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Identification of Three Novel QTLs Associated with Yellow Rust Resistance in Wheat (*Triticum aestivum* L.) Anong-179/Khaista-17 F_2 Population

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Abstract: Wheat yellow rust (YR) caused by *Puccinia striiformis* is lethal for the leaf photosynthetic process, which substantially affects yield components and ultimately causes drastic yield reduction. The current study aimed to identify all-stage YR resistance linked QTLs in the best cross-combination. Experimental materials were phenotyped for disease severity in YR-hot spot area at Cereal Crops Research Institute, Pirsabak Pakistan in Khyber Pakhtunkhwa province in 2019 and 2020 and 2020 and 2021 Rabi seasons. The AN179 × KS17 was found to be the best cross combination, which showed high resistance to YR, whereas crosses AN179 × PK15 and PR129 × PK15 demonstrated susceptibility to YR with high disease severity. The recombinant inbred lines (RIL) F_2 wheat population Annong-179/Khaista-17 demonstrated highly desirable YR resistance and yield component traits. Simple sequence repeat (SSR) markers were used to genotype the RIL population and their parents. Three novel QTLs linked to all-stage YR resistance were found on chromosomes 2BS, 3BS and 6BS, which explained 1.24, 0.54, and 0.75 phenotypic variance, respectively. Incorporation of the newly identified novel YR-resistance associated QTLs into hybridization wheat breeding program could be effective for marker-assisted selection of the improved and sustainable resistance.

Keywords: *Puccinia striiformis*; disease severity; linkage analysis; recombinant inbred lines; heritability

1. Introduction

Yellow rust is a deadly wheat (*Triticum aestivum*) crop disease caused by *Puccinia striiformis* that can become epidemic if the conditions are favorable. Yield losses due to yellow rust disease ranged from 10% to 40% and could reach 100% in early infection and favorable environmental conditions [1]. Under favorable climatic conditions, this disease may turn into an epidemic. Rust has been detected in over sixty countries worldwide, spanning all continents [2]. The major wheat-producing countries have experienced severe yellow rust outbreaks, resulting in significant losses [3,4]. The severity of the disease is determined by the stage of infection, pathotypes, and the presence of inherent genetic resistance [5].

Wheat cultivars with long-term resistance are the most cost-effective and environmentally friendly way to combat wheat rusts. The challenge with the cultivar-based resistance is the lack of a durable and specific source of resistance for widespread deployment. The exploration of yellow rust resistance genes is a continuous process because of the changing behavior of rust pathotypes. To date, approximately 80 YR resistance genes have been cataloged in wheat, and about 327 YR QTL have been reported [6–8]. QTL mapping is an efficient method to segment potential quantitative and genetic variation, including yellow rust resistance, hence, detecting the chromosomal regions strongly associated with YR resistance and delimiting the associated linked with markers of interest [3].

Wheat YR genes are expressed at various stages of development. So far, 84 YR resistance genes have been reported and majority of them are all-stage with some exceptions i.e., (Yr11-14), (Yr-16), (Yr-18/Lr-34/Sr-57/Pm-38/Ltn-1), (Yr-29/Lr-46/Sr-58/Pm-39/Ltn-2), (Yr-30/Lr-27/Sr-2), Yr-36, Yr-39, Yr-46/Lr-67/Sr-55/Pm-46/Ltn-3, Yr-52,Yr-59, Yr-62, Yr-68, Yr-71, Yr-75, Yr-77/80, and Yr-82 [4,9–13].

Protection against wheat yellow rust is based on a combination of resistance genes obtained through marker-assisted selection and breeding. Hence, identification of closely linked DNA markers is required for breeding as well as basic research and understanding of the genetic nature of diverse resistance genes. Single nucleotide polymorphism (SNP), restriction-fragment-length-polymorphism (RFLP), and simple-sequence-repeat (SSR) markers are used to map the genome in order to assess the linkage of yellow rust resistance genes. The SSR are the ideal markers for comprehensive linkage mapping due to the advantage of high repeatability, accuracy, polymorphism, chromosome specificness, co-dominance, and easy handling [2]. The current study was undertaken with the objective of identifying QTLs associated with yellow rust resistance using SSR markers.

Materials and Methods.

The current research was conducted at the Cereal Crops Research Institute (CCRI), Pirsabak, Pakistan during 2019 and 2020 and 2020 and 2021. CCRI is located on the left bank of the River Kabul in Khyber Pakhtunkhwa Pakistan, at 32N latitude and 74E longitude, and is 945 feet above mean sea level (MSL). It has a hot, humid climate and is declared a hotspot for wheat yellow rust.

1.1. Breeding Material

Two Chinese viz. Annong-179 (AN179) and Annong-837 (AN837), five CIMMYT viz. PR123, PR125, PR127, PR129, and PR130, two Pakistani viz. PR-126 and PR-128 advance wheat lines were used as a female parent. Five Pakistani wheat cultivars viz. Pirsabak-13 (PS13), Pirsabak-15 (PS15), Pakhtunkhwa-15 (PK15), Khaista-17 (KS17) and waddan-17 (WD17) were used as male testers (Table 1). Experimental seeds were procured from the CCRI-Pakistan, CIMMYT-Mexico, and the Key State Laboratory of the Department of Crop Genetics and Breeding, Anhui Agricultural University Hefei, People's Republic of China.

Table 1. List of wheat breeding material used in the experiment.

