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Evaluating the contribution of Yr genes to stripe rust resistance breeding through marker-assisted detection in wheat

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Abstract Numerous stripe rust resistance genes have been identified from wheat, and new virulent races of *Puccinia striiformis* f. sp. *tritici* have also emerged in recent years. Deployment of diverse combinations of resistance genes is an efficient way to combat virulent evolution of strip rust pathogen. In this study, publically available molecular markers were used to identify the distribution of 36 Yr genes in 672 wheat accessions. The effectiveness of Yr genes individually and in combinations was also evaluated in field conditions. The result showed effective resistance of some recently applied genes, such as *Yr15* and *Yr65*. It also showed the lost efficacy of some once widely used genes, such as *Yr9* and *Yr10*. Moreover, significant additive effects were observed in some gene combinations, such as *Yr9* + *Yr18* and *Yr30* + *Yr46*. Proper deploying of Yr genes and utilizing the positive interactions will be helpful for durable resistance breeding in wheat.

Keywords Stripe rust · Resistance gene · Gene pyramiding · Marker-assisted selection · Wheat breeding

Introduction

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a serious fungal disease for wheat, especially in hypothermal and moist environments (Chen et al. 2014). To control this disease genetically, numerous Yr (yellow rust) genes have been found (McIntosh et al. 2016). The formally designated Yr genes were up to *Yr76* (Dracatos et al. 2016; Xiang et al. 2016). *Yr15*, *Yr24/Yr26*, *Yr35*, *Yr36*, *Yr53*, *Yr64* and *Yr65* were derived from tetraploid wheat, *Yr8* from *Aegilops. comosa* (Niu et al. 2004), *Yr9* from *Secale cereale* (Mago et al. 2005), *Yr17* from *Ae. ventricosa* (Jia et al. 2011), *Yr28* and *Yr48* from *Ae. tauschii* (Singh et al. 2000; Lowe et al. 2011), *Yr37* from *Ae. kotschyi* (Heyns et al. 2011), *Yr38* from *Ae. sharonensis* (Marais et al. 2010), *Yr40* from *Ae. geniculata* (Kuraparthy et al. 2009), *Yr42* from *Ae. neglecta* (Marais et al. 2009), *Yr50* from *Thinopyrum intermedium* (Liu et al. 2013), *Yr70* from *Ae. umbellulata* (Bansal et al. 2016), and others mainly from hexaploid landraces (McIntosh et al. 2016).

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To date, *Yr10*, *Yr18*, *Yr36* and *Yr46* have been cloned (Liu et al. 2014; Krattinger et al. 2009; Fu et al. 2009; Moore et al. 2015). *Yr10* encodes a NBS–LRR protein; *Yr18* encodes an ATP-binding cassette (ABC) transporter; *Yr36* encodes a wheat Kinase-START (WKS) protein; *Yr46* encodes a hexose transporter. Fine mapping of *Yr9*, *Yr15* and *Yr26* was also conducted (Mago et al. 2005; Zhang et al. 2013; Abdollahi Mandoulakani et al. 2015). Moreover, a lot of functional genes involved in the wheat-stripe rust responses have been identified, such as *TaHLRG*, *TaMDHAR* and *TaADF7* (Liu et al. 2008a, b; Feng et al. 2014; Fu et al. 2014). Concerning coevolution of plant and pathogen, the effectiveness of single gene to the resistance is limited and short-term. Gene pyramiding will be an effective way to improve plant durable resistance (Ellis et al. 2014).

When proposing pyramiding strategies, distribution of Yr genes needs to be identified in wheat germplasms and breeding lines. Marker-assisted detection (MAD) is the most commonly used method to identify the presence of Yr genes (Goutam et al. 2015). Since most markers are linkage markers instead of gene markers, the validity of them remains to be assessed. The presence of *Yr5*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr18*, *Yr26* or *Yr36* has been identified in a limited number of wheat cultivars and breeding lines (Tabassum et al. 2010; Yuan et al. 2012; Zeng et al. 2014). There are a great number of *Pst* resistance genes, especially newly reported ones (McIntosh et al. 2016), having not been detected in diverse germplasms and breeding lines. Moreover, the effectiveness of most Yr genes against the newly emerged *Pst* races, such as PST-V26 (Tian et al. 2016), is not very clear. The function of resistance gene also depends on the genetic background (Ellis et al. 2014). So, it is meaningful to evaluate the effectiveness of Yr genes in diverse germplasms not just in the near-isogenic lines (NILs).

Many researches have indicated that additive effects exist extensively among Yr genes. For example, complex additive interactions were observed by Yang et al. (2013). *Yr31* suppressed the additive effect of *Yr30* and a 3D locus, but not of *Yr18* in Mexican. The 3D and 5BL loci were generally not additive with each other, but were additive when combined with other loci in China. Additive effects were also observed between *Yr58* and *Yr46*. The recombinant inbred lines (RILs) carrying both genes showed a lower IT than those carrying *Yr58* or *Yr46* individually (Chhetri et al. 2016). Studies also showed that rust resistance can be enhanced by combining all stage resistance (ASR) or seedling resistance (SR) genes with adult plant resistance (APR) or slow rusting resistance (SLR) genes (Chen et al. 2013; Ellis et al. 2014). It is necessary to evaluate the interactions between different Yr genes pyramided together.

The Sichuan Province in China is an important overwintering area for stripe rust races. The prevalent *Pst* races are PST-CYR32 and PST-CYR33 (Zhou et al. 2014a, b). New virulent races also emerged frequently, such as PST-G22 (Xiang et al. 2013). If stripe rust could not be controlled in the Chengdu Plain, it would put a threat on the wheat production of the middle and lower reaches of the Yangtze River. To control epidemics of stripe rust, the evaluation for the efficacy of Yr genes in wheat germplasms and breeding lines should be timely assayed.

Materials and methods

Plant materials

A total of 672 wheat accessions (Table S1) were collected, including 17 Yr gene NILs of Avocet "S" (AvS), 21 cultivars and 147 breeding lines of "Chuanyu" wheats, 170 landraces and 140 cultivars in China, 148 accessions from all over the world, and 29 synthetic wheats. Among these materials, the NILs were received from the Sichuan Academy of Agricultural Sciences (SAAS), the 200 China core collections and 84 foreign germplasms were obtained from the Chinese Academy of Agricultural Sciences (CAAS, http://www.cgris.net/), and 23 accessions containing specific Yr genes were acquired from USDA-ARS (http://www.ars-grin.gov/).

