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Development and characterization of a novel wheat-rye T2DS·2DL-2RL translocation line with high stripe rust resistance

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Abstract

Rye (*Secale cereale* L.), a close relative of common wheat, represents a valuable genetic resource for enhancing the disease resistance of common wheat. Introducing novel rye-derived genes into wheat can potentially improve disease resistance. In this study, we successfully developed a novel wheat-rye derivative line LCR4 through hybridization between hexaploid triticale line Currency and common wheat cultivar Jimai 22 (JM22). We confirmed that LCR4 was a T2DS·2DL-2RL translocation line via comprehensive molecular cytogenetic analyses, including genomic in situ hybridization, multi-color fluorescence in situ hybridization, molecular marker analysis, and wheat SNP-arrays genotyping. Notably, upon inoculation with *Puccinia striiformis* f. sp. *tritici* (*Pst*) race V26 at the seedling stage and mixed *Pst* races at the adult stage, LCR4 exhibited robust resistance against stripe rust infection at both stages. Subsequent genetic analysis further elucidated that the translocated 2RL chromosome segment is responsible for this resistance. Consequently, LCR4 harboring elite agronomic traits can be effectively employed in breeding programs against stripe rust.

Keywords Common wheat, Triticale, Translocation line, Stripe rust, Agronomic performance

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Background

Common wheat (*Triticum aestivum* L., 2n = 6× = 42, AABBDD) is a staple crop that ensures food security worldwide. With the continuously growing population, achieving higher crop yields has become increasingly imperative, and a minimum growth rate of approximately 70% is required to meet future demands by 2050 (Hickey et al. 2019). However, wheat breeding involves intricate trait exchange, genetic recombination, and harmonious integration of parental traits/genes (Whitford et al. 2013). Notably, many elite genes cannot be directly used in wheat production due to the linkage drags, particularly when alien genes are involved. Furthermore, the challenge lies in reconciling antagonistic interactions between the elite genes; for instance, high yield and resistance traits frequently do not coexist effectively (Singh et al. 2016; Li et al. 2022; Qian et al. 2024). Therefore, it holds immense value to develop and explore wheat germplasms that facilitate collaborative improvement in disease resistance and high yield potential.

Climate change facilitates the emergence of new pathogen virulence, resulting in the loss of utility for certain resistance genes (An et al. 2024; Jin et al. 2024). This can lead to substantially reduced yield and pose a threat to global food security (Chen et al. 2005; Wu et al. 2022; Wang et al. 2023). Among wheat diseases, stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), can remarkably reduce yield and endanger the safety of wheat production. Stripe rust has been reported in over 60 countries worldwide, causing yield losses of up to 50% in susceptible cultivars (Zhao et al. 2023). The main measures to control this disease include cultivation management, chemical prevention, and host resistance. Host resistance is undoubtedly the most preferred measure due to its high-efficiency, low-cost, and environmental-friendly characteristics.

Up to now, 86 formally named stripe rust resistance (*Yr*) genes (*Yr1*–*Yr86*) have been identified, together with many other temporary names or designated genes or quantitative trait loci (Zhu et al. 2023). However, most of the *Yr* genes are race-specific and easy to be defeated by the pathogen due to the co-evolution between the host and the pathogen. Therefore, it is urgent to identify more *Yr* genes, especially from the wheat relatives, and introduce the alien chromosome segments carrying the resistance genes into the wheat genome to develop disease-resistant germplasm resources for controlling wheat stripe rust (Li et al. 2020). Rye (*Secale cereale* L.), a close relative of common wheat possessing numerous valuable traits, has been extensively utilized to enhance various resistances and tolerances of wheat against drought, saline-alkali conditions, cold stress, and diseases. Extensive research efforts have focused on broadening the genetic basis by mining useful genes from rye for the establishment of innovative breeding strategies (Lukaszewski et al. 2015; Li et al. 2021; Rabanus-Wallace et al. 2021; Han et al. 2024a, b).

A convenient and clear characterization is the prerequisite when the alien chromosome segments are transferred into wheat backgrounds. After years of development, researchers have established powerful tools, such as genomic in situ hybridization (GISH), fluorescence in situ hybridization (FISH), and specific molecular marker technologies, for detecting and determining the identity of alien chromatin in wheat backgrounds (An et al. 2019). These technologies have been successfully applied to characterize a large number of wheat-alien translocation, substitution, and addition lines (An et al. 2013, 2015, 2019, 2022; Ye et al. 2015; Rahmatov et al. 2016). Besides, high-accuracy rye-specific markers were developed to accurately detect and transfer its effective genes in breeding practices (Han et al. 2020a).

