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STS markers for powdery mildew resistance gene *Pm6* in wheat

Jianhui Ji · Bi Qin · Haiyan Wang · Aizhong Cao · Suling Wang · Peidu Chen · Lifang Zhuang · Yu Du · Dajun Liu · Xiue Wang

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Abstract The powdery mildew resistance gene *Pm6*, transferred to common wheat from the tetraploid *Triticum timopheevii*, is effective in most epidemic areas for powdery mildew in China. RFLP probe BCD135 was previously associated with *Pm6*. In the present research, four STS primers (NAU/$STS_{BCD135-1}$, NAU/$STS_{BCD135-2}$, STS003 and STS004) were designed from the sequence data of BCD135. These primers were used for PCR amplification using the genomic DNA of resistant near-isogenic lines with *Pm6* and their recurrent parent, cv. Prins. No polymorphic product was observed using primers STS003 and STS004; however, primers NAU/$STS_{BCD135-1}$ and NAU/$STS_{BCD135-2}$ amplified two and one bands, respectively, polymorphic between the resistant near-isogenic-lines and Prins. The two primers were then used to amplify the F_2 population from the cross IGV1-465 (FAO163b/7*Prins) × Prins. The amplification and the powdery mildew resistance identification data were analyzed using the software Mapmaker 3.0. The results indicated that both NAU/$STS_{BCD135-1}$ and NAU/$STS_{BCD135-2}$ were closely linked to *Pm6* with a genetic distance of 0.8 cM. A total of 175 commercial varieties without *Pm6* from different ecological areas of China were tested using marker NAU/$STS_{BCD135-2}$ and none of them amplified the 230 bp-specific band. This marker thus has high practicability and can be used in MAS of *Pm6* in wheat breeding programs for powdery mildew resistance.

Keywords Marker assisted selection · MAS · Powdery mildew · Sequence Tagged Site · STS · *Triticum aestivum* · *Triticum timopheevii*

Introduction

Powdery mildew, caused by *Blumeria graminis* f.sp. *tritici*, is one of the most important diseases of wheat (*T. aestivum* L.) worldwide. Development and utilization of wheat varieties with powdery mildew resistance is the most economical and effective strategy for disease control. To date, 34 powdery mildew resistance gene loci have been identified in common wheat (McIntosh et al. 2003; Hsam et al. 2003; Zhu et al. 2005; Miranda et al. 2006). Accumulating multiple resistance genes within a single genotype, commonly referred to as "gene pyramiding," is one strategy for increasing durability of resistance (Kameswara Rao et al. 2002). However, gene pyramiding can be difficult without utilysing markers. Restriction Fragment Length Polymorphism (RFLP) and Random Amplified Polymorphic DNA

Jianhui Ji and Bi Qin contributed equally to this work.

J. Ji · B. Qin · H. Wang · A. Cao · S. Wang · P. Chen · L. Zhuang · Y. Du · D. Liu · X. Wang (✉)
Cytogenetics Institute and National Key Lab for Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Jiangsu 210095, P.R. China
e-mail: xiuew@njau.edu.cn

(RAPD) have been used to generate wheat genetic maps and to find molecular markers for resistance genes, but screening of RFLP probes for closely linked markers is laborious and time-consuming, even though the position of the target gene is known. Also, RAPD markers identified in one population are not always useful for another, and may lack reliability (Weeden et al. 1992).

To address the above problems, it is useful to convert a RFLP or RAPD polymorphic fragments into sequence tagged site (STS) or sequence-characterized amplified region (SCAR) markers (Olson et al. 1989). STSs are relatively easy to develop from DNA fragments identified by arbitrarily primed PCR, although they do not always result in detectable polymorphisms. STSs, as specific PCR products, are reproducible across a relatively wide range of reaction conditions in different laboratories thus overcoming the shortcomings of RAPDs.

