Physical mapping of a powdery mildew resistance related gene *Hv-S/TPK* by FISH with a TAC clone in wheat

Yang XM[1,3], Sun YL[1], Cao AZ[1], Xing LP[1], Cheng ZK[2], Ma HX[3], Yao JB[3], Chen PD[1*]

[1]Agricultural College, Nanjing Agricultural University, Nanjing, Jiangsu210095, China.

[2]Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing100101,China.

[3]Institute of Agricultural Biotechnology, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu210014, China

**Author for correspondence*

ABSTRACT

Powdery mildew, caused by *Erysiphe graminis* DC.f. sp. *Tritic* Marchl., is one of the most serious diseases for wheat production worldwide. *Pm21* is a broad-spectrum resistance gene to powdery mildew and has been located on the short arm of the chromosome 6V of *Haynaldia villosa* L (2n=2x=14, VV). In the previous study, a resistance related gene, *Hv-S/TPK* (serine/threonine protein kinase), has been cloned and showed enhanced resistance to powdery mildew when over-expressed in the transgenic common wheat. A TAC library was constructed using wheat-*H.villosa* translocation line 6VS/6AL. Screening the library with a gene-based marker specific to the gene *Hv-S/TPK* identified a positive clone, TAC15. Sequence analysis of TAC15 confirmed that it contained *Hv-S/TPK* gene. In this study, TAC15 was used to determine the physical location of the *Hv-S/TPK* gene by sequential fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH). The result indicated that TAC15 detected a single locus on 6VS. The FL values of this locus varied between 0.566 to 0.587 when assessed using different genetic stocks including 6Vaddition line, 6V(6A) substitution line and 6VS/6AL translocation lines.

INTRODUCTION

Wheat powdery mildew disease, caused by *Erysiphe graminis* DC, is one of the most serious diseases for wheat production worldwide. Identifying, localizing, and cloning more resistant genes conferring powdery mildew resistance are becoming more and more important. *Pm21,* a gene located on chromosome arm 6VS of *H. villosa*, confers high level of resistance to all known races of *E. graminis*[1,2]. A serine/threonine kinase gene (or *Hv-S/TPK*), which is up-regulated by *E. graminis* inoculation, was cloned by using barley genechip in the Cytogenetics Institute, Nanjing Agricultural University[3]. This gene was located on the short arm of chromosme 6V by PCR technique[4]. To study the relationship of this gene with *Pm21*, more precise location of this gene on 6VS should be conducted.

In plant, fluorescence *in situ* hybridization (FISH) has been widely used for mapping repetitive DNA sequences and multi-copy gene families. The use of genomic DNA cloned in large-insert vectors such as bacterial artificial chromosomes (BACs) in combination with FISH (BAC-FISH) has been used for mapping specific DNA sequences and for identifying chromosomes in many plant species including rice, wheat, maize, potato, and *Arabidopis*. Genomic *in situ* hybridization (GISH), a modification of the FISH using total genomic DNA from one species as the probe, has been widely used in the detection of introgressed alien chromatin. BAC-FISH with sequential GISH can be used to locate the specific sequence to individual alien chromosomes[5-8].

We have constructed a TAC (Transformation-competent artificial chromosome) library using a 6VS/6AL translocation line harbouring the *Pm21*[9]. A sequence-specific PCR marker, Xcinau15-$_{902}$(NAU/xibao15$_{902}$), linked to *Pm21*, was designed based on the sequence of *Hv-S/TPK* gene[4]. A positive clone, TAC15, containing the sequence of *Hv-S/TPK* gene was identified by screening the TAC library with PCR marker Xcinau15-$_{902}$ [10]. In this study, physical map of the *Hv-S/TPK* gene was conducted by sequential FISH with TAC15 as probe and GISH with genomic DNA of *H. villosa* as probe in *T. aestivum-H. villosa* 6V addition, 6V(6A)substitution and 6VS/6AL translocation lines .

