Introgression of Two Quantitative Trait Loci for Stripe Rust Resistance into Three Chinese Wheat Cultivars

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Abstract: Wheat stripe rust, caused by Puccinia striiformis f. sp. tritici (Pst), is one of the most devastating diseases in wheat. Due to the large-scale and widely-distributed planting pattern of wheat, the directional selection pressure of the pathogen is very strong. Therefore, it is urgent to pyramid more stripe rust resistance genes in wheat cultivars to enhance resistance durability and ensure wheat production safety. In this study, two quantitative trait loci (QTL) for adult plant resistance (APR) to stripe rust, QYr.nafu-2BL and QYr.nafu-3BS, were validated and introgressed from wheat line P9897 into three Chinese elite wheat cultivars, Chuanmai 42, Xiangmai 25, and Zhengmai 9023, through marker validation. The three Chinese elite varieties were used as the female parent to cross with wheat line P9897, and they were selfed to the F6 generation. A total of 114 lines were then selected based on field agronomic traits and stripe rust resistance. Four markers (Xcfd73, Xgwm120, Xbarc87 and Xbarc133) linked with the QTL’s regions were employed to screen the 114 F6 lines. Subsequently, 27 lines combining two target QTL from P9897 were selected. The combination of agronomic traits and disease resistance results showed that 13 of these selected lines had favorable application prospects. The promising lines selected in this study could enrich the genetic resources of wheat stripe rust resistance genes, as well as provide material support and a theoretical basis for the prevention and control of wheat stripe rust in China.

Keywords: wheat; stripe rust resistance; quantitative trait loci (QTL); marker-assisted selection (MAS)

1. Introduction

Wheat is a cereal crop widely cultivated all over the world. It is planted in over 200 million hectares per year with a production of 751.1 million tons, and human consumption of wheat is increasing every year [1]. By 2050, the global population will exceed 9.5 billion, according to the World Bank, and global wheat production needs to increase by 60% to meet food demand [2,3]. Wheat stripe rust is caused by Puccinia striiformis f. sp. tritici (Pst). The fungi can spread long distances on air currents, and its occurrence and damage can be characterized as long-term, fulminant, epidemic, or variable. The disease is distributed throughout global wheat-producing areas. In China, wheat stripe rust occurs...
with varying degrees of severity every year, with an average of about 4 million hm² and a yield loss of more than 1 billion kg [4–6]. Due to wheat’s large-scale and widely-distributed planting pattern, the directional selection pressure of the pathogen is very strong. Once a strong new virulent race appears, it may make the wheat variety lose its resistance [6]. For example, with the prevalence of CYR34 in China, some leading varieties that contain Yr26 have gradually lost their disease resistance [7–9]. Traditionally, commonly used methods for the prevention and control of stripe rust have been the utilization of chemical agents and breeding resistant varieties. However, use of fungicides adds significant cost to production, has the potential to select fungicide-tolerant populations of the pathogen, and is potentially harmful to humans, animals, and the environment [6,10]. Directing research toward breeding resistant varieties and utilizing disease resistance genes to control stripe rust is the most cost-effective and environmentally-friendly approach [11–13].

Wheat stripe rust resistance genes can be classified into race-specific (also refer to all-stage or major genes) and non-race-specific (also refer to adult plant resistance genes) categories [6,14–17]. Adult plant resistance (APR) genes often present incomplete resistance and have historically been more durable than race-specific genes. Therefore, one of the promising approaches to solve the problem of the loss of disease resistance is using APR, which is effective in the adult plant stage and is considered to have a broader range of resistance and longer durability [18–20]. In a wide range of applications, the life span of genes and varieties can be increased by gene pyramiding [21–23]. However, conventional breeding is a time-consuming and labor-intensive process, since successfully cultivating a new wheat variety often takes 7 to 20 years. Marker-assisted selection (MAS) is a method of screening out individuals using molecular markers linked to the target gene, or quantitative trait locus (QTL), as a substitute for or to assist in phenotypic selection [24]. With the emergence of more advanced molecular markers techniques, such as simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs), denser maps can be developed [25], enabling more accurate pyramiding of target genes while reducing linkage drag [23]. The use of molecular markers greatly improves the efficiency of the breeding process and reduces the consumption of human and material resources [26]. For example, Guinong 19, Yangmai 22, Shannong 20, and some other high-yield varieties are bred through marker-assisted selection (MAS) [27–29]. By MAS, some important genes can be conferred by introgression or pyramiding in plants. For example, MAS has been used in pyramiding the rym1 and rym5 genes in barley, increasing the resistance of barley to barley yellow mosaic virus [30]; enhancing the resistance to bacterial blight by pyramiding xa5, xa13, and Xa21 genes in the deepwater rice variety Jamagna [31]; and improving the resistance to powdery mildew by the introgression of Pm2b gene in three high-yield wheat cultivars [32]. Compared to conventional breeding based on phenotypic assessment, MAS provides a more reliable, precise assessment method, and can be applied on a larger scale in a shorter period of time [33].

