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ORIGINAL PAPER

Genetic analysis and molecular mapping of stripe rust resistance genes in Chinese native wheat (*Triticum aestivum*) Lankao 5

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Abstract Lankao 5 is a common wheat (*Triticum aestivum*) landrace collected from Henan province, China, which exhibits a high level of resistance to stripe rust (*Puccinia striiformis* f.sp. *tritici*, 'Pst') in field trials over many years. To analysegenetic of resistance in Lankao 5, F_1 and F_2 progenies were obtained from a cross between wheat genotypes Lankao 5 and Chinese Spring (CS). Parents, F_1 and F_2 plants, were planted in greenhouse and tested at the seedling stage with Pst races CYR29, CYR30, CYR31, CYR32, Sun11-4, and Sun11-11. Genetic analysis showed that the resistance of Lankao 5 to race CYR30 were conferred independently all by one dominant gene and one recessive gene, independently, resistance to Sun11-11 by one dominant gene, resistance to CYR31, CYR32, and Sun11-4 all by two independent dominant genes, and resistance to CYR29 by two recessive genes of independent action. Using F_2 progenies from crosses between Lankao 5 and a set of 20 CS monosomic lines, the resistance gene in Lankao 5 to Pst race Sun11-11 was cytogenetically located on chromosome 7B. Using 141 F_2 plants and their F_3 generations with 7B specific simple sequence repeat (SSR) markers, a linkage map consisting of five SSR loci and the resistance gene locus *YrLk* was constructed. The linkage map spanned a genetic distance of 21.6 cM, and the SSR markers *Xwmc396* and *Xbarc267* were closely linked to *YrLk* with genetic distances of 3.3 and 4.4 cM, respectively. Analyses of chromosomal positions, reaction types to Pst race, and gene origin suggested that *YrLk* was very likely a novel resistance gene for stripe rust in wheat. Genetic analysis of the stripe rust resistance gene in Lankao 5 and closely linked markers for *YrLk* will facilitate the use of resistance in breeding programs to control wheat stripe rust.

Keywords Stripe rust · *Puccinia striiformis* · Resistance gene mapping · Genetic analysis · Wheat · *Triticum aestivum*

Introduction

Wheat stripe rust (yellow rust), an airborne disease caused by *Puccinia striiformis* f. sp. *tritici* (Pst), is one of the most damaging diseases of wheat (*Triticum aestivum* L.) worldwide (Chen 2005; Wellings 2011). In China, the disease occurs annually in most wheat growing areas, especially in northwest, southwest, and the Huang-Huai wheat production region, which is considered as the largest stripe rust epidemiologic region in the world (Wan et al. 2007). Although the use of fungicides can prevent the disease, the application of fungicides adds a significant extra cost to farmers. The chemicals may cause adverse effects to the environment and may not be fully effective if missing the best application time. Growing resistant cultivars is the most effective, environmentally sound, economic, and consistently used method of controlling stripe rust. To improve stripe rust resistance, two types of resistance are generally used in wheat breeding programs.

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One type is race specific, also known as all-stage resistance, and the other is non-race specific or adult plant resistance. All-stage resistance expresses from seedling through all developmental stages of wheat, facilitates breeding selection, and is widely employed in wheat cultivars. However, race-specific resistance can be easily overcome by new virulent races, due to the high variability of the stripe rust pathogen, resulting in rust epidemics (Li and Zeng 2002; Line 2002). Adult plant resistance is durable but may not be adequate when rust infection is too severe. Both resistance types need to be combined to attain the better disease control (Chen 2005). With the variation of Pst virulence, the resistance genes in the majority of breeding programs in China have varied at least eight times since the first nation-wide stripe rust epidemics in the 1950s (Hou et al. 2013; Li and Zeng 2002; Wan et al. 2003, 2007). In the past decade, resistance in wheat cultivars Hybrid 46, Suwon 11, and '*Yr24*' were overcome by the predominant Pst races, and only a few resistance genes are effective against the current Chinese Pst population (Cheng et al. 2006; Han et al. 2010, 2012; Wan et al. 2003; Wang et al. 2007). The need is great to identify new, effective resistance genes for breeding programs in stripe rust epidemic areas, such as Sichuan, Yunnan, Shaanxi, and Gansu, China (Wan et al. 2004; Cheng et al. 2006; Yang et al. 2003).

Landraces constitute a valuable genetic resource, possessing various desirable agronomic traits, including high-level resistance to wheat stripe rust. In the 1950s, Prof. Zhao used a Chinese landrace Maza crossed with an elite cultivar Biyu to develop a set of wheat cultivars with good resistance to stripe rust in China (Li and Zeng 2002; Wan et al. 2007). However, the landrace has some poor agronomic traits, requiring an extended period for selective introgression of desirable traits through the breeding process. In the past several decades, molecular techniques have provided useful tools for modern wheat breeding. Identification of molecular markers associated with resistance genes then using these markers in breeding process will facilitate successful selection. Molecular markers have been developed for many stripe rust resistance genes, such as *Yr5*, *Yr10*, *Yr15*, *Yr26*, *Yr45*, *Yr53*, *Yr64*, and *Yr65* (Yan et al. 2003; Wang et al. 2002; Li et al. 2011; Wang et al. 2008; Xu et al. 2013; Yaniv et al. 2015; Cheng et al. 2014). Among the molecular markers, simple sequence repeat (SSR) marker is most widely utilized in wheat disease resistance gene mapping and genetic improvement, due to its high polymorphism, repeatability, and low cost (Li et al. 2009; Ren et al. 2010; Wang et al. 2009; Zhang et al. 2010; Cheng et al. 2014).

Lankao 5, a wheat landrace collected in Henan province of China, showed a high-level resistance to stripe rust in our field trials over many years. In this study, we analyzed the resistance inheritance of Lankao 5 to the prevalent races in the Chinese Pst population and constructed a genetic map of the stripe rust resistance gene in Lankao 5 with SSR markers using F_1 plants and $F_{2:3}$ populations from cross of Lankao 5 and Chinese Spring. These findings will be important for understanding the genetic basis of rust resistance in Lankao 5 and for using marker-assisted selection to transfer this resistance gene to other wheat cultivars in improving control of wheat stripe rust.

