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ORIGINAL PAPER

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Mapping a resistance gene in wheat cultivar Yangfu 9311 to yellow mosaic virus, using microsatellite markers

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Abstract Wheat yellow mosaic disease, which is caused by wheat yellow mosaic bymovirus (WYMV) and transmitted by soil-borne fungus, results in severe damage on wheat (*Triticum aestivum* L.) production in China. For development of resistant cultivars to reduce wheat yield losses due to wheat yellow mosaic disease, resistance test and genetic analysis indicated that a single dominant gene in wheat cultivar Yangfu 9311 contributed to the resistance. Bulk segregant analysis was used to identify microsatellite markers linked to the resistance gene in an F_2 population derived from the cross Yangfu 9311 (resistant) × Yangmai 10 (susceptible). Microsatellite markers *Xwmc41*, *Xwmc181*, *Xpsp3039*, and *Xgwm349* were co-dominantly or dominantly linked with the gene responsible for WYMV resistance at a distance of 8.1–11.6 cM. Based on the wheat microsatellite consensus map and the results from amplification of the cultivar Chinese Spring nulli-tetrasomic stocks, the resistance gene to wheat yellow mosaic disease derived from Yangfu 9311, temporarily named as *YmYF*, was thus mapped on the long arm of chromosome 2D (2DL).

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Introduction

Since the mid 1970s, yellow mosaic disease has been reported in wheat (*Triticum aestivum* L.) grown in different regions of China, especially in the middle and lower reaches of Yangtze River, the Sichuan Basin in the southwest, Huanghuai winter wheat belt, and the Wei River Basin of Shanxi Province. Due to the disease, wheat yield losses were 20–30%, even up to 50–70% when the disease occurred severely on some susceptible varieties (Li et al. 1997). Wheat yellow mosaic is caused by wheat yellow mosaic bymovirus (WYMV) that is soil-borne and transmitted by fungus *Polymyxa graminis* (Inouye 1969). Symptoms of yellow-striped leaves and stunted plants developed on infected wheat are similar to those caused by wheat spindle streak mosaic virus (WSSMV) in North America and Europe (Chen et al. 2000), which differs from WYMV in nucleotide and amino acid sequences (Yu et al. 1999). Because the resting spores of *P. graminis* carrying WYMV survive in plant residues for a long period, the fungal inoculum in the contaminated fields is hardly to be eliminated through conventional crop management or fungicides. Furthermore, a mass of foster cultivars in local regions and the immoderate cultivation fashion of rice–wheat rotation aggravated the incidence of the disease. In recent years, to manage the crop damage by this disease, several wheat cultivars were bred for resistance to WYMV, such as cultivars Yangfu 9311 and Ningmai 9 (Yao et al. 1999). Genetic analyses of different wheat varieties showed that the inheritance of resistance to WYMV is complex and influenced by many factors, generally controlled by one to three genes (Qin et al. 1986; Zhou et al. 2000). Development of molecular markers closely associated with resistance genes to wheat yellow mosaic disease would be necessary for marker-assisted selection breeding in the future.

Fig. 1 Phenotypes of the parental wheat cultivars by wheat yellow mosaic bymovirus infection. *Left* Susceptible cultivar Yangmai 10, *right* resistant cultivar Yangfu 9311