P9897 (CI 14312, P-9897-8T-2B-1T-1B) is a spring wheat line developed by Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) with APR to stripe rust, with two QTL (QYr.nafu-2BL and QYr.nafu-3BS) conferring resistance to it, as reported in previous research [34]. Chuanmai 42, Xiangmai 25, and Zhengmai 9023 are three Chinese elite wheat varieties with yields per hectare of more than 6000 kg, which have accumulated more than 1 million mu of commercial planting area in China, and have demonstrated good resistance to stripe rust in the past [35–37]. However, in 2008, a new strain (code V26, now called CYR34) that is infectious to Gui Nong 22, the identification host of Chinese wheat, was found in Sichuan [7,38]. Preliminary gene analysis found that it contained VYr26 (= YYr24) and VYr10 genes not available in CYR32 and CYR33 [7,8]. With the prevalence of CYR34 in recent years, the elite varieties’ resistance has been lost to varying degrees. In a previous study, we found that wheat variety P9897 has two resistant QTL on 2BL and 3BS, and its linked molecular markers are Xcfd73 and Xgwm120 on 2BS and Xbarc87 and Xbarc133 on 3BL [34]. It is also known that Chuanmai 42 contains the resistance gene Yr26 (flanked by WE-173 and Xbarc181) [39]. The objective of this study is the introgression of two resistant QTL from P9897 into three leading varieties by conventional breeding methods, and using marker validation to select the lines which combine two
QTL or more. The agronomic traits and disease resistance will be evaluated to obtain which lines have high application value.

2. Materials and Methods

2.1. Plant Material

The donor parent was the wheat line P9897, which carries two adult plant stripe rust-resistant QTL (QYr.nafu-2BL and QYr.nafu-3BS) and has maintained a good APR to stripe rust over many years of testing [34]. Its stability and resistance QTL make it an excellent resistant germplasm resource. The Chinese elite varieties Chuanmai 42 (from Sichuan Province and approved by the National Crop Variety Approval Committee (NCVAC) in 2004, and known to carry the Yr26 gene), Xiangmai 25 (from Hubei Province and approved by the NCVAC in 2008), and Zhengmai 9023 (from Henan Province and approved by the NCVAC in 2003) were the recipient parents. These three varieties have had high yields and strong disease resistance in the past, but with the emergence of new races of stripe rust, especially the emergence and prevalence of CYR34, their resistance to stripe rust has gradually been lost. However, due to their exceptional agronomic traits, they can continue to be used as recipient parents [35–37].

2.2. Cross Combination and Offspring Screening

P9897 was crossed with Chuanmai 42, Xiangmai 25, and Zhengmai 9023, respectively, with P9897 as the male parent and the three elite varieties as the female parents. The F1 seeds were obtained and planted in the field. The F2, F3, F4, and F5 seeds of each cross combination were planted over 30 rows, with approximately 80 seeds in a 2 m row with 30 cm between rows. We performed a bulk harvest before the F5 generation for each cross to retain all possible genotypes, and after several generations of accumulation, we selected the F5 generation plants with resistance to stripe rust, moderate plant height, and a high number of tiller and spikelet numbers. A total of 114 F6 lines were obtained from the F5 generation of three cross combinations in 2018. Through artificial screening and selecting in this way, the agronomic traits of the F6 lines we obtained became stable (Figure 1).