Materials and methods

Plant and pathogen materials

Lankao 5 is a wheat landrace collected from Henan province of China, which is resistant to most races of stripe rust in China. Chinese Spring (CS) is a wheat germplasm susceptible to all Pst races at the seedling stage. Lankao 5 was crossed with CS and a set of 21 monosomic CS lines (3D monosomic line was absent in further analyses due to F_1 failed to produce F_2 seeds). Chinese Spring monosomic lines and F_1 plants were confirmed cytologically using the Feulgen staining method (Xu et al. 2013). For each of the 21 crosses, the F_1 plants were selfed to develop F_2 populations; then, the F_2 plants were grown to maturity and harvested for $F_{2:3}$ seeds. The seeds of parents, F_1, F_2, and $F_{2:3}$ generations, were propagated in a controlled greenhouse.

Six predominant Chinese Pst races (Chen et al. 2009; Li et al. 2016), Sun11-4, Sun11-11, CYR29, CYR30, CYR31, and CYR32, were used to test Lankao 5, CS, and F_1 plants and F_2 populations of CS monosomic lines at the seedling stage in greenhouse.

Seedling tests

Each F_2 population from crosses between Lankao 5 and the 20 CS monosomic lines were tested in parallel with race Sun11-11 to analyze the chromosome location of the resistance gene in Lankao 5. $F_{2:3}$ generations from Lankao5 × CS 1D monosomic which were selected to construct a genetic linkage map were tested with race Sun11-11.

Seedling tests were conducted under controlled greenhouse conditions. About 15 seeds from each parent and F_1 generations, about 200 seeds from each F_2 population, and 20 seeds from each of the F_3 lines were used for tests. Fifteen to 20 seeds were planted per 10 cm diameter × 12 cm deep pot. Plants were grown at day/night temperatures of 25/15 °C and day/night lights of 16/8 h cycle.

Pathogen inoculations were performed when the first wheat leaves were fully expanded. The inoculum was made of spore/talc mixture at a 1/20 ratio and gently dusted on leaves. Inoculated plants were kept in a dew chamber at 10 °C for about 24 h, then moved to a growth chamber under a daily cycle of 16 h of light at 18 °C and 8 h of darkness at 10 °C. Plant infection type (IT) were scored based on the scale of 0,

0;, 0;$^+$, 1, 1$^+$, 2, 2$^+$, 3$^-$, 3, 3$^+$, and 4, where 0 means no visible infection, 0; means necrotic/chlorotic flecks without sporulation, 0;$^+$ means necrotic/chlorotic stripes without sporulation, 1 means necrotic/chlorotic stripes with scattered small sporulation, 1$^+$ means necrotic/chlorotic stripes with many small sporulation, 2 means a few moderate sporulation with necrotic/chlorotic flecks, 2$^+$ means many moderate sporulation with necrotic/chlorotic stripes, 3$^-$ means abundant sporulation with necrotic stripes, 3 means abundant sporulation with chlorotic stripes, 3$^+$ means abundant sporulation with chlorosis, and 4 means abundant sporulation without chlorosis (Bariana and McIntosh 1993; Li et al. 2006a). Plants with IT of 0 to 2$^+$ were considered resistant, while those with ITs of 3$^-$ to 4 were treated as susceptible (Liu 1988; Li et al. 2006a). The susceptible wheat cv. Chinese Spring served as a universal test control.

DNA extraction and bulk segregant analysis

The parents and 141 F_2 plants from Lankao 5 × Chinese Spring 1D monosomic were selected for SSR analysis. Genomic DNA was extracted from leaves of parents and F_2 plants using the CTAB method (Riede and Anderson 1996). Based on $F_{2:3}$ phenotypic data, resistant and susceptible bulks were made with equal amounts of DNA by ten homozygous resistant and ten homozygous susceptible F_2 plants, respectively.

Microsatellite marker analysis

Because the resistance gene in Lankao 5 to Pst race Sun11-11 was cytogenetically located on chromosome 7B by CS monosomic lines, 44 SSR markers specific to wheat chromosome 7B were used to identify polymorphism and to confirm the chromosomal region of the resistance gene in Lankao 5. SSR primer sequences were obtained from Grain Genes (http://wheat.pw.usda.gov), then synthesized by Sangon Biotech, Inc. (Shanghai, China). The PCR method for each SSR marker followed previous reports (Röder et al. 1998; Somers et al. 2004).

Each 15 μL PCR mixture consisted of 1.5 μL 10× buffer, 2.1 μL DNA template (25 ng/μL), 0.3 μL dNTPs (2.5 mM each), 1.2 μL $MgCl_2$ (25 mM), 1.5 μL each of the forward and reverse primers (10 μM each), 0.15 μL Taq DNA polymerase (5 U/μL) and 6.75 μL ddH_2O. All applied PCR reagents were purchased from Sangon Biotech, Inc. PCR amplification was performed using a MJ Research PTC-200 thermal cycler by the following protocol: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 30 s each under denaturation at 94 °C, 1 min of annealing at 50–68 °C (depending on primers), and 45 s at 72 °C for extension, then, a finally extension at 72 °C for 10 min. After amplification, 6 μL of formamide loading buffer [98% formamide, 10 mM EDTA (pH 8.0), 0.5% (*w*/*v*) xylene cyanol, and 0.5% (*w*/*v*) bromophenol blue] was added to the PCR product. About 5–7 μL mixture of the PCR product and loading buffer for each sample was loaded for electrophoresis on 8% polyacrylamide gel, then visualized by applying silver stain (Bassam et al. 1991; Chen et al. 1998).

Data analysis

Segregation of the markers and resistance was both tested for fitness to expected ratios using the chi-squared (χ^2) tests on Excel data analysis program (Microsoft Office 2010). Linkage analysis and map construction of SSR markers and resistance locus were performed with Mapmaker 3.0 (Lander et al. 1987). The recombination rate was converted to map distance (in centimorgan, cM) according to the Kosambi mapping function (Kosambi 1944). The linkage map was drawn using Mapdraw V2.1 (Liu and Meng 2003).

Results

Resistance evaluation and inheritance analysis of Lankao 5 resistance to stripe rust

Infection types (ITs) of two races (CYR32 and Sun11-4) on seedlings of Lankao 5 were resistant with no apparent symptoms (IT 0), while those of four Pst races (CYR29, CYR30, CYR31, and Sun11-11) exhibited necrotic flecks without sporulation (IT 0;).The results indicated that Lankao 5 was highly resistant to the test Pst races. In contrast, ITs of six Pst races on Chinese Spring displayed full sporulation and absent in any necrosis (IT 4), indicating Chinese Spring was highly susceptible (Table 1).