Molecular markers are widely used in identification and localization of resistance genes to various wheat diseases caused by fungi, such as leaf rust (*Puccinia triticina* Eriks.), stripe rust (*P. striiformis* Westend.), and powdery mildew (*Erysiphe graminis* f. sp. *tritici* DC) (Autrique et al. 1995; Sun et al. 1997; Chagué et al. 1999; Robert et al. 1999; Shi et al. 2001; Yan et al. 2003). However, only a few molecular markers associated with virus resistance genes were identified in wheat. A resistance gene for wheat streak mosaic virus derived from *Agropyron intermedium* was tagged using random amplified polymorphic DNA (RAPD) and sequence-tagged site (STS) techniques (Talbert et al. 1996). Restriction fragment length polymorphic (RFLP) markers linked with wheat resistance gene for yellow dwarf disease were mapped on the end of chromosome 7XL derived from *Thinopyrum intermedium* (Zhang et al. 1999). In parallel, a co-dominant PCR marker linked to the gene *Yd2* for resistance to barley yellow dwarf virus deriving from *Th. intermedium* was developed for application in marker-assisted barley introgression program (Stoutjesdijk et al. 2001; Jefferies et al. 2003). An RFLP marker tightly linked with a resistance gene against to WSSMV infection was obtained by Khan et al. (2000), which was presumed to be useful to WYMV that was closely related to WSSMV. In order to obtain molecular tagging of resistance gene to WYMV, by genetic analyses with 37 wheat cultivars from China, Japan, and the United States, a single dominant resistance gene responsible for the resistance to WYMV was revealed only in the local wheat cultivar Yangfu 9311, whereas the resistances in other cultivars were controlled by multiple genes predominately (Liu et al. 2004). In this study, microsatellite markers developed by different researchers were used to identify molecular markers linked to the WYMV resistance gene in the wheat variety.

Materials and methods

Plant materials

Wheat cultivar Yangfu 9311 (Yangmai 3 × Gaojiasuo) resistant to WYMV and the susceptible cultivar Yangmai 10 (Yuma/8*Cc × Yangmai 5) were developed in the Institute of Agricultural Sciences in Lixiahe District, Jiangsu Province. These cultivars were crossed to develop of an F_2 mapping population composed of 186 plants. Young leaves were collected from each of the F_2 plants for DNA extraction. Cultivar Chinese Spring, nulli-tetrasomic (NT) stocks N2AT2D and N2DT2A of Chinese Spring were from the Institute of Crop Germplasm Resources, CAAS.

Table 1 Segregation analysis for the resistance gene and SSR markers in the F_2 population

Gene or markers	Number of F_2 plants	Observed number			Expected ratio	χ^2	*P*
		$X_1X_1^a$	X_1X_2	X_2X_2			
YmYF (resistance gene)	186	140[b]		46	3:1	0.007	>0.90
Xwmc41	186	50	95	41	1:2:1	0.96	0.50–0.75
Xwmc181	186	51	94	41	1:2:1	1.31	0.50–0.75
Xpsp3039	186	145[b]		41	3:1	0.87	0.30–0.50
Xgwm349	186	142[b]		44	3:1	0.18	0.50–0.75

[a]Genotype: X_1X_1 = cultivar Yangfu 9311; X_1X_2 = heterozygous; X_2X_2 = cultivar Yangmai 10
[b]Pooled values from homozygous and heterozygous classes

