Molecular Marker Based Design for Breeding Wheat Lines with Multiple Resistance and Superior Quality

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Abstract

There has not been a major wheat stem rust epidemic worldwide since the 1970s, but the emergence of race TTKSK of *Puccinia graminis* f. sp. *tritici* in 1998 presented a great threat to the world wheat production. Single disease-resistance genes are usually effective for only several years before the pathogen changes genetically to overcome the resistance. Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is one of the most common and persistent wheat diseases worldwide. The development of varieties with multiple resistance is the most economical and effective strategy for preventing stripe rust and stem rust, the two main rust diseases constraining wheat production. Plateau 448 has been widely used in the spring wheat growing region in northwest China, but it has become susceptible to stripe rust and is susceptible to TTKSK. To produce more durable resistance to race TTKSK as well as to stripe rust, four stem rust resistance genes (*Sr33*, *Sr36*, *Sr-Cad*, and *Sr43*) and three stripe rust

resistance genes (*Yr5*, *Yr18*, and *Yr26*) were simultaneously introgressed into Plateau 448 to improve its stem rust (Ug99) and stripe rust resistance using a marker-assisted backcrossing strategy combined with phenotypic selection. We obtained 131 BC₁F₅ lines that pyramided two to four Ug99 resistance genes and one to two *Pst* resistance genes simultaneously. Thirteen of these lines were selected for their TTKSK resistance, and all of them exhibited near immunity or high resistance to TTKSK. Among the 131 pyramided lines, 95 showed high resistance to mixed *Pst* races. Nine lines exhibited not only high resistance to TTKSK and *Pst* but also better agronomic traits and high-molecular-weight glutenin subunit compositions than Plateau 448.

Keywords: Ug99, disease resistance, gene pyramiding, HMW-GS, stripe rust

Wheat is a major food crop worldwide and plays a crucial role in human society (Kiszonas and Morris 2017). The most important biotic constraints to wheat production in temperate regions of the world are rust diseases caused by *Puccinia triticina* Erikss. (leaf rust), *P. striiformis* Westend. f. sp. *tritici* Erikss. (stripe rust, *Pst*), and *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Erikss. & E. Henn. (stem rust, *Pgt*) (Jin et al. 2007). Stripe rust is one of the most devastating diseases threatening wheat production in all winter wheat growing regions as well as in the spring wheat areas in northwestern China (Chen et al. 2009; Wan et al. 2004). In recent years, with the increase of rainfall, stripe rust is becoming more and more serious in Qinghai Province. Stem rust mainly attacks leaves, sheaths, and stems of wheat approximately 3 weeks before grain filling, leading to reduced photosynthetic area and blockage of organic transport, causing devastating grain yield losses (Singh et al. 2008). In the 1950s, every few

years, a stem rust outbreak occurred somewhere in the world, and the countries or regions close to the outbreak had almost no harvest (Stokstad 2007). After the “Green Revolution”, the effective stem rust (*Sr*) resistance gene *Sr31* was introduced to wheat breeding programs worldwide through the utilization of 1BL/1RS translocation (Purnhauser et al. 2011; Singh et al. 2006). There were no reports of stem rust as a large-scale epidemic that occurred over the following three decades (Hiebert et al. 2011; Singh et al. 2011).

In 1998, a large number of cultivars with resistance against stem rust were severely infected by a new race of *Pgt* in nurseries in Uganda in Eastern Africa (Ghazvini et al. 2012). The new race was virulent to *Sr31* and designated Ug99 (TTKSK). Previous works indicated that TTKSK showed strong infection in almost all Asian wheat cultivars (Ghazvini et al. 2012; Han et al. 2013; He et al. 2008), and it was virulent to most cultivars with *Sr* genes (Wanyera et al. 2006). Among the currently known wheat stem rust resistance genes, only a small number, including *Sr13*, *Sr14*, *Sr22*, *Sr28*, *Sr33*, *Sr35*, *Sr36*, *Sr-Cad*, *Sr43*, *Sr45*, and *Sr60*, are resistant to Ug99 (Chen et al. 2018; Ghazvini et al. 2012; Jin et al. 2009; Mago et al. 2009; Niu et al. 2014; Purnhauser et al. 2011). With certain biotic or abiotic conditions, more than 13 countries have suffered from devastating wheat production losses because of the invasion of Ug99 and its newly evolved virulent races (Chen et al. 2018). To date, seven Ug99 variants have been detected in Africa and the Middle East, including TTKSK, TTKSF, TTKST, TTTSK, TTKSP, PTKSK, and PTKST (Rautela and Dwivedi 2018).