Field testing

All 672 wheat accessions were evaluated for stripe rust reaction at Shuangliu, Shifang and Jitian in Sichuan Province in 2013, 2014 and 2015. Twenty seeds of each accession were planted in a row, and susceptible wheat strains "Minxian169" and "Chuanyu12" were inserted after every 9 rows. Mixed *Pst* spores of races PST-CYR32, PST-CYR33, PST-SU11, PST-Hybrid46 and PST-G22 (provided by SAAS), were suspended in 0.05% Tween 20 and were sprayed on wheat seedling

leaves at trefoil stage. The infection types (ITs) were recorded at the adult plant stages (twice for each environment at 150 and 164 days after seeding) by a modified method according to the standard classification system from 0 to 4 (McIntosh et al. 1995).

Molecular markers

Two closely linked (usually flanking) markers of each Yr gene were chosen to identify its presence/absence in the wheat accessions, except a few genes for which only one closely linked marker was reported. As shown in Table 1, a total of 77 markers (37 SSR markers, 15 STS markers and 25 EST or gene based markers) for 39 stripe rust resistance genes were employed in this study. The primer sequences were synthesized by GENEWIZ Biotech (China).

DNA extraction

A total of 100 mg fresh leaf tissue was collected from each accession, frozen in liquid nitrogen, and ground to powder with a high-throughput tissue grinder. Then, 2× hexadecyltrimethylammonium bromide (CTAB) extraction buffer containing 1.4 M NaCl, 100 mM Tris–HCl (pH 8.0), 2% CTAB and 20 mM EDTA was added to extract the genomic DNA according to Riede and Anderson (1996). Finally, the DNA was dissolved in 100 µl TE buffer with 10 mM Tris–HCl (pH 8.0), 1 mM EDTA and 20 µg/ml RNase, and was incubated for 1 h at 37 °C before storing at −20 °C.

PCR and electrophoresis

Polymerase chain reaction (PCR) was performed using a Master Cycler Pro PCR System (Eppendorf, Germany). A 10 µl PCR mixture consisted of 100 ng template DNA, 5 µl 2× Es Taq MasterMix (CWBIO Biotech, China), 0.3 µl 10 µM forward primer and 0.3 µl 10 µM reverse primer. Amplifications were programmed with 5 min of denaturation at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 40–60 °C (depending on the primer pair) and 30 s at 72 °C; and 72 °C for 10 min followed by a 4 °C hold step. PCR products were separated by 1.5% agarose gel, 8% non-denaturing polyacrylamide gel or 6% denaturing polyacrylamide gel, and were stained with ethidium bromide or silver nitrate (An et al. 2009), respectively. For the primer pairs STS-7/8, STS-9/10 and URIC/LN2, the PCR products were digested with *DpnII* (New England Biolabs, USA) according to Chen et al. (2003) before electrophoresis.

Phenotyping and genotyping

The immune plant with no visible symptoms was scored as IT 0, the highly resistant plant with little necrotic flecks and no sporulation was scored as IT 1, the moderately resistant plant with a few necrotic flecks and trace sporulation was scored as IT 2, the moderately susceptible plant with necrotic blotches and moderate sporulation was scored as IT 3, the highly susceptible plant with chlorotic stripes and abundant sporulation was scored as IT 4. The average ITs from the scores of all environments was used to represent the resistant/susceptible (R/S) phenotype of a wheat accession. Few wheat accessions in some environments were not scored because of their absence, but it did not affect the scores collected from other environments. While analyzing genotyping results, the wheat accession was counted for carrying a specific Yr gene only when both flanking markers were presented, except for a few genes where one closely linked marker has been reported.

Data analysis

All data of phenotypes and genotypes were recorded in Microsoft office excel 2010 for statistical analysis. To compare the effective of each gene in diverse genetic backgrounds, the data was divided into two groups (presence of a gene and absence of a gene). To evaluate the effective of different combinations of two resistance genes, the data was divided into four groups (group one, where both selected genes were present; group two and group three, where only one of the selected genes was present; group four, where none of the two genes was present). One-way analysis of variance (ANOVA) was conducted to evaluate the variance and significance among these groups.

Results

Detection of stripe rust resistance genes in 672 wheat accessions

The accuracy of MAD is affected by the distance between the target gene and the linkage markers. So,

Table 1 The information of molecular markers for the Yr genes used in this study

Gene	Marker	Type	Distance (cM)	Primer sequence	Annealing (°C)	+ (bp)	− (bp)	References
Yr1	*stm673acag*	STM	1.1	TAACTCACAACACGTTCTGGTCGT ACACACACACACACAGAGAGAG	56	120	124	Bansal et al. (2009)
Yr1	*BU099658*	EST-SSR		TGAATCAATACAAGGACGCC GTCACAACACTGGCAACCC	54	206		Hasancebi et al. (2014)
Yr4/ YrRub	*cfb3530*	SSR	2.9	TTGTGCTTGTGCTACTATTACC CAACATCTTACTGCTAACGTCC	50	150	155	Bansal et al. (2010)
Yr4/ YrRub	*barc75*	SSR	5.3	AGGGTTACAGTTTGCTCTTTTAC CCCGACGACCTATCTATACTTCTCTA	50	132	135/136/ 139	Bansal et al. (2010)
Yr5	*STS-7/8*	STS/CAPS	0.3	GTACAATTCACCTAGAGT GCAAGTTTTCTCCCTATT	46	478	472	Murphy et al. (2009)
Yr5	*STS-9/10*	STS/CAPS	0.7	AAAGAATACTTTAATGAA CAAACTTATCAGGATTAC	42	439		Zhang et al. (2009)
Yr5	WC6 WC5	STS		AAAGGTGCCTTAGGGTAG GTTGCGCATCATATTCAAATGCCG	46	250		Tabassum et al. (2010)
Yr5	*S19M93*	STS	0.54	TAATTGGGACCGAGAGACG TTCTTGCAGCTCCAAAACCT	55	100	62/275	Smith et al. (2007)
Yr5	*barc349*	SSR	0.4	CGA ATA GCC GCT GCA CAA G TAT GCA TGC CTT TCT TTA CAA T	46	105		Murphy et al. (2009)
Yr6	*wmc76*	SSR		CTTCAGAGCCTCTTTCTCTACA CTGCTTCACTTGCTGATCTTTG	51	256		Li and Niu (2007)
Yr6	*wmc276*	SSR		GACATGTGCACCAGAATAGC AGAAGAACTATTCGACTCCT	47	292		Li and Niu (2007)
Yr7	*gwm526*	SSR	5.3	CAATAGTTCTGTGAGAGCTGCG CCAACCCAAATACACATTCTCA	48	212	217	Yao et al. (2006)
Yr8	*SC-OPD11*	SCAR		AGCGCCATTGGGAGCTCTATGGTG AGCGCCATTGCCAATACATTTAAT	57	1265		Niu et al. (2004)
Yr9	*H20*	Rye chromosome specific		GTTGGAAGGGAGCTCGAGCTG GTTGGGCAGAAAGGTCGACATC	56	1598		Liu et al. (2008a, b)