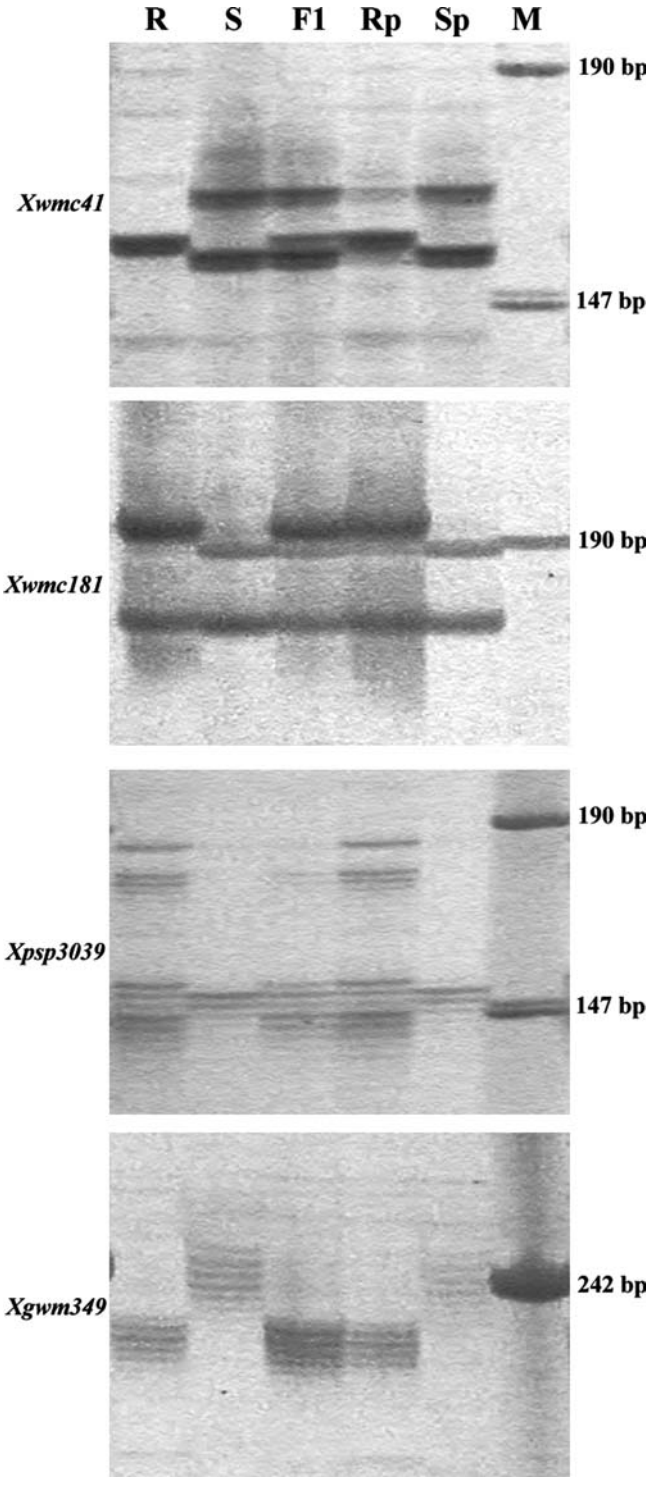

Fig. 2 Polymorphic amplification with the microsatellite primers. Silver-stained polyacrylamide gel patterns amplified with the primer pairs (from *top* to *bottom*) of *Xwmc41*, *Xwmc181*, and *Xgwm349* reported previously (Röder et al. 1998; Gupta et al. 2002), and the *Xpsp3039* primer from the John Innes Centre (unpublished). *R* Resistant parent, *S* susceptible parent, *F1* F_1 progenies, *Rp* resistant F_2 pool, *Sp* susceptible F_2 pool, *M* pUC Mix Marker (Sangon, Shanghai, China)

Assessment of resistance to WYMV

Field experiments of resistance to WYMV were carried out at a disease nursery located in Baimi Town, Jiangyan County, Jiangsu Province, where the soil has been contaminated by WYMV since late 1980s and the susceptible parental cultivar, Yangmai 10, was 100% infected without additional inoculation. To identify the phenotypes of parents, F_1 and F_2 progeny plants by natural infection in the disease nursery, wheat seed of the tested cultivars were sown in 30-cm row pitches and 10 cm between individual plants in late October 2001. In early March 2002, when the wheat plants started to tiller and elongate, the evaluation for resistance to WYMV was conducted. Infection types (ITs) were rated using a 0–3 scale, where 0 = no visible symptom, IT 1 = lightly streak mosaic leaf but plant not dwarfed, IT 2 = distinct mosaic streak covering one half of the diseased leaf, and IT 3 = mosaic area covering three quarters of the diseased leaf and the plant dwarfed obviously.

