#### Research Article

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# Evaluation of Genetic Parameters for Yield Traits and Stripe Rust Resistance in Bread Wheat Recombinant Inbred Lines

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#### **Abstract**

Stripe rust, caused by Puccinia striiformis f. sp. tritici, is a significant threat to wheat production worldwide, impacting grain yield, quality, and nutritional value. Therefore, it becomes essential to study the genetic variability among diverse lines. In order to, explore the genetic variability a complete set of RILs (Recombinant inbred lines) derived from cross 'W8627/PBW343' was evaluated against stripe rust for seedling and adult plant resistance. This study was conducted at ICAR- Indian Institute of Wheat and Barley Research, Regional Station, Shimla for seedling stage and experimental field of ICAR-IIWBR, Karnal, for adult plant stage during the year 2021-22 and 2022-23. Disease severity and infection type data was recorded and then was used to compute the coefficient of infection (CI) and Area Under Disease Progress Curve (AUDPC). Also, the data for various agromorphological traits was recorded. Significant differences were observed among the RILs after employing analysis of variance across years for all the traits. The combined analysis of variance across years revealed the presence of significant differences for all the traits in RIL population. The correlation analysis displayed the negatively significant association among disease severity and yield contributing traits. The findings revealed extensive genetic variability and high heritability, as well as favorable associations among various traits, facilitating the selection of promising RILs exhibiting disease resistance, elevated yield, and broad adaptability.

**Keywords:** *Triticum aestivum*, Stripe rust, genetic variability, seedling and adult plant resistance

#### 1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most significant crops ensuring the food and nutritional security globally. In past few decades, there are several reports depicting the emerging challenges due to climate change leading to adverse effects of biotic and abiotic stresses adversely impacting the wheat production (Trethowan *et al.*, 2018). Among biotic stresses, stripe rust caused by *Puccinia* 

striiformis f.sp. tritici is the most devastating disease that may cause upto 100% losses in case of severe epidemics (Afzal et al., 2007). This disease not only reduces grain yield but also affects the quality and nutritional value of the wheat, posing a serious challenge to farmers. Wheat stripe rust caused by *Puccinia striiformis* f.sp. tritici (Pst) has been explicitly known for inducing substantial loss in wheat



cultivars throughout the world. Yellow rust (YR) infection notably affects wheat leaves, interrupting photosynthesis, which results in reduced light absorption and radiation use efficiency, ultimately leading to decreased yields. Moreover, it can also affect the glumes, lemma, and palea of the wheat ear, especially during moderate to severe epidemics, resulting in significant loss in crop yield and quality and hence threatening food security (Bouvet *et al.*, 2022; Wellings, 2007).

YR is highly prevalent in India, particularly affecting the sub-mountainous regions of Punjab, Haryana, and Western Uttar Pradesh, which are important wheatgrowing areas in the country. Significant outbreaks of stripe rust were observed during 2006 and 2012-2013, resulting in considerable crop losses (Prashar et al., 2007; Saharan et al., 2013; Pradhan et al., 2020). PBW343 gained prominence as a major wheat variety due to its ability to thrive in diverse environments, leading to its widespread cultivation for more than a decade. The highly virulent pathotype '78S84' against gene Yr27 rendered PBW343 susceptible to yellow rust (Prashar et al., 2007; Sharma et al., 2021). The main cause of stripe rust epidemics is the occurrence of several virulent pathotypes. New pathotypes continue to emerge in nature due to their ability to mutate and sexually recombine. To date, among the 140 pathotypes recognized globally, India has documented more than Pst 28 pathotypes (Bhardwaj et al., 2014; Prasad et al., 2021).

