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Marker-assisted selection for combining stem rust and stripe rust resistance in wheat using rye derived genes

Mahboobeh Yazdani[1] | **Rimsha Ashraf**[1] | **Eva Johansson**[1] | **Pernilla Vallenback**[2] | **Mogens S. Hovmøller**[3] | **Mehran Patpour**[3] | **Mahbubjon Rahmatov**[1]

[1]Department of Plant Breeding, Swedish University of Agricultural Sciences, Lomma, Sweden

[2]Lantmännen Lantbruk, Svalöv, Sweden

[3]Department of Agroecology, Aarhus University, Slagelse, Denmark

Correspondence
Mahbubjon Rahmatov, Department of Plant Breeding, Swedish University of Agricultural Sciences, P.O Box 190, SE-234 22 Lomma, Sweden.
Email: Mahbubjon.Rahmatov@slu.se

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Abstract

Stem rust and stripe rust are among the most devastating wheat (*Triticum aestivum* L.) diseases globally. This study used marker-assisted selection to incorporate two resistance genes, *Sr59* and *YrSLU* (where SLU is Swedish University of Agriculture Science), derived from rye (*Secale cereale* L.), into elite wheat backgrounds. The initial cross combined *Sr59* from line *TA5095* and *YrSLU* from line #392 using kompetitive allele-specific PCR (KASP) markers. A three-step crossing scheme integrated these genes into adapted wheat lines. F_1 plants were crossed with the commercial wheat variety Linkert, followed by top-crossing with Navruz. Selected progeny were double top-crossed with an elite breeding line, SLU-Elite, producing generations TT_1 to TT_4 through self-pollination. Plants containing *Sr59* and *YrSLU* were identified at each generation using KASP markers. Field trials in the TT_5 generation assessed agronomic performance and increased seed production. In the TT_6 and TT_7 generations, seedling resistance tests confirmed that *Sr59* conferred robust resistance to *Pgt* races TTKSK, TTRTF, and TTTTF. *YrSLU* provided resistance against *Pst* races *Psts10*, *Psts16*, *Psts7*, and *Psts13*. However, TT_6 remained segregated for resistance to *Pst* race *Psts7* (Warrior). By TT_7, consistent resistance to *Psts7* was observed in pyramided lines. This study shows the effectiveness of crossing schemes integrating rye-derived resistance genes into wheat. KASP markers enabled precise selection, combining enhanced disease resistance with elite agronomic traits. These findings demonstrate a practical approach to improving wheat's resilience to rust diseases through targeted breeding.

Abbreviations: CSA, Chinese Spring; DT, double top-cross; GBS, genotyping-by-selection; KASP, kompetitive allele-specific PCR; LD, linkage disequilibrium; MAS, marker-assisted selection; *Pgt*, *Puccinia graminis* f. sp. *tritici*; *Pst*, *Puccinia striiformis* f. sp. *tritici*; SLU, Swedish University of Agriculture Science; TT, triple top-cross.

Mahboobeh Yazdani and Rimsha Ashraf contributed equally to this work and share first authorship.

Plain Language Summary

Stem rust and stripe rust are serious wheat diseases that threaten global wheat production. This study focused on improving wheat's resistance to these diseases by combining two resistance genes, *Sr59* and *YrSLU* (where SLU is Swedish University of Agriculture Science), from rye into elite wheat varieties. Using advanced genetic tools called kompetitive allele-specific PCR (KASP) markers, the researchers successfully identified and tracked these genes during breeding. The process began by crossing two wheat lines, one carrying *Sr59* and the other carrying *YrSLU*. The offspring were then crossed with commercial wheat varieties Linkert and Navruz, followed by further crossing with an elite breeding line, SLU-Elite. This multi-step breeding method developed new wheat lines with improved resistance. This study describes how traditional plant breeding and modern genetic tools can develop disease-resistant and high-performing wheat varieties. As a result, wheat's resilience to stem and stripe rust can be enhanced, providing hope for more sustainable wheat production.

1 | INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the three major crops (together with maize and rice) globally, contributing around 20% of the calories and proteins consumed by the entire human population (Hawkesford et al., 2013). In total, more than 780 million metric tons of wheat was produced in 2023 alone (USDA Foreign Agricultural Service, 2023/2024). Therefore, wheat evidently plays a critical role in global food security (Reynolds & Braun, 2022). However, yield loss caused by pathogens and pests is a serious threat to this. The Food and Agriculture Organization estimates that plant diseases and pests contribute to approximately 40% of annual global food production losses, with an economic impact of around $220 billion (IPPC-Secretariat, 2021). Fungal diseases are major contributors, responsible for over 20% of annual global wheat yield loss (Savary et al., 2019). Thus, there is an urgent need for researchers and breeders to identify and develop wheat varieties that are resistant to these diseases.