Table 1 continued

Gene	Marker	Type	Distance (cM)	Primer sequence	Annealing (°C)	+ (bp)	− (bp)	References
Yr9	*P6M12-P*	STS	0.35	GTACTAGTATCCAGAGGTCACAAG CAGACAAACAGAGTACGGGC	56	250/350		Mago et al. (2005)
Yr10	*E51100*	SCAR		TCAAGGAGGTCAGTGACAG TCAGGGAGGTGTAGCCTAAT	56	1085		Liu et al. (2014)
Yr10	Yr10 F Yr10 R	Gene specific		TCAAAGACATCAAGAGCCGC TGGCCTACATGAACTCTGGAT	51	543		Liu et al. (2014)
Yr15	*barc8*	SSR	4.2	GCGGGAATCATGCATAGGAAAACAGAA GCGGGGGCGAAACATACACATAAAAACA	56	200	280	Murphy et al. (2009)
Yr15	*gwm413*	SSR	3.5	TGCTTGTCTAGATTGCTTGGG GATCGTCTCGTCCTTGGCA	55	96	98	Murphy et al. (2009)
Yr16	*wmc18*	SSR		CTGGGGCTTGGATCACGTCATT AGCCATGGACATGGTGTCCTTC	56	~390		Agenbag et al. (2012)
Yr16	*wmc245*	SSR		GCTCAGATCATCCACCAACTTC AGATGCTCTGGGAGAGTCCTTA	55	~670		Agenbag et al. (2012)
Yr17	*URIC/LN2*	*T. ventricosum* chromosome specific		GGTCGCCCTGGCTTGCACCT TGCAGCTACAGCAGTATGTACACAAAA	64	285	275	Jia et al. (2011)
Yr17	*SC-385*	SCAR	3.4	CTGAATACAAACAGCAAACCAG ACAGAAAGTGATCATTTCCATC	50	385		Jia et al. (2011)
Yr18	*csLV34*	STS	0.4	GTTGGTTAAGACTGGTGATGG TGCTTGCTATTGCTGAATAGT	51	150	229	Krattinger et al. (2009)
Yr18	L34DINT9F L34PLUSR	Gene specific		TTGATGAAACCAGTTTTTTTTCTA GCCATTTAACATAATCATGATGGA	51	517		Krattinger et al. (2009)
Yr26	*we173*	STS	1.4	GGGACAAGGGGAGTTGAAGC GAGAGTTCCAAGCAGAACAC	57	451/500	730	Zhang et al. (2013)
Yr26	*CON-4*	STS	0.08	GTGCTGTACCTGACGACGGA GTGGAGATGTTGGGCTTGG	58	~390		Zhang et al. (2013)
Yr28	*cib-Yr28M1*	STS			51	449		Developed from cloned gene sequence close to Xmwg634 locus, unpublished[a]
Yr28	*cib-Yr28M2*	SSR			55	245	290	

Table 1 continued

Gene	Marker	Type	Distance (cM)	Primer sequence	Annealing (°C)	+ (bp)	− (bp)	References
Yr29	*bac17R*	STS	2.1/4.2	CCCATGCTGACATGGCCACAT CTCTGCTCTTTAGTAGTTGCC	55	1700	500	Rosewarne et al. (2006)
Yr29	*wmc44*	SSR	3.6/10.9	GGTCTTCTGGGCTTTGATCCTG TGTTGCTAGGGACCCGTAGTGG	57	260	>260	Rosewarne et al. (2006)
Yr30	*stm598tgag*	STM		GTTGCTTTAGGGGAAAAGCC TCTCTCTCTCTCTCACACACAC	54	56	62/58	Hayden et al. (2004)
Yr30	*wms533*	SSR	2	AAGGCGAATCAAACGGAATA GTTGCTTTAGGGGAAAAGCC	55	120	105/155	Hayden et al. (2004)
Yr33	*gwm111*	SSR		TCTGTAGGCTCTCTCCGACTG ACCTGATCAGATCCCACTCG	52	184	206	Zahravi et al. (2003)
Yr33	*gwm437*	SSR		GATCAAGACTTTTGTATCTCTC GATGTCCAACAGTTAGCTTA	46	111	109	Zahravi et al. (2003)
Yr35	*cfd1*	SSR	4.1	ACCAAAGAACTTGCCTGGTG AAGCCTGACCTAGCCCAAAT	56	225	275	Dadkhodaie et al. (2011)
Yr35	*gwm508*	SSR	7.5	GTTATAGTAGCATATAATGGCC GTGCTGCCATGATATTT	51	135		Dadkhodaie et al. (2011)
Yr36	*Yr36E1a*	Gene specific		AAGGCAAAGGCAAAGTGG TGATCTTTACCAAGCATTCG	57	911		Fu et al. (2009)
Yr36	*Yr36START*	Gene specific		GGCCACACTGCAATACTATACC CACAAATCCTGGCTGTGGAC	52	871/537		Fu et al. (2009)
Yr39	*wgp36*	RGAP	0.8	GCATTGGAACAAGGTGAA GAYGTNAARCCIGARAA	45	830		Lin and Chen (2007)
Yr39	*wgp45*	RGAP	1.9	GCATTGGAACAAGGTGAA CCGTTGGACAGGAAGGAG	45	940		Lin and Chen (2007)
Yr41/ YrCN19	*gwm410*	SSR	0.3	GCTTGAGACCGGCACAGT CGAGACCTTGAGGGTCTAGA	58	410/367/ 151	157/335/ 338	Luo et al. (2008)
Yr41/ YrCN19	*gwm374*	SSR	7.9	ATAGTGTGTTGCATGCTGTGTG TCTAATTAGCGTTGGCTGCC	60	192	210/212	Luo et al. (2008)
Yr44	pWB5/ pWN1R1	STS	9.4	GGTGCAATTTGAGTTTGGAGT GGTGTTGACTGGAGAATCCG	49	380		Sui et al. (2009)

