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Mapping of leaf rust resistance genes in common wheat 'Guinong08-6'

Mapa de genes de resistência à ferrugem da folha em trigo 'Guinong08-6'

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Highlights

Leaf rust resistance and susceptible wheat lines were crossed to develop hybrids.
The resistance wheat was controlled by two complementary dominant genes.
LrGn08-6A was inherited from *Aegilops ventricosa* and may be the resistance gene *Lr37.*
LrGn08-6B may be a newly leaf rust resistance gene.

Abstract

Wheat leaf rust (*Puccinia triticina* Eriks.), a devastating disease of wheat in the world, causes severe yield losses and therefore the development of resistant cultivars is very important. Here, a Chinese wheat line (Guinong08-6) showed adult-plant resistance against mixed fungal isolates of leaf rust, which is common in Guiyang region. It was crossed with a susceptible wheat line (Guinong19) to develop F1, F2, and F3 hybrids. Combined SSR and STS markers were used to map leaf rust resistance genes in Guinong08-6, and the resistance phenotype of Guinong08-6 was co-regulated by two complementary dominant genes, named *LrGn08-6A* and *LrGn08-6B. LrGn08-6A* was mapped to chromosome 2AS with markers *URIC-LN2* and *Xgpw2204,* which flanked the gene at distances of 1.8 centimorgan (cM) and 14.83 cM, respectively. *LrGn08-6B* was mapped to chromosome 4DL with markers *Xgpw342* and *Xbarc93,* which both flanked the gene at a distance of 26.57 cM. Genetic and molecular marker analyses demonstrated that *LrGn08-6A,* which was inherited from *Aegilops ventricosa* may be the resistance gene *Lr37,* while *LrGn08-6B* may be a newly discovered leaf rust resistance gene.
Key words: *Puccinia triticina.* SSR. Complementary dominant genes. Resistance gene.

Resumo

A ferrugem da folha do trigo (*Puccinia triticina* Eriks.), importante doença do trigo em todo o mundo, causa graves perdas de rendimento e, portanto, o desenvolvimento de cultivares resistentes é muito

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importante. Nesta pesquisa, uma linhagem chinesa de trigo (Guinong08-6) mostrou resistência de plantas adultas a uma mistura de isolados do patógeno, na região de Guiyang, China. Essa linhagem foi cruzada com uma linhagem suscetível de trigo (Guinong19) para desenvolver híbridos F1, F2 e F3. Combinados de marcadores SSR e STS foram usados para mapear genes de resistência à ferrugem da folha em Guinong08-6, e o fenótipo de resistência de Guinong08-6 foi co-regulado por dois genes dominantes complementares, chamados LrGn08-6A e LrGn08-6B. LrGn08-6A foi mapeado para o cromossomo 2AS com marcadores URIC-LN2 e Xgpw2204, que flanquearam o gene em distâncias de 1,8 centimorgano (cM) e 14,83 cM, respectivamente. LrGn08-6B foi mapeado para o cromossomo 4DL com marcadores Xgpw342 e Xbarc93, e ambos flanquearam o gene a uma distância de 26,57 cM. As análises genéticas e moleculares de marcadores demonstraram que LrGn08-6A, que foi herdado de *Aegilops ventricosa,* pode ser o gene de resistência Lr37, enquanto LrGn08-6B pode ser um gene recentemente descoberto de resistência à ferrugem da folha do trigo.
Palavras-chave: Puccinia triticina. SSR. Genes dominantes complementares. Gene de resistência.

Introduction

Leaf rust (LR), caused by *Puccinia triticina,* is a common and widespread wheat (*Triticum aestivum* L.) disease. Depending on the developmental stage of wheat, leaf rust (LR) can lead to yield losses of 7% to 30% (Basnet et al., 2013; Bolton et al., 2008). The development of resistant cultivars is recognized as the most efficient, economical, and environmentally safe way to control this disease in wheat (Millet et al., 2014). However, *P. triticina* is highly variable to evolve new virulent races, allowing the evolution of new virulent races, which results in the resistant wheat cultivars losing their resistance (Bolton et al., 2008; Juliana et al., 2017). Therefore, identifying and introducing new resistance genes into elite commercial cultivars have been emphasized by international wheat breeding groups (Kole et al., 2015).