Sr36 was considered a widely used gene before TTKST and TTTSK were identified to be virulent to *Sr24* and *Sr36*, respectively (Jin et al. 2009). The fact that new pathogenic mutants of Ug99 were virulent to wheat cultivars carrying *Sr31* and *Sr36* indicated that a single resistance gene cannot effectively cope with future variation in the Ug99 race group and other races. A cost-effective and long-lasting measure to control stem rust is to develop cultivars that have multiple effective resistance genes pyramided using marker-assisted selection (MAS) (He et al. 2019; Jin et al. 2007; Rimbaud et al. 2018;

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Rouse et al. 2012). As a large wheat producing country, control of rusts is important for China's food security.

The geographic information system data of the International Maize and Wheat Improvement Center showed that a continuous 72 h airflow over the Hamada wheat region of Iran, where TTKSK was detected in 2007, passed into Northern Tibet, Qinghai, and Gansu provinces of China between January and July 2008 (Li 2012), indicating the possibility of rust spreading from Central Asia to China. Qinghai Province is located in the northeastern Qinghai-Tibet Plateau and belongs to the spring wheat region. He et al. (2008) found that only one of 118 commonly planted wheat cultivars in China showed high resistance to Ug99, and cultivars from southern China were susceptible to Ug99 (Xu et al. 2018). Plateau 448, the most commonly grown wheat cultivar in Qinghai Province, is susceptible to race TTKSK of *Pgt* and also susceptible to major races of *Pst*. It is possible that Qinghai may become an incubation area for the onset of the Ug99 races once they appear in the region. If this occurs, it would be a serious threat to the wheat production in China and other eastern Asian countries.

The appearance of Ug99 attacking *Sr31* in 1998 and a new variant with virulence to *Sr36* in 2007 indicated that cultivars carrying a single stem rust resistance gene are not highly effective against *Pgt* races. Jin et al. (2007) proposed that developing cultivars with different pyramided *Sr* genes could be an efficient way to increase durable and broad-spectrum resistance to stem rust.

In the present research, we successfully stacked the stem rust resistance genes *Sr33*, *Sr36*, *Sr-Cad*, and *Sr43* and stripe rust resistance genes *Yr5*, *Yr18*, and *Yr26* into Plateau 448 by combining a marker-assisted backcrossing (MABC) strategy with phenotypic selection and developed new spring wheat lines with improved Ug99 and stripe rust resistance as well as better yield and milling quality than those of Plateau 448.

Materials and Methods

Wheat materials. Wheat lines RL5405 (*Sr33*, *Yr5*), Lang (*Sr36*, *Yr5*, *Yr18*), AC Cadillac (*Sr-Cad*, *Yr18*, *Yr26*), and RWG34 (*Sr43*, *Yr10*, *Yr18*) from our laboratory were used in this study. Wheat genotype 'Ps4-1' is a doubled haploid line derived from hybridization of AC Cadillac/Lang/RWG34/RL5045 with pyramided *Sr33*, *Sr36*, *Sr-Cad*, *Sr43*, *Yr5*, *Yr18*, and *Yr26*. Ps4-1 has been confirmed to be resistant to *Pgt* race TTKSK and also to tested *Pst* races. Plateau 448 is a spring wheat cultivar currently widely grown in Qinghai Province and is susceptible to TTKSK and stripe rust. Isolate SY95-71 of *Pst* from the Triticeae Research Institute, Sichuan Agricultural University, was used to evaluate stripe rust response.

