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研究论文

应用GISH与STS标记鉴定小麦-中间偃麦草抗黄矮病端体系

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摘要 由大麦黄矮病毒引起的小麦黄矮病毒病是一个严重病害，至今在小麦属内还没有发现抗源。中间偃麦草2Ai-2染色体携带一个高抗黄矮病基因，对该基因的染色体臂定位将为制定抗病基因向小麦转移策略，筛选、开发特定的、与抗性连锁的分子标记的研究提供重要信息。本文对由小麦-中间偃麦草二体附加系Z6衍生的3个抗黄矮病端体系进行鉴定，通过分析端体的遗传构成、筛选与端体共分离的STS标记以确定端体在遗传上的染色体臂归属，从而明确BYDV抗病基因的染色体位置。以拟鹅冠草基因组[*Pseudoroegneria strigosa* (M. Bieb.) Löve，St]DNA为探针，中国春基因组(*Triticum aestivum* L., ABD) DNA作封阻分别对抗病亲本Z6及抗病端体系N530的根尖体细胞染色体进行原位杂交，结果表明，N530体细胞中有2个端体显示出与Z6中外源染色体2Ai-2短臂相似，而与长臂不同的杂交信号。以小麦第2同源群的5个RFLP探针的DNA序列为基础，设计了6对PCR引物，对小麦-中间偃麦草二体异附加系、二体代换系和端体系进行扩增，结果表明，基于短臂探针psr_{126}，psr_{131}序列设计的2对引物，可在含有2Ai-2染色体及端体的抗黄矮病材料中特异扩增，而基于长臂探针psr_{112}序列设计的1对引物，可在含有2Ai-2染色体的抗黄矮病材料中特异扩增，但不能在端体系进行特异扩增，证明外源端体为2Ai-2染色体的短臂。本研究不仅将黄矮病抗性基因定位于2Ai-2染色体的短臂上，而且由RFLP探针psr126、psr_{131}和psr_{112}序列转化的标记STS_{126} (sequence tagged site) STS_{131}和STS_{112}还可分别作为追踪2Ai-2染色体短臂和长臂的分子标记，用于抗病易位系辅助选择。

关键词 端体系 基因组原位杂交(GISH) STS标记 小麦黄矮病 中间偃麦草

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Identification of Wheat-*Thinopyrum intermedium* Telosomic Lines Resistant to Barley Yellow Dwarf Virus by GISH and STS Markers Converted from RFLP

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Abstract Wheat yellow dwarf virus caused by barley yellow dwarf virus (BYDV) is one of the most serious diseases in wheat, and no resistant resource has been found in all wheat germplasms tested so far. According to our previous study, Wheat-Thinopyrum intermedium amphiploid Zhong 5 and its three derivatives, disomic addition lines Z1, Z2 and Z6, as well as three ditelosomic lines N523, N530 and N545 derived from Z6 showed high level resistance to BYDV and the resistance is associated with the alien chromosome of 2Ai-2. The objectives of this study were: i) to analyze the genetic composition of the telosome; ii) to screen and/or develop molecular markers co-segregated with the telosome; and therefore, iii) to determine the arm to which the telosome genetically belongs and consequently the location on which the BYDV resistance gene(s) and located. By using Pseudoroegneria.strigosa (St) genomic DNA as probe and Triticum astivum (ABD) genomic DNA as blocker, the results of GISH showed that two telosomes in line N530 were similar to the short arm but quite different from the long arm of 2Ai-2 chromosomes in Z6. Based on DNA sequences of five RFLP probes of chromosome homoeologous group 2, six PCR primers were designed and used to develop molecular markers. One of the primers, STS126, which was based on the sequence of psr126 from the short arm, showed that an about 730bp fragment was amplified specifically in BYDV resistant addition lines, substitution lines and ditelosomic lines but didn’t in wheat parents. It accordingly supported the GISH result that the telosome was the short arm of 2Ai-2 chromosome. In addition, the primer amplified three fragments in wheat parent which were determined by chromosome 2AS, 2BS and 2DS, respectively, according to the PCR patterns of different substitutions. The result of the tolesome being the short arm of 2Ai-2 chromosome was further confirmed by evidences from other two primers of STS131 and STS112. Primer STS131 from short arm of group 2 amplified an alien chromosome-specific fragment which present in all alien addition lines and substitution lines including disomic and ditelosomic lines, while p

rimer STS112 from long arm of group 2 amplified an alien chromosome-specific fragment only in alien disomic addition and substitution lines, but neither in alien ditelosomic lines nor in wheat parents. This study not only localized the resistant gen e on the short arm of 2Ai-2 chromosomes, but also successfully converted three RFLP probes into STS markers, named as STS126, STS131 and STS112. They could be used to track the different arms of 2Ai-2 chromosome and would facilitate the screening for the translocation lines with BYDV resistance effectively.

Key words Telosomic lines Genomic in situ hybridization (GISH) Sequence tagged site (STS) Whe at yellow dwarf virus (BYDV) Thinopyrum intermedium

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