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Genetic analysis and SSR mapping of stem rust resistance gene from wheat mutant D51

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Abstract Wheat (*Triticum aestivum* L.) stem rust caused by *Puccinia graminis* f. sp. *tritici* is one of the main diseases of wheat worldwide. Wheat mutant line D51, which forms a highly susceptible cultivar ‘L6239’ to the three races notated and cultured with immature embryos, shows resistance to prevailing races 21C3CPH, 21C3CKH, and 21C3CTR of *P. graminis* f. sp. *tritici* in China. In this study, the number and the expression stages of the resistance genes in mutant D51 were studied using inoculation identification and microsatellite (SSR) marker analysis. Two F₁ populations from the crosses of D51 × L6239 (60 individuals) and D51 × Chinese Spring (60 individuals), their F₂ populations (185 and 175 individuals respectively) at the seedling stage, and one F₂ population derived from the cross of D51 × L6239 (194 individuals) at the adult stage were inoculated with pathogen race 21C3CPH to test for resistance. All F₁ individuals of the two crosses were immune to stem rust at both seedling and adult stages. The response pattern of the three F₂ populations showed that the R:S segregation ratio was 3:1, suggesting that the stem rust resistance of D51 is controlled by a single dominant gene, and is expressed during the entire growth period. The identification of the stem rust resistance by the F₃ progeny test confirmed the credibility of the F₂ population test. Segregating populations and small population analyses were used to identify chromosomal regions

and molecular markers linked to the gene by the SSR marker method. A total of 675 SSR markers and 185 individuals of the D51 × L6239 F₂ population were used to search genetically linked markers to the target gene. Using Mapmaker 3.0 and Map-draw with Kosambi’s function and other options set at default values, molecular mapping revealed that the gene was located on chromosome 5DS, linked with and flanked by two SSR markers, Xgwm190 and Xwmc150, at 18.58 and 21.33 cM, respectively. It has been reported that only one stem rust resistant gene, *Sr30*, is located on the wheat chromosome 5DL, and that it has no resistance to 34C2MKK and 34C2MFK, while the parent L6239 of mutant D51 has no resistance to 21C3CPH, 21C3CTK and 21C3CTR, but has resistance to 34C2MKK and 34C2MFK. The results above indicate that the gene identified in the study might be a novel resistance gene to stem rust, tentatively designated as *SrD51*.

Keywords wheat (*Triticum aestivum* L.), stem rust, resistance gene, SSR marker

1 Introduction

Wheat (*Triticum aestivum* L.) stem rust is one of the major diseases worldwide. Many stem rust-resistant cultivars have been developed and stem rust genes have been passed on, which made the control of wheat stem rust successful in the 1970s (Yao et al., 1997), and which have provided a good productive environment for growing wheat of high yield, high efficiency, and good quality in China.

However, wheat stem rust may appear again due to the use of a single resistance source causing a new pathogen race to come into being and making the resistant gene disappear (Chen and Wang, 1997). Recently, a new pathogen, Ug99, was found in eastern Africa, which infected wheat cultivars with the *Sr31* gene that has been extensively used worldwide. There could be huge losses in wheat production if Ug99 is transmitted to the Middle

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East, Asia and America. At present, about 80 stem rust resistance genes have been discovered around the world. Some Chinese wheat cultivars were analyzed for stem rust resistance genes. Most of them carried *Sr9e*, *Sr13*, *Sr14*, *Sr22*, *Sr23*, *Sr26*, *Sr31*, *Sr32*, *Sr36*, *Sr37* and *GT* genes, however, the virulence frequency of *Sr13*, *Sr14*, *Sr22*, *Sr23*, *Sr32* and *Sr36* were above 70% (Yao et al., 1997). *Sr11*, which was an effective stem rust resistance gene against the pathogen 21C3CTR in China, has lost its resistance (Cao et al., 1996). Therefore, discovering new resistant sources and markers for the stem rust gene is the most important task in wheat breeding. These new resistant sources and markers are preconditions and form the basis for molecular breeding and realizing permanent resistance. With the use of microsatellite markers, also known as SSRs, some important resistant genes of wheat have been marked (Roder et al., 1998; Somers et al., 2004; Hayden et al., 2004; Yang et al., 2005; McCartney et al., 2005). SSR makers have been widely used for location and genetic mapping because its locality is known along with codominance and polymorphism.