The breeding program started in the spring of 2014. Plateau 448 was used as the recurrent parent and Ps4-1 as the donor of *Sr33*, *Sr36*, *Sr-Cad*, *Sr43*, *Yr5*, *Yr18*, and *Yr26*. The breeding procedure is shown in Figure 1. F_1 plants were planted in Yuanmou (101.80° E, 25.72° N) of Yunnan Province, and F_2 seeds were harvested and planted in Xining (101.75° E, 36.62° N) of Qinghai Province. Eight F_2 plants with three to four *Pgt* resistance genes and two to three *Pst* resistance genes selected by markers were backcrossed with Plateau 448 in the summer of 2015. The BC_1F_1 seeds were sown in Yuanmou in October 2015, and the harvested BC_1F_2 seeds were sown in Xining in spring 2016. By measuring and comparing the tiller number (TN), plant height (PH), and kernels per spike (KS) in the field and 1,000-grain weight (TGW) in the laboratory, plants with improved agronomic traits were selected from the BC_1F_2 population and screened using molecular markers. MAS combined with agronomic character selection was applied from BC_1F_2 to BC_1F_5 to enhance the accuracy and efficiency of breeding.

Molecular markers screening. Genomic DNA samples were extracted from the positive control wheat lines RL5405, Lang, AC Cadillac, RWG34, two parental lines of Ps4-1, Plateau 448, and plants or lines with improved agronomic traits in the F_2 to BC_1F_6 generation using the cetyl trimethyl ammonium bromide method (Devi et al. 2013). A total of 1 μ l of DNA solution was used to test the DNA quality in a 10 g/liter agarose gel by electrophoresis, and the DNA concentration was measured using a NanoDrop 2000C

spectrophotometer (Thermo Scientific, Waltham, MA). Molecular markers were selected according to our previous studies and corresponding references (Table 1) (Lagudah et al. 2009; Liu et al. 2017; Marchal et al. 2018).

Evaluation of selected lines with an Ug99 race. The selected 13 pyramided lines, two parents, and two stem rust-susceptible wheat varieties were inoculated with TTKSK (Ug99, isolate SA31) inside a biocontainment facility at the Modern Research and Development Center, Agriculture and Agri-Food Canada. Seedlings were rated for their infection types (ITs) 14 days postinoculation using the 0 to 4 scale. ITs from 0 to 2+ were considered resistant, whereas 3 to 4 were considered susceptible.

Evaluation of selected lines for stripe rust infection type. The selected lines were evaluated for stripe rust response in the experimental farm of the Triticeae Research Institute, Sichuan Agricultural University, Wenjiang, Chengdu, China. Seedlings of SY95-71 and pyramided lines were inoculated 6 weeks after planting with a mixture of the prevalent *Pst* races in China comprising CYR32, CYR33, CYR34, Zhong4, and HY46, which were provided by the Research Institute of Plant Protection, Gansu Academy of Agriculture Sciences. ITs were recorded using the 0 to 9 scale (Line and Qayoum 1992). ITs 0 to 4 showing necrotic symptoms without or with slight rust sporulation were considered resistant, whereas 5 to 9 were susceptible.

Evaluation of selected lines for agronomic traits. From 2014 to 2018, the pyramided lines of every generation and their parents were planted in Xining (101.75° E, 36.62° N) and Yuanmou (101.80° E, 25.72° N), respectively. Each of the pyramided lines and the parents was planted with 100 seeds in a single 2-m row with 0.2 m between rows in Xining, and with 15 seeds in a single 0.8-m row with 0.25 m between rows in Yuanmou. Nitrogen and superphosphate fertilizers were applied at a rate of 80 and 100 kg/ha, respectively, at sowing. Field management was performed according to the common practices. Six plants with consistent growth status of each line were randomly selected to investigate agronomic traits at the maturity stage. PH was measured from the base of a plant to the top of the main spike (awns were not included). KS was counted as the number of kernels per spike. Panicle length (PL) was measured as the length from the base of the main spikes to the top except the awns. TN was determined as the number of productive tillers per plant. All above traits were measured in the field. TGW was determined by weighting 1,000 kernels with precision of 0.01 g in the laboratory after harvest. The phenotypic data were statistically analyzed using SPSS 18.0 software.

Fig. 1. Elite wheat breeding lines with Ug99 resistance developed by a rational design.

