Improving Stripe Rust Resistance and Agronomic Performance in Three Elite Wheat Cultivars by Using a Combination of Marker Detection and Phenotypic Selection

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Abstract

Wheat is an important cereal crop globally and improvement programs reach a plateau from ever-evolving stripe rust pathogen. Stripe rust, caused by *Puccinia striiformis* f. sp. *Tritici* (*PST*), is a fungal disease that devastates global wheat production. This disease is most effectively restrained by developing and deploying cultivars with highly durable resistance. The utilization of resistant genes is essential for breeding resistant cultivars. To improve stripe rust resistance and achieve favorable agronomic performance in commercial cultivars, an adult plant resistance gene *Yr48* was introgressed from PI610750 into three Chinese elite wheat cultivars Chuanmai 42, Lunxuan 987, and Bainongaikang 58 using a combination of marker detection and phenotypic selection.The three Chinese elite cultivars as the recipient parent were used to cross with the donor parent PI610750. Eighty-seven introgression lines (ILs) were gained from all three crosses in the F_5 generation. To validate the introgression lines possessing *Yr48*, SSR markers previously reported to be linked to the resistance gene loci were used to detect its presence or absence in different genetic backgrounds. Resistance and effect of introgressed *Yr48* were studied using F_6 and F_7 grain grown in the field. The ILs from three crosses were evaluated the agronomic traits and the stripe rust reaction following stringent phenotypic criteria. Both phenotype and genotype data confirmed the presence of *Yr48*. A combination of phenotypic selection and marker-assisted breeding revealed that 9 selected lines had more application prospects. Those ILs had increased TGW (4.5–31.1%) in infested environments compared to the recipient parents. New lines with improved disease resistance and better agronomic performance should have more advantages than the original cultivars in wheat breeding programs.

Introduction

Wheat, as one of the three most important cereal crops in the world, is the staple food of about 40% of the world's population (Li et al. 2019). The global people will exceed 9.5 billion by 2050, and global wheat yield will need to grow by 60% to satisfy food requirements (Savadi et al. 2017; Tian et al. 2003). However, wheat production is often affected by pathogenic fungi, resulting in huge yield loss. Stripe rust or yellow rust (*Yr*) of wheat caused by *Puccinia striiformis* f. sp. *Tritici* (*PST*), is one of the most globally devastating fungal diseases that significantly reduces yield and quality (Tao et al. 2018). It damages virtually all winter wheat varieties and limits the realization of the full genetic potential of modern improved wheat cultivars considered the most important fungal disease to decrease wheat production in China (Wan et al. 2004; Zhou et al. 2019).As the adaptability and rapid evolution of the stripe rust pathogen, previously used resistant varieties usually last only a few years. Then the ineffective resistance and heavy yield losses will render popular wheat varieties phased out of farmers' fields. Due to the ineffectiveness of ASR genes, many stripe rust epidemics have taken place all over the world, resulting in enormous yield losses (Chen et al. 2009; Wellings et al. 2007). In general, effective fungicides are available for preventing or reducing yield losses from stripe rust. However, the use of fungicides adds a significant cost for wheat production and adversely affects the environment and human health. Hence, it is compulsory to produce more high level, durable resistance resource to reduce the threat of the widespread epidemic. The deployment of new effective varieties has always been the most economical, effective, and environmentally friendly approach for combating the disease (Chen. 2005). Breeders across the whole world are engaged in the creation and cultivation of new resources of resistance to control the continuously evolving disease (Bariana et al. 2006). Improving resistance to the fungal disease with stripe rust-

resistant genes is usually the highest priority in various regions of the world where stripe rust epidemics infest frequently (Qie et al. 2018).

Seeding/all stage resistance (ASR) and adult plant resistance (APR) are the most common types of stripe rust resistance (Chen. 2005; Rosewarne et al. 2013). Seeding resistance is characterized by racial specificity and qualitative inheritance (Carter et al. 2009). This type of resistance is prone to be detected during the seedling stage of the wheat and is frequently expressed at all ranges of temperatures, and continues throughout the life cycle of all growth stages of plant development. As the specific nature and the strong selection pressure placed on the pathogen, all-stage resistance is frequently defeated by race changes in the pathogen population (Chen. 1995). On the other hand, APR which expresses at the post-seedling growth stages and often is effective to a broader range of races, confer partial resistance and encode a more varied set of proteins (Chen. 2013; Fu et al. 2009; Lowe et al. 2011a). New virulent variants with constant evolution damage resistance of popular cultivars especially for which carry ASR genes. Therefore, it is necessary to continuously identify and deploy more effective resources in wheat breeding. Naturally, the introgression of the APR gene into modern and popular cultivars seems to be favorable to combat the fungal disease and yield loss. Recent developments in the wheat genome facilitate the identification of molecular markers associated with resistant genes that can be deployed to develop more durable resistant cultivars.

A typical application of molecular markers is the marker-assisted breeding (MAB), which is based on the concept of molecular biology. This process has been practiced to improve disease resistance in various crops including barley, cotton, pea, rice, soybean, and wheat (Chemayek et al. 2017; Chen. 2013; Cox et al. 1994; Gahan et al. 2005). MAB has gained considerable attraction as a breeding tool in wheat improvement. Over 60 previously reported genes/quantitative trait loci (QTL) have been used in wheat breeding (Gupta et al. 2010). Many wheat cultivars, such as Clear white 515, Expresso, Patwin 515, Scarlet 09, Summit 515, and Seahawk grown in the western United States, were developed by MAB. Moreover, there have been many successful cases in peanuts where disease-resistant QTLs have been introduced into advanced varieties that have lost resistance (Varshney et al. 2014). Likewise, three elite wheat cultivars successfully improved resistance to stripe rust in China by MAB (Hu et al. 2020). Here, we describe MAB progress in a wheat breeding program aimed at improving stripe rust resistance and agronomic traits in three Chinese advanced cultivars. Marker-assisted breeding for disease resistance is performed routinely in many crop-breeding programs. Phenotypic selection and marker-assisted methods have been used for breeding stripe rust-resistant wheat cultivars in the Southwest University of Science and Technology wheat-breeding program. The MAB methodology has proved more successful in producing cultivars with introgression of adult plant resistance genes. *Yr48*, as an APR gene, was located in the long arm of chromosome 5A by Lowe et al Lowe et al. (2011b). Despite the effectiveness of *Yr48* was clearly confirmed by the previous report (Lan et al. 2017; Lowe et al. 2011b), this APR gene has not been adequately adopted in a wheat breeding program. Gene-linked markers recommended for marker-assisted breeding programs are *BE495011* (0.09 cM proximal of *Yr48*) and *CFA2149* (0.06 cM distal of *Yr48*) (Lowe et al. 2011b).