Although fungicides and appropriate agronomical practices aid in controlling rust infection, genetic resistance is still one of the best and environmentally safe means for minimizing losses (Kumar et al., 2019; Wellings, 2011). Resistance to cereal rusts is categorized into two types: Seedling resistance, also known as all-stage resistance (ASR), targets one or a few Pst races. These seedling resistance genes are active both at the seedling and adult plant stages, and they are marked by a strong to moderate immune response. Adult plant resistance (APR) combats more than one race of pathogens is very well documented. Additionally, using resistant cultivars is probably a reliable, environmentally beneficial strategy. The degree of genetic diversity for target traits is one of the most crucial factors in the development and selection of stripe rust resistant cultivars by employing resistance breeding approach. Several parameters, including the

mean, range and heritability of agro-morphological features attributing rust resistance, are helpful in estimating the degree of genetic diversity and have been applied to a variety of crops (Singh et al., 2005). Heritability, a helpful quantitative metric for choosing genotypes with resistance and other qualities, determines how a character manifests. Selection from segregating generations is aided by Greater heritability and high genetic advancement aids in selection from segregating progenies (Sarfraz et al., 2016). Additionally, the trait association reveals the strength of correlation between disease resistance and yield contributing traits which is found to be advantageous while identifying preferred genotypes. When choosing desired genotypes, the character association reveals the strength of the correlation between resistance and yield components with yield as well as among themselves.

The present study was attempted to phenotype the RILs derived 'W8627/PBW343' in order to evaluate the genetic parameters such as genetic variability, heritability and genetic advancement that can assist breeders make progress toward stripe rust resistance.

### 2. Materials and Methods

The plant material consisted of a RIL population of 212 ( $F_8$ ) lines derived from the resistant parent W8627, which is a multiple disease resistant wheat genetic stock and the agronomically superior but susceptible megavariety PBW343 (Nord Desprez/VG9144//Kalyansona/Bluebird/3/Yaco/4/ Veery#5). The donor parent W8627 has already been confirmed as a multiple-disease resistant line (Virdi *et al.*, 2016). The RIL population derived from PBW343 x W8627 were further advanced to fixed stage ( $F_8$ ) and evaluated for stripe rust resistance.

### 2.1. Evaluation in greenhouse at seedling stage

For seedling stage experiment, RIL population along with both the parents and differential sets were raised in aluminium bread pan/trays using a mixture of fine loam and farmyard manure in the ratio of 3:1 (autoclaved) with each tray holding 10 rows of seedlings with a susceptible check (Agra Local) (Gangwar *et al.*, 2016). Following one week, the seedlings were treated with uredospore of four distinct races namely 110S119, 238S119, 110S84 and 78S884, individually, by spraying the inoculum in Soltrol. After inoculation, the trays were shifted in the glasshouse at  $22\pm2^{\circ}\mathrm{C}$  and maintained with a photoperiod of 16 h light



and 8 h of darkness. The inoculated plants were subjected to misting with water and subsequently incubated for 24 hours within glass chambers saturated with water. In order to prevent, the powdery mildew infection the plants were dusted with elemental dust powder of Sulfur and shifted to the greenhouse. The greenhouse conditions were meticulously optimized (temperature maintained at  $15\pm1^{\circ}$ C to promote robust plant growth and ensure effective stripe rust infection (Prasad *et al.*, 2021). Fourteen days following inoculation, the host's reaction to seedling infection types was assessed using a 0–4 scale, as described by Nayar *et al.* (1997).

### 2.2. Field Evaluation

The adult plant stage experiment for the evaluation of stripe rust resistance was conducted at ICAR- Indian Institute of Wheat and Barley Research, Karnal. Fifteen seeds from each line were planted in single rows of 1m length with 20 cm spacing, with two replications. The crop experiment was conducted by adhering to the recommended practices for the specific agro-climatic zone. Inoculum was sprayed at GS31-GS41 (on Zadok's scale) to create artificial epiphytotic conditions. To ensure the consistent infection across the field susceptible check wheat variety 'Agra Local', was sown every 20 rows of experimental set. Susceptible infector lines were sown around the experimental material, which comprised a mixture of susceptible varieties: Agra Local, WL711, C306, and Kharchia.