Powdery mildew resistance gene *Pm6* was introgressed from tetraploid wheat *T. timopheevii* into common wheat (*T. aestivum*) and mapped in the long arm of chromosome 2B (Nyquist 1963; Jorgensen and Jensen 1972, 1973). *Pm6* has been widely used in wheat breeding for powdery mildew resistance, and is still one of the most effective resistance genes in many areas of the world, including China, Europe and North America, especially when it is used combined with other *Pm* genes, such as *Pm2* (Jorgensen and Jensen 1972; Cai et al. 2005). Tao et al. (2000) defined the lengths of the introgressed *T. timopheevii* segments in six different introgression lines and identified several RFLP markers linked with *Pm6*. Among them, *Xbcd135* was the closest, with a genetic distance of 1.6 ± 1.5 cM from *Pm6*. Ji et al. (2007) further discriminated the *Pm6*-carrying *T. aestivum*-*T. timopheevii* introgression lines using PCR-based molecular markers. The objectives of this study were to convert the RFLP marker *Xbcd135* into STS markers, and to evaluate those markers for application in marker-assisted selection of *Pm6* in wheat breeding programs.

Materials and methods

Plant materials

The Swedish Spring common wheat cultivar (cv.) Prins and eight introgression lines with *Pm6* (listed in Table 1) were provided by Dr. J. MacKey, Swedish Agricultural University, Uppsala. The lines were developed through backcrossing for seven to eight generations with the recurrent parent Prins. The donor lines of *Pm6* are listed in Table 1. Four wheat cultivars and lines carrying *Pm6* (Coker 747, Coker 983, Timgalen, and *T. timopheevii*) (Nyquist 1963; Leath and Heun 1990; Bennett and Kints 1983) were also used to assess the applicability of the markers for identifying *Pm6* in different wheat backgrounds. Prins was crossed with line IGV1-465. The resulting F_1 population was selfed and an F_2 population of 374 individuals was generated from which leaf tissue was harvested for genomic DNA extraction. The F_2 plants were grown to maturity and selfed; the responses of the F_3 families, viz. homozygous resistant, homozygous susceptible, or segregating for resistance were used to deduce F_2 genotypes.

A total of 175 commercial wheat varieties from different ecological areas of China, kindly provided by Mr Ruiqi Zhang, Nanjing Agricultural University, were used to confirm that the STS markers would be useful for marker assisted selection.

Evaluation of powdery mildew response

Materials evaluated for powdery mildew response included the eight introgression lines, four wheat cultivars and lines carrying *Pm6*, the recurrent parent Prins, and F_2 and F_3 progenies of IGV1-465 × Prins, the F_3 test comprising a progeny test of 20 seedlings from each F_2 individual. All of the plants were inoculated with a local *Blumeria graminis* mixture at the four-leaf stage in the greenhouse, and inoculations were repeated after 48 h. Host responses were

Table 1 *Pm6* near-isogenic lines

Designation	Pedigree	Gene
IGV1-448	Suwon 92/9*Prins	*Pm6*
IGV1-458	CI 13250/7*Prins	*Pm6*
IGV1-463	PI 170914/7*Prins	*Pm6*
IGV1-464	262618/7*Prins	*Pm6*
IGV1-465	FAO 163b/7*Prins	*Pm6*
IGV1-466	Kenya Lemphi 50-13596/7*Prins	*Pm6* + ?
IGV1-468	SC 60-5723/7*Prins	*Pm6*
IGV1-474	PI 170913*Prins	*Pm6*

recorded 7–14 days after inoculation when the susceptible control showed obvious disease symptoms and the resistant control was powdery mildew-free. The reactions of individual seedlings were rated as either resistant or susceptible. Chi-squared tests for goodness of fit were used to test for deviation of observed data from theoretically expected segregation ratios.

STS primer design

Barley probe BCD135 (773K14.2) derived from barley BAC clone AF474072 (Rostoks et al. 2002; Park et al. 2004) was downloaded from the NCBI website (http://www.ncbi.nlm.nih.gov/), and analyzed using the computer program SSRHUNTER developed by Li and Wan (2005). Three SSRs (simple sequence repeats) were identified within the BCD135 sequence, and four STS primers were designed using Primer 3 Software (http://www.genome.wi.mit.Edu/cgi-bin/primer/primer3.cgi). The primers were synthesized at Genebase Biotechnology Co. Ltd., Shanghai. The sequences and annealing temperatures of the four primers are shown in Table 2.