Since the expression of resistance to stripe rust of *Yr48* and the reliability of closely linked molecular markers, this study was undertaken to select a set of significantly improved *PST* resistance wheat lines with *Yr48* and important agronomic traits. These selected lines will be useful for cultivating new wheat varieties with highly durable resistance to stripe rust.

Materials And Methods

Plant material

Three Chinese elite wheat varieties that were popular in farmers' land, namely Chuanmai 42 (CM42), Lunxuan 987 (LX987), and Bainongaikang 58 (BNAK58), all were selected for introgression of stripe rust resistance. CM42 is a widely adapted, high-yielding variety. This variety, a derivative of the cross SynCD768/SW3243//C6415, was bred at Sichuan Academy of agricultural science, China, and released for cultivation in 2004. CM42 is China's first accredited product bred from synthetic genetic resources Species SynCD768, an introduction from International Maize and Wheat Improvement Center (CIMMYT). LX987 is a cold-resistant and drought-tolerant variety with high and stable yields. It was cultivated at the Chinese Academy of Agricultural Sciences following the method of recurrent selection and pedigree method, released for agriculture during 2003 and has been very prevalent in China. BNAK58, a popular variety, is a high-yielding, cold-resistant and drought-tolerant variety with high harvest index is used extensively for confectionery purpose. All three elite varieties were popular in farmers' land for high yield and wide suitability in china. However, these elite cultivars succumbed to new races of CYR34 thus it is imperative to restructure the genetic makeup of these varieties to achieve better yield and stripe rust resistance. PI610750, spring wheat, is a synthetic derivative developed by CIMMYT. PI610750 is a resistant variety to stripe rust and was selected as a donor parent in the breeding program as it was used to identify the *Yr48* gene for stripe rust resistance. The reliable markers and resistance of PI610750 were confirmed in America and Mexico made it a prominent resistant germplasm resource (Lan et al. 2017; Lowe et al. 2011b).

Developing three crosses presumably possessing *Yr48* with favorable agronomic performance

The schematic procedure for crossing, screening, and selection is illustrated in Fig. 1. To combine *Yr48* and yield-related traits, all three target cultivars were used as female parents to make an independent cross (CM42 × PI610750, LX987 × PI610750, and BNAK58 × PI610750). Initially, The F_1 seeds were planted and harvested in the field. Subsequently, the F_2, F_3, F_4, and F_5 of each wheat cross combination were planted over 30 rows with about 80–100 seeds in a 2 m row with 25 cm between rows. We executed a modified bulk method before the F_5 for each cross to collect all promising genotypes (Hu et al. 2020), and after the accumulation of several generations, we selected the F_5 generation plants with resistance to stripe rust and important agronomic traits including plant height, productive tiller numbers, spike length, grain number per spike, and thousand-grain weight. These populations were selected at Mianyang in Sichuan province (31.682N, 104.663E) under a natural *PST* infection environment, together with MX 169, PI610750, CM42, LX987, and BNAK58 during the 2017–2018 cropping seasons.

Phenotyping for stripe rust reaction and agronomic traits

Three populations of F_5 plants were selected and advanced to F_7. Such F_6 and F_7 generations were subjected to a selection of lines with rust resistance and excellent agronomic traits. Phenotyping of parents, F_6 and F_7 lines for adult plant stripe rust response and agronomic traits were done in China fields in Mianyang (31.682N, 104.663E) of Sichuan province during the 2018–2019 and 2019–2020 cropping seasons (2019MY and 2020MY). The F_7 lines and their parents were also evaluated for APR to stripe rust in China fields in Yangling

(34.292N, 108.077E) of Shanxi province during the 2019–2020 (2020YL) cropping seasons. These lines were arranged in randomized complete blocks with three replications. About 80–100 seeds per line were planted in a row of 2 m long with 25cm space between rows. Spreader rows containing the *PST*-susceptible MX169 were planted around the experimental plot to produce an epidemic environment. These experimental fields are ideal over-wintering and over-summering regions for stripe rust in China and nurseries naturally become infected without creating artificial disease epiphytotic.

The stripe rust infection type (IT) of each plant or each line was rated based on the 0 to 9 scale (Line and Qayoum. 1992). Disease severity (DS) was scored by using the modified Cobb scale (Peterson et al. 1948). Both IT and DS were collected two times during each season. The first note was recorded when the susceptible check MX169 exhibited approximately 80% severity and repeated about a week later (Nsabiyera et al. 2018). Mean IT and DS over test environments were used to analyze.

Besides, stringent visual phenotypic criteria were used to select promising plants with important traits such as plant height, productive tiller numbers, spike length, grain number per spike, and thousand-grain weight in Mianyang of Sichuan province during the 2018–2019 and 2019–2020 cropping seasons. Plant height (PH) was calculated as the mean height of five plants from the plant base to the tip of the spike (excluding awns) in each line. Productive tiller number (PTN) from five independent plants of each line was counted. Spike length (SL) and grain number per spike (GNS) were measured as the mean of the main spikes of five independent plants from each line. TGW was calculated as tenfold of an average weight of 100 seeds from five different plants of each line (Ma et al. 2019). For the F_5 plants, we selected resistant plants based on the stripe rust reactions with full grains, plant height less than 1 m, and a tiller number of 4 or more. For the F_6 and F_7 lines, we randomly selected 5 plants from each line to evaluate agronomic traits. Mean agronomic traits data over two years were used to analyze.