In this study, we aimed to develop elite wheat-rye derivatives exhibiting high resistance against stripe rust while maintaining elite agronomic and yield performances. Consequently, we generated a wheat-rye translocation line LCR4 through cross and backcross between the hexaploid triticale line Currency and the wheat cultivar Jimai 22 (JM22). The subsequent tasks encompassed: (1) analyzing the chromosome composition of LCR4 using comprehensive molecular cytogenetic and high throughput genotyping techniques; (2) evaluating stripe rust resistance and identifying the source of this resistance; (3) assessing agronomic and yield traits of LCR4. The novel derivative line could serve as a valuable germplasm resource for wheat disease resistance breeding.

Results

Cytological analysis of LCR4

GISH and FISH were performed to determine the chromosomal composition of LCR4. GISH results showed that LCR4 contained 42 wheat chromosomes, of which a pair of wheat chromosomes showed a bright-green signal in the terminal regions (Fig. 1a, c), indicating that these regions were introduced from the rye chromosomes. After GISH analysis, the same metaphase cell was subjected to FISH analysis using eight probes. All the wheat chromosomes were distinguished. The translocated wheat chromosomes were identified as 2D that were translocated by rye chromosome segments in the terminal regions in the long arms (Fig. 1b, d).

Molecular marker analysis of LCR4

After marker screening, the newly developed marker GW02, located on chromosome arm 2RL, can amplify the targeted fragments of approximately 262 bp in