Marker analysis

STS primers linked to *Pm6* were identified by bulked segregant analysis (BSA). Resistant and susceptible bulks were made by pooling equal amounts of DNA from ten resistant and susceptible F_2 plants, respectively.

DNA extraction was according to Qi et al. (1997) and PCR amplifications were performed in reaction mixture volumes of 25 μl, each containing 1 × PCR buffer, 1.8 mM $MgCl_2$, 0.2 mM dNTPs, 0.24 μM of each STS primer, 80 ng template DNA and 1U Taq DNA polymerase (Takara Bio Inc., Japan). The PCR profile was: initial denaturation at 94°C for 4 min, 31 cycles at 94°C for 30 s, 56°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 1 min. The PCR analyses were carried out in a PE 9600 thermal cycler (Perkin Elmer, Norwalk, CT, USA), and the products were separated in 8% non-denaturing polyacrylamide gels run in a 1 × TBE buffer.

Linkage analysis was performed using MAPMAKER/Exp Version 3.0b (Lincoln et al. 1993). Map distances were determined using the Kosambi mapping function (Kosambi, 1944).

Results

Evaluation of powdery mildew reactions

A total of 374 F_2 individuals were obtained from the cross of (IGV1-465 × Prins), 279 were resistant, and 95 were susceptible, which was in agreement with the expected 3:1 segregation ratio for resistant and susceptible plants ($\chi^2_{3:1}$= 0.03, $P > 0.95$). Based on the F_3 data, the F_2 genotypes were 101 *Pm6Pm6*, 178 *Pm6pm6*, and 95 *pm6pm6*, an acceptable fit to a 1:2:1 segregation ($\chi^2_{1:2:1}$= 1.06, $P > 0.50$).

The recurrent parent Prins was susceptible, and the various introgression lines showed resistant reactions.

Linkage of the STS markers with *Pm6*

BSA was used to determine the relationship of the four markers with *Pm6*. No polymorphisms occurred

Table 2 Details for the BCD135-derived STS primers

Marker name	Left primer	Right primer	Annealing temperature Tm (°C)[a]	SSR Motif
$NAU/STS_{BCD135-1}$	ATTTGGATGAGGCAAAGGTG	TCTGCTGGTCCTCTGATGTG	55	$(AAG)_7$ $(AAG)_5$
$NAU/STS_{BCD135-2}$	GCTCCGAAGCAAGAGAAGAA	TCTGCTGGTCCTCTGATGTG	55	$(AAG)_5$
STS003	ATTTGGATGAGGCAAAGGTG	TTTCTTCACCTTCTTCTCTTGCTT	55	$(AAG)_7$
STS004	GGGCCTCTTCTTCTGCTTCT	GGCGGTTGAGGAGGTAGAAC	60	$(CCG)_5$

[a] Tm = annealing temperature

between the two parents and the two pools using STS003 and STS004 as primers. However, primers NAU/$STS_{BCD135-1}$ and NAU/$STS_{BCD135-2}$ amplified bands polymorphic between IGV1-465 and Prins and between the two bulks. NAU/$STS_{BCD135-1}$ amplified two bands (300 and 350 bp), present in the resistant lines and the resistant bulk, but absent in the susceptible lines and the susceptible bulk (Fig. 1). Primer NAU/$STS_{BCD135-2}$ amplified one polymorphic band (230 bp), again present in the resistant lines and the resistant bulk, but absent in the susceptible lines and the susceptible bulk (Fig. 2).

To determine the genetic linkage of the STS markers with the *Pm6* gene, NAU/$STS_{BCD135-1}$ and NAU/$STS_{BCD135-2}$ were further used to screen the entire F_2 population (Fig 3). Both markers behaved identically. Bands were observed in all 278 homozygous resistant and heterozygous plants, and in two of the 95 homozygous susceptible plants. Linkage analysis indicated a genetic distance of 0.8 cM between the markers and *Pm6*.