Table 1 continued

Gene	Marker	Type	Distance (cM)	Primer sequence	Annealing (°C)	+ (bp)	− (bp)	References
Yr44	*wgp100*	RGAP	3.9	GCATTGGAACAAGGTGAA GGTGGGGTTGGGAAGACAACG	45	820		Sui et al. (2009)
Yr46	*gwm165*	SSR	0.4	TGCAGTGGTCAGATGTTTCC CTTTTCTTTCAGATTGCGCC	49	~236		Herrera-Foessel et al. (2014)
Yr46	*gwm192*	SSR	0.4	GGTTTTCTTTCAGATTGCGC CGTTGTCTAATCTTGCCTTGC	54	~130		Herrera-Foessel et al. (2014)
Yr48	*BE495011*	EST-SSR	0.09	TGATTACTGTAGCTACCTCCTCCT GGTGCAAGATGTGCCTGTAA	56	236	228	Lowe et al. (2011)
Yr48	*SNF-A2*	STS	0.18	TCCGTCTCCATCATTCAACA GTGTTGCGCAAGTTTGTGAC	49	150	200	Lowe et al. (2011)
Yr49	*gpw7321*	SSR	0	TGAGACCATCTCTTGCTCCA TCACAGTCGCTGCATAAAGG	57	165		McIntosh et al. (2016)
Yr49	*gwm161*	SSR	1	GATCGAGTGATGGCAGATGG TGTGAATTACTTGGACGTGG	57	154	145	McIntosh et al. (2016)
Yr51	*sun106*	DArT-STS	1.8	TGCACACAAGGAGAGGAGTG AGAGGACAGTGCCCGTGTAG	56			Randhawa et al. (2014)
Yr51	*Owm45F3R3*	Gene specific	1.2	CGCAACAGGGACCGGTAT GAGCTGCTGGTCGGAACTC	56	~300/ 250		Randhawa et al. (2014)
Yr51	*sun104*	DArT-STS	2.5	TGCTATGTGCGTGATGATGA TTACATGCTCCAGCGACTTG	56	225		Randhawa et al. (2014)
Yr52	*wgp5258*	RGAP	1.1	GGCAAGACCACATTA CCATATGTCATCAATGAG	45	650	<650	Ren et al. (2012)
Yr52	*barc182*	SSR	1.2	CCATGGCCAACAGCTCAAGGTCTC CGCAAAACCGCATCAGGGAAGCACCAAT	59	75	>75	Ren et al. (2012)
Yr53	*wmc441*	SSR	5.6	TCCAGTAGAGCACCTTTCATT ACGAAGATAAACAAACGG	47	151/159		Xu et al. (2013)
Yr53	*STS2F/1R*	STS	4.1	AGGGATAGGGCGTGGTT CCACCACGTAGCAACACTTA	50	219		Xu et al. (2013)
Yr57	*gwm389*	SSR	2	ATCATGTCGATCTCCTTGACG	51	128	117	Randhawa et al. (2015)

Table 1 continued

Gene	Marker	Type	Distance (cM)	Primer sequence	Annealing (°C)	+ (bp)	− (bp)	References
				TGCCATGCACATTAGCAGAT				
Yr57	*mag2095*	STS	2	AAGAAGCTGAGGCTCACCAA	56	~660		Randhawa et al. (2015)
				TTCGTCCTGGCTCTTCTGTT				
Yr59	*barc32*	SSR	1.1	GCGTGAATCCGGAAACCCAATCTGTG	55	165	175	Zhou et al. (2014a, b)
				TGGAGAACCTTCGCATTGTGTCATTA				
Yr59	*wgp5175*	RGAP	2.1	GGAGGCTTAGGGAAG	45	450		Zhou et al. (2014a, b)
				TGGTAGGTCCTTGTA				
Yr60	*wmc313*	SSR	0.6	GCAGTCTAATTATCTGCTGGCG	53	197		Herrera-Foessel et al. (2014)
				GGGTCCTTGTCTACTCATGTCT				
Yr60	*wmc776*	SSR	0	CCATGACGTGACAACGCAG	52	194		Herrera-Foessel et al. (2014)
				ATTGCAGGCGCGTTGGTA				
Yr61	*STS5765b*	STS	3.9	GATTTGCGATTACTCTACTA	50	300	285	Zhou et al. (2014a, b)
				GGCTTTCGTGTCCGTCTC				
Yr61	*STS5467*	STS	1.9	CACACACCTCAGCTCTTGGATG	55	175		Zhou et al. (2014a, b)
				CAAGAAGGCGACGAGGATGTTG				
Yr62	*gwm192*	SSR	2	GGTTTTCTTTCAGATTGCGC	51	222		Lu et al. (2014)
				CGTTGTCTAATCTTGCCTTGC				
Yr62	*gwm251*	SSR	3.3	CAACTGGTTGCTACACAAGCA	51	133		Lu et al. (2014)
				GGGATGTCTGTTCCATCTTAG				
Yr64	*gwm413*	SSR	3.5	TGCTTGTCTAGATTGCTTGGG	52	95	91	Cheng et al. (2014)
				GATCGTCTCGTCCTTGGCA				
Yr64	*gdm33*	SSR	2	GGCTCAATTCAACCGTTCTT	55	~130		Cheng et al. (2014)
				TACGTTCTGGTGGCTGCTC				
Yr65	*gwm18*	SSR	1.2	TGGCGCCATGATTGCATTATCTTC	53	182	188	Cheng et al. (2014)
				GGTTGCTGAAGAACCTTATTTAGG				
Yr65	*gwm11*	SSR	2.1	GGATAGTCAGACAATTCTTGTG	50	213	202	Cheng et al. (2014)
				GTGAATTGTGTCTTGTATGCTTCC				
Yr67/ YrC591	*SC-P35M48*	SCAR	11.7	GTTCAGACACAGCACAACTCGG	53	373		Li et al. (2009)
				CCTGAGTAACACAGTACGGATG				

