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# Genetic mapping of the powdery mildew resistance gene *Pm6* in wheat by RFLP analysis

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**Abstract** *Pm6* in bread wheat (*Triticum aestivum* L.), which was transferred from *Triticum. timopheevii* L., is a gene conferring resistance to the powdery mildew disease caused by *Erysiphe graminis f.* sp. *tritici*. Six near-isogenic lines ( NILs ) of *Pm6* in a cultivar 'Prins' background were analyzed to map this gene using restriction fragment length polymorphism (RFLP). Each of the six NILs possessed a *T. timopheevii*-derived segment, varying in length, and associated with powdery mildew resistance. Lines IGV1–465 (FAO163b/ 7*Prins) and IGV1–467 (Idaed 59B/7*Prins) had the shortest introgressed segments, which were detected only by DNA probes BCD135 and PSR934, respectively. The polymorphic loci detected by both probes were mapped to the long arm of chromosome 2B. Lines IGV1–458 (CI13250/7*Prins) and IGV1–456 (CI12559/8*Prins) contained the longest *T. timopheevii* segments involving both arms of donor chromosome 2G across the centromere. All these introgressed segments had an overlapping region flanked by the loci *xpsr934* and *xbcd135* on 2BL. Thus, *Pm6* was located in this region since the powdery mildew resistance in all the NILs resulted from the introgressed fragments. Using the $F_2$ mapping population from a cross of IGV1–463 (PI170914/7*Prins)×Prins, *Pm6* was shown to be closely linked to the loci *xbcd135* and *xbcd266* at a genetic distance of 1.6 cM and 4.8 cM, respectively. BCD135 was successfully used in detecting the presence of *Pm6* in different genetic backgrounds.

**Key words** *Triticum aestivum · Tritiam timopheevii · Pm*6 · Introgression lines · RFLP

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## Introduction

Powdery mildew disease caused by *Erysiphe gramminis f.* sp. *tritici* has posed an increasing threat to wheat production worldwide. The use of resistant cultivars is the most economical and environmentally safe means for its control. As the resistance of *Pm8,* which is the major resistance gene used in China wheat breeding program, has already broken down, rational utilization of genes still effective in resistance as well as novel resistance genes has become an urgent task for the wheat scientists in this country. Studies have shown that *Pm*2, *Pm4* and *Pm*2+*6* still have effective resistance in most wheat-growing areas in China (Xiang et al. 1995; Zhuang 1993).

*Pm6* has been widely and successfully used in wheat powdery mildew resistance breeding (Bennett 1984). It was transferred to wheat from *Triticum timopheevii* most likely through recombination between the B genome of common wheat and the G genome of *T. timopheevii*. Several common wheat cultivars and lines carrying powdery mildew resistance and rust resistance genes from *T. timopheevii* have been developed (Bennett 1984; Tomar et al. 1988). *Pm6* was assigned to chromosome 2B on the basis of its close linkage to the stem rust resistance gene *Sr9c*(*Sr36*) from *T. timopheevii* (Jorgensen and Jensen 1973). McIntosh (personal communication) suggested, however, that *Sr36* may be on the short arm of 2B, with *Pm6* on the long arm.

*Pm*6 is best expressed from the three-leaf stage onwards and moderately effective, but recognizable, at the seedling stage (Bennett 1984). However, the identification of the specific powdery mildew resistance gene, based on the interaction between the host and mildew isolate, is frequently inconclusive because of the com-

**Table 1** *Pm6* near-isogenic lines

| Id.no | Near-isogenic line | Genes |
|---|---|---|
| IGV1–456 | CI 12559/8*Prins | *Pm6* |
| IGV1–458 | CI 13250/7*Prins | *Pm6* |
| IGV1–463 | PI170914/7*Prins | *Pm6* |
| IGV1–464 | PI262618/7*Prins | *Pm6* |
| IGV1–465 | FAO 163b/7*Prins | *Pm6* |
| IGV1–467 | Idaed 59B/7*Prins | *Pm6*+? |

plexity of gene expression. On the other hand, molecular markers linked to the resistance gene make it feasible to select resistant genotypes by following the transmission of the markers generation by generation, instead of relying on its variable phenotypic expression. RFLP markers have been identified for *Pm2* and *Pm4* (Ma et al. 1994; Hartl et al. 1995), but not for *Pm6*. In the present paper we report on the comparison of different wheat-*T. timopheevii* introgression lines and the mapping of *Pm6* with RFLP markers.