Among the various wheat diseases, the three rust diseases, stem rust (caused by *Puccinia graminis* f. sp. *tritici* Erikss. & E. Henning [*Pgt*]), stripe rust (*Puccinia striiformis* West-end. f. sp. *tritici* Eriks. [*Pst*]), and leaf rust (*Puccinia triticina* Eriks.), are known to pose substantial challenges to global wheat production. These diseases have triggered numerous epidemics throughout history, resulting in widespread crop losses (Hovmøller et al., 2011; Szabo et al., 2014). In susceptible wheat cultivars, *Pgt* and *Pst* can lead to up to 100% yield losses (Singh et al., 2015), while leaf rust can result in up to 70% (Huerta-Espino et al., 2011), causing significant economic losses, for example, $979 million per year for *Pst* infections (Beddow et al., 2015). The frequent emergence of new *Pgt* races, such as the Ug99 race group (TTKSK), and *Pst* races, such as Warrior (*PstS7*) and Kranich (*PstS8*), present considerable challenges for the breeding of wheat with resistance to *Pgt* and *Pst* (Hovmøller et al., 2021; Hovmøller et al., 2016; Patpour, Hovmøller et al., 2022; Patpour, Rahmatov et al., 2022). Thus, the identification of novel resistance genes to the emerging races, along with the development of new wheat varieties holding broad-spectrum resistance, is crucial (Rahmatov, 2016). The incorporation of novel resistance genes into adapted genotypes is an effective, economic, and environmentally friendly approach to sustain wheat production despite the emergence of novel *Pgt* and *Pst* races (Babu et al., 2020; Yazdani et al., 2023). In this context, cultivated and wild relatives of wheat serve as reservoirs of valuable genes with the potential to contribute significantly to wheat improvement (Ashraf et al., 2023; Johansson, Henriksson, et al., 2020; Rahmatov, 2016). The opportunity to combine multiple genes into a single genotype for the simultaneous expression of multiple desirable traits (Lombardo et al., 2016) provides additional benefits and extensive protection from a wide range of pathogens. In fact, the assembly of several resistance genes into the same cultivar is a practical tool to sustainably control crop diseases (Zhang et al., 2018). Several studies have demonstrated that the presence of multiple resistance genes to different or race-specific diseases in the same cultivar contributes to resistance durability and efficiency (Ellis et al., 2014; Paillard et al., 2012). The multi-gene pyramiding approach has been proven effective in resistance breeding in various crops such as rice (Y. Liu et al., 2016), maize (Zhu et al., 2018), wheat (Huang et al., 2012; I. J. Mackay et al., 2014), and soybean (D.-G. Wang et al., 2017). Furthermore, combining several resistance genes in the same

cultivar against the same disease has exhibited beneficial results in wheat. For example, a combination of *Pgt* resistance genes, that is, *Sr22, Sr25, Sr26, Sr33, Sr35, Sr45*, and *Sr50*, were found to contribute potential resistance against Ug99 in Africa and Asia (Hatta et al., 2021; Sibikeev et al., 2021; Wu et al., 2020). Combining multiple resistance genes, such as *Yr15*, *Yr65*, *Yr9* + *Yr18*, *Yr30* + *Yr46*, *Yr26* + *Yr48*, *Yr30* + *Yr64*, *Yr30* + *Yr48*, *Yr26* + *Yr64*, and *Yr5* + *YrSP*, has significantly improved wheat varieties' resistance to *Pst* (R. Liu et al., 2020; Zheng et al., 2017). These findings indicate that the combination of several specific effective resistance genes is an efficient method for establishing long-lasting resistance.

As described in previous studies (I. Mackay & Powell, 2007), linkage disequilibrium (LD) mapping has proven to be a valuable tool for locating quantitative trait loci in plants by correlating a trait with a genetic marker. However, the effectiveness of LD mapping in populations of modern cultivars and wild relatives is dependent on how LD decays with genetic distance. Recently, novel genome-based and marker-assisted selections (MASs) have been developed (Kondić-Špika et al., 2023), providing opportunities to identify new resistance genes for transfer into wheat from alien species. The use of Chinese Spring *ph1b*-induced lines along with genotyping-by-selection (GBS) and the development of kompetitive allele-specific PCR (KASP) markers has proven to be highly valuable (Rahmatov, 2016; Rahmatov, Rouse, Nirmala, et al., 2016; Yazdani et al., 2023). Thus, the *ph1b*-induced lines promote DNA recombination, thereby allowing the transfer of target genes to a suitable genetic background (Rahmatov, 2016). GBS provides detailed genomic information, which is used for selecting a suitable genetic background (Yazdani et al., 2023) and mapping genes, and for developing specific KASP markers. The KASP markers enable efficient tracking of target genes across different generations in breeding (Rahmatov, Rouse, Nirmala, et al., 2016).

This study aimed to (1) develop a set of novel wheat lines with enhanced resistance to *Pgt* and *Pst* by combining recently identified resistance genes: *Sr59* (Rahmatov, Rouse, Nirmala, et al., 2016) for *Pgt* and *YrSLU* (where SLU is Swedish University of Agriculture Science) (Ashraf et al., 2023) for *Pst*; (2) use MAS to transfer these genes into an adapted genetic background; and (3) evaluate the resistance of lines carrying both genes against the currently prevalent *Pst* and *Pgt* races and their field performance.

Core Ideas

- Employing marker-assisted selection to develop wheat lines with novel resistance genes to stem rust and stripe rust.
- Transfer of the resistance genes from rye into an adapted through multiple crossing with commercial cultivars.
- Using kompetitive allele-specific PCR (KASP) markers for selection of candidate plants containing *Sr* and *Yr* resistance genes.