Wheat mutant line D51, derived from immature embryo culture and radiation of the cultivar 'L6239', which is highly susceptible to the prevailing races 21C3CPH, 21C3CKH, and 21C3CTR, showed resistance to these three races. It was a good line with high yield, disease resistance, and fine quality. In this study, two sets of F₂ populations derived from the crosses of D51 × L6239 and D51 × Chinese Spring were used for genetic analysis and mapping of stem rust gene by microsatellite (SSR) marker for the mutant D51. The aim of this research was to determine the number of resistance genes in the mutant D51, the genotype location on the chromosome, and its relations with other known genes. This study showed the significance of discovering new genes, the reasonable use of known stem rust genes, and resistant breeding in wheat.

2 Methods

2.1 Plant materials and pathogen of *P. graminis* f. sp. *tritici*

Plant materials used were of the resistant mutant D51, the highly susceptible cultivar 'L6239', Chinese Spring, and the F₁, F₂, and F₃ populations from the crosses of D51 × L6239 and D51 × Chinese Spring. 21C3CPH of *P. graminis* f. sp. *tritici* was from the Plant Pathological Institute of Shenyang Agricultural University.

2.2 Resistant identification of wheat stem rust

From 2005 to 2006, inoculations were done at the seeding and adult stages by using the stem rust race 21C3CPH.

Identifications at the seeding stage for 60 F₁ individuals from the crosses D51 × L6239 and D51 × Chinese Spring, their parents, and their F₂, revealed 185 and 175 individuals, respectively. Identification at the adult stages in the F₁ and F₂ from crosses D51 × L6239 revealed 60 and 194 individuals, respectively. Because the F₂ populations were highly infected and almost all died during seedling identification, seeds could not be kept. However, part of the F₂ populations survived to the adult stage, although a lot of them were highly infected. Fourteen F₂ individual lines from D51 × L6239 were selected randomly (8 resistant and 6 susceptible plants) and cultivated in a greenhouse. Their resistance to stem rust was tested using 21C3CPH at the seedling stage.

Identified plant materials were cultured in pots with a diameter of 25 cm. Meanwhile, the susceptible cultivars L6239 and Chinese Spring were used as controls. There were 7 individuals per pot. Fresh pathogen was dissolved in 0.04% Tween-20 water solution. When the wheat seedlings had developed two leaves and one central leaf, the leaves were sprayed with the water solution, inoculated by smearing with the fingers, then sprayed with the water solution again until dewdrops formed on the leaves. Inoculated individuals were covered with wet cloth and plastic film for 16 hours, the temperature was kept at 25°C/15°C (day/night) with a photoperiod of 12 to 13 hours for 16 days or so. Resistance was analyzed according to Roelfs (1988) method with seven classes of standards including 0, 0, 1, 2, 3, 4 and Mixing (X) type when control cultivars L6239 and Chinese Spring were fully susceptible. 0, 0, 1, and 2 were resistant types. 3, 4 and X were susceptible types. The R:S segregation ratio fit of the three F₂ populations was estimated by χ^2 test. The experiments were repeated 25 days after inoculation.

Fresh pathogen 21C3CPH in 0.04% Tween-20 water solution in the same volume was injected into the internodes under the spikes of adult plants using a needle when the plants had grown to the flag stage - boot stage. They were kept wet for 16 days with the other factors kept the same as those at the seedling stage.