![Figure 1](image.png)

**Figure 1.** The scheme of development, validation, and selection of the wheat lines approach for the introgression of stripe rust-resistance QTL in three elite cultivars (Chuanmai 42, Xiangmai 25, and Zhengmai 9023) using the same donor (P9897). Chuanmai 42 is red, Xiangmai 25 is yellow, Zhengmai 9023 is blue, and P9897 is green; the color of their cross offspring is the same as their respective female parents.
2.3. Genotyping Wheat Lines by Molecular Markers

The parents and 114 F\textsubscript{6} lines of the three cross-combinations were genotyped using molecular markers linked to the two QTL (QYr.nafu-2BL and QYr.nafu-3BS) in P9897 to selected lines with a single QTL or two QTL (Table 1). The SSR markers Xcfd73 and Xgwm120, which are closely linked to QYr.nafu-2BL, and Xbarc87 and Xbarc133, which are closely linked to QYr.nafu-3BS, were used to detect the F\textsubscript{6} lines with QYr.nafu-2BL, QYr.nafu-3BS, or both QTL. Two molecular markers, WE-173 and Xbarc181, which are linked to Yr26 [39], were used to detect the lines containing Yr26 in the Chuanmai 42/P9897 cross-combination (Table 1).

| Marker | Primer Sequence | Tm \textsuperscript{d} (°C) | References |
|--------|----------------|-----------------|------------|
| Xcfd73 \textsuperscript{a} | F:GATAGATCAATGTGGGCCGT<br>R:AACCTGTTCGCCCATCTGAGC | 60 | [40] |
| Xgwm120 | F:GATCCACCTTCCTCTCTCT<br>R:GATTATACTGGTGCCGAAAC | 60 | [41] |
| Xbarc87 \textsuperscript{b} | F:GCTCACCGGGCATTGGGATCA<br>R:GCGATGACGAGATAAAGGTGGAGAAC | 55 | [42] |
| Xbarc133 | F: AGCGCTCGAAAAGTCAG<br>R:GGCAGGTCCAACTCCAG | 50 | [43] |
| WE-173 \textsuperscript{c} | F:GGGACAAGGGGAGTTGAAGC<br>R:GAGAGTTCCAAGCAGAAC | 55 | [39] |
| Xbarc181 | F:CGCTGGAGGGGGTAAGTCATCAC<br>R:CCCGAATCAAGAACGGGAGAAAAA | 58 | [42] |

\textsuperscript{a} Xcfd73 and Xgwm120 are linked to the QYr.nafu-2BL; \textsuperscript{b} Xbarc87 and Xbarc133 are linked to the QYr.nafu-3BS; \textsuperscript{c} WE-173 and Xbarc181 are linked to the Yr26. \textsuperscript{d} Annealing temperature.

2.4. DNA Extraction and PCR

In 2019, the fresh leaves of the 114 F\textsubscript{6} lines and four parents were collected in a Mianyang experimental field. The DNA were extracted by a modified cetyltrimethyl ammonium bromide (CTAB) method [44]. The polymerase chain reaction (PCR) program followed conditions described in previous research [45,46]. PCR was performed in 10\textmu L reaction mixtures containing 2\textmu L (100ng/\textmu L) template DNA, 1\textmu L 10\times PCR buffer (containing mg\textsuperscript{2}+), 0.8\textmu L 2.5 mM of each dNTP, 1\textmu L (2\muM) of each primer solution, 0.2\textmu L Taq DNA polymerase solution (2.5 unit/\mu L), and 4\textmu L sterilized dd H\textsubscript{2}O. The PCR amplification profile consisted of a denaturation (4 min at 94 °C) followed by 35 cycles (94 °C for 30 s, 55 °C for 20 s, and 72 °C for 30 s) and an extension for 8 min at 72 °C. The PCR amplification products of the linked markers were separated using 6% polyacrylamide gel electrophoresis (PAGE) (Beijing Solarbio Science & Technology Co., Ltd. Beijing, China) [47].