## Materials and methods

### Plant material

The Swedish Spring common wheat variety Prins and its *Pm6* NILs kindly provided by Dr. James Mac Key were used for RFLP analysis and for screening polymorphic markers linked to the gene. The NILs were developed through backcrossing seven to eight generations with the recurrent parent Prins. The donor lines of *Pm6* are listed in Table 1.

A mapping population was produced by crossing Prins with line IGV1–463. One single $F_1$ Plant (heterozygous for *Pm6*) was selfed to produce 73 $F_2$ plants. Prins and the original *Pm6* donor *T. timopheevii* were used as controls for polymorphic analysis. The chromosomal locations of the polymorphic bands were determined by nulli-tetrasomic lines of Chinese Spring.

Fifteen wheat cultivars and lines reportedly carrying *Pm6* (Nyquist 1963; Leijerstam 1972; Wolfe and Wright 1972; Jorgensen and Jensen 1973; Bennett and Van Kints 1983; Leath and Heum 1990) were collected to assess the applicability of the markers for the identification of *Pm6* in different wheat backgrounds.

### RFLP analysis

Based on the chromosomal location of *Pm6* (McIntosh, personal communication), thirty six clones mapped to homoeologous group 2 were used to detect the polymorphism. Clones from PSR, a wheat cDNA and genomic library, BCD, a barley cDNA library, CDO, an oat cDNA library, and WG, a wheat genomic DNA library; KSU, a *Triticum tauschii* gDNA library, were provided by Dr. M.D.Gale, Norwich, UK, Dr. M.E.Sorrells, Ithaca, USA, and Dr. B.S.Gill, Manhattan, USA, respectively.

DNA isolation followed the method of Gill et al. (1991) with slight modifications. Two grams of ground leaf tissue were incubated in 20 ml of 2% CTAB DNA extraction buffer at 65°C for about 2 h. The slurry was extracted with 16 ml of phenol-chloroform-isoamyl alcohol (25:24:1, v/v), followed by chloroform-isoamyl (24:1 v/v). The emulsion was centrifuged at 8000 rpm for 10 min at 15–20°C. The DNA was precipitated with 2/3 vol of cold ethanol and 1/10 vol of 3 M sodium acetate, rinsed in 70% ethanol several times, and dissolved in TE (pH 7.9–8.0). DNA restriction digestion, Southern blotting, probe labelling and the hybridization methods used are as described by Sharp et al. (1988).

### Powdery mildew resistance test

Powdery mildew inoculation of the 73 $F_2$ plants was carried out in the greenhouse at the seedling stage using a field population of *E. graminis f.* sp. *tritici* randomly collected in Yangzhou and Nanjing, Jiangsu province, as inoculum. Prins was employed as a susceptible control, the NILs and $F_1$ as resistant controls. Repeated inoculations were made at intervals of 48 h. Disease readings were assessed 7–10 days later as described by Sheng (1988), when symptoms were fully developed in the susceptible control.

Inoculation was also carried out at the three-leaf stage for evaluating the response types of the plants to powdery mildew infection.

### Linkage analysis

Recombination values were calculated according to maximum-likelihood estimates (Allard 1956), and were converted into map units (centi Morgan, cM) by the Kosambi function (Kosambi 1944).