Evaluation of milling quality. Half of the seeds of each line were used for glutenin extraction (Danno 1981). Subunits were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for 6 h at a constant current of 30 mA. The high-molecular-weight glutenin subunit (HMW-GS) constitutions of each line were acquired by statistical subunit bands, and the quality scores were evaluated by their HMW-GS constitutions. For easy describing, “UP” was used to indicate “Ug99 resistance gene pyramiding”.

Results

Pyramiding of stem rust and stripe rust resistance genes. After comparing the agronomic traits, 183 plants with improved agronomic traits were selected from the BC₁F₂ population. Using molecular markers, 25 plants with three to four *Pgt* resistance genes and two to three *Pst* resistance genes were obtained (Fig. 2). Two hundred plants were selected with improved agronomic traits in the BC₁F₅ population. Finally, 131 lines from these 200 plants were verified to have pyramided two to four *Pgt* resistant genes and one to two *Pst* resistance genes.

Ug99 resistance. Thirteen randomly selected BC₁F₅ lines putatively containing *Sr-Cad*, *Sr33*, *Sr36*, *Sr43*, *Yr5*, *Yr18*, and *Yr26*, the two parents, and two stem rust-susceptible cultivars were tested

with race TTKSK in the Modern Research and Development Center, Agriculture and Agri-Food Canada. All of the 13 lines and Ps4-1 were near immune or highly resistant to TTKSK, whereas the standard check cultivars Little Club and Shaw as well as the negative control Plateau 448 displayed susceptible ITs (Table 2).

Stripe rust resistance. Of the 131 UP lines evaluated using the mixed *Pst* races, 95 were immune or highly resistant, 17 were moderately resistant, six were moderately susceptible, 12 were susceptible, and one was highly susceptible (Supplementary Table S2). Figure 3 demonstrates the *Pst* ITs of the corresponding lines that were tested for TTKSK reaction. When tested with *Pst*, UP3 and UP7 were immune; UP26, UP71, UP127, UP237, UP253, UP254, and UP275 highly resistant; UP217 and UP235 moderately resistant; UP196 and UP210 susceptible; and Plateau 448 highly susceptible.

The 89 BC₁F_{5,6} lines that were selected from 131 BC₁F₆ UP lines as having improved agronomic traits were tested with the markers for *Yr5*, *Yr18*, and *Yr26*. The results showed that 60 lines had *Yr5*, two lines contained *Yr18*, six lines harbored *Yr26*, one line had *Yr5* and *Yr18*, and one line had both *Yr5* and *Yr26* (Table 3, Fig. 4).

Agronomic traits. PH, PL, KS, TN, and TGW were compared between 13 Ug99-resistant lines and their parent Plateau 448 in 2017 and 2018 (Table 4, Supplementary Table S1). All of the pyramided

Table 1. Simple sequence repeat markers used for identifying genes for resistance to stem rust and stripe rust

Resistance genes	Primers	Primer sequences	Annealing temperature (°C)	Product size (bp)	
				Positive	Negative
<i>Sr33</i>	Xcfd15	5'-CTCCCGTATTGAGCAGGAAG-3' 5'-GGCAGGTGTGGTGATGATCT-3'	62	210	150
<i>Sr36</i>	STM773-2	5'-ATGGTTTGTGTGTGTGTGTAGG-3' 5'-AAACGCCCAACCACTCTCTC-3'	61	170	200
<i>Sr-Cad</i>	FSD-RSA	5'-GTTTTATCTTTTATTTC-3' 5'-CTCCTCCCCCA-3'	47	300	Null
<i>Sr43</i>	Cfa2040	5'-TCAAATGATTTCAGGTAACCACTA-3' 5'-TTCCTGATCCCAACCAACAT-3'	58	250	270
<i>Yr5</i>	Yr5_B	5'-GGGAACACTTCACGATCA-3' 5'-AATTCCTTCATGCCTTCC-3'	53	909	Null
<i>Yr18</i>	csLv34	5'-GTTGGTTAAGACTGGTGATGG-3' 5'-TGCTTGCTATTGCTGAATAGT-3'	55	150	250
<i>Yr26</i>	CD77	5'-CGACGAAGCCGTTGTTAT-3' 5'-TCAAGCAAAGACGAGAGGAT-3'	55	480	620