Table 1 continued

Gene	Marker	Type	Distance (cM)	Primer sequence	Annealing (°C)	+ (bp)	− (bp)	References
Yr67/ YrC591	*cfa2040*	SSR	8	TCAAATGATTTCAGGTAACCACT	52	286		Li et al. (2009)
				TTCCTGATCCCACCAAACAT				
YrSP	*dp269*	STS	1.5	CTGCTGTCACCGCTCTCC	55	198	209	Feng et al. (2015)
				AGTCACACGCCCTACTCTCC				
YrZH84	*Xrga-1*	RGAP	0.8	TAGGGCCTCTTGCATCGT	45	343		Yin et al. (2009)
				GGAATGGGNGGNGTNGGNAARAC				
TaHLRG	*THR1*	SSR	0	GTAAGCTTCTCCTCCCACACCCACCCCTCTCC	60	157	145	Liu et al. (2008a, b)
				CTTGGCGCCTGCCCCCGTCATACTTGTC				

CAPS cleaved amplified polymorphism sequences, *DArT* diversity arrays technology, *EST* expressed sequence tag, *RGAP* resistance gene analogs polymorphisms, *SCAR* sequence characterized amplified regions, *SSR* simple sequence repeats, *STM* sequence tagged microsatellites, *STS* sequence tagged site, *cM* centimorgan, *bp* base pair, "+" the PCR product indicating the existence of corresponded gene, "−" the PCR product indicating the absence of corresponded gene

[a] The primers for *Yr28* available via a Material Transfer Agreement

closely linked markers are desired. For example, a recent report indicated that *YrSP* was different from *Yr5/Yr7* (Feng et al. 2015). Both of them were located on the 2BL chromosome, and were hard to be distinguished. Seven published markers were screened in CS, Avocet and Yr-NILs of *Yr5*, *Yr7* and *YrSP* (Fig. 1). *S19M93* and *STS7/8* can be used to identify *Yr5*; *gwm526* and *barc349* can be used to identify *Yr7*; *dp269* and *WC5/WC6* can be used to identify *YrSP*. Moreover, some markers were easy to be distinguished, such as STS (*S19M93* for *Yr5*) and SCAR (*SC385* for *Yr17*), and were helpful for high-throughput detection. By using the NILs or donors of the corresponding gene as the positive control, the detection of 36 Yr genes in 672 wheat accessions was accomplished (Fig. S1).

Distribution of 36 *Pst* resistance genes in different taxon groups

Totally, *Yr10*, *Yr17* and *Yr18* were identified positively in numerous accessions, while *Yr8*, *Yr36*, *Yr61* and *YrZH84* were detected positively only in a few accessions. As shown in Table 2, *Yr5*, *Yr9*, *Yr15*, *Yr17*, *Yr30*, *Yr48*, *Yr65*, *Yr67*, *YrSP* and *TaHLRG* showed high frequency in "Chuanyu" breeding lines; *Yr10*, *Yr18*, *Yr33*, *Yr51*, *Yr59* and *Yr62* showed high frequency in Chinese landraces; *Yr5*, *Yr9*, *Yr29*, *Yr30*, *Yr39*, *Yr41*, *Yr48*, *Yr49*, *Yr57* and *Yr59* showed high frequency in Chinese modern cultivars; *Yr4*, *Yr5*, *Yr7*, *Yr9*, *Yr29*, *Yr30*, *Yr33*, *Yr41*, *Yr52*, *Yr57*, *Yr60* and *YrSP* showed high frequency in introduced foreign germplasms. These results revealed broad diversity of resistance genes in different wheat taxon groups. Moreover, the accessions carrying multiple Yr genes identified in this study might be useful as parental lines for diversifying *Pst* resistance sources in wheat breeding.

Stripe rust reactions in field testing

By analyzing the IT scores collected from three places and three years, environmental variation was non-significant ($p = 0.87$), but significant variances were observed among the average ITs of different wheat accessions from all environments ($p < 0.01$). The overview for the percentages of each *Pst* infection phenotype and the average ITs of whole wheat accessions and four taxon groups are shown in Fig. S2. The ratio of resistant to susceptible phenotype

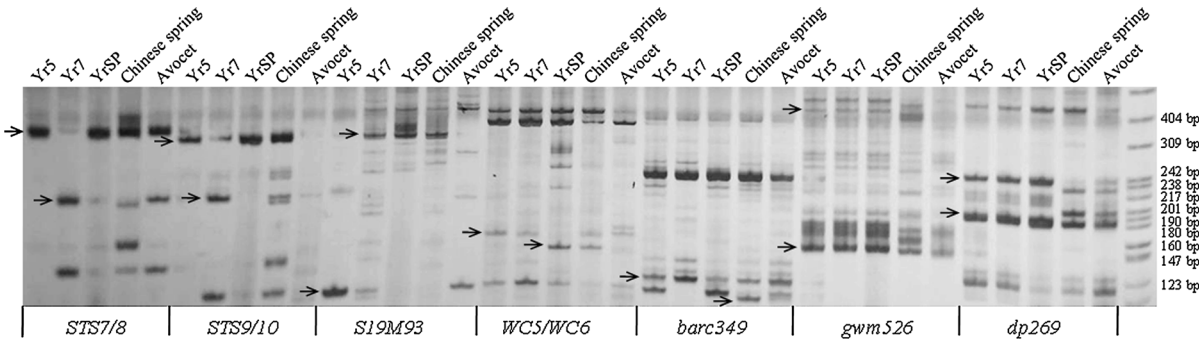

Fig. 1 Electrophoretogram of seven PCR based markers for the *Yr5*/*Yr7*–*YrSP* locus. An 8% non-denaturing polyacrylamide gel was used to separate the PCR products. A molecular weight standard of base pairs was listed in the *right*. *Right arrow* indicates the polymorphism bands of each molecular marker among the five wheat accessions

in the total wheat accessions is near to 1:1, and the average IT of the total wheat accessions is between 2.5 and 3.