To prepare the inoculum, rust uredospores from the most prevalent pathotypes were suspended in 10 liters of water, with a few drops of Tween-20 added to the mixture. Spray inoculations were carried out in the evenings using an ultralow volume sprayer on alternate days from December until the appearance of stripe rust. Disease severity and host response were assessed for differential sets and the RIL population at the seedling stage after 21 days. Stripe rust was monitored weekly from January to March using the modified Cobb's Scale (Peterson et al., 1948). Host reactions were categorized: S = susceptible, characterized by large uredia with necrotic tissues; MS = moderately susceptible, with small uredia and necrotic tissues; MR = moderately resistant, also with small uredia and necrotic tissues; R = resistant, indicated by very small uredia with necrotic tissues. The area under the disease progress curve (AUDPC) (Wilcoxson et al., 1975) and the

Genetic variability and stripe rust association in bread wheat RILs coefficient of infection (CI) were determined using the specified formulas:

AUDPC= 
$$\sum_{n=1}^{\infty} {X_i + X_{i+1}(x) \choose 2} (a_{i+1} - t_i)$$

where, Xi is the rust intensity on date i,  $t_i$  is the time in days between i and date i+1 and n is the number of dates on which disease was recorded. Coefficient of infection (CI) was calculated by constant values for infection types based on Immune = 0, R = 0.2, MR = 0.4, M = 0.6, MS = 0.8, S = 1 (Stubbs *et al.*, 1988).

Observations for various agro-morphological traits namely days to heading (DH), days to anthesis (DA), days to maturity (DM), plant height (PH), Spike length (SL), No. of effective tillers (ET), No. of grains per spike (NGS), grain weight per spike (GWS), 1000-grain weight (TGW) and grain yield (GY), were recorded. At Zadoks growth stage 87, days to heading were noted at the hard dough stage (Zadoks et al., 1974). The number of days from sowing until 50% of the ear fully emerges from the boot of the flag leaf in each row was taken into account to compute the DH. At maturity, the height of the plant (measured in centimeters) was recorded from the ground to the tip of the tagged tiller spike, without the awns. The spike length (cm) was measured from the base up to the tip of the spike excluding awns. The numbers of days from sowing till the grains were completely hard and retained less than 18% moisture was used to calculate the dry matter (DM). Five main shoot spikes were tagged in each row and were harvested separately and threshed manually. The grains so collected were weighed to determine the grain weight/ spike in grams and counted to obtain grain number/spike. 1000 seeds were counted randomly by using seed counter for TGW (grams), and an electric balance was used to weigh the seeds. Weighing all of the collected and cleaned seeds from each row was employed to calculate the grain yield per row length (grams).

#### 2.3. Statistical Analysis

PROC GLM of SAS software (SAS Institute Inc. v. 9.1.) was employed to perform analysis of variance for the design of experiment for all traits under study. Data were subjected to transformation for traits measured in ratio and percent. To verify if data were normally frequency distribution curve analysis was done using 'ggplot' package. Pearson's correlation coefficient among



agromorphological traits and AUDPC was determined by using 'corrplot' package in R Studio (R version 4.3.2). To calculate the heritability broad sense ( $H^2$  (bs) (%)) 'variability' package was used.

#### 3. Results and Discussion

The results showed that W8627 (resistant parent) accounted for high resistance against 78S84 and 110S84 while it showed susceptible infection type against 238S119 and 110S119 including PBW343 (susceptible parent) and Agra Local (susceptible check). Among the four identified pathotypes, 238S119 demonstrated the highest virulence, exhibiting a susceptibility rate of 89.6%. This was followed by the 110S119 pathotype, which displayed a susceptibility rate of 78.5%. The 110S84 pathotype showed a susceptibility rate of 66.5%, while the 78S84 pathotype had the lowest susceptibility rate at 63%, as illustrated in Figure 1. Resistance that is effective throughout all stages of plant development, referred to

as all-stage resistance or seedling resistance, can initially be identified during the seedling phase. This type of resistance provides strong protection against Pst infection throughout the plant's development. However, it may not be able to withstand attacks from highly virulent pathotypes, making the cultivar vulnerable to infection. The resistant parent, W8627, exhibited strong resistance to both 78S84 and 110S84 Pst pathotypes, indicating its potential for continued use as a dependable source of resistance in breeding initiatives. Our findings further validate the existence of stripe rust resistance in W8627 (resistant parent) as per earlier reports by Virdi et al., 2016. On the basis of IT scores, our results displayed the stripe rust severity against four distinct pathotypes in the order: 238S119>110S119>110S84> 78S84 suggesting that 238S119 and 110S119 were the recently evolved and most virulent pathotypes (Pradhan et al., 2020; Kumar et al., 2020).