2 | MATERIALS AND METHODS

2.1 | Plant materials and population development

The plant material used in this study comprised (i) line TA5095 carrying the *Sr59* gene as a Robertsonian translocation on chromosome 2R, providing resistance to a wide range of *Pgt* races (Rahmatov, Rouse, Nirmala, et al., 2016); (ii) line #392 carrying *YrSLU* contributing resistance to *Pst*, as a small translocation on chromosome 6R (Ashraf et al., 2023); and (iii) three spring wheat varieties, Linkert (Reg. No. CV-1137, PI 672164; provided by Prof. Jim Anderson from the University of Minnesota), Navruz (from the National Wheat Breeding Program in Tajikistan), and SLU-Elite (from the collection of the late Professor Arnulf Merker at SLU). The lines TA5095 (T2DS·2RL translocation) and #392 (6DS·6DL·6RL·6DL translocation) are both Chinese Spring *ph1b*-induced lines, which resulted in wheat–rye translocations (Ashraf et al., 2023; Rahmatov, Rouse, Nirmala, et al., 2016). Previous studies have shown that TA5095, which carries the *Sr59* gene, provides stable resistance to all known *Pgt* races, including the newly emerged Ug99 races and Sicily races, as confirmed by three KASP markers: 20194C2, 21825C, 375C1, and 387C2 (Rahmatov, Rouse, Nirmala, et al., 2016; Yazdani et al., 2023). Line #392, an offspring of family 29-N3-5 derived from SLU126, has displayed resistance to *Pst* races PSTv-14, PSTv-37, PSTv-40, and PSTv-221, as confirmed by KASP markers 375C1, 387C2, and 392CA (Ashraf et al., 2023). Three spring wheat varieties were used in a crossing strategy to combine the *Sr59* gene (from TA5095) and *YrSLU* gene (from #392) into an adapted genetic background within the same genotype (Figure 1). Linkert is a leading commercial wheat variety in Minnesota (Anderson et al., 2018), Navruz is a prominent commercial variety in Tajikistan, and SLU-Elite is a Swedish elite breeding line. The top-crossing strategy, involving wheat lines from Minnesota, Tajikistan, and Sweden, was designed to introduce genetic diversity simultaneously. An F_1 progeny (60 F_1 seeds) was first generated from a cross between TA5095 and #392. This F_1 progeny was then crossed with Linkert, followed by a subsequent cross with the Navruz variety (top-cross [T]). The resulting offspring (double top-cross [DT]) were crossed with SLU-Elite (Figure 1). The triple top-cross (TT) progeny was self-pollinated to both increase homozygosity and reduce *ph1bM* background. For KASP marker assay, plant genomic DNA was extracted from young leaves using

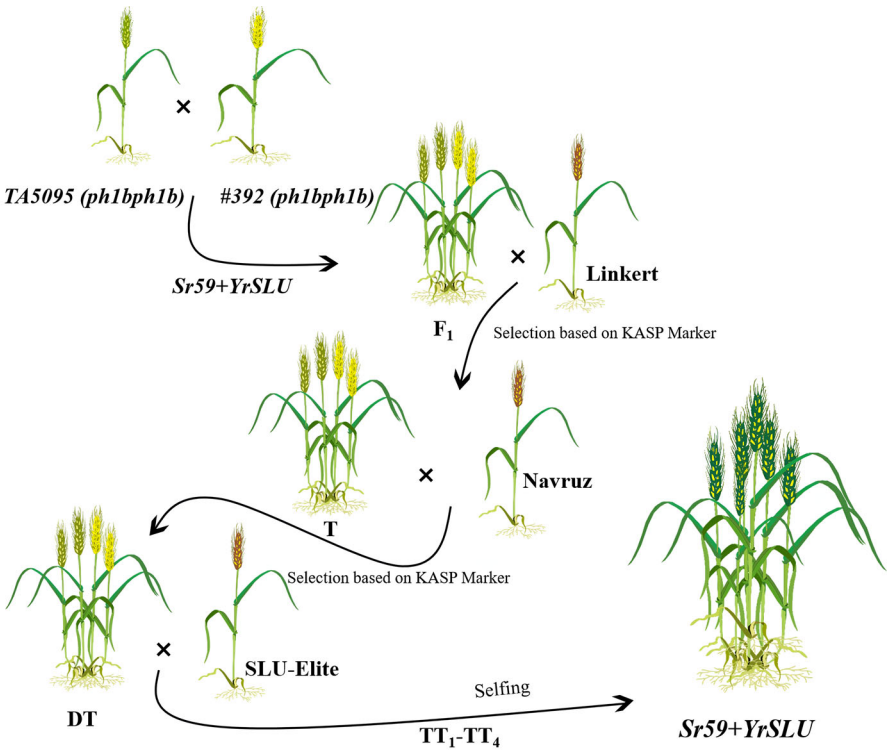

FIGURE 1 Scheme marker-assisted gene pyramiding performed for stem rust and stripe rust resistance, including top-cross (T), double top-cross (DT), triple top-cross 1 (TT_1), and triple top-cross 4 (TT_4). KASP, kompetitive allele-specific PCR.