2.5. Disease Resistance and Agronomic Trait Evaluation in the Field

The F\textsubscript{5} plants, F\textsubscript{6} lines, and parents were evaluated for adult plant stripe rust response in Mianyang in the Sichuan province (31°33′N, 104°55′E, altitude 485 m) during the 2017–2018 and 2018–2019 cropping seasons. The F\textsubscript{5} plants of each cross combination were planted across 30 rows, with approximately 80 seeds in a 2 m row with 30 cm between rows. The F\textsubscript{6} lines were arranged in randomized complete blocks with three replications. Each line, with approximately 80 seeds, was planted in a 2 m row with 30 cm between rows. The parents and the susceptible control Mingxian 169 (M169) were planted after every 20 rows throughout the field. To increase field inoculum, one column of Mingxian169 was also planted perpendicularly and adjacent to the test rows. Mianyang is natural over-wintering region for stripe rust in China, and nurseries regularly become infected without artificial inoculation. The main prevalent races in Sichuan Province currently include CYR32, CYR33, and CYR34. In recent years, CYR34 has gradually become the predominant race [9]. Infection type
(IT) data were collected based on the 0-9 scale of Line and Qayoum [48]. Disease severity (DS) scores were based on the modified Cobb scale [49]. Both IT and DS were recorded two times for parents, all F5 plants, and F6 lines when stripe rust severity on M169 reached between approximately 50% and 90% around 1 to 20 April in Mianyang. IT numbers of 0, 1–3, 4–6, and 7–9 were considered immune, resistant, intermediate, and susceptible, respectively. DS was recorded as the percentage of the diseased leaf area [43,50].

For the F5 plants, we selected resistant plants with full spikes, plant height less than 1 m, and a tiller number of 4 or greater. For the F6 lines, we selected 10 plants from the middle row of each repeating plot to evaluate agronomic traits. After the milk stage, we measured the average plant height of the selected plants with a ruler. The plant height of each plant was measured from the ground to the top of the spike. After maturity, the spikelets on each main spike, including the sterile spikelets, and the effective tillers of each plant were counted, and the average was taken. After harvest, we randomly selected 200 seeds from each wheat line and recorded their weight. The procedure was repeated three times for each line, and the average weight was taken as the 1000 grain weight.

3. Results

A total of 114 F6 lines were selected due to their resistance to stripe rust, moderate plant height, and high tiller and spikelet numbers. Of these, 19 lines were selected from the cross of Chuanmai 42/P9897, 62 lines were selected from the cross of Xiangmai 25/P9897, and 33 lines were selected from the cross of Zhengmai 9023/P9897.

3.1. Quantitative Trait Locus Detection

Corresponding to the QTL detection results (Figure S1), the 114 F6 lines from three cross-combinations were divided into four groups: carrying \( QYr.nafu-2BL \), carrying \( QYr.nafu-3BS \), carrying both QTL, and carrying no target QTL (Table 2). The results of QTL detection showed that among the F6 lines of cross-combination Chuanmai 42/P9897, four lines carried \( QYr.nafu-2BL \), three lines carried \( QYr.nafu-3BS \), seven lines carried both QTL, and five lines did not carry a QTL; the mean IT and mean DS for the lines were 1.5, 1.7, 1.6, 2.8 and 4.3, 3, 3, and 40, respectively (Figure 2). In addition, two markers (\( WE-173 \) and \( Xbarc181 \)) linked to \( Yr26 \) were used to detect the F6 lines of Chuanmai 42/P9897, identifying that 63.2% (12 in total) of the lines contained \( Yr26 \) in the Chuanmai 42/P9897 cross-combination. Among them, a total of six lines combined \( Yr26 \) with the two target QTL from P9897. The F6 lines of cross-combination Xiangmai 25/P9897 consisted of 25 lines carrying \( QYr.nafu-2BL \), 9 lines carrying \( QYr.nafu-3BS \), and 15 lines carrying both QTL, while 13 did not carry a QTL. The mean IT and mean DS were 2.1, 1.8, 1.6, 3.6 and 8, 6.7, 5.5, and 34.3, respectively (Figure 2). In cross-combination Zhengmai 9023/P9897, consisted of eight lines carrying \( QYr.nafu-2BL \), 10 lines carrying \( QYr.nafu-3BS \), and five lines carrying both QTL, while 10 did not carry a QTL. The mean IT and mean DS were 2.3, 1.7, 1.6, 3.4 and 8, 6.3, 4.8, and 23.3, respectively (Figure 2). Among the F6 lines of the three cross-combinations, the total number of lines containing two QTL was 27. Apart from this, at least one QTL was detected in a total of 86 F6 lines, and in 28 F6 lines a QTL was not detected. As an example, Figure S1 shows that marker \( Xgwm120 \) linked to \( QYr.nafu-2BL \) (a), marker \( Xbarc87 \) linked to \( QYr.nafu-3BS \) (b), and marker \( WE-173 \) linked to \( Yr26 \) was present or absent in P9897, Chuanmai 42, Xiangmai 25, and Zhengmai 9023, and segregated in the F6 lines.