Genotypes	Parentage/Pedigree	Source/Origin
Lines		
PR123	ND643/2*WBLL1/4/WHEAR/KUKUNA/3/C80.1/ 3*BATAVIA//2*WBLL1	CIMMYT-Mexico
PR125	KACHU/3/PBW343*2/KUKUNA//PBW343*2/KUKUNA	CIMMYT-Mexico
PR126	SIREN2010/PIRSABAK-04	CCRI, Pakistan

Table 1. *Cont.*

Genotypes	Parentage/Pedigree	Source/Origin
PR127	SOKOLL/3/PASTOR//HXL7573/2*BAU*2/4/PASTOR//MILAN/KAUZ/3/BAV92	CIMMYT-Mexico
PR128	SALEEM-2000/PIRSABAK-05	CCRI, Pakistan
PR129	PASTOR//HXL7573/2*BAU/3/SOKOLL/WBLL1/4/HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07/5/WHEAR/SOKOLL	CIMMYT-Mexico
PR130	MILAN/S87230//BAV92*2/3/AKURI	CIMMYT-Mexico
AN179	JIMAI22/SANYOU2018	AAU Hefei China
AN837	XINONG822///LUO3429//ANNONG0807/YANNONG5	AAU Hefei China
Testers		
Pirsabak-13 (PS13)	CS/TH.SC//3*PVN/3/MIRLO/BUC/4/MILAN/5/	CCRI, Pakistan
Pirsabak-15 (PS15)	MILAN/S87230//BABAX	CCRI, Pakistan
Pakhtunkhwa15 (PK15)	WBLL1*2/4/YACO/PBW65/3/KUAZ*2/TRAP//KAUZ	CCRI, Pakistan
Khaista-17 (KS17)	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES/7/AL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR	CCRI, Pakistan
Wadaan-17 (WD17)	YAV79//DACK/RABI/3/SNIPE/4/AE. SQUARROSA	CCRI, Pakistan

Key: CIMMYT = Centro Internacional de Mejoramiento de Maíz y Trigo (International Maize and Wheat Improvement Center, Mexico D.F., Mexico); AAU = Anhui Agricultural University; CCRI = Cereal Crops Research Institute.

1.2. Crossing Block Design and Development of F_1 Hybrids

For making crosses via line × tester matting format, the experimental lines and testers were sown in November and December, 2019 at the CCRI in two crossing blocks, with two rows per genotype, at 10 days gap for optimum pollen availability. The row length was maintained at 2 m, with 10 cm plant-plant distance. The second crossing block was sown a little bit late, in December, in order to protect seedlings from frost injury and speedy growth, proper plastic tunnels were made for safety and synchronization of the diverse breeding material. To obtain quantity of F_1 hybrid seeds, 20 to 30 best spikes from each line were correctly emasculated and then pollinated by a specified tester in each cross combination. Each of the abovementioned female lines was crossed with all the five male testers. The set seeds from individually pollinated spikes were threshed and packed in separate seed envelopes. Emamectin was applied in field crossing block to avoid post-harvest seed larvae infestations (Figure S1).

1.3. Yellow Rust Inoculation and Disease Resistance Categorization

Fresh yellow rust inoculum was acquired from the Cereal Diseases Research Institute (CDRI) Murree, Pakistan. Inoculation of the parental material with mixed race yellow rust spores was done by reconstitution of spores with talcum powder in 1:100, using a turbo air sprayer (Figure S3). Tween-20 was added to the spore mixture to ensure spore germination and infiltration of the fungus-hyphae. The target wheat lines in the field were categorized as, Resistant (R), Moderately Resistant (M.R), Moderately Susceptible (M.S), and Susceptible (S) (Figure S3). The rust severity was determined using a modified Cobb scale [14], which was used to visually evaluate the amount of leaf area infected by yellow rust (1 to 100 percent). The host's response was detected and recorded by disease reaction and explained as follows.

Immune, no infection.

R: Resistant, having observable necrotic spots or no pustules.
MR: Moderately resistant, having tiny pustules with necrotic spots.

MS: Moderately susceptible, intermediate pustules, zero necrosis, mild chlorosis.
S: Susceptible; bulky pustules, having no necrosis or chlorosis.

Coefficient of infection (CI) was obtained by multiplying disease severity and host response using protocol of Pathan and Park [15]. Coefficient of infection was calculated by crossing the infection severity value by its host reaction response. Like, for S: 1.00, MS: 0.8, M: 0.6, MR: 0.4 and R: 0.1.

$$\text{Coefficient of infection} = \text{Disease severity} \times \text{infection type.}$$

1.4. Generation's Advancement

To develop segregating F_2 population, F_1 hybrid seeds were grown under the Shuttle Wheat Breeding Program (SWBP) at the Summer Agriculture Research Station (SARS) Kaghan, Pakistan, in June–October, 2019. Kaghan is a mountainous valley located at 34°50′ N 73°31′ E with an elevation of 650 m (2134 ft) above sea level. This shuttle breeding station is established for wheat generation advancement due to its suitable climate for wheat during summer. The average summer temperature ranges from 20 °C to 26 °C with an average humidity of 59%. To ensure satisfactory germination of F_1 hybrid seed, the seeds were sown in two-meter rows, with a row-to-row distance of 30 cm. Shallow sowing was done with the help of a dibbler. Spaced plantation was done with a plant-to-plant distance of 10 cm (Figure S2).

1.5. Phenotyping of Parents and F_2 Population for YR

For plant phenotyping against yellow rust, morphological and yield traits, parental lines, and F_2 population were grown at the CCRI, Pirsabak, in November, 2019. A randomized complete block design in triplicate, with a net experimental block size of 4.5 m^2 (13 × 5 m × 0.30 m) was used. For YR disease scoring, the experimental materials were inoculated with yellow rust spores, as described above. Morocco, the universally susceptible wheat line, was planted around the trial as a disease spreader.