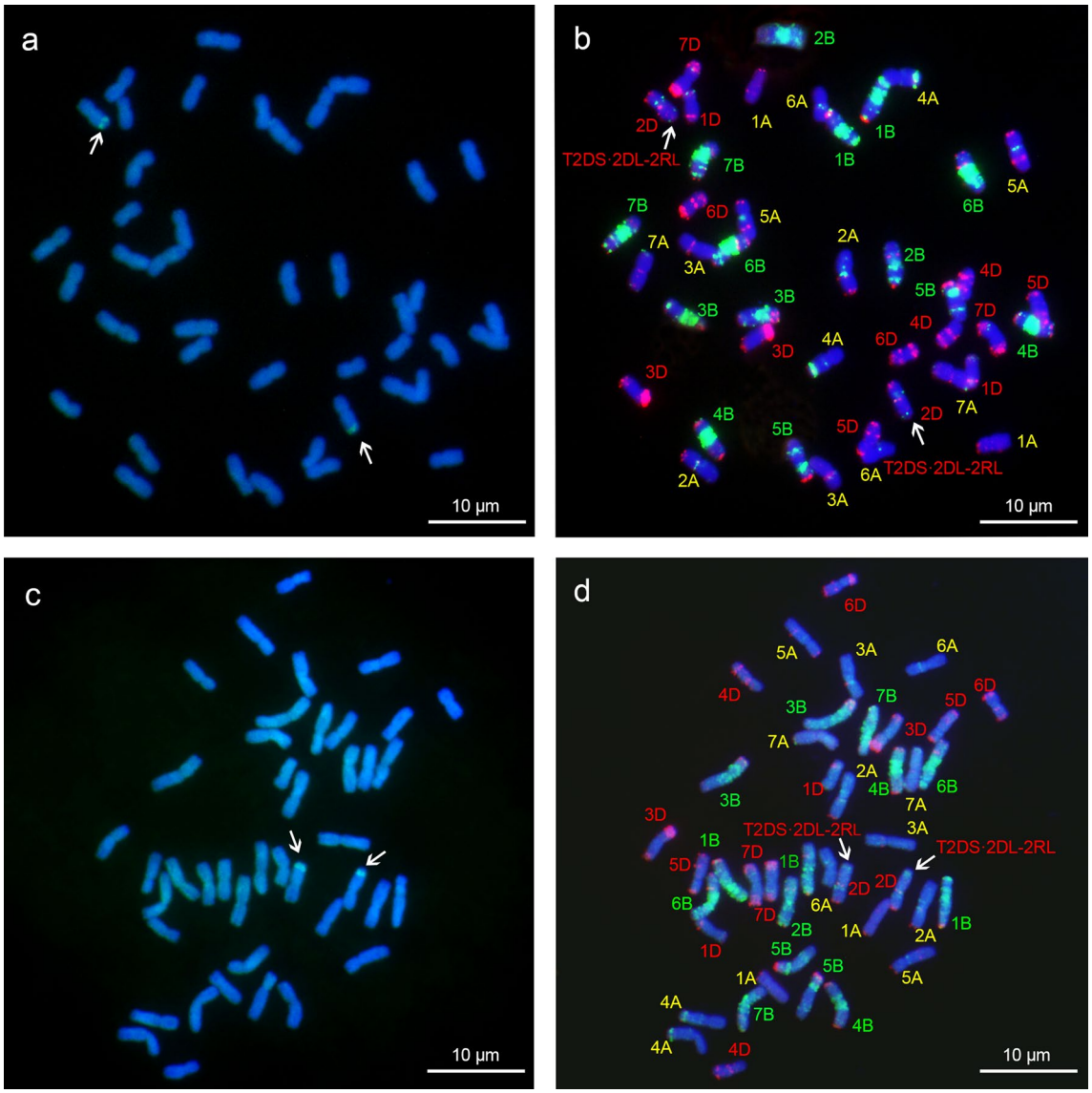


Fig. 1 Genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH) analyses of chromosomes in LCR4. **a**, **b** GISH analysis of LCR4. **c**, **d** FISH analysis of LCR4. The arrows indicate the rye chromosome segments of 2RL

Jingzhou rye, Currency, and LCR4, but were absent in the wheat accessions JM22, Mianyang 11 (MY11), Jimai 4075 (JM4075), and Chinese Spring (CS) (Fig. 2; Table 1). So, the translocated segment in LCR4 can be confirmed from rye chromosome arm 2RL, and GW02 can also be used to detect this translocated segment. To further confirm this result and investigate the translocated segment, 50 reported 2RL-specific markers were further screened, and four markers were found to detect the translocated 2RL segment (Fig. 2; Table 1). Taking the results of GISH, FISH, and molecular markers detection, we confirmed that LCR4 is a T2DS·2DL-2RL translocation line. From the locations of the five specific markers, we also found that the translocated segment covered 316.1 Mb (Chr2R: 603.3–919.4 Mb) based on the rye reference genome Lo7 (Rabanus-Wallace et al. 2021).

Wheat single nucleotide polymorphism (SNP)-arrays analysis of LCR4

After genotyping, we detected 158,508 high-quality SNPs in LCR4, and among them, 613 SNPs without physical location were subsequently removed. Considering that LCR4 is a T2DS·2DL-2RL translocation line, we chose 8326 SNPs located on chromosome 2D to analyze the translocation event in LCR4. We found that the vast majority of missing SNPs on chromosome 2D (90.5%)

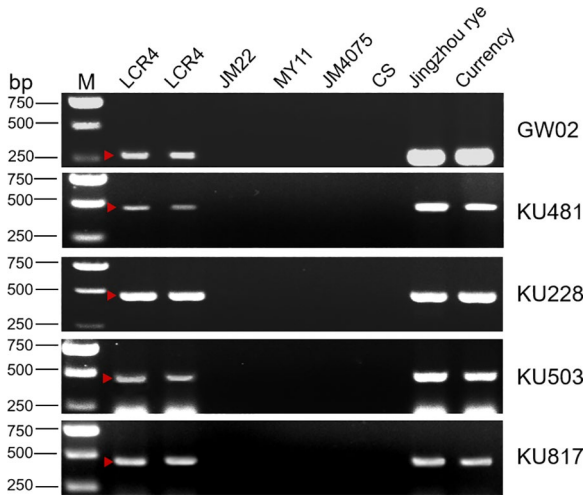


Fig. 2 Molecular markers analysis of LCR4, Jimai 22 (JM22), Jimai 4075 (JM4075), Chinese Spring (CS), Mianyang 11 (MY11), hexaploid triticale Currency, and Jingzhou rye using the rye chromosome 2RL-specific markers GW02, KU481, KU228, KU503, and KU817. The arrows indicate the specific bands

were within the terminal region (574.4–656.6 Mb), and the remaining (9.5%) were distributed in other vast region in this chromosome (0–574.4 Mb) (Fig. 3). This result suggested that the breakpoint for chromosome 2D was most likely located near the 574.4 Mb locus.

Analysis of the stripe rust resistance in LCR4

At the seedling stage, Jingzhou rye, Currency, and LCR4 were all resistant to the *Pst* race V26 with infection type (IT) 0, whereas the wheat parent JM22 and susceptible control Huixianhong (HXH) were all highly susceptible with IT 4 (Fig. 4). Furthermore, disease assays at adult-stage also revealed the consistent results with IT 0 for Jingzhou rye, Currency, and LCR4, whereas IT 7–9 for JM22 and HXH (Fig. 4). These results implied that the stripe resistance in LCR4 may derived from the hexaploid triticale parent Currency.