To data, more than 100 LR resistance (*Lr*) genes have been documented in wheat, and ~80 *Lr* genes have been formally designated (McIntosh et al., 2017; Prasad et al., 2020). These genes include all stage resistance genes or seedling resistance genes (often considered race specific), which can be overcome easily by new pathogen virulence, and adult-plant resistance (*APR*) genes (often considered race nonspecific), which are more durable against the rapidly evolving rust pathogens (Pasam et al., 2017). Although all stage resistance genes are apt to lose resistance to *P. triticina,* individual genes can be combined with other genes to impart LR disease resistance. On the other hand, APR genes exhibit partial resistance or slow-rusting and also need to be combined with other resistance genes against LR disease (Singh et al., 2000). The slow-rusting APR genes have durable, as well as additive and pleiotropic effects, which make the adult-plant resistance more long lasting than qualitative resistance. At the seedling stage, cultivars with adult-plant resistance typically display a compatible infection type (IT); however, following plant growth, the infected plants have greater resistance levels against rust pathogens (Singh et al., 2011; Lu et al., 2017). To date, *Lr34, Lr46, Lr67, Lr68,* and *Lr77* have been characterized as well designated adult-

plant resistance genes on chromosomes 7D, 1B, 4DL, 7BL, 1BL, respectively (Herrera-Foessel et al., 2012, 2014; Peng & Yang, 2017; Kolmer et al., 2018). Some APR genes, such as *Lr12, Lr13, Lr22a,* and *Lr22b,* are race specific (Ellis et al., 2014; McIntosh et al., 2017). Wheat cultivar resistance to *P. triticina* occurs temporary because the pathogens can evolve new pathotypes that have virulence against the resistance genes (Wu et al., 2017). Therefore, both the identification of new *Lr* genes and the deployment of a suitable combination of different *Lr* genes are very important.

The Aegilops species with C, U, and M genomes exhibit a high frequency of LR, and new genetic resources could be exploited. Bariana and McIntosh demonstrated that *Lr37* was located in the short arm of chromosome 2A (Bariana & McIntosh, 1993). *Lr37,* which originated from *Aegilops ventricosa,* was transferred onto wheat chromosome 2AS (Tanguy et al., 2005) and combinations with other genes provided effective protection against all Australian *P. triticina* pathotypes (Bansal et al., 2011). Lr37 was also found in *Aegilops kotschyi, Aegilops ovate,* and *Aegilops biuncialis* (Kwiatek et al., 2012). Recently, Gebrewahid et al. (2020) reported that *Lr37* conferred resistance to leaf rust through mapping leaf rust resistance quantitative trait loci in wheat line Mianyang351-15 (Gebrewahid et al., 2020).

Our research has focused on a genetic analysis of a Chinese wheat line (Guinong 08-6), which showed adult-plant resistance against leaf rust disease according to several years of field observations. Then the genomic locations of potential resistance genes of Guinong 08-6 were explored.

Materials and Methods

Plant materials

Chinese wheat line (Guinong08-6 and Guinong19) was stored in the Wheat Research Center at the College of Agriculture in Guizhou University. Guinong08-6, generated from Guinong13 × Guinong775, is highly resistant against LR, and Guinong775, as a highly resistant material to LR, is cultivated from a complex wide cross of the hybrids of *Aegilops tauschii* Coss × *A. fatua* Linn*//A. ventricosa* Tausch × Sauwne20. Guinong 19 generated from Zhongyan963 × Guinong21 is susceptible to LR. The $F_{2:3}$ population of hybrids contained 201 lines.

