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Assessment of *Triticosecale*-Derived Wheat Introgressions for Karnal Bunt (*Tilletia Indica* Mitra) Resistance

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Abstract

Karnal bunt (KB) caused by *Tilletia indica* is a quarantine disease of wheat that reduces grain quality and hinders international trade. Rye (*Secale cereale*) has long been harnessed as a valuable source of resistance to diverse biotic and abiotic stresses. Its transfer into bread wheat is facilitated through triticale (*Triticosecale*), which often carries KB resistance. The present study involves evaluation of 337 introgression lines for KB under artificially created epiphytotic conditions during two consecutive crop seasons (2020-21 and 2021-22). The lines were developed by introgressing KB resistant triticale accessions (TL 2908, TL 2065, TL 3021 and TL 3048) into elite wheat cultivars (PBW 343, PBW 550 and DBW 17) serving as recipient backgrounds. Screening for KB resistance revealed that 26 lines were highly resistant (HR) and 151 lines were resistant (R). The highest proportion of highly resistant (HR) lines was observed among the introgression lines derived from the cross TL 3048/2*CS(S)//PBW 343 (15.05%), followed by TL 3021/2*CS(S)//PBW 550 (13.11%). In contrast, the maximum proportion of resistant (R) lines was recorded in introgressions derived from TL 2908/2*CS(S)//DBW 17 (83.33%) and TL 3048/2*CS(S)//PBW 550 (70.00%). To determine the chromosomal basis of resistance, the 177 lines identified as highly resistant (HR) and resistant (R) were further analyzed using expressed sequence tag (EST)-based markers specific to rye chromosomal regions. Marker analysis resulted in identification of 4 lines carrying both 4RL and 4RS chromosome arms, while 53 lines possessing introgressed segments of 4RL. These results indicate that the rye chromosome arms 4RL and 4RS may contribute to KB resistance in the evaluated material. The findings therefore suggest that 4RL and 4RS are potential carriers of KB resistance loci. These rye chromosomal regions could be further validated and fine-mapped to identify the genetic determinants underlying KB resistance. Moreover, the identified KB resistant introgression lines will be a valuable pre-breeding material and potential donor sources for the development of KB resistant wheat cultivars in future breeding programs.

Keywords: Karnal bunt, wheat, *Tilletia indica, Triticosecale,* introgressions, EST-based rye markers

1. Introduction

Wheat is a major cereal crop that plays a crucial role in global food security by significantly contributing substantially to caloric intake (Bromand *et al.,* 2024). Biotic and abiotic stresses significantly reduce the yield and quality of wheat production. Among the biotic constraints, fungal diseases cause approximately 22 per cent of the total yield loss in wheat (Kayim *et al.,* 2022). Karnal bunt (KB), incited by *Tilletia indica* Mitra, is regarded as one of the most important fungal disease to its impact on grain quality and trade restrictions. KB is a floret-infecting disease that threatens bread wheat, durum wheat, triticale and several related species. The disease leads to partial bunt as the infection is typically confined to only a few kernels within a spike and often affects just a portion of the grain, thus, rarely colonizing the entire kernel (Pandey *et al.,* 2019). The disease spreads not only through seed and soil but also *via* an airborne sporidial stage, making its management particularly challenging (Bala *et al.,* 2022). Infected grains develop a distinct fishy odor, caused by trimethylamine and show flour discoloration, which makes the wheat unsuitable for chapatti preparation and other food uses (Bishnoi *et al.,* 2020). Even 3 per cent of bunted grains render the produce unfit for consumption (Ullah *et al.,* 2012; Kaur *et al.,* 2022). Although the disease does not cause heavy yield losses however, its real impact lies in trade restrictions. More than 77 countries, including major importers such as Russia, China, USA and Canada, enforce strict quarantine regulations that demand zero KB infection in imported wheat thus, making KB a disease of major international concern (Gupta *et al.,* 2019; Aasma *et al.,* 2022). In India, the disease epidemic was first reported in Punjab during 1953-1954 (Agarwal *et al.,* 1977). Subsequently, the disease spread to other wheat-growing areas of north-western plains and foothill regions. Punjab often referred to as the "wheat bowl of India", has historically faced the maximum impact of KB and the repeated outbreaks of the disease are largely linked to the widespread cultivation of susceptible wheat varieties and weather conditions that favour the pathogen during the susceptible stage of the crop (Bala *et al.,* 2022).

Among the various management approaches, host resistance remains the most sustainable and environmentally friendly approach, necessitating the identification and exploitation of reliable resistant donors. Rye (*Secale cereale*, 2n=2x=14) has been extensively harnessed as a valuable reservoir of resistance to a wide range of biotic and abiotic stresses (Crespo-Herrera *et al.,* 2017; Spetsov and Daskalova 2022; Wallace *et al*., 2024). Among the related species, rye has been reported to possess a comparatively higher level of resistance to KB and therefore represents a valuable genetic resource for improving resistance in wheat (Bishnoi *et al.,* 2020). Warham (1988) suggested rye as a promising source of KB resistance, due to its morphological characters *viz*., pubescence and tight glumes, which may reduce pathogen entry. However, the direct introgression of rye chromatin into bread wheat is often limited by cytogenetic barriers and poor agronomic compatibility. To overcome these limitations, triticale (*Triticosecale*), a synthetic hybrid between wheat and rye, has been widely used as a bridge species for transferring useful genes from rye into wheat backgrounds (Khare *et al.,* 2018). Under natural infection conditions, triticale generally exhibits lower susceptibility to KB compared with bread wheat and durum wheat, reflecting the contribution of rye-derived resistance (Bishnoi *et al.,* 2020; Iquebal *et al.,* 2021). Recent advances in genomic resources, particularly the development of chromosome-specific molecular markers covering all seven rye chromosomes and their arms**,** have facilitated the precise detection and characterization of rye chromatin segments introgressed into wheat backgrounds (Xu *et al.,* 2012). Despite the availability of such tools, the genetic basis of rye-derived resistance to KB has not been extensively explored. Therefore, the present study focuses on the development of triticale-wheat introgression lines, their artificial screening for KB resistance and the identification of associated rye chromosome segments using molecular markers**.**

