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Identification of SRAP and RGA markers linked to powdery mildew (*Blumeria graminis*) resistance gene *PmZB90* in common wheat

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Abstract

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (Bgt), is one of the major diseases of common wheat (*Triticum aestivum* L.) worldwide. Easy-to-use and more closely linked markers can accelerate the use of powdery mildew resistance genes in the genetic improvement for this disease resistance. Thus, the sequence-related amplified polymorphism (SRAP) and the resistance gene analog (RGA) techniques combined with the bulk segregant analysis (BSA) were employed to develop tightly linked and usable molecular markers of the *PmZB90* gene, which confers good resistance to powdery mildew in common wheat. Two hundred and forty SRAP and 11 RGA primer combinations were used to test the 144 $F_{2:3}$ families of the *PmZB90*. At the result, 2 SRAP markers Me5/Em5 and Me8/Em16 and 1 RGA marker R11F/R11R linked to the *PmZB90* with the genetic distances of 12.9cM, 9.7cM and 9.2cM, respectively. Then, Me8/Em16 and R11F/R11R were used to amplify 31 wheat cultivars/lines. The results showed that Me8/Em16 can be effectively applied in the marker-assisted selection (MAS) for the powdery mildew resistance gene *PmZB90* in wheat breeding programmes.

Keywords: *Triticum aestivum*; powdery mildew resistance; *PmZB90*; SRAP; RGA.
Abbreviations: Pm_powdery mildew; SRAP_sequence-related amplified polymorphism; RGA_resistance gene analog; BSA_bulk segregant analysis; MAS_marker-assisted selection.

Introduction

Powdery mildew (*Blumeria graminis* (DC) E.O. Speer f. sp. *tritici*, Bgt) is one of the most important diseases of wheat. Controlling the disease through usage of chemicals besides being costly to the farmer is also harmful to the environment. Whereas incorporation of the resistance genes is eco-friendly way and does not place any cost burden on the growers. At present, more than 60 powdery mildew resistance gene on 42 resistance loci (*Pm1-Pm46*, *Pm18=Pm1c*, *Pm22=Pm1e*, *Pm23=Pm4c*, *Pm31=Pm21*) have been identified on different chromosomes (Huang et al., 2004; McIntosh et al., 2006; Miranda et al., 2006; Luo et al., 2009; He et al., 2009; Ma et al., 2011; Alam et al., 2011; Xie et al., 2012; Gao et al., 2012). Though many resistance genes have been introgressed but many of them have lost their resistance because of variation in the prevalent *Bgt* races. Because of the changing nature of the races, the identification and utilization of new broad-spectrum resistance genes continues to be the most effective and economical approach to control this disease (Yi et al., 2007; Ma et al., 2011). Nowadays, various molecular markers have been widely used in plant genetic mapping and marker-assisted selection (MAS), such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), sequence tagged site (STS), simple sequence repeat (SSR), inter-simple sequence repeat (ISSR) (Ma et al., 2004; Miranda et al., 2006). For example, RAPD are PCR-based dominant markers revealed using a single DNA primer with random sequences of usually 10 bases, indicating the simplicity of RAPD markers, but the relatively low reproducibility limits their use. Because of the radioactivity and complexity, RFLP markers need to be transferred to STS markers for MAS. SSRs are co-dominant markers, however, the development of SSR markers is time consuming and costly. AFLP markers have been used for gene mapping and cloning but the operation is very complex (Yi et al., 2008). Each of these marker types has its own advantages and limitations. To improve few shortcomings of these markers, two kinds of markers namely resistance gene analog (RGA) (Xie et al., 2004; Chen et al., 2006; McFadden et al., 2006) and sequence-related amplified polymorphism (SRAP) were developed. Resistance gene analogs, which have been demonstrated to be closely linked to known resistance genes, can be helpful for mapping and isolation resistance gene.

Sequence-related amplified polymorphism that targets coding sequences in the genome and results in a moderate number of co-dominant markers (Li and Quiros, 2001), combines simplicity, reliability, moderate throughput ratio and facile sequencing of selected bands. SRAP markers were used to amplify genomic DNA and cDNA to show their broad applications such as in constructing a detailed linkage map of *Brassica oleracea* (Okazaki et al., 2007), mapping QTL for cotton fibre quality (Lin et al., 2005) and the genes for lateral branch traits in cucumber (Wang et al., 2005) as well as analysing the genetic diversity in plants (Ferriol et al., 2003).