the Thermo Scientific GeneJET Plant Genomic DNA Purification Kit. Starting from the F_1 × Linkert cross, all lines obtained, as well as those from each subsequent crossing, were tested using KASP markers (20194C2, 21825C, 375C1, and 387C2) specifically developed to identify the *Sr59* and *YrSLU* genes according to Rahmatov, Rouse, Nirmala et al. (2016) and Ashraf et al. (2023). Plants carrying the desired genes were accordingly selected. After crossing with the three spring wheat varieties, selected plants from the KASP marker analysis were grown using a modified speed breeding procedure in a standard greenhouse with high-pressure sodium lamps for the subsequent generations. To accelerate growth, the plants were grown in small pots (4 cm × 3.8 cm × 5.2 cm), self-pollinated, and harvested at the Zadoks-87 stage (Zadoks et al., 1974). The harvested spikes were dried in an oven at 35°C for 3 days, then replanted in similar small pots and subjected to a cold treatment at 4°C for 4–5 days. The plants were then transferred to a greenhouse with a 20-h photoperiod and temperatures ranging from 20 to 24°C until harvest. In all subsequent generations, KASP markers were used to select plants carrying the *Sr59* and *YrSLU* resistance genes (Ashraf et al., 2023; Rahmatov, Rouse, Nirmala, et al., 2016). The TT 6 and 7 ($TT_{6,7}$) were tested for their resistance against *Pgt* and *Pst* races and resistance planes. The TT_7 generation was assessed using markers linked to the *ph1b* allele to confirm its elimination in the selected wheat lines (Roberts et al., 1999).

2.2 | Seedling resistance assessment for *Sr* and *Yr*

The stem rust seedling resistance tests were conducted at the Biosafety Level-3 containment greenhouse at the Global Rust Reference Center, Aarhus University, Flakkebjerg, Denmark. The African races TTKSK (Ug244a_19), TTRTF (IT16a_18), and TTTTF (TZ92a_16) were used to evaluate the resistance of 15 selected lines for *Pgt* in the TT_6 population (10 plants per pot for each line), according to a modified method described by Patpour, Hovmøller, et al. (2022) and Patpour, Rahmatov, et al. (2022). Similarly, all 15 lines of the TT_6 population (10 plants per pot for each line) were evaluated for the presence of *Pst* resistance, using the races DK229_19 (*Psts10*), ET58_21 (*Psts16*), DK09_11sp (*Psts7*), and DK69_15 (*Psts13*) in a previously developed procedure (Sørensen et al., 2016) with slight modifications. Thus, stored *Pgt* and *Pst* urediniospores were removed from a −80°C freezer and heat-shocked for 10 min at 42°C. Following this, the spores were suspended in light mineral oil (Novec 7100) and inoculated on 10-day-old seedlings. Inoculated plants were kept in a dark, moist chamber at 18°C for *Pgt* and 10°C for *Pst* for 24 h before being transferred to the Biosafety Level-3 containment greenhouse with supplemental sodium light (200 µmol s^{-1} m^{-2}) for 16 h day^{-1} at 20–22°C day/18–20°C night temperature. Sixteen days after inoculation, seedling

infection of *Pgt* was scored using a scale of 0–4 based on Stakman et al. (1962). Infection of *Pst* on wheat seedlings was also scored after 16 days of inoculation using a scale from 0 to 9 (McNeal et al., 1971).

2.3 | Field evaluations

The TT_5 generation was sown at SITES Lönnstorp Research Station (55.925621° N, 13.096742° E) in field conditions for seed multiplication and phenotypic evaluation. The lines were sown in small plots ($2m^2$) in one replicated field trial to assess various phenotypic traits, with comparisons made against the control (parental) varieties: Linkert, Navruz, SLU-Elite, and Chinese Spring (CSA). Data on multiple characteristics were collected, such as the number of days to reach 50% flowering and maturity, plant height, tillering capacity, lodging tendency, grain color, and susceptibility to *Pgt*, *Pst*, and powdery mildew. The CSA landrace, which is susceptible to *Pgt*, *Pst*, and powdery mildew, was included in the field trial. Once the CSA plants showed full susceptibility, the disease severity was evaluated at the booting, heading, and dough stages using the Zadoks scale (Zadoks et al., 1974). The percentage of leaf tissue infected by *Pgt*, *Pst*, and powdery mildew (0%–100%) was assessed using the modified Cobb scale (Peterson et al., 1948), while infection responses were evaluated following Roelfs et al. (1992).

3 | RESULTS

3.1 | Generation of double and triple top-crossings combining *Sr59* and *YrSLU* genes on an elite wheat background

A base population containing resistance genes for stem rust (*Sr59*) and stripe rust (*YrSLU*) was successfully developed by crossbreeding the lines TA5095 and #392, resulting in 60 F_1 seeds. These F_1 seeds were then crossed with Linkert, which produced 200 T seeds. KASP marker analysis identified 140 plants ($x^2 = 2.66$) carrying the *Sr59* gene, and among these, 11 plants ($x^2 = 0.617$) also possessed the *YrSLU* resistance gene. The selected 11 plants with both *Sr59* and *YrSLU* were crossed with Navruz, resulting in 210 DT seeds. Of these, 27 plants were verified by KASP markers to contain both *Sr59* and *YrSLU* resistance genes (Figures 1 and 2). These 27 lines were then crossed with the SLU-Elite variety, producing 350 TT_1 seeds. The top-crossing strategy combined resistance genes from different genetic backgrounds into a single population. Using KASP markers at each crossing stage facilitated the accurate selection of lines with both *Sr59* and *YrSLU*, demonstrating the effectiveness of MAS in developing wheat lines with combined resistance to *Pgt* and *Pst*.