2. Materials and methods

2.1. Plant material and experimental design

The present investigation was conducted in the Department of Plant Breeding and Genetics, PAU, Ludhiana. A total of 337 triticale-wheat introgression lines were developed using four triticale donors (TL 2908, TL 2065, TL 3021, TL 3048) crossed with cultivated wheat backgrounds, namely PBW 343, PBW 550 and DBW 17. These introgression lines were derived from 7 different crosses *viz*., TL 2908/2*Chinese Spring (CS) (S)//PBW 343 (96 lines)**,** TL 3048/2*CS (S)//PBW 343 (93 lines)**,** TL 2065/2*CS (S)//PBW 343 (15 lines)**,** TL 3021/2*CS (S)//PBW 550 (61 lines)**,** TL 3048/2*CS (S)//PBW

550 (30 lines), TL 3048/2*CS (S)//DBW 17 (36 lines) and TL 2908/2*CS (S)//DBW 17 (6 lines).The triticale-wheat introgression lines were screened for KB resistance under artificial epiphytotic conditions during two cropping seasons, namely, 2020-21 and 2021-22. The lines were sown during the last week of November in plots measuring 2×2 m^2 with a row-to-row spacing of 22.5 cm. Susceptible checks (PBW 550, PBW 343, TL 2908, Chinese Spring and DBW 17) and resistant check (white rye), along with commonly grown wheat cultivars (PBW 621, HD 3086, HD 2967, PBW 723, PBW 761, PBW 677, PBW 1 Zn) were also included to validate the screening. All recommended agronomic practices were followed as per the package of practices (Anonymous, 2020).

2.2. Inoculum preparation, inoculation and data recording

A total of 54 *T. indica* isolates collected from grain markets across different agro-climatic regions of Punjab were used for artificial inoculation of the introgressions lines. A composite mixture of teliospores obtained from these isolates was used as inoculum to ensure greater pathogen heterogeneity, which may provide a more reliable assessment of resistance (Sirari *et al.,* 2008). The pathogen was isolated, purified and multiplication as followed by George *et al.,* 2026. Inoculum was prepared by harvesting sporidia from 10-day-old cultures by adding sterile distilled water to the test tubes and gently scraping the growth with an inoculating rod. The suspension was filtered through a double layer of muslin cloth and adjusted to a concentration of approximately 10,000 sporidia/ml. Inoculation was carried out at the boot leaf stage (Zadoks growth stage 49; Zadoks *et al.* (1974), using the hypodermic syringe method as described by Aujla *et al.* (1982). In each plant, five ears were inoculated, tagged and harvested separately at maturity (Fig 1a and 1b). Data on number of infected and total number of grains were recorded. The per cent of KB infection (KBI%) was calculated using formula:

$$\text{KB infection (KBI) \%} = \frac{\text{No. of infected grains}}{\text{total number of grains}} \times 100$$

Based on KBI (%), the lines were classified into six disease reaction categories. Lines showing 0 per cent infection were categorized as highly resistant (HR), 0.1-5 per cent as resistant (R), 5.1-10 per cent as moderately resistant (MR), 10.1-15 per cent as moderately susceptible (MS), 15.1-20 per cent as susceptible (S) and greater than 20 per cent as highly susceptible (HS). Lines exhibiting susceptible reactions in the first year were discarded, whereas the resistant lines were advanced and screened again in the subsequent crop season.

Fig 1: Artificial inoculation of Tilletia indica isolates (a) and tagging of inoculated ear heads (b)

2.3. DNA isolation and quality assessment

DNA was isolated from young leaf samples of highly resistant and resistant triticale-wheat introgression lines collected during early morning hours. DNA was isolated following the CTAB method (Saghai-Maroof *et al*., 1984). DNA quality and quantity were assessed by 0.8 per cent agarose gel electrophoresis and nanodrop, respectively.

2.4. Marker analysis

Marker analysis was performed on 177 resistant introgression lines using a total of 14 rye-specific markers (7 from the long arm and 7 from the short arm) that consisted of 17 EST-derived primers (Table 1).

PCR was performed by preparing the total reaction volume of 13 µl consisting of 5 µl PCR master mix, 1 µl forward primer, 1 µl reverse primer, 3 µl nuclease-free water and 3 µl template DNA using a BIORAD MyCycler™ thermal cycler. Initial denaturation was done at 94°C for 4 min, followed by 37 cycles of 94°C for 1 min, annealing at 50-60°C (primer-specific) for 1 min and 72°C for 2 min; followed by a final extension at 72°C for 10 min. PCR products were resolved on a 2.5 per cent agarose gel. Some rye markers were monomorphic, preventing identification of specific rye chromosome segments associated with KB resistance. Therefore, higher-resolution analysis was conducted using polyacrylamide gel electrophoresis (PAGE).

3. Results and discussion

3.1. Screening of introgression lines

A total of 337 triticale-bread wheat introgression lines were screened for KB resistance, along with susceptible as well as resistant checks, and commonly grown wheat cultivars. The commonly grown cultivars *viz*., PBW 621, HD 3086, HD 2967, PBW 723, PBW 761, PBW 677 and PBW 1 Zn were found to be susceptible to susceptible (HS) with mean per cent KBI ranging from 19.78 per cent (WH 1105) to 25.7 per cent (PBW 725) (Table 2). This emphasizes the need to strengthen KB-resistance breeding programs to safeguard wheat productivity and trade. Among the susceptible checks, PBW 550 recorded the highest mean KBI of 37.1 per cent. The resistant check white rye recorded only 3.5 per cent KBI, confirming its stable resistance to KB. The contrasting reactions of the susceptible and resistant checks confirmed that the artificial epiphytotic conditions successfully generated adequate disease pressure for reliable screening. The results of screening for each cross in the present study are discussed under the following subheadings:

3.2. TL 2908/ 2*CS (S) // PBW 343

Of the 96 lines 2 lines, *viz.,* TL 2908/ 2* CS(S)//PBW 343-33 and TL 2908/ 2* CS(S)//PBW 343-90 showed highly resistant (HR) response and 28 lines were found resistant (R) (Fig 2; Table 3). The mean KBI (%) in this cross ranged from 0.00 to 44.78 per cent and the derived lines showed a relatively broad distribution of disease reactions. It was found that 2.08, 29.17, 15.63, 12.50 and 17.71 per cent of the lines were highly resistant (HR), resistant (R), moderately susceptible (MS), susceptible (S) and highly susceptible (HS), respectively indicating partial susceptibility within this background.