2.3 SSR marker analysis

Total wheat genomic DNA was isolated from fresh or frozen leaf tissues according to the CTAB method (Aldrich, 1993). The concentration and purity of the extracted DNA were determined on a 1% agarose gel and by UV-spectrophotometry. SSR markers including 500 wmc, 125 gwm, 50 Xbarc were synthesized according to sequences from the website <http://wheat.pw.usda.gov/ggpages/> and Röder et al. (1998). PCR followed the method of Röder et al. (1998). The mutant D51 and L6239 were initially screened for polymorphic bands. A small F₂ population which included 12 resistant and susceptible individuals, and 185 F₂ individuals from D51 × L6239 were used to search genetically linked

makers to the target gene. Primary reaction products were resolved by gel electrophoresis on 6% polyacrylamide (PAGE) gels followed by silver staining.

2.4 Estimation of genetic distance and mapping

Based on the polymorphic bands of the PCR reaction products and the separated parameters of the D51 × L6239 F₂ population, molecular markers linked with the target gene and mapping were analyzed using Mapmaker 3.0 and Map-draw software.

3 Results

3.1 Identifying of stem rust resistance and genetic analysis

The results from the seedling stage showed that all individuals of the mutant D51 were resistant to stem rust. All individuals of L6239 and Chinese Spring were highly susceptible. The F₁ population of D51 × L6239 was immune. Resistance and susceptible lines were segregated in the F₂ population of D51 × L6239, with 139 resistant and 46 susceptible individuals, respectively. The F₁ population (60 individuals) of D51 × Chinese Spring were all immune. Resistance and susceptible individuals were segregated from the F₂ population of D51 × Chinese Spring, There were 130 resistant individuals and 45 susceptible individuals. The response pattern of the two F₂ populations to 21C3CPH showed that the R:S segregation ratio was 3:1, suggesting that the stem rust resistance of D51 was controlled by a single dominant gene. One hundred and ninety-four F₂ individuals of D51 × L6239 used for inoculation at the adult stage showed an R:S segregation ratio of 3:1 ($\chi^2_{3:1} = 0.557 < \chi^2_{0.05}$) using the χ^2 test, with 150 resistant and 44 susceptible individuals. The stem rust resistance of D51 controlled by a single dominant gene was proven again, and D51 had a manifestation of resistance at the adult stage (Table 1). The result of the experiment done after 25 days of inoculation was the same, suggesting that the stem rust resistance of D51 was controlled by a single

dominant gene, and expressed during the entire growth period.

Two F₁ populations from the crosses of D51 × L6239 (60 individuals) and D51 × Chinese Spring (60 individuals), their F₂ populations (185 and 175 individuals, respectively) at the seedling stage and one F₂ population derived from the cross of D51 × L6239 (194 individuals) at the adult stage were inoculated with the pathogen race 21C3CPH to test their resistance to the disease. All F₁ individuals of the two crosses were immune to stem rust. The response pattern of the three F₂ populations showed that the R:S segregation ratio was 3:1, suggesting that the stem rust resistance of D51 was controlled by a single dominant gene, and expressed during the entire growth period. The identification of stem rust resistance by the F₃ progeny test confirmed the credibility of F₂ population test.

3.2 Resistance identification of F₃ individuals to stem rust

All F₃ lines, derived from six F₂ susceptible individuals of the cross D51 × L6239 (plant Nos.:17, 22, 26, 33, 34, and 35) were susceptible to 21C3CPH. Three F₃ lines from eight F₂ resistant individuals of the cross D51 × L6239 showed resistance. The results showed that they carried a homozygous resistant gene; the other five lines represented the R:S segregation, which included 35 resistant and 12 susceptible lines. These F₂ individuals carried a heterozygous gene (Table 2). The above analyses proved the reliability of the result in the identification of F₂ individuals from D51 × L6239 for their stem rust resistance to 21C3CPH. The resistant gene was tentatively named *SrD51*.