To further analyze the source of the stripe rust resistance, we made a cross between LCR4 and the susceptible wheat cultivar MY11 to obtain an F_2 population. After evaluation of the stripe rust resistance, the population was segregated with 44 resistant and 13 susceptible plants, which fits a theoretical ratio of 3:1 for the monogenic segregation ($\chi^2 = 0.15$; $P = 0.70$). Using the 2RL-specific marker GW02 that can trace the 2RL translocated segment in LCR4, we confirmed that all the resistant plants had the 2RL translocated segment and showed a reaction pattern similar to that of LCR4, and all the susceptible ones lacked the 2RL translocated segment (Fig. 5). Therefore, the stripe rust resistance in LCR4 should be due to the 2RL translocated segment.

Agronomic and yield performance

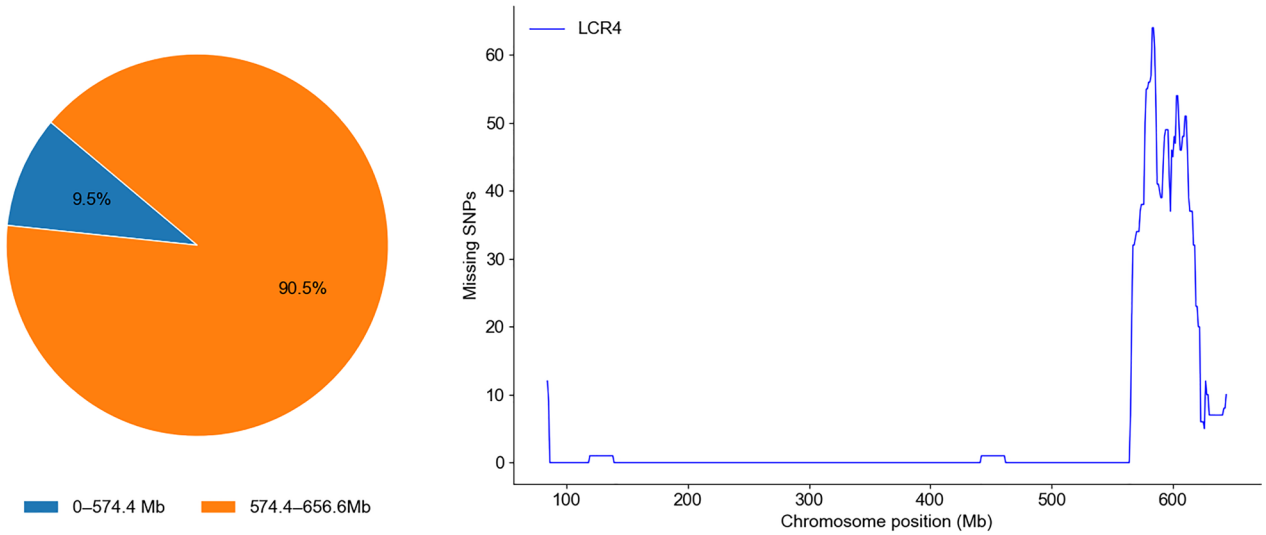
After being bagged for self-pollinating for more than five consecutive generations, LCR4 became stable in either morphological or genetic performance. Two years of field investigation further confirmed the favorable agronomic traits of LCR4, surpassing even its wheat parent JM22, a renowned wheat cultivar in China (Fig. 6; Table 2). Specifically, LCR4 exhibited noticeable improvements in spike length (SL) and spike number per plant (SNPP). LCR4 also has higher plant height (PH) than JM22 but in a reasonable lodging-resistant range. For other traits, LCR4 also showed satisfactory performance compared to JM22. In summary, LCR4 has an elite agronomic and yield performance in the field and can be used as a high-potential parent in wheat improvement against stripe rust.