Table 2. Summary of the F6 lines of the three crosses for no QTL, 2B QTL, 3B QTL, 2B+3B QTL and contains quantitative trait locus (QTL) totals.

| Cross Combination | No QTL | 2B QTL | 3B QTL | 2B+3B QTL | Contains QTL Totals |
|-------------------|--------|--------|--------|-----------|-------------------|
| Chuanmai 42/P9897 | 5      | 4      | 3      | 7         | 14                |
| Xiangmai 25/P9897 | 13     | 25     | 9      | 15        | 49                |
| Zhengmai 9023/P9897 | 10   | 8      | 10     | 5         | 23                |

*2B QTL means the QTL of \( QYr.nafu-2BL \); 3B QTL means the QTL of \( QYr.nafu-3BS \).*
The phenotypes of all of the lines in which a QTL was detected were resistant, as the ITs of Chuanmai 42, Xiangmai 25, and Zhengmai 9023 were 7, 6, and 7 (Figure 3), respectively. These results demonstrate that introgression of the QTL from P9897 into the three leading varieties significantly enhanced the disease resistance of its offspring (Figure 2).

The combined results of two surveys of the lines showed different degrees of disease resistance. P9897 showed a high level of resistance (IT = 2; DS = 5%) (Figure 3); however, Chuanmai 42 (IT = 7; DS = 60%), Xiangmai 25 (IT = 6; DS = 50%), and Zhengmai 9023 (IT = 7; DS = 50%) were shown to be susceptible (Figure 4A). Based on our many years of disease resistance evaluation in the field, these three recipient parents are still partially resistant to stripe rust (IT 5-8; DS 50%-80%). Li and Xu
reported that the ITs of Chuanmai 42, Xiangmai 25, and Zhengmai 9023 were 0, 2, and 2, respectively at seeding stage [51,52]. Therefore, we speculate that these three varieties contain unknown resistance genes. In the F₆ lines of Chuanmai 42/P9897 (19 total), 18 lines were resistant, 1 line was of intermediate resistance, and no line was immune (Table 3, Figure 4B). In the F₆ lines of Xiangmai 25/P9897 (62 total), the number of immune, resistant, and intermediate lines were 3, 55, and 4, respectively (Table 3, Figure 4C). In addition, in the F₆ lines of Zhengmai 9023/P9897 (33 total), the number of immune, resistant, and intermediate lines were 1, 31, and 1, respectively (Table 3, Figure 4D). There were no susceptible lines in the three cross-combinations, except for Xiangmai 25/P9897-30 (IT = 6, DS = 70%), in which the DS of all the F₆ lines were less than 50%.

Table 3. Summary of the infection type (IT) of the F₆ lines of the three crosses as immune, resistant, intermediate, susceptible.

| Cross Combination | Immune (IT = 0) | Resistant (IT = 1–3) | Intermediate (IT = 4–6) | Susceptible (IT = 7–9) |
|-------------------|-----------------|----------------------|-------------------------|------------------------|
| Chuanmai 42/P9897 | 0               | 18                   | 1                       | 0                      |
| Xiangmai 25/P9897 | 3               | 55                   | 4                       | 0                      |
| Zhengmai 9023/P9897 | 1           | 29                   | 3                       | 0                      |

Figure 4. Stripe rust reactions on leaves of four parents and susceptible controls (A), Chuanmai42/P9897 F₆ lines (B), Xiangmai25/P9897 F₆ lines (C), and Zhengmai9023/P9897 F₆ lines (D).
The plant height of the four parents—P9897, Chuanmai 42, Xiangmai 25, and Zhengmai 9023—were 112 cm, 100 cm, 93 cm, and 91 cm respectively, and the lines of Chuanmai 42/P9897, Xiangmai 25/P9897, and Zhengmai 9023/P9897 were mainly distributed in the range of 71~100 cm (Figure 5). The spikelet numbers of P9897, Chuanmai 42, Xiangmai 25, and Zhengmai 9023 were 15, 17, 19, and 17, respectively, and the number of spikelets in the F_6 lines of the three cross-combinations was more than 16, basically (Figure 5). The number of tillers of P9897, Chuanmai 42, Xiangmai 25, and Zhengmai 9023 were 4, 6, 4, and 6, respectively. The number of tillers of the lines of Xiangmai 25/P9897 were mainly concentrated in the range of 3~5, while in the lines of Chuanmai 42/P9897 and Zhengmai 9023/P9897 they were mainly concentrated in the range of 4~7 (Figure 5).

