

IDENTIFICATION OF BROWN RUST TOLERANT AND DEVELOPMENT OF DISEASE TOLERANT HIGH YIELDING SPRING WHEAT (*TRITICUM AESTIVUM L.***) GENOTYPES**

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(Received, 04th June 2024, Revised 19th August 2024, Published 31st August 2024)

Abstract: *In the agricultural sector of Pakistan, wheat cultivation confronted a substantial obstacle in the form of leaf rust disease caused by the fungus Puccinia triticina. During the crop year 2021-22, a comprehensive screening was conducted on a hundred wheat genotypes to evaluate their resistance to this brown rust affliction. Results indicated six wheat genotypes tolerant to brown rust. Line into Tester mating design was used to assess combining ability. Grain yield and other related characters were studied in 18 F1 generations along with nine parents (six lines and three testers), which were evaluated for combining ability with three replications in a randomized complete block design (RCBD). The results indicated that among various lines, line 9479 was identified as a good general combiner for the character's spike length, spikelets per spike, and 1000-grain weight. In contrast, Line 9486 appeared as a good general combiner for the characters, flag leaf area, plant height, peduncle length, number of tillers per plant, and 1000-grain weight. Line 9515 is for flag leaf area, plant height, and spike length. Line 9519 for the number of tillers per plant, grain yield per plant, and plant height; line 9520 for peduncle length, number of tillers per plant, grains per spike, and grain yield per plant; and 9521 was identified as a good general combiner for the characters spike length, spikelet's per spike, number of grains per spike and spike density. Tester Punjab-11 emerged as an excellent general combiner for the character's peduncle length, plant height, flag leaf area, and number of tillers per plant. In contrast, Tester Ass-11 appeared as a good combiner for the spike length, peduncle length, 1000-grain weight, and flag leaf area. Tester Chakwal-50 identified an excellent general combiner for the number of tillers per plant, plant height, grains per spike, spikelets per spike, grain yield per plant, 1000-grain weight, and spike density. Out of 18 cross combinations, seven crosses viz. 9479 × Aas-11, 9486 × Aas-11, 9515 × Aas-11, 9519 × Chakwal-50, 9520 × Punjab-11, 9521 × Punjab-11, and 9521 × Aas-11 emerged with significant positive SCA effects for grain yield per plant. Thus, a biparental mating system can exploit these crosses for grain yield per plant.*

Keywords: Brown Rust, Combining Ability, Yield, Line × Tester Analysis, Spring Wheat

Introduction

Wheat, specifically *Triticum aestivum* L., is the principal cereal grain that feeds a vast portion of the global population. Pakistan's agricultural sector represents an 8.2% increase in value, accounting for 1.9% of the nation's GDP. The cultivation area for wheat had a modest rise of 0.7%, expanding from 8,977 to 9,043 thousand hectares in the 2022-2023 period compared to the previous year. This period also witnessed a significant 5.4% surge in wheat production, climbing from 26.208 million tonnes to 28.634 million tonnes, as the Pakistan Economic Survey reported for 2023-24. Ranking as the world's eighth-largest wheat producer, Pakistan's contribution to the wheat market is noteworthy. Aestivum wheat is the predominant species cultivated worldwide, occupying 90% of the wheat-growing area. It is followed by durum wheat, which constitutes roughly 9%, while *T. diccoum* and *T. monococcum* collectively cover less than 1% of the global wheat cultivation area, as noted by (Abou-Elwafa & Shehzad, 2021). Wheat is a staple for nearly 40% of the global

population, and it is crucial for the dietary protein requirements of over 4.5 billion individuals in developing nations, accounting for 21% of their daily intake (Giraldo et al., 2019). Annually, it contributes about 55% of the carbohydrate consumption and 20% of the caloric intake worldwide (Widyaratne & Zijlstra, 2007). With projections indicating a surge to 9.3 billion in the global population by 2050 (Cleland, 2013), the urgency to breed enhanced wheat varieties to meet the escalating food demand is evident. In Pakistan, wheat production lags behind developed countries (Ahmad et al., 2013). Addressing this deficit necessitates the development of high-yield wheat strains suited to various agricultural and climatic conditions. Consequently, genetic diversity was evaluated in breeding stock to determine the correlation between the performance of firstgeneration hybrids and the extent of genetic variation, utilizing their combining abilities. The yield potential of recent wheat varieties is diminishing over time due to various factors such as biotic and abiotic stresses, genetic drift, cross-pollination contamination, and seed mixing with