Molecular markers, DNA extraction, PCR amplification, and electrophoresis

The SSR markers *CFA2149* and STS markers *BE495011*, which are closely linked to *Yr48*, were used to detect its presence or absence in the wheat genotypes. Plant genomic DNA was isolated from leaf tissues of the parental lines (PI61750, CM42, LX987, and BNAK58) and the F_6 and F_7 lines of CM42 × PI61750, BNAK58 × PI61750, and LX987 × PI61750, employing the modified cetyltrimethylammonium bromide (CTAB) extraction method (Riede et al. 1996). DNA quality and quantity were measured using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies) and diluted to a concentration of 50ng/ul for further genotyping with linked markers. The tightly linked markers were used for amplification using polymerase chain reaction (PCR) following conditions mentioned in the previous report (Lowe et al. 2011b). PCR reactions were prepared by mixing 2 ul of genomic DNA, 1 μl 10×PCR buffer (including $Mg^{2}+$), 0.2 μl Taq DNA polymerase solution, 1 μl of each primer solution, 0.8 μl of each dNTP, and 4 μl sterilized ddH_2O. The PCR reaction was performed at 94.0°C for 3 min, 40 cycles of (95.0°C for 30 sec, 59°C or 60°C (according to each primer pair) for 30 sec, 72°C for 30 sec), and the final extension at 72°C for 5 min. The PCR products of the linked markers were separated in 6% polyacrylamide gel electrophoresis (PAGE).

Results

Development of common wheat lines presumably possessing *Yr48*

Marker detection and phenotypic selection with elite cultivars including genotyping and phenotyping was undertaken as mentioned in Fig. 1. For the transfer of *Yr48* to wheat varieties CM42, LX987, BNAK58, the crosses were made with PI610750 as a male parent. Before the F_5 generation, we executed a bulk harvest for each cross to maintain all potential genotypes. After several generations of accumulation, Eighty-seven F_5 plants according to their resistance to stripe rust, moderate plant height, and the high number of tiller and grain numbers were selected following stringent visual phenotypic criteria. Of these, 64 lines were selected from the cross of CM42/PI610750, 11 lines were selected from the cross of LX987/PI610750, and 12 lines were selected from the cross of BNAK58/PI610750. Progenies of the 87 selected lines from three crosses were advanced to further genotype and phenotype.

Stripe rust and marker detection

During the 2018−2019 and 2019−2020 screening season, Stripe rust IT of ILs ranged from 0 to 8, and stripe rust DS of ILs ranged from 0 to 80% over 2 years. The response of donor parent was consistently low (IT 1−3, DS < 20%), and the recipient parents CM42, LX987, and BNAK58 were higher (IT 7−8, DS 50−80%) (Fig. 2). The evaluation results of several fields revealed that PI610750 expressed excellent stripe rust resistance in different environments. The majority of the tested ILs showed a disease score of 3 or 6 in different environments, a lower score compared with that of the recipient parents. Similarly, the DS was much lower than the recipient parents. Disease reaction for stripe rust resistance on parents and introgression lines (ILs) possessing resistant gene introgressed through marker detection in wheat (Fig. S1). The donor parent (PI610750) and its ILs (CM42 + *Yr48*, CM42 + *Yr48*, and CM42 + *Yr48*) showed favorable resistant features.

According to the absence or presence of *Yr48*, the 87 lines from three crosses were divided into two groups: possessing *Yr48*, lacking *Yr48*. As a result, 45 lines were found to be positive lines carrying the *Yr48*. Among those, Thirty-six lines from CM42/PI610750, 4 lines were from the cross of LX987/PI610750, and 5 lines were from the cross of BNAK58/PI610750. We compared IT and DS between two groups in different environments and recorded the disease score of parents to illustrate the effectiveness of *Yr48*. The mean adult plant stage IT ranged and DS ranged from 1 to 5 and 5 to 50% for lines carrying *Yr48*, respectively, whereas it was 4 to 7 and 10 to 80% for lines lacking this resistance gene, respectively (Fig. 3A, 3B). To further assess stripe rust was successfully restrained by the wheat breeding program, we compared the disease score in different genetic backgrounds. Student's t-test results showed that the lines lacking the *Yr48* alleles had much higher phenotypic values than those carrying *Yr48* at all the three crosses (P < 0.05, Fig. 3C, 3D).

Phenotyping of agronomic traits of ILs under disease infection

During 2018−2019 and 2019−2020 cropping season same seasons, evaluation trials were conducted at Mianyang to compare agronomic traits including plant height, productive tillers, grain number per spike, spike length, and thousand-grain weight of ILs with their respective recipient and donor parent genotypes (Fig. 4). A good variation in TGW and other agronomic traits was observed among the 87 ILs. PTN of parents were 3 or 4 while the majority of the tested lines showed a much higher number (Fig. 4A). Similarly, the SL of parents at around 9 cm, which is much lower than the tested lines (Fig. 4B). The GNS ranged from 35 to 65 among the selected lines whereas their parents ranged from 35 to 45. More importantly, the majority of these lines

recorded higher GNS than the recipient parents (Fig. 4C). The TGW in ILs ranged from 39–67 g in the genetic background of CM42, 34–54 g in the genetic background of LX987 while that of BNAK58 ranged from 36–46 g. The PH of CM42, LX987, and BNAK58 was 101cm, 91cm, and 85cm, respectively. Enhanced thousand-grain weight and better agronomic performance were observed in some ILs compared to their parent (Fig. 4).

A combination of marker detection and phenotypic selection

Selection for stripe rust resistance was done in a combined phenotypic and genotypic strategy. *Yr48* positive plants were selected by amplifying gene-specific markers *BE495011* and *CFA2149*. Screening for stripe rust was also done in the field along with marker-assisted selection to validate the effectiveness of *Yr48*. F_6 and F_7 of 87 ILs were screened against stripe rust at the adult plant stage in the field during season 2018–2019 and 2019–2020. Initially, 45 positive ILs for *Yr48* were selected by marker detection. According to the disease reaction for stripe rust over two years, IT less than 5 and DS lower than 50% were selected from promising uniform stripe rust resistant lines. Thirty-three lines were selected following the standard disease score.