3.3 Analysis of SSR marker and linkage mapping

A total of 675 SSR markers were used for the primary screening separation of polymorphism of the mutants D51 and L6239. Forty pairs of primers were polymorphic. Further screening separation was done for 12 resistant individuals and 12 susceptible individuals in the F₂ generation. Four primers, gwm190, gwm205, wmc150 and wmc96 (Fig. 1), which were located in the 5D chromosome,

Table 1 Resistance identification of D51/L6239 and D51/Chinese Spring F₂ populations infected by the pathogen race 21C3CPH of *P. graminis* f. sp. *tritici* at the seedling and adult stages

material	infection type						total number of plants	expected ratio	χ^2 value	P-value
	0	0;	1	2	3	4				
D51	60						60			
L6239						60	60			
Chinese Spring						60	60			
D51/L6239 F ₁	60						60	1:0		
D51/Chinese Spring F ₁	60						60	1:0		
D51/L6239 F ₂	135	4			6	40	185	3:1	0.0018	0.900
D51/L6239F ₂ (adult)	144	3	2	1	4	40	194	3:1	0.557	0.500–0.250
D51/Chinese Spring F ₂	121	9			8	37	175	3:1	0.0476	0.900–0.750

Table 2 Disease reaction of D51/L6239 F₃ lines to 21C3CPH isolate

code for plants of the F ₂ population	resistant reaction of F ₂ population	resistant reaction of F ₃ population	
		resistance (R)	susceptible (S)
6	R	11	0
7	R	10	0
8	R	8	3
9	R	9	0
10	R	9	3
11	R	6	2
13	R	5	2
14	R	7	2
17	S	0	6
22	S	0	5
26	S	0	5
33	S	0	4
34	S	0	7
35	S	0	6

showed polymorphic amplification. The F₂ population (185 individuals) DNA and the polymorphic primer were used again for PCR to identify genetic linkage and estimate the genetic distance between SSR makers and the target gene. Mapmaker3.0 and Map-draw were used for linkage analysis. Molecular mapping revealed that the gene was located on the chromosome 5DS, linked and flanked by two SSR markers, Xgwm190 and Xwmc150, at 18.58 and 21.33 cM, respectively (Table 3, Fig. 2). The marker sequence and established SSR genetic map of wheat were uniform (<http://www.shigen.nig.ac.jp/>).

4 Discussion

Forty five *Sr* genes have been given formal designations (Li et al., 2002; Zhang, 2002), 58 genes are located on wheat chromosomes (<http://www.umn.edu/res-gene/wsr>).

htm), and 12 wheat *Sr* genes including *Sr2*, *Rpg1*, *Sr5*, *Sr9e*, *Sr9g*, *Sr22*, *Sr26*, *Sr31*, *Sr35*, *Sr36*, *Sr38* and *Sr39* (Kilian et al., 1994; Mago et al., 2005; Bariana and McIntosh, 1993; Gold et al., 1999; Margo et al., 2004; Druka et al., 2000) have been developed as molecular markers for about 80 stem rust resistant (*Sr*) genes against the fungal pathogen *Puccinia graminis* f. sp. *tritici*. These genes are distributed in different wheat chromosomes. The SSR molecular marker Xgwm533/120 bp for stem rust resistant gene *Sr2* was first identified by Hayden et al. (2004), followed by the SSR molecular markers of Cfa2019 and Cfa2123 with genetic distance 5.9 and 6.0 cM for *Sr22* that were identified by Khan et al. (2005). Other genes have been marked using RFLP.

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It has been reported that only one stem rust resistant gene, *Sr30*, is located on the wheat chromosome 5DL and it has not been marked yet. The 34C2 race of *Puccinia graminis* f. sp. *tritici* comprises five pathogenic types: MKH, MKR, MKK, MFK and MFR (Yao et al., 1995, 1997). *Sr30* has no resistance to 34C2MKK and