Practicability of NAU/$STS_{BCD135-2}$ in MAS for powdery mildew resistance

In order to evaluate the applicability of the STS markers for marker assisted selection of *Pm6* in breeding programs, a total of 175 commercial varieties or elite lines were amplified using NAU/$STS_{BCD135-2}$. The results indicated that none showed the specific 230 bp band, which was present in *T. timopheevii* and IGV1-465 (Fig. 4), thus indicating that this marker could be used in MAS of *Pm6* over a wide range of genetic backgrounds.

Discussion

Bennett (1984) reported that *Pm6* was moderately effective and best expressed from the three-leaf stage onwards, but was also recognizable at the first-leaf stage. In preliminary experiments we inoculated the Prins materials at various growth stages from the second to fifth leaves and found that the fourth leaf stage was optimal for clear differences between resistant and susceptible genotypes. This growth stage was then used in our genetic experiments. *Pm6* was inherited as a single Mendelian factor.

The precision of the measured genetic distance is related to the number of individuals in the mapping population. Primary genetic maps are typically based on 50–100 individuals, permitting the detection of recombination between markers 1 and 3 cM apart. For experiments requiring a higher degree of precision, additional individuals must be analyzed. Compared with Tao et al. (2000) and Wang et al. (2000), in which only 73 F_2 individuals were analyzed, we used 374 F_2 genotypes in the present research. Therefore our estimate of genetic distance should be more precise.

Pm6 was introduced into common wheat from *T. timopheevii* and its incorporation into the wheat genome involved recombination between related B and G chromosomes. Due to the differentiation of the 2G segment and the equivalent region of chromosome 2B, recombination near *Pm6* may be reduced. Thus the physical distance between the marker and *Pm6* might be larger than suggested by the recombination value. For map-based cloning of *Pm6*, a larger F_2 mapping population (such as more than 1,000 F_2 plants) could be generated by crossing the smallest introgression (IGV1-465) with Prins, followed by fine mapping for further recombinants using

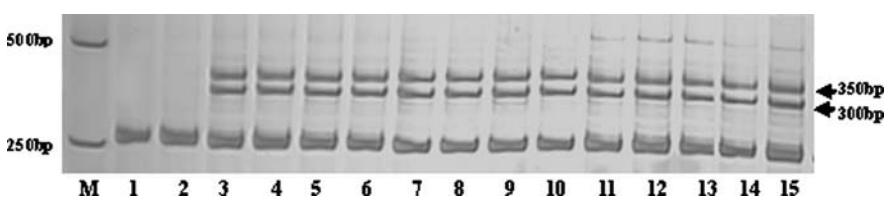

Fig. 1 PCR Results using primer NAU/$STS_{BCD135-1}$ (arrows show the polymorphic bands) M: the size standard marker, to the *left* is the marker band size in bp, Lanes 1–15 represent Prins, S pools from (IGV1-465 × Prins) F_2, R pools from (IGV1-465 × Prins) F_2, IGV1-448, IGV1-458, IGV1-463, IGV1-464, IGV1-465, IGV1-466, IGV1-468, IGV1-474, Coker747, *T. timopheevii*, Coker983, Timgalen, respectively

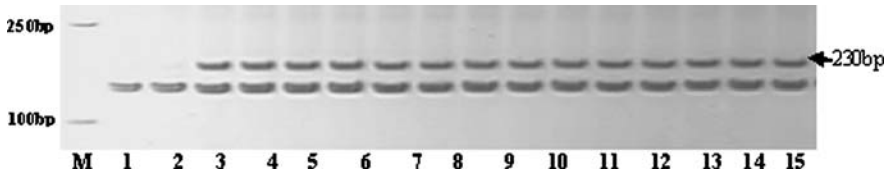

Fig. 2 PCR Results using primer NAU/STS$_{BCD135-2}$ (arrows show the polymorphic bands) M: the size standard marker, to the *left* is the marker band size in bp, Lanes 1–15 represent Prins, S pools from (IGV1-465 × Prins) F_2, R pools from (IGV1-465 × Prins) F_2, IGV1-448, IGV1-458, IGV1-463, IGV1-464, IGV1-465, IGV1-466, IGV1-468, IGV1-474, Coker747, *T. timopheevii*, Coker983, Timgalen, respectively