3.3. TL 3048/ 2* CS(S)//PBW 343

The mean KBI (%) among the lines ranged from 0.00 to 53.25 per cent. Out of 93 lines derived from the cross TL 3048/2*CS(S)//PBW 343, 14 lines (15.05%) were classified as highly resistant (HR) and 39 lines (41.94%) as resistant (R). In addition, 17 lines exhibited a moderately resistant (MR) reaction, 7 lines were moderately susceptible (MS), 5 lines were susceptible (S) and 11 lines were highly susceptible (HS) to KB (Fig 2; Table 3). Thus, relatively higher proportion of HR and R lines were found in this cross.

3.4. TL 2065 / 2*CS (S)//PBW 343

Fifteen introgression lines derived from the cross were screened for resistance to KB under field conditions. Based on disease reaction, 6 lines were categorized as resistant (R), 7 as moderately resistant (MR) and 2 as highly susceptible (HS) (Fig 2; Table 3). The mean KBI (%) varied from 2.70 to 34.86 per cent. Overall, the majority of the lines exhibited a moderately resistant (MR) reaction (46.67%), while 40.00 per cent of the lines were categorized as resistant (R).

3.5. TL 3021/2* CS (S)//PBW 550

The results showed that out of the 61 introgression lines evaluated 8, 34 and 17 lines exhibited highly resistant (HR), resistant (R) and moderately resistant (MR) reactions, respectively, while 1 line each was categorized as moderately susceptible (MS) and susceptible (S) (Fig 2; Table 3). KBI (%) among these lines ranged from 0.00 to 15.32 per cent. The introgression lines derived from this

Table 1: List of rye chromosome specific markers, forward and reverse primer, annealing temperature and amplicon size (Xu *et al.*, 2012)

S. No.	Specific Marker	EST	Chromosome arm	Forward primer 5'-3'	Reverse primer 5'-3'	Annealing temp. (°C)	Size (bp)
1	SWES999	CJ548455	1RS	ACGCTGCGGATGAAGATG	GCTGGCAATGCTGAAAGG	57	800
2	SWES1119	CJ653076	1RL	GAAACCCACCTCCCTTAC	AGAGCCTACCATCCCATC	51	400
3	CGG62	BE587051	2RS	GCCCTCGACGACATGAAA	CGCTTGCCGGTCTTGTAT	60	290
4	CGG9	BE588133	2RL	CAGAGCAACAGCGACATCTTC	TCAACCCAAGGCAAAAGG	56	200
5	SWES228	CD899455	3RS	GCATCTTCTTCCTCTGCTCCTG	CGGGCTTCTTGTCCTCGTC	62	150
6	CGG32	BE705766	3RL	GGAGGTGAGACAACAAGAC	CAGACGGCAATGTGATAG	55	480
7	KSUM62	BE585783	4RS	GGAGAGGATAGGCACAGGAC	GAGAGCAGAGGGAGCTATGG	62	160
8	CGG49	BE586668	4RL	GAACGCAAGCACTTCTCA	GCTCCTTTCTAAGCCTGTC	55	1000
9	MAG1242	TC235573	5RS	GCCACCGACTGTTAGGTTTCACTC	CGAGGGTTCTTGGGAATGACAC	55	500
10	CGG4	BE493824	5RL	CGAGGTGAACGATGATGACAGC	TAGCAATGAGCAGCAACGGC	60	200
11	CGG16	BE586813		ACCGTTCATCCATCGTTCC	ACCAACCAATGTTGCTCCC	57	250
12	CGG143-6R*	AF136486	6RS	TCGGATCACATTCACTTA	TGGCGTCTTGACTACATT	50	100
13	CGG23	BE704532		TCGTCTCGCAGACCTTGCAC	CAGCAACGCATCGACTGAGC	62	480
14	CGG59	BE586431	6RL	AGCTACGTGGAGCACAAG	TGATGATACGCACAACAAA	51	200
15	SWES231	CD91608		AAGCATCCTACATAGCCCTCA	GGAGACCACTCGGCAGAAA	58	260
16	CFE228	BM13616	7RS	CACACCACCACTGTCCTCC	GAACTCGTGCATCCCGTC	60	1000
17	CGG26	BE496047	7RL	GCTGGTTGGAATAACGCTGATG	CGGCAAGCAATGAGAGACAGAG	60	150

Table 2: Reaction of commonly grown wheat cultivars for Karnal bunt

Wheat cultivar	Mean KBI (%)	Disease reaction
PBW 725	25.70	HS
PBW 621	21.40	HS
HD 3086	22.64	HS
PBW 723	21.28	HS
HD 2967	22.91	HS
PBW 761	21.51	HS
PBW 677	21.74	HS
PBW 1 Zn	21.58	HS
WH 1105	19.78	HS
PBW 343 (susceptible check)	19.73	S
PBW 550 (susceptible check)	37.10	HS
CS(S) (susceptible check)	20.50	HS
TL 2908 (susceptible check)	15.30	S
DBW 17 (susceptible check)	1.60	MS
White rye (resistant check)	3.50	R

cross exhibited a high proportion of resistant reactions, with 13.11, 55.74 and 27.83 per cent of the lines classified as HR, R and MR, respectively. These results suggested that the introgressed segments from this genetic background may carry stable and effective resistance genes against KB.

*3.6. TL 3048/2*CS (S)// PBW 550*

Among the 30 introgression lines derived from this cross, none were classified as highly resistant (HR), whereas 21 and 6 lines were categorized as resistant (R) and moderately resistant (MR), respectively (Fig 2; Table 3). The mean infection ranged from 0.29 to 34.96 per cent. Overall, most lines demonstrated a desirable level of resistance, with 70 per cent lines showing resistant (R) reactions and 20 per cent lines moderately resistant (MR), while only a small proportion exhibited susceptibility.