Microsatellite analysis

Young leaf tissues were used for total DNA extraction using the cetyltrimethyl ammonium bromide method as described by Saghai-Maroof et al. (1984). The DNA samples from ten F_2 plants with resistant phenotype and ten F_2 individuals susceptible to the disease were bulked separately for bulked segregant analysis according to Michelmore et al. (1991). Three hundred and twenty-seven wheat microsatellite primer pairs were synthesized according to the sequences previously reported (Röder et al. 1998; Pestsova et al. 2000; Gupta et al. 2002; Somers et al. 2004) and additional 42 *Xpsp* primers were provided by John Innes Centre, UK (unpublished). The DNA amplifications were carried out in each 20-μl volume of the reaction mixtures (50 ng template DNA, 2 μ*M* each of the microsatellite primer pairs, 2.5 m *M* each dNTPs, 2 m *M* $MgCl_2$, 1X PCR buffer, and 1 U *Taq* DNA polymerase), using a thermal cycler (PTC-200, MJ Research). The PCR was programmed at 95°C for 3 min, followed by 35 cycles of 94°C denaturing for 30 s, annealing of different primers at 50, 55, or 60°C for 45 s at a ramp rate of 0.5°C/s, 72°C for 1 min, and finally extended at 72°C for 5 min. The PCR products were separated on 6% denatured polyacrylamide gels, followed by silver staining (Bassam et al. 1991).

Linkage analysis

Linkage between DNA markers and the resistance gene was established with MAPMAKER/EXP, version 3.0b (Lander et al. 1987). Markers were placed with a LOD threshold of 3.0 and a maximum distance of 50 cM. The Kosambi function was applied to convert recombination fractions into map distance (Kosambi 1944).