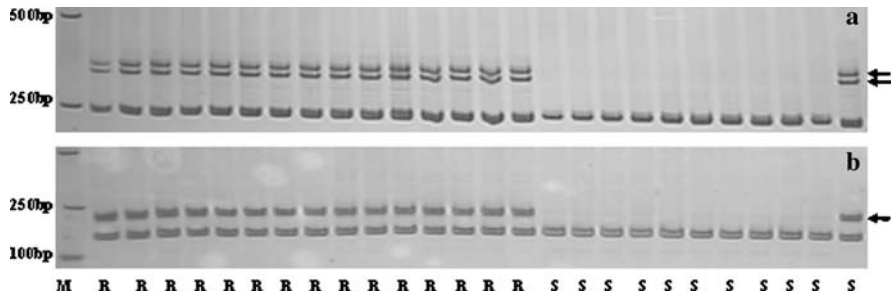

Fig. 3 PCR results using primers NAU/STS$_{BCD135-1}$ (**a**) and NAU/STS$_{BCD135-2}$ (**b**) on the IGV1-465 × Prins F_2 population (arrows show the polymorphic bands). R represents the resistant F_2 strain and S represents the susceptible F_2 strain

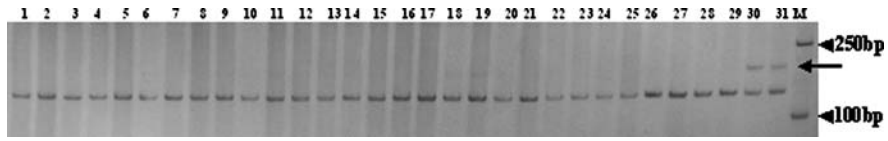

Fig. 4 PCR results using primer NAU/STS$_{BCD135-2}$ on 28 commercial varieties or elite lines and controls.M: the size standard marker (lane 32), to the *right* is the marker band size in bp, Lanes 1–31: 1. Ailiduo, 2. Zhengzhou 24, 3. Nongda 183, 4. Youbao, 5. Jinan 12, 6. Funo, 7. Taishan 5, 8. Yannong 15, 9. Xiaoyan 6, 10. Shannongfu 63, 11. Lumai 10, 12. Xuzhou 21, 13.Yumai 21, 14. Shan 229, 15. Jinan 17, 16. Zheng 9023, 17. Huanyin 9628, 18. Xiaoyan 54, 19. Yuanfeng 898, 20. Jimai 20, 21. Xuzhou 438, 22. Shannong 6521, 23. Bainong 3217, 24. Nanyang 756, 25. Xiannong 151, 26. Chinese Spring, 27. Yangmai 5, 28. 92R137, 29. Prins, 30. IGV1-465, 31. *T. timopheevii* (arrow shows the polymorphic band. Arrow heads show the fragment size)

the STS marker isolated in the present study as one of the markers for *Pm6*. Even then, the remaining *T. timopheevii* segment may be physically too large and alternative procedures such as resistance gene analogues or subtractive hybridization might be more rewarding.

To facilitate the use of molecular markers in large-scale screening, it is necessary to convert RFLP markers into PCR-based markers. Talbert et al. (1994) successfully converted RFLP probes of wheat into STS markers. Later, many laboratories attempted to generate STS primers from either wheat genomic clones (Feuillet et al. 1995; Talbert et al. 1996; Seyfarth et al. 1999; Ma et al. 2004), RAPD PCR products (Hu et al. 1997; Naik et al. 1998; Dweikat et al. 2002) or AFLP bands (Parker and Langridge 2000; Prins et al. 2001; Smith et al. 2002). The conversion of heterologous probes, such as barley clones or oat clones, is more difficult because sequence polymorphisms may exist between the homoeologous loci of wheat and barley or oat. In addition, the amplified products may not originate from the same loci where the RFLP probes originally mapped. Byran et al. (1997) pointed out that PCR primers based on wheat cDNA have a tendency to produce several PCR products in similar size. In this paper, we firstly designed STS primers according to the three SSR loci found in the sequence of BCD135. This method for the STS primer design combined the advantages of the RFLP, SSR and STS.