3.7. TL 2908/2 CS (S)//DBW 17*

Among the 6 introgression lines derived from the cross TL 2908/2*CS (S)//DBW 17, five lines TL 2908/2*CS(S)//DBW 17-1, TL 2908/2*CS(S)//DBW 17-2, TL 2908/2*CS(S)//DBW 17-4, TL2908/2*CS(S)//DBW 17-5 and TL 2908/2*CS(S)//DBW 17-6 exhibited a resistant (R) reaction to KB, whereas one line, TL 2908/2*CS(S)// DBW 17-3, showed a moderately susceptible (MS) response (Fig 2; Table 3). The mean KBI (%) across the two years ranged from 1.75 to 14.00 per cent, with resistant lines accounting for 83.33 per cent of the total.

3.8. TL 3048/2 CS (S)//DBW 17*

Among the 36 introgression lines derived from this cross 2, 18 and 3 lines exhibited highly resistant (HR), resistant (R) and moderately resistant (MR) reactions, respectively. In contrast, 5, 4 and 4 lines were classified as moderately susceptible (MS), susceptible (S) and highly susceptible (HS), respectively (Fig 2; Table 3). The per cent KBI (%) ranged from 0.0 to 44.50. Overall, the cross demonstrated a moderate level of resistance, with 55.56 per cent of the lines falling in the highly resistant (HR) and resistant (R) categories.

Rye has been extensively harnessed as a valuable reservoir of resistance to a wide range of biotic and abiotic stresses (Crespo-Herrera *et al.*, 2017; Spetsov and Daskalova, 2022; Wallace *et al*., 2024). However, the direct introgression of resistance into bread wheat is often limited by cytogenetic barriers and poor agronomic compatibility. Thus, triticale was used as a bridge and a total of 337 triticale-bread wheat introgression lines were developed from 7 crosses. The screening of 337 lines for KB resistance resulted in the identification of 26 highly resistant (HR) and 151 resistant (R) lines. Among all the crosses, the highest percentage of highly resistant (HR) lines was observed in TL 3048/2*CS(S)//PBW 343 (15.05%), followed

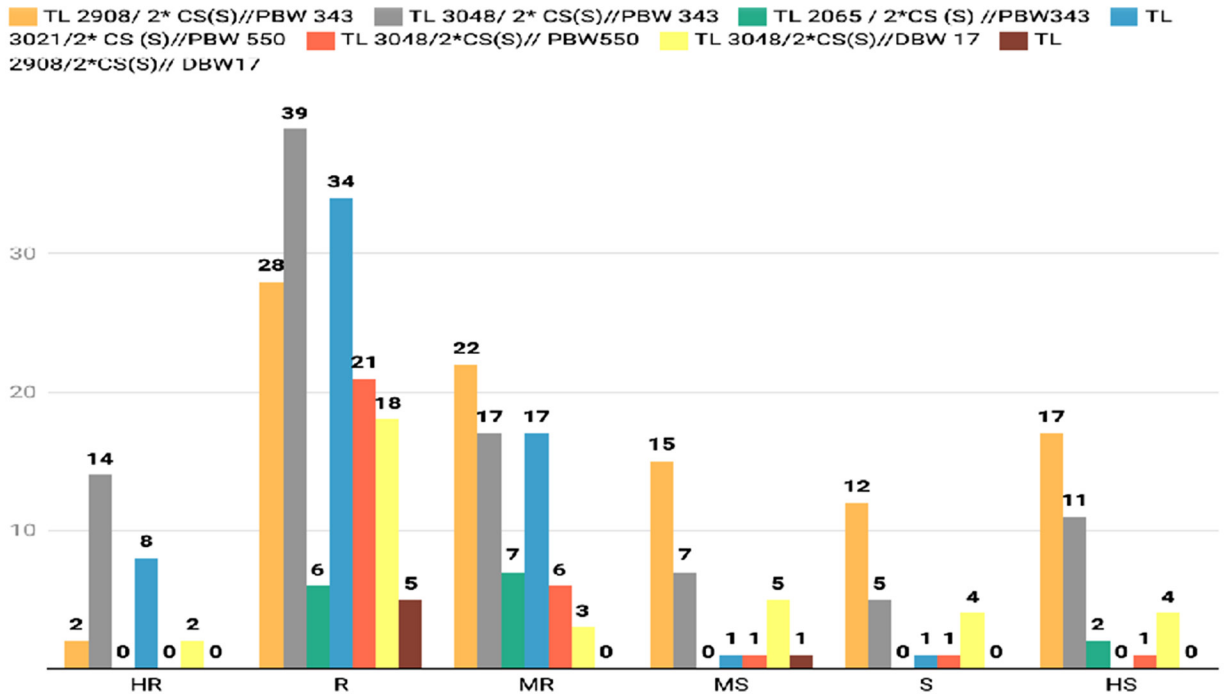

Fig 2: Disease reaction of triticale-derived wheat introgression lines against Karnal bunt across different genetic backgrounds viz., PBW 343, PBW 550, DBW 17

by TL 3021/2*CS(S)//PBW 550 (13.11%). The highest percentage of resistant (R) lines was recorded in the population derived from TL 2908/2*CS(S)//DBW 17 (83.33%), followed by TL 3048/2*CS(S)//PBW 550 (70.00%). The occurrence of a large proportion of highly resistant (HR) and resistant (R) lines across different crosses indicates the successful transfer of resistance from triticale-derived introgressions into wheat backgrounds. Earlier studies have also demonstrated the potential of triticale as a source of resistance to KB. Bahadur *et al.* (1998) reported resistance of triticale to multiple diseases including KB along with other diseases of wheat. Similarly,

Table 3: Highly resistant and resistant *Triticosecale*-derived wheat introgressions lines