Relationship between the number of pyramided genes and *Pst* resistance

To obtain a realistic relationship between *Pst* resistance and the pyramided gene number, the results of MAD and field testing were combined for analysis. As shown in Fig. 2, a significant positive correlation ($R^2 > 0.8$, $p < 0.01$) between the number of Yr genes and the *Pst* resistance was observed in the 672 wheat accessions. This high correlation suggested that pyramiding of Yr genes will be an effective way to improve stripe rust resistance in wheat breeding. Moreover, the accessions carrying multiple Yr genes might be useful as intermediate materials for introducing *Pst* resistance sources into commercial varieties.

Contribution of Yr genes individually to *Pst* resistance

The contribution of each Yr gene to stripe rust resistance was evaluated in diverse genetic backgrounds. As shown in Fig. 3, the wheat accessions carrying *Yr15*, *Yr17*, *Yr18*, *Yr65* and *Yr67* had significantly lower ITs than those without the corresponding gene, whilst the situation for *Yr29* was opposite. The other genes did not meet a significant level to stripe rust resistance in this study. Moreover, the contribution of eleven genes to *Pst* resistance in the four taxon groups was shown in Fig. S3. These results revealed some Yr genes contributed effective resistance to the current *Pst* races. The other genes that did not show significance but still had lower ITs, such as *Yr46* and *Yr60*, might contribute to partial resistance in pyramids, which will be analyzed in the following section.

Comparing different combinations of two genes with respect to *Pst* resistance

A total of 183 combinations of each two genes were suitable for the statistical analysis, and partial results of them were shown in Fig. 4. 30.23% combinations showed a similar average ITs with those carrying either one gene, 27.13% combinations showed a higher average ITs than those either one gene present, and 42.64% combinations showed a lower average ITs than those either one gene present. The results revealed that some combinations of two genes, one conferring significant resistance and another conferring non-significant resistance (Fig. 3), such as *Yr17* + *Yr26* and *Yr9* + *Yr18*, effectively improved the *Pst* resistance in the field trails. The results also showed that some combinations of two genes conferring non-significant resistance, such as *Yr30* + *Yr46*, effectively improved the *Pst* resistance when pyramided together. But in some other combinations, there was a reduction of resistance compared with those carrying either of the two genes, such as *Yr48* + *Yr67*. These results revealed the additive effects or epistatic effects between the resistance genes when pyramided.

Table 2 The distribution percentage of each resistance gene in different wheat panels

Genes	Total wheat accessions (%)	"Chuanyu" breeding lines (%)	Chinese landraces (%)	Chinese modern cultivars (%)	Introduced foreign germplasms (%)	Average
Yr4/YrRub	8.18	7.74	6.47	5.71	**14.19**[a]	8.46
Yr5	12.65	**13.69**	4.71	**21.43**	**14.86**	13.47
Yr6	5.36	1.79	6.47	8.57	6.08	5.65
Yr7	8.48	10.12	3.53	8.57	**13.51**	8.84
Yr8	0.15	0.00	0.00	0.00	0.00	0.03
Yr9	16.07	**23.21**	4.12	**20.00**	**18.92**	16.46
Yr10	20.83	16.67	**38.82**	13.57	15.54	21.09
Yr15	3.87	**12.50**	0.00	1.43	0.68	3.69
Yr16	5.65	8.33	2.35	2.86	10.14	5.87
Yr17	**24.26**	**74.40**	2.35	12.86	6.76	24.13
Yr18	**22.77**	0.60	**59.41**	14.29	15.54	22.52
Yr24/Yr26	4.32	6.55	0.59	7.14	3.38	4.39
Yr28	3.72	0.00	0.59	2.86	12.16	0.06
Yr29	9.08	0.00	2.35	**15.71**	**18.92**	9.21
Yr30	24.11	**25.60**	7.06	**31.43**	**35.81**	24.80
Yr33	14.29	5.95	**27.65**	9.29	**17.57**	14.95
Yr36	0.45	0.00	0.00	0.15	0.30	0.18
Yr39	6.10	0.00	7.06	**13.57**	6.76	6.70
Yr41/YrCN19	10.27	7.14	7.65	**14.29**	**14.86**	10.84
Yr46	3.27	1.19	1.18	1.43	7.43	2.90
Yr48	18.90	**39.29**	5.88	**25.00**	6.08	19.03
Yr49	6.55	4.17	4.12	**15.71**	4.73	7.05
Yr51	15.63	0.60	**41.76**	10.71	12.16	16.17
Yr52	7.14	0.00	1.76	7.86	**22.97**	7.95
Yr53	2.23	0.00	0.00	0.71	8.78	2.35
Yr57	9.67	2.98	4.71	**19.29**	**10.81**	9.49
Yr59	15.18	2.98	**36.47**	**17.86**	6.76	15.85
Yr60	5.65	1.79	2.35	4.29	**15.54**	5.92
Yr61	0.45	0.00	0.00	0.00	0.45	0.18
Yr62	6.99	5.36	**11.18**	7.14	4.73	7.08
Yr64	6.10	4.17	5.29	7.86	8.78	6.44
Yr65	6.25	**14.29**	0.00	2.14	7.43	6.02
Yr67/YrC591	17.71	**41.67**	7.06	7.86	15.54	17.97
YrSP	17.26	**33.93**	10.59	7.86	**18.92**	17.71
YrZH84	0.45	0.00	0.00	0.45	0.00	0.18
TaHLRG	16.22	**35.71**	4.05	16.07	10.59	16.53
Average	9.80	11.18	8.81	9.81	10.44	10.00

[a] The values more than the average of corresponded row and the average of corresponded column simultaneously were highlighted in bold

Discussion

Although numerous Yr genes have been reported, *Yr11*, *Yr12*, *Yr13*, *Yr14*, *Yr19*, *Yr20*, *Yr22*, *Yr23* and *Yr25* have not been mapped, *Yr27*, *Yr31*, *Yr32*, *Yr37*, *Yr38*, *Yr40*, *Yr42*, *Yr45*, *Yr50*, *Yr54*, *Yr56*, *Yr63*, *Yr66*, *Yr70, Yr73* and *Yr74* have no appropriate markers, *Yr2*, *Yr3*, *Yr21*, *Yr34*, *Yr43*, *Yr47*, *Yr55* and other temporarily named Yr genes have no closely linked markers. So, 36 Yr genes were finally screened and