The 1000 grain weights of P9897, Chuanmai 42, Xiangmai 25, and Zhengmai 9023 were 40.47 g, 41.03 g, 44.37 g, and 42.37 g, respectively. In the lines of Chuanmai 42/P9897 and Xiangmai 25/P9897, the 1000 grain weight was mainly concentrated in the range of 40~50 g, while the 1000 grain weight of the lines of Zhengmai 9023/P9897 was distributed in the range of 30~60 g (Figure 5).

Figure 5. Frequency distribution of plant height, spikelet number, number of tillers, and 1000 grain weight for the 114 F_6 lines of the three cross-combinations.

The three recipient parents, as the elite cultivars in China, have very good agronomic traits, which are used as standard references. Lines with a plant height between 80 and 100 cm (considering factors such as lodging resistance and easy harvesting), a spikelet number of 17 or greater, a tiller number of 4 or greater, and 1000 grain weight larger than 40 g were selected. At last, 13 lines were selected according to the above criteria (Table 4).

In summary, in the 114 F_6 lines of the three cross combinations, we utilized the MAS method to select 27 lines which combined two QTL from P9897, including some lines combining Yr26 or some unknown resistance QTL. All the selected lines were resistant to stripe rust, according to the results of the disease resistance evaluation. Then, combining the evaluation of agronomic traits, we narrowed our selection to 13 lines, including Chuanmai 42/P9897-3, Chuanmai 42/P9897-14, Xiangmai 25/P9897-25, Xiangmai 25/P9897-26, Xiangmai 25/P9897-27, Xiangmai 25/P9897-39, Xiangmai 25/P9897-40, Xiangmai 25/P9897-56, Xiangmai 25/P9897-59, Xiangmai 25/P9897-60, Xiangmai 25/P9897-78, Zhengmai 9023/P9897-92, and Zhengmai 9023/P9897-105 (Table 4). These lines possessed high resistance to stripe rust, moderate plant height, and high tiller and spikelet numbers, and their 1000 grain weights were similar or surpassed the parent lines. These final selected lines have promising application prospects.
Table 4. The results of the evaluation of resistance to stripe rust and agronomic traits and QTL detection of the susceptible control, the parents, and the selected lines.