The resistance inheritance patterns to wheat stripe rust were controlled by various genes in Lankao 5. In tests on Pst races Sun11-11 and CYR30, F_1 plants were resistant, while F_2 populations were segregating as at a ratio 3R:1S when tested on Sun11-11and at a ratio 13R:3S when tested on CYR30 (Table 2). The preceding tests indicated that resistance in Lankao 5 to Pst race Sun11-11 was controlled by a dominant gene (temporarily designated *YrLk*), while resistance to Pst race CYR30 was controlled by one dominant gene and one recessive gene, independently.

In tests on Pst races CYR31, CYR32, and Sun11-4, all F_1 plants exhibited resistance, while F_2 populations segregated at a ratio 15R:1S (Table 2), demonstrating that resistance in Lankao 5 to these three races all was controlled by two independently inherited dominant genes.

In terms of resistance to CYR29, all F_1 plants were susceptible, while F_2 populations segregated at a ratio of 7R:9S (Table 2), indicating that resistance to CYR29 in Lankao 5 was controlled by two recessive genes of independent action.

Table 1 Reaction types of wheat cultivars Lankao 5 and Chinese Spring to different races of the wheat stripe rust fungus, *Puccinia striiformis* f. sp. *tritici* (Pst)

Parents	Races					
	CYR29	CYR30	CYR31	CYR32	Sun11-4	Sun11-11
Lankao 5	0;	0;	0;	0	0	0;
Chinese Spring	4	4	4	4	4	4

Chromosomal location of the resistance gene

To determine the chromosomal location of resistance gene *YrLk* in Lankao 5 to Pst race Sun11-11, monosomic analysis was applied. Because F_1 plants of Lankao 5 × CS 3D monosomic line failed to produce F_2 seeds, none further analysis was conducted on their F_2 populations. The other 20 crosses of Lankao 5 and CS monosomic lines were examined with race Sun11-11. Each F_2 population from crosses between Lankao 5 and 19 CS monosomic lines was segregating at a ratio of 3 resistant:1 susceptible, except the Lankao 5 × CS 7B monosomic line (Table 3). The corresponding Lankao 5 × CS 1D monosomic line (chose by which showed lowest χ^2 value on monosomic analysis (Table 3)) F_3 populations was segregating at a ratio of 1R:2Seg:1S. Plant observations fit the expected ratio, complying with the chi-squared test. For example, Table 3 shows the test results for the F_2 Lankao 5 × CS 1D monosomic line population ($\chi^2 = 0.36 < \chi^2_{0.05} = 5.99$). The preceding results further supported the conclusion that one dominant gene in Lankao 5 conferred resistance to Sun11-11. Segregation of F_2 plants from crosses of Lankao 5 × CS 7B monosomic line significantly deviated from the 3R:1S ratio ($\chi^2 = 43.64 > \chi^2_{0.05} = 3.84$), indicating that chromosome 7B carries the resistance gene *YrLk*.

Table 2 Genetic analysis of stripe rust resistance to six races of *Puccinia striiformis* f.sp. *tritici* in Lankao 5 (P1) × Chinese Spring (P2) F_1 and F_2 populations

Races	Populations	No. of plants			Expected ratio	χ^2	*P* value
		Total	Res.	Sus.			
Sun11-11	F_1	8	8	0			
	$F_{2\text{-}1}$	178	122	56	3:1	3.63	0.05
	$F_{2\text{-}2}$	174	139	35	3:1	1.96	0.14
CYR30	F_1	5	5	0			
	$F_{2\text{-}1}$	163	131	32	13:3	0.04	0.77
	$F_{2\text{-}2}$	169	128	41	13:3	3.02	0.07
CYR31	F_1	8	8	0			
	$F_{2\text{-}1}$	152	144	8	15:1	0.11	0.62
	$F_{2\text{-}2}$	145	139	6	15:1	0.77	0.29
CYR32	F_1	9	9	0			
	$F_{2\text{-}1}$	163	154	9	15:1	0.05	0.70
	$F_{2\text{-}2}$	124	112	12	15:1	1.94	0.11
Sun11-4	F_1	11	11	0			
	$F_{2\text{-}1}$	154	147	7	15:1	0.50	0.38
	$F_{2\text{-}2}$	176	167	9	15:1	0.22	0.53
CYR29	F_1	8	0	8			
	$F_{2\text{-}1}$	135	52	83	7:9	1.30	0.22
	$F_{2\text{-}2}$	150	61	89	7:9	0.46	0.45

Table 3 Expected ratios, observed resistant (R) and susceptible (S) F_2 plants from crosses of resistant cultivar Lankao 5 with Chinese Spring (CS) monosomic lines, inoculated with the Chinese wheat stripe rust *Puccinia striiformis* f.sp. *Tritici* race Sun11-11, and probabilities of chi-squared test for fit to theoretical ratios

Monosome types	No. of plants[b]				Expected ratio[c]	χ^2 value[d]
	Total	Res	Seg	Sus		
1A	129	99		30	3:1	0.13
2A	113	87		26	3:1	0.14
3A	87	60		27	3:1	1.38
4A	122	88		34	3:1	0.39
5A	100	78		22	3:1	0.33
6A	59	48		11	3:1	0.95
7A	109	83		26	3:1	0.03
1B	111	81		30	3:1	0.15
2B	107	78		29	3:1	0.15
3B	69	50		19	3:1	0.12
4B	96	69		27	3:1	0.35
5B	114	84		30	3:1	0.05
6B	116	91		25	3:1	0.56
7B	121	110		11	3:1	15.50**
1D	141	107		34	3:1	0.02
2D	105	84		21	3:1	1.15
4D	87	69		18	3:1	0.65
5D	103	76		27	3:1	0.03
6D	68	49		19	3:1	0.18
7D	84	64		20	3:1	0.02
All F_2 plants	2041	1555	70	486	3:1	1.47
Lankao 5/CS 1D F_3[a]	141	33	70	38	1:2:1	0.36