Table 1. Reaction of F_2 population and F_{2-3} progenies of ZB90 × SF42 to isolate Bgt5.

Cross	Isolates	F_2 population		F_3 progeny test	
		R:rr	$\chi^2_{3:1}$	RR: Rr:rr	$x^2_{1:2:1}$
ZB90 × SF42	Bgt5	112:32	0.45	30:81:33	2.38

RR=homozygous resistant; Rr=heterozygous resistant; rr=homozygous susceptible; $\chi^2_{0.05,1}$=3.84; $\chi^2_{0.05,2}$=5.99.

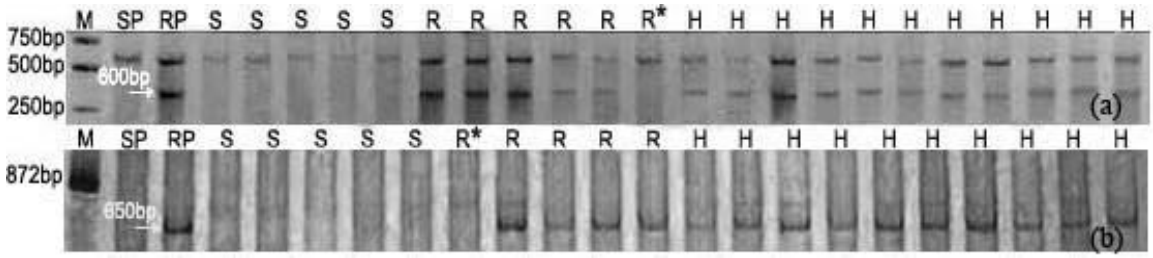

Fig 1. PCR bands of the F_2 population amplified by SRAP markers ME8/Em16-600(a) and ME5/Em5-650(b). M: DNA Marker; SP: SF42; RP: ZB90; S: susceptible plant; R: homozygous resistant plant; H: heterozygous resistant plant; *= Recombinant plant. Arrows indicated the 1102bp band linked to *PmZB90* gene.

Present study deals with development of these markers for newly identified powdery mildew gene. Chinese native wheat variety ZB90 confers a good resistance to Bgt at both the seedling and adult stages. The powdery mildew resistance gene from ZB90 has been characterized and molecular mapped with SSR markers (Yi et al., 2007). In the present studies, SRAP and RGA markers were screened for developing more tightly linked and easy-to-use markers of the powdery mildew resistance gene *PmZB90*. Then, they will be expected for using in the MAS programs and gene pyramiding in wheat.

Results and discussion

Powdery mildew tests

The resistant parent ZB90 was immune to the Bgt isolate Bgt5 and the susceptible parent SF42 was highly susceptible with a score of 4. To test the inheritance of the ZB90, 144 F_2 individuals from the cross ZB90 × SF42 were challenged by the Bgt5 with detached-leaf-segment at the seedling stage. The result showed that of 144 individuals 112 were resistant and 32 were susceptible (Table 1), which fit the expected 3:1 ratio on the basis of the χ^2-test ($\chi^2_{3:1}$ = 0.45, df = 1, P = 0.50). To determine the genotypes of the F_2 plants, their derived F_3 lines were tested in the greenhouse with Bgt5. The result indicated that 30 families were homozygous susceptible (IT=4), 81 families were heterozygous resistant (IT=1) and 33 families were homozygous resistant (IT=0 to 0;), which fit a 1:2:1 segregation ratio ($\chi^2_{1:2:1}$ = 2.38, df = 2, P = 0.31). These results showed that the *PmZB90* was a single dominant gene. It is necessary to search for novel disease resistance genes and combine multiple resistance genes into each cultivar, in wheat breeding programs, in order to be able to protect wheat yields and secure the world's food supply (Xie et al., 2012). Therefore, the powdery mildew resistance gene *PmZB90* could be developed a series of introgression lines by crossing ZB90 with other cultivars by marker-assisted selection (MAS).