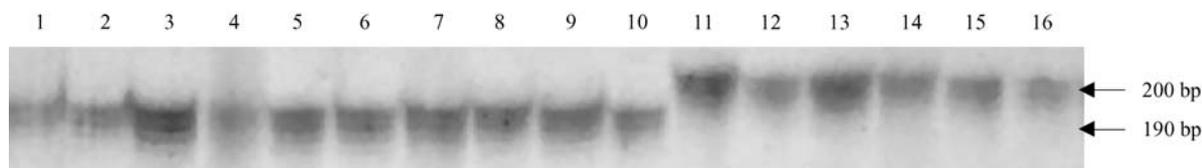


Fig. 1 Distribution of SSR marker Xgwm190 in the F₂ segregation population
 Note: 1–9: phenotypically resistant individuals; 10: D51; 11: L6239; 12–16: phenotypically susceptible individuals.

Table 3 Segregation of D51/L6239 F₂ population for the stem rust resistance gene and the SSR markers

SSR maker	resistance genotype	marker genotype			total number of plants	genetic distance/cM
		A	H	B		
gwm190	R	44	74	16	185	18.58
	S	4	15	32		
wmc150	R	40	74	20	185	21.33
	S	5	16	30		

Note: R: resistant; S: susceptible; A: resistant parent genotype; H: heterozygous genotype; B: susceptible parent genotype.

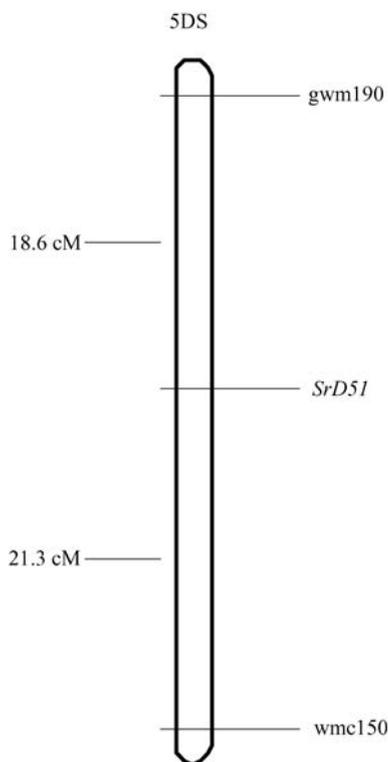


Fig. 2 Linkage map of stem rust resistance gene *SrD51* on chromosome 5DS

34C2MFK. In this study, mutant D51 was resistant to 34C2MFK and 34C2MFK, and parent L6239 of mutant D51 had no resistance to 21C3CPH, 21C3CTK and 21C3CTR, but had resistance to 34C2MFK and 34C2MFK. So L6239, from the wheat cultivars Webster and Festiguay, has no *Sr30* which is located on chromosome 5DL (Yao, 1997; Knot and McIntosh, 1978) and is the only resistant stem rust gene in Webster expressing close recessive resistance to the pathogen races 19, 29 and 15B. However, Qiu et al. (1999) believes that *Sr30* has dominant resistance to 21C3CTR and 34C2CKR. In our study, it was shown that mutant D51 had the dominant resistant gene to 21C3CPH with two F_2 populations from D51/Chinese Spring and D51/L6239 being carried on the resistant and susceptible separation tests. All F_1 individuals from our KANG 151-1/D51 cross combination for assistance breeding had resistance to 21C3CPH, and proved further that the gene *SrD51* was a dominant gene. Resistant stem rust gene *SrD51* from mutant line D51 might be a novel gene, but this has yet to be verified further using allelic analysis according to the above procedure, resistance identification, and the results of the molecular biology experiments.

Most of the *Sr* genes were expressed during the entire growth period. However, resistance of *Sr2*, *Sr23*, *Sr25* and *Sr40* changed at different growth stages. *Sr2* was susceptible at the seedling stage and resistant at the adult stage, *Sr23*, *Sr25* and *Sr40* were resistant at the seedling stage

and susceptible at the adult stage (Li and Zeng, 2002). In this study, it was shown that the *SrD51* gene was expressed during the entire growth period through inoculation appraisal at the seedling and adult stages.