[a] The Lankao 5/4CS 1D F_3 lines, which were used to construct the mapping population were derived from Lankao 5/CS 1D F_2 plants

[b] The F_2 ratios are for resistant and susceptible plants, and the F_3 ratios are for homozygous resistant, segregating and homozygous susceptible lines

[c] The segregating lines included resistant and susceptible plants

[d] For F_2 ratios $\chi^2_{0.05} = 3.84$, For F_3 ratios $\chi^2_{0.05} = 5.99$

** indicated significance level: $P < 0.01$

Molecular mapping of the stripe rust resistance gene

Forty-four SSR markers covering chromosome 7B were screened on Lankao 5, resistant bulk, the susceptible parent Chinese Spring, and susceptible bulk to identify those associated with the *YrLk* locus. Five markers, *Xwmc476*, X*barc267*, *Xwmc396*, *Xgwm131*, and *Xwmc517*, generated polymorphism bands between the resistant and susceptible bulks, as well as their parents. All of the five markers are located on the long arm of chromosome 7B, based on the wheat consensus SSR map (Somers et al. 2004).

These five SSR markers were further used to genotype the 141 $F_{2:3}$ plants. The results showed that each of the five markers was co-dominant and well fitted a segregating ratio of 1 (homozygous resistant parental allele):2 (heterozygous allele):1 (susceptible parental allele), indicating each marker is a single locus and reliable for use to construct a linkage map (Table 4). Linkage analysis showed that resistance gene *YrLk* was linked with the five SSR loci; therefore, *YrLk* was also located on the long arm of chromosome 7B. A linkage map was composed of *YrLk* locus and five SSR loci, spanning 21.6 cM. The marker order obtained for the five SSRs was compatible with that determined by Somers et al. (2004). The two closest-flanking loci of *YrLk* were *Xwmc396* and *Xbarc267*, with genetic distances of 3.3 and 4.4 cM, respectively (Fig. 1). For example, Fig. 2 displays the segregation of marker alleles in the $F_{2:3}$ populations of the two closest-flanking markers. *Xwmc396* and *Xbarc267* are located near the centromere, based on wheat SSR maps (Somers et al. 2004), delineating the position of *YrLk* as proximal to the centromere of chromosome 7BL.

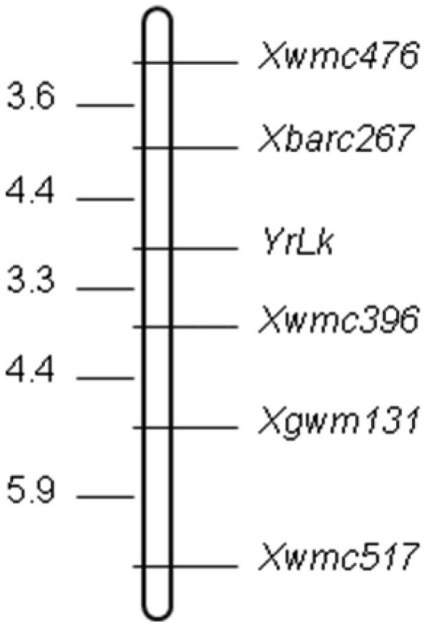

Fig. 1 Linkage map of stripe rust resistance gene *YrLk*

Discussion

In addition to *YrLk*, seven officially named *Yr* genes (*Yr2*, *Yr6*, *Yr39*, *Yr52*, *Yr59*, *Yr63*, and *Yr67* reported by McIntosh et al. 1998; El-Bedewy and Robbelen 1982; Lin and Chen 2007; Ren et al. 2012; Zhou et al. 2014; McIntosh et al. 2014), five temporarily named *Yr* genes (*YrZh84*, *YrC591*, *YrShan515*, *yrMY37*, and *YrSu-1*, reported by Li et al. 2009; Li et al. 2006b; Zhang et al. 2011; Ren et al. 2015; Yan et al. 2009), and 11 quantitative-trait loci (QTL) (Maccaferri et al. 2015) have been reported on chromosome 7B. *Yr6* and *Yr63* are located on the short arm of chromosome 7B (El-Bedewy and Robbelen 1982; McIntosh et al. 2014), a different chromosomal arm from *YrLk*. The *Yr6* near-isogenic line AvSYr6NIL and *Yr6* carrier cultivars Heines Peko and Heines Kolben (Calonnec et al. 1997) were susceptible to Pst race Sun11-11 in our greenhouse test, while *YrLk* is resistant. *YrLk* should be different from *Yr6*.

Yr39, *Yr52*, and *Yr59* are high-temperature adult-plant (HTAP) resistance genes, which are susceptible to Pst races at the seedling stage (Lin and Chen 2007; Ren et al. 2012; Zhou et al. 2014). *Yr52*, *Yr59*, *Yr67*, *YrZh84*, and *YrC591* are located on the telomere of the long arm of chromosome 7B (Li et al. 2006b; McIntosh et al. 2014; Ren et al. 2012; Zhou et al. 2014; Xu et al. 2014), while *YrLk* is proximal to the centromere of 7B. Based on the resistance type and chromosome location, *YrLk* may be different from these four genes.

In 1962, *Yr2* was first reported as located on chromosome 7B in Heines VII (McIntosh et al. 1998) and was later mapped on 7BL, with a genetic distance to *Xwmc364* of 5.6 cM (Lin et al. 2005). *Xwmc364* does not exhibit polymorphism between Lankao 5 and Chinese Spring, and cannot be mapped in our population. *YrSu-1* was reported on chromosome 7BL with the genetic distance from *Xwmc396* to *YrSu-1* of 21.1 cM (Yan et al. 2009). The distance from *YrLk* to *Xwmc396* is 3.3 cM. When Heines VII was tested with Sun11-11 at the seedling stage in greenhouse condition, it was susceptible (IT 3–4), and the carrier cultivar of *YrSu-1*, Suwon11, was susceptible to Sun11-11 (Chen et al. 2009). The rust response reaction

Table 4 Observed molecular marker numbers and alleles of Lankao 5 (A), Chinese Spring (B) and their heterozygous (H) progeny in F_2 population from Lankao 5 × Chinese Spring, and probabilities of chi-squared test of fit to theoretical ratios