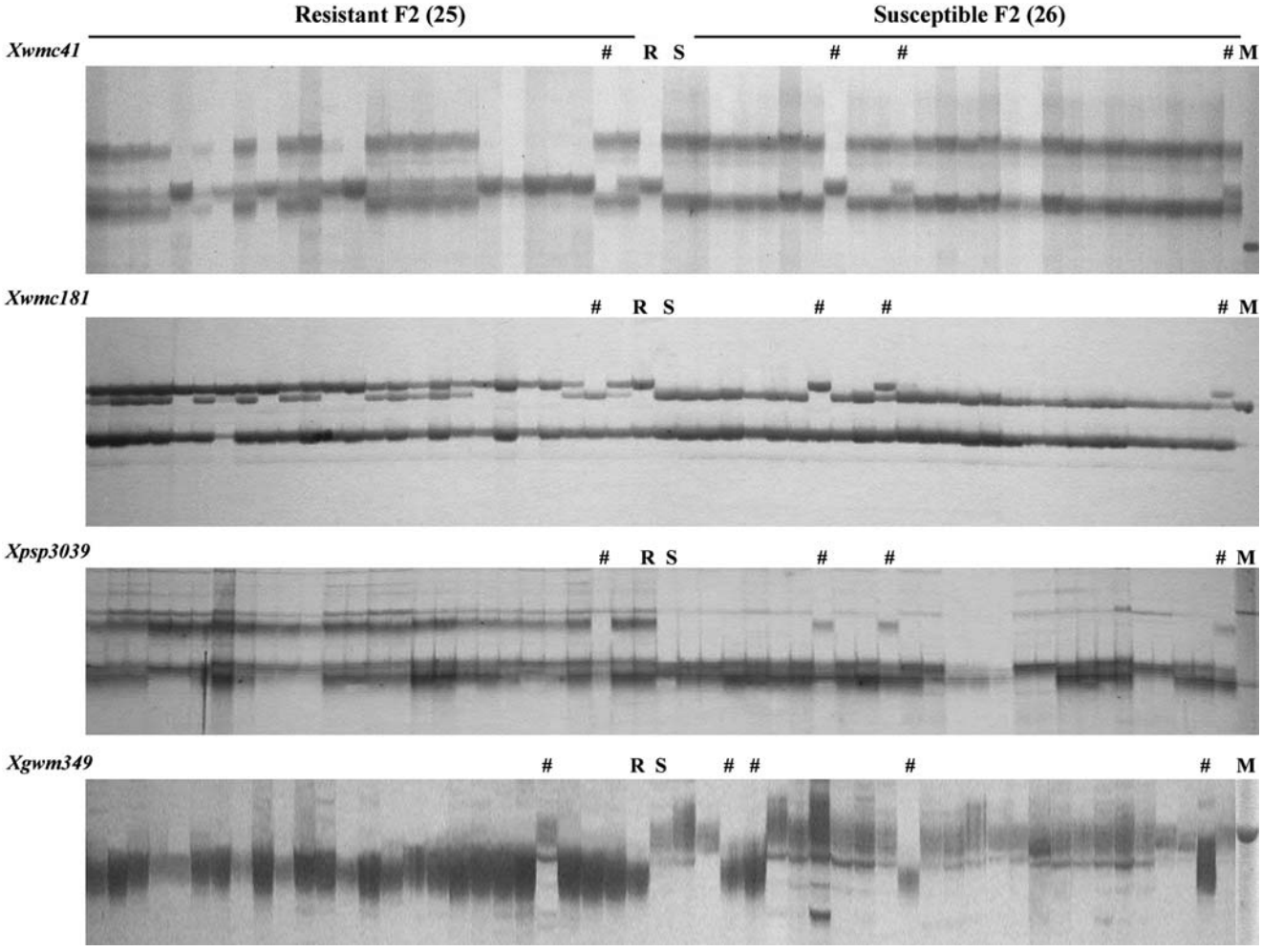

Fig. 3 Amplification of the F_2 individuals with the polymorphic primers. The resistant and susceptible F_2 individuals were amplified with the primer pairs of *Xwmc41*, *Xwmc181*, *Xpsp3039*, and *Xgwm349* (from *top* to *bottom*), respectively. The samples extracted from different F_2 individuals were loaded in parallel for the four primers. *R* and *S* resistant or susceptible parent, respectively, # recombinant plants, *M* pUC Mix Marker (Sangon)

Results

Genetic analyses and development of mapping population

By natural infection as described above, all 108 F_1 plants derived from the cross of Yangfu 9311 × Yangmai 10 were identically resistant to WYMV at the same level as the resistant parent, cultivar Yangfu 9311 (Fig. 1, right). Of 186 F_2 plants, 46 were susceptible to WYMV as the susceptible parent, cultivar Yangmai 10, showing a phenotype of IT 3 (Fig. 1, left). Considering that the remaining 140 F_2 plants showed infection type to WYMV same as the resistant parent at IT 0, the segregation ratio of 3:1 ($\chi^2 = 0.007 < \chi^2_{0.05,\ 1}$ 3.84) indicated that Yangfu 9311 wheat carried a dominant gene responsible for resistance to WYMV (Table 1).

Identifying and mapping of SSR markers linked to the resistance gene

With this F_2 population, 369 microsatellite primer pairs that distributed over the whole wheat genome were used to identify polymorphic SSR markers between the resistant and susceptible parents. Among the 132 primer pairs (36.26% of the total) that were polymorphic between the resistant and susceptible parents, 46 (12.64% of the total) displayed diversities between the DNA bulks derived from the resistant or susceptible individuals in the F_2 population. Four of the primer pairs, *Xwmc41*, *Xwmc181*, *Xpsp3039*, and *Xgwm349*, were proven to correlate with the resistance in the DNA bulks and the parents (Fig. 2), but other primers did not show association with the phenotypes by WYMV infection.

In further screening of the individuals in the F_2 population, the SSR markers *Xwmc41* and *Xwmc181* showed segregation ratio of 1:2:1 (χ^2 test), indicating that these two microsatellite markers are linked with the resistance gene in a co-dominant manner (Table 1; Fig. 3). The 3:1 segregation ratio of resistant to susceptible progeny in the F_2 population was exhibited by the primer pair *Xpsp3039* from the John Innes Centre