Fig. 2. Detection of the gene pyramided wheat lines with molecular markers. M₁ = 1-kb-plus DNA ladder; M₂ = 50-bp ladder; 1 = RL5405; 2 = Ps4-1; 3 = blank; 4 = Plateau 448; 5 = Lang; 6 = AC Cadillac; and 7 = RWG34.

lines were shorter than Plateau 448 in PH in both years. Among them, UP127, UP196, UP210, and UP217 were more than 20 cm shorter than Plateau 448. UP196, UP217, UP235, and UP254 had higher TN than Plateau 448 in both years. The mean TGWs of UP26, UP71, UP237, UP253, UP254, and UP275 in 2017 were 45.77, 46.49, 53.05, 53.76, 52.44, and 46.50 g, respectively. In 2018, the TGWs were 44.73, 47.27, 49.47, 49.60, 52.60, and 49.53 g, respectively. All of these values were significantly increased compared with Plateau 448. In particular, the TGW value of UP254 (52.60 g) was increased by 25.63% compared with that of Plateau 448 (41.87 g).

Milling quality. The nutritional and flour processing quality of wheat lines are largely determined by their subunit composition and content of glutenin. The HMW-GS compositions of the two parental lines and 89 BC₁F_{5,6} lines were evaluated. Five HMW-GS types were detected in all 89 BC₁F_{5,6} lines and the parents (Table 5, Supplementary Table S1). Null and 1, as two subunit types of *Glu-A1* locus, account for 20.88 and 79.12%, respectively. For the *Glu-B1* locus, the 7+8 subunit type was detected only in Ps4-1, whereas 89 lines and Plateau 448 exhibited the 7+9 subunit type. For *Glu-D1*, the 5+10 subunit was detected in all 89 lines and the two parents. The HMW-GS composition of Ps4-1 was 1/7+8/5+10, and that of Plateau 448 was Null/7+9/5+10 (Fig. 5, Supplementary Table S1). Five lines of the 13 Ug99-resistant lines exhibited the Null/7+9/5+10 subunit type, and eight exhibited the 1/7+9/5+10 subunit type (Fig. 5).

Table 2. Infection types (ITs) of wheat lines tested with an Ug99 race (TTKSK) of *Puccinia graminis* f. sp. *tritici*

Order	Cultivar/line	IT
1	Little Club	4
2	Shaw	4
3	Plateau 448	4
4	Ps4-1	1-
5	UP3	0;
6	UP7	0;
7	UP26	0;
8	UP71	1-
9	UP127	1-
10	UP196	0;
11	UP210	0;
12	UP217	0;
13	UP235	0;
14	UP237	0;
15	UP253	0;
16	UP254	0;
17	UP275	0;

According to the standard quality score system of the subunit composition (Nakamura 2000), Chinese Spring had a score of 6, Ps4-1 of 10, and Plateau 448 of 7. Among the 89 BC₁F_{5,6} lines, 18 had scores of 7, and 71 had scores of 9 (Supplementary Table S2).

Discussion

Wheat is the second largest crop in China and a staple food in northwest China. Additionally, steady wheat yields are of great importance to ensure food security. The Ug99 race group of the stem rust pathogen is a threat to wheat production (Olivera et al. 2018). Should Ug99 races cross the Red Sea, approximately a quarter of the wheat production area worldwide would be threatened (Rautela and Dwivedi 2018). Plateau 448, the most commonly planted wheat cultivar in Qinghai Province as well as other parts of northwest China, is susceptible to *Pgt* race TTKSK and predominant Chinese *Pst* races. Plateau 448 has been widely used as a parent in breeding programs during the past 10 years in northwest China. For example, Qingmai 10 (Plateau 437/Plateau 448) has become one of the most popular varieties, with wide adaptability and high yield potential. However, Plateau 448 and the derived varieties are susceptible to TTKSK and *Pst*, and there is a potential for big yield losses due to occasional outbreaks of TTKSK and *Pst*. There is a potential that ure-diniospores of the Ug99 races may be spread to China by the prevailing summer wind from central Asia. Once reaching China, Ug99 races would occur regionally and gradually spread to other wheat growing regions in China. Thus, it is important to develop wheat cultivars with resistance to Ug99 races of *Pgt*.