Subsequently, 33 lines were also evaluated TGW and other agronomic traits to select better lines. The three popular recipient parents in china have very good agronomic traits, which are regarded as related references. Lines with plant height between 80 and 100 cm (considering factors such as lodging resistance and easy harvesting), productive tiller number equal to or more than 5, Spike length over 8 cm, grain number of per spike of 30 or more, and thousand-grain weight larger than 40g were selected. Ultimately we found 9 best introgression lines from three crosses, including CM42/PI610750-12, CM42/PI610750-16, CM42/PI610750-20, LX987/PI610750-70, LX987/PI610750-71, LX987/PI610750-72, BNAK58/PI610750-77, BNAK58/PI610750-83 and BNAK58/PI610750-86.

Yield parameters for nine of the best ILs in three genetic backgrounds are given in Table 2. The thousand-grain weight in ILs ranged from 50.4–56.1 g in the genetic background of CM42, 42.1–45.8 g in the genetic background of LX987 while that of BNAK58 ranged from 42.3–46.4 g. enhanced-thousand grain weight was observed in these ILs compared to their respective recipient parent genotypes (Table 2). More accurately, 3 ILs of CM42/PI610750 recorded 17.7–31.1% higher TGW than the recipient parent genotype CM42 (42.8 g). Three ILs of LX987/PI610750 recorded a higher TGW of 4.5–13.6% compared to LX987 (40.3 g). Three ILs of BNAK58/PI610750 recorded higher TGW of 9.9–20.2% compared to BNAK58 (38.6 g). These selected lines showed lower disease score to stripe rust, appropriate plant height, more productive tiller and grain number, better spike length and thousand grain weight compared with their recipient parent, respectively. The new developed lines will be good for wheat breeders to cultivate stripe rust resistant wheat varieties with high yield.

Table 1
The information of molecular markers for the *Yr48* used in this study

Marker	Primer Sequence	Tm(℃)	References
BE495011	F-TGATTACTGTAGCTACCTCCTCCT	60	Lowe et al. (2011b)
	R-GGTGCAAGATGTGCCTGTAA		
CFA2149	F-CTTGGAGCTCGGGTAGTAGC	59	Lowe et al. (2011b)
	R-AAGGCAGCTCAATCGGAGTA		

Table 2
Details of three of the best lines in each parent background of CM42, LX987, and BNAK58.

Parent/line	aTGW (g)	TGW gain (%)	bPH (cm)	cSL (cm)	dPTN (NO.)	eGNS (NO.)	2019MY		2020MY		2020YL	
							IT	DS	IT	DS	IT	DS
CM42	42.8	-	101	7.3	4	31	8	70	8	80	7	70
CM42/PI61750-12	50.4	17.7	94	9.2	7	45	4	20	2	10	3	20
CM42/PI61750-16	54.0	26.1	95	10.0	7	47	3	10	4	20	2	5
CM42/PI61750-20	56.1	31.1	96	10.4	7	43	2	10	3	10	4	10
LX987	40.3	-	93	7.6	3	34	7	50	7	60	7	60
LX987/PI61750-70	45.8	13.6	93	9.2	5	44	4	30	3	10	3	20
LX987/PI61750-71	43.0	6.7	88	10.1	5	41	3	20	2	5	3	10
LX987/PI61750-72	42.1	4.5	90	10.6	5	43	2	5	3	10	4	10
BNAK58	38.6	-	85	7.7	4	43	7	50	8	70	7	60
BNAK58/PI61750-77	46.4	20.2	98	9.6	7	46	4	20	4	20	3	20
BNAK58/PI61750-83	42.3	9.9	95	9.9	6	48	3	20	2	10	2	20
BNAK58/PI61750-86	42.7	10.6	90	8.4	5	34	3	10	3	5	3	10

aTGW, thousand-grain weight

bPH, plant height

cSL, spike length

dPTN, productive tiller number

eGNS, grain number per spike

Discussion

The conventional breeding method has been widely used to develop cultivars with improved disease resistance agronomic traits, but it is labor-intensive and time-consuming. Cultivating new cultivars in this method can take

between 7 and 20 years, depending on plant species. The use of molecular markers improves the efficiency of breeding. A major application of molecular markers in this study is genotype screening. The development of selection technologies to achieve a genotype-based selection of resistance genes in the absence of bioassays has evolved rapidly in the last decades. Robust molecular markers are now available for several commercially important agronomic traits. Marker-assisted breeding has been proved to be a quick way to develop new cultivars with favorable agronomic traits and improved disease resistance (Bariana et al. 2007; Singh et al. 2000), but introgressing disease resistant genes into the existing preferred wheat cultivars has been rarely attempted.

In the present study, we first developed three wheat populations by crossing a wheat line carrying *Yr48* and another three leading wheat lines with special features such as high yield, enough grain number and moderate plant height, and others. Their progenies presumably carry *Yr48* as some of them expressed lower disease scores than recipient parents. To confirm these lines carry the gene, we validated the presence of *Yr48* by further phenotyping with the tightly linked markers (*CFA2149* and *BE495011*). We genotyped the F_6 and F_7 lines to collect genotype data and evaluated the stripe rust reaction and other important agronomic traits to record phenotype data during 2018–2019 and 2019–2020 cropping seasons over multi-locations. Thus, the introgressing and selecting strategy practiced in the present study is accurate, effective, and economic. Both phenotype and genotype data confirmed the presence of *Yr48*. Though some QTLs for stripe rust resistance have also been reported in the PI610750 (Lowe et al. 2011b), the previously reported markers were found to be non-polymorphic between the receeipient and donor parents used in the present study. Thus a conventional method can be a complement for marker assisted breeding.

Besides increase stripe rust resistance, progress was also made in improving TGW and some other agronomic traits. Replicated yield related traits assessment under infection environment showed an increase in thousand grain weight by up to 31.1 % higher than CM42, 13.6 % more than LX987 and 20.2 % higher than BNAK58. All these ILs showed a disease score on par with that of the donor parent genotype while displaying yield related traits better than that of the recipient parent genotypes. The higher thousand grain weight in ILs compared to that in the recipient parent may be partly attributed to the guaranty provided by the resistance gene against the fungal disease and stringent visual phenotypic criteria. The results against the theory that an active resistant reaction results in yield penalties for plants combating with pathogens. Thus, highlight the value of conventional phenotypic selection in crop breeding.