S. No.	Cross	Highly resistant (HR) lines	Resistant (R) lines
1.	TL 2908/ 2*CS (S) // PBW 343	TL 2908/ 2* CS(S)//PBW 343-33, TL 2908/ 2* CS(S)//PBW 343-90	TL 2908/ 2* CS(S)//PBW 343-3, TL 2908/ 2* CS(S)// PBW 343-4, TL 2908/ 2* CS(S)//PBW 343-5, TL 2908/ 2* CS(S)//PBW 343-7, TL 2908/ 2* CS(S)//PBW 343-13, TL 2908/ 2* CS(S)//PBW 343-24, TL 2908/ 2* CS(S)// PBW 343-26, TL 2908/ 2* CS(S)//PBW 343-30, TL 2908/ 2* CS(S)//PBW 343-31, TL 2908/ 2* CS(S)//PBW 343-32, TL 2908/ 2* CS(S)//PBW 343-37, TL 2908/ 2* CS(S)// PBW 343-38, TL 2908/ 2* CS(S)//PBW 343-41, TL 2908/ 2* CS(S)//PBW 343-42, TL 2908/ 2* CS(S)//PBW 343-44, TL 2908/ 2* CS(S)//PBW 343-47, TL 2908/ 2* CS(S)// PBW 343-49, TL 2908/ 2* CS(S)//PBW 343-50, TL 2908/ 2* CS(S)//PBW 343-54, TL 2908/ 2* CS(S)//PBW 343-55, TL 2908/ 2* CS(S)//PBW 343-56, TL 2908/ 2* CS(S)// PBW 343-58, TL 2908/ 2* CS(S)//PBW 343-59, TL 2908/ 2* CS(S)//PBW 343-81, TL 2908/ 2* CS(S)//PBW 343-84, TL 2908/ 2* CS(S)//PBW 343-85, TL 2908/ 2* CS(S)// PBW 343-88, TL 2908/ 2* CS(S)//PBW 343-96
2.	TL 2065/2*CS (S)// PBW 343	-	TL 2065/2* CS (S)// PBW 343-2, TL 2065/2* CS (S)// PBW 343-6, TL 2065/2* CS (S)// PBW343-7, TL 2065/2* CS (S)// PBW 343-9, TL 2065/2* CS (S)// PBW 343-10, TL 2065/2* CS (S)// PBW 343-14

3.	TL 3048/ 2* CS(S)//PBW 343	TL 3048/ 2* CS(S)//PBW 343-3, TL 3048/ 2* CS(S)//PBW 343-4, TL 3048/ 2* CS(S)// PBW 343-5, TL 3048/ 2* CS(S)//PBW 343-16, TL 3048/ 2* CS(S)//PBW 343-17, TL 3048/ 2* CS(S)//PBW 343-21, TL 3048/ 2* CS(S)//PBW 343-22, TL 3048/ 2* CS(S)//PBW 343-23, TL 3048/ 2* CS(S)// PBW 343-32, TL 3048/ 2* CS(S)//PBW 343-33, TL 3048/ 2* CS(S)//PBW 343-35,TL 3048/ 2* CS(S)//PBW 343-36, TL 3048/ 2* CS(S)//PBW 343-37, TL 3048/ 2* CS(S)//PBW 343-47	TL 3048/ 2* CS(S)//PBW 343-12, TL 3048/ 2* CS(S)// PBW 343-14, TL 3048/ 2* CS(S)//PBW 343-18, TL 3048/ 2* CS(S)//PBW 343-19, TL 3048/ 2* CS(S)//PBW 343-20, TL 3048/ 2* CS(S)//PBW 343-24, TL 3048/ 2* CS(S)// PBW 343-27, TL 3048/ 2* CS(S)//PBW 343-31, TL 3048/ 2* CS(S)//PBW 343-34, TL 3048/ 2* CS(S)//PBW 343-39, TL 3048/ 2* CS(S)//PBW 343-40, TL 3048/ 2* CS(S)// PBW 343-41, TL 3048/ 2* CS(S)//PBW 343-42, TL 3048/ 2* CS(S)//PBW 343-43, TL 3048/ 2* CS(S)//PBW 343-44, TL 3048/ 2* CS(S)//PBW 343-45, TL 3048/ 2* CS(S)// PBW 343-54, TL 3048/ 2* CS(S)//PBW 343-55, TL 3048/ 2* CS(S)//PBW 343-56, TL 3048/ 2* CS(S)//PBW 343-61, TL 3048/ 2* CS(S)//PBW 343-63, TL 3048/ 2* CS(S)// PBW 343-64, TL 3048/ 2* CS(S)//PBW 343-65, TL 3048/ 2* CS(S)//PBW 343-71, TL 3048/ 2* CS(S)//PBW 343-72, TL 3048/ 2* CS(S)//PBW 343-73, TL 3048/ 2* CS(S)// PBW 343-75, TL 3048/ 2* CS(S)//PBW 343-76, TL 3048/ 2* CS(S)//PBW 343-77, TL 3048/ 2* CS(S)//PBW 343-78, TL 3048/ 2* CS(S)//PBW 343-79, TL 3048/ 2* CS(S)// PBW 343-80, TL 3048/ 2* CS(S)//PBW 343-81, TL 3048/ 2* CS(S)//PBW 343-82, TL 3048/ 2* CS(S)//PBW 343-86, TL 3048/ 2* CS(S)//PBW 343-89, TL 3048/ 2* CS(S)// PBW 343-90, TL 3048/ 2* CS(S)//PBW 343-91, TL 3048/ 2* CS(S)//PBW 343-92
4.	TL 3021/2* CS (S)//PBW 550	TL 3021/2* CS (S)//PBW 550-1, TL 3021/2* CS (S)//PBW 550-2, TL 3021/2* CS (S)// PBW 550-3, TL 3021/2* CS (S)//PBW 550-6, TL 3021/2* CS (S)//PBW 550-8, TL 3021/2* CS (S)//PBW 550-9, TL 3021/2* CS (S)//PBW 550-11, TL 3021/2* CS (S)//PBW 550-13	TL 3021/2* CS (S)//PBW 550-4, TL 3021/2* CS (S)// PBW 550-5, TL 3021/2* CS (S)//PBW 550-7, TL 3021/2* CS (S)//PBW 550-10, TL 3021/2* CS (S)//PBW 550-12, TL 3021/2* CS (S)//PBW 550-14, TL 3021/2* CS (S)// PBW 550-15, TL 3021/2* CS (S)//PBW 550-16, TL 3021/2* CS (S)//PBW 550-17, TL 3021/2* CS (S)//PBW 550-18, TL 3021/2* CS (S)//PBW 550-20, TL 3021/2* CS (S)//PBW 550-21, TL 3021/2* CS (S)//PBW 550-24, TL 3021/2* CS (S)//PBW 550-26, TL 3021/2* CS (S)//PBW 550-28, TL 3021/2* CS (S)//PBW 550-29, TL 3021/2* CS (S)//PBW 550-30, TL 3021/2* CS (S)//PBW 550-32, TL 3021/2* CS (S)//PBW 550-33, TL 3021/2* CS (S)//PBW 550-34, TL 3021/2* CS (S)//PBW 550-37, TL 3021/2* CS (S)//PBW 550-40, TL 3021/2* CS (S)//PBW 550-41, TL 3021/2* CS (S)//PBW 550-46, TL 3021/2* CS (S)//PBW 550-47, TL 3021/2* CS (S)//PBW 550-48, TL 3021/2* CS (S)//PBW 550-49, TL 3021/2* CS (S)//PBW 550-51, TL 3021/2* CS (S)//PBW 550-52, TL 3021/2* CS (S)//PBW 550-53, TL 3021/2* CS (S)//PBW 550-54, TL 3021/2* CS (S)//PBW 550-55, TL 3021/2* CS (S)//PBW 550-56, TL 3021/2* CS (S)//PBW 550-61
5.	TL 3048/2*CS (S)// PBW550	-	TL 3048/2* CS (S)// PBW550-1, TL 3048/2CS(S)// PBW550-2, TL 3048/2* CS (S)// PBW550-3, TL 3048/2* CS (S)// PBW550-4, TL 3048/2* CS (S)// PBW550-6, TL 3048/2* CS (S)// PBW550-7, TL 3048/2CS(S)// PBW550-10, TL 3048/2CS(S)// PBW550-11, TL 3048/2* CS(S)// PBW550-12, TL 3048/2* CS(S)// PBW550-13, TL 3048/2* CS(S)// PBW550-14, TL 3048/2* CS(S)// PBW550-15, TL 3048/2* CS(S)// PBW550-16, TL 3048/2* CS(S)// PBW550-18, TL 3048/2* CS(S)// PBW550-19, TL 3048/2* CS(S)// PBW550-21, TL 3048/2* CS(S)// PBW550-24, TL 3048/2* CS(S)// PBW550-25, TL 3048/2* CS(S)// PBW550-26, TL 3048/2* CS(S)// PBW550-27, TL 3048/2*CS(S)// PBW550-28