Pyramiding resistance genes is an efficient strategy to stack several of the same or different kinds of resistance genes against the same or different pathogens in a single breeding line or cultivar and is a

Table 3. Presence of *Yr* genes in wheat genotype Ps4-1 and its 89 BC₁F₆ lines

Genes	Cultivar/line
<i>Yr5</i>	Ps4-1, UP3, UP7, UP26, UP71, UP127, UP168, UP196, UP210, UP217, UP235, UP275, UP6, UP9, UP10, UP11, UP17, UP24, UP25, UP29, UP31, UP32, UP37, UP44, UP49, UP51, UP55, UP56, UP57, UP58, UP65, UP66, UP67, UP72, UP73, UP82, UP86, UP87, UP94, UP99, UP101, UP112, UP126, UP141, UP145, UP151, UP157, UP159, UP164, UP167, UP181, UP189, UP194, UP208, UP209, UP215, UP224, UP239, UP240, UP276, UP294
<i>Yr18</i>	Ps4-1, UP240, UP266
<i>Yr26</i>	Ps4-1, UP235, UP236, UP237, UP253, UP254, UP260

Fig. 3. Identification of stripe rust resistance in Ug99-resistant lines.

Fig. 4. Detection of stripe rust resistance genes in BC₁F₆ lines. M₁ = 1-kb-plus DNA ladder; M₂ = 50-bp ladder; 1 = RL5405; 2 = Lang; 3 = AC Cadillac; 4 = RWG34; 5 = Ps4-1; 6 = Plateau 448; 7 = blank; and a to o represent the partial of BC₁F₆ lines.

Table 4. Comparison of agronomic traits between the parental Plateau 448 and 13 *Sr* gene pyramided lines^a

Year	Lines	PH	PL	KS	TN	TGW
2017	Plateau 448	103.63 ± 2.17	7.42 ± 0.38	41.00 ± 2.26	8.76 ± 3.00	43.33 ± 0.94
	UP3	93.00	9.00	59.00	7.00	44.02 ± 0.16
	UP7	79.00	11.00	66.00	6.00	43.04 ± 0.18
	UP26	90.00	10.00	58.00	7.00	45.77 ± 0.32**
	UP71	88.00	9.00	60.00	6.00	46.49 ± 0.30**
	UP127	67.00	8.00	50.00	9.00	31.16 ± 0.40**
	UP196	75.00	8.00	61.00	11.00	38.28 ± 0.18**
	UP210	65.00	8.00	50.00	9.00	36.43 ± 0.22**
	UP217	70.00	8.00	50.00	14.00	38.54 ± 0.14**
	UP235	100.00	10.00	57.00	15.00	40.67 ± 0.36**
	UP237	98.00	10.00	64.00	9.00	53.05 ± 0.26**
	UP253	90.00	9.00	60.00	8.00	53.76 ± 0.25**
	UP254	96.00	8.00	58.00	10.00	52.44 ± 0.42**
	UP275	102.50	9.00	56.00	7.00	46.50 ± 0.34**
2018	Plateau 448	109.17 ± 2.32	10.83 ± 0.98	42.00 ± 2.37	6.50 ± 1.05	41.87 ± 0.58
	UP3	94.88 ± 2.38**	9.58 ± 0.49*	42.17 ± 6.68	9.50 ± 3.08	44.87 ± 1.10**
	UP7	88.67 ± 3.57**	9.58 ± 0.92*	42.83 ± 5.46	10.50 ± 2.43*	37.20 ± 0.80**
	UP26	95.25 ± 1.89**	9.17 ± 0.98**	44.50 ± 9.40	11.17 ± 5.91**	44.73 ± 0.61**
	UP71	98.67 ± 4.08**	9.50 ± 0.55**	43.33 ± 2.34	12.67 ± 7.28**	47.27 ± 0.31**
	UP127	80.17 ± 3.60**	7.75 ± 0.88**	45.00 ± 3.79	12.33 ± 3.98**	31.80 ± 0.60**
	UP196	74.83 ± 2.79**	9.17 ± 0.75**	41.67 ± 6.50	10.50 ± 1.05*	35.20 ± 0.53**
	UP210	75.83 ± 2.23**	8.17 ± 0.75**	35.33 ± 6.15	9.33 ± 1.03	37.67 ± 1.36**
	UP217	74.83 ± 2.04**	8.67 ± 0.82**	42.17 ± 5.78	13.50 ± 1.87**	36.93 ± 0.95**
	UP235	100.83 ± 2.64**	10.50 ± 1.05	46.17 ± 8.06	14.17 ± 0.75**	51.33 ± 1.70**
	UP237	103.67 ± 3.20**	9.83 ± 0.98*	40.67 ± 9.67	9.17 ± 0.75	49.47 ± 2.23**
	UP253	100.83 ± 4.36**	9.33 ± 1.03**	43.17 ± 8.47	8.17 ± 0.75	49.60 ± 0.92**
	UP254	102.17 ± 2.71**	9.50 ± 0.84**	45.67 ± 10.95	9.83 ± 1.72	52.60 ± 0.72**
	UP275	98.50 ± 1.05**	8.83 ± 0.41**	45.17 ± 7.41	7.00 ± 0.89	45.93 ± 0.99**