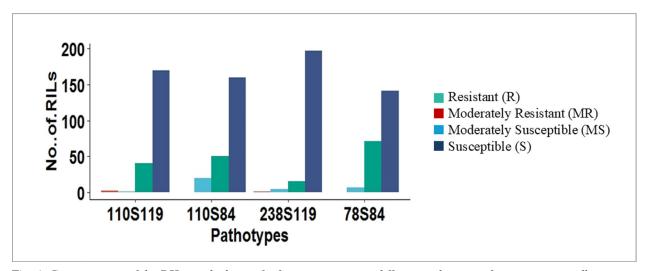


Fig. 1: Categorization of the RILs on the basis of infection response to different pathotypes of stripe rust at seedling stage: resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S).

Adult plant resistance (APR) is considered to be more persistent due to the fact that a distict genetic change manifests not enough to overpower this kind of resistance during asexual stage of pathogen (Schwessinger, 2017). Usually, APR stall infection and spore formation, which leads to slow rusting mechanism in plants rather than total immunity. In the present study, the field evaluation of RILs and their parental lines was conducted based on host response, disease severity, and AUDPC. The results showed that the mean value of W8627 (resistant parent) and susceptible parent (PBW343) consistently exhibited

a resistant and moderately susceptible response over the two consecutive seasons with ACI values of 2.85 and 50, AUDPC values of 52.5 and 487.5, respectively. For the RILs, mean value of the AUDPC ranging from 0 to 880.4 (Figure 2). W8627 consistently demonstrated stable resistance to the mixture of Pst pathotypes, whereas the PBW343, exhibited a moderate level of infection across years. Our results displayed the mean value of RILs for disease severity was much wider (beyond parental range) suggesting that segregation of RILs had been stabilized during initial generations. Some lines exhibited the



Genetic variability and stripe rust association in bread wheat RILs

immune response (no reaction) which further signifies that these RILs might have transgressive segregation toward stripe rust resistance. Also, revealing the direct utilization of highly resistant lines which are performing better than the resistant parent (W8627). A high susceptibility to disease was observed in a significant number of lines, suggesting a strong disease pressure.

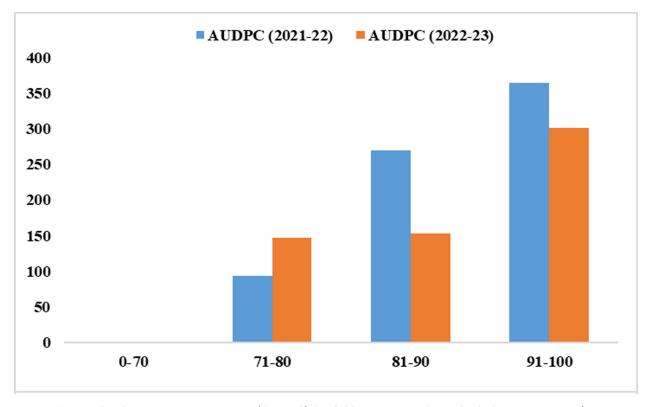
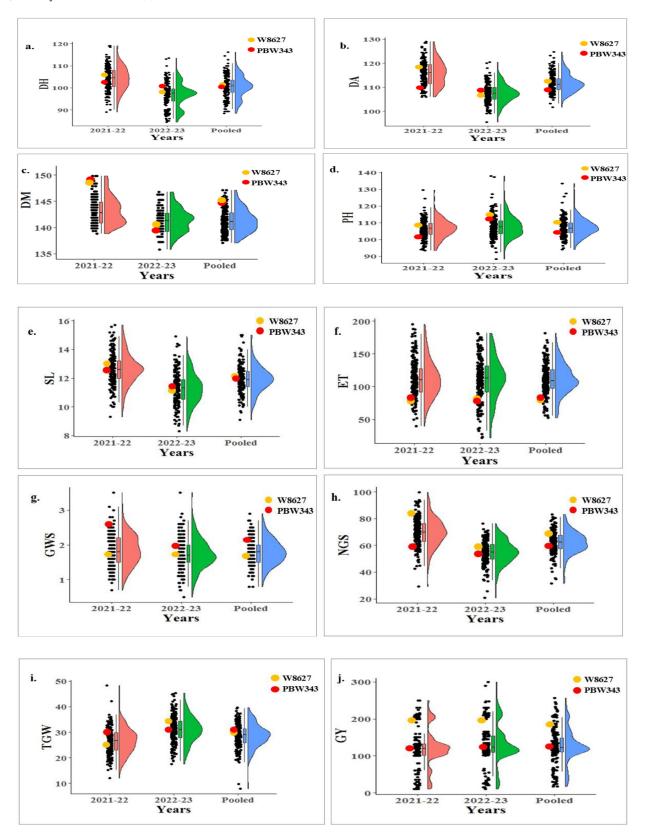