3.2 | Optimizing a speed breeding approach for accelerated wheat development

An efficient speed breeding method was developed and applied under standard greenhouse conditions in the present study, thereby enabling rapid progression to the next selection and evaluation stage. This approach involved growing wheat plants in small pots, which significantly reduced maturation time. Plants were harvested early at the hard dough stage (Zadoks-87), and seeds were immediately dried at 35°C for 3–4 days. The speed breeding process was carried out from generations TT_1 to TT_4. After planting, seeds underwent a cold treatment at 4°C for 4–5 days to break dormancy and enhance uniform germination. The plants were then grown in a greenhouse at 20–24°C and self-pollinated until harvest, enabling efficient generation turnover. All TT_1 to TT_4 plants were tested using KASP markers to confirm the presence of *Sr59* and *YrSLU* resistance genes. The *Sr59* gene was identified using markers KASP_2RL_c20194C2 and KASP_2RL_c21825C1, while the *YrSLU* gene was detected with marker KASP_6RL_387C2. Only plants carrying both resistance genes were selected for the next generation. Both the speed breeding and KASP marker approaches enabled the rapid development of wheat lines with both resistance genes, demonstrating their efficiency in accelerating breeding cycles.

3.3 | Pyramiding *Sr59* and *YrSLU* into elite wheat backgrounds

For the development of the wheat lines herein, self-pollination combined with the described speed breeding method was used from the TT_1 to the TT_4 generations. Each generation was screened for *Sr59* and *YrSLU* resistance genes using KASP markers (Figure 2; Table 1). From these screenings, 14 families with a stable inheritance of both resistance genes were identified and confirmed by KASP markers in the TT_4 generation. The remaining families showed segregation, indicating an incomplete stability of resistance traits. The 14 selected TT_5 families were evaluated in the field in 2023 and showed a consistent performance similar to parental wheat varieties (Linkert, Navruz, and SLU-Elite). The evaluated traits included plant stand, tillering capacity, height, spike phenotype (awned or awnless), lodging resistance, days to maturity, and seed fertility, compared with CSA. No natural infections of *Pgt*, *Pst*, or powdery mildew were observed in any of the families compared to CSA, demonstrating resistance to these naturally occurring diseases. These findings confirm the successful development of adapted wheat lines with combined resistance to *Pgt* and *Pst*, providing valuable genetic resources for wheat breeding programs.

TABLE 1 Seedling resistance of various lines against races of stem rust, stripe rust, and kompetitive allele-specific PCR (KASP) analysis.

Lines	*Sr* seedling			*Yr* seedling				Molecular markers					Notes
	TTKSK	TTRTF	TTTTF	*Psts10*	*Psts16*	*Psts7*	*Psts13*	*ph1b*	KASP_2RL_c20194C2	KASP_2RL_c21825C1	KASP_6RL 375_C2	KASP_6RL _387C2	
CSA	4	3+/4	4	7	7	7	7	–	–	–	–	–	Parental
Linkert	33+	3	;1	7	7	7	7	–	–	–	–	–	Parental
Navruz	4	3+	3+	7	7	7	7	–	–	–	–	–	Parental
SLU-Elite	3+	3+	3+	7	7	7	7	–	–	–	–	–	Parental
TA5094	;1	11+	11+	–	–	–	–	+	+	+	–	–	Parental
SLU238	;1	11+	11+	23	67	7	7	–	+	+	–	–	Parental
SLU126	4	4	4	12	12	12	12	–	–	–	+	+	Parental
SLU284	1+2-	11+	1+2-	–	–	–	–	+	+	+	–	–	Parental
#392	3+	3+	4	12	12	12	23	+	–	–	+	+	Parental
TT_7-3-11	1+2-	11+	1+2-	12	12	34	12	–	+	+	+	+	TT_7
TT_7-5-33	1+2-	11+	1+2-	12	12	7	12	–	+	+	+	+	TT_7
TT_7-5-38	1+2-	11+	1+2-	23	12	7	12	–	+	+	+	+	TT_7
TT_7-6-29	1+2-	11+	1	12	12	7	12	–	+	+	+	+	TT_7
TT_7-6-32	1+2-	11+	1	12	12	7	12	–	+	+	+	+	TT_7

Note: *Sr* seedling: Infection types observed based on 0–4 scale (Stakman et al., 1962). Plants with ;1 to 2- IT types were considered as resistant, and plants with 33+ to 4 infection types were considered as susceptible. *Yr* seedling: Infection type observed based on 0–9 scale (McNeal et al., 1971). Plants with 0, 1-2, and 2-3 are considered resistant while plants with 6-7 are considered susceptible. The signs "+" and "–" indicate presence and absence of the alleles of corresponding, individually.

Abbreviation: CSA, Chinese Spring.