SRAP markers analysis

To identify markers linked to the *PmZB90*, SRAP analysis was carried out with BSA. A total of 240 primer combinations were tested in the two bulks. The size of SRAP amplified fragments ranged from 50 to 2000bp. The result showed that 16 primer combinations yielded polymorphic amplicons between the resistant and the susceptible bulks. However, only 2 primer combinations Me5/Em5 and Me8/Em16, which amplified 650-bp and 600-bp polymorphic bands respectively, showed linkage with the *PmZB90* (Fig.1a, 1b). The polymorphic bands are designated with the primer combination and the fragment size (Yi et al., 2008). $Me5/Em5_{-650}$ indicates that the polymorphic fragment is generated using the primer combination Me5/Em5 with a size of 650bp and $Me8/Em16_{-600}$ indicates that the polymorphic fragment is generated using the primer combination Me8/Em16 with a size of 600bp. SRAP markers combine reliability and genomic abundance with high levels of polymorphism. They do not require sophisticated DNA extraction methods and are ideally suited for high-throughput automated scoring and multiplexing. Thus, MAS using SRAP markers will likely become a valuable tool in wheat breeding (Yi et al., 2008). In the current study, two SRAP linked markers were dominant markers, the resistance-related band were clear and would also be interesting to investigate the resistance gene.

RGA markers analysis

Among the 11 RGA primer pairs, 3 primer pairs could amplify ploymorphisms between SF42 and ZB90, but only one primer pair R11F/R11R showed linked to the *PmZB90* with a genetic distance of 9.2cM when tested with the 144 F_2 plants. An RGA fragment with 1102 bp was generated only in the resistant parent and most of resistant plants, but absent in the susceptible parents and F_2 plants (Fig.2). The markers were linked to the resistance locus and the distances were shown in Fig.3. Accumulation of a considerable number of sequences related to the resistance genes (R-genes) allowed the identification of candidate R-gene sequences (Bozkurt et al., 2007). In the chromosome of wheat, more RGAs were found since the genome sequence could be accessed. This would help us to explore the exact position of the *PmZB90*.

Mapping of PmZB90

All three markers were used to construct the genetic map of the *PmZB90* (Fig.3). The SRAP marker $Me8/Em16_{-600}$ was mapped on one side of the *PmZB90* gene locus with a genetic distance of 9.7cM, whereas the RGA marker RGA_{-1102} and the SRAP marker $Me5/Em5_{-650}$ were located on other side of

Table 2. Marker detection of wheat cultivars/lines possessing different *Pm* genes.

Wheat lines	*Pm* gene	Markers	
		RGA$_{-1102}$	*Me8/Em16*$_{-600}$
Yumai13	no	-	-
Yumai49	no	-	-
Bainong3217	no	-	-
Chancellor	no	-	-
Axminster/8*Cc	*Pm1*	-	-
Ulka/8*Cc	*Pm2*	-	-
Asosan/8*Cc	*Pm3a*	-	-
CI14121	*Pm3b*	-	-
Chul/8*Cc	*Pm3b*	-	-
Sonora/8*Cc	*Pm3c*	-	-
Khapli/8*Cc	*Pm4a*	-	-
Yuma/8*Cc	*Pm4a*	-	-
VPM	*Pm4b*	-	-
CI14125	*Pm5*	-	-
Timgalen	*Pm6*	+	-
Coker747	*Pm6*	-	-
CI14189	*Pm7*	-	-
PI361879	*Pm8*	-	-
96-282	*Pm13*	-	-
Amigo	*Pm17*	-	-
96-286	*Pm19*	-	-
96-287	*Pm20*	-	-
6VS/6AL	*Pm21*	-	-
81-7241	*Pm23*	-	-
Chiyacao	*Pm24*	-	-
Normandie	*Pm1+2+9*	-	-
Arthur	*Pm2+6*	-	-
Coker983	*Pm5a+6*	-	-
2654-7R	*PmDR147*	+	
Am9	*Pm33*	-	-
ZB90	*PmZB90*	+	+

Cc= Chancellor; Pm= Powdery mildew; += present; -= absent.

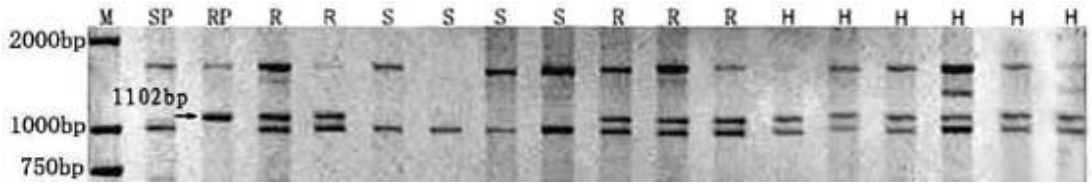

Fig 2. PCR bands of the F_2 population amplified by RGA marker RGA-1102. M: DNA Marker; SP: SF42; RP: ZB90; S: susceptible plant; R: homozygous resistant plant; H: heterozygous resistant plant; Arrows indicated the polymorphic bands linked to *PmZB90* gene.

the *PmZB90* gene locus, with a linkage distance of 9.2cM and 12.9cM, respectively.