Discussion

Wheat breeding is a complex process involving gene recombination and trait exchange. In recent years, wheat breeding has encountered a genetic bottleneck, making it increasingly challenging to develop breakthrough cultivars due to the scarcity of innovative germplasm resources (Xiao et al. 2022). Rye is a naturally cross-pollinating relative of common wheat and has emerged as a promising source for developing novel germplasms (Li et al. 2021; Rabanus-Wallace et al. 2021). In the past, numerous rye chromatin segments have been introgressed into the wheat background through distant hybridization techniques, noticeably enhancing disease

Table 1 Primer sequences and the location (based on the rye reference genome Lo7) of the molecular markers specific for the alien translocated segment of 2RL in LCR4

Markers	Forward primer (5′–3′)	Reverse primer (5′–3′)	Location	References
GW02	GGTGGAGCTCAGCCCATATG	CATCACGCCCTCAAGGTACA	919,438,248–919,438,510	Newly developed
KU228	TTGCCACGATTCATGTTGAT	CGTCCGTTTAACCAGTCCTC	919,438,196–919,438,625	Qiu et al. (2016)
KU481	AGCACACCGGTGAAAAAGTT	AGACATCATCTGCCGTTCAC	879,526,071–879526482	Qiu et al. (2016)
KU503	GGCGTCTACCACCTAGCTCA	GCACCATTATCGTGACATCG	603,333,550–603333965	Qiu et al. (2016)
KU817	CGTCAATGGACTTTCATCCTG	AAATCCTTACAAAAACACCCTACC	603,395,057–603395499	Qiu et al. (2016)



a b

Fig. 3 SNP analysis of LCR4 using wheat 120 K SNP array. **a** Proportion of deleted SNPs in different physical regions of chromosome 2D in LCR4. **b** Number of missing SNPs on chromosome 2D in LCR4 using 20-Mb sliding window, with 1-Mb step

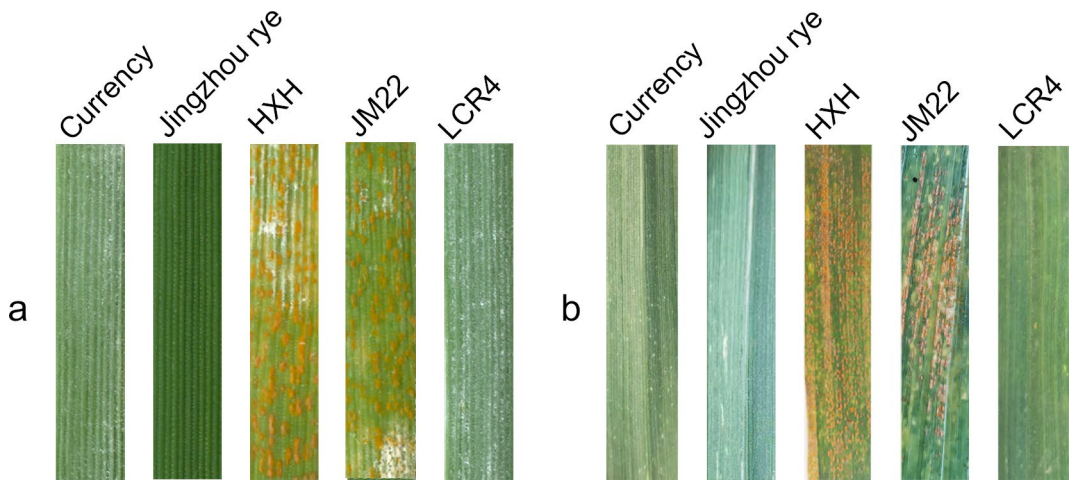


Fig. 4 **a**, **b** Stripe rust disease assays of hexaploid triticale Currency, Jingzhou rye, Huixianhong (HXH), Jimai 22 (JM22), and LCR4 infected with *Puccinia striiformis* f. sp. *tritici* (*Pst*) race V26 at seedling (**a**) and adult (**b**) plant stages

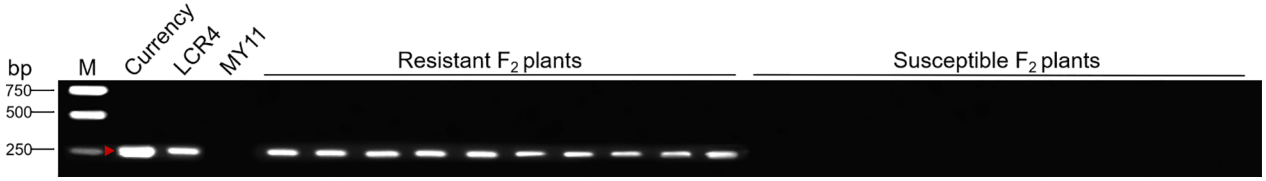


Fig. 5 Molecular detection of the 2RL translocated fragment from LCR4 using the tracing marker GW02 in F_2 plants of LCR4 × Mianyang11 (MY11) for genetic analysis of the stripe rust resistance. The red arrow indicates the targeted band of GW02