6.	TL 2908/2*CS(S)// DBW 17	-	TL 2908/2*CS(S)// DBW 17-1, TL 2908/2*CS(S)// DBW 17-2, TL 2908/2*CS(S)// DBW 17-4, TL 2908/2*CS(S)// DBW 17-5, TL 2908/2*CS(S)// DBW 17-6
7.	TL 3048/2*CS (S)//DBW 17	TL 3048/2* CS(S)//DBW 17-8, TL 3048/2* CS(S)//DBW 17-9	TL 3048/2* CS(S)//DBW 17-4, TL 3048/2* CS(S)//DBW 17-5, TL 3048/2* CS(S)//DBW 17-6, TL 3048/2* CS(S)// DBW 17-12, TL 3048/2* CS(S)//DBW 17-15, TL 3048/2* CS(S)//DBW 17-17, TL 3048/2* CS(S)//DBW 17-19, TL 3048/2* CS(S)//DBW 17-20, TL 3048/2* CS(S)//DBW 17-22, TL 3048/2* CS(S)//DBW 17-23, TL 3048/2* CS(S)// DBW 17-24, TL 3048/2* CS(S)//DBW 17-25, TL 3048/2* CS(S)//DBW 17-29, TL 3048/2* CS(S)//DBW 17-30, TL 3048/2* CS(S)//DBW 17-32, TL 3048/2* CS(S)//DBW 17-33, TL 3048/2* CS(S)//DBW 17-35, TL 3048/2* CS(S)// DBW 17-36

Gaudet *et al.* (2001) observed less than 3 per cent KBI (%) in triticale cultivars evaluated in Mexico, indicating a high level of resistance. In contrast, Riccioni *et al.* (2008) reported that several European bread wheat and durum wheat cultivars were susceptible to KB, while triticale genotypes showed comparatively higher resistance. Further, Emebiri *et al.* (2019) confirmed the presence of KB resistance in several triticale genotypes under Australian conditions and Fuentes-Dávila *et al.* (2021) reported comparatively low levels of KB infection in 21 advanced triticale lines evaluated under artificial inoculation. These reports collectively suggested that triticale represents an important genetic resource for improving resistance to KB in wheat breeding programmes. The identification of a substantial number of HR and R introgression lines in the present study further reinforces the effectiveness of triticale-wheat introgressions in conferring KB resistance. At the same time, the variable frequencies of resistance observed among different crosses suggest that the genetic background of the wheat parent plays a crucial role in the expression and stabilization of resistance in the introgression lines. Such variation may result from interactions between the introgressed rye chromatin and the wheat genome, influencing the expression of resistance genes in different genetic backgrounds.

3.9. Marker analysis

A total of 177 introgression lines exhibiting HR and R disease reaction were used for the identification of rye chromosomal segments associated with resistance, using 17 EST-derived markers. Marker analysis revealed the presence of rye chromosome segments among KB-resistant introgression lines (Table 4). Four lines *viz.*, TL 2908/2*CS(S)//PBW 343-59, TL 3021/2*CS(S)// PBW 550-1, TL 3021/2*CS(S)//PBW 550-4 and TL 3048/2*CS(S)//DBW 17-5 showed the presence of rye chromosomal segments corresponding to both 4RL and 4RS arms and 53 lines carried introgressed regions specifically from 4RL (Fig 3). These findings suggest that introgressions from rye chromosome arms 4RL and 4RS may play an important role in conferring KB resistance in wheat. The presence of these rye chromatin segments indicates that useful resistance factors from rye may have been successfully transferred into the wheat background through triticale-mediated introgression.