Pm6 is an effective resistance gene in many powdery mildew epidemic areas of China, especially for adult resistance in the field. However, lines with *Pm6* may be susceptible at the seedling stage, making

it difficult to identify the presence of *Pm6* in seedling tests. The strategy of marker-assisted selection is useful for combining resistance genes in a single genotype. Such a strategy is thought likely to increase the durability of resistance, but requires that the individual genes not to be used simultaneously in other cultivars. We found that the specific PCR products were only amplified in *Pm6*-carrying genotypes, indicating that NAU/$STS_{BCD135-2}$ should be helpful for marker assisted selection of plants with *Pm6*.

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References

Bennett FGA (1984) Resistance to powdery mildew in wheat: a review of its use in agricuture and breeding programmes. Plant Pathol 33:279–300

Bennett FGA, van Kints T (1983) Mildew of wheat U.K. Cereal Pathogen Virulence Survey Annual Report. National Institute of Agricultural Botany, Cambridge, UK, pp 7–21

Bryan GK, Collins AJ, Stephenson P, Orry A, Smith JB, Gale MD (1997) Isolation and characterization of microsatellites from hexaploid bread wheat. Theor Appl Genet 94:557–563

Cai SB, Cheng SH, Wu JZ, Yan W (2005) Evaluation, improvement and utilization of introducted wheat reserve resource resistant to powdery mildew. Acta Tritical Crops 25:116–120

Dweikat I, Zhang W, Ohm H (2002) Development of STS markers linked to Hessian fly resistance gene *H6* in wheat. Theor Appl Genet 105:766–770

Feuillet C, Messmer M, Schachermayr G, Keller B (1995) Genetic and physical characterization of the *Lr1* leaf rust resistance locus in wheat (*Triticum aestivum* L.). Mol Gen Genet 248:553–562

Hsam SLK, Lapochkina IF, Zeller FJ (2003) Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). 8. Gene *Pm32* in a wheat-*Aegilops speltoides* translocation line. Euphytica 133:367–370

Hu XY, Ohm HW, Dweikat I (1997) Identification of RAPD markers linked to the gene *Pm1* for resistance to powdery mildew in wheat. Theor Appl Genet 94:832–840

Ji JH, CAO AZ, Wang HY, Qin B, Wang SL, Kong F, Chen PD, Liu DJ, Wang XE (2007) Discrimination of Triticum aestivum-T. timopheevii introgression lines using PCR-based molecular markers. Hereditas (Beijing) (In Press) (In Chinese with English abstract)

Jorgensen JH, Jensen CJ (1972) Genes for resistance to wheat powdery mildew in derivatives of *Triticum timopheevi* and *T carthlicum*. Euphytica 21:121–128

Jorgensen JH, Jensen CJ (1973) Gene *Pm6* for resistance to powdery mildew in wheat. Euphytica 22:423

Kameswara Rao K, Lakshminarasu M, Jena KK (2002) DNA markers and marker-assisted breeding for durable resistance to bacterial blight disease in rice. Biotechnol Adv 20:33–47

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Leath S, Heun M (1990) Identification of powdery mildew resistance genes in cultivars of soft red winter wheats. Plant Dis 74:747–752

Li Q, Wan JM (2005) SSRHunter. Development of a local searching software for SSR sites. Hereditas (Beijing) 27:808–810

Lincoln SE, Daly MJ, Lander ES (1993) Constructing linkage maps with MAPMAKER/Exp Version 3.0. A tutorial reference manual, 3rd edn. Whitehead Institute for Medical Research, Cambridge, MA

Ma ZQ, Wei JB, Cheng SH (2004) PCR-based markers for the powdery mildew resistance gene *Pm4a* in wheat. Theor Appl Genet 109:140–145