Resistance test for LR in the field

Guinong08-6, Guinong19, and the $F_{2:3}$ population from the Guinong08-6 × Guinong19 were tested for the presence of *APR* genes associated with LR, in the field in Guiyang, China, which is a hotspot for LR. The field inoculation was performed according to Zhou et al. (2014) with modifications in a 2-m row. Equal amounts of *P. triticina* pathotypes common in Guiyang were mixed and sprayed onto rows at tilling. The inoculated plants grew until the susceptible lines completed rust development, and then the ITs were investigated. Based on Roelfs (1984), the ITs were scored on a 0-4 scale.

DNA extraction

Fresh leaf tissues at 5-leaf stage were cut and stored at -80 °C. The DNA extraction was carried out using the CTAB method.

PCR analysis

PCR amplification was performed in 25-μL reaction volume containing 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 2.0 mM $MgCl_2$, 0.08% Nonidet P40, 0.2 mM dNTPs, 1 unit *Taq* DNA polymerase, 0.4 μM of each primer (Sangon Biotech, Shanghai, China), and 20-50 ng genomic DNA. Primer screening was performed with the following program: 94 °C for 3 min (pre-denaturation), followed by 4 cycles of 1 min at 94 °C, 1 min at 38 °C, and 2 min at 72 °C for pre-amplification, then followed by 39 cycles of 10 s at 94 °C, 1 min at 38 °C, 2 min at 72 °C, and a final extension at 72 °C for 5 min. Specific primer amplification was performed with the following program: 94 °C for 3 min (pre-denaturation), followed by 4 cycles of 1 min at 94 °C, 1 min at 39 °C, and 2 min at 72 °C for pre-amplification, then followed by 35 cycles of 10 sec at 94 °C, 1 min at 39 °C, 2 min at 72 °C, and a final extension at 72 °C for 5 min. The PCR products were detected by 5% non-denatured PAGE combined with silver staining. Gels were visualized using a UVP imaging analysis system.

Inheritance analysis and linkage map construction of resistance genes

The chi-squared (χ^2)-tests were used to detect the segregation of the resistance genes for the $F_{2:3}$ populations. The differential primers were used to amplify and classify the genotypes of the populations, in which 'A' indicated that the molecular marker band type was the same as that of Guinong08-6, 'B' indicated that the molecular marker band type was the same as that of Guinong19, 'H' indicated heterozygous lines and '–' represented data missing or not detected. MAPMAKER, version 3.0 was used to carry out molecular marker and resistance linkage analyses, Kosambi mapping function was used to calculate the genetic distance (cM), and the linkage map was constructed using Map Draw V2.1.

Results and Discussion

Parental infection types (Its) and an inheritance analysis

Wheat seedlings were infected with mixed fungal isolates of LR that are common in Guiyang, and the degree of infection was scored on a 0-4 scale (Figure 1). Testing results for three years demonstrated that Guinong08-6 was highly resistant (IT = 1), and Guinong19 was susceptible (IT = 4). The F_1 population of the Guinong08-6 × Guinong19 cross exhibited high resistance (IT = 1), whereas the $F_{2:3}$ population, with 201 lines, exhibited segregation of LR resistance. Among the 201 lines, 111 lines were highly resistant (IT = 1), and 90 lines were susceptible (IT = 4). In 200 reciprocal lines, 109 lines were highly resistant (IT = 1) and 91 lines were susceptible (IT = 4) (Table 1). This result corroborated the identified results of the Chinese Academy of Sciences (tgliu@ippcaas.cn). The χ^2 results ($\chi^2_{9:7} = 0.24 < \chi^2_{0.05} = 3.84$) indicated that two independent dominant complementary genes participated in the LR resistance of Guinong08-6. These two genes were named *LrGn08-6A* and *LrGn08-6B.*