Also, we compared the agronomic traits in two groups (Possessing *Yr48* and lacking *Yr48*) to illustrate the benefits of combination using a marker assisted and phenotypic selection. Given the sheer magnitude of its effect reduction in disease severity (Fig. 5A), a clear evidence that *Yr48* is an excellent resistance gene. Considering the function of *Yr48*, specifically in stripe rust resistance at the adult plant stage, we wondered that introgressing the *Yr48* into wheat may confer resistance in different genetic backgrounds whether affecting yield traits. As expected, the introgression of a short fragment on wheat 5AL resulted in wheat lines with broad resistance to stripe rust (Fig. S1). Significant thousand-grain weight reduction caused by *Yr48* resistance was also not detected when it was introgressed into three different genetic backgrounds (Fig. 5B). Detailed characterization of the population (CM42 background) in field conditions showed no significant difference in agronomic yield related traits (e.g., plant height, spike length, etc.; Fig. 5C). And, the stable agronomic performance and stripe rust resistance made the selected lines more reliable and valuable. These results

demonstrated the high advantages of *Yr48* resistance without yield reduction, and thus emphasized the practical utility of the *Yr48* locus in wheat breeding for improved stripe rust resistance and good yield traits. So the improved agronomic performance is partially attributed to the phenotypic selection. Although, the *Yr48* didn't clone yet. But with the tightly linked markers (less than 1cM), a preliminary diagnosis about the gene may be partly helpful to characterize *Yr48* (Fig. 5C).

Yield penalty is a major concern in utilizing stripe rust resistance or any other favorable traits for wheat breeders. Disease resistance is frequently costly and physically detrimental for plant fitness (Tian et al. 2003; Büschges et al. 1997; Denancé et al. 2013). For example, a previous found that one beneficial gene, *PigmR*, confers *Pigm* blast resistance in the rice variety Gumei4. However, the decreased yield was observed through a reduction of grain weight and size with the expression of *PigmR* and the absence of a gene named *PigmS* (Deng et al. 2017). In the present study, the yield difference caused by *Yr48* resistance was also not detected when it was introgressed into three different genetic backgrounds, which appeared to be novel, as the usual negative correlation between yield and an active resistance response (Wang et al. 2018). The beneficial effects on TGW and other traits could be due to stringent visual phenotypic selection as reported in the previous study (Varshney et al. 2014, Hu et al. 2020).

The importance of genetic resistance to restrain fungal diseases was first illustrated by Biffen (Biffen et al. 1905). Currently, the utilization of *Yr* genes has been widely practiced to develop cultivars with improved disease resistance. The introgression of the stripe rust resistance gene *Yr15* has cultivated several commercial wheat cultivars (Yaniv et al. 2015). Breeding of stripe rust-resistant cultivars using conventional and marker-assisted breeding technologies in wheat was demonstrated for creating a wheat line with pyramided genes *Yr64* and *Yr15* for resistance to stripe rust (Qie et al., 2019). Pyramiding of multiple genes including *YrZH84* using MAB was demonstrated by Li et al. in a high quality and high-yielding wheat cultivars Zhengmai 7698 (Li et al. 2019). Liu et al. recommended that breeders may use some effective *Yr* genes (such as *Yr15*, *Yr62*, and *Yr65*) to achieve durable resistance to *PST* in wheat breeding (Liu et al. 2020). Hu et al. reported that two QTLs for stripe rust resistance were introgressed into three Chinese leading wheat cultivars (Chuanmai 42, Xiangmai 25, and Zhengmai 9023) to improve stripe rust resistance. Apart from stripe rust, several studies have reported QTLs and genes for improving important traits in wheat. For example, marker assisted selection was used to improve protein content and grain weight in Indian leading wheat cultivar HUW234 by introducing favorable genes from other wheat (Vishwakarma et al. 2016). Roy et al. demonstrated that introgression of an expressed HMW 1Ay glutenin subunit allele into bread wheat can improve storage protein composition and bread-making quality without decreasing grain yield (Roy et al. 2020).

To our knowledge, this study is a rareness of its kind for developing elite cultivars for stripe rust resistance in a wheat breeding program. The introgressing and selecting strategy was practiced in the present study is effective and economic. It is helpful for molecular breeding study for developing Chinese superior lines in wheat. Nevertheless, the selected lines with *Yr48* and beneficial agronomic traits in the present study should be more useful than the previously developed cultivars. It should be better to pyramid more useful genes through backcrossing and other breeding methods for increasing stripe rust resistance and wheat production. Advanced breeding of the selected lines can lead to the release of new cultivars and also serve as valuable recourse for crop breeders to be utilized in future breeding programs.

Conclusion

In the present study, *Yr48* has been transferred into three Chinese elite wheat cultivars CM42, LX987, and BNAK58 from a resistant genotype, PI610750. In the background of CM42, LX987, and BNAK58, 9 ILs with an increase in TGW up to 31.1%, 13.6% and 20.2% respectively (with respect to recipient parents), other better yield trait and improved stripe rust resistance was selected, through a combination of marker detection and phenotypic selections in 2 years replicated field trials.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author contribution statement

QY detected the QTL, analyzed the data and prepared the first draft of the manuscript. THF contributed to collected samples and phenotype data. XLZ and XL contributed to the crosses and revised the manuscript. THF, and SZY contributed to the selected of target lines and evaluated the populations. XLZ and ZSK suggested the project and generated the final version of the manuscript. All authors provided suggestions during revision of the manuscript.