## Results

### Detection of the introgressed segments in different NILs with *Pm6*

As *Pm6* has been mapped on chromosome 2B by genetic analysis (McIntosh, personal communication), 36 probes assigned to homoeologous group-chromosomes by Devos (1993) and Nelson (1995) were used to detect the differences among the individual NILs IGV1–456, IGV1–458, IGV1–463, IGV1–464, IGV1–465 and IGV1–467 (Table 1), the original *Pm6* donor *T. timopheevii*, and the recurrent parent Prins. Introgression lines IGV1–456 and IGV1–458 were distinct from the others. Nineteen long arm-specific and 6 short-arm-specific probes detected RFLPs between resistant IGV1–456, IGV1–458 and susceptible Prins. Based on the positions of these polymorphic clones in the wheat genetic maps (Devos et al. 1993; Nelson 1995), it was concluded that the introgressed *T. timopheevii* 2G chromatin in IGV1–456 and IGV1–458 was flanked by *xcdo405* and *xpsr934* and involved both arms spanning the centromere. Polymorphism resulting from *T. timopheevii* chromatin introgression in the other NILs could only be detected by the probes mapped to the long arm. Six probes (BCD307, BCD1095, BCD135, BCD266, PSR934, PSR932 plus BCD292 and CDO678) showed RFLPs between Prins and IGV1–463, implying that the introgressed *T. timopheevii* segment in IGV1–463 was smaller than those detected in IGV1–456 and IGV1–458, and involved only the long-arm region flanked by *xbcd307* and *xcdo678*. Polymorphism between IGV1–464 and Prins was revealed by PSR934 and BCD135. The introgressed segments in lines IGV1–465 and IGV1–467 are the smallest among the six NIls, since RFLP between IGV1–465, IGV1–467 and Prins was detected only by BCD135 and PSR934, respectively.

As *Pm6* was carried by the introgessed fragments in all the introgression lines, it can be reasonably located in the region of 2BL flanked by the loci *xbcd135*–2BL and

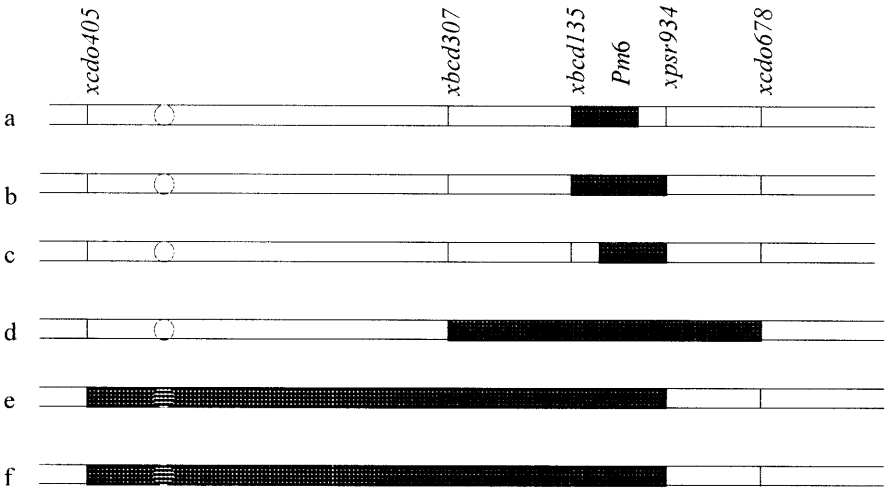


**Fig. 1** The graphic genotypes of chromosome 2B in the wheat-*T. timopheevii* introgression lines; *a* IGV1-465, *b* IGV1–464, *c* IGV1–467, *d* IGV1–463, *e* IGV1–458, *f* IGV1–456. The segments substituted by the 2G chromatin are shown by *dark regions*