^a Asterisk (*) indicates that $P \leq 0.05$, and ** indicates that $P \leq 0.01$. PH = plant height; PL = panicle length; KS = kernels per spike; TN = tiller number; and TGW = thousand grain weight.

Table 5. High-molecular-weight glutenin subunit (HMW-GS) type and composition in 89 BC₁F_{5,6} lines and parents

Locus	HMW-GS	Varieties	Frequency (%)	Subunit composition	Quality score
<i>Glu-A1</i>	Null	19	20.88	Null/7+9/5+10	7
	1	72	79.12	1/7+9/5+10	9
<i>Glu-B1</i>	7+8	1	1.10	1/7+8/5+10	10
	7+9	90	98.90		
<i>Glu-D1</i>	5+10	91	100		

Fig. 5. SDS-PAGE electropherogram of HMW-GS in BC₁F_{5,6} families. CK1, CK2, P1, and P2 represent Chinese Spring, Plateau 356, Ps4-1, and Plateau 448, respectively, and a to m represent the 13 lines with Ug99 resistance gene pyramiding.

potentially economical strategy to create more durable and broad-spectrum resistance (Liu et al. 2000; Mago et al. 2010). Successful marker-assisted pyramiding of genes with resistance to either leaf rust, stripe rust, powdery mildew, or stem rust has been reported previously (Kloppers and Pretorius 1997; Liu et al. 2000; Mago et al. 2010; Zhang et al. 2019). In the present study, stem rust and stripe rust resistance genes, as well as genes related to improved agronomic traits and superior HMW-GS, were pyramided in advanced breeding lines. Previous studies showed that *Sr33*, *Sr36*, *Sr-Cad*, and *Sr43* were effective genes against Ug99 and most of its variants (Chemayek et al. 2017; Ghazvini et al. 2012; Hiebert et al. 2016; Li et al. 2018; Periyannan et al. 2013), and *Yr5*, *Yr18*, and *Yr26* are either still effective or have some value when in combination for resistance to stripe rust (Rani et al. 2019). A large number of advanced lines harboring two to four *Sr* and one to two *Yr* genes as well as new HMW-GS were developed and proved to be resistant by MABC and MAS, indicating that MABC and MAS can accurately define target plants and improve breeding accuracy and efficiency. There is a certain probability that the markers and genes are not always linked after recombination, which may result in false positives. Therefore, the screening plants must be complemented by phenotyping. Approximately 10% of the BC₁F₅ UP lines were randomly selected for TTKSK testing, and all of them showed near immunity. Therefore, we could assume that all of the *Sr* gene pyramiding lines would be highly resistant to TTKSK. In the meantime, the present study used mixed *Pst* races to evaluate stripe rust resistance in the 131 lines at the adult-plant stage, and 95 were highly resistant or immune to the mixed races. *Yr5* confers all-stage resistance (ASR) to stripe rust. As an adult plant resistance (APR) gene, *Yr18* alone does not confer adequate resistance under high disease pressure, whereas *Yr18* and *Yr36* in combination increase stripe rust resistance (Hale et al. 2013; Yuan et al. 2012). ASR genes combined with some minor resistance genes can increase the durability and level of resistance to disease in cereal (Feng et al. 2018). Our data indicate that there may be other APR or minor *Yr* genes in some UP lines. Some of the parents used for pyramiding might contain unknown *Pst* resistance genes, and the unknown *Pst* resistance genes might be pyramided in these UP lines. *Yr26* has lost resistance to *Pst* race CYR34. In the present study, CYR34 was included in the race mixture for inoculating the experimental field in Wenjiang, Sichuan Province. It is difficult to confirm which race dominated in the field because natural *Pst* races might occur. It remains to be determined if the five pyramided UP lines (UP236, UP237, UP253, UP254, and UP260) with only *Yr26* indicated by markers and identified as highly resistant have any other genes for resistance to stripe rust as discussed above. Notably, in the past 3 years, *Yr18* appeared to be not as effective as before in Wenjiang, Sichuan Province (data not published). Thus, in the present study, we eliminated the lines with only *Yr18* or *Yr26* in the early stage with MAS. So many of the final selected lines have *Yr5* lines, and few lines have two of the three *Yr* genes with *Yr26* and *Yr18*. Moreover, *Yr18* was linked to *Lr34* and *Pm38*, indicating that some UP lines may be resistant to leaf rust and powdery mildew. Pyramiding different disease resistance genes is a useful strategy to reduce the loss of crop production due to multiple pathogens.