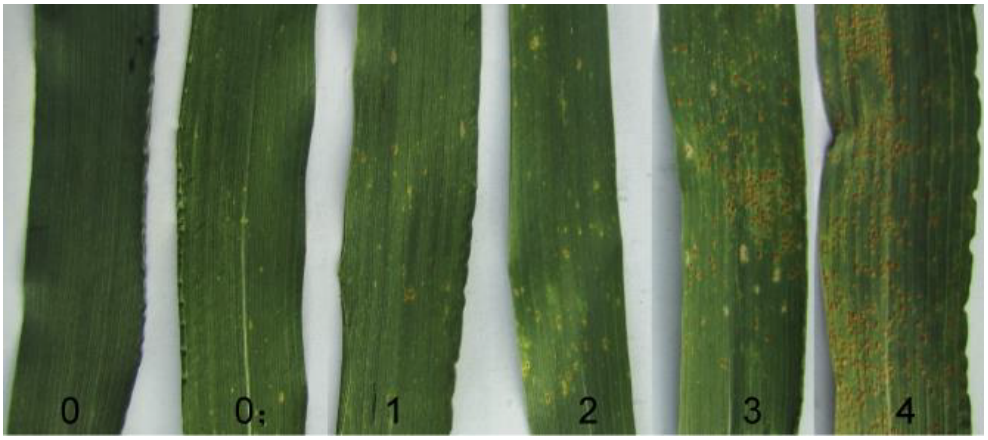

Figure 1. Standard infection types (IT) of wheat leaves infected with leaf rust.
0, indicates immune; 0:, indicates nearly immune; 1, indicates highly resistant; 2, indicates mildly resistant; 3, indicates mildly susceptible; and 4, indicates highly susceptible.

Table 1
Genetic maps of *LrGn08-6A* and *LrGn08-6B.* Leaf rust infection levels of parents and a genetic analysis of the $F_{2:3}$ population of the Guinong19 × Guinong08-6 cross

Population	Progeny	Number of seedlings						Expected ratio	χ^2
		0	0:	1	2	3	4		
Guinong19	P_1						40		
Guinong08-6	P_2			42					
Guinong19 × Guinong08-6	F_1			19					
Guinong19 × Guinong08-6	$F_{2:3}$	31	19	34	27	57	33	9:7	0.05

Note: χ^2 represents the chi-square value of 9:7, $\chi^2_{0.05}$ = 3.84.

Screen molecular markers

Molecular markers used for mapping resistance genes of Guinong08-6 were screened using 27 primer pair specific for LR resistance genes and 243 pairs of SSR primers. Molecular marker, URIC-LN2, which is closely linked to *Lr37,* and 40 pairs of SSR primers, displayed polymorphisms in the parents. Then, the high resistance and susceptibility bulked segregant analyses were constructed using a genomic DNA mixture of eight family lines from the homozygous F_2 lines with IT = 1 and IT = 4, respectively. Four codominant markers (URIC-LN2, Xgpw2204, Xbar93, and Xgpw342) were specifically amplified in the high resistance bulked segregants and Guinong08-6 (Table 2). These were used to generate the $F_{2:3}$ genotype containing 201 lines (Table 3).

Table 2
Sequences of four specific markers for bulked segregant analyses

Marker name	Primer sequence (5´-3´)	Location	Tm (℃)
URIC-LN2	GGTCGCCCTGGCTTGCACCT TGCAGCTACAGCAGTATGTACACAAAA	2NS/2AS	63
Xgpw2204	ACGTGCCTCGGTCGATATAC ACCCCGAGCATGTCAAATAG	2AS	50
Xbarc93	GCCGGACGGATTTAGGTGGAGGAGA CGCAACCTCACCATCACCGCCTCATC	4DL	62
Xgpw342	AGAGCCATGAGTTGGTCGC CACAATCGTCCCTTCATCCT	4DL	62

Table 3
Band types of four specific markers in the $F_{2:3}$ lines.