Markers	No. of F_2 plants			Expected ratio	χ^2	*P* value
	A	H	B			
Xwmc476	33	70	38	A:H:B = 1:2:1	0.36	0.83
Xbarc267	36	72	33	A:H:B = 1:2:1	0.19	0.91
Xwmc396	32	79	40	A:H:B = 1:2:1	1.17	0.56
Xgwm131	34	73	34	A:H:B = 1:2:1	0.18	0.92
Xwmc517	34	77	30	A:H:B = 1:2:1	1.43	0.49

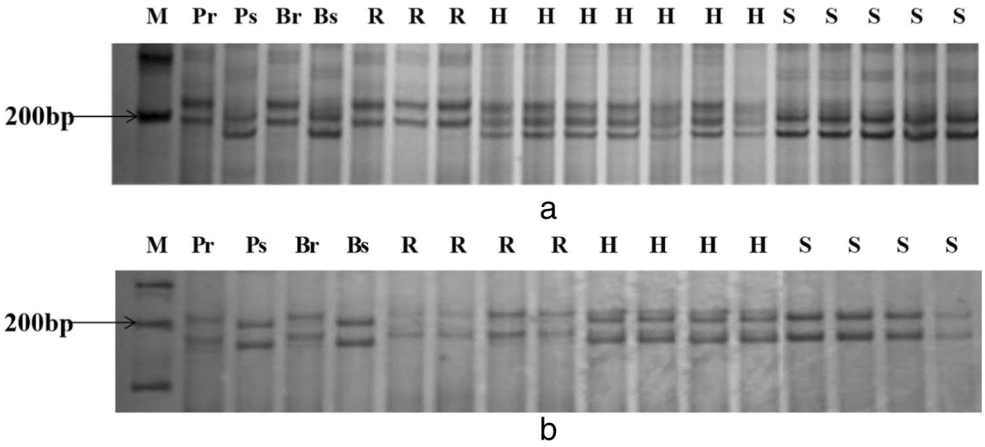

Fig. 2 PCR profiles for the F_2 segregated population and parents, amplified by the *YrLk* flanking markers *Xbarc267* (**a**) and *Xwmc396* (**b**) in polyacrylamide gel. *M* DNA size ladder, *Pr* resistant parent Lankao 5, *Ps* susceptible parent Chinese Spring, *Br* resistant DNA bulk, *Bs* susceptible DNA bulk, *R* resistant plant, *S* susceptible plant, *H* heterozygous resistant plants

and relative chromosomal position of three genes suggested that *Yr2* and *YrSu-1* are different from *YrLk*.

YrShan515 was identified in Chinese landrace Shan 515, conferring resistance to Pst race Sun11-4 (Zhang et al. 2011). The genetic distances of flanking markers *Xbarc267* and *Xwmc653* to *YrShan515* are 3.0 and 3.4 cM, respectively (Zhang et al. 2011), while the genetic distance from *Xbarc267* to *YrLk* is 4.4 cM. It appears that *YrShan515* and *YrLk* are genetically proximal. However, resistance by Shan 515 to race Sun11-11 is reported as controlled by two

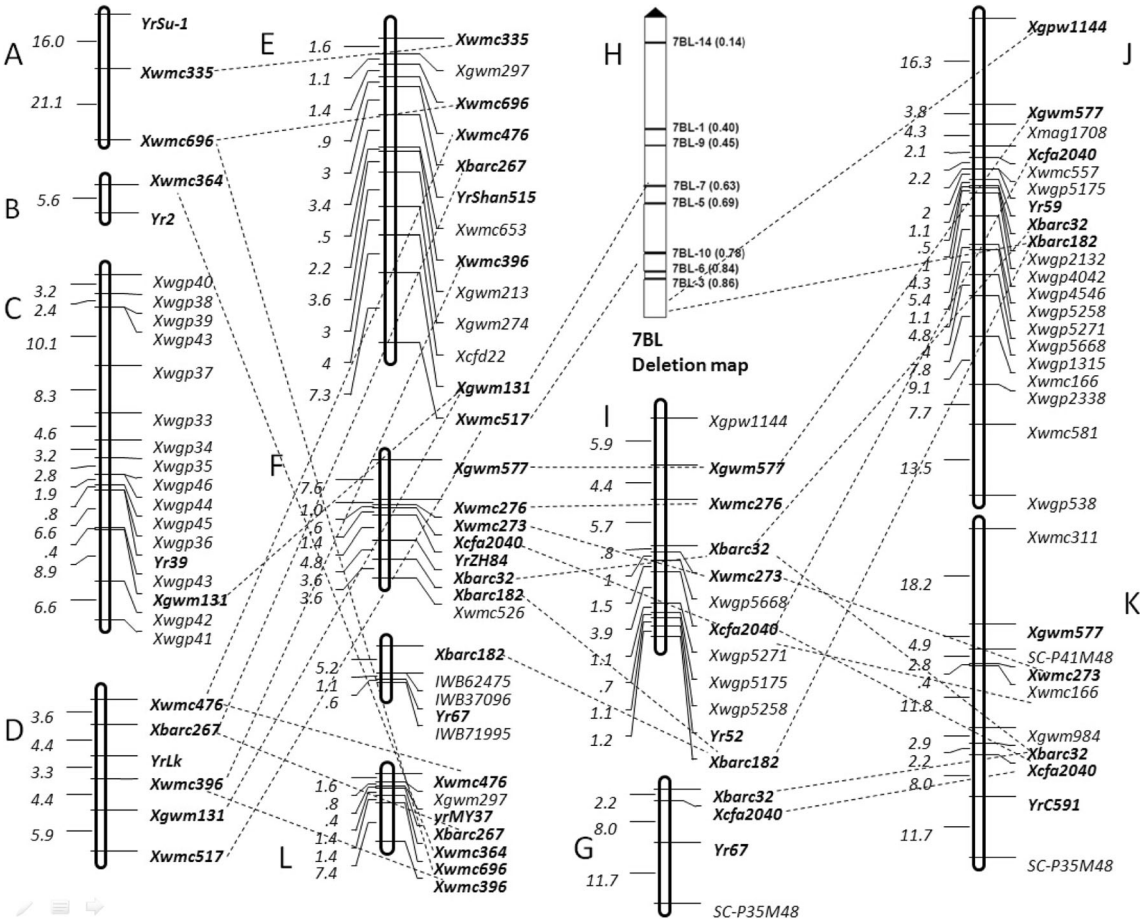

Fig. 3 Linkage maps for stripe rust resistant genes on the long arm of chromosome 7B and comparisons with the consensus map. The *dashed lines* connect common markers on different maps. Genetic map showing the relative genetic locations of *YrLk* (*D*) with other *Yr* genes in this study. *Yr2* in Lin et al. (2005) (*B*), *Yr39* in Lin and Chen (2007) (C), *Yr52* in Ren et al. (2012) (*I*), *Yr59* in Zhou et al. (2014) (*J*), *Yr67* in McIntosh et al. (2014) (*G*), *YrC591* in Xu et al. (2014) (*K*), *YrZh84* in Li et al. (2006b) (*F*), *YrSu-1* in Yan et al. (2009) (*A*), *YrShan515* in Zhang et al. (2011) (*E*), and *yrYM37* in Ren et al. (2015) on chromosome 7BL, and the shared markers in each linkage map and the corresponding chromosomal bin locations (*H*)