Fig. 6 Morphology of wheat-rye chromosome T2DS·2DL-2RL translocation line LCR4 and its wheat parent Jimai 22 (JM22) (sampled on May 12, 2024, Tai'an, China). **a** Plants of LCR4 (left) and JM22 (right). **b** Spikes of LCR4 (left) and JM22 (right)

Table 2 Agronomic and yield performance of LCR4 and its wheat parent Jimai 22 (JM22) in years 2021–2023

Year	Genotypes	PH (cm)	SL (cm)	KNS	TKW (g)	SNPP
2021–2022	LCR4	69.94 ± 4.80^{b}	10.19 ± 0.98^{b}	57.50 ± 6.53^{b}	48.50 ± 3.16^{a}	18.88 ± 5.02^{a}
	JM22	63.63 ± 5.75^{b}	8.63 ± 0.54^{c}	56.55 ± 6.52^{b}	46.59 ± 1.09^{a}	13.88 ± 2.80^{b}
2022–2023	LCR4	68.12 ± 3.46^{b}	9.90 ± 0.70^{a}	61.63 ± 5.01^{b}	49.59 ± 1.59^{a}	18.63 ± 6.00^{a}
	JM22	59.21 ± 4.30^{c}	7.81 ± 0.69^{b}	57.75 ± 4.03^{b}	47.28 ± 1.26^{b}	13.63 ± 2.33^{b}

Values with the same letters in the same column were not significantly different at the $P < 0.05$ according to the LSD test. PH, Plant height; SL, Spike length; KNS, Kernel number per spike; TKW, Thousand kernel weight; SNPP: Spike number per plant

resistance, stress tolerance, adaptation capacity, and yield potential (Howell et al. 2014; Crespo-Herrera et al. 2017; Hackauf et al. 2022; Liu et al. 2022). However, most of these introgressions cannot be directly utilized in breeding programs due to the linkage drag and competition lag associated with addition or substitution lines. Therefore, it is crucial to develop or identify small segment translocation lines that exhibit high breeding coordination ability while minimizing linkage drags. In this study, we successfully developed a wheat-rye small-segment translocation line with high disease resistance and elite agronomic and yield performance using hexaploid triticale through hybridization (Figs. 4, 6). Compared with rye, triticale has better compossibility with the common wheat in distant hybridization, making it a better bridge for transferring the alien chromosomal segments/genes.

Among all the previous wheat-rye germplasms developed through distant hybridization, the 1RS translocation line has been widely recognized as the most successful application in wheat improvement using wheat relatives. Since the 1950s, when the chromosome arm 1RS from the German rye cultivar Petkus was firstly introgressed into common wheat (Schlegel1 and Korzun 1997), thousands of wheat cultivars have been bred utilizing this type of germplasm due to their harmonious disease resistance and superior yield traits. This breakthrough could even be considered a second revolutionary milestone in wheat breeding history after the introduction of the green revolution genes (Han et al. 2020b). Unfortunately, resistance genes located on the chromosome arm 1RS have gradually lost their effectiveness due to the continuous evolution of virulent isolates within pathogen populations. The issue of limited genetic diversity resulting from a single source origin has been extensively addressed. Despite numerous attempts to transfer various rye chromosomes and chromosome segments into

the wheat genome apart from 1RS translocation lines, no successful breeding practices have been achieved in commercial cultivars primarily due to unbreakable linkage drags or adverse pleiotropic effects on the recipient wheat genome. Therefore, it is highly valuable to develop novel breakthrough germplasms that rival or surpass the performance of existing 1RS translocation lines. Fortunately, in this study, we developed and identified a valuable wheat-rye T2DS·2DL-2RL translocation line LCR4 (Figs. 1, 2). Based on the rye reference genome Lo7, the five specific markers covered 316.1 Mb (Chr2R: 603.3–919.4 Mb) (Table 1). However, the number of specific markers is relatively limited and cannot cover the full length of the translocated segment. Meanwhile, rye is a naturally cross-pollinating crop, resulting in heterogeneity between different rye and triticale genotypes. This characteristic creates huge variations and hence has high genetic diversity between different rye and triticale genotypes. So, the size and location of the translocated segment based on the rye reference genome Lo7 can only be used as a reference for evaluating the translocated segment from the specific hexaploid triticale line Currency. LCR4 carries a relatively small chromosome segment of chromosome arm 2RL from the cytological result, thereby potentially alleviating numerous linkage drags. Notably, LCR4 exhibits favorable agronomic and yield traits comparable to the wheat parent JM22, the predominant wheat cultivar nationwide with an accumulated promotion of 23 million hectares (Fig. 6; Table 2). Especially, due to the translocation of small chromosome segments, LCR4 demonstrated normal chromosome pairing behavior and can be directly employed in expedited wheat breeding programs. Apart from LCR4, several other 2R or 2RL derivatives with stripe rust resistance have also been identified. For example, a T2BS.2RL translocation line from Chaupon rye was susceptible to three stripe rust pathotypes at the seedling stage but showed adult plant resistance under natural conditions, and meanwhile it conferred resistance to powdery mildew, leaf rust, and stem rust (Hysing et al. 2007); a $2R^{a}$ (2D) substitution line LF24 from *S. africanum* was immune to stripe rust, and the resistance was confirmed to be derived from the chromosome $2R^{a}$ (Lei et al. 2011); a T2DS.$2R^{afr}$L translocation line from *S. africanum* carried gene(s) for dwarfing and stripe rust resistance (Lei et al. 2013). These 2R or 2RL derivatives, together with LCR4, effectively unleashed the genetic potential of rye chromosome 2RL against wheat stripe rust.