Marker	1			4			$\chi^2_{1:2:1}$
	A	H	B	A	H	B	
URIC-LN2	45	64	2	11	35	44	1.04<5.99
Xgpw2204	42	53	16	13	42	35	0.75<5.99
Xgpw342	25	59	27	23	51	16	2.48<5.99
Xbarc93	31	53	27	20	52	18	0.76<5.99

Note: 'A' indicates that the molecular marker belt type was the same as that of Guinong08-6; 'B' indicates that the molecular marker band type was the same as that of Guinong19; 'H' indicates heterozygous lines.

Genomic locations of LrGn08-6A and LrGn08-6B

Based on the GrainGenes2.0 database, Xgpw2204 was located in chromosome 2AS, and Xgpw342/Xbarc93 were located in chromosome 4DL. In the $F_{2:3}$ population (Figure 2; Table 3), the dominant *LrGn08-6A* gene was located in chromosome 2AS, which was 1.8 cM from marker URIC-LN2, and 14.83 cM from Xgpw2204 (Figure 3A). Furthermore, URIC-LN2 was a CAPS marker closely linked to the *Lr37* gene, which originated from *A. ventricose* (Helguera et al., 2003). *Lr37*, as a single resistance gene, was introgressed into common wheat for the cultivation of cultivars with resistance to LR, and it is present in 2AS of VPM1-derived wheat in the form of the closely linked Lr37/Yr17/Sr38 group (Stepień et al., 2003). *Lr37* was first reported to be overcome in the USA. Then, Huang et al. reported that *Lr37* combined with *Lr10, Lr13* and *Lr14a* in European cultivars showed resistance to *P. triticina* (Błaszczyk et al., 2004). Our resistance test indicated that *Lr37* was widely present (31.4%) in cultivars of Guizhou Province and that combined resistance genes (*Lr37+Lr34*) were detected in cultivars, such as 97012-4 and 163-4-1-3 (data not shown).

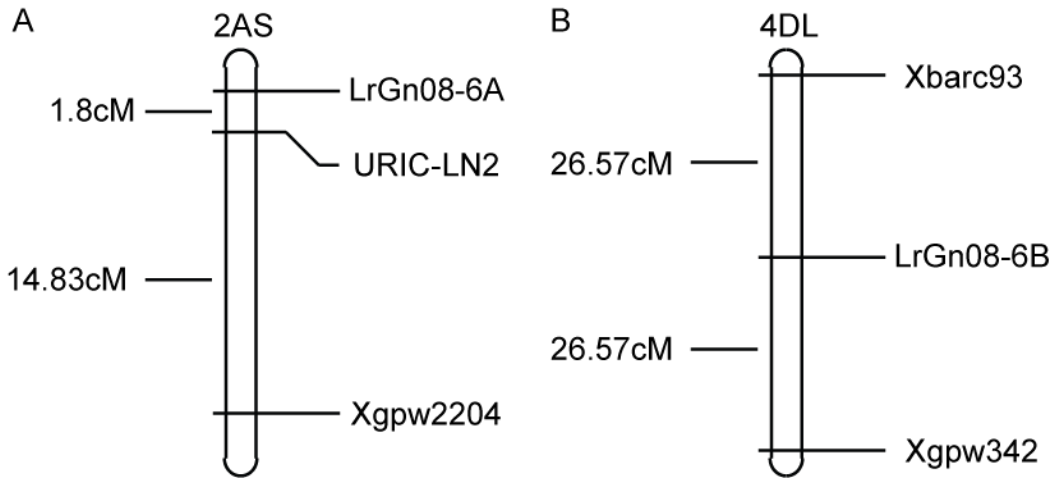

Figure 2. Genetic maps of *LrGn08-6A* and *LrGn08-6B.*
(A) genetic map of *LrGn08-6A*; (B) genetic map of *LrGn08-6B.*

The dominant *LrGn08-6B* gene was located in chromosome 4DL, which was 26.57 cM from both marker Xbar93 and Xgpw342 (Figure 3B). Until now, only one resistance gene, *Lr67,* was reported to localize in chromosome 4DL (Hiebert et al., 2010). However, the linkage of the Xcfd71 marker with *Lr67* was not detected in the parents, B1 and B4 (data not shown). Therefore, *LrGn08-6B* may be a new resistance gene to LR.