Molecular marker-based approaches have greatly facilitated the identification and tracking of rye chromatin introgressions in wheat breeding programmes. Several studies have demonstrated the effectiveness of rye-specific molecular markers for detecting alien chromatin in wheat backgrounds (Hackauf and Wehling, 2002; Khlestkina *et al.,* 2004; Milczarski *et al.,* 2007). Earlier, Koebner (1995) developed PCR-based markers using rye-specific oligonucleotide primers for detecting rye sequences in wheat, enabling efficient screening of wheat–rye introgression lines. Subsequently, Hackauf and Wehling (2002) and Khlestkina *et al.* (2004) identified microsatellite polymorphisms and mapped numerous SSR loci in the rye genome, while Milczarski *et al.* (2007) constructed detailed genetic maps of rye using PCR-based markers. Similarly, Silkova *et al.* (2007) identified rye chromosomes in wheat-rye substitution lines and determined the corresponding wheat chromosomes replaced by rye chromatin using SSR markers. Feltaous (2015) utilized the rye-specific marker SCM9, associated with the 1B/1R translocation, to screen 2,651 lines for the presence of rye chromatin. Likewise, Khare (2017) employed rye-specific SSR markers to confirm the presence of rye chromosomes in wheat lines derived from triticale crosses.

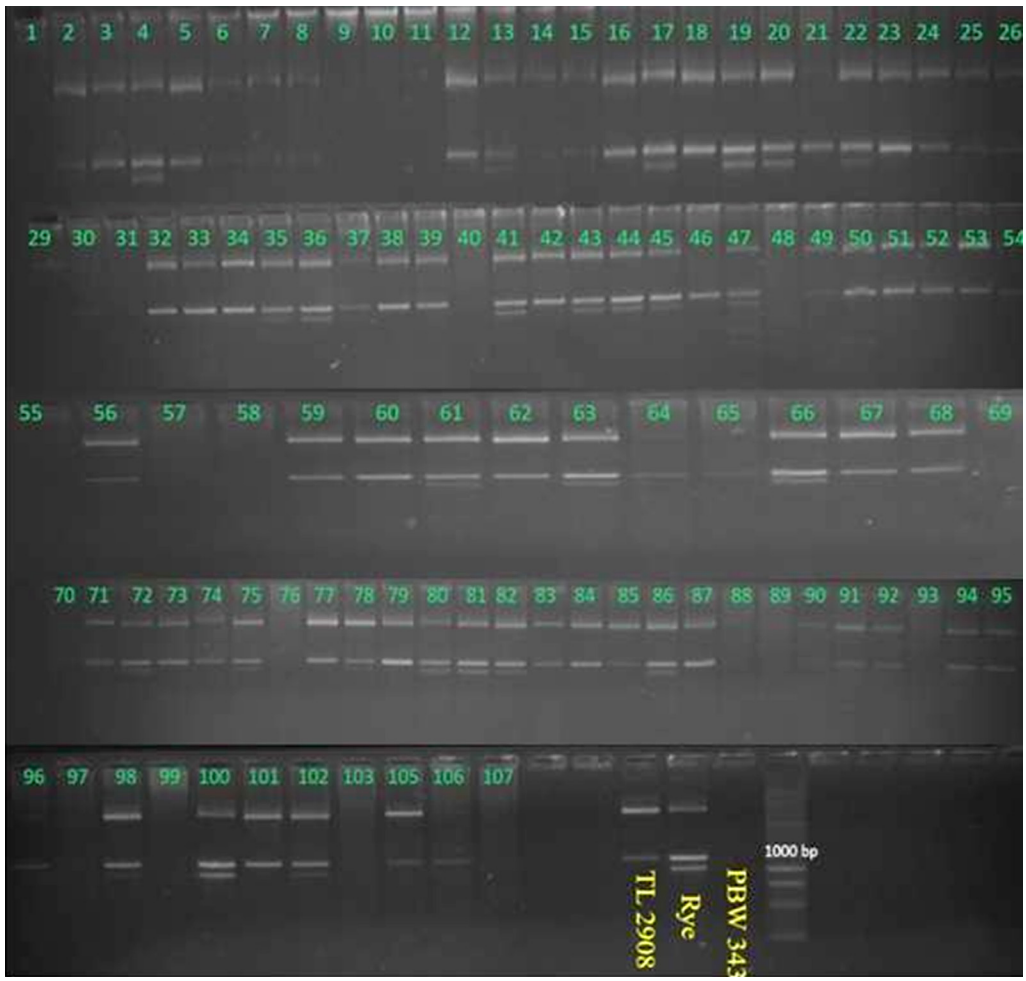

Fig 3: Agarose gel view of rye chromosome specific markers CGG 49 (4RL) on resistant triticale-bread wheat introgression lines.

Table 4: Introgression lines having rye chromosome segment for KB resistance

S. No.	Introgression line	CGG 49 (4RL)	KSUM 62 (4RS)	Disease score
1	TL 2908/ 2* CS(S)//PBW 343-4	P	A	2.40
2	TL 2908/ 2* CS(S)//PBW 343-5	P	A	1.67
3	TL 2908/ 2* CS(S)//PBW 343-7	P	A	4.80
4	TL 2908/ 2* CS(S)//PBW 343-13	P	A	1.13
5	TL 2908/ 2* CS(S)//PBW 343-24	P	A	1.44
6	TL 2908/ 2* CS(S)//PBW 343-37	P	A	2.64
7	TL 2908/ 2* CS(S)//PBW 343-38	P	A	3.56
8	TL 2908/ 2* CS(S)//PBW 343-41	P	A	2.27
9	TL 2908/ 2* CS(S)//PBW 343-42	P	A	3.34
10	TL 2908/ 2* CS(S)//PBW 343-44	P	A	4.50
11	TL 2908/ 2* CS(S)//PBW 343-47	P	A	4.50
12	TL 2908/ 2* CS(S)//PBW 343-49	P	A	1.50
13	TL 2908/ 2* CS(S)//PBW 343-50	P	A	4.25
14	TL 2908/ 2* CS(S)//PBW 343-55	P	A	3.61
15	TL 2908/ 2* CS(S)//PBW 343-56	P	A	4.00