1.6. Standard Cultural Practices

The experiment followed standard agronomic and cultural practices, such as sowing with a dibbler in a 10 cm space plantation at a rate of single seeds per hill. Nitrogen (80 kg ha^{-1}), phosphorus (58 kg ha^{-1}), potash (63 kg ha^{-1}), zinc (33 percent) 15 kg ha^{-1}, and boron (17 percent) 7.5 kg ha^{-1} were applied at the time of sowing, except for nitrogen fertilizer, which was applied in four splits. With the first irrigation, zinc and boron were applied.

1.7. Leaf Sampling, DNA Extraction, and Genotyping

The recombinant inbred lines (RIL) population of Annong-179 (AN179) and Khaista-17 (KS17) were selected for further molecular assessment. Fresh leaf samples were taken and ground in liquid N2, and genomic DNA was extracted with cetyl-trimethyl ammonium-bromide [16].

1.8. Primer Selection, PCR Cycle, and SDS-PAGE Electrophoresis

Initially, the parental lines were tested for polymorphism using a core set of 1500 pairs of SSR primers (http://wheat.pw.usda.gov; accessed on 15 September 2019). After that, all populations were genotyped using primer pairs showing polymorphisms between the two parents of F_2 population. In a MJ Research (PTCe200) thermocycler, PCR reactions were run at a volume of 25 µL. A 25-µL reaction mixture including 1X PCR buffer, 200 nM of each primer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 1 unit of Taq polymerase (Thermo-Fischer Scientific Waltham USA), and 60 ng of template DNA was used to perform PCR amplifications. The following was the amplification profile: Step1: 3 min denaturation at 94 °C, Step 2: 38 cycles of denaturation at 94 °C for 45 s, annealing at 50–60 °C for 45 s (depending on primers), extension for 1 min at 72 °C, and a final extension for 10 min at 72 °C. To visualize the PCR products, 2 µL loading buffer was added to each sample and denatured for

10 min at 95 °C and chilled on ice. A 6% polyacrylamide gel was prepared (19:1; acrylamide: bisacrylamide), which contained 8M Urea in 1× TBE [90 mM Tris-Borate (PH 8.3), 2 mM EDTA]. 4 to 6 µL DNA samples (depending on well size) were loaded in individual wells. Then, for nearly 1 h and 20 min, a PAGE gel was resolved at 55 W in a Bio-Rad Mini-PROTEAN Tetra Cell assembly (Bio-Rad, Hercules, CA, USA). The resolved gel was stained with silver stain and then visualized under UV light in a gel-doc system [10].

1.9. Genetic Map Construction and QTLs Analysis

The Join Map 4.0 V2.5 was used to perform primary linkage map analysis utilizing composite interval mapping (CIM) with both backward and forward regressions set to 0.1 [17,18]. The presence of significant QTL was determined using a threshold logarithm of odds (LOD) score of 2.50.

1.10. Statistical Analysis

Basic and advanced statistical analysis were performed through MicroSoft Excel and IBM SPSS statistical software packages. Data on disease scoring, morphological, and yield components traits were subjected to statistical tests for calculating population means and standard error. All the data across genotypes were analyzed for significant variations using post-hoc Tukey's honestly significant differences (HSD) test at 95% confidence level [19]. Data were presented as bar graphs using MS Excel program.

2. Results

2.1. Primary Linkage Mapping

A linkage map of the Annong-179/Khaista-17 RIL population was generated to identify the YR-resistance QTL locus on chromosomes 2B, 3B, and 6B. A total of 150 F_2 segregating plants were genotyped using 22, 27, and 21 SSR markers on chromosomes 2BS, 6BS, and 3BS, respectively. The primary linkage analysis revealed three novel QTLs related with YR-resistance, one each on chromosomes 2BS, 3BS, and 6BS. QTL on chromosome 2BS was mapped at the marker interval X2cd18-Xmwg546 (Figure 1). The LOD score 4.51 was recorded for the flanking markers X2cd18 and Xmwg546, each of which accounts for 1.24 of the phenotypic variation (Table 2).

Similarly, a novel QTL was detected on 3BS with a marker interval of X12rc73-Swm13 (Figure 1). The LOD score 5.64 was recorded for the flanking markers X12rc73 and Swm13, accounting for 0.54 of the phenotypic variation (Table 2). Likewise, a novel QTL associated to YR-resistance was detected on 6BS with a marker interval of IW21254-IW24290 (Figure 1). The flanking markers IW21254 and IW24290 had an LOD score of 2.50, with each accounting for 0.75 of the phenotypic variation.

Table 2. Chromosome number, markers, LOD score, PVE percentage with additive and dominant effect in wheat F_2 population.

Chromosome	Markers Interval	[1] LOD	[2] PVE %	Additive Effects	Dominant Effects
2BS	X2cd18-Xmwg546	4.51	1.24	−17.45	−15.93
3BS	X12rc73-Swm13	5.64	0.54	−15.64	−23.40
6BS	IW21254-IW24290	2.50	0.75	−18.31	−18.04

[1] Logarithm of odds score. [2] Percentages of phenotypic variance explained by the QTL.

2.2. Genetic Variability and Heritability Analysis

Analysis of variance revealed that genotypes differed significantly in yield and its components (Table 3). For all yield traits, there were significant phenotypic differences between parents. A highly significant variation ($p < 0.01$) was recorded for all the phenotypic parameters. Except for grain spike^{-1} ($p < 0.05$), the testers showed significant differences in other attributes. A similar tendency of variation for studied parameters was observed among crosses. Moreover, peduncle length (0.87) had high broad-sense heredity, whereas grain yield (0.75), spike length (0.74), and grains spike-1 (0.71) had moderate heritability; however, yellow rust coefficient of infection had a low inheritance magnitude (0.1) (Table 3).