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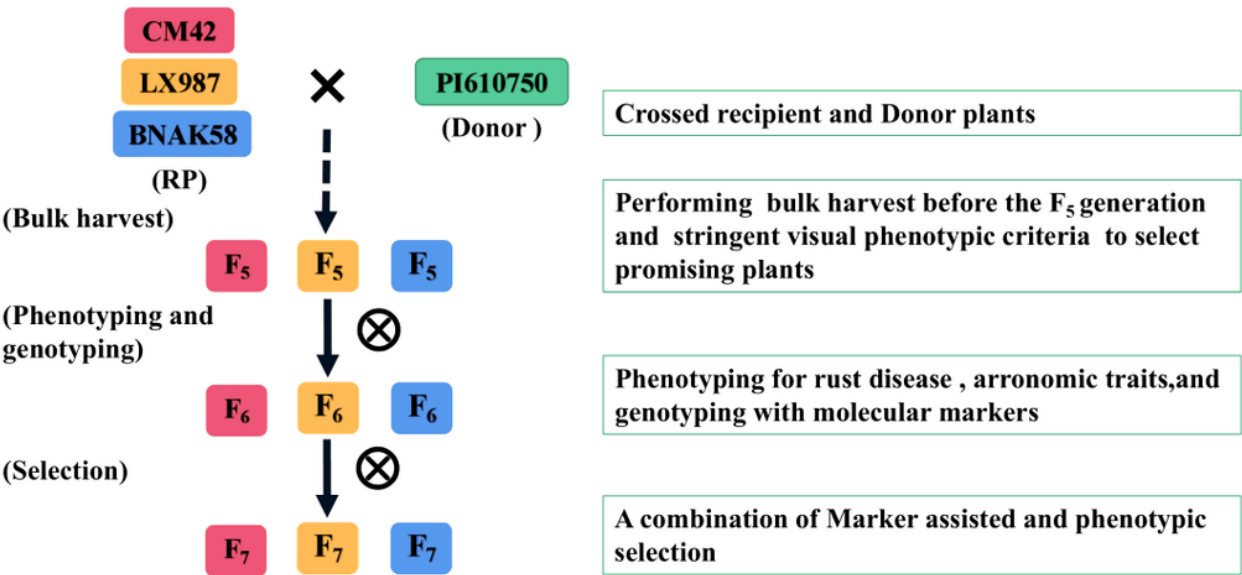
Figures

Figure 1

The scheme of development, validation, and selection to improve stripe rust resistance in three elite cultivars (CM42, LX987, and BNAK58). CM42 is shown in red, LX987 is shown in yellow, BNAK58 is shown in blue, and PI610750 is shown in green; the color of their cross offspring is the same as their respective female parents. RP, recipient parent
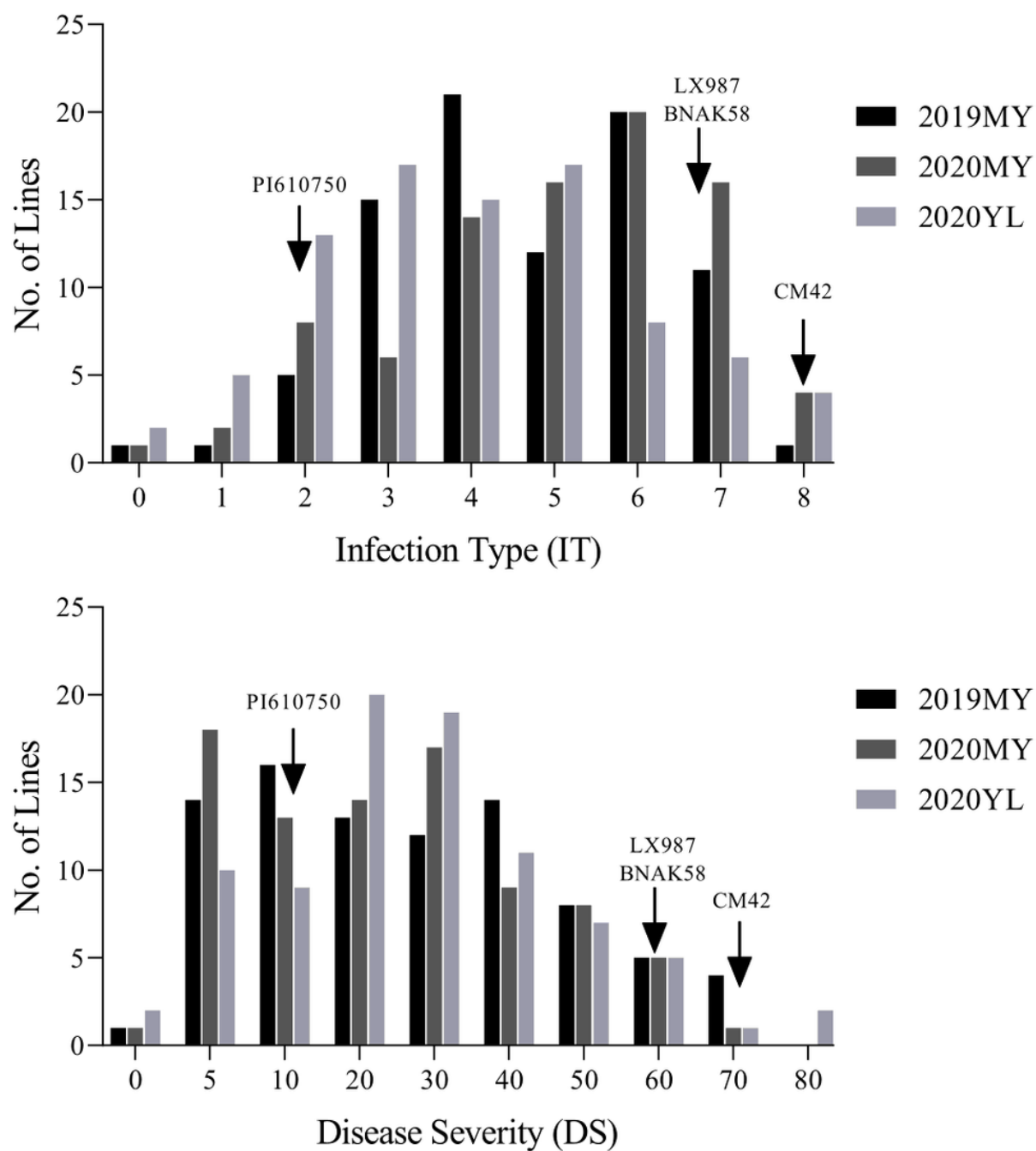
Figure 2