Fig. 2: Area under disease progression curve (AUDPC) for field experiment during both the crop seasons (2021-22 & 2022-23) of RILs.

The range and mean values of RILs and parents for various traits are presented in figure 3a-3j. The analysis of variance (ANOVA) estimates indicated that the present RILs exhibited significant variation for the different traits revealing the effect of environment on population which further complements the phenotyping of such material with genotyping validate the accuracy of results over the environments (Table 1). In our study, both the parents and the RILs exhibited high degree of variability as evident by the mean performance for the studied traits during both the crop seasons (2021-22 and 2022-23). The present study revealed that PBW343 (the parent susceptible to disease) exhibited a shorter time to reaching the flowering stage compared to W8627 (the parent resistant to disease). This finding suggests that the recombinant inbred lines (RILs) with early flowering tendencies might exhibit more severe disease symptoms compared to lines that flower late. This phenomenon has also been observed in previous studies by Murray et al. (1995) and Naseri et al. (2019). Heritability

plays a crucial role as a quantitative measurement that considers the impact of both environment and genetics on the expression of a trait. It allows for the analysis of the differing influences of genes and environments on the variability of characteristics within and across populations (Allard et al., 1960). Based on the categorization suggested by Johnson et al. (1955), significant heritability rates greater than 60% for all traits were observed in this study. This suggests that the genotypes with high heritability, of the traits being studied may hold promise for evaluation across different environments. These genotypes demonstrate potential advancements in terms of yield, resistance, and adaptability to varying environmental conditions. Further, these outcomes coincide with the research conducted by Kokhmetova et al., 2023. The RIL population exhibited high broad-sense heritability (≥0.8) for all traits across the years (Table 2), indicating that breeding can effectively enhance rust resistance.







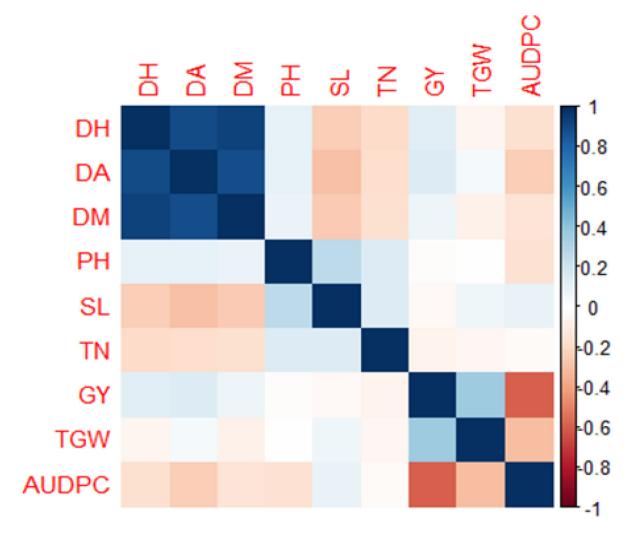


Fig. 4: Pearson correlation coefficients between AUDPC and different agro-morphological traits in the RIL population during crop season 2021–22 and 2022–23.