| Parent/Line                        | Trait | QTL a          |
|-----------------------------------|-------|----------------|
|                                   | IT    | PH | SPN | TLN | TGW |                   |
| Mingxian169/P9897                  | 9     | 120 | 15  | 5   | 38.13 | 0                 |
| Chuanmai 42                        | 7     | 112 | 15  | 4   | 40.47 | 2BS+3BL           |
| Xiangmai 25                        | 6     | 93  | 21  | 4   | 44.37 |                   |
| Zhengmai 9023                      | 7     | 91  | 15  | 6   | 42.37 |                   |
| Chuanmai 42/P9897-2                | 2     | 74  | 21  | 5   | 44.93 | 2BS+3BL+Yr26      |
| Chuanmai 42/P9897-3                | 2     | 81  | 20  | 7   | 45.37 | 2BS+3BL+Yr26      |
| Chuanmai 42/P9897-4                | 2     | 77  | 21  | 7   | 33.67 | 2BS+3BL           |
| Chuanmai 42/P9897-5                | 1     | 80  | 20  | 3   | 34.60 |                   |
| Chuanmai 42/P9897-7                | 2     | 80  | 23  | 6   | 33.37 | 2BS+3BL+Yr26      |
| Chuanmai 42/P9897-9                | 1     | 80  | 21  | 6   | 33.37 | 2BS+3BL+Yr26      |
| Chuanmai 42/P9897-14 c             | 2     | 88  | 19  | 5   | 40.77 | 2BS+3BL+Yr26      |
| Chuanmai 25/P9897-25 c             | 2     | 88  | 18  | 4   | 50.47 | 2BS+3BL           |
| Chuanmai 25/P9897-26 c             | 1     | 90  | 17  | 7   | 42.60 | 2BS+3BL           |
| Chuanmai 25/P9897-27 c             | 2     | 90  | 20  | 5   | 44.43 | 2BS+3BL           |
| Chuanmai 25/P9897-34               | 0     | 117 | 21  | 5   | 41.03 | 2BS+3BL           |
| Chuanmai 25/P9897-39 c             | 1     | 90  | 21  | 4   | 49.27 | 2BS+3BL           |
| Chuanmai 25/P9897-40 c             | 2     | 94  | 21  | 4   | 43.20 | 2BS+3BL           |
| Chuanmai 25/P9897-41               | 2     | 95  | 22  | 3   | 47.80 | 2BS+3BL           |
| Chuanmai 25/P9897-42               | 2     | 95  | 22  | 3   | 36.50 | 2BS+3BL           |
| Chuanmai 25/P9897-56 c             | 2     | 80  | 17  | 4   | 43.03 | 2BS+3BL           |
| Chuanmai 25/P9897-59 c             | 2     | 83  | 21  | 4   | 47.80 | 2BS+3BL           |
| Chuanmai 25/P9897-60 c             | 0     | 80  | 20  | 6   | 42.80 | 2BS+3BL           |
| Chuanmai 25/P9897-62               | 2     | 80  | 18  | 3   | 41.46 | 2BS+3BL           |
| Chuanmai 25/P9897-63               | 2     | 75  | 18  | 3   | 43.48 | 2BS+3BL           |
| Chuanmai 25/P9897-65               | 2     | 70  | 14  | 3   | 38.75 | 2BS+3BL           |
| Chuanmai 25/P9897-78 c             | 2     | 85  | 22  | 4   | 46.27 | 2BS+3BL           |
| Zhengmai 9023/P9897-92 c           | 1     | 95  | 17  | 6   | 51.00 | 2BS+3BL           |
| Zhengmai 9023/P9897-96             | 2     | 90  | 19  | 6   | 39.93 | 2BS+3BL           |
| Zhengmai 9023/P9897-102            | 1     | 86  | 20  | 5   | 39.87 | 2BS+3BL           |
| Zhengmai 9023/P9897-105 c          | 2     | 97  | 17  | 7   | 44.20 | 3BL+3BL           |
| Zhengmai 9023/P9897-107            | 2     | 103 | 19  | 4   | 50.50 | 2BS+3BL           |

IT: Infection type; PH: plant height; SPN: spikelet number, TLN: number of tillers, TGW: 1000-grain weight. a QTL means the lines contain the QYr.nafu-2BL and QYr.nafu-3BS, Yr26I, or all three. b “?” means these varieties contain some unknown resistance genes. c means the eventually-selected lines, which possessed good resistance and excellent agriculture traits.

4. Discussion

In this study, we combined conventional breeding methods with molecular marker validation and the introgression of two QTL from P9897 into three domestic leading varieties: Chuanmai 42, Xiangmai 25, and Zhengmai 9023. After the QTL were detected, we selected 27 lines from the three crosses that showed good stripe rust resistance, and of those, 13 that also possessed excellent agronomic traits. These results illustrate the application value of these germplasm resources.

In the results, some lines that contained only one QTL also showed a high level of resistance, but considering the more durable resistance and wider resistance spectrum, the lines that combined two or some unknown QTL from Chuanmai 42, Xiangmai 25, and Zhengmai 9023 have a higher application value in the long term. According to previous research [53], Chuanmai 42 contains the Yr26 resistance gene, which confers seedling resistance but has been overcome by the CYR34 PST race, which has recently increased in prevalence [54]. Therefore, combining the ASR gene (Yr26) with the APR genes (QYr.nafu-2BL and QYr.nafu-3BS) may form a complementary effect that can widen the resistance spectrum [55] and enhance resistance. Studies focused on Xiangmai 25 and Zhengmai 9023 have not
identified genes for stripe rust resistance; however, they are presumed to contain unknown resistance genes based on field resistance evaluation (Figure 4A). Combining these unknown resistance genes with the two QTL from P9897 can provide a long-lasting and high-level resistance to stripe rust [2]. For example, Xiangmai 25/P9897-34 has a higher resistance (IT = 0, DS = 0%) than the donor parents P9897 (IT = 2, DS = 10%), so we speculate that this line combines the unknown resistance genes from Xiangmai 25 with the two QTL from P9897.