Frequency distributions of infection types (IT) and disease severities (DS) for 87 ILs from the crosses of CM42/PI610750, LX987/PI610750, and BNAK58/PI610750 grown at Yangling and Mianyang in 2019−2020. Arrows indicate the values of the parental lines
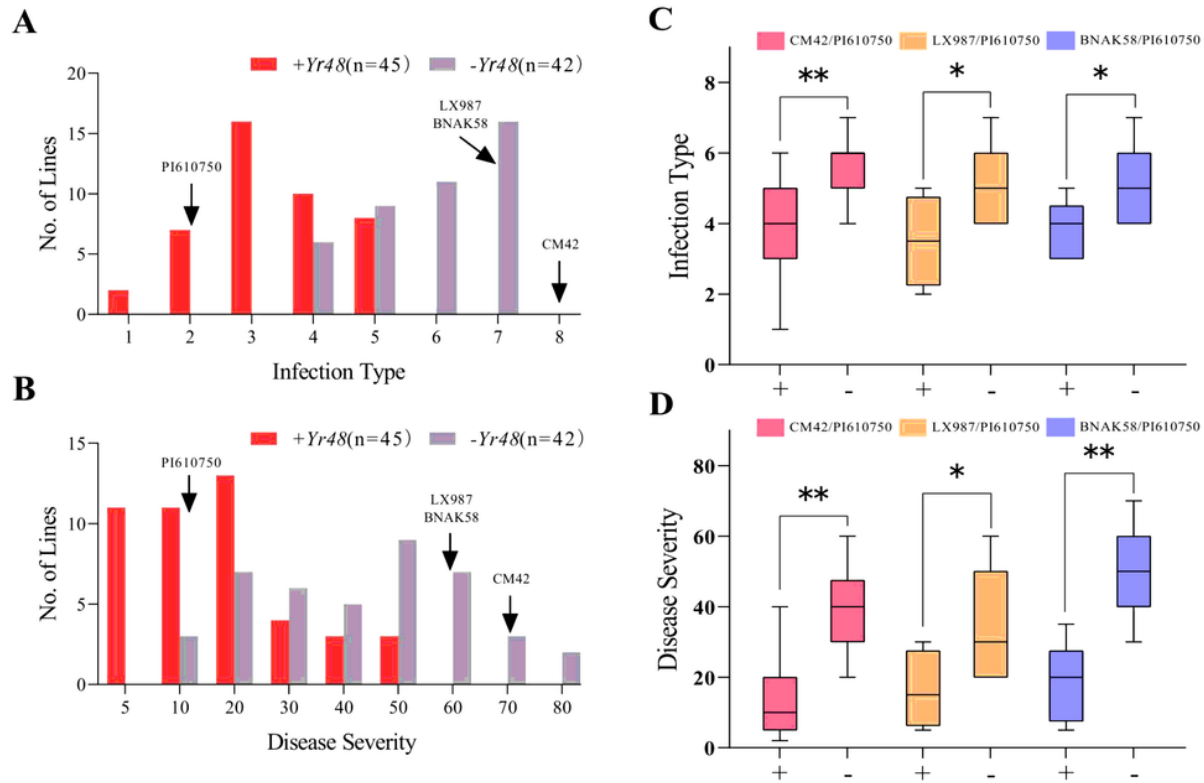
Figure 3

Yr48 improves stripe rust resistance in three populations. (A) Frequency distributions of mean infection type in the 87 ILs possessing and lacking Yr48 at adult growth stages over 2 years of test environments. (B) Frequency distributions of mean disease severity in the 87 ILs possessing and lacking Yr48 at adult growth stages over 2 years of test environments. (C and D) Effect of Yr48 in three different populations. "+" represents the presence of Yr48, "-" represents the absence of Yr48. P-value was generated by the Student's t-test. n.s., no significance at P > 0.05 (* Significance at P < 0.05, **Significance at P < 0.01).
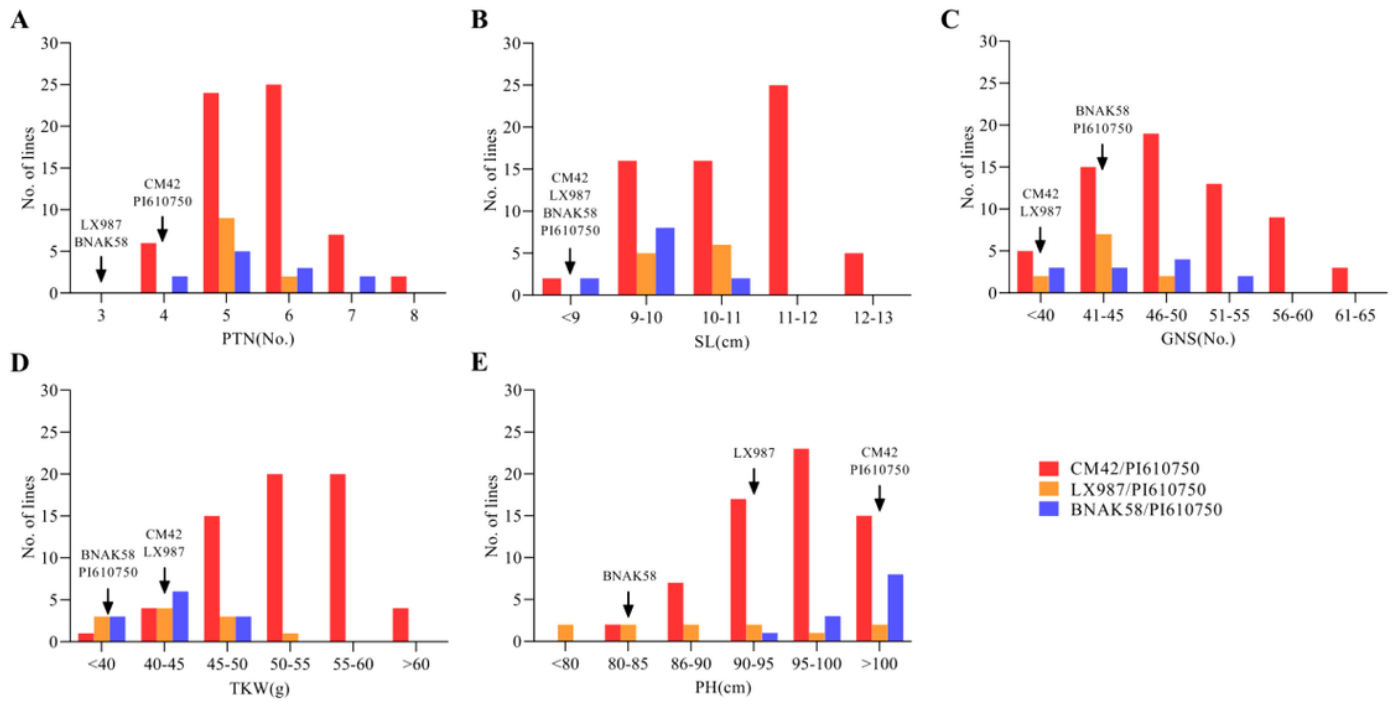
Figure 4