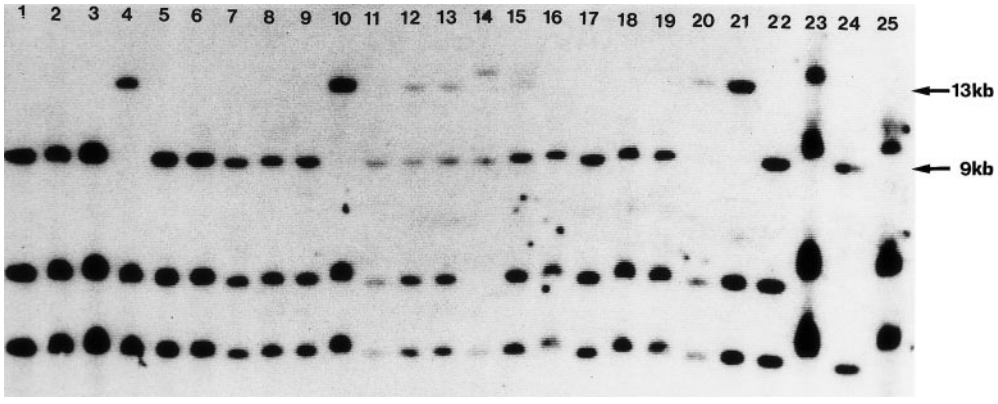


**Fig. 2** Banding patterns of probe BCD135 hybridized to *Eco*RV-cleaved genomic DNA from wheat cultivars and lines supposedly carrying *Pm6*. (*1*) IGV1–456; (*2*) IGV1–458; (*3*) IGV1–463; (*4*) Prins; (*5*) CI13381/8*Prins; (*6*) CI13399/7*Prins; (*7*) IGV1–464; (*8*) IGV1–465; (*9*) Kenya Lemphi 50–13596/7*Prins; (*10*) IGV1–467; (*11*) SC 60–5723/7*Prins (*Pm6*); (*12*) Prins×PI170914/7*Prins; (*13*) Prins×CI12559/8*Prins; (*14*) Maris Huntsman; (*15*) PI405718; (*16*) Oasis; (*17*) CI12633; (*18*) CI12632; (*19*) TP114; (*20*) CI12632/Cc; (*21*) Timgalen; (*22*) Coker 747; (*23*) Coker 983; (*24*) *T. timpheevii*; (*25*) Suwon 92/9*Prins

*xpsr934*–2BL, based on the overlapping portion of the introgressed segments detected in different lines (Fig. 1).

### Identification of Markers closely linked to *Pm6*

The eight probes (BCD307, BCD135, BCD266, PSR934, CDO678, PSR932, BCD292 and BCD1095) detecting polymorphism between NIL IGVI-463 and Prins were hybridized to DNA blots from *T. timopheevii* and a set of wheat cultivars and lines reportedly carrying *Pm6*. BCD135 detected a 9-kb band under *Eco*RV restriction in almost all the resistant lines and *T. timopheevii*, and an allelic 13-kb band in all the susceptible lines, IGV1–467, and two other *Pm6*-carriers CI12632/Cc and Timgalen (Bennett and VanKints 1983) in which the presence of *Pm6* was questionable in China (Table 1, Fig. 2). This *xbcd135* locus was mapped on chromosome 2B using nulli-tetrasomic lines. RFLP patterns revealed by probes BCD307 and BCD266 in most lines tested were identical to those obtained in IGVI-463. The polymorphism detected by BCD1095 could hardly be identified due to its weak signal.

A population of 73 $F_2$ plant from Prins×IGVI-463 showed clear cut segregation in powdery mildew resistance and was employed to estimate the genetic distances between the polymorphic markers and *Pm6*. The 9-kb allele derived from IGVI-463 and revealed by BCD135 were present in all 61 resistant plants. One susceptible plant (no. 35, Fig. 3) was in a heterozygous state at this locus. *xbcd135* was mapped at a distance of 1.6±1.5 cM from *Pm*6. *Xbcd307* and *Xbcd266* were mapped at a distance of 1.6±1.5 and 4.8±2.6 cM from *Pm6*, respectively (Fig. 4).

## Discussion

The powdery mildew resistance gene *Pm6* carried by chromosome 2G of the tetraploid *T. timopheevii* has been transferred into hexaploid common wheat on several occassions (McIntosh and Gyarfas 1971; McIntosh and Luig