We observed that Guinong08-6 exhibited persistent resistance to LR isolates that were common in Guizhou Province after 2008. Our results confirmed that the resistance of the wheat line Guinong08-6 was controlled by two dominant complementary genes *LrGn08-6A* and *LrGn08-6B.* This function of complementary *Lr* genes was similar to the complementary *Yr* genes (*Yr73* and *Yr74*) reported by Dracatos et al (2006). Genetic populations will be enlarged to confirm the specific localizations of these two genes, especially *LrGn08-6B.* Meanwhile, allele detection should also be carried out to allow for the separation and cloning of both genes.

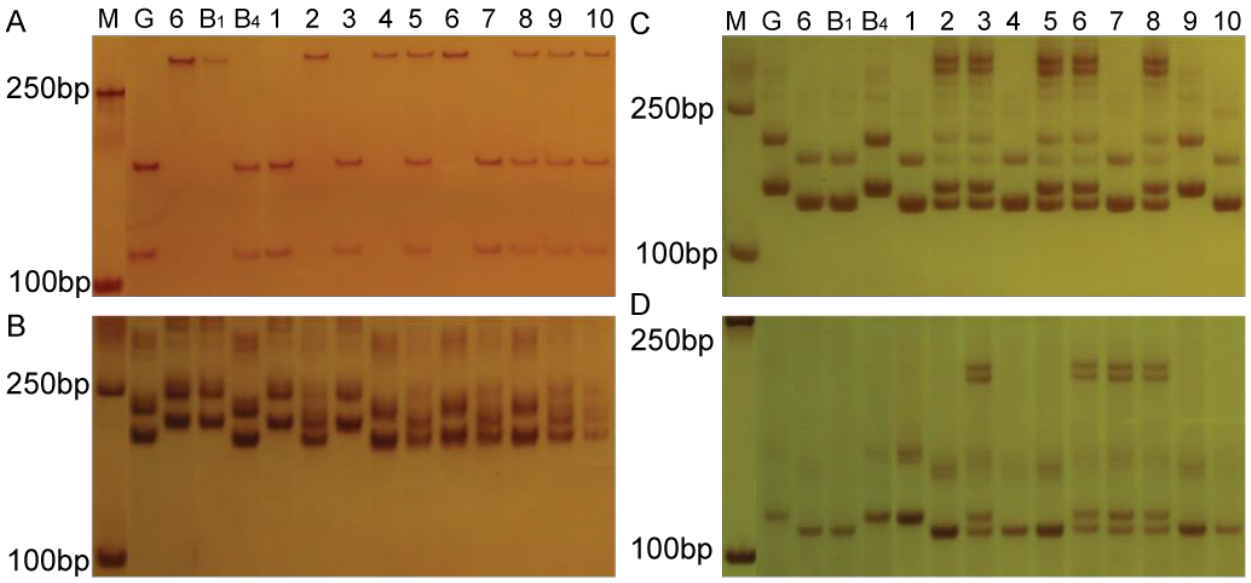

Figure 3. Marker analysis of the $F_{2:3}$ population.
(A-D) Detection with URIC-LN2, Xgpw2204, Xgpw342, and Xbarc93 primers, respectively. M: D2000 Marker; G: Guinong19; 6: Guinong08-6; B_1: Highly resistant pool; B_4: Highly susceptible pool; 1-10: individual $F_{2:3}$ lines.