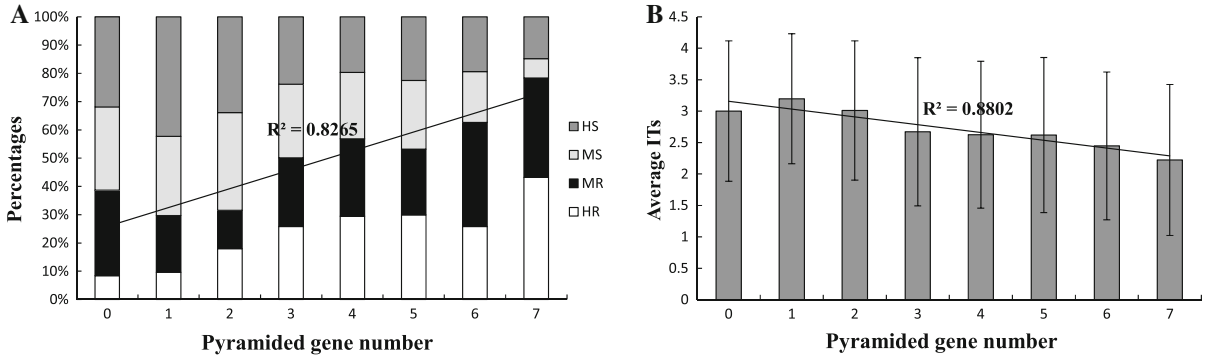

Fig. 2 The relationship of stripe rust resistance and pyramided gene number. The trend line was added based on the sum percentages of MR and HR, **a** or the average ITs, **b** of each pyramided gene number. *HS* highly susceptible phenotype, *MS* moderately susceptible phenotype, *MR* moderately resistant phenotype, *HR* highly resistant phenotype. *Each bar* represents the mean value of the stripe rust IT (0, 1, 2, 3, 4) and standard deviation (SD; n ≥ 17) in **b**

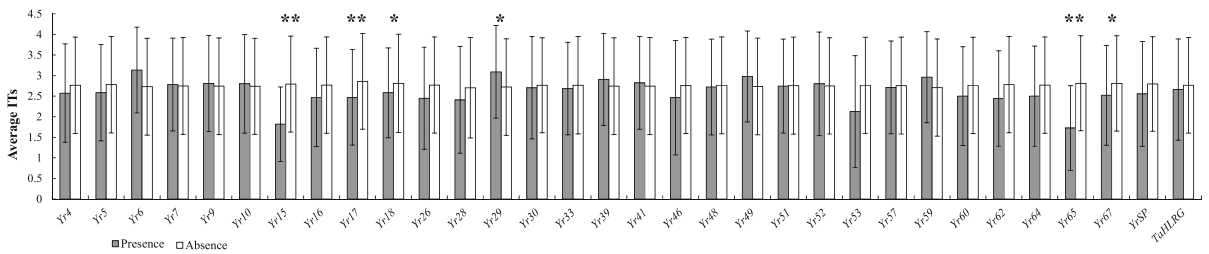

Fig. 3 Contribution of each gene to *Pst* resistance. *Each bar* represents the mean value of the stripe rust IT (0, 1, 2, 3, 4) and SD (n ≥ 15). One-way ANOVA was used to determine the significance level between the presence and absence groups of each Yr gene. Significance at $*p < 0.05$. Significance at $**p < 0.01$

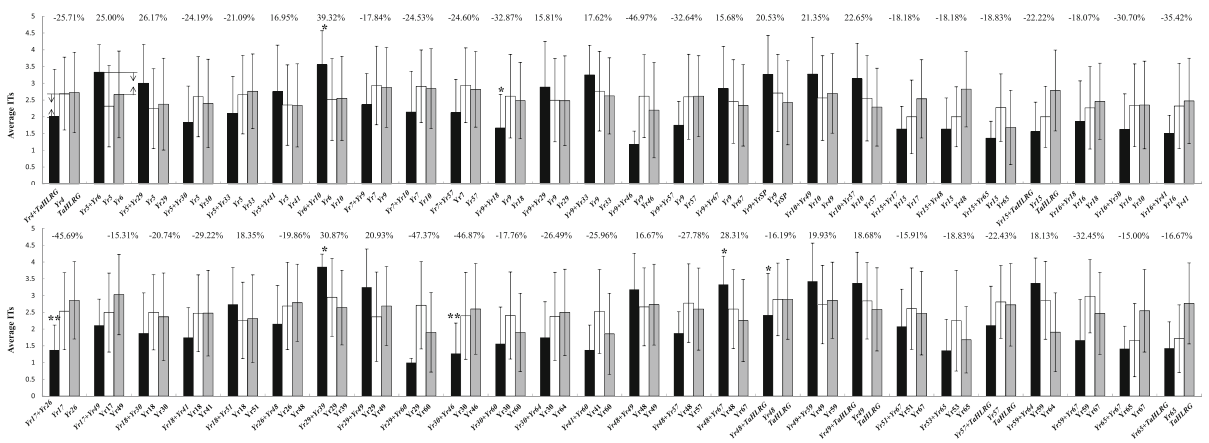

Fig. 4 Contribution of different combinations of two Yr genes to stripe rust resistance. The difference value (D value) of the ITs between the combination group and the higher or lower group (indicated by ↕) are shown on the column to represent the resistance improvement (negative D value) or reduction (positive D value). The results of a absolute D value >15% are shown here. *Each bar* represents the mean value of the stripe rust IT (0, 1, 2, 3, 4) and SD (n ≥ 10). One-way ANOVA was used to determine the significance between the combination group and the higher or lower group of each pair of Yr genes. Significance at $*p < 0.05$, significance at $**p < 0.01$

used to evaluate their contribution to the current *Pst* resistance in this study.

When conducting marker-assisted selection (MAS) in breeding, validity and convenience of a molecular marker should be considered. Here, PCR based markers were employed (Table 1) for identification of the corresponding gene (Fig. S1). There are still some markers used to map a gene but not suitable for detecting the gene in diverse genetic backgrounds, such as *Owm45F3R3* for *Yr51* (Randhawa et al. 2014). It might be because the polymorphism of a marker showed in the mapping population could not be distinguished in some wheat germplasms. Some RGAP markers also have this problem, such as the RGAP markers for *Yr44* (Sui et al. 2009), *Yr45* (Li et al. 2011) and *Yr59* (Zhou et al. 2014a, b). So, it is hard to apply these markers directly for MAD, even with the donor lines. Moreover, the brightness of the target band of some markers was too weak to be distinguished from polymorphic bands even with a touchdown PCR program, such as *stm673acag* for *Yr1* (Bansal et al. 2009) and *gwm508* for *Yr35* (Dadkhodaie et al. 2011).