Applicability of markers

Pyramiding or combination of additional resistance genes to virulent pathotypes should be considered to provide durable resistance in breeding practice (Hua et al., 2009). In order to investigate the validation of markers Me8/Em16$_{-600}$ and RGA$_{-1102}$ for MAS, 4 cultivars/lines without any known *Pm* genes and 27 cultivars/lines with known *Pm* genes or *Pm* gene combinations were amplified using primer pairs Me8/Em16 and R11F/R11R. The 1102 bp RGA fragment was produced only in ZB90 (*PmZB90*), 2654-7R (*PmDR147*) and Timgalen (*Pm6*) by primer pair R11F/R11R. The 600 SRAP fragment was produced only in ZB90 (*PmZB90*) by primer pair (Me8/Em16). Therefore, marker Me8/Em16$_{-600}$ can be used in combination with RGA$_{-1102}$ to detect the resistance gene *PmZB90* in wheat. It would greatly increase the efficiency of the *PmZB90*. Molecular markers that are closely linked to these genes have greatly facilitated their utilization in breeding programs (Ma et al., 2011). The markers Me8/Em16$_{-600}$ and RGA$_{-1102}$ would provide a powerful tool for combination of *PmZB90* and other valid genes in one cultivar, which could provide the durable resistance for wheat breeding.

Materials and methods

Plant materials

A total of 144 F_2 individuals derived from the cross of powdery mildew resistance variety ZB90 and susceptible

Table 3. List of RGA primer pairs used in the study.

Primer name	Sequence (5'-3')	Conserved region
S2	5'-GGTGGGGTTGGGAAGACAACG-3'	NBS
A2	5'-CAACGCTAGTGGCAATCC-3'	NBS
S3	5'-GGNATGGGNGGNTTNGGNAARACNCAN-3'	P-loop
AS3	5'-TCNCGNATNATNTTNACNACNCGN-3'	Kinase-3a
F	5'-GGNATGGGNGGNNTNGGNAA(A/G)CANAC-3	Kinase-1a
R	5'-NCA(T/A)TTNAGNGCNAGNGGNAGNCC-3'	Domain2
R11F	5'-AACCCAATTCCACCTCTTTTACA-3'	NBS-LRR
R11R	5'-TTCCCCTTGCAATAGTCACCATAG-3'	NBS-LRR
R2F	5'-CTATGGTGACTATTGCAAGGGGAA-3'	NBS-LRR
R2R	5'-ATTGTGATTGATGGCATGTCTACG-3'	NBS-LRR
OF	5'–CCTCGATGCAATAACTAATTT-3'	NBS
OR	5'-TCTGTTTGCATCATCAATGT-3'	NBS
NF	5'-TAGGGCCTCTTGCTACGT-3'	LRR
NR	5'-TATAAAAAGTGGCGGACT-3'	LRR
3LF	5'-CCTT(G/T)CCTT(C/A)GAGCTTTGTAT-3'	NBS-LRR
3LR	5'-GCTTCCTTTGCCTCCCC(A/C)AC-3'	NBS-LRR
R4-2F	5'-CAGCAGCCTAAGATTCCTCCTA-3'	LRR
R4-2R	5'-TGTGCAGAACCTCCAATGATAC-3'	LRR
R4-3F	5'-AGGGCTTGCAAATATTAGACCTC-3'	Kinase
R4-3R	5'-CTCTAGCAGATGTTTGTGTGTGC-3'	Kinase
R4-4F	5'-ACCTCTCTGGCACAATGATAAAA-3'	LRR
R4-4R	5'-AGACTTGGCCTTGTTCATCATAA-3'	LRR