In this study, some lines did not detect resistance QTL of P9897, but their field phenotype still showed resistance or immunity. The first likely reason for this is because the molecular markers applied to MAS should be co-segregated or closely linked to the target trait (1 cM or less) [56], while the molecular markers used in this study had a genetic distance greater than 1 cM. This means the probability of recombination between the marker and the gene increased, so the detection rate was low. Secondly, the population used in this study differed from the population used in the P9897 resistance QTL mapping, so different recombinations occurred between the genes and the molecular markers. As a result, the amount of lines containing QTL that were detected by molecular markers was less than the actual number that were present. Therefore, these resistant offspring lines without detectable target QTL still have further application value.

According to the results of 1000 grain weight analysis of the lines of the three cross combinations, the lines with higher level resistance (IT = 0–2) had a lower mean 1000 grain weight than the lines with lower level resistance (IT = 3–6). These results support the theory that an active immune response results in yield penalties for crops fighting pathogens [57]. Therefore, in the breeding process, it is necessary to consider the disease resistance of the line, and also to screen other agronomic traits in order to select varieties suitable for wide application.

At present, in the study of using marker validation to screen target genes in wheat breeding in order to eliminate undesirable donor traits, many backcrossing and selection steps are required, making the introgression of resistance genes more complicated [58]. However, since the target genes to be introgressed in this study were two QTL located on different chromosomes, we performed a bulk harvest before the F5 generation, retaining all possible genotypes and reducing the loss of ideal genes that were still highly heterozygous [59]. After several generations of accumulation, we used artificial screening to select the F5 generation plants according to phenotypes, such as disease resistance and agronomic traits. By the F6 generation, the traits had stabilized and combined with molecular marker detection, so the selected target lines have promising application prospects. Although F6 was not tested in multiple environments in this study, the agronomic traits and resistance to stripe rust of these cross combinations became stable after the preliminary evaluation and selection of F5. Then, through the screening and evaluation of F6r, the agronomic traits and resistance of the recombinant inbred lines (RILs) were finally screened out to be stable in inheritance and expression, so the results of this experiment were reliable. With the emergence of new races of stripe rust, especially the emergence and prevalence of CYR34, these three elite varieties have gradually lost their resistance to stripe rust, which impacted yield. In this study, two resistance QTL were introgressed into wheat cultivars to enhance their resistance without compromising their excellent agronomic traits, so that they can continue to be used commercially.

5. Conclusions

This study combines conventional breeding with marker validation. Through the evaluation of the field agronomic traits and molecular detection results of resistant QTL, 27 wheat lines with two or more QTL and showing high resistance to wheat stripe rust were successfully selected.

Among the 27 lines selected, a total of 13 lines also possessed several excellent agronomic traits, such as moderate plant height, spikelet number, number of tillers, and 1000 grain weights that were similar to or better than the three leading wheat varieties. This will enrich the genetic resources of wheat stripe rust resistance in China and provide material support and a theoretical basis for the prevention and control of wheat stripe rust in China.
**Supplementary Materials:** The following are available online at [http://www.mdpi.com/2073-4395/10/4/483/s1](http://www.mdpi.com/2073-4395/10/4/483/s1), Figure S1: SSR marker Xgwm120 linked to QYr.nafu-2BL (a). SSR marker Xharc87 linked to QYr.nafu-3BS (b). SSR marker WE-173 linked to Yr26 (c). The PCR products were run in 6% polyacrylamide gels. P: P9897, C: Chuangmai 42, X: Xiangmai 25, Z: Zhengmai 9023.

**Author Contributions:** T.H. detected the QTL, analyzed the data, and prepared the first draft of the manuscript. X.Z. (Xiao Zhong) and Q.Y. (Qiang Yao) contributed to collected samples and phenotype data. X.Z. (Xinli Zhou) and X.L. contributed to the crosses and revised the manuscript. X.Z. (Xiao Zhong), Q.Y. (Qiang Yao), L.H., and S.Y. contributed to the selection of target lines and evaluated the populations. Q.G., X.Z. (Xinli Zhou), and Z.K. suggested the project and generated the final version of the manuscript. All authors provided suggestions during revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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