To enhance the reliability of molecular markers, much improvement has been made from polymorphic markers to specific markers, to gene specific markers, and to functional markers. There were many types of marker conversion having been conducted, such as amplified fragment length polymorphism (AFLP) to SCAR (*SC-OPD11*, Niu et al. 2004), SSR to SCAR (*gwm415* to *SC-372*, Jia et al. 2011), SSR to STM (*gwm533* to *stm559tgag*, Hayden et al. 2004), EST to SSR (*bu099658*, Hasancebi et al. 2014), DArT to STS (*sun104*, Randhawa et al. 2014) and RGAP to STS (*wgp5467* to *STS5467*, Zhou et al. 2014a, b). Still, fine mapping of some Yr genes is required for valid detection, such as distinguishing the closely linked loci *Yr5/Yr7-YrSP* (Fig. 1). Moreover, sequence specific marker could indicate the presence of the cloned *Yr10*, *Yr18* and *Yr36* in the genomic DNA accurately (Fig. S1). But, whether it could be used to indicate the functional type still needs to be considered. Because there are many haplotypes existed in the hexaploid wheat, such as the resistant (*Lr67res*) and susceptible types (*Lr67sus*) of *Yr46* (Moore et al. 2015). So, function specific markers were developed to indicate the functional mutant. For example, the marker *THR1* developed in the non-coding region was used to identify the association of *TaHLRG* and stripe rust resistance (Liu et al. 2008a, b). Two markers *cib-Yr28M1* and *cib-Yr28M2* developed in the gene coding region were used to distinguish the resistance associated *Yr28* (unpublished). To utilize these cloned *Pst* resistance-associated genes, such as *TaMDHAR* (Feng et al. 2014) and *TaADF7* (Fu et al. 2014), more functional markers are required.

Through MAD, the distribution of 36 resistance genes in 672 wheat accessions was illustrated. The result showed consistence with previous studies. *Yr9*, *Yr10*, *Yr17* and *Yr18* were the mostly identified genes, while *Yr8* and *Yr36* were identified in a few accessions (Tabassum et al. 2010; Yuan et al. 2012; Zeng et al. 2014). Many "Chuanyu" cultivars and advanced breeding lines were released in Sichuan and other provinces of China. They showed a good adaptability to the local *Pst* races (Fig. S2). This might be related to the introduction of new Yr genes (such as *Yr15*, *Yr65* and *Yr67*) and the reduction of previously widely applied Yr genes (such as *Yr9*, *Yr10* and *Yr24/Yr26*). However, there are two prominent problems. One problem is the over-use of a few Yr genes, such as *Yr17* presenting in 74.40% of "Chuanyu" breeding lines (Table 2). This will lead to diversity reduction of resistance genes in breeding population and is unfavourable for breeding durable resistance varieties. The other problem is that the durable genes have not been widely deployed in "Chuanyu" wheat. The Chinese landraces maintaining rust resistance chronically (Fig. S2) showed a high frequency of *Yr18* to 59.41% (Table 2). Many reports revealed the importance of durable resistance genes in combining with other Yr genes (Krattinger et al. 2009; Yang et al. 2011). The enhancement effect was also confirmed in this study, such as *Yr9* + *Yr18* (Fig. 4). Therefore, through MAD, the overuse of a few Yr genes can be avoided, and durable genes with low frequency can be introduced intentionally. Additionally, the newly reported heterogenous genes, such as *Yr37* (Heyns et al. 2011), *Yr40* (Kuraparthy et al. 2009) and *Yr50* (Liu et al. 2013), should also be applied properly. To perform resistance breeding for a long period, extensive resistance resources are required to broaden the genetic basis of breeding materials.

As prevalent *Pst* races were variant with environments, it is necessary to understand the effective of Yr

genes under a specific condition. Here, a case study was performed in Sichuan province, and we hope that it would attract enough attention to the breeders and researchers in the world-wide. Some Yr genes identified in this study showed significant effects under current conditions, such as *Yr15* and *Yr65*, while some once massively used Yr genes did not confer significant resistance, such as *Yr9* (Fig. 3). These results were basically consistent with the rust testing results of some available NILs. Only the NILs of *Yr5*, *Yr15* and *Yr18* showed resistance, others (such as the NILs of *Yr9* and *Yr26*) were susceptible to *Pst* in this study. But variances existed in the results of *Yr17*. *Yr17* contributed significantly to the current *Pst* resistance in this study (Fig. 3), but *Yr17*-NIL was susceptible. Further analysis showed that the contribution significance of *Yr17* varied with different taxon groups, non-significant in "Chuanyu" breeding lines but significant in Chinese modern cultivars and introduced foreign germplasms (Fig. S3). Other genes, such as *Yr67*, also showed similar results. The function of one gene depends on the genetic background, so it is meaningful to evaluate the contribution of Yr genes to stripe rust resistance in diverse genetic backgrounds rather than in their NILs. MAD combined with rust testing results could be used to select the most effective genes against current *Pst* races when breeding high resistant varieties.

The genes conferring significant resistance will be applied widely in breeding, such as *Yr15*, *Yr17* and *Yr65* (Table 2; Fig. 3). But other genes showing non-significant effectiveness under current conditions still need to be utilized in gene pyramiding. Because the results identified in this study (Fig. 2) and the presence of multi-QTLs in resistant wheat (Lowe et al. 2011; Rosewarne et al. 2013; Yang et al. 2013) suggested that gene pyramiding could improve the durability of rust resistance. Additive effects and epistatic effects exist extensively in Yr gene pyramids (Fig. 4). And 42.64% of the combinations of two Yr genes improved *Pst* resistance than those either one present. Although showing non-significance, these combinations with substantially lower ITs (negative D value, Fig. 4) than those carrying either one gene still need to be considered. Moreover, some durable resistance genes conferring partial resistance to *Pst* showed enhancing effect to race-specific genes in this study, such as *Yr17*, *Yr18*, *Yr30* and *Yr46* (Fig. 4). Taking advantage of the positive interactions and avoiding the negative interactions should be carefully considered in resistance gene pyramiding.

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