根据已克隆植物抗病（R）基因编码蛋白质的保守结构设计简并引物，利用同源序列法PCR扩增、克隆到9个具有开放阅读框的中间偃麦草R基因同源片段（Resistance Gene Analogs，RGAs）。利用抗黄矮病材料(含Bdv2)、感黄矮病材料（无Bdv2）进行RFLP分析，筛选到1个NBS类RGA序列TirgaZ1与Bdv2连锁。根据TirgaZ1的序列重新设计1对引物，优化PCR扩增条件，将其转化为经典特异PCR标记（SC-TZ1）。利用该特异PCR标记（SC-TZ1）和克隆池-PCR法筛选抗黄矮病小麦-中间偃草易位系HW642基因组的可转化人工染色体（Transformation-competent Artificial Chromosome，TAC）文库，分离到4个阳性TAC克隆T1～T4。限制酶切图谱分析结果表明，T1～T3为1类、插入片段约23 kb，T4为另1类、插入片段约为25 kb。以TirgaZ1为探针，通过Southern杂交证实了阳性TAC克隆T1、T4为含有TirgaZ1序列的抗病基因候选克隆。分别以中间偃麦草、HW642和小麦亲本为探针对阳性克隆T1、T4进行Southern分析，结果表明，阳性TAC克隆T1、T4的插入片段均具有抗黄矮病易位系的中间偃麦草易位染色体片段7XL，T1、T4为抗黄矮病基因候选克隆。测定和分析阳性克隆T1插入片段5′端－6448 bp部分的序列，表明其最长完整开放阅读框(Open Reading Frame, ORF)为2675 bp,其编码产物具有信号肽、低复杂性结构、NB-ARC、跨膜域等结构，与已克隆的NBS-LRR类抗病基因RPP13、I2C等具有同源性。抗黄矮病基因候选克隆T1、T4的生物学功能有待通过转化、抗病鉴定进行验证。

关键词 中间偃麦草 黄矮病抗性 抗病基因同源序列 可转化人工染色体（TAC） 克隆池PCR

分类号 Q78

Isolation of Resistance Gene Candidates by a Resistance Gene Analog of Thinopyrum intermedium and Pooled-PCR

ZHANG Zeng-Yan,XU Jing-Sheng,LIU Yao-Guang,WANG Xiao-Ping,LIN Zhi-Shan,XIN Zhi-Yong

Key Lab of Crop Genetics and Breeding，Ministry of Agriculture，Institute of Crop Breeding and Cultivation，CAAS，Beijing 100081

Abstract Based on the known R gene conserved domains, 10 pairs of degenerate primers were synthesized and used to amplify genomic DNAs of Th. intermedium and translocation line HW642. The PCR products of Th. intermedium were excised and cloned. Clones were sequenced and compared to sequence homolog with gene databases in Genbank by Blastx and blastn. Nine resistance gene analogs (RGAs) of Th. intermedium with ORF were obtained. Using the RGAs as probes，results of RFLP analyses indicated that 1 RGA of TirgaZ1 was associated with Bdv2 gene. Based on the sequence of TirgaZ1, a pair of specific PCR primers of TZ1U and TZ1L were designed, synthesized and could amplify only one band present in the resistance materials with Bdv2 but absent in the susceptible materials without Bdv2. According to the pooled-PCR protocol, the primers of TZ1U and TZ1L were used to screen the genomic DNA TAC library of HW642. By three rounds of screening， four positive TAC clones of T1, T2, T3 and T4 were isolated from the library. By restriction enzymes analysis, four positive clones T1-T4 were classified into two categories, of which T1, T2 and T3 belonged to one group with the insert about 23kb, and T4 belonged to another with the insert about 25kb. The clones T1 and T4 were further confirmed to be resistance candidates for Bdv2 by Southern analysis with probes of TrgaZ1, genomic DNA of Th. intermedium and of Zhong8601 respectively.

Key words Thinopyrum intermedium； resistance to barley yellow dwarf virus；resistance gene analog（RGA）； transformation-competent artificial chromosome（TAC）； pooled-PCR

DOI:

通讯作者 张增艳 zyzh-68@163.com