The correlation coefficients were appraised to find out the associations among disease severity and different agromorphological traits at phenotypic level which signifies about the degree of association of traits. It was observed that grain yield displayed the positive correlation with DH, DA, DM and TGW while significantly negative correlation was observed with AUDPC (Figure 4). Similarly, significant positive association of GY with the other traits *viz*, TGW and GNS reported by Nazem and Arzani (2013). Verma *et al.* (2024) screened wheat germplasm for stripe rust resistance through field and molecular studies, identifying genotypes with combined resistance to yellow rust and powdery mildew that show potential as varieties or donors for breeding resistant

sources. Zhou et al., 2022 evaluated two F2 populations under controlled and field conditions, showing similar disease response rankings. Phenotyping under controlled conditions with continuous light could expedite the development of resistant wheat varieties (Singroha et al., 2017). In conclusion, present study revealed the presence of wide variability for stripe rust resistance in RIL population; further some lines exhibited the immune response (no reaction) further suggesting the direct utilization of highly resistant RILs which are performing better than the resistant parent. The cross holds great promise in not only improving the grain yield parameters but also stripe rust resistance.



**Table 1.** Analysis of variance for stripe rust severity and other traits during 2021-22 and 2022-23.

Traits	ts	DH	DA	DM	PH	SL	LN	GNS	GWS	GWS TGW	$\mathbf{G}\mathbf{X}$	DS
	DF											
Geno.	211	56,49***	40.08***	10.54***	74.71***	2.67***	1631.863***	188.69***	188.69*** 0.405***	155.67	155.67 0.006***	902.05***
Reps.		71.85**	29.53	94.88***	333.37***	1.64	7945.86**	39.64	1.634**	509.31	0.019**	9.03
Error	211	86.8	9.24	3.04	23.69	1.19	1109.97	108.80	0.22	141.74	0.00	20.73

significant at (P<0.1) per cent LSD; where DH: Days to heading, DA: Days to anthesis, DM: Days to maturity, PH: Plant height, SL: Spike ength, ET: No. of effective tillers m2, GWS: Grain weight per spike), NGS: No. of grains per spike, TGW: Thousand grain weight, GY: Grain yield m2, DS: Disease Severity. significant at (P<0.05) per cent LSD and \* Significant at (P < 0.01) per cent LSD,

Estimates of heritability among RILs for stripe rust severity and other traits during 2014-15 and 2015-16. Table 2.

Traits	DH	DA	DM	PH	SL	ZI	CNS	GWS	IGW	CY.
$\mathrm{H}^{2}\left( \mathrm{bs}\right)$	0.97	0.94	0.97	0.89	0.84	0.84	98.0	0.82	0.76	0.87
DH: Days to headin	DH: Days to heading, DA: Days to anthesis, DM: Days to n weight GY: Grain yield m <sup>2</sup> . DS: Disease Severity.	sis, DM: Days to r.	naturity, PH: Plant	height, SL: Spike l	nt, $\mathbf{SL}.$ Spike length, $\mathbf{ET}.$ No. of effective tillers $m^2,$ $\mathbf{GWS}.$ Grain	effective tillers m	<sup>2</sup> , <b>GWS:</b> Grain we	ight per spike), NGS: No	GS: No. of grains	per spike, TGW: Thou

## **Author Contributions**

R, SK, PP conducted the experiment, recorded data & prepared the manuscript; IS, RT, PS conceptualized the research including plant material & finalized the manuscript; AV did statistical analysis

#### Conflict of Interest

The authors declare no conflict of interest.

# **Ethical Approval**

This article does not contain any studies involving human or animal participants performed by any of the authors.

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