recessive genes (Zhang 2010), different from results for *YrLk* in this study. Furthermore, Shan 515 is susceptible to race CYR32 while Lankao 5 is resistant to CYR32. These results indicate high likelihood that *YrLk* and *YrShan515* are different genes.

yrMY37 was identified in Chinese wheat cultivar Mianmai 37, conferring resistance to Pst race v26 (Ren et al. 2015). The genetic distances of flanking markers *Xgwm297* and *Xbarc267* to *yrMY37* are 0.78 and 0.38 cM, respectively (Ren et al. 2015), while the genetic distance from *Xbarc267* to *YrLk* is 4.4 cM. But *yrMY37* was reported as a recessive gene, while *YrLk* is a dominant gene. The reaction type of Mianmai 37 (*yrMY37*) to race CYR32 is 1, and to CYR31 is 2 (Ren et al. 2015), while reaction type of Lankao 5 to race CYR32 is 0, and to CYR31 is 0:. These results indicate that *YrLk* and *yrMY37* are different genes.

The above cultivars content of *Yr* genes originate in very different places. *Yr2*, *Yr6*, *Yr63*, and *Yr67* are derived from European cultivars. *Yr39* was identified from the US cultivar Alpowa (Lin and Chen 2007). The carrier cultivars for *Yr52*, *Yr59*, and *YrC591* originate in Pakistan, Iraq, and India, respectively (Ren et al. 2012; Zhou et al. 2014; Li et al. 2009). *YrZhou84* was identified from wheat cultivar, Zhou8425B. The stripe rust resistance in Zhou8425B originated in an Italian cultivar Milan (Li et al. 2006b). *YrSu-1* is derived from a Korean cultivar Suwon 11. Shan 515, *YrShan515* gene carrier, is a wheat landrace from Shaanxi, China (Zhang 2010). *yrMY37* is derived from Chinese wheat cultivar Mianmai 37.

Overall, *YrLk* shows great differences in various aspects from other *Yr* genes on chromosome 7B, including chromosomal position, origin of gene carrier, and resistance against Pst races. It is very likely that *YrLk* is a novel, effective stripe rust resistance gene. Figure 3 shows the relative locations of *YrLk* (D) and *Yr2* (B), *Yr39* (C), *Yr52* (I), *Yr59* (J), *Yr67* (G), *YrC591* (K), *YrZh84* (F), *YrSu-1* (A), *YrShan515* (E), and *yrMY37* (L) on 7BL compared with common markers on the chromosome 7BL linkage maps.

Landraces are important genetic sources and may harbor valuable stripe rust resistance (Sthapit et al. 2014; Kertho et al. 2015). Wang et al. (2012) identified over 200 stripe rust resistance landraces from 28 countries and developed 70 germplasms in the background of the elite spring wheat cultivar 'Avocet', and these germplasms have various resistance types to the Pst populations in the USA. Most of these germplasm lines have high resistance to current Chinese Pst races (Zhou et al. 2015). The resistance genes *Yr45*, *Yr52*, *Yr53*, *Yr59*, and *Yr62* in these landraces were mapped with molecular markers (Li et al. 2011; Xu et al. 2013; Ren et al. 2012; Zhou et al. 2014; Lu et al. 2014). Some Chinese wheat landraces play important roles in the stripe rust epidemic areas where modern cultivars are unable to adapt to the local ecosystem, such as Pingyan 50, Caoxuan 5, and Baidatou (Lan et al. 2010; Zhou et al. 2015; Ma et al. 2015). However, most landraces have poor agronomic traits or low yield potential and are thus not suited to modern cultivation conditions. It is necessary to develop techniques to transfer desirable traits, such as stripe rust resistance, from these landraces to new cultivars, while excluding negative traits. Undoubtedly, the closely linked molecular markers of resistance genes constitute an efficient tool for selection during wheat breeding. The genomic region of *YrLk* is expressed as a gene hotspot, which allows gene exchange to occur. As a result, combining different *Yr* genes into a new germplasm may prove to be relatively easy.

In this study, results confirm that Lankao 5 is resistant to most of the currently prevalent Chinese Pst races at the seedling stage. At least four genes in Lankao 5 conferring resistance to stripe rust were genetically determined, one dominant gene (*YrLk*) resistant to race Sun11-11, one dominant gene and one recessive gene to CYR30, two dominant genes resistant to races CYR31 and CYR32 (one of which maybe *YrLk*), and two recessive genes resistant to CYR29. Lankao 5 is an excellent stripe rust resistance wheat landrace, which is one of the best candidates for disease resistance; furthermore, the closely flanking markers, *Xwmc396* and *Xbarc267*, of *YrLk* allow *YrLk* for high efficient use in marker-assisted selection for stripe rust resistance in breeding programs.