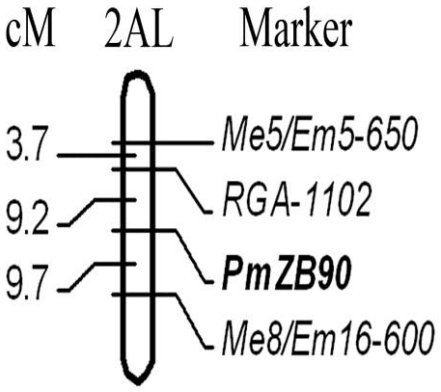

Fig 3. Genetic linkage map of *PmZB90* and the linked markers on wheat chromosome 2AL

variety SF42 were selected for genetic analysis of the powdery mildew resistance. 10 resistant plants and 10 susceptible plants from F_2 segregating population were mixed respectively to produce resistant and susceptible bulks for bulked segregation analysis (BSA). Some materials carrying the known *Pm* genes (Table 1) were kindly provided by Dr. Y.L. Zhou, Institute of Plant Protection, and Dr. Z.D. Zhu, Institute of Crop Science, Chinese Academy of Agricultural Sciences. Total genomic DNA was extracted from young leaf tissue using CTAB method, as previously described by Al-Humaid et al. (2011).

Powdery mildew resistance evaluation

One prevailing local Bgt isolate Bgt5 was used to test the powdery mildew resistance of the ZB90, the SF42 and the F_2 plants and $F_{2:3}$ families derived from ZB90 × SF42. The methods and conditions of incubation were according to Yi et al. (2008). The disease assessments were according to Huang et al. (2003). Powdery mildew resistance reactions were classified into two groups, with resistant (IT = 0, 0; and 1) and susceptible (IT=2-4) infection types. Chi-square tests for goodness of fit were used for the deviation of observed data from theoretically expected segregation ratios.

Molecular markers analysis

A total of 240 primer combinations for SRAP were used for screening of polymorphism between the two bulks at first. The polymorphic markers between the bulks were further checked for their linkage to the resistance gene in the F_2 mapping population consisting of 144 plants. PCR content and reaction conditions were same as Yi et al., (2008). Primer combinations used for linkage analysis were:
Me5: 5′-TGAGTCCAAACCGGAAG-3′
Em5: 5′-GACTGCGTACGAATTAAC-3′
Me8: 5′-TGAGTCCAAACCGGTGC-3′
Em16: 5′- GGCTTGAACGAGTGACTGA-3′

Based on the conserved sequences of known resistance genes, eleven pairs of degenerate primers (Table 3) were designed for amplifying the RGAs between two bulks at first. The polymorphic markers between the bulks were further checked for their linkage to the resistance gene with the 144 F_2 plants. PCR reaction contained 60ng of template DNA, 0.4μmol/L of primers, 0.15mmol/L of dNTPs, 2.5mmol/L of $MgCl_2$, 2.5μl of 10× PCR buffer and 1U of Taq DNA polymerase in a total volume of 25μl. The amplification was programmed at 94°C for 3min, followed by 35 cycles at 94°C

for 40sec, 50°C-60°C (depending on the different primers) for 1 min and 72°C for 1 min, with a final extension at 72°C for 5min. The 1.5% agarose gels (containing ethidium bromide) was used to separate the PCR products, and visualized by UV light.

Linkage analysis

The linkage relationship between markers and the resistance gene *PmZB90* was analyzed using Mapmaker/Exp Version 3.0 (Lander et al., 1987). The Kosambi function was used for calculating the genetic distance, and a LOD score of 3.0 was used as the threshold for declaration of linkage.

Conclusion

Me5/Em5$_{-650}$ and *Me8/Em16*$_{-600}$ were associated with resistance gene *PmZB90* and the genetic distances were 12.9cM and 9.7cM, respectively. *RGA*$_{-1102}$ had close genetic distance (9.2cM) to *PmZB90*. Combination of the linked markers Me8/Em16$_{-600}$ and RGA-$_{1102}$ could be used for marker-assisted selection of the resistance gene *PmZB90* in wheat breeding programmes.

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References

Al-Humaid A, Ibrahim GH, Motawei MI (2011) Molecular identification of some turfgrass cultivars and their resistance to *Fusarium graminearum*. Aust J Crop Sci 5: 1754-1759.

Alam MA, Xue F, Wang CY, Ji WQ (2011) Powdery mildew resistance genes in wheat: identification and genetic analysis. J Mol Biol Res 1: 20-39.

Bozkurt O, Hakki EE, Akkaya MS (2007) Isolation and sequence analysis of wheat NBS-LRR type disease resistance gene analogs using degenerate PCR primers. Biochem Genet 45: 469-486.

Chen YP, Wang HZ, Cao AZ, Wang CM, Chen PD (2006) Cloning of a resistance gene analog from wheat and development of a codominant PCR marker for *Pm21*. J Integr Plant Biol 48: 715-721.

Ferriol M, Pico B, Nuez F (2003) Genetic diversity of a germplasm collection of Cucurbita pepo using SRAP and AFLP markers. Theor Appl Genet 107: 271-282.

Gao H, Zhu F, Jiang Y, Wu J, Yan W, Zhang Q, Jacobi A, Cai S (2012) Genetic analysis and molecular mapping of a new powdery mildew resistant gene *Pm46* in common wheat. Theor Appl Genet 125: 967-973.

He RL, Chang ZJ, Yang ZJ, Yuan ZY, Zhan HX, Zhang XJ, Liu JX (2009) Inheritance and mapping of powdery mildew resistance gene *Pm43* introgressed from *Thinopyrum intermedium* into wheat. Theor Appl Genet 118: 1173-1180.

Hua W, Liu ZJ, Zhu J, Xie CJ, Yang T, Zhou YL, Duan XY, Sun QX, Liu ZY (2009) Identification and genetic mapping of *Pm42*, a new recessive wheat powdery mildew resistance gene derived from wild emmer (*Triticum turgidum var. dicoccoides*). Theor Appl Genet 119: 223-230.

Huang XQ, Röder MS (2004) Molecular mapping of powdery mildew resistance genes in wheat: a review. Euphytica 137: 203-223.

Huang XQ, Wang LX, Xu MX, Röder MS (2003) Microsatellite mapping of the powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestevum* L.). Theor Appl Genet 106: 858-865.

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg LA (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174-181.

Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor Appl Genet 103: 455-461.

Lin Z, He D, Zhang X, Nie Y, Guo X, Feng C, Stewart JM (2005) Linkage map construction and mapping QTL for cotton fibre quality using SRAP, SSR and RAPD. Plant Breeding 124: 180-187.

Luo PG, Luo HY, Chang ZJ, Zhang HY, Zhang M, Ren ZL (2009) Characterization and chromosomal location of *Pm40* in common wheat: a new gene for resistance to powdery mildew derived from *Elytrigia intermedium*. Theor Appl Genet 118: 1059-1064.

Ma HQ, Kong ZX, Fu BS, Li N, Zhang LX, Jia HY, Ma ZQ (2011) Identification and mapping of a new powdery mildew resistance gene on chromosome 6D of common wheat. Theor Appl Genet 123: 1099-1106.

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.

McFadden H Lehmensiek A, Lagudah ES (2006) Resistance gene analogues of wheat: molecular genetic analysis of ESTs. Theor Appl Genet 113: 1-16.

McIntosh RA, Devos KM, Dubcovsky J, Rogers WJ, Morris CF, Appels R, Anderson OA (2006) Catalogue of gene symbols for wheat: supplement. Ann Wheat Newsl 52: 208-230.

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.

Okazaki K, Sakamoto K, Kikuchi R, Saito A, Togashi E, Kuginuki Y, Matsumoto S, Hirai M (2007) Mapping and characterization of FLC homologs and QTL analysis of flowering time in *Brassica oleracea*. Theor Appl Genet 114: 595-608.

Wang Pan JS, Li X He HL, Wu AZ, Cai R (2005) Construction of a cucumber genetic linkage map with SRAP markers and location of the genes for lateral branch traits. Sci China C Life Sci 48: 213-220.

Xie CJ, Sun QX, Ni ZF, Yang T, Nevo E, Fahima T (2004) Identification of resistance gene analogue markers closely linked to wheat powdery mildew resistance gene *Pm31*. Plant Breeding 123: 198-200.

Xie WL, Ben-David R, Zeng B, Distelfeld A, Röder MS, Dinoor A, Fahima T (2012) Identification and characterization of a novel powdery mildew resistance gene *PmG3M* derived from wild emmer wheat, *Triticum dicoccoides*. Theor Appl Genet 124: 911-922.

Yi YJ, Hu N, Liu HY, Wang JM, Wang R, Gao SX (2007) Chromosomal location of a new powdery mildew resistance gene in broad-spectrum resistance wheat using microsatellite markers. J Triti Crop 27: 565-569.

Yi YJ, Liu HY, Huang XQ, An LZ, Wang F, Wang XL (2008) Development of molecular markers linked to the wheat powdery mildew resistance gene *Pm4b* and marker validation for molecular breeding. Plant Breeding 27: 116-120.