[Citation Ullah, A., Islam, B., Samad, R.A., Ghuffar, S., Ahmad, K., Saeed, A., Ahmad, M., Usman, U., Nusrat, A.T. (2024). Identification of brown rust tolerant and development of disease tolerant high yielding spring wheat (*TRITICUM AESTIVUM L.*) Genotypes. *Biol. Clin. Sci. Res. J.,* **2024***: 1087*. doi: [https://doi.org/10.54112/bcsrj.v2024i1.1087\]](https://doi.org/10.54112/bcsrj.v2024i1.1087)

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different varieties during threshing, all contributing to a progressive decline in yield. Wheat fields are vulnerable to numerous pathogens, including various rusts—such as leaf, stripe, and stem rusts—alongside smut, karnal bunt, and powdery mildew, which all contribute to reduced harvests according to Soliman et al. (2012). The fungus *Puccinia triticina*, responsible for leaf rust, poses a considerable risk to wheat production. It is an airborne pathogen that can spread to areas far from its initial outbreak. This rust type alone can cause up to 10% wheat yield losses. Although leaf rust is more detrimental than stem and yellow rust, its impact varies depending on the wheat's growth phase and resistance level. The likelihood of rust proliferation is highest in conditions of elevated humidity and temperatures between 15-22°C, with spore germination most favorable at 20˚C during a short 6–8 hour period. Infections of leaf rust at the early growth stages can cut down yields significantly by over 50% in some cases. Despite leaf rust's less conspicuous symptoms compared to stripe rust, its more frequent occurrence suggests it may be responsible for larger cumulative losses worldwide. Hussain *et al*. (2006) pointed out the lack of wheat strains in Pakistan that are disease-resistant and high-yielding. Economically, leaf rust has caused substantial losses, such as a 20 million dollar deficit in Western Australia. Pakistan also faced a 10% decrease in wheat production in 1978, as recorded by Hassan in 1979. In severe infestations, leaf rust can yield reductions as drastic as 50%. To counteract this reduction in yield, it is essential to reorganize the genetic material of these varieties and genotypes. This reorganization aims to achieve an optimal genetic composition, enabling them to perform more effectively across diverse and changing environmental conditions. Since wheat is grown in a broad spectrum of climatic scenarios, a deep comprehension of genetics is invaluable for plant breeding. The Line \times tester mating design is a statistical approach used to assess parental genotypes and their hybrid combinations for creating new varieties, allowing for testing more parental genotypes with fewer cross combinations. The study encompasses both primary and secondary statistical analyses, which are crucial for assessing the combining ability—general and specific (GCA and SCA)—across various crops such as wheat, rice, barley, sugarcane, maize, and other significant cash crops. This is instrumental in deciphering the genetic factors influencing yield and its associated characteristics. The experiment aimed to unravel the genetic mechanisms and pinpoint the superior combining genotypes contributing to seed yield and its

associated attributes. Consequently, the insights gained could be instrumental in selecting the appropriate parents and their hybrid combinations, laying the groundwork for a robust breeding program.

Methodology

The experiment was conducted at the research area of Plant Breeding and Genetics, College of Agriculture, University of Sargodha, to find out the potential varieties showing resistance against brown leaf rust during 2021–22, 100 wheat genotypes screened against brown leaf rust. Row to row distance maintained at 30 cm. The length of each line was 5 m. Weather data was recorded regularly to observe which temperature and humidity favor brown leaf rust incidence. Wheat genotypes were sown early in October to find out the disease severity. All the agronomic practices were kept the same. Rust inoculations of the spreaders and check lines were carried out using the hypodermic syringe method using aqueous urediospore suspension, to which 1 to 2 drops of Tween-20 were added to break the surface tension. The severity of leaf rusts was recorded according to Loegering (1959). The modified Cobb scale recorded the severity as the percent of plant rust infection (Peterson *et al*., 1948). As severity is determined by visual observation, readings cannot be correct. Therefore, the intervals for a severity below 5% are trace (T) to 2. Usually, 5 percent intervals are used from 5 to 20 percent severity and 10 percent intervals for higher readings. The response of a variety refers to the type of infection recorded by the following capital letters.

O - No visible infection

R - Resistant Necrotic areas with or without minute uremia

MR - Moderately resistant. Small credit present surrounded by necrotic areