The Crop Journal 13 (2025) 909–918

Contents lists available at ScienceDirect

The Crop Journal

journal homepage: www.keaipublishing.com/en/journals/the-crop-journal/

Identification and characterization of *Sr59*-mediated stem rust resistance in a novel wheat-rye translocation T2BL·2BS-2RL

Mahboobeh Yazdani [a], Matthew N. Rouse [b,c], Prabin Bajgain [d], Tatiana V. Danilova [e,f], Ivan Motsnyi [g], Brian J. Steffenson [h], Mehran Patpour [i], Mahbubjon Rahmatov [a,*]

[a] *Department of Plant Breeding, Swedish University of Agricultural Sciences, 23053 Alnarp, Sweden*
[b] *United States Department of Agriculture, Agricultural Research Service, Cereal Disease Laboratory, St. Paul, MN 55108, USA*
[c] *United States Department of Agriculture, Agricultural Research Service, Sugarcane Field Station, Canal Point, FL 33438, USA*
[d] *Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA*
[e] *USDA-ARS, Wheat, Sorghum & Forage Research Unit, Lincoln, NE 68583, USA*
[f] *Department of Plant Pathology, Wheat Genetic Resources Center, Throckmorton Plant Sciences, Manhattan, KS 66506, USA*
[g] *Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation, Ovidiopolska Dor., 3, Odesa 65036, Ukraine*
[h] *Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA*
[i] *Department of Agroecology, Aarhus University, Flakkebjerg, 4200 Slagelse, Denmark*

ARTICLE INFO

Article history:
Received 19 October 2024
Revised 7 February 2025
Accepted 18 February 2025
Available online 1 April 2025

Keywords:
Cytogenetic analysis
Marker-assisted selection
NLR
Resistance gene
Wheat-rye introgression

ABSTRACT

Emerging new races of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) are threatening global wheat (*Triticum aestivum* L.) production. Host resistance is the most effective and environmentally friendly method of controlling stem rust. The stem rust resistance gene *Sr59* was previously identified within a T2DS·2RL wheat-rye whole arm translocation, providing broad-spectrum resistance to various stem rust races. Seedling evaluation, molecular marker analysis, and cytogenetic studies identified wheat-rye introgression line #284 containing a new translocation chromosome T2BL·2BS-2RL. This line has demonstrated broad-spectrum resistance to stem rust at the seedling stage. Seedling evaluation and cytogenetic analysis of three backcross populations between the line #284 and the adapted cultivars SLU-Elite, Navruz, and Linkert confirmed that *Sr59* is located within the short distal 2RL translocation. This study aimed physical mapping of *Sr59* in the 2RL introgression segment and develop a robust molecular marker for marker-assisted selection. Using genotyping-by-sequencing (GBS), GBS-derived SNPs were aligned with full-length annotated rye nucleotide-binding leucine-rich repeat (NLR) genes in the parental lines CS *ph1b*, SLU238, SLU-Elite, Navruz, and Linkert, as well as in 33 BC_4F_5 progeny. Four NLR genes were identified on the 2R chromosome, with Chr2R_NLR_60 being tightly linked to the *Sr59* resistance gene. *In-silico* functional enrichment analysis of the translocated 2RL region (25,681,915 bp) identified 223 genes, with seven candidate genes associated with plant disease resistance and three linked to agronomic performance, contributing to oxidative stress response, protein kinase activity, and cellular homeostasis. These findings facilitate a better understanding of the genetic basis of stem rust resistance provided by *Sr59*.

1. Introduction

Bread wheat (*Triticum aestivum* L., 2*n* = 6*x* = 42, AABBDD) is one of the staple food crops worldwide that provides a major source of daily protein and essential nutrients for the global population [1]. Wheat production is increasingly threatened by emerging diseases and pests, which pose significant challenges to meeting the food demands of a growing population [2]. One of the most devastating threats leading to substantial economic losses is stem rust, *Puccinia graminis* f. sp. *tritici* (*Pgt*) which can cause up to 100% yield loss on susceptible varieties [3]. The emergence of the Ug99 virulent *Pgt* race, which overcame the *Sr31* resistance gene and first identified in Africa, has raised significant concerns for global wheat production [4]. Over the last two decades, Ug99 has evolved into new virulent races, continuously overcoming resistance genes in new cultivars [5]. As a result of the Ug99 race group, outbreaks and epidemics have also occurred in countries such as Egypt, Eritrea, Iran,

* Corresponding author.
E-mail address: mahbubjon.rahmatov@slu.se (M. Rahmatov).

https://doi.org/10.1016/j.cj.2025.02.012

Nepal, etc. posing a threat to global food security [5,6]. The continuous emergence and spread of new *Pgt* races in Europe, such as TTRTF, TKKTF, etc., is a growing concern [7], with increasing outbreaks recorded in more countries each year. This ongoing expansion demonstrates stem rust pathogens' adaptability to different environments and ability to overcome existing resistance genes. In addition, other highly virulent *Pgt* races, such as TRTTF, TKTTF, TTKST, PRCTM, and TTKTT have been detected with additional virulence combinations (i.e., *Sr9e + Sr13*, *Sr22 + Sr24*, *Sr24 + Sr31*, *Sr13b + Sr35 + Sr37*, and *Sr24 + Sr31 + SrTmp*) [8–10]. Breeding for host resistance has proven the most cost-effective and environmentally sustainable way to manage wheat rust diseases in environmentally responsible efforts to achieve zero agricultural pollution [11,12]. However, the genetic diversity of cultivated wheat is limited due to a narrow genetic bottleneck [13], which hinders the diversification of stem rust resistance genes needed to combat emerging new races. Wild and cultivated relatives of wheat represent a valuable source of genetic diversity, providing beneficial traits that can diversify and strengthen the genetic base of cultivated wheat. This diversity is crucial for developing durable resistance to stem rust and other wheat diseases, ensuring for sustainable wheat production.

Rye (*Secale cereale* L., $2n = 2x = 14$, RR) has been a vital source of genetic variability for improving bread wheat, particularly in enhancing resistance to both abiotic and biotic stresses [14]. Several stem rust resistance genes, such as *Sr27*, *Sr1R*^{Amigo}, *SrSatu*, and *Sr31*, *Sr50* have been successfully introgressed from rye into wheat [15–17]. Moreover, rye was utilized to enhance a number of agronomical and physiological traits, including insect resistance and yield [18,19]. The late Professor Arnulf Merker developed a large number of wheat-rye introgression lines at the Swedish University of Agricultural Sciences [20,21]. One wheat–rye 2R (2D) chromosome substitution line 'SLU238' was identified as providing effective resistance to diverse *Pgt* races [22]. Later, a line containing a T2DS·2RL Robertsonian translocation TA5094 was developed and proven to possess the stem rust resistance gene, designated as *Sr59* [23]. We also identified line #284, which has small introgressions confirmed by two 2RL-specific KASP markers [23].

Methods that overcome the presence of the *Ph1* locus must be employed to develop new chromosomal translocations that incorporate desired traits from related species into wheat, such as rye. On the 5BL chromosome of wheat, the *Ph1* locus plays a guarding role in homologous chromosome pairing during meiosis by keeping the AABBDD chromosomes pairing with homologous chromosomes [24,25]. Mutations in the *Ph1* locus (*ph1b* in hexaploid wheat and *ph1c* in tetraploid wheat) promote pairing between homoeologous chromosomes during meiosis [26,27]. This has resulted in the CS *ph1b* mutant being highly effective at inducing recombination between wheat and wild relatives chromosomes, and it has already been widely used as a tool for deploying rust resistance genes [15,23,28–30]. Plants employ the gene-for-gene hypothesis as a defense mechanism against pathogens, wherein resistance in a plant is conditional upon the presence of specific resistance (*R*) genes corresponding to specific avirulence (*Avr*) genes in the pathogen [31], for which many *R* genes encode nucleotide-binding leucine-rich repeat (NLR) proteins [32]. More than 67 stem rust *R* genes have been identified in wheat and its relatives [33], and some of them, such as *Sr26*, *Sr33 Sr35*, *Sr61*, etc. have been characterized as NLR genes [34–36]. Studies have successfully predicted potential NLR genes from the wheat and rye reference genomes using the NLR-Annotator tool [37–39]. Thus, this approach can enhance our understanding of the genetic basis of disease resistance in rye, which can be used to improve resistance traits in wheat. The objectives of this study are to (1) evaluate and determine the size of the 2RL segment in the introgression line #284 using stem rust seedling assessment and cytogenetic analysis; (2) identify NLR gene(s) within the 2RL segment and develop corresponding Kompetitive Allele Specific PCR (KASP) markers; and (3) conduct physical mapping of the 2RL segment to locate the NLR gene(s) associated with stem rust resistance.

2. Materials and methods

2.1. Plant materials and stem rust seedling evaluations

Line #284 was derived from a cross between Chinese Spring (CS) *ph1b* and SLU238, as previously described by Rahmatov et al. [23]. Line #284 was assessed for its seedling response to *Pgt* race TTTTF (isolate 01MN84A-1–2) [40,41], and after each seedling assessment, the resistance plants were subjected to two KASP markers linked with *Sr59* [42]. The segregation for resistance to *Pgt* races TTTTF (IT 2-/3+), TPMKC (IT;1-/4+), RKQQC (IT 2-/4+) and RCRSC (IT 2-/3+) was observed in line #284. Molecular marker analysis using rye markers (*Xscm43*, *Xscm75*, *Xrems1251*, *Xrems1203*, *Xgrm0676*, *Xgrm0837*, *Xgrm0315*, *Xgrm1265*, *Xcgg8*, *Xcgg62*, *Xtnac1142*, and *Xgrm1265*) did not detect the presence of rye chromatin, but the two KASP markers co-segregated with resistance to race TTTTF within the line #284 [42]. In the F_5 generation, approximately 60 seeds from resistant plants in line #284 were subjected to the two KASP markers. The F_6 and F_7 generations were assessed for seedling response against *Pgt* race TTTTF at the USDA-ARS Cereal Disease Laboratory and the University of Minnesota. Resistant plants were tested with the two KASP markers and subsequently self-pollinated to produce the next generation. Additionally, line #284 in the F_7 generation was tested against various *Pgt* races including TTTTF (isolate RU118b/16), QTHJC (C25; isolate 1541), TPMKC (C53; isolate 1373), RKQQC (C35; isolate 1312), RCRSC (isolate 77ND82A), TTRTF (isolate IT14a/16), TKTTF (isolates IQ115a/14 and SE27121), TTKTT (isolate 14KEN58-1), TTKSK (isolate 04KEN156/04), TTKST (isolate 06KEN19v3), TTTSK (isolate 07KEN24-4), TRTTF (isolate 06YEM34-1), LTBDC (Australian *Pgt* race 98–1,2,3,5,6), and JRCQC (isolate 08ETH03-1). Three adapted cultivars, Linkert (provided by Prof. Jim Anderson from the University of Minnesota), Navruz (from the National Wheat Breeding Program in Tajikistan) and SLU-Elite (from SLU), were used to develop backcross populations. TA5018 (T2BS.2RL) and TA5084 (T2BS.2BL-2RL), derived from Chaupon rye [43] were also included in the seedling screening.

2.2. Population development, stem rust seedling evaluations, molecular marker analysis, and field evaluations

Line #284 was completely homozygous for resistance in the F_7 generation, and the #284–7 line was crossed with three recurrent parents: SLU-Elite (producing 62 F_1 seeds), Navruz (producing 58 F_1 seeds), and Linkert (producing 52 F_1 seeds) (Fig. S1). The F_1 plants were backcrossed to their respective recurrent parents, generating over 300 BC_1F_1 seeds for each backcross population (Fig. S1). The BC_1F_1 plants were tested to *Pgt* races TTTTF and TTKSK [40,41], and resistant plants were checked with the two KASP markers (KASP_2RL_c20194C2 and KASP_2RL_c21825C1) [42]. The selected resistant BC_1F_1 plants were further backcrossed to produce the BC_2F_1 generation, which was also assessed with *Pgt* race TTTTF and the two KASP markers to select resistant plants for additional backcrossing (Fig. S1). The derived resistant BC_3F_1 and BC_4F_1 plants were again assessed against TTTTF race and two KASP markers (Fig. S1), and then self-pollinated. The BC_4F_2 to BC_4F_4 generations were tested against race TTTTF, and selected resistant plants from each family were checked with the two KASP markers. Homozygous BC_4F_4 was also evaluated against multiple races: QTHJC, TPMKC, RKQQC, RCRSC, TKTTF, TTKSK, TTKST, TTTSK, TRTTF,

TTRTF, and TTKTT. BC_4F_5 and BC_4F_6 generations were sown at SITES Lönnstorp Research Station (55.668638°N, 13.104622°E) for phenotypic evaluations in 2020 and 2021. In a one-replicate field trial, these lines were planted in 2 m^2 plots to assess phenotypic traits compared with the recurrent parents. The data collected included traits such as days to 50% flowering and maturity, plant height, awn/awnless spikes, tillering, lodging, and natural susceptibility to diseases (for example, rusts, powdery mildew, septoria, FHB). After harvest, grain color and 1000-kernel weight (TKW) were also measured. The harvested seeds were further analyzed for protein profiling and protein content.

2.3. Genotyping and physical mapping

Genomic DNA was isolated from leaf tissue of CS *ph1b*, SLU-Elite, Navruz, and Linkert, SLU238, line #284, and all 33 homozygous BC_4F_4 plants (11 plants form each backcrossing population) using the Qiagen BioSprint 96 instrument and the Qiagen BioSprint DNA Plant kit. The isolated DNA was digested with *PstI* (5′-CTGCAG-3′) and *MspI* (5′-CCGG-3′) to create double digested libraries, which were then sequenced on an Illumina HiSeq2500 platform (Illumina, San Diego, CA, USA) at the University of Minnesota Genomics Center [44,45]. The generated sequences in FASTQ files were passed through a quality filter of Q > 30, and then de-multiplexed to obtain reads for each individuals. These reads were aligned to the International Wheat Genome Sequencing Consortium Reference Sequence v2.0 (RefSeq v2.0) and International Rye Genome Sequencing Consortium reference sequence (Rye_Lo7) using the Burrow–Wheelers Alignment tool (BWA) v0.7.4 [46], and SNPs calling and annotation were accomplished using Samtools + Bcftools [29]. SNPs with minor allele frequency (MAF) of less than 5% and more than 20% missing data were removed, resulting in 40,584 SNP markers for wheat and 15,116 SNP markers for chromosome 2R being retained for further analysis. Allele frequencies and genetic relationship between donor and recurrent parental lines were calculated using TASSEL v5.2.65 [47]. The physical map was drawn using MapChart (https://www.wur.nl/en/show/mapchart.htm).

2.4. Identifying NLR genes and development of Kompetitive allele specific PCR (KASP) markers

The 2R chromosome contains 95 full-length annotated rye NLR genes [38]. We aligned these 95 NLR genes with the GBS dataset using the BWA tool to identify relevant sequences. Polymorphic markers (SNPs) between donor and recurrent parental lines in these NLR genes were also identified using Samtools + Bcftools. The polymorphic SNPs situated within NLR genes were converted into KASP markers and validated. The KASP assays were analyzed according to Rahmatov et al. [23].

2.5. Identification of deleted 2BL and translocated 2RL regions

To investigate the genomic status of the deleted 2BL (78,519 bp) region and the translocated 2RL (25,681,915 bp) region, *in-silico* analyses were performed using the wheat (RefSeq v2.0) and rye (Rye_Lo7) reference genomes available in the EnsemblPlants database (https://plants.ensembl.org/index.html). The BLASTN program was utilized to align sequences from these regions to the reference genomes, enabling the identification of positional changes and gene content. This analysis detected structural variations corresponding to the extent of the 2BL deletion and the boundaries of the 2RL translocation. It also evaluated potential candidate genes associated with plant disease resistance or other agronomically important traits in the translocated intervals. Gene Ontology (GO) enrichment analysis was performed using the g:Profiler web tool (https://biit.cs.ut.ee/gprofiler/gost) to evaluate the identified genes' functional roles [48]. GO terms were considered significant based on an adjusted *P*-value threshold ($P_{adj} \leq 0.05$), providing insights into key biological processes, molecular functions, and cellular components linked to the candidate genes. Gene annotations derived from the RefSeq v2.0 and Rye_Lo7 reference sequences pinpointed specific genes within the affected intervals.

2.6. Fluorescent in situ hybridization (FISH)

To visualize wheat–rye introgressions, the parents and lines from three selected BC_4F_4 families, which were fixed for resistance to the races TTTTF, TTKSK, QTHJC, TPMKC, RKQQC, RCRSC, TTTSK, and TRTTF, were analyzed with FISH using probes specific to rye and wheat repetitive DNA sequences. Rye chromosomes were painted using the combination of probes to dispersed repeat UCM600 [49] synthesized by SGI DNA, La Jolla, CA, USA; centromere specific pAWRC.1 [50], and subtelomeric repeat pSc74 [51,52] labeled with Fluorescein-12-dUTP (PerkinElmer, cat. NEL413001EA). Cy5-(GAA)9 and TEX615-pAs1-2 oligonucleotide probes synthesized by IDT, Coralville, IA were used to identify wheat chromosomes [53]. Somatic chromosomes preparation, direct probe labeling by nick translation, and the FISH process was performed as described by Kato et al. [54] with slight modifications [53]. Images were captured using a Zeiss Axioplan 2 microscope with a cooled charge-coupled device camera CoolSNAP HQ2 (Photometrics, Tuscon, AZ) and AxioVision 4.8 software (Carl Zeiss AG, Germany) and processed with Adobe Photoshop software (Adobe Systems Incorporated, San Jose, CA, USA).

2.7. Protein extraction using SE-HPLC

The seeds of the BC_4F_5 and BC_4F_6 generations, along with all parental lines tested for HPLC and DUMAS analysis, were produced in the field at SITES Lönnstorp Research Station. Protein profiling and protein content analysis were performed on seeds from parental lines, recurrent parents, rye Petkus, BC_4F_5 and BC_4F_6 populations, as well as Dragon HPLC standard samples. The whole grain from all seeds was milled into three in triplicate using an Ultra Centrifugal Mill ZM 200 (Retsch) at 6000 rpm and then freeze-dried for 72 h using a Cool Safe Pro (LaboGene). Polymeric proteins for SE-HPLC analysis were extracted from the freeze-dried wheat flour in two stages [55,56], with minor modifications. Gluten proteins extracted in the first and second steps were classified as SDS-extractable and SDS-unextractable, respectively. For each HPLC run, 20 μL of extracted supernatant was loaded onto a BIOSEP SEC-4000 Phenomenex column. The separation time was set to 30 min, using a mobile phase of 50% HPLC-grade acetonitrile with 0.1% trifluoroacetic acid (TFA) and 50% DD H2O with 0.1% TFA. Gluten proteins were detected using a Waters 996 Photodiode Array Detector at an absorption wavelength of 210 nm. TOTE (Total Protein Concentration) and % UPP (percentage of unextractable polymeric protein in total polymeric protein) were measured according to Johansson et al. [57]. Three mg of each flour were then used to measure DUMAS and the conversion factor 5.7 to determinate the protein content of the genotypes according to Hussain et al. [58].

3. Results

3.1. Producing homozygous #284 selections and multi stem rust seedling test

In 60 F_5 plants of line #284, subsequent analyses with two KASP markers identified the SLU238 allele in 41 resistant plants. The 22 F_6 progenies were tested at the seedling stage for resistance to *Pgt* race TTTTF, and the resistant F_6 plants were further confirmed

using two KASP markers (KASP_2RL_c20194C2 and KASP_2RL_c21825C1). Seven F_7 lines of #284 (#284–4, #284–7, #284–18, #284–21, #284–33, #284–42, and #284–49) were identified as homozygous for resistance to the TTTTF race and were further tested against the races QTHJC, TPMKC, RKQQC, RCRSC, TKTTF (Ethiopia), LTBDC, JRCQC, TRTTF, TTKST, and TTTSK (Table S1). Line #284 was also evaluated against a range of other races, such as TTTTF (Russia), TTRTF, TKTTF (Iraq and Sweden), and TTKTT, with infection types (ITs) ranging from;1- to 1+2- observed across all races tested (Table S1). The ITs of SLU238 and TA5094 to TTKTT, TTRTF, TTKSK, TTKST, TTTSK, and TRTTF races is;1- to;11+, while the ITs of line #284 is 11+ to 1+2- (Table S1; Fig. 1). Seedling resistance was also tested in three recurrent cultivars (SLU-Elite, Navruz, and Linkert), with only Linkert showing resistance to some races (Table S1; Fig. 1). The ITs revealed distinct differences in resistance and susceptibility between line #284 and lines TA5018 and TA5084 (Table S1; Fig. 1).

3.2. Population development using line #284–7

After identifying homozygous line #284–7 in the F_7 generation, which exhibited seedling ITs ranging from;1- to 1+2- against multiple races (QTHJC, TPMKC, RKQQC, RCRSC, TKTTF (Ethiopia), LTBDC, JRCQC, TRTTF, TTKST, and TTTSK) (Table S1), three populations were developed through marker-assisted crossing and backcrossing (Fig. S1). Of the 304 BC_1F_1 plants tested with race TTTTF, 146 plants in the SLU-Elite*1/#284–7 population exhibited 1+2- ITs. Similarly, of the 261 BC_1F_1 plants tested with race TTTTF, 129 in the Navruz*1/#284–7 population displayed 1+2- ITs. In the Linkert*1/#284–7 population, 202 of 399 BC_1F_1 plants tested with the race TTKSK exhibited 1+2- ITs. Segregation ratios in all three populations fit the 1:1 expectation for a single gene (χ^2 = 0.47, P = 0.491 for SLU-Elite*1/#284–7, χ^2 = 0.03, P = 0.853 for Navruz*1/#284–7, and χ^2 = 0.06, P = 0.802 for Linkert*1/#284–7) (Table 1). Thirty selected resistant BC_1F_1 plants were used to develop BC_2F_1 populations following confirmation with two KASP markers (KASP_2RL_c20194C2 and KASP_2RL_c21825C1). The SLU-Elite*2/#284–7 (χ^2 = 0.23, P = 0.629) and Navruz*2/#284–7 (χ^2 = 0.01, P = 0.915) BC_2F_1 seedlings also exhibited 1+2- ITs to *Pgt* race TTTTF and were subsequently genotyped with two KASP markers (KASP_2RL_c20194C2 and KASP_2RL_c21825C1) to select plants for further backcrossing (Table 1). Linkert*2/#284–7 (χ^2 = 0.09, P = 0.761) BC_2F_1 plants were selected solely on the two KASP markers, as Linkert (carrying *Sr7a*) showed resistance to race TTTTF (Table 1). The BC_3F_1 (SLU-Elite*3/#284–7 and Navruz*3/#284–7) and BC_4F_1 (SLU-Elite*4/#284–7 and Navruz*4/#284–7) populations were selected based on IT 1+2 in responses to race TTTTF, with resistant plants further confirmed using two KASP markers for the presence of the resistance gene. The Linkert BC_3F_1 (Linkert*3/#284–7) and BC_4F_1 (Linkert*4/#284–7) populations were selected based on genotyping with KASP_2RL_c20194C2 and KASP_2RL_c21825C1 markers. SLU-Elite*4/#284–7 and Navruz*4/#284–7 from BC_4F_2 to BC_4F_4 populations were selected based on seedling resistance to race TTTTF, while Linkert*4/#284–7 from BC_4F_2 to BC_4F_4 population were selected for resistance to race TTKSK, with all selections confirmed using the two KASP markers. The homozygous BC_4F_4 families resistant to races TTTTF and TTKSK exhibited similar seedling responses to races QTHJC, TPMKC, RKQQC, RCRSC, TKTTF, TTRTF, TTKTT, TTKSK, TTKST, TTTSK, TRTTF, LTBDC, and JRCQC, with ITs ranging from; 1- to 1+2- for resistant seedlings, whereas susceptible seedlings exhibited IT 3+4 (Fig. 1).

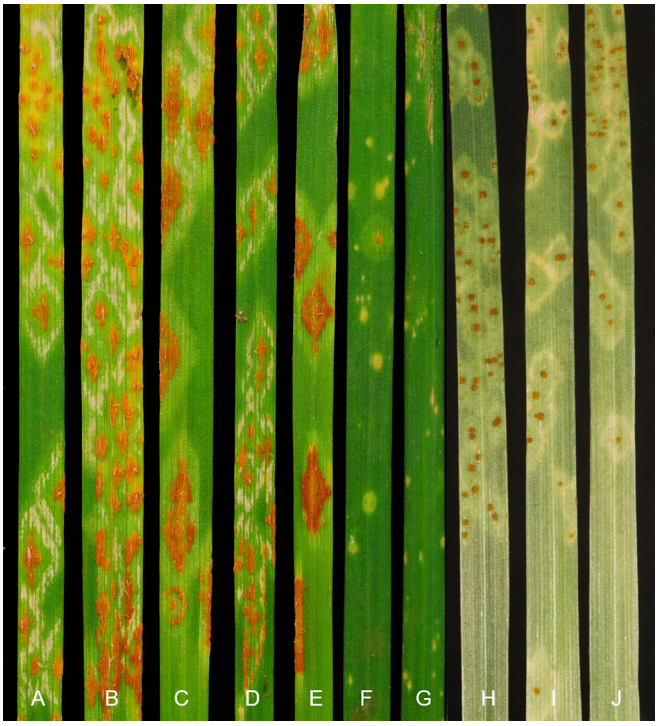

Fig. 1. Reaction to *Pgt* race TTKSK to A) SLU-Elite, B) Navruz, C) Linkert, D) TA5018 (T2BS.2RL), E) TA5084 (T2BS.2BL-2RL), F) SLU238 [2R (2D) wheat–rye disomic substitution], G) TA5094 (T2DS·2RL), H) Line #284 (2BS.2BL-2RL), I) BC_4F_4 Navruz*4/#284–7, and J) BC_4F_4 Linkert*4/#284–7.

3.3. Greenhouse and field phenotyping

Phenotypic selection from BC_1F_1 to BC_4F_4 populations was based on awn absence (line #284–7) or presence (SLU-Elite, Navruz, and Linkert) during crossing and backcrossing. Of the 30 BC_1F_1 plants, those with the greatest phenotypic similarity to the recurrent parents with awns were selected for developing the BC_2F_1 population in the greenhouse (Table 1). Plants in the BC_4F_2 to BC_4F_4 populations were selected based on their days to maturity and plant fertility performance in the greenhouse. No significant differences were observed between the recurrent parents and the BC_4F_5 and BC_4F_6 populations in the field for plant height, presence of awns on spikes, maturity, fertility, and lodging. Plant height ranged from 74 cm in the wheat variety Linkert to 165 cm in the rye line Petkus (Table 2). The BC_4F_2 to BC_4F_4 populations exhibited moderate heights between 86 cm and 92 cm, comparable to their recurrent parents, demonstrating that the introgression of the 2RL segment did not negatively affect plant height (Table 2). As for the days to maturity, Linkert (115 d), SLU-Elite (120 d), and Navruz (125 d) showed early maturity, whereas the BC_4F_5 and BC_4F_6 populations consistently showed early maturity (115 d) (Table 2).

3.4. Protein compositions of the 2BS.2BL-2RL wheat-rye translocation lines

HPLC analysis of wheat flour from the BC_4F_5 and BC_4F_6 generations of the 2BS.2BL-2RL wheat-rye translocation demonstrated stable protein composition, with no adverse effects of the translocated 2RL rye chromosome on end-use quality. Statistical comparisons of total protein concentration (TOTE) and gluten strength (% UPP) showed no significant differences between the two years (P > 0.05), confirming stability and uniformity of protein parameters across these BC_4F_5 and BC_4F_6 generations (Table 2). The lowest TOTE and %UPP values were observed in the rye Petkus (P < 0.05), which were significantly lower than in the BC_4F_5 and BC_4F_6 lines carrying the 2BS.2BL-2RL translocation in all three genetic backgrounds

Table 1
Segregation for resistance to *Pgt* races TTKSK and TTTTF in BC_2F_1 to BC_4F_1 generations in three adapted cultivars.

Cross	Seedling race	Generation	Resistant	Susceptible	$\chi^2_{(1:1)}$	*P* value	KASP_2RL_c20194A1 and KASP_2RL_c21825A1	No. of transplanted plants	No. of plants with awn spikes used for backcrossing
SLU-Elite*1/#284–7	TTTTF	BC_1F_1	146	158	0.474	0.491	+	30	23
SLU-Elite*2/#284–7	TTTTF	BC_2F_1	178	169	0.233	0.629	+	30	19
SLU-Elite*3/#284–7	TTTTF	BC_3F_1	189	172	0.801	0.371	+	30	25
SLU-Elite*4/#284–7	TTTTF	BC_4F_1	157	160	0.028	0.866	+	30	30
Navruz*1/#284–7	TTTTF	BC_1F_1	129	132	0.034	0.853	+	30	18
Navruz*2/#284–7	TTTTF	BC_2F_1	174	176	0.011	0.915	+	30	26
Navruz*3/#284–7	TTTTF	BC_3F_1	194	190	0.042	0.838	+	30	24
Navruz*4/#284–7	TTTTF	BC_4F_1	143	137	0.129	0.72	+	30	28
Linkert*1/#284–7	TTKSK	BC_1F_1	202	197	0.063	0.802	+	30	21
Linkert*2/#284–7	KASP	BC_2F_1	198	192	0.092	0.761	+	30	22
Linkert*3/#284–7	KASP	BC_3F_1	169	154	0.697	0.404	+	30	27
Linkert*4/#284–7	TTKSK	BC_4F_1	224	220	0.036	0.849	+	30	30

Table 2
The ANOVA table presents data on TOTE, %UPP, nitrogen content (%) from DUMAS, and TKW (g) in parental lines, the BC_4F_6 generation, and rye.

Line	Plant height (cm)	Days to maturity (d)	Spike phenotype awn/awnless	TOTE	UPP (%)	Protein content (%)	TKW (g)	Chromosome constitutions
SLU238	105	127	Awnless	131,948,727 bc	30.03 ab	12.65 b	24.01 bc	2R (2D)
CSA	111	135	Awnless	202,794,707 a	23.91 c	12.43 b	24.00 bc	AABBDD
#284	107	135	Awnless	169,342,198 ab	34.33 ab	12.65 b	24.90 bc	2BL.2BS-2RL
TA5094	107	135	Awnless	168,754,443 b	35.19 ab	13.57 b	23.81c	2DS·2RL
SLU-Elite	87	120	Awn	161,491,675 b	45.28 a	15.39 a	43.40 a	AABBDD
Navruz	90	125	Awn	164,549,454 b	36.4 ab	15.62 a	36.10 ab	AABBDD
Linkert	74	115	Awn	171,750,176 a	49.44 a	15.05 a	33.50 ab	AABBDD
SLU-Elite*4/#284–7 (BC_4F_5)	89	115	Awn	184,792,178 a	34.7 ab	14.42 a	37.20 ab	2BL.2BS-2RL
SLU-Elite*5/#284–7 (BC_4F_6)	90	115	Awn	185,681,080 a	34.6 ab	14.52 a	38.00 ab	2BL.2BS-2RL
Navruz*4/#284–7 (BC_4F_5)	92	115	Awn	179,806,737 a	33.35 ab	14.31 a	33.69 ab	2BL.2BS-2RL
Navruz*5/#284–7 (BC_4F_6)	92	115	Awn	180,504,636 a	32.95 ab	14.42 a	33.50 ab	2BL.2BS-2RL
Linkert*4/#284–7 (BC_4F_5)	86	115	Awn	188,977,834 a	29.63 b	14.25 a	31.90 b	2BL.2BS-2RL
Linkert*5/#284–7 (BC_4F_6)	86	115	Awn	196,993,102 a	30.01 ab	14.23 a	32.00 b	2BL.2BS-2RL
Petkus	165	120	Awn	104,026,848 c	13.37 d	12.14 c	25.12 bc	RR
Mean ± SE	98.64 ± 5.8	121.57 ± 2.21		170,815,271 ± 6,927,291	33.08 ± 2.27	13.97 ± 0.3	31.51 ± 1.67	

Numbers followed by the same letters within a column do not differ significantly, as determined by Tukey's post-hoc test at $P < 0.05$. TOTE, total protein concentration; %UPP, gluten strength; TKW, thousand-kernel weight. Nitrogen content (%) was calculated using the nitrogen amount from the DUMAS method and a conversion factor of 5.7.

(Table 2). The BC_4F_5 and BC_4F_6 lines exhibited consistent TOTE and % UPP values in all three backgrounds, indicating the heritability of protein quality (Table 2). DUMAS analysis confirmed the HPLC results, showing no significant differences in total nitrogen (organic and inorganic) or carbon content between the 2BS.2BL-2RL translocation lines and the recurrent parental lines (SLU-Elite, Navruz, and Linkert) (Table 2). The recurrent parents exhibited the highest TKW, while the BC_4F_5 and BC_4F_6 populations showed comparable TKW values, maintaining acceptable grain size and quality (Table 2). These results confirm that the 2BS.2BL-2RL translocation exhibits stable protein composition and desirable agronomic traits, making this translocation well-suited to wheat breeding programs.

3.5. Cytogenetic analysis of line #284 and its progeny

The chromosome constitution of line #284 was examined using FISH. A new T2BS.2BL-2RL wheat-rye translocation chromosome with a short rye segment located at the distal end of the long arm of chromosome 2B was found (Fig. 2C). Cytogenetic analysis was applied to plants from homozygous BC_4F_4 families, selected after extensive testing against multiple *Pgt* races. FISH results confirmed the presence of the T2BS.2BL-2RL wheat-rye translocation chromosome in all three homozygous BC_4F_4 families (Fig. 2D).

3.6. Identification of NLR genes, KASP markers analysis, and the physical positioning of the T2BS.2BL-2RL wheat-rye translocation

We used all parental lines, recurrent parents, and 33 homozygous BC_4F_4 plants (11 from each BC_4F_4 population) for GBS analysis. The GBS reads were aligned to the RefSeq v2.0 and Rye_Lo7 reference genomes to map the putative SNPs for the 2B and 2R chromosomes in wheat and rye, respectively. A total of 4067 SNPs for 2B (ranging from physical positions 11,067 bp to 800,998,610 bp) and 15,116 SNPs for 2R (ranging from physical positions 347,694 bp to 945,773,747 bp) were mapped to their chromosomal positions (Fig. 3). The FISH pattern indicates that the T2BS.2BL-2RL wheat-rye translocation occurred in the distal region of chromosome arm 2RL (Fig. 2). A BLASTN search of three KASP markers (KASP_2RL_c25837C1, KASP_2RL_c20194C2, and KASP_2RL_c21825C1) against the Rye_Lo7 genome placed them between 914,812,226 bp and 943,109,279 bp on chromosome 2R, covering a region of 28 Mb (Fig. 3A). The marker KASP_2RL_c25837C1 located at 914,812,226 bp on 2RL was not amplified in line #284–7,

while the other two KASP markers, KASP_2RL_c20194C2 (928,886,030 bp) and KASP_2RL_c21825C1 (943,109,279 bp), were successfully amplified. The distance between these two amplified KASP markers (KASP_2RL_c20194C2 and KASP_2RL_c21825C1) is 14 Mb (Fig. 3A). Ninety-five full-length annotated rye NLR genes were aligned against the 15,116 SNPs on the 2R chromosome, mapping four NLR genes to 2R chromosome in line SLU238. Among these, the NLR gene Chr2R_NLR_60, located at 945,483,852 bp on 2R in lines SLU238 and TA5094, was also mapped in line #284 and all 33 homozygous BC_4F_4 plants (Fig. 3; Table 3). A 25,681,915 bp region of the distal 2RL was broken and translocated to the 2BL chromosome at position 800,911,418 bp, forming the T2BS.2BL-2RL translocation (Figs. 2C, D, 3). The distance between the KASP_2RL_c21825C1 marker (943,109,279 bp) and the NLR gene Chr2R_NLR_60 (945,483,852 bp) was 2,374,573 bp, indicating that the KASP_2RL_c21825C1 marker is closely located to the Chr2R_NLR_60 gene. Thus, we postulate that the NLR gene Chr2R_NLR_60 is tightly linked with the *Sr59* resistance gene. The SNPs located around the NLR Chr2R_NLR_60 gene positions on 2RL were selected and used to develop KASP markers. KASP markers (KASP_2RL_chr2R_nlr_60_10, KASP_2RL_chr2R_nlr_60_12C1, and KASP_2RL_chr2R_nlr_60_103) clearly identified the 'SLU238 and #284' allele in all three BC_4F_4 populations (Table 3; Fig. 4).

3.7. In-silico functional analysis of genes in the deleted 2BL and translocated 2RL regions

A total of 223 genes were identified in the translocated 2RL region (25,681,915 bp). Ten genes were categorized as potentially significant ($P_{adj} \leq 0.05$) based on functional annotations performed using the g:Profiler web tool (Fig. S2). Among these, seven candidate genes namely, *SECCE2Rv1G0139700* (protein kinase activity), *SECCE2Rv1G0140930* (response to oxidative stress), *SECCE2Rv1G0140960* (reactive oxygen species metabolic process), *SECCE2Rv1G0140850* (hydrogen peroxide catabolic process), *SECCE2Rv1G0141010* (antioxidant activity), *SECCE2Rv1G0141270* (peroxidase activity), and *SECCE2Rv1G0141020* (oxidoreductase activity) were associated with essential functions related to plant disease resistance. The remaining three candidate genes, *SECCE2Rv1G0139530* (phosphotransferase activity), *SECCE2Rv1G0140430* (catabolic process) and *SECCE2Rv1G0140470* (cellular catabolic process) were associated with agronomic performance, contributing to roles in energy production, nutrient recycling, and cellular homeostasis necessary for plant growth and productivity. The analysis of the deleted 2BL region (78,519 bp) revealed only two genes, *TraesCS2B03G1532600* and *TraesCS2B03G1532100*, which encode an unknown protein with no annotated function. Compared to the translocated 2RL region, the deleted 2BL region likely contributes minimal to plant disease resistance.

4. Discussion

We have developed a new wheat-rye introgression line novel translocation chromosome T2BS.2BL-2RL with shortened distal 2RL segment carrying the *Sr59* resistance gene. The line was characterized through stem rust seedling assessments, molecular marker assays, and FISH analysis. Our study identified the NLR gene Chr2R_NLR_60, which is tightly linked to the *Sr59* resistance gene. The *Sr59* gene within this translocation provides effective resistance against multiple stem rust races, making it a valuable genetic resource for enhancing disease resistance in wheat. Previously, the T2DS·2RL whole arm Robertsonian translocation was reported to carry the *Sr59* resistance gene, providing broad-spectrum resistance against stem rust races [23]. The line #284 was identified as possessing putative wheat-2RL homoeologous recombinants in resistant plants, leading to shortened rye chromatin segments detected by two KASP markers [23], though the chromosomal constitution was not determined. The T2BS.2BL-2RL translocation in line #284 conferred broad-spectrum resistance

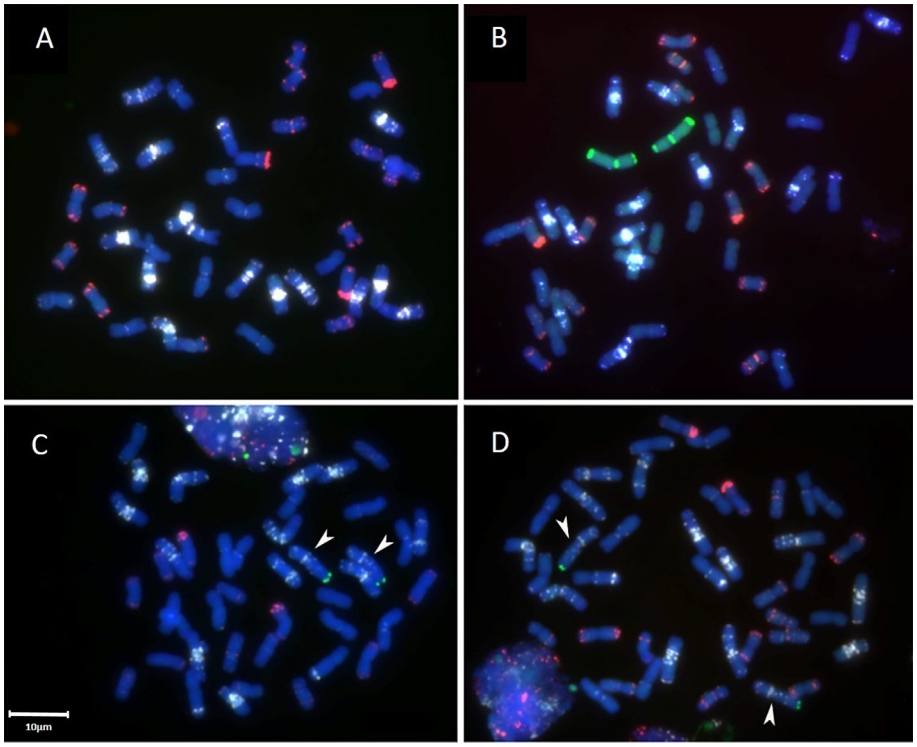

Fig. 2. FISH patterns of mitotic chromosomes in T2BS.2BL-2RL wheat-rye translocation. (A) CS *ph1b*. (B) SLU238 2R (2D). (C) Line #284 with two translocation chromosomes T2BS.2BL-2RL. (D) BC_4F_4 generation with two T2BS.2BL-2RL. Rye chromatin is shown in green, pAs1 repeats are red, (GAA)n repeats are shown in white; arrows are pointing rye introgression segment in translocation chromosome T2BS.2BL-2RL. Scare bar, 10 µm.

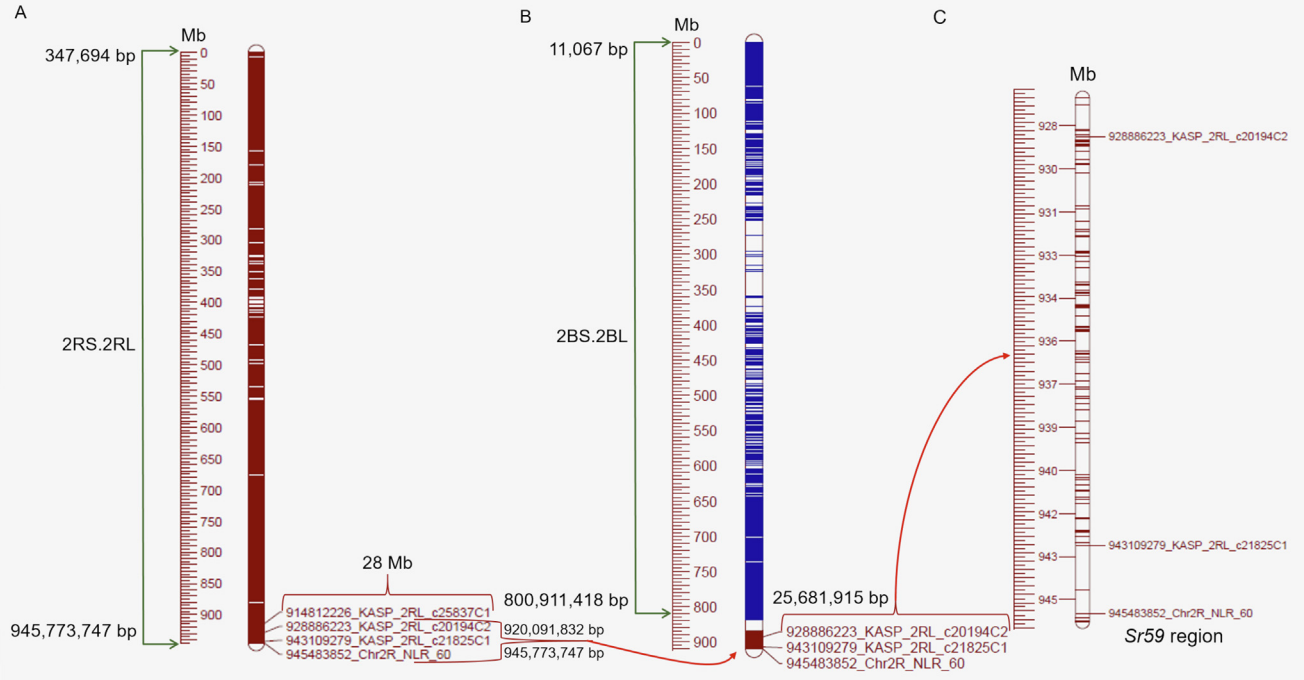

Fig. 3. Physical positions of the *Sr59* resistance gene. (A) Physical position of the 2R chromosome (from 347,694 bp to 945,773,747 bp) with the *Sr59*-linked region in the distal region of 2RL. (B) Physical position of the 2B chromosome (from 11,067 bp to 800,911,418 bp), with the translocated distal 2RL segment with *Sr59* (from 920,091,832 bp to 945,773,747 bp) into the 2B chromosome. (C) Distances between linked SNPs (from 927,534,770 bp to 945,773,747 bp) for *Sr59* in the 2RL segment.

Table 3
KASP markers linked with *Sr59* in the 2RL chromosome.

Primer name	Primer sequence (5′–3′)	SLU2381 Line #284	SLU-Elite Navruz Linkert	SLU-Elite*4/ #284–7	Navruz*4/ #284–7	Linkert*4/ #284–7
KASP_2RL_c20194A1	CCAGCTAGGACAAACTTTGCCTAAA	+	–	+	+	+
KASP_2RL_c20194A2	CAGCTAGGACAAACTTTGCCTAAG	+	–	+	+	+
KASP_2RL_c20194C2	CTTGTGGGCGCTCGTGGCTTT	+	–	+	+	+
KASP_2RL_c21825A1	ACATTTCGGTTGGTATTGATTCTAACG	+	–	+	+	+
KASP_2RL_c21825A2	ACATTTCGGTTGGTATTGATTCTAACC	+	–	+	+	+
KASP_2RL_c21825C1	CCAGCCATGAAGAAAATAACAATTCGAGAT	+	–	+	+	+
KASP_2RL_chr2R_nlr_60_10A1	CGCTCTCACTTACGTAAGCTAGA	+	–	+	+	+
KASP_2RL_chr2R_nlr_60_10A2	CGCTCTCACTTACGTAAGCTAGT	+	–	+	+	+
KASP_2RL_chr2R_nlr_60_10C1	GTGAGGAAGAAGGCCGAAGA	+	–	+	+	+
KASP_2RL_chr2R_nlr_60_12A1	CCTTCTCTTCTCGCTCTCACTTG	+	–	+	+	+
KASP_2RL_chr2R_nlr_60_12A2	CCTTCTCTTCTCGCTCTCACTTA	+	–	+	+	+
KASP_2RL_chr2R_nlr_60_12C1	AAGAACAAGTCTGCAGCAGC	+	–	+	+	+
KASP_2RL_chr2R_nlr_60_103A1	CGTTGGCCATAGTAGCGGC	+	–	+	+	+
KASP_2RL_chr2R_nlr_60_103A2	CGTTGGCCATAGTAGCGGT	+	–	+	+	+
KASP_2RL_chr2R_nlr_60_103C1	TTCTTCTAGCTAGACGGCGC	+	–	+	+	+

For the A1 primer sequence, the FAM tail and for the A2 primer sequence, the HEX tail were added.

to various stem rust races (Table S1), demonstrating the utility of rye chromatin in wheat breeding programs while retaining the *Sr59* resistance gene without adverse effects such as quality, and agronomic performances (Table 2). Wheat chromosome engineering employing homoeologous meiotic recombination between chromosomes of wheat and related species in CS *ph1b* mutant background. It allows significantly shorten alien chromosome introgression segments. Many valuable rust resistance genes, such as *Sr26*, *Sr39*, *Sr43*, *Sr61*, and *Yr83* [29,30,35,36,59] were introgressed to wheat through chromosome engineering. CS does not provide acceptable agronomic performance, thus transferring the desired gene to a wheat genetic background with better agronomic performance is necessary [60]. The new shortened rye translocation segment, discovered in our study was transferred into three elite wheat varieties (SLU-Elite, Navruz, and Linkert) through stem rust seedling screening and marker-assisted backcrossing. Transferring small rye chromatin segments is beneficial as it minimizes linkage drag and reduces the introduction of unwanted traits, making it more suitable for breeding programs for cultivar development [28]. This approach integrates beneficial traits, such as disease resistance, into elite wheat lines while preserving their desirable agronomic characteristics. Background selection on line TA5094 2DS·2RL Robertsonian translocations used marker-assisted backcrossing to recover recurrent parent phenotypes, ensuring desirable traits were retained while incorporating the T2DS·2RL Robertsonian translocations [60]. This approach enabled the accurate selection of plants that maintained key agronomic characteristics, optimizing the integration of the T2BS.2BL-2RL translocation for enhanced resistance and performance, including TKW, protein profiling using HPLC and protein content analysis via the DUMAS method demonstrated that the 2BS.2BL-2RL wheat-rye translocation lines are stable and do not negatively impact end-use quality. T2BS.2RL translocations enhance grain yield and increase resistance to biotic

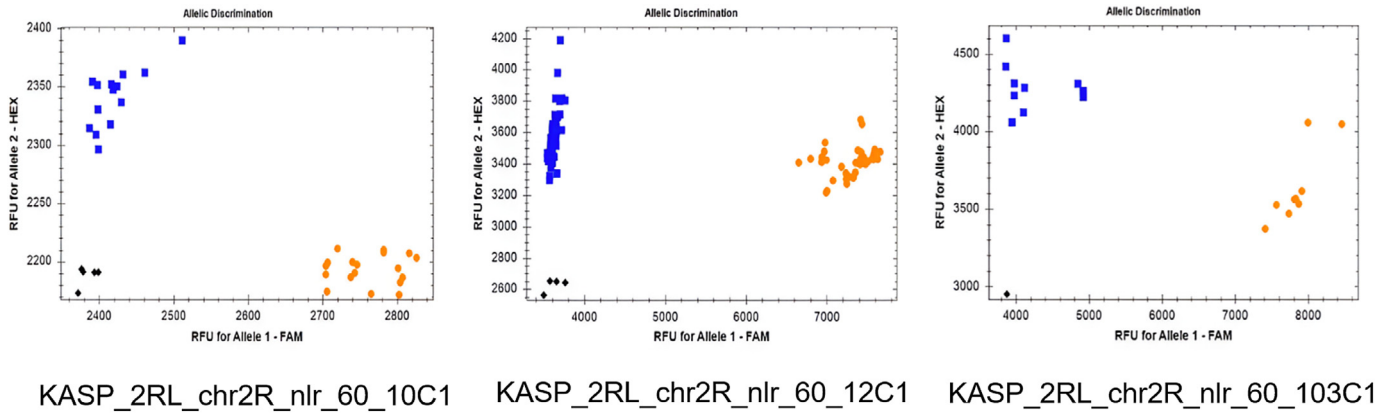

Fig. 4. Allele discrimination plots for the KASP markers were used. For each of the three markers, 'SLU238' and resistant BC_4F_6 plants displayed the 'SLU238' allele, represented by blue squares. The SLU-Elite, Navruz and Linkert and BC_4F_6 susceptible plants displayed a different allele, represented by orange circles, while the no template controls are indicated with black "diamonds".

and abiotic stresses without negatively affecting end-use quality [61]. Also, the T2DS·2RL Robertsonian translocations in elite genetic backgrounds exhibited positive characteristics [60]. Rye contains four loci encoding secalin proteins: *Sec-1* on 1RS, *Sec-2* on 2RS, *Sec-3* on 1RL, and *Sec-4* on 1RS [37]. The absence of secalin genes on the 2RL segment means that transferring this 2RL chromosome region into wheat is less likely to disrupt wheat's gluten properties and end-use quality, making it a more favourable candidate for introgression without compromising baking qualities.

We observed difference in response to rust races between lines SLU238 containing the whole chromosome 2R; TA5094, TA5018 containing rye chromosome arm 2RL of different origin and two lines with the shortened 2RL introgression segments of different origin #284 and TA5084. The ITs of SLU238 and TA5094 are ;1- to ;11+, while line #284 shows ITs ranging from 11+ to 1+2- against the TTKTT, TTRTF, TTKSK, TTTSK, and TRTTF races. Similar differences were observed in a previous study, where SLU238 and TA5094 showed different ITs between TTTTF (IT 2-) and TTKSK (IT ;1) [23]. ITs 3+4 observed in TA5018 (T2BS.2RL) and TA5084 (T2BS.2BL-2RL) indicate their response to *Pgt* races TTTTF, TTRTF, TTKTT, TKTTF, TTKSK, TTTSK, TTKST, and TRTTF (Table S1; Fig. 1). Based on this variation, the 2RL segment from SLU238 may contain one more resistance gene in addition to *Sr59* or an enhancer of *Sr59* resistance. With its high resolution and capacity to generate extensive data, GBS successfully identified the physical locations of the markers KASP_2RL_c25837C1 (914,812,226 bp), KASP_2RL_c20194C2 (928,886,030 bp), and KASP_2RL_c21825C1 (943,109,279 bp). These three KASP markers were mapped within a 28 Mb region in the distal part of the 2RL chromosome, indicating the markers span a physical distance of 28 Mb when aligned to the rye genome (Fig. 3). In line #284, only two of these markers (KASP_2RL_c20194C2 and KASP_2RL_c21825C1) were amplified. A FISH assay was also performed to confirm the presence of the translocation T2BS.2BL-2RL (Fig. 2). Out of 95 full-length annotated 2R-specific NLR genes [38], four NLR genes were identified on the 2R chromosome in the SLU238 line. The NLR gene Chr2R_NLR_60 (945,483,852 bp) is associated with the *Sr59* resistance gene in line #284 and all 33 homozygous BC_4F_4 plants. The results show that the stem rust resistance gene *Sr59* is associated with the distal region of the 2RL chromosome within the T2BS.2BL-2RL translocation and is tightly linked to the NLR gene Chr2R_NLR_60. The KASP assay targeting the NLR Chr2R_NLR_60 gene was used to confirm the presence of the *Sr59* resistance gene (Table 3). Thus, the T2BS.2BL-2RL translocation contains a 25 Mb rye chromatin containing the *Sr59* gene, which is tightly linked to the NLR gene Chr2R_NLR_60. This provides valuable insights into the genetic structure of the small distal region of the 2RL segment with the *Sr59* resistance gene. Previous studies have shown that GBS is an efficient and robust method for discovering genome-wide markers and NLR genes to detect small resistance genes on rye chromosome segments, such as those conferring yellow rust resistance [28], including the *Ph1* deletion (*ph1b*) on the 5BL chromosome [60].

The *in-silico* analysis highlighted the functional importance of the translocated 2RL region, which harbors genes with diverse roles such as in putative plant disease resistance mechanisms, whereas the deleted 2BL region appears to have an unknown genetic contribution to resistance. Candidate genes such as *SECCE2Rv1G0139700*, *SECCE2Rv1G0140930*, *SECCE2Rv1G0140960*, *SECCE2Rv1G0140850*, *SECCE2Rv1G0141010*, *SECCE2Rv1G0141270*, and *SECCE2Rv1G0141020* in the translocated 2RL region encode proteins that are involved in enhancing the plant's ability to recognize, respond and recover from pathogen attacks. These genes are linked to key processes involving reactive oxygen species detoxification, oxidative stress response, and immune signaling, all of which are essential for efficient plant defence mechanisms [62,63]. The other three candidate genes in the translocated 2RL region, *SECCE2Rv1G0139530*, *SECCE2Rv1G0140430*, and *SECCE2Rv1G0140470*, are primarily associated with agronomic performance. These proteins were associated with phosphotransferase activity, catabolic processes, and cellular homeostasis, contribute to plants' energy balance, nutrient recycling, and overall productivity [64,65]. This functional diversity underlines the utility of the 2R chromosome as a valuable genetic resource for wheat improvement [22,23,60]. The identified functions, consisting of peroxidase activity, protein kinase activity, hydrogen peroxide catabolic processes, cysteine-type endopeptidase inhibitor activity, and alpha-tubulin binding, have been consistently linked to plant resistance and stress management mechanisms [66–70]. These genes collectively enhance the plant's ability to mitigate oxidative stress, activate immune signaling pathways, and maintain structural stability under pathogen-induced stress conditions [65]. These functions contribute to plant disease resistance mechanisms and agronomic performance, demonstrating the dual utility of the translocated 2RL chromosome segment for enhancing disease resistance and crop productivity. The lack of annotated resistance-related genes in the 2BL region replaced by the 2RL translocation further suggests that the deleted 2BL interval contributes minimally to the plant's defense mechanisms. This underlines the relevance of the translocated 2RL chromosome segment to improve stem rust resistance in wheat.

This study reports the development of a new wheat-rye T2BS.2BL-2RL translocation carrying the *Sr59* resistance gene and

its associated NLR gene, Chr2R_NLR_60, for wheat improvement. Our findings show that transferring small rye chromatin segments minimizes linkage drag and reduces unwanted traits, rendering these lines suitable for wheat breeding programs. The absence of secalin genes in the 2RL segment maintains wheat's gluten properties and end-use quality attributes. The broad-spectrum resistance and genetic stability of the *Sr59* gene make it a valuable resource for enhancing wheat resistance without compromising agronomic performance or quality attributes. These insights into the genetic structure of the T2BS.2BL-2RL translocation demonstrate its potential for improving disease resistance in practical breeding programs, aiding the development of high-performance, stem rust-resistant wheat varieties.

CRediT authorship contribution statement

Mahboobeh Yazdani: Writing – original draft, Methodology, Investigation. **Matthew N. Rouse:** Data curation. **Prabin Bajgain:** Formal analysis, Data curation. **Tatiana V. Danilova:** Formal analysis, Data curation. **Ivan Motsnyi:** Validation. **Brian J. Steffenson:** Data curation. **Mehran Patpour:** Validation, Investigation. **Mahbubjon Rahmatov:** Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We gratefully acknowledge the financial support from FORMAS (2018-01029). We thank the Swedish Institute (01132-2022) for supporting Ivan Motsnyi's visit and research at Swedish University of Agricultural Sciences. We also thank the Global Rust Reference Center at Aarhus University, the University of Minnesota, and the USDA Agricultural Research Service's Cereal Disease Laboratory for their support with stem rust seedling resistance analysis. We appreciate the genotyping services provided by the University of Minnesota Genomics Center and the computational resources supplied by the University of Minnesota Supercomputing Institute.

Appendix A. Supplementary data

Supplementary data for this article can be found online at https://doi.org/10.1016/j.cj.2025.02.012.

References

[1] International Wheat Genome Sequencing Consortium, Shifting the limits in wheat research and breeding using a fully annotated reference genome, Science 361 (2018) eaar7191.
[2] S. Savary, L. Willocquet, S.J. Pethybridge, P. Esker, N. McRoberts, A. Nelson, The global burden of pathogens and pests on major food crops, Nat. Ecol. Evol. 3 (2019) 430.
[3] P. Pardey, J. Beddow, D. Kriticos, T. Hurley, R. Park, E. Duveiller, R. Sutherst, J. Burdon, D. Hodson, Right-sizing stem-rust research, Science 340 (2013) 147–148.
[4] Z. Pretorius, R. Singh, W. Wagoire, T. Payne, Detection of virulence to wheat stem rust resistance gene Sr31 in *Puccinia graminis* f. sp. *tritici* in Uganda, Plant Dis. 84 (2000) 203.
[5] R.P. Singh, D.P. Hodson, Y. Jin, E.S. Lagudah, M.A. Ayliffe, S. Bhavani, M.N. Rouse, Z.A. Pretorius, L.J. Szabo, J. Huerta-Espino, B.R. Basnet, C. Lan, M.S. Hovmøller, Emergence and spread of new races of wheat stem rust fungus: continued threat to food security and prospects of genetic control, Phytopathology 105 (2015) 872–884.
[6] M. Patpour, S. Baidya, R. Basnet, A.F. Justesen, D. Hodson, D.B. Thapa, M.S. Hovmoller, First report of Ug99 wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* in south asia, Plant Dis. 108 (2024), https://doi.org/10.1094/Pdis-03-24-0644-Pdn.
[7] S. Bhattacharya, Deadly new wheat disease threatens Europe's crops, Nature 542 (2017) 145.
[8] P. Olivera, M. Newcomb, L.J. Szabo, M. Rouse, J. Johnson, S. Gale, D.G. Luster, D. Hodson, J.A. Cox, L. Burgin, M. Hort, C.A. Gilligan, M. Patpour, A.F. Justesen, M.S. Hovmoller, G. Woldeab, E. Hailu, B. Hundie, K. Tadesse, M. Pumphrey, R.P. Singh, Y. Jin, Phenotypic and genotypic characterization of race TKTTF of *Puccinia graminis* f. sp *tritici* that caused a wheat stem rust epidemic in southern ethiopia in 2013-14, Phytopathology 105 (2015) 917–928.
[9] P.D. Olivera, Z. Sikharulidze, R. Dumbadze, L.J. Szabo, M. Newcomb, K. Natsarishvili, M.N. Rouse, D.G. Luster, Y. Jin, Presence of a sexual population of *Puccinia graminis* f. sp. *tritici* in georgia provides a hotspot for genotypic and phenotypic diversity, Phytopathology 109 (2019) 2152–2160.
[10] M.S. Hovmøller, M. Patpour, J. Rodriguez-Algaba, T. Thach, A.F. Justesen, J.G. Hansen, GRRC Report of Yellow and Stem Rust Genotyping and Race Analyses 2020, Aarhus University, Flakkebjerg, Denmark, 2021.
[11] R. Johnson, Durable resistance - definition of, genetic-control, and attainment in plant-breeding, Phytopathology 71 (1981) 567–568.
[12] R. Johnson, A critical analysis of durable resistance, Ann. Rev. Phytopathol. 22 (1984) 309–330.
[13] C. Sansaloni, J. Franco, B. Santos, L. Percival-Alwyn, S. Singh, C. Petroli, J. Campos, K. Dreher, T. Payne, D. Marshall, B. Kilian, I. Milne, S. Raubach, P. Shaw, G. Stephen, J. Carling, C. Saint Pierre, J. Burgueno, J. Crosa, H.H. Li, C. Guzman, Z. Kehel, A. Amri, A. Kilian, P. Wenzl, C. Uauy, M. Banziger, M. Caccamo, K. Pixley, Diversity analysis of 80,000 wheat accessions reveals consequences and opportunities of selection footprints, Nat. Commun. 11 (2020) 4572.
[14] K. Moskal, S. Kowalik, W. Podyma, B. Łapiński, M. Boczkowska, The pros and cons of rye chromatin introgression into wheat genome, Agronomy 11 (2021) 456.
[15] R. Mago, P. Zhang, S. Vautrin, H. Šimková, U. Bansal, M. Luo, The wheat *Sr50* gene reveals rich diversity at a cereal disease resistance locus, Nat. Plant 1 (2015) 15186.
[16] G.F. Marais, A.S. Marais, The derivation of compensating translocations involving homoeologous group-3 chromosomes of wheat and rye, Euphytica 79 (1994) 75–80.
[17] T.T. The, R.B. Gupta, P.L. Dyck, R. Appels, U. Hohmann, R.A. Mcintosh, Characterization of stem rust resistant derivatives of wheat cultivar amigo, Euphytica 58 (1991) 245–252.
[18] E. Johansson, T. Henriksson, M.L. Prieto-Linde, S. Andersson, R. Ashraf, M. Rahmatov, Diverse wheat-alien introgression lines as a basis for durable resistance and quality characteristics in bread wheat, Front. Plant Sci. 11 (2020) 1067.
[19] S. Rabinovich, Importance of wheat-rye translocations for breeding modern cultivars of *Triticum aestivum* L., Euphytica 100 (1998) 323–340.
[20] A. Merker, The rye genome in wheat breeding, Hereditas 100 (1984) 183–191.
[21] A. Merker, The breeding behaviour of some rye wheat chromosome substitutions, Hereditas 91 (1979) 245–255.
[22] M. Rahmatov, M.N. Rouse, B.J. Steffenson, S.C. Andersson, R. Wanyera, Z.A. Pretorius, A. Houben, N. Kumarse, S. Bhavani, E. Johansson, Sources of stem rust resistance in wheat-alien introgression lines, Plant Dis. 100 (2016) 1101–1109.
[23] M. Rahmatov, M.N. Rouse, J. Nirmala, T. Danilova, B. Friebe, B.J. Steffenson, E. Johansson, A new 2DS·2RL Robertsonian translocation transfers stem rust resistance gene *Sr59* into wheat, Theor. Appl. Genet. 129 (2016) 1383–1392.
[24] T. Mellosampayo, Genetic regulation of meiotic chromosome pairing by chromosome-3d of *Triticum-aestivum*, Nat. New Biol. 230 (1971) 22–23.
[25] R. Riley, V. Chapman, Genetic control of the cytologically diploid behaviour of hexaploid wheat, Nature 182 (1958) 713–715.
[26] B. Giorgi, F. Barbera, Chromosome pairing in hybrids between a ph mutant of triticum durum cultivar cappelli and 4 tetra ploid species of aegilops, Genet. Agraria 35 (1981) 68–69.
[27] E.R. Sears, Induced mutant with homoeologous pairing in common wheat, Can. J. Genet. Cytol. 19 (1977) 585–593.
[28] R. Ashraf, E. Johansson, P. Vallenback, B.J. Steffenson, P. Bajgain, M. Rahmatov, Identification of a small translocation from 6R possessing stripe rust resistance to wheat, Plant Dis. 107 (2023) 720–729.
[29] H. Li, A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data, Bioinformatics 27 (2011) 2987–2993.
[30] Z.X. Niu, D.L. Klindworth, T.L. Friesen, S.M. Chao, Y. Jin, X.W. Cai, S.S. Xu, Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering, Genetics 187 (2011) 1011–1068.
[31] H.H. Flor, Host-parasite interaction in flax rust - its genetics and other implications, Phytopathology 45 (1955) 680–685.
[32] J.D.G. Jones, J.L. Dangl, The plant immune system, Nature 444 (2006) 323–329.
[33] J.S. Sharma, M.Z. Che, T. Fetch, B.D. Mccallum, S.S. Xu, C.W. Hiebert, Identification of Sr67, a new gene for stem rust resistance in KU168-2 located close to the *Sr13* locus in wheat, Theor. Appl. Genet. 137 (2024) 30.
[34] Y.Q. Zhang, D. Edwards, J. Batley, Comparison and evolutionary analysis of nucleotide binding site leucine rich repeat (NLR) genes and importance for disease resistance breeding, Plant Genome 14 (2021) e20060.
[35] J.P. Zhang, T.C. Hewitt, W.H.P. Boshoff, I. Dundas, N. Upadhyaya, J.B. Li, M. Patpour, S. Chandramohan, Z.A. Pretorius, M. Hovmoller, W. Schnippenkoetter, R.F. Park, R. Mago, S. Periyannan, D. Bhatt, S. Hoxha, S. Chakraborty, M. Luo, P.

Dodds, B. Steuernagel, B.B.H. Wulff, M. Ayliffe, R.A. McIntosh, P. Zhang, E.S. Lagudah, A recombined *Sr26* and *Sr61* disease resistance gene stack in wheat encodes unrelated NLR genes, Nat. Commun. 12 (2021) 3378.
[36] J.P. Zhang, P. Zhang, T. Hewitt, J.B. Li, I. Dundas, W. Schnippenkoetter, S. Hoxha, C.H. Chen, R. Park, E. Lagudah, A strategy for identifying markers linked with stem rust resistance in wheat harbouring an alien chromosome introgression from a non-sequenced genome, Theor. Appl. Genet. 132 (2019) 125–135.
[37] G.W. Li, L.J. Wang, J.P. Yang, H. He, H.B. Jin, X.M. Li, T.H. Ren, Z.L. Ren, F. Li, X. Han, X.G. Zhao, L.L. Dong, Y.W. Li, Z.P. Song, Z.H. Yan, N.N. Zheng, C.L. Shi, Z.H. Wang, S.L. Yang, Z.J. Xiong, M.L. Zhang, G.H. Sun, X. Zheng, M.Y. Gou, C.M. Ji, J.K. Du, H.K. Zheng, J. Dolezel, X.W. Deng, N. Stein, Q.H. Yang, K.P. Zhang, D.W. Wang, A high-quality genome assembly highlights rye genomic characteristics and agronomically important genes, Nat. Genet. 53 (2021) 574–584.
[38] M.T. Rabanus-Wallace, B. Hackauf, M. Mascher, T. Lux, T. Wicker, H. Gundlach, M. Baez, A. Houben, K.F. Mayer, L. Guo, Chromosome-scale genome assembly provides insights into rye biology, evolution and agronomic potential, Nat. Genet. 53 (2021) 564–573.
[39] B. Steuernagel, K. Witek, S.G. Krattinger, R.H. Ramirez-Gonzalez, H.J. Schoonbeek, G. Yu, E. Baggs, A.I. Witek, I. Yadav, K.V. Krasileva, J.D.G. Jones, C. Uauy, B. Keller, C.J. Ridout, B.B.H. Wulff, The NLR-annotator tool enables annotation of the intracellular immune receptor repertoire, Plant Physiol. 183 (2020) 468–482.
[40] M.N. Rouse, R. Wanyera, P. Njau, Y. Jin, Sources of resistance to stem rust race Ug99 in spring wheat germplasm, Plant Dis. 95 (2011) 762–766.
[41] E.C. Stakman, D.M. Stewart, W.Q. Loegering, Identification of Physiologic Races of *Puccinia graminis* var. *tritici*, United States Department of Agriculture, Agricultural Research Service, Beltsville, MD, USA, 1962.
[42] M. Rahmatov, M.N. Rouse, J. Nirmala, T. Danilova, B. Friebe, B.J. Steffenson, E. Johansson, A new 2DS.2RL Robertsonian translocation transfers stem rust resistance gene *Sr59* into wheat, Theor. Appl. Genet. 129 (2016) 1383–1392.
[43] J. Cainong, L. Zavatsky, M. Chen, J. Johnson, B. Friebe, B. Gill, A. Lukaszewski, Wheat-rye T2BS·2BL-2RL recombinants with resistance to hessian fly (H21), Crop Sci. 50 (2010) 920–925.
[44] J.A. Poland, P.J. Brown, M.E. Sorrells, J.L. Jannink, Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach, PLoS ONE 7 (2012) e32253.
[45] R.J. Elshire, J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler, S.E. Mitchell, A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species, PLoS ONE 6 (2011) e19379.
[46] H. Li, R. Durbin, Fast and accurate short read alignment with burrows–wheeler transform, Bioinformatics 25 (2009) 1754–1760.
[47] P.J. Bradbury, Z. Zhang, D.E. Kroon, T.M. Casstevens, Y. Ramdoss, E.S. Buckler, TASSEL: software for association mapping of complex traits in diverse samples, Bioinformatics 23 (2007) 2633–2635.
[48] L. Kolberg, U. Raudvere, I. Kuzmin, P. Adler, J. Vilo, H. Peterson, g:Profiler-interoperable web service for functional enrichment analysis and gene identifier mapping (2023 update), Nucleic Acids Res. 51 (2023) W207-W212.
[49] M. González-García, M. Cuacos, M. González-Sánchez, M.J. Puertas, J.M. Vega, Painting the rye genome with genome-specific sequences, Genome 54 (2011) 555–564.
[50] M.G. Francki, Identification of a diverged centromeric *Ty1* retrotransposon family from cereal rye, Genome 44 (2001) 266–274.
[51] Bedbrook JR, Jones J, O'Dell M, Thompson RD, F. RB, A molecular description of telomeric heterochromatin in Secale species, Cell 19 (1980) 545–560.
[52] Lapitan NLV, Sears RG, Rayburn AL, G. BS, Wheat-rye translocations: detection of chromosome breakpoints by in situ hybridization with a biotin-labeled DNA probe, J. Hered. 77 (1986) 415–419.
[53] T.V. Danilova, B. Friebe, B.S. Gill, Single-copy gene fluorescence in situ hybridization and genome analysis: acc-2 loci mark evolutionary chromosomal rearrangements in wheat, Chromosoma 121 (2012) 597–611.
[54] A. Kato, P.S. Albert, J.M. Vega, J.A. Birchler, Sensitive fluorescence hybridization signal detection in maize using directly labeled probes produced by high concentration DNA polymerase nick translation, Biotech. Histochem. 81 (2006) 71–78.
[55] S.K. Gupta, H. Singh, A.C. Varshney, P. Prakash, S.P. Singh, Biochemical-alterations during wound-healing under the influence of natural honey and ampicillin in buffalos, Indian Vet. J. 70 (1993) 45–47.
[56] E. Johansson, M.-L. Prieto-Linde, R. Kuktaite, A. Andersson, J. Jönsson, G. Svensson, Breeding for improved stability in bread-making quality, in: S.P. Cauvain, S.S. Salmon, L.S. Young , Woodhead Publishing Series in Food Science, Technology and Nutrition, Using Cereal Science and Technology for the Benefit of Consumers, Woodhead Publishing, Cambridge, UK, 2005, pp. 44–48.
[57] E. Johansson, A.H. Malik, A. Hussain, F. Rasheed, W.R. Newson, T. Plivelic, M.S. Hedenqvist, M. Gallstedt, R. Kuktaite, Wheat gluten polymer structures: the impact of genotype, environment, and processing on their functionality in various applications, Cereal Chem. 90 (2013) 367–376.
[58] A. Hussain, H. Larsson, R. Kuktaite, E. Johansson, Mineral composition of organically grown wheat genotypes: contribution to daily minerals intake, Int. J. Environ. Res. Public Health 7 (2010) 3442–3456.
[59] Z. Niu, D.L. Klindworth, G. Yu, T.L. Friesen, S. Chao, Y. Jin, X. Cai, J.B. Ohm, J.B. Rasmussen, S.S. Xu, Development and characterization of wheat lines carrying stem rust resistance gene Sr43 derived from *Thinopyrum ponticum*, Theor. Appl. Genet. 127 (2014) 969–980.
[60] M. Yazdani, M.N. Rouse, B.J. Steffenson, P. Bajgain, M. Patpour, E. Johansson, M. Rahmatov, Developing adapted wheat lines with broad-spectrum resistance to stem rust: introgression of through backcrossing and selections based on genotyping-by sequencing data, PLoS ONE 18 (2023) e0292724.
[61] S.C. Hysing, S.L.K. Hsam, R.P. Singh, J. Huerta-Espino, L.A. Boyd, R.M.D. Koebner, S. Cambron, J.W. Johnson, D.E. Bland, E. Liljeroth, A. Merker, Agronomic performance and multiple disease resistance in T2BS.2RL wheat-rye translocation lines, Crop Sci. 47 (2007) 254–260.
[62] K. Apel, H. Hirt, Reactive oxygen species: metabolism, oxidative stress, and signal transduction, Annu. Rev. Plant Biol. 55 (2004) 373–399.
[63] R. Mittler, S. Vanderauwera, M. Gollery, F. van Breusegem, Reactive oxygen gene network of plants, Trends Plant Sci. 9 (2004) 490–498.
[64] B. Heinemann, T.M. Hildebrandt, The role of amino acid metabolism in signaling and metabolic adaptation to stress-induced energy deficiency in plants, J. Exp. Bot. 72 (2021) 4634–4645.
[65] Y. Poirier, A. Jaskolowski, J. Clúa, Phosphate acquisition and metabolism in plants, Curr. Biol. 32 (2022) R623–R629.
[66] D. Breviario, P. Nick, Plant tubulins: a melting pot for basic questions and promising applications, Transgenic Res. 9 (2000) 383–393.
[67] G. Ievinsh, Characterization of the peroxidase system in winter rye seedlings - compartmentation and dependence on leaf development and hydrogen donors used, J. Plant Physiol. 140 (1992) 257–263.
[68] M. Martínez, I. Cambra, P. González-Melendi, M.E. Santamaría, I. Díaz, C1A cysteine-proteases and their inhibitors in plants, Physiol. Plant. 145 (2012) 85–94.
[69] L.J. Niu, W.B. Liao, Hydrogen peroxide signaling in plant development and abiotic responses: crosstalk with nitric oxide and calcium, Front. Plant Sci. 7 (2016) 230.
[70] M. Tomita, H. Tanaka, K. Takahashi, ABA-induced serine/threonine protein kinase gene transcribed in rye (*Secale cereale* L.), Cereal Res. Commun. 49 (2021) 21–30.