The particularly notable advantage in LCR4 was coordinated improvement between high stripe rust resistance and agronomic and yield traits (Figs. 4, 6). Previous studies have reported the development of wheat-rye lines with resistance to stripe rust from 1R, 2R, 6R, and 7R (Lei et al. 2011, 2013; Qi et al. 2016; Schneider et al. 2016; Ren et al. 2017, 2018, 2020, 2022; Li et al. 2020). However, most of these lines exhibit significant agronomic performance drawbacks and have, therefore, not been utilized in wheat production. In this study, LCR4 demonstrated high resistance to stripe rust at all growth stages, whereas its wheat parent JM22 showed susceptibility. To expedite the breeding utilization of the 2RL chromosome segments, marker-assisted selection (MAS) is a powerful tool at the present stage (Wang et al. 2024). For this purpose, we screened five tracing markers specific to the small translocated fragments of chromosome 2RL that can be used for MAS (Fig. 2). Moving forward, our future efforts will focus on transferring them into more diverse genetic backgrounds to develop stable and industrialized 2RL translocation lines while selecting breakthrough cultivars.

Conclusions

In this study, we developed a wheat-rye T2DS·2DL-2RL translocation line LCR4 with enhanced resistance against stripe rust, evaluated the translocation event in LCR4, and screened tracing markers for the rye chromosome 2RL segment that can be used for MAS. Furthermore, LCR4 exhibited robust resistance to stripe rust along with exceptional agronomic traits, thereby establishing its potential to be a valuable germplasm for enhancing disease resistance in wheat chromosome engineering breeding.

Methods

Plant materials

The wheat-rye derivative line LCR4 was produced by crossing hexaploid triticale line Currency ($2n=6\times=42$, AABBRR) with predominant wheat cultivar JM22, which was bred and maintained by our lab and backcrossed with the resulting F_1 progeny, and then stable lines were selected in the generation BC_1F_6. Currency and wheat cultivars MY11 and CS were kindly provided by Prof. Zujun Yang (University of Electronic Science and Technology of China, Chengdu, China). The wheat cultivar HXH, provided by Prof. Xiaojun Zhang (Shanxi Agricultural University, Jinzhong, China), was used as the susceptible control in phenotypic assessment and served as the *Pst* inoculum spreader. Wheat cultivar JM4075, bred and maintained by our lab, was used as the control without rye chromatin in detecting chromosome segments in wheat background.

GISH and FISH analyses

The chromosomes were prepared following the method described by Kato et al. (2004). The total genomic DNA of rye was labeled with fluorescein-12-dUTP (green) and

used as a probe, while the genomic DNA of JM22 was used as a blocker with a ratio of 1:30 for the probe. GISH analysis was performed as described in the study by Fu et al. (2012).

FISH analysis was performed using the probe combination: AFA-3 (red), AFA-4 (red), pAs1-1 (red), pAs1-3 (red), pAs1-4 (red), pAs1-6 (red), pSc119.2-1 (green), and GAA_{10} (green) (Du et al. 2017). All probes were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The chromosomes were counterstained with 4,6-diamidino-2-phenylindole, and the images were captured with a fluorescence microscope (Olympus BX60, Tokyo, Japan) equipped with a charge-coupled device camera.