Wheat cultivars from Qinghai are generally characterized by weak gluten and poor noodle quality (Liu et al. 2004; Lukow et al. 1990). Glutenin is one of the major storage proteins in wheat grain (Mohd et al. 2007), and it is divided into HMW-GS and low-molecular-weight glutenin subunit. Although the content of HMW-GS is approximately 12% of the total protein of wheat grain, it has a great influence on the processing quality of food products, especially the processing quality of bread. Specifically, the contribution of HMW-GS to bread quality can be as high as 70%. Therefore, the flour quality and gluten quality of wheat are dramatically influenced by the type and composition of HMW-GS (Yang et al. 2018). Previous research has shown that varieties carrying subunits 1 and 5+10 have an advantage in gluten strength and bread-making quality, whereas subunit 7+8 has a significant positive effect on increasing wheat gluten strength. Among the 89 BC₁F_{5,6} lines, *Glu-B1c*

(Bx7 + By9), and *Glu-D1d* (Dx5 + Dy10) were in all lines. Among them, 18 had *Glu-A1c* (Null), and 71 had *Glu-A1a* (Ax1). A series of research showed that the 1 and 2* subunits at the *Glu-A1* locus have significantly more positive effects on all quality parameters than the subunit Null (Liu et al. 2005). The positive effects of subunits 7+8 and 7+9 were better than those of other subunits at the *Glu-B1* locus, and the effect of 5+10 at the *Glu-D1* locus was better than that of 2+12 (Gobaa et al. 2008; Zhang et al. 2009). In other words, approximately 80% of the tested descendants carried the subunits or combinations that result in higher quality than that of Plateau 448.

In the present study, many UP lines were not only resistant to stem rust and stripe rust but were also greatly improved in yield and quality compared with the recurrent parent Plateau 448. For example, UP24, UP25, UP37, UP44, UP55, UP56, UP57, UP61, and UP66 were highly resistant to the *Pgt* race and mixed *Pst* races, and their TGW exceeded 48.0 g, especially those of UP37 (50.73 g) and UP61 (51.40 g), which were above 50 g, and all of their HMW-GS quality scores were 9.

In the past half-decade, it has been observed that the precipitation in Qinghai has increased during the spring wheat grain filling stage. Excessive rainfall during the grain filling stage leads to large areas of lodging in Qinghai spring wheat production regions, especially in the fields grown with the main cultivar, Plateau 448. Breeding relatively shorter wheat cultivars is another aim in Qinghai Province. In the present study, most of the selected UP lines showed significantly decreased PH compared with that of Plateau 448, which can be an additional advantage to grow the stem rust and stripe rust resistant lines in Qinghai and other areas with similar climates.

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