Origins of LrGn08-6A and LrGn08-6B

Four codominant markers (URIC-LN2, Xgpw2204, Xbar93, and Xgpw342) were used to identify the pedigree parents (Figure 4). The URIC-LN2 primers amplified a specific band in Guinong08-6 and *Aegilops ventricosa* Tausch, while Xgpw2204 primers amplified a specific band in Guinong08-6 and *A. tauschii* Coss. The detection result for *LrGn08-6A* using the URIC-LN2 marker indicated that *LrGn08-6A* may be *Lr37.* However, the result from Guinong08-6 using the Xgpw2204 marker may have been caused by the long genetic distance between the Xgpw2204 marker and the *LrGn08-6A* gene. We hypothesized that *LrGn08-6A* originated from *A. ventricosa* Tausch, and the detection failure by the Xgpw2204 primers may be the result of the long genetic distance between the Xgpw2204 marker and the resistance gene. The Xbar93 primers could amplify a specific band in Guinong 08-6 and *A. ventricosa* Tausch. However, the Xgpw342 primers did not amplify any specific bands in the five pedigree parents.

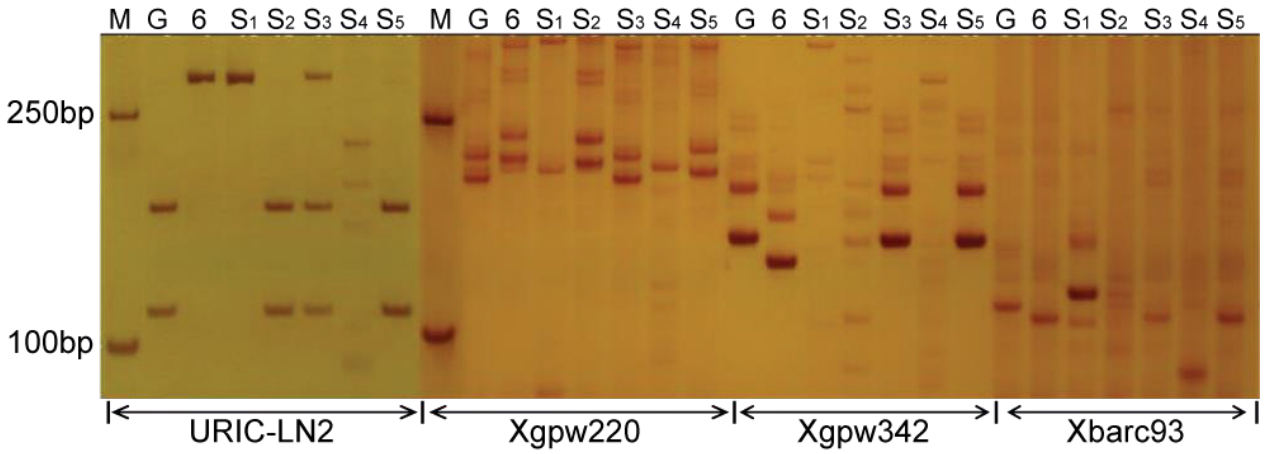

Figure 4. PCR amplification by four codominant primers in pedigree parents.
M: D2000 Marker; G: Guinong19; 6: Guinong08-6; S_1: *Aegilops ventricosa* Tausch; S_2: *Aegilops tauschii* Coss; S_3: Guinong 13; S_4: *Avena fatua* Linn; S_5: Guinong 775.

In summary, this study confirmed the adult-plant resistance to *P. triticina* in Guinong08-6 wheat is controlled by a pair of complementary dominant genes. The genomic location analysis indicated that *LrGn08-6A,* which localized in 2AS, may be *Lr37* and that *LrGn08-6B,* which localized in 4DL, may be a newly discovered LR resistance gene.

Authors' contributions

Jianshu Sui performed experiments; Mingjian Ren did data collection; Ruhong Xu and Luhua Li designed the study. Luhua Li wrote the manuscript.

Acknowledgments

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