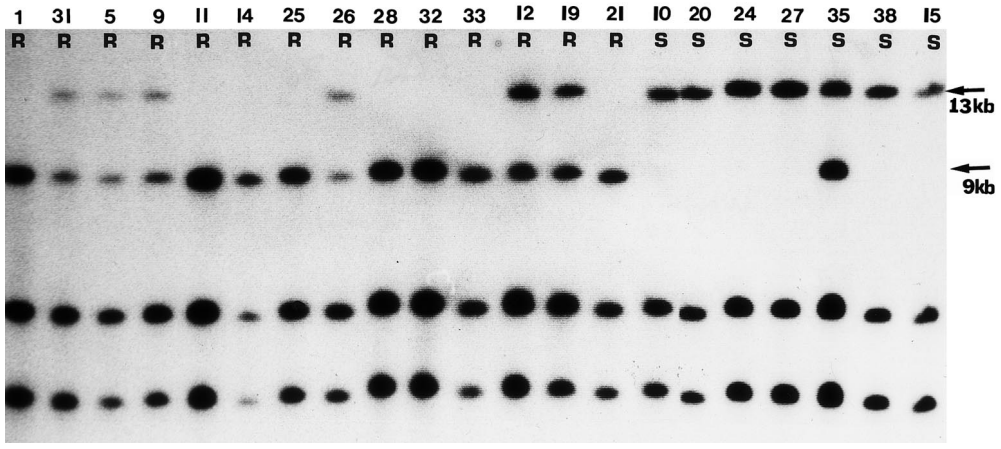


**Fig. 3** Segregation pattern of the *xbcd135* locus in a subsample of the $F_2$ progenies from the cross Prins×IGV1–463, *R* resistance, *S* susceptible. The DNA was digested with *Eco*R

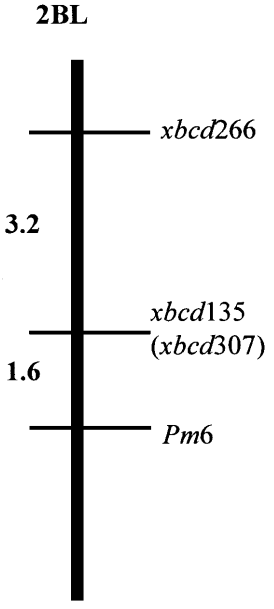


**Fig. 4** A linkage map of *Pm6* on 2BL. *Numbers* to the left of the map represent the genetic distance (cM)

1973; Jorgensen and Jensen 1973; McIntosh 1991). *Pm6* is linked to *Sr36* in most cultivars, which is a stem rust resistance gene originating also from *T. timopheevii* (Jorgensen and Jensen 1973). However, the dissociation of these two genes has been reported in some cases. Genetic analysis showed that *Sr36* was located in the short arm, and *Pm6* in the long arm of chromosome 2B (McIntosh, personal communication). Thus, the size of the introgressed *T. timopheevii* chromosomal segments in these cultivars or lines could be different. A backcross program was adopted to develop *Pm*6 NILs in order to avoid the potential linkage drag caused by a long 2G segment (Mac Key, personal communication). The *Pm6* NILs possessing the smallest introgressed 2G segment demonstrated by us most likely represented the success of such a program.

To determine the alien segments in different lines, *T. timopheevii* and the NILs have been examined by means of chromosome C-banding and in situ hybridization, which is, however, rather difficult to discriminate the normal 2B from its analogue when the inserted alien chromatin is small. Using banding methods, Friebe et al. (1996) revealed that in the Australian variety 'Timvera' the inserted *T. timopheevii* segment on 2B involved both arms of 2G. Nevertheless, they were not able to locate the exact breakpoints of the translocation. Genomic in situ hybridization has been unsuccessful due to extensive cross hybridization between the wheat and *T. timopheevii* DNA. RFLP analysis has been shown to be very useful in the identification of the introgressed alien chromatin which is hardly detectable by cytogenetic approaches (Young and Tanksley 1989; Autrique et al. 1995; Donini et al. 1995). In the present study a total of six NILs supposedly containing a *T. timopheevii* 2G segment were screened with homoeologous group-2 RFLP probes to investigate the size of the introgressed fragments and map the powdery mildew resistance gene. The size of the introgressed segments in 2B was highly variable in these lines. *Pm6* was located in the region delimited by *xpsr934*–2BL and *xbcd*135–2BL.

Our results may be of importance for the positional cloning of *Pm6*. Linkage analysis demonstrated that the alien introgressed segments were conserved as linkage blocks (Donini et al. 1995). The RFLP markers around the *T. timopheevii* fragments can be useful in tagging *Pm6*. As *Pm6* is readily expressed from the three-leaf stage onwards, linked markers *xbcd135*, *xbcd266* and *xbcd307* can be used in the selection of genotypes possessing *Pm6* in the early stage of growth. The RFLP marker *xbcd135* was shown to be applicable under different genetic backgrounds. The fact that no polymorphism was detected between CI12632/Cc, Timgalen and Prins by BCD135 supported Chinese pathologists' doubts about the existence of *Pm6* in these two lines which were introduced from IWWPMN (Li and Huang 1990; Xiang et al. 1996).

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