MATERIALS AND METHODS

Materials

T. aestivum-H. villosa 6V addition line 06R33, 6V(6A) substitution line 06R41 and 6VS/6AL translocation line 92R137 were developed and provided by Cytogenetics Institute, Nanjing Agricultural University. A positive clone TAC15 containing *Hv-S/TPK* gene was screened by Sun et al[10].

Chromosome preparation and Fluorescence *in situ* hybridization

The traditional squash method was used for chromosome preparations in wheat. FISH and sequential GISH were conducted as described[6, 7] with a few modifications. TAC15 clone and *H. villosa* genomic DNA was labelled with digoxygenin-11-dUTP (Roche) by standard nick translocation reactions. Total genomic DNA of common wheat Chinese Spring and *H. villosa* were sheared to an average size of 300 bp as the blocking DNA. A 300-fold

excess of sheared genomic DNA of Chinese Spring and *H. villosa* were added as blocking DNA in hybridization solution in TAC-FISH. The same preparations were used in sequential FISH with TAC15 as probe and GISH with genomic DNA of *H.villosa* as probe. The fraction length of hybridization sites was calculated as the distance from the centromere to the hybridization signal relative to the total length of the chromosome arm. More than ten chromosomes were measured for each FL calculation and standard deviations were estimated.

RESULTS

T.aestivum-H.villosa addition, substitution and translocation lines were used to physically map the TAC15 which contained the gene of *Hv-S/TPK*. After TAC-FISH, clear double fluorescent signals were detected at a pair of short arms of metaphase chromosomes. No significant non-specific fluorescent noise was observed when the blocking DNA of total wheat and *H. villosa* genomic DNA were added to the hybridization solution (Figure 1a, 1c, 1e). Re-probing of chromosome preparations with *H. villosa* genomic DNA showed that chromosomes with strong hybridization signals were 6V or 6VS (Figure 1b, 1d, 1f). The FL position of the *Hv-S/TPK* loci was 0.573±0.033, 0.587±0.040 and 0.566±0.034 in *T.aestivum-H.villosa* 6V addition line 06R33, 6V(6A) substitution line 06R41 and 6VS/6AL translocation line 92R137, respectively.