Frequency distribution of agronomic traits for the 87 ILs of the three populations. (A) productive tiller number (PTN); (B) spike length (SL); (C) grain number per spike (GNS); (D) thousand-grain weight (TGW); (E) plant height (PH).
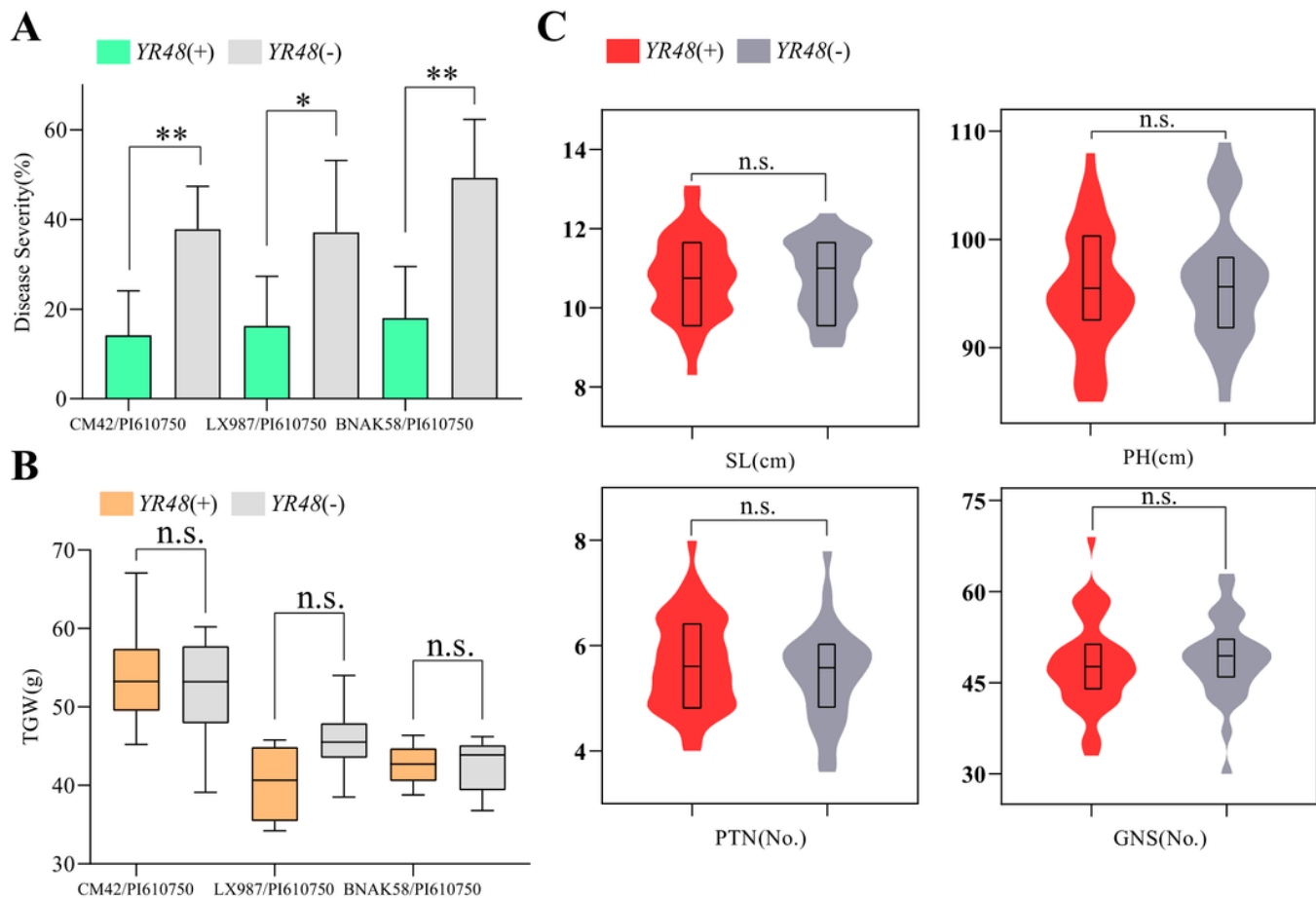
Figure 5

Stable agronomic performance in different genetic backgrounds. (A) Stripe rust resistance of Yr48 in three different wheat genetic backgrounds evaluated in field conditions. (B) Comparison of thousand-grain weight (TGW) in different wheat genetic backgrounds. The TGW was calculated as ten-fold of the average weight of 100 seeds from five different samples of each line. (C) Comparison of the agronomic traits among Yr48 (+) lines and Yr48 (−) lines in the CM42 background evaluated in the 2019 and 2020 field experiment. SL, spike length (cm); PH, plant height (cm); PTN, productive tiller number; GNS, grain number per spike. P-value was generated by the Student's t-test. n.s., no significance at P > 0.05 (* Significance at P < 0.05, **Significance at **P < 0.01)