Wheat mutant line D51 was derived from 'L6239', a highly susceptible cultivar to stem rust, using light-stimulated mutation and tissue culture of immature embryos. This mutant had a slight difference in yield and quality, and a close genetic relationship with L6239. Because light-stimulated mutation is indirect and can penetrate into the structure of multitier cells, it has an effect on the DNA and the chromosome. The common effects of light-stimulated mutation and tissue culture of immature embryos includes resistance to the stem rust gene of the mutant D51 and other little changes in the effect of the genes. These were the reasons why 40 pairs of primers for the 675 pairs of SSR markers expressed polymorphism among their parents, while the 675 pairs of SSR markers used to screen for D51 and L6239 for higher mutation were stimulated by such effects. The artificially controlled culture surrounding, and the chemical components jointly affected the cell during culture (Wu et al., 2005).

In our study, 185 individuals of D51 \times L6239 and the small population analysis were used to search genetically linked makers to the target gene by the SSR method. Mapmaker3.0 molecular mapping analysis revealed that the gene was linked to and flanked by two SSR markers, Xgwm190 and Xwmc150, at 18.58 and 21.33 cM, respectively with a slightly larger distance of the SSR marker from *Sr* gene. Screening for polymorphic primers to refine mapping needs to be done for map-based cloning of *Sr* gene and marker assisted breeding.

The main characteristics of wheat stem rust has not changed in the species and the frequency of the main epidemic race in China, especially race 21, has not changed with an 80% and above frequency of occurrence. For race 34, it is about 10% (Yao et al., 1995). From 2003 to 2005, the identification of stem rust resistance in Shenyang Agricultural University showed that mutant D51 was not only immune to 21C3CPH, but also immune or closely immune to 21C3CFH, 21C3CTH, 21C3CKH, 21C3CTR, 34C2 and 34MKGe. Therefore, the discovery, identification and application of the resistant gene *SrD51* has positive practical applications.

5 Conclusion

The wheat stem rust resistance of mutant D51 was controlled by a single dominant gene which was expressed throughout the growth period. 675 pairs of SSR markers and 185 individuals of D51 \times L6239 F_2 population were used to search genetic linkage markers of the target gene. Molecular mapping revealed that the gene is located on the chromosome 5DS, linked to and flanked by two SSR

markers, Xgwm190 and Xwmc150, at 18.58 and 21.33 cM, respectively. Identification of resistance to stem rust and the SSR markers' location indicated that the gene identified in this study might be a novel resistant gene to stem rust, tentatively named *SrD51*. Wheat mutant line D51 showed resistance to prevailing races 21C3CPH, 21C3CTR, and 34C2 of *P. graminis* f. sp. *tritici* highlighting its important value in wheat breeding for resistance to stem rust by genetic analysis, *Sr* gene discovery, and the discovery of molecular markers in China.