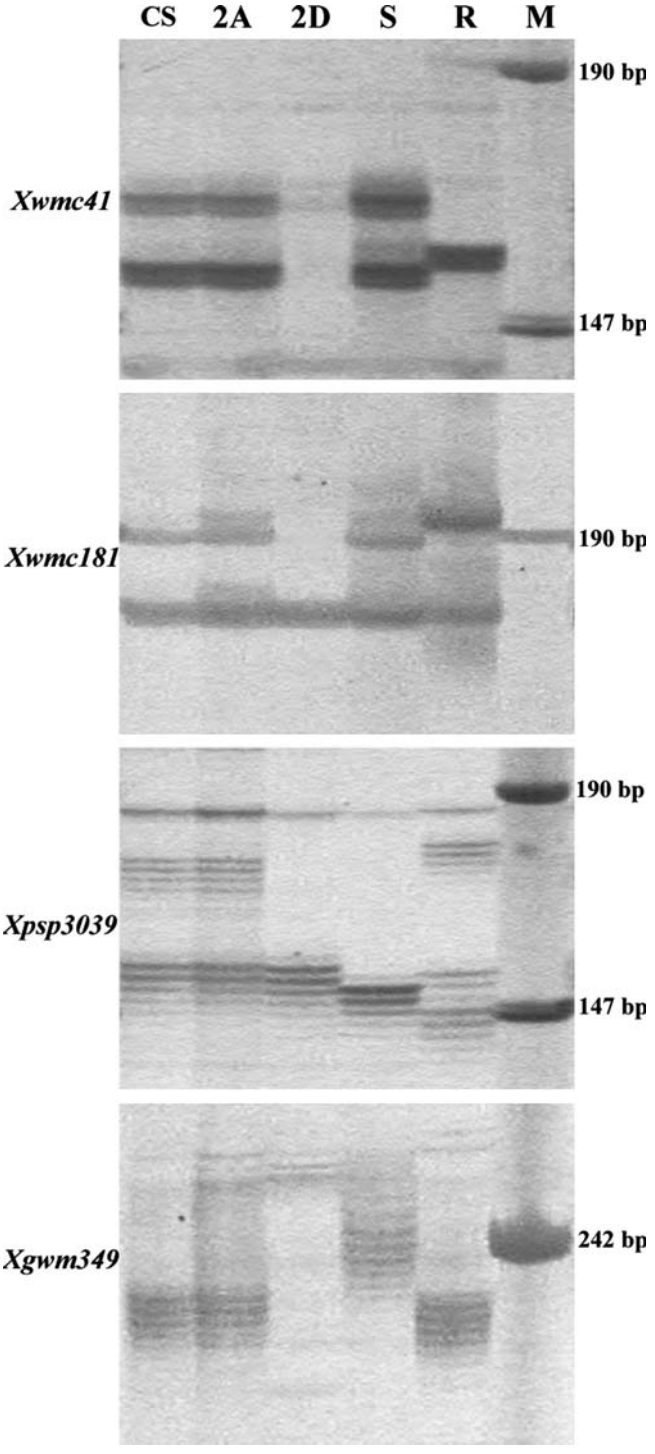

Fig. 4 Nulli-tetrasomic (NT) analysis of the chromosome loci segregating with the resistance gene. The DNA samples extracted from cultivar Chinese Spring (*CS*), NT stocks N2AT2D (*2A*) and N2DT2A (*2D*) of CS, the susceptible (*S*) and resistant (*R*) parent were amplified with the primer pairs of *Xwmc41*, *Xwmc181*, *Xpsp3039*, and *Xgwm349* (from *top* to *bottom*). *M* pUC Mix Marker (Sangon)

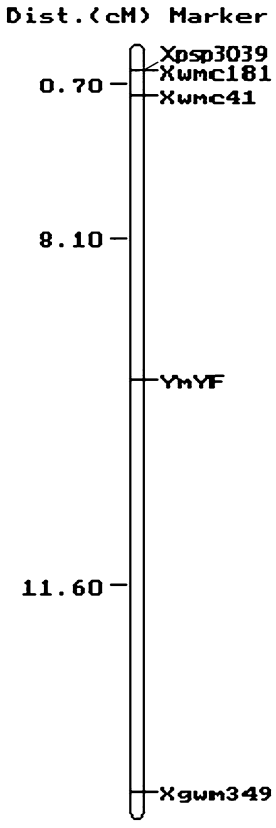

Fig. 5 Genetic linkage map of the *YmYF* gene and the SSR markers segregating in F_2 population in linear order