S. No.	Introgression line	CGG 49 (4RL)	KSUM 62 (4RS)	Disease score
16	TL 2908/ 2* CS(S)//PBW 343-58	P	A	2.87
17	TL 2908/ 2* CS(S)//PBW 343-59	P	P	3.82
18	TL 2908/ 2* CS(S)//PBW 343-81	P	A	2.93
19	TL 2908/ 2* CS(S)//PBW 343-84	P	A	4.50
20	TL 3048/ 2* CS(S)//PBW 343-3	P	A	0.00
21	TL 3048/ 2* CS(S)//PBW 343-4	P	A	0.00
22	TL 3048/ 2* CS(S)//PBW 343-5	P	A	0.00
23	TL 3048/ 2* CS(S)//PBW 343-12	P	A	3.68
24	TL 3048/ 2* CS(S)//PBW 343-14	P	A	2.62
25	TL 3048/ 2* CS(S)//PBW 343-16	P	A	0.00
26	TL 3048/ 2* CS(S)//PBW 343-17	P	A	0.00
27	TL 3048/ 2* CS(S)//PBW 343-18	P	A	0.94
28	TL 3048/ 2* CS(S)//PBW 343-19	P	A	2.22
29	TL 3048/ 2* CS(S)//PBW 343-20	P	A	2.50
30	TL 3048/ 2* CS(S)//PBW 343-21	P	A	0.00
31	TL 3048/ 2* CS(S)//PBW 343-22	P	A	0.00
32	TL 3048/ 2* CS(S)//PBW 343-23	P	A	0.00
33	TL 3048/ 2* CS(S)//PBW 343-27	P	A	1.59
34	TL 3048/ 2* CS(S)//PBW 343-31	P	A	1.53
35	TL 3048/ 2* CS(S)//PBW 343-32	P	A	0.00
36	TL 3048/ 2* CS(S)//PBW 343-33	P	A	0.00
37	TL 3048/ 2* CS(S)//PBW 343-34	P	A	2.36
38	TL 3048/ 2* CS(S)//PBW 343-35	P	A	0.00
39	TL 3048/ 2* CS(S)//PBW 343-37	P	A	0.00
40	TL 3048/ 2* CS(S)//PBW 343-41	P	A	1.87
41	TL 2065 / 2*CS (S) //PBW343-2	P	A	2.70
42	TL 2065 / 2*CS (S) //PBW343-9	P	A	4.58
43	TL 2065 / 2*CS (S) //PBW343-10	P	A	4.50
44	TL 3048/2*CS(S)// PBW550-1	P	A	2.72
45	TL 3048/2*CS(S)// PBW550-2	P	A	1.67
46	TL 3048/2*CS(S)// PBW550-3	P	A	4.17
47	TL 3048/2*CS(S)// PBW550-4	P	A	4.34
48	TL 3048/2*CS(S)// PBW550-6	P	A	4.00
49	TL 3048/2*CS(S)// PBW550-10	P	A	4.09
50	TL 3021/2* CS (S)//PBW 550-1	P	P	0.00
51	TL 3021/2* CS (S)//PBW 550-2	P	A	0.00
52	TL 3021/2* CS (S)//PBW 550-3	P	A	0.00
53	TL 3021/2* CS (S)//PBW 550-4	P	P	0.88
54	TL 3021/2* CS (S)//PBW 550-5	P	A	0.60
55	TL 3021/2* CS (S)//PBW 550-6	P	A	0.00
56	TL 3048/2*CS(S)//DBW 17-4	P	A	3.46
57	TL 3048/2*CS(S)//DBW 17-5	P	P	4.25

Earlier cytogenetic studies also support the importance of rye chromosomes as sources of disease resistance in wheat. For instance, An *et al.* (2013) identified a wheat-rye 4R chromosome translocation line exhibiting resistance to powdery mildew using molecular cytogenetic characterization. Rye chromosomes are known to harbor several important resistance genes against major wheat diseases. Notably, genes such as *Yr9*, *Lr26*, *Sr31* and *Pm8* conferring resistance to yellow rust, leaf rust, stem rust and powdery mildew, respectively, have been mapped on rye chromosome 1R (Mago *et al.,* 2002), while *Pm56*, a powdery mildew resistance gene, has been identified on 6RS (Hao *et al.,* 2018). Recently, Ma *et al.* (2020) and Dyda *et al.* (2022) reported wheat lines carrying rye chromosome 4R translocations to exhibit strong powdery mildew resistance. In 2021, Moskal *et al.* also reported rye chromosome 4R harbors genes conferring resistance against rusts and Fusarium head blight which has been successfully utilized in wheat-rye translocation breeding programmes. Thus, highlighting the considerable potential of rye particularly chromosome arm 4RL as reservoirs of resistance genes for wheat improvement. The use of triticale as a genetic bridge has proven to be an effective strategy for transferring beneficial rye genes into wheat (Saulescu *et al.,* 2011).

Earlier studies also suggest that rye chromosomes contribute KB resistance in wheat. Two disomic addition lines carrying rye chromosomes 4R and 6R of cultivar Imperial in the background of *T. aestivum* cultivar Chinese Spring were reported to be completely free from KB under artificial inoculation. Furthermore, the transfer of these two chromosome into the high-yielding but KB-susceptible bread wheat cultivar WL 711 through back crossing maintained resistance under both monosomic and disomic addition conditions (Datta *et al.,* 1995). Similarly, the present findings also highlight the role of rye chromosome arms 4RL and 4RS as a valuable genetic resource for enhancing KB resistance in wheat. Further validation and fine mapping of these introgressed segments using advanced genomic tools such as high-density SNP markers, next-generation sequencing approaches may help to identify the underlying KB resistance loci. The identified KB-resistant introgression lines could serve as a valuable pre-breeding material for wheat improvement programmes aimed at developing high-yielding KB resistance cultivars.

Author contributions

All the authors contributed in the generation of data and development of the variety.

Conflict of interest

The authors declare no conflict of interest.

Ethical Approval

The article doesn't contain any study involving ethical approval.

Generative AI or AI/Assisted Technologies use in Manuscript Preparation

No

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