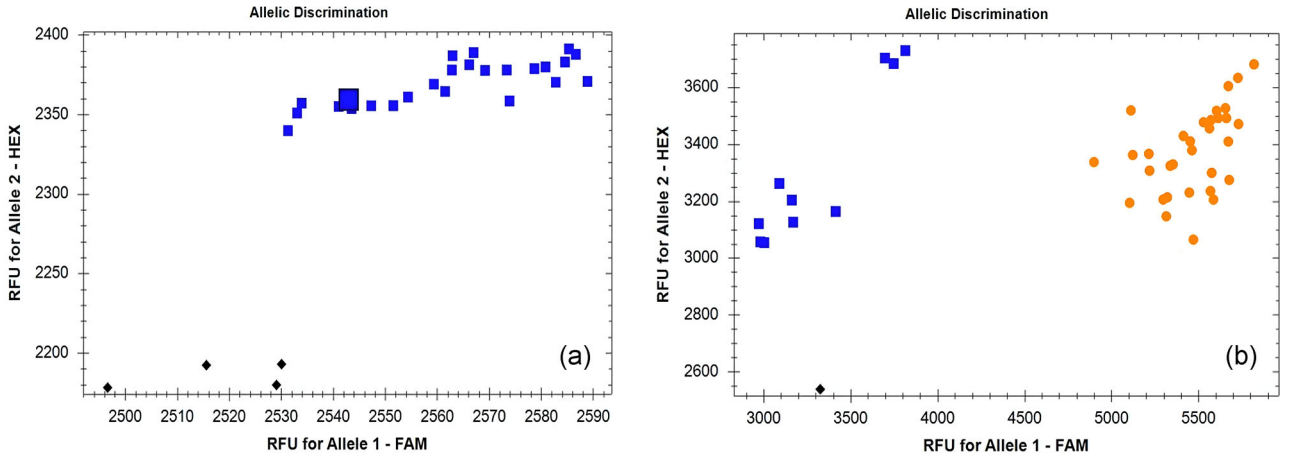

FIGURE 2 Genotyping results of kompetitive allele-specific PCR (KASP) markers on pyramided lines in the double top-cross (DT). (a) *Sr59* KASP (KASP_2RL_c20194C2): Blue points indicate lines with the *Sr59* in lines, while black points indicate negative controls and an adapted parental line (Linkers, Navruz, and SLU-Elite). (b) *YrSLU* KASP (KASP_6RL_387C2): blue points indicate plants with *YrSLU* and orange points represent plants without *YrSLU*.

3.4 | Seedling resistance assays for stem rust and stripe rust and KASP markers selection

The seedling test was performed on the TT_6 and TT_7 families using three *Pgt* races (TTKSK, TTRTF, and TTTTF) and four *Pst* races (*Psts10*, *Psts16*, *Psts7*, and *Psts13*). The results show that SLU238 and TA5094 exhibited broad resistance to all tested *Pgt* races with infection types (ITs) ranging from ;1 to 11+ (Table 1). The cultivars Navruz and SLU-Elite were susceptible to all three *Pgt* races, while Linkert showed an ITs of ;1 to the TTTTF race (Table 1). The TT_6 families demonstrated consistent resistance to all three *Pgt* races, showing ITs from 11+ to 1+2, due to the presence of the *Sr59* gene (Table 1; Figure 3). Lines SLU126 and #392 showed resistance to all four *Pst* races with an ITs of 12, while the varieties Linkert, Navruz, and SLU-Elite were highly susceptible (Table 1; Figure 3). Among the TT_6 families, seedling tests showed segregation in their response to *Pst* races *Psts10*, *Psts16*, and *Psts13*, with ITs ranging from 12 to 67, while most families were susceptible to the *Psts7* race. For all four *Pst* races in each TT_6 families, most plants (8–9 plant) showed resistance while 1–2 plants were susceptible. Following this, the resistant TT_6 plants were subjected to the KASP markers (KASP_2RL_c20194C2, KASP_2RL_c21825C1 for *Sr59*, and KASP_6RL_387C2 for *YrSLU*), and the selected resistant plants were advanced to the TT_7 generation. The TT_7 plants were tested again against the same three *Pgt* and four *Pst* races, showing consistent reactions with no segregation, confirming stable resistance in the TT_7 generation (Table 1). These results demonstrate that the combination of *Sr59* and *YrSLU* genes in the TT_7 families provides effective and stable resistance to both *Pgt* and *Pst*, confirming the successful integration of resistance genes through MAS and seedling analysis.

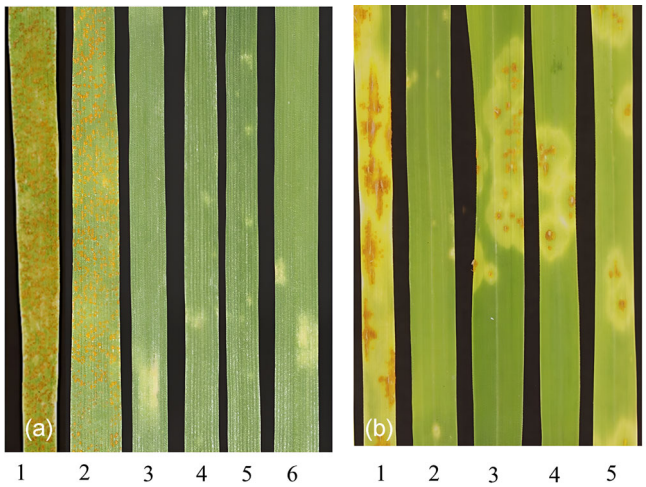

FIGURE 3 Seedling resistance reaction to (a) *Pst* race *Psts13*, (1) Chinese Spring (CSA), (2) Linkert, (3) SLU126, (4) #392, (5) TT_7-3-11, and (6) TT_7-5-33; and (b) *Pgt* race TTKSK, (1) CSA, (2) SLI238, (3) TA5094, (4) TT_7-3-11, and (5) TT_7-5-33.