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References

- Aldrich C (1993). CTAB DNA extraction from plant tissues. *Plant Mol Biol Rep*, 11: 128–141
- Bariana H S, McIntosh R A (1993). Cytogenetic studies in wheat: XV. Chromosome location of rust resistance genes in VPM1. *Genome*, 36: 476–482
- Cao Y Y, Yao P, Liu W Z, Wu Y S (1996). Pathogenic spectrum analysis of 21C3CTR of *Puccinia graminis* f. sp. *tritici* in China. *J Shenyang Agric Univ*, 27(1): 26–30 (in Chinese)
- Chen W Q, Wang J X (1997). Genes for leaf and stem rust resistance in 76 wheat genetic resources. *Acta Agron Sin*, 23(6): 655–662 (in Chinese)
- Druka A, Kudrna D, Han F, Kilian A, Steffenson B, Frisch D, Tomkins J, Wing R, Kleinhofs A, Eversmeyer M G, Kramer C L (2000). Physical mapping of the barley stem rust resistance gene *rpg4*. *Mol Gen Genet*, 264: 283–290
- Gold J, Harder D, Townley-Smith F, Aung T, Procnier J (1999). Development of a molecular marker for rust resistance genes *Sr39* and *Lr35* in wheat breeding lines. *Electronic Biotechnol*, 2: 1–7
- Hayden M J, Kuchel H, Chalmers K J (2004). Sequence tagged microsatellites for the Xgwm533 locus provide new diagnostic markers to select for the presence of stem rust resistance gene *Sr2* in bread wheat (*Triticum aestivum* L.). *Theor Appl Genet*, 109(8): 1641–1647
- Khan R R, Bariana H S, Dholakia B B, Naik S V, Lagu M D, Rathjen A J, Bhavani S, Gupta V S (2005). Molecular mapping of stem and leaf rust resistance in wheat. *Theor Appl Genet*, 111: 846–850
- Kilian A, Steffenson B J, Saghai Maroof M A, Kleinhofs A (1994). RFLP markers linked to the durable stem rust resistance gene *Rpg1* in barley. *Mol Plant Microbe Interact*, 7: 298–301
- Knot D R, McIntosh R A (1978). Inheritance of stem rust resistance in “Webster” wheat. *Crop Sci*, 18: 365–369
- Li Z Q, Zeng S M (2002). Wheat Rust in China. Beijing: China Agriculture Press, 56–61 (in Chinese)
- Mago R, Bariana H S, Dundas I S, Spielmeyer W, Lawrence G J, Pryor A J, Ellis J G (2005). Development of PCR markers for the selection of wheat stem rust resistance genes *Sr24* and *Sr26* in diverse wheat germplasm. *Theor Appl Genet*, 111(3): 496–504
- Margo R, Spielmeyer W, Lawrence G J, Lagudah E S, Ellis J G, Pryor A (2004). Resistance genes for rye stem rust (*SrR*) and barley powdery mildew (*Mla*) are located in syntenic regions on short arm of chromosome. *Genome*, 47: 112–121
- McCartney C A, Somers D J, McCallum B D, Thomas J, Humphreys D G, G Menzies J, Brown P D (2005). Microsatellite tagging of the leaf rust resistance gene *Lr16* on wheat chromosome 2BS. *Mol Breed*, 15: 329–337
- Qiu Y C, Zhang S S, Liu Y L (1999). Monosomic analysis of the stem rust resistant genes in Garuda “s”. *J Shenyang Agric Univ*, 30(4): 416–419 (in Chinese)
- Röder M S, Korum V, Wendehake K, Plaschke J, Tixier M H, Leroy P, Ganal M W (1998). A microsatellite map in wheat. *Genetics*, 149: 2007–2023
- Roelfs A P (1988). An international system of nomenclature for *Puccinia graminis* f. sp. *tritici*. *Phytopathology*, 78: 526–533
- Somers D J, Isaac P, Edwards K (2004). A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theor Appl Genet*, 109: 1105–1114
- Wu W G, Liu G R, Yang X J (2005). Applications of the mutation in connection with in vitro culture for plant breeding. *Chin Agric Sci Bull*, 21(11): 197–201 (in Chinese)
- Yang X Q, Liu Q, Han Z F, Sun Q X (2005). Genetic diversity revealed by genomic-SSR and EST-SSR markers among common wheat, spelt and compatum. *Prog Nat Sci*, 15(1): 24–33
- Yao P, Cao Y Y, Liu W Z, Wu Y S (1995). Race dynamic of *Puccinia graminis* f. sp. *tritici* in China in 1993. *Acta Phytohyalacica Sin*, 22(4): 303–308 (in Chinese)
- Yao P, Cao Y Y, Liu W Z, Wu Y S (1997). Race dynamic of *Puccinia graminis* f. sp. *tritici* in China (1990–1994). *Acta Phytohyalacica Sin*, 24(4): 297–302 (in Chinese)
- Zhang Z B (2002). Wheat Genetics. Beijing: China Agriculture Press, 122–186 (in Chinese)