and *Xgwm349*, suggesting dominant SSR markers (Table 1; Fig. 3). In total of the population, the same 13 recombinant individuals were identified coincidently among the *Xwmc41*, *Xwmc181*, and *Xpsp3039* primers in repeated amplifications (Fig. 3). However, these four markers were localized on the different loci of wheat chromosomes in previous reports, in which *Xwmc41* and *Xgwm349* were located on the chromosome 2D, and the primer *Xwmc181* was located on the long arms of both wheat chromosomes 2A and 2D (Röder et al. 1998, Gupta et al. 2002; Somers et al. 2004), but the microsatellite *Xpsp3039* was displayed only on the chromosome 2A in the CeResDB database of the John Innes Centre (http://jic-bioinfo.bbsrc.ac.uk/cereals/). To verify the chromosome localization of the resistance gene, the DNA extracted from Chinese Spring and NT stocks N2AT2D and N2DT2A of Chinese Spring were amplified with these four primer pairs. As shown in Fig. 4, these four markers associated with the resistance gene were all tagged on the wheat chromosome 2D. Among them, the primer pair *Xpsp3090* showed more than one locus by the amplification of Chinese Spring, and the 180-bp locus of the marker on 2DL was linked with the resistance gene (Fig. 4), in stead of the 152 bp locus that was the only locus amplified by the primers on 2AL of Chinese Spring presented in the CeResDB database (http://jic-bioinfo.bbsrc.ac.uk/cereals/).

By analyzing with MAPMAKER/EXP, these four microsatellite markers were closely linked to the resistance gene, temporarily named as *YmYF*, in the linkage distances of 8.1 cM for *Xwmc41*, 8.8 cM for *Xwmc181*

and *Xpsp3039* that are bordered next to each other, and 11.6 cM for *Xgwm349* that was flanked from the other three markers in different direction (Fig. 5). Based on the genetic map of these four markers, the resistance gene *YmYF* is located on the long arm of chromosome 2D (2DL).

Discussion

In addition to wheat yellow dwarf disease caused by barley yellow dwarf virus, yellow mosaic disease is another severe disease of wheat in China. The phenotypes of disease severity vary significantly among wheat genotypes, the seasonal climates, and the farming systems in different regions. Early sowing in autumn, cool temperatures of drizzle prevernal weather, and overfertilizing would increase the disease severity.

Unlike most other experiments of wheat resistance, it is difficult to mechanically inoculate wheat plants with WYMV. Compared to the natural transmission of WYMV by the soil-borne fungus, mechanical inoculation showed low infection, in which maximum infection rate was no more than 70% on the highly susceptible parent (data not shown). Due to the fragile filamentary virus particles easily to be degraded during the process in vitro, it was infeasible to identify wheat resistance by mechanical inoculation.

Attributed to the same *Bymovirus* genus of Potyviridae and very high similarities in morphology, serology, fungal vector (Usugi et al. 1989), and symptoms in wheat, WYMV and WSSMV were previously considered as the same viral pathogen (Zhou et al. 1990; Hou 1993). However, molecular evidence indicated that WYMV was a different species from WSSMV in its regional distribution and heterogeneous RNA sequences (Yu et al. 1999). Compared to WSSMV found in North America and Europe (Chen et al. 2000), WYMV was identified as the only *Bymovirus* species occurred in wheat in China using RT-PCR primers specific to WYMV or WSSMV to test susceptible wheat samples from different regions (Li et al. 1997). In addition to a resistance gene to WSSMV mapped on the long arm of wheat chromosome 2D (2DL) (Khan et al. 2000), results of the *YmYF* on chromosome 2DL in this study would be practically useful in marker-assisted selection breeding of wheat resistance to WYMV, perhaps also to WSSMV.

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