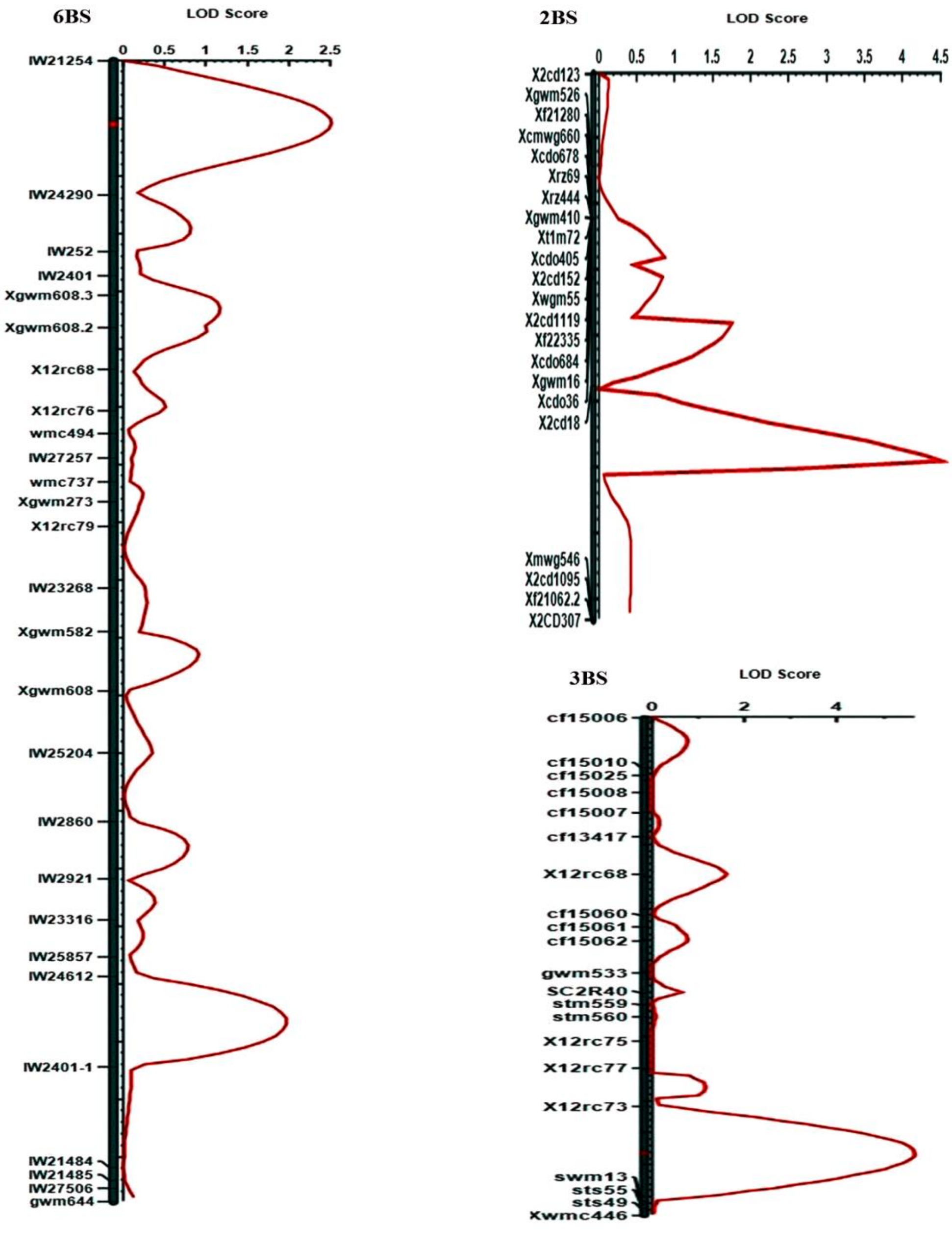

Figure 1. Linkage map of Chromosomes 2BS, 3BS, and 6BS of the hexaploid wheat. Strength of linkage is depicted by intensity of the peaks.

Table 3. Analysis of variance for yellow rust coefficient of infection (CI), spike length, peduncle length, grain spike^{-1}, and 1000 grain weight in parent and F_2 population.

SOV	DF	YR (CI)	Spike Length	Peduncle Length	Grains Spike^{-1}	1000 Grain Weight	Grain Yield Plant^{-1}
Rep	2	29.90 ^{ns}	1.62 ^{ns}	7.80 ^{ns}	57.2 ^{ns}	11.45 ^{ns}	1252.59 ^{ns}
Genotype	58	359.72 **	2.36 **	64.02 **	137.9 **	83.12 **	160.05 **
Parents	13	453.16 **	5.01 **	96.54 **	278.12 **	126.63 **	257.15 **
Crosses	44	330.72 **	1.25 **	51.54 **	96.46 **	68.42 **	105.24 **
Lines (L)	8	212.8 *	4.5 *	111.3 *	219.0 *	126.7 *	45.42 **
Tester (T)	4	1817.8 **	2.4 **	168.9 **	178.0 *	57.3 **	84.3 **
L x T	32	174.32 ^{ns}	0.29 **	21.94 **	55.61 **	55.24 **	200.4 *
Error	116	26.10	0.12	2.18	18.47	10.78	102.70
h^2	–	0.10	0.74	0.87	0.72	0.70	0.75

** Significant at 1% probability level; * Significant at 5% probability level; ^{ns} Non-significant.

2.3. Association of Yellow Rust Infection with Yield Traits

Regression analysis revealed a negative relationship for yellow rust coefficient of infection (CI) for all studied traits (Figure 2). A downward trend between yellow rust infection and other yield traits, i.e., spike length, peduncle length, grains spike^{-1}, grain weight, were observed (Figure 2A).

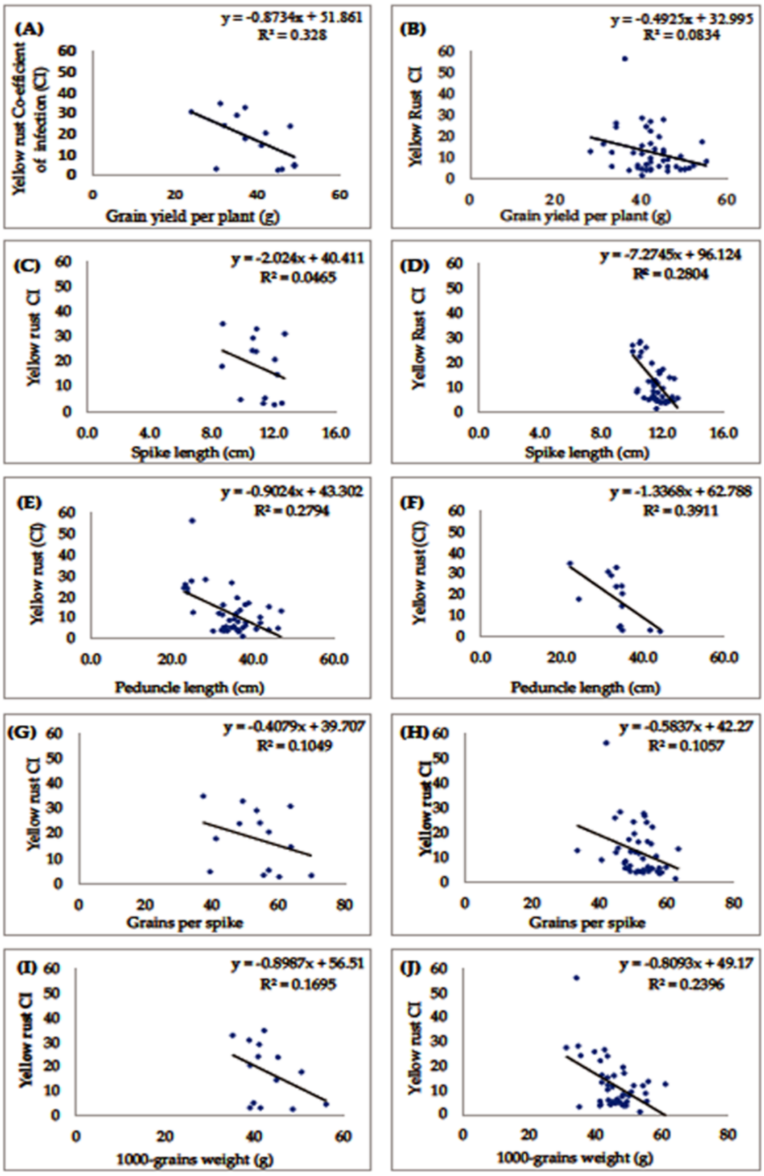

Figure 2. (**A**–**J**). Graphical representation of regression analysis of yellow rust coefficient of infection (CI) with different yield component traits, i.e., grain yield per plant (**A**,**B**), spike length (**C**,**D**), peduncle length (**E**,**F**), grains per spike (**G**,**H**), and 1000-grains weight (**I**,**J**). Graphs in left panel represent data from parents and in right panel from F_2 wheat population.

2.4. Yellow Rust Disease Severity

A total of 59 test genotypes (including 14 parents and 45 F_2 crosses) were evaluated through triplicated trials, and the data were taken for yellow rust final disease severity (FDS), 1000-grain weight, grain yield, peduncle length, spike length, and number of grains per spike. In parents, the final disease severity ranged from 3.8% to 33.0% and 5.5% to 56.3% in the F_2 crosses population (Figure 3). KS17, WD17, P125, P127, and PR128 were recorded as a resistant parent with <10% FDS. A total of 24 crosses were considered resistant with FDS values <10% among which cross PR128 × PS15, PR126 × WD17, and AN179 × KS17 were highly resistant crosses with only <5% FDS. Maximum FDS were observed for the cross AN179 × PK15 (56.3%).

The YR-resistant genotypes demonstrated the least visible YR pustule symptoms in cross combinations. Longer peduncle lengths (PDL) with better resistance to yellow rust infection were observed in the parents WD17 (41.8 ± 1.4 cm) and KS17 (44.3 ± 0.5 cm) and other resistant parents with an average PDL of 37.9 ± 1.2 cm. The resistant crosses also showed a higher mean value (36.3 ± 0.5 cm) for PDL with the longest PDLs recorded for P127 × WD17 (41.8 ± 0.3 cm), PR128 × PS13 (43.9 ± 1.3 cm), PR128 × PK15 (46.9 ± 0.5 cm), PR128 × WD17 (46.1 ± 0.4 cm), P130 × WD17 (41.2 ± 0.7 cm), and AN837 × WD17 (40.8 ± 0.4 cm) (Figure 3).

In comparison, YR-susceptible parent AN179 (22.0 ± 2.1 cm), P130 (33.5 ± 1.5 cm), and PK15 (31.5 ± 1.3 cm) showed significantly shorter PDL with an average length of 29.2 ± 2.0 cm. The crosses showing sensitivity and moderate sensitivity to YR also developed shorter PDL (24.7 ± 1.5 and 26.4 ± 1.0 cm, respectively) compared to the resistant crosses. Spike length was not affected by disease severity in parental genotypes. However, in crosses, the moderately sensitive and sensitive genotypes showed a significantly shorter spike length (10.8 ± 0.3 cm) compared to the resistant genotypes (11.8 ± 0.5 cm).

For number of grains per spike, it was observed that resistant parents PR128 (70.0 ± 4.6 grains) and KS17 (60.3 ± 1.4 grains), as well as the resistant crosses PR128 × PS15 (62.7 ± 0.7 grains), PR123 × PS15 (59.9 ± 1.2 grains), PR126 × PS15 (58.6 ± 2.6), PR125 × PS15 (54.7 ± 1.3 grains), PR126 × WD17 (57.9 ± 1.4 grains), AN837 × PS15 (54.1 ± 8.1 grains) and AN837 × WD17 (53.2 ± 3.2 grains) showed a higher number of grains per spike with average values of 56.5 ± 2.9 grains for parents and 52.3 ± 0.7 grains for crosses (Figure. 3). On the other hand, YR-sensitive parent and crosses with high disease severity showed a smaller number of grains per spike (49.1 ± 3.6 and 42.1 ± 2.1 grains, respectively) compared to the resistant genotypes. Grain yield per spike as well as grain yield per plant did not show variation for different disease severity classes.

Maximum 1000-grain weight was recorded in parental genotypes PR125 (56.1 ± 4.5 g) and AN837 (51.0 ± 5.6 g), which were categorized as YR-resistant genotypes. The YR-sensitive genotypes PR130 (35.1 ± 1.7 g) and PK15 (38.8 ± 0.7 g) showed the least 1000-grain weight. It was depicted from the results that parental lines like, PR125, AN837, and KS17 (48.6 g), and crosses viz. PR127 × PK15 (55.9 ± 2.1 g), PR128 × PS15 (53.4 ± 2.5 g), PR125 × WD17 (49.5 ± 0.8 g), PR126 × KS17 (48.6 ± 6.9 g), PR129 × WD17 (55.5 ± 2.2 g), AN179 × KS17 (48.8 ± 2.8 g) had more grains weight and better resistance capability against yellow rust. In contrast, parent PR130 and crosses viz. PR123 × PS13 (35.5 ± 0.7 g), PR123 × PK15 (31.2 ± 0.9 g), PR129 × PK15 (34.8 ± 1.0 g), and AN179 × PK15 (34.3 ± 1.5 g) had the least 1000 grain weight and highest disease severity compared to resistance lines.

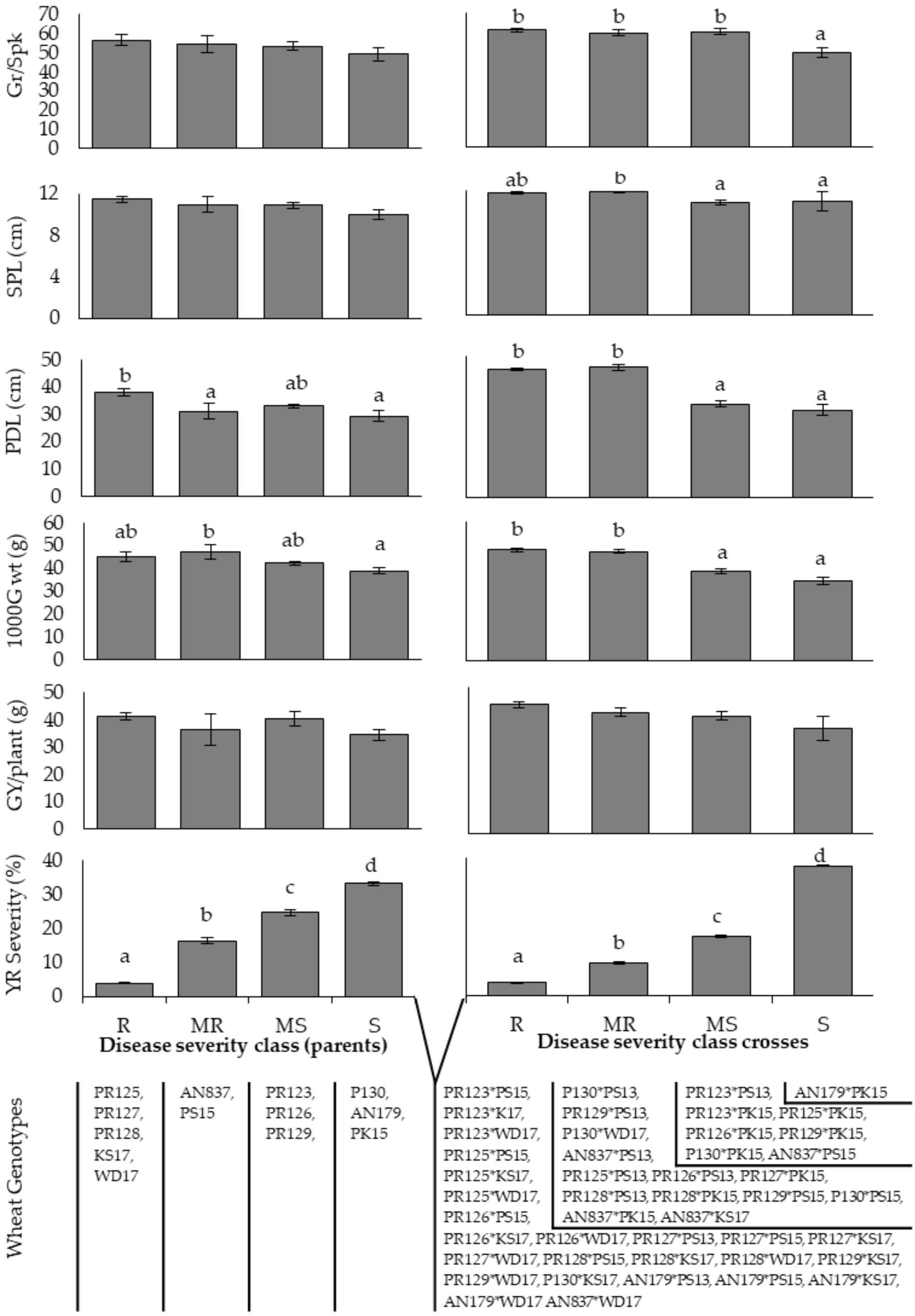

Figure 3. Effect of yellow rust disease severity (R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible) on different morphological and yield component traits. Bar graphs represent mean values of percent disease severity (YR severity), grains yield per plant (GY/plant), 1000-grains weight (1000 G wt), peduncle length (PDL), spike length (SPL), and number of grains per spike (Gr/Spk). Respective genotypes are grouped based on disease severity classes along the *X*-axis. Data on parental (left panel) and F_2 cross genotypes (right panel) are mentioned. Error bars represent standard error. Letters on bars represent differences within an individual graph (Post-hoc Tukey's HSD test at $p = 0.05$).

3. Discussion

3.1. Choosing the Parental Genotypes

The wheat germplasms (genotypes) used in the current research included advanced lines and cultivars from Pakistan, China, and CIMMYT (Mexico). The test germplasms were chosen based on different disease resistance, yield and/or morphological parameters assessed in advance trials conducted by the CCRI Wheat Breeding Program in three to five locations across the Khyber Pakhtunkhwa province. For example, parental genotypes PR125, PR127, PR128, KS17, and WD17 were primarily selected due to YR resistance in prescreen trials. Other parents, including AN837, PS15, PR123, PR126, PR129, PR130, AN179, and PK15, were chosen for their better yield performance, with compact spikes and attractive plant appearance.

3.2. QTLs Associated with Yellow Rust

Understanding the genetic diversity and population structure are primary requirements for the initiation and utilization of plant genetic resources in breeding programs [20]. Previous research has also found Yr genes/QTLs on nearly all wheat chromosomes, indicating that both major and minor genes are involved in conferring resistance to yellow rust pathogens [21–23]. Yellow rust resistance QTLs were identified on 1B, 2A, 2B, 3B, 3D, 5A, 5B, 6D, and 7A [21]. Yellow rust resistance QTLs were identified on 1B, 2A, 2B, 2D, 5B, and 7B [22], whereas 12 QTLs were identified on the long arms of 1B, 3D, 5A, 5B, and 7B and the short arms of chromosomes 1A, 5A, 6A, 6B, and 7A [23].

Based on a high probability of finding QTLs associated with yellow rust resistance, this study was undertaken to screen a recombinant inbred line AN179 × KS17. In a field screening, the parental line KS17 showed resistance to yellow rust, while the parental line AN179 showed sensitivity to yellow rust at both seedling and adult plant stages. Population originating from the cross AN179 × KS17 showed promising disease resistance, morphological and yield parameters. Subsequent genetic analysis of the DNA samples from AN179 × KS17 revealed the presence of three novel QTLs on chromosomes 2BS, 3BS, and 6BS, associated with disease severity.

One of the identified QTLs was on chromosome 2BS, which is flanked by SSR markers X2cd18 and Xmwg546, and accounted only for 1.24% of the phenotypic variation for disease severity. Several QTLs have previously been identified on the long arm of wheat chromosome 2B, e.g., QTL was detected on the 2B, Qyr.stripe-2BL.2 close proximity to the SSR marker Xwmc361 that is close to the yellow rust resistance QTL Naxos (QYr.cass-2BL) [24]. A recent study has also reported two QTLs on the 2BL (QYr.uaf.2BL.1 and QYr.uaf.2BL.2) spanning the genomic region containing three important genes Yr5, Yr43, and Yr54 [25]. In TAM111, QYr.tam-2BL was discovered on the long arm of chromosome 2B, flanked by *wPt6242* and *wPt6471* and it showed PVEs of 13–63 percent in adult plants and 40.5 percent in seedling plants for stripe rust, respectively. Camp Remy [5,26] discovered *QYr.inra*-2B.1 on the centromere of chromosome 2B, which explained PVEs of 42–61 percent in adult plants.

In consistence with a previous study [5] which found a minor-effect QTL, i.e., QYrhm.nwafu-3BS, located on the chromosomal arm 3BS, near the SSR marker Xbarc87. The current study also detected a QTL on chromosome 3BS with a marker interval of X12rc73-Swm13 (Figure 1). The identified QTL on 3BS accounts only for a minute (PVE = 0.54) fraction of the disease phenotype variation. Many QTLs or genes (Yr30, Yr57, Yrns-B1, and QYr.uga-3BS) have been identified on chromosome 3BS in previous investigations, and various loci have been identified as Yr30 in different wheat varieties [27–30]. In the terminal region of chromosome 3BS, the APR gene Yr30/Sr2/Lr27 with a morphological marker for pseudo-black chaff was discovered. Yrns-B1, a key APR gene derived from Lgst.79–74, is found near the SSR marker Xgwm493 [31]. A QTL identified on chromosomes 6BS flanking by IW21254 and IW24290 accounted for 0.75% of yellow rust resistance. Dong and colleagues [32] mapped the wheat F2 population and found a QTL for YR resistance, QYr.ucw-6B, on chromosomal arm 6BS, between flanking markers IWA7257 and wmc737/IWA4408.

In addition to the QTLs on 2B and 3B, yet another QTL associated to yellow rust disease resistance was detected on 6BS with a marker interval of IW21254-IW24290, which accounts for 0.75 of the phenotypic variation for disease severity. Previously, QTL only on the chromosome 6BS, Qyr.stripe-6BL.2 at 408 Mb was found in close proximity to the stripe rust resistance associated SSR markers Xwmc397 and Xwmc105b [33]. However, no QTL has so far been detected on 6BS. This study recommends further screening of the chromosomal region containing the YR resistance QTLs reported in the current study with an aim for an in-depth understanding and detailed functional analysis of these QTLs' association with yellow rust resistance.

3.3. Variability and Heritability

Most wheat breeding initiatives aim to boost grain yield by introducing diversity into the existing germplasm. In order to choose the ideal parents, it is paramount to identify superior crosses with the best yield and related traits [34]. However, it is important to understand that yield is a quantitative parameter and is impacted by several variables. El-Murshedy and colleagues found strong genetic variability for grain yield and yield component traits studied [35]. Selection for grain yield plant^{-1} in the F_2 population would be highly effective, as genetic variability is reduced after the second cycle of selection, with about 50% of F_2 crosses manifesting better performance for grain yield. The current study found significant and high genetic variability for yellow rust resistance and grain yield both in parent and the cross combinations. Because of the diversity in parental genotypes, such a large variation was expected. Our findings are consistent with other studies [36], which found sufficient genetic diversity for yellow rust infection in spring wheat genotypes and 24 $F_{1:2}$ populations. The high broad-sense heritability estimates for peduncle length, followed by grain yield, indicated that selection among recombinants could improve this essential characteristic [34]. Although heritability estimates for peduncle length and grain yield are rather high in this study, genotypic differences in grain yield plant^{-1} are challenging to interpret due to the polygenic nature of yield. Wheat scientists should, therefore, test wheat genotypes YR resistance as well as genotypic variance yield attributes in the controlled conditions to reduce the impact of environment for a more robust interpretation of YR impact on yield.

3.4. Association of Yellow Rust Infection with Yield Traits

Generally, a negative relationship between yellow rust infection and yield parameters was seen, indicating that wheat genotypes were impacted by the YR infection strong negative association of YR infection with grain yield has been previously observed [8]. However, a weak association of YR with grain yield was demonstrated in the current yellow rust assessment trials. This anomaly may be explained by the late onset of disease, which was restricted to smaller areas around the spore landing sites. However, 1000 grains weight, peduncle length, and spike length showed a fair negative correlation with YR, which may have resulted from a decline in photosynthetic capacity due to YR on leaves, leading to incomplete grain filling. It is very important to comprehend that yellow rust resistance breeding targets must adapt to deal with the rapidly changing pathotypes threat and that sources of genetic resistance are constantly required for the development of enhanced wheat cultivars. Wheat genotypes with desirable characteristics could be used in wheat hybridization programs to improve yellow rust resistance.

3.5. Disease Severity and Impact on Plant Traits

Under the current climatic change scenario, yellow rust is a prevalent fungal disease and a constant threat to the wheat crop, which results in substantial grain yield losses [37]. Yellow rust not only affects the grain spike^{-1} and grain weight but causes considerable yield losses due to undersized shriveled grain [38]. The field performance of fifty-nine wheat genotypes, including 14 parental lines and 45 F_2 crosses, was assessed for yellow rust disease severity and its effect on the yield component traits. Among the parents, KS17, WD17, PR128, and PR126 were the most promising wheat genotypes for yield and low YR infection, while, among the crosses, PR123 × PS15, PR125 × KS17, PR126 × WD17, PR127 × PS15, AN179 × KS17, AN179 × WD17, and AN837 × WD17 were top ranked with low YR infection rate and better grain yield. Wheat parent line AN179 had the worst genotype (with an ACI of 35), followed by PK15 (with an ACI of 31), which may carry YR resistance genes, now overwhelmed by YR pathogen (*P. striiformis)*. The current study's findings are consistent with those obtained previously by Chen and colleagues, who reported that YR major gene expression can be assessed in field via disease scoring, using final disease severity (FDS) as a tool in segregating wheat lines for identifying slow rusting phenomena (APR) for durable resistance [39]. On the whole, the parental genotypes PR129, PR130, AN179, and PK15 underperformed, showing high YR susceptibility, shorter peduncle, spike, and low grain weight. On the contrary, KS-17 had an overall better average performance, with low ACI, FDS, higher grain weight, and grain yield per plant, as well as longer peduncle and spikes. The parental genotypes, i.e., AN179 and PK15, as well as the resultant cross combination AN179 × PK15, underperformed showing high YR infection rate, lower 1000 grains weight, grain yield per plant, whereas shorter peduncle and spikes. Cross combinations, such as AN179 × PK15 should be discouraged in selection from among the transgressive segregants in subsequent generations [40]. In contrast AN179 × PK15, among the tested germplasm, the cross combination AN179 x KS17 showed better genetic performance, with healthy grains, better grain yield, longer peduncle for photosynthate and spikes and slow rust ability is highly recommended for selection from the recombinants. Longer peduncle lengths expose flag leaf to more light and thus higher photosynthetic ability, whereas longer spikes allow more grains setting and thus should contribute to higher yield.

4. Conclusions

The current study successfully identified three novel YR-resistance associated QTLs on chromosomes 2BS, 3BS, and 6BS of the hexaploid wheat cross AN179 × KS17. These novel QTLs could be employed in wheat hybridization program to aid in marker-assisted selection and pyramiding of all-stage YR resistance genes, for improving yellow rust resistance. It is recommended to screen for the presence of these QTL in more populations under diverse environments. An overall fair negative correlation between the YR and yield component traits was observed, with the most noticeable changes observed in the peduncle length, spike length and grains per spike. The application of both classical genetic and genomics approaches, disease assessment, and pathogen monitoring should provide resources, which may help in boosting the genetics for yellow rust resistance. The classical and molecular breeding techniques will benefit from the current discovery, and incorporation of these QTLs via wheat breeding should render resistance against YR, thus helping to protect future wheat from this potentially devastating disease.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14127454/s1, Figure S1: Pictorial glimpse of the wheat crossing block (Photo: Saeed, M.).; Figure S2; Pictorial glimpse of generation advancement (Photo: Saeed, M.) Figure S3: Infection types produced by AN179 and KS17 when inoculated with mixed YR-spores at the seedling stage (Photo: Saeed, M).

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