4 | DISCUSSION

The study demonstrated the effectiveness of using MAS combined with speed breeding to rapidly and efficiently transfer multiple resistance genes into a single wheat genotype. KASP markers were used to confirm the *Sr59* and *YrSLU* genes, thereby both accelerating selection and maintaining their presence over successive generations. Developing breeding lines that combine *Sr59* and *YrSLU* genes provided resistance to *Pgt* and *Pst*, respectively. Speed breeding allowed for rapid generation turnover and efficient selection of plants with both resistance genes. The results validate the potential of MAS and speed breeding to improve breeding programs' efficiency by enabling the rapid selection of wheat lines with desired

resistance traits. Field evaluations of the TT_5 generation and seedling assays for the TT_6 and TT_7 generations confirmed the effectiveness of this approach. The TT_5 families displayed similar phenotypic characteristics to their parental cultivars (Linkert, Navruz, and SLU-Elite), indicating that combining *Sr59* and *YrSLU* resistance genes did not compromise key agronomic performances. Maintaining desirable characteristics such as plant height, tillering capacity, lodging resistance, and days to maturity will ensure that developed lines are suitable for future breeding efforts.

The development and characterization of the Chinese Spring *ph1b*-induced lines TA5095 and #392, used as starting material in the present study, have been previously reported (Ashraf et al., 2023; Rahmatov, Rouse, Nirmala et al., 2016). These lines were also instrumental in developing KASP markers for the studied resistance genes, which is extremely valuable for plant breeding. However, the agronomic performance of Chinese Spring *ph1b*-induced lines is known to be poor, which underscores the necessity of transferring these resistance genes into a more suitable genetic background to enhance their potential in wheat resistance breeding. A recent study, using GBS to select offspring resembling parental traits and KASP markers to identify offspring with the target gene, has demonstrated new opportunities for transferring genes from alien sources into wheat (Yazdani et al., 2023). The *Pst* resistance gene *Sr59*, transferred to an elite genetic background in this study, previously demonstrated strong resistance to a wide range of *Pgt* races (Rahmatov, Rouse, Nirmala et al., 2016). In this study, *Sr59* provided complete resistance to all tested *Pgt* races (TTKSK, TTRTF, and TTTTF). KASP marker analysis confirmed that the *YrSLU* resistance gene was successfully transferred to generation TT_5 through MAS. However, the seedling assays revealed segregation in *Pst* resistance against *Pst* races *Psts10*, *Psts16*, and *Psts13*, and most TT_6 families were susceptible to *Pst* race *Psts7* (except line 11 TT_6). Line #392, used as the parental line in the present study, has previously been shown to carry the *YrSLU Pst* resistance gene, conferring resistance toward the PSTv-14, PSTv-37, PSTv-40, PSTv-218, and PSTv-221 races (Ashraf et al., 2023). Its parental line, SLU126 (from which family 29-N3-5 and #392 originated), has been shown to hold resistance toward a wide range of *Pst* races (Ashraf et al., 2023). The *Pst* resistance in line #392 was identified as a small translocation from chromosome 6RL, while the broader resistance observed in the parental line SLU126 likely resulted from additional rye introgressions (Ashraf et al., 2023). In the present study, line #392 exhibited resistance to all four *Pst* races used in this study, unlike the TT_6 progenies, which did not show complete resistance. The selection of resistant TT_6 plants for self-pollination to produce a TT_7 progeny resulted in a homozygous resistant TT_7 generation that did not show segregation for the *Pst* resistance trait. This confirmed the stable inheritance of the *YrSLU* gene(s) (Table 1).

The emergence of novel virulent races, such as the Ug99 race group, and *Pst* races, such as Warrior (*PstS7*) and Kranich (*PstS8*), has rendered ineffective many previously effective *Yr* and *Sr* resistance genes (Hovmøller et al., 2016; T. L. Liu et al., 2017; Singh et al., 2011). This highlights the urgent need to discover new resistance genes, with alien genetic resources being a valuable tool to secure future wheat resistance and end-use quality attributes (Johansson, Henriksson, et al., 2020, 2024). The present study also showed the potential of alien resistance genes to broaden our understanding of how different genes contribute to resistance mechanisms. Several studies have demonstrated that pyramiding of several resistance genes is an effective strategy to enhance wheat resistance to *Pgt* and *Pst*. However, majority of this work has focused on combining genes for a single disease, either *Pgt* or *Pst* (Charpe et al., 2012; Mallick et al., 2015; Randhawa et al., 2019; Revathi et al., 2010). There is an increasing need to combine genes for resistance to multiple diseases and to better understand the response mechanisms of these genes. New marker-assisted technologies provide promising opportunities for these studies. Pyramiding genes is not only cost-effective but also environmentally sustainable, as it can lead to reduced fungicide use (R. Liu et al., 2020; Mundt, 2018; F. Wang et al., 2023). The gene pyramiding approach used in this study has the potential to significantly reduce pesticide use by targeting resistance to multiple pathogens in a single genotype. In successful cases, this strategy could lead to durable, broad-spectrum wheat resistance. This study also employed an adapted process of speed breeding to accelerate population development, even under standard greenhouse conditions. Growing plants in small pots with optimized light and temperature substantially speeds up plant development compared to normal conditions. Similar outcomes have been reported in previous studies that used speed breeding to shorten breeding cycles and accelerate the evaluation and selection of desirable traits (Ghosh et al., 2018; Hickey et al., 2014; Watson et al., 2018). Moreover, KASP markers played a key role in this study, aiding to determine the presence of the *Sr59* and *YrSLU* genes in wheat lines and populations. As reported in previous research (I. Mackay & Powell, 2007), identifying genes for specific traits is essential, particularly when combining wild and adapted lines. Thus, the pyramiding of *Sr59* and *YrSLU* in this study and their associated KASP markers is necessary for future breeding efforts. This study also demonstrated the importance of using KASP markers to track *Sr59* and *YrSLU* gene transfer. Combining speed breeding with KASP markers significantly accelerated selection, which improved efficiency and effectiveness. This approach can drastically reduce the time needed to develop disease-resistant varieties with novel resistance genes from 10–12 years to 5–6 years while also enabling more precise identification of the most promising candidates. Previous research has shown that MAS provides numerous benefits in plant breeding, such as a more targeted

