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黄萎病不同抗性陆地棉品种抗病基因同源序列生物信息学分析

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Bioinformatics Analysis of NBS Type Resistance Gene Analogs in Different Upland Cotton Varieties Resistant to Verticillium Wilt

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摘要 根据已知抗病基因NBS(Nucleotide-binding sites)保守区的P-loop和GLPL区设计一对简并引物F1/R1， 以已鉴定黄萎病抗性的9个陆地棉品种基因组DNA为模板进行PCR扩增。在9个品种中均扩增出500 bp左右的条带。对目的条带进行回收，连接、转化克隆得到350个阳性克隆，进行测序。在8个棉花品种中克隆到74条具有完整开放读码框的棉花RGAs序列。这74条序列共有64种不同的基因型，有10条与其它品种中的RGAs序列相同。用MEGA软件对8个棉花品种的74条RGA序列以及12个已知的抗病基因的NBS区域进行聚类分析，结果分为TIR和nonTIR两大类，而nonTIR类又细分为Ⅰ、Ⅱ、Ⅲ 3类。4类RGAs之间的氨基酸序列相似性较低，而各大类之内来自不同品种的RGAs的相似度却非常高。推测各大类中相似性非常高的这部分序列分别属于同一个基因家族，从位点上说可能处于同一个基因簇。

关键词： 棉花 抗病基因同源序列(RGA) 核苷酸结合位点(NBS) 生物信息学分析 多态性

Abstract： A specific fragment of about 500 bp was cloned from the genomic DNA of the nine identified cultivars of upland cotton(*Gossypium hirsutum*) by PCR degenerate primer designed according to the conserved domains P-loop and GLPL in the NBS(nucleotide-binding sites) region of reported R genes. The fragments are recycled and inserted into pGM-T vector, and then transformed into *E. coli* DH5α. 350 recombination bands were obtained through cloning and restriction digestion identification. The clones are sequenced and 74 RGAs with complete open reading frames (ORF) from eight varieties of cotton are finally obtained. Comparing with the reported R genes, the 74 RGAs all contain P-loop (Kinase1a), Kinase2, GLPL region, and motif RNBS-A, B, C defined by Meyers. Cluster analysis of their putative amino acid shows that the RGAs could be sorted into two distinct types, TIR-NBS type and nonTIR-NBS type, and nonTIR-NBS could be subdivided into three types, Ⅰ, Ⅱ, Ⅲ. The TIR type RGAs all contain the RNBS-A-TIR motif, while the nonTIR type RGAs all contain RNBS-A-nonTIR motif. This result consists with the early report that NBS-LRR gene has two major groups in dicotyledon. The deduced amino acid of the RGAs have high similarity with the reported R genes. NonTIR type RGAs have a similarity of 32%～50% with *L6*, *M*, *Gro*1-4, N, while TIR type RGAs have a similarity of 23.2%～56.5% with *I2C*-1, *Mi*-1.1, *RPM*1, *RPP*8, *RPS*2, *RPS*5, *XA*1, *Prf*. The similarity among deduced amino acids of the different type RGAs is lower, but that among deduced amino acids of the same type RGAs is very high, from which we deduced that the RGAs with high similarity belong to the same gene family, and maybe locate at the same gene cluster.

Keywords： Gossypium hirsutum resistance gene analog(RGA) nucleotide-binding sites(NBS) analysis polymorphism

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