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References

Bariana HS, McIntosh RA (1993) Cytogenetic studies in wheat XV. Location of rust resistance genes in VPM1 and their genetic linkage with other disease resistance genes in chromosome 2A. Genome 36: 476–482

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196: 80–83

Calonnec A, Johnson R, De Vallavieille-Pope C (1997) Genetic analysis of resistance to *Puccinia striiformis* in the wheat differential cultivars Heines VII, Heines Peko and Strubes Dickkopf. Plant Pathol 46:373–386

Chen XM (2005) Epidemiology and control of stripe rust [*Puccinia striiformis f. sp. tritici*] on wheat. Can J Plant Pathol 27:314–337

Chen WQ, Wu LR, Liu TG, Xu SC (2009) Race dynamics, diversity, and virulence evolution in *Puccinia striiformis* f. sp. *tritici*, the causal

agent of wheat stripe rust in China from 2003 to 2007. Plant Dis 93: 1093–1101

Chen XM, Line RF, Leung H (1998) Genome scanning for resistance-gene analogs in rice, barley, and wheat by high-resolution electrophoresis. Theor Appl Genet 97:345–355

Cheng P, Xu LS, Wang MN, See DR, Chen XM (2014) Molecular mapping of genes *Yr64* and *Yr65* for stripe rust resistance in hexaploid derivatives of durum wheat accessions PI 331260 and PI 480016. Theor Appl Genet 127:2267–2277

Cheng Y, Song W, Liu ZY, Xie CJ, Ni ZF, Peng HR, Nie XL, Yang ZM, Sun QX (2006) Microsatellite markers for a yellow rust resistant gene in wheat cultivar Guinong 21. Acta Agronom Sin 32:1867–1872 (In Chinese)

El-Bedewy R, Robbelen G (1982) Chromosomal location and change of dominance of a gene for resistance against yellow rust, *Puccinia striiformis* West., in wheat, *Triticum aestivum* L. Zeitschrift fur Pflanzenzuchtung 89:145–157

Han DJ, Wang N, Jiang Z, Wang QL, Wang XJ, Kang ZS (2012) Characterization and inheritance of resistance to stripe rust in the wheat line Guinong 775. Hereditas 34:1607–1613 (In Chinese)

Han DJ, Wang QL, Zhang L, Wei GR, Zeng QD, Zhao J, Wang XJ, Huang LL, Kang ZS (2010) Evaluation of resistance of current wheat cultivars to stripe rust in northwest China, north China and the middle and lower reaches of Changjiang river epidemic area. Sci Agric Sin 43:2889–2896 (In Chinese)

Hou L, Ma DF, Hu ML, He MM, Lu Y, Jing JX (2013) Genetic analysis and molecular mapping of an all-stage stripe rust resistance gene in *Triticum aestivum–Haynaldia villosa* translocation line V3. J Integr Agr 12:2197–2208

Kertho A, Mamidi S, Bonman JM, McClean PE, Acevedo M (2015) Genome-wide association mapping for resistance to leaf rust and stripe rust in winter-habit hexaploid wheat landraces. PLoS One 10:e0129580. doi:10.1371/journal.pone.0129580

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

Lan CX, Liang SS, Zhou XC, Zhou G, Lu QL, Xia XC, He ZH (2010) Identification of genomic regions controlling adult-plant stripe rust resistance in Chinese landrace Pingyuan 50 through bulked segregant analysis. Phytopathology 100:313–318

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

Li ZQ, Zeng SM (2002) Wheat rust in China. Chinese Agricultural Press, Beijing. p 41–50, 164–173. (In Chinese)

Li GQ, Li ZF, Yang WY, Zhang Y, He ZH, Xu SC, Singh RP, Qu YY, Xia XC (2006a) Molecular mapping of stripe rust resistance gene *YrCH42* in Chinese wheat cultivar Chuanmai 42 and its allelism with *Yr24* and *Yr26*. Theor Appl Genet 112:1434–1440

Li ZF, Zheng TC, He ZH, Li GQ, Xu SC, Li XP, Yang GY, Singh RP, Xia XC (2006b) Molecular tagging of stripe rust resistance gene *YrZH84* in Chinese wheat line Zhou 8425B. Theor Appl Genet 112:1098–1103

Li Y, Niu YC, Chen XM (2009) Mapping a stripe rust resistance gene *YrC591* in wheat variety C591 with SSR and AFLP markers. Theor Appl Genet 118:339–346

Li Q, Chen XM, Wang MN, Jing JX (2011) Yr45, a new wheat gene for stripe rust resistance on the long arm of chromosome 3D. Theor Appl Genet 122:189–197

Li Q, Li GB, Yue WY, Du JY, Yang LJ, Kang ZS, Jing JX, Wang BT (2016) Pathogenicity changes of wheat stripe rust fungus and disease resistance of wheat cultivars (lines) in Shaanxi province during 2002-2014. Acta Phytophyl Sin 46:374–383

Lin F, Chen XM (2007) Genetics and molecular mapping of genes for race-specific all-stage resistance and non-race-specific high-temperature adult-plant resistance to stripe rust in spring wheat cultivar Alpowa. Theor Appl Genet 114:1277–1287

Lin F, Xu SC, Zhang LJ, Miao Q, Zhai Q, Li N (2005) SSR marker of wheat stripe rust resistance gene *Yr2*. J Triticeae Crop 25:17–19 (In Chinese)

Line RF (2002) Stripe rust of wheat and barley in North America: a retrospective historical review. Annu Rev Phytopathol 40:75–118

Liu RH, Meng JL (2003) Map Draw: a Microsoft Excel macro for drawing genetic linkage maps based on given genetic linkage data. Hereditas 25:317–321 (In Chinese)

Liu XK (1988) A preliminary study on the inheritance of resistance to stripe rust in wheat. Acta Phytophyl Sin 15:33–39 (In Chinese)

Lu Y, Wang MN, Chen XM, See DR, Chao SM, Jing JX (2014) Mapping of *Yr62* and a small-effect QTL for high-temperature adult-plant resistance to stripe rust in spring wheat PI192252. Theor Appl Genet 127:1449–1459

Ma DF, Li Q, Tang MS, Chao KX, Li JC, Wang BT, Jing JX (2015) Mapping of gene conferring adult-plant resistance to stripe rust in Chinese wheat landrace Baidatou. Mol Breeding 35:157–165

Maccaferri M, Zhang JL, Bulli P, Abate Z, Chao SM, Cantu D, Bossolini E, Chen XM, Pumphrey M, Dubcovsky J (2015) A genome-wide association study of resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in a worldwide collection of hexaploid spring wheat (*Triticum aestivum* L.) G3: Genes Genom Genet 5:449–465

McIntosh RA, Hart GE, Devos KM, Gale MD, Rogers WJ, Slinkard A E (1998) Catalogue of gene symbols for wheat. In: Proceedings of the 9th International Wheat Genetics Symposium. vol. 5. University Extension Press, University of Saskatchewan, Saskatoon, p 1.125.

McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Appels R, Xia XC (2014) Catalogue of gene symbols for wheat: 2013–2014 supplement. http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp

Riede CR, Anderson JA (1996) Linkage of RFLP markers to an aluminum tolerance gene in wheat. Crop Sci 36:905–909

Ren Q, Liu HJ, Chen Y, Xu SC, He MZ, Xin ZY, Zhang ZY (2010) Molecular tagging of a stripe rust resistance gene in a *Triticum durum-Aegilops squarrosa* synthetic wheat CI191. Acta Agronom Sin 36:721–727 (In Chinese)

Ren RS, Wang MN, Chen XM, Zhang ZJ (2012) Characterization and molecular mapping of *Yr52* for high-temperature adult-plant resistance to stripe rust in spring wheat germplasm PI183527. Theor Appl Genet 125:847–857

Ren Y, Li SR, Xia XC, Zhou Q, He YJ, Wei YM, Zheng YL, He ZH (2015) Molecular mapping of a recessive stripe rust resistance gene yrMY37 in Chinese wheat cultivar Mianmai 37. Mol Breeding 35: 97

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A micro-satellite map of wheat. Genetics 149: 2007–2023

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.) Theor Appl Genet 109:1105–1114

Sthapit J, Newcomb M, Bonman JM, Chen XM, See DR (2014) Genetic diversity for stripe rust resistance in wheat landraces and identification of accessions with resistance to stem rust and stripe rust. Crop Sci 54:2131–2139

Wan AM, Wu LR, Jia QZ, Jin SL, Wang BT, Yao G, Yang JX, Yuan ZY, Bi YQ (2003) Pathogenic changes of stripe rust fungus of wheat in China during 1997-2001. Acta Agronom Sin 33:261–266 (In Chinese)

Wan AM, Zhao ZH, Chen XM, He ZH, Jin SL, Jia QZ, Yao G, Yang JX, Wang BT, Li GB, Bi YQ, Yuan ZY (2004) Wheat stripe rust epidemic and virulence of *Puccinia striiformis* f. sp. *tritici* in China in 2002. Plant Dis 88:896–904

Wan AM, Chen XM, He ZH (2007) Stripe rust in China. Aust J Agric Res 58:605–619

Wang LF, Ma JX, Zhou RH, Wang XM, Jia JZ (2002) Molecular tagging of the yellow rust resistance gene *Yr10* in common wheat, PI178383 (*Triticum aestivum* L.) Euphytica 124:71–73

Wang BT, Li GB, Li Q, Wang F, Kang ZS (2007) Population changes of stripe rust fungus of wheat in Shaanxi province during 2001-2005. Journal of Northwest A & F University (Nat Sci Ed) 35:209–216 (In Chinese)

Wang CM, Zhang YP, Han DJ, Kang ZS, Li GP, Cao AZ, Chen PD (2008) SSR and STS markers for wheat stripe rust resistance gene *Yr26*. Euphytica 159:359–366

Wang LM, Zhang ZY, Liu HJ, Xu SC, He MZ, Liu HX, Veisz O, Xin ZY (2009) Identification, gene postulation and molecular tagging of a stripe rust resistance gene in synthetic wheat CI142. Cereal Res Commun 37:209–215

Wang MN, Chen XM, Xu LS, Cheng P, Bockelman HE (2012) Registration of 70 common spring wheat germplasm lines resistant to stripe rust. Journal of Plant Registrations 6(1):104–110

Wellings CR (2011) Global status of stripe rust: a review of historical and current threats. Euphytica 179:129–141

Xu LS, Wang MN, Cheng P, Kang ZS, Hulbert S, Chen XM (2013) Molecular mapping of *Yr53*, a new gene for stripe rust resistance in durum wheat accession PI480148 and its transfer to common wheat. Theor Appl Genet 126:523–533

Xu HX, Zhang J, Zhang P, Qie YM, Niu YC, Li HJ, Ma PT, Xu YF, An DG (2014) Development and validation of molecular markers closely linked to the wheat stripe rust resistance gene YrC591 for marker-assisted selection. Theor Appl Genet 198:317–323

Yan GP, Chen XM, Line RF, Wellings CR (2003) Resistance gene analog polymorphism markers co-segregating with the *Yr5* gene for resistance to wheat stripe rust. Theor Appl Genet 106:636–643

Yan YD, Lu LL, Zhou XL, Wang MN, Jing JX (2009) The genetic analysis and SSR molecular markers of stripe rust resistance gene in Chinese differential cultivar Suwon 11. J Plant Protect 36:285–286 (In Chinese)

Yang ZM, Xie CJ, Sun QX (2003) Situation of sources of stripe rust resistant of wheat in the post-CYR32 era in China. Acta Agronom Sin 29:161–168 (In Chinese)

Yaniv E, Raats D, Ronin Y, Korol AB, Grama A, Briana H, Dubcovsky J, Schulman AH, Fahima T (2015) Evaluation of marker-assisted selection for stripe rust resistance gene *Yr15*, introgressed from wild emmer wheat. Mol breeding 35:43

Zhang H, Ren ZL, Hu YG, Wang CY, Ji WQ (2010) Characterization of wheat stripe rust resistance genes in Shaanmai 139. Acta Agronom Sin 36:109–114 (In Chinese)

Zhang SY, Xu ZQ, Wang R, Li Q, Yao Q, JING JX (2011) Genetics and molecular mapping of stripe rust resistance gene *YrShan515* in Chinese wheat cultivar Shan 515. J Integr Agr 10:553–559

Zhang SY (2010) Genetic analysis of stripe rust resistance gene in wheat varieties Shan515, Xiaoyan016 and Xiaoyan9366. M.S. Thesis, Northwest A&F University, page 24–25. (In Chinese)

Zhou XL, Wang MN, Chen XM, Lu Y, Kang ZS, Jing JX (2014) Identification of *Yr59* conferring high temperature adult plant resistance to stripe rust in wheat germplasm PI178759. Theor Appl Genet 127:935–945

Zhou XL, Zhan GM, Huang LL, Han DJ, Kang ZS (2015) Evaluation of resistance to stripe rust in eighty abroad spring wheat germplasms. Sci Agric Sin 48:1518–1526 (In Chinese)