approach to breeding goals and an overall improved breeding efficiency (Collard & Mackill, 2008; Hasan et al., 2021).

When selecting founder lines, both phenotypic and genotypic characteristics must be considered. Key parameters such as yield stability, winter hardiness, and resistance to other diseases (e.g., net blotch and leaf rust) are important for effective selection (Bülow et al., 2019; Huang et al., 2012; Samantara et al., 2021). The preliminary field evaluation carried out in this study (1 year in small plots) indicated that the selected lines performed similarly to the parental varieties (Linkert, Navruz, and SLU-Elite). Thus, the incorporation of the *Sr59* and *YrSLU* resistance genes did not appear to negatively impact key agronomic traits, such as plant stand density, tillering capacity, plant height, lodging resistance, days to maturity, and seed fertility. Additionally, 2DS.2RL translocation carrying *Sr59* did not negatively affect the quality attributes of lines with this translocation (unpublished data). Protein composition in wheat, particularly high molecular weight-glutenin subunits (HMW-GS), is key in determining bread-making quality (Johansson, Branlard, et al., 2020). The genes encoding the HWW-GS and gliadins are on chromosome groups 1 and 6, respectively (Johansson et al., 2013). Since *Sr59* and *YrSLU* are located on chromosomes 2RL and 6RL, respectively (Rahmatov, Rouse, Steffenson et al., 2016; Ashraf et al., 2023), their presence should not compete with the HMW-GS composition on wheat chromosome group 1. The *Sec-1* and *Sec-3* loci encoding the secalins are located on the short arm of the chromosome 1R (Lawrence & Shepherd, 1980; Shepherd, 1968), while the *Sec-2* and *Sec-4* loci are located on the 2RS and 3RS chromosomes (Carillo et al., 1992; Shewry et al., 1984). This distribution of loci indicates that the 2RL chromosome, carrying *Sr59*, which is distinct from these chromosome arms, does not contain loci known to negatively affect quality. The 2BS.2RL translocations in wheat have been shown to positively affect agronomic and quality performance (Hysing et al., 2007). Thus, combining *Sr59* and *YrSLU* into a single wheat background will enhance disease resistance without negatively affecting essential quality parameters, yield, or overall plant health.

5 | CONCLUSIONS

For this study, we developed wheat lines containing rye-derived resistance genes to combat two of the most destructive wheat diseases globally: *Pgt* and *Pst*. Combining effective resistance genes for multiple diseases on a single wheat background is a key strategy for high yields. We transferred newly discovered resistance genes from the rye genome into wheat using Chinese Spring *ph1b*-induced lines. These lines were then crossed and top-crossed with adapted varieties to establish an adapted wheat genetic background. KASP markers were used throughout the generations to accelerate the selection process and successfully pyramided the*Sr59* and *YrSLU* resistance genes to an adapted wheat background. This study recommends breeders to combine effective resistance genes, such as *Sr59* and *YrSLU*, as part of their disease resistance breeding programs. As new pathogen races increasingly overcome existing resistance genes, breeders and researchers must focus on identifying and harnessing novel resistance genes from related and alien species. This approach is vital for maintaining long-term resistance in wheat breeding because it broadens the genetic base for resistance and counteracts the evolving nature of pathogens. Hence, resistance genes from related species can be crucial in developing high-yielding wheat varieties resilient to biotic stresses, thereby directly contributing to food security.

AUTHOR CONTRIBUTIONS

Mahboobeh Yazdani: Formal analysis; investigation; methodology; validation; visualization; writing—original draft. **Rimsha Ashraf**: Investigation; methodology; writing—original draft. **Eva Johansson**: Conceptualization; data curation; formal analysis; project administration; resources; supervision; writing—review and editing. **Pernilla Vallenback**: Conceptualization; supervision. **Mogens S. Hovmøller**: Conceptualization; investigation; methodology; supervision; writing—review and editing. **Mehran Patpour**: Investigation; methodology; supervision; validation; writing—review and editing. **Mahbubjon Rahmatov**: Conceptualization; data curation; formal analysis; project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The dataset is openly available with the corresponding author and can be accessed on reasonable request.

ORCID

Mahbubjon Rahmatov https://orcid.org/0000-0001-7491-2836

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