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers WJ, Appels R (2003) Catalogue of gene symbols for wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp vol 4. Instituto Sperimentale per la Cerealcoltura, Rome

Miranda LM, Murphy JP, Marshall D, Leath S (2006) *Pm34*: a new powdery mildew resistance gene transferred from *Aegilops tauschii* Coss. to common wheat (*Triticum aestivum* L.). Theor Appl Genet 113:1497–1504

Naik S, Gill KS, Prakasa Rao VS, Gupta VS, Tamhankar SA, Pujar S, Gill BS, Ranjekar PK (1998) Identification of a STS marker linked to the *Aegilops speltoides*-derived leaf rust resistance gene *Lr28* in wheat. Theor Appl Genet 97:535–540

Nyquist WE (1963) Inheritance of powdery mildew resistance in hybrids involving a common wheat strain derived from *Triticum timopheevi*. Crop Sci 3:40–43

Olson M, Hood L, Cantor C, Botstein D (1989) A common language for physical mapping of the human genome. Science 245:1434–1435

Park YJ, Dixit A, Yoo JW, Bennetzen J (2004) Further evidence of microcollinearity between barley and rice genomes at two orthologous regions. Mol Cells 17:492–502

Parker GD, Langridge P (2000) Development of a STS marker linked to a major locus controlling flour colour in wheat (*Triticum aestivum* L.). Mol Breed 6:169–174

Prins R, Groenewald JZ, Marais GF, Snape JW, Koebner RMD (2001) AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. Theor Appl Genet 103:618–624

Qi LL, Wang SL, Chen PD, Liu DJ, Friebe B, Gill BS (1997) Molecular cytogenetic analysis of *Leymus racemosus* chromosomes added to wheat. Theor Appl Genet 95:1084–1091

Rostoks N, Park YJ, Ramakrishna W, Ma J, Druka A, Shiloff BA, SanMiguel PJ, Jiang Z, Brueggeman R, Sandhu D, Gill K, Bennetzen JL, Kleinhofs A (2002) Genomic sequencing reveal gene content, genomic organization, and recombination relationships in barley. Funct Integr Genomics 2:51–59

Seyfarth R, Feuillet C, Schachermayr G, Winzeler M, Keller B (1999) Development of a molecular marker for the adult plant leaf rust resistance gene *Lr35* in wheat. Theor Appl Genet 99:554–560

Smith PH, Koebner RMD, Boyd LA (2002) The development of a STS marker linked to a yellow rust resistance derived from the wheat cultivar Moro. Theor Appl Genet 104:1278–1282

Talbert LE, Blake NK, Chee PW, Blake TK, Magyar GM (1994) Evaluation of "sequence-tagged-site" PCR products as molecular markers in wheat. Theor Appl Genet 87:789–794

Talbert LE, Bruckner PL, Smith LY, Sears R, Martin TJ (1996) Development of PCR markers linked to resistance to wheat streak mosaic virus in wheat. Theor Appl Genet 93:463–467

Tao WJ, Liu JY, Liu DJ, Chen PD (2000) Genetic mapping of the powdery mildew resistance gene *Pm6* in wheat by RFLP analysis. Theor Appl Genet 100:564–568

Wang XY, Qi ZJ, Chen PD, Liu DJ (2000) Identification of RAPD markers tightly linked to wheat powdery mildew resistance gene *Pm6*. Acta Genetica Sin 27:1072–1079

Weeden NF, Tierman GM, Hemmat M, Kneen BE, Lodhi MA (1992) Inheritance and reliability of RAPD markers. In: Hoisington D, McNab A (eds) Proc symp on application of RAPD technology to plant breeding (Joint Plant Breeding Symp. Ser.), CSSA, ASHS, AGA, Minneapolis, pp 31–35

Zhu ZD, Zhou RH, Kong XY, Dong YC, Jia JZ (2005) Microsatellite markers linked to two genes conferring resistance to powdery mildew in common wheat introgressed from *Triticum carthlicum* acc. PS5 Genome 48:585–590