Molecular marker analysis

To determine the rye chromosomes introduced to LCR4, sequences of chromosome 2R of rye were extracted from the rye reference genome Lo7 (http://202.194.139.32/). The sequences were aligned with the Chinese Spring reference genome v2.1 (Zhu et al. 2021) to obtain differences in sequence. Then, 15 molecular markers were designed using the PrimerServer function in WheatOmics 1.0 (http://202.194.139.32/). Meanwhile, 50 specific markers of chromosome 2R of rye (Qiu et al. 2016) were also used to screen markers for the translocated rye chromosome segment.

PCR amplification system was a 10-μL reaction mixture, containing 40 ng genomic DNA, 2 μM each of forward and reverse primers, 2.5 mM each of the dNTPs, 2.5 mM $MgCl_2$, 1×PCR buffer (10 mM Tris-HCl, pH 8.5, 50 mM KCl), and 0.5 U Taq DNA polymerase. The amplification reactions were performed with a PTC-200 thermal cycler (Bio-Rad, Hercules, CA) and carried out using the following profile: 94°C for 5 min; followed by 36 cycles of 94°C for 30 s, 55–60°C (based on primer annealing temperature) for 30 s and 72°C for 30 s; and a final elongation at 72°C for 10 min before cooling to 4°C. The PCR products were resolved on 2% agarose gel electrophoresis and then visualized by band patterns.

Wheat SNP-arrays genotyping

To explore the translocation events and homology between alien segments and wheat chromosomes, genomic DNA was isolated from LCR4, and then a 130 K SNP array, conducted by Chengdu Tiancheng Future Technology Co., LTD, Chengdu, China, was used for genotyping LCR4. The SNPs with exact physical positions on the LCR4 were used to analyze deletion and translocation events. The deletion rate for each chromosome was calculated using a 20-Mb sliding window with a 1-Mb step. Chromosomal region(s) with significantly higher deletion rates were considered to be affected by deletion and translocation events, and hence as the area(s) that happened the translocation event(s).

Evaluation of stripe rust resistance

LCR4, its parents Currency and JM22, and the Jingzhou rye as a control were tested for their reactions to stripe rust. The seedling resistance test was performed in a growth chamber using the *Pst* race V26, provided by Prof. Jiajie Wu (Shandong Agricultural University, Tai'an, China). The seedling inoculation and evaluation were performed according to the method of Zhou et al. (2023) using HXH as a susceptible control. At the adult stage, the accessions were tested with a mixture of *Pst* races and evaluated following the procedure described in Wu et al. (2018) using HXH as susceptible control.

To assess the inheritance of the stripe rust resistance in LCR4, it was crossed with the susceptible cultivar MY11 to obtain F_2 population. The evaluation of disease resistance was performed as described above. Meanwhile, the genomic DNA of each F_2 plant was isolated and genotyped using the 2RL-specific marker GW02 for the rye chromosome segment. The relationship between the alien chromosome and the disease resistance was analyzed to confirm the source of stripe rust resistance.

Evaluation of agronomic traits

To evaluate the agronomic and yield traits, LCR4 and JM22 were planted at the field in Shandong Academy of Agricultural Sciences, Jinan, China, in two consecutive growth cycles (2021–2023). Three replicates for each genotype were set in each sowing season. For each genotype in a replicate, 20 seeds were planted in 1.5-m-long rows, spaced 0.25 m apart. At physiological maturity, PH, SL, SNPP, thousand-kernel weight (TKW), and kernel number per spike (KNS) of 20 randomly selected plants in the middle of the two internal rows were assessed. The analysis of variance and least significant difference (LSD) test were performed using software SPSS 22.0 (SPSS Inc., Chicago, IL, USA) to test the significance of differences between LCR4 and JM22 for each agronomic trait.

Abbreviations

CS	Chinese spring
FISH	Fluorescence in situ hybridization
GISH	Genomic in situ hybridization
HXH	Huixianhong
IT	Infection type
JM22	Jimai 22
JM4075	Jimai 4075
KNS	Kernel number per spike
LSD	Least significant difference
MAS	Marker-assisted selection
MY11	Mianyang 11
PH	Plant height
Pst	*Puccinia striiformis* F. sp. *tritici*
SL	Spike length
SNP	Single nucleotide polymorphism

SNPP Spike number per plant
TKW Thousand-kernel weight

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Author contributions
CL and PM conceived the research. YJ, GH, WG, RH, XW, YB, JL, AL, HL, and JL performed the experiments and collected data. PM and GH performed data analyses. CL developed the experimental materials. YJ, PM, and GH wrote and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed in this study are available from the corresponding authors on request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no conflicts of interest.

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