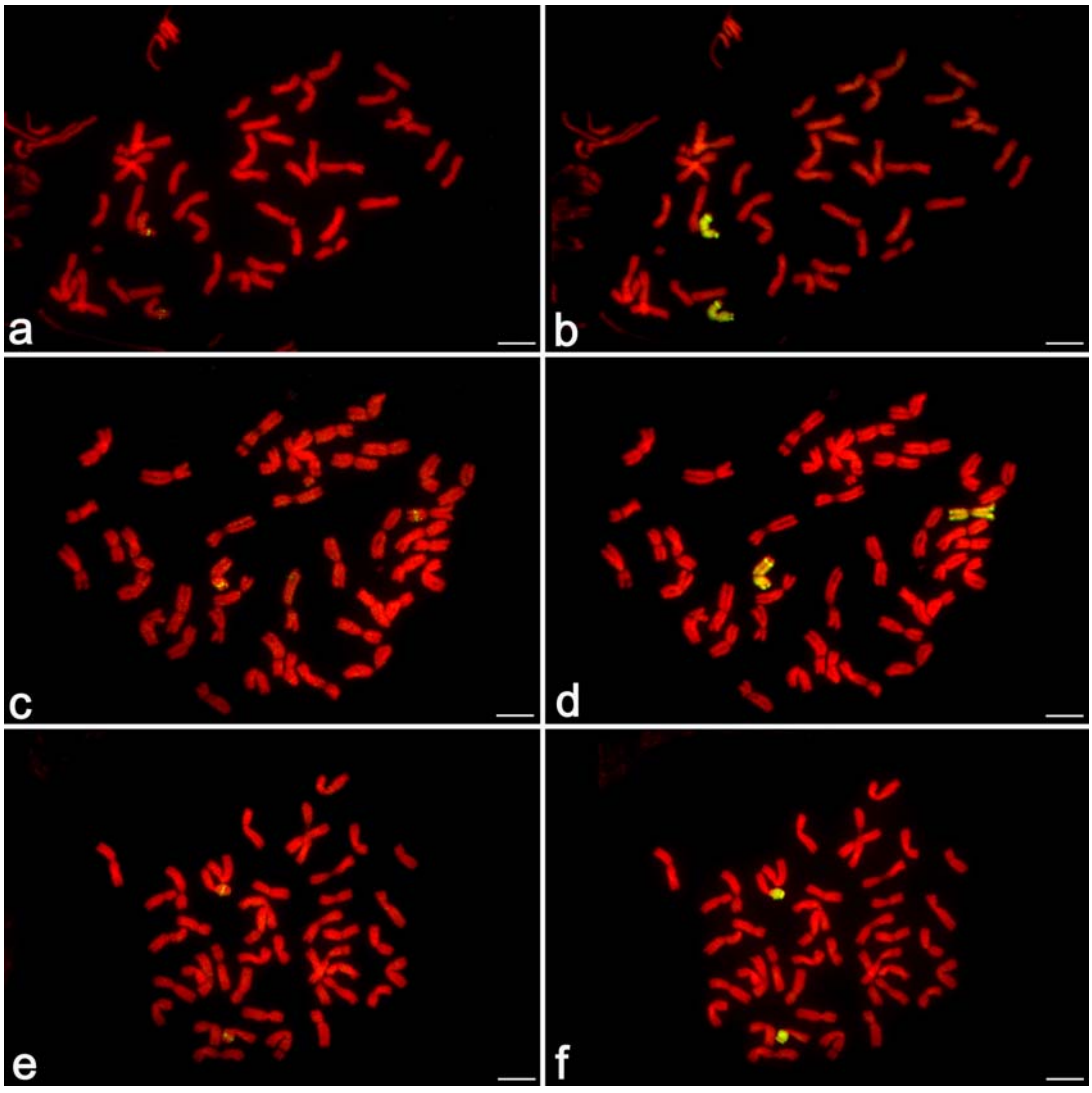

Figure 1. FISH with TAC15 as the probe (a,c,e) and GISH with the genomic DNA of *H.villosa* as the probe (b,d,f) hybridized to cells with complete mitotic metaphase chromosomes of different genetic stocks containing 6V. Hybridization signals were detected with FITC-anti-digoxigenin, and chromosomes were counterstained with DAPI. Chromosomes are pseudocolored as red. Scale bars equal 10 μm.

a,b. *T.aestivum-H.villosa* 6V addition line 06R33

c,d. *T.aestivum-H.villosa* 6V substitution line 06R41

e,f. *T.aestivum-H.villosa* 6VS/6AL translocation line 92R137

DISCUSSION

Physically mapping DNA on chromosomes or on DNA fibres will give the most useful information in map-based cloning, because actual physical lengths could not be determined by the recombination values only. Recently, BACs with single- or low-copy sequences have been successfully mapped in plant species with small genomes, such as *Arabidopsis*, rice, sorghum, *Ipomoea trifida* and *Beta vulgaris*[5]. However, BACs from wheat are difficult to generate unique locus-specific FISH signals, because in wheat more than 80% of the genome consists of repeated DNA sequences of varying degree of repetition and lengths and only a small percentage (12%) consists of low-copy number or unique sequences[11, 12]. In this study, we demonstrated that FISH and sequential GISH could be used to localize the alien gene from *H. villosa* chromosome 6V with resistance to powdery mildew. Strong non-specific noise, produced from repetitive sequences of the TAC clone, could be blocked by adding excessive blocking DNA of CS and *H. villosa* in TAC-FISH, and the hybridization signals of TAC15 were easily detected. Thus, combining FISH and sequential GISH, clones of BAC or TAC with low- or single DNA sequences from wheat relatives can be mapped on the alien chromosomes. The physical mapping of *Hv-S/TPK* to the position of average FL 0.575±0.037 in 6VS will be helpful in better understanding *Pm21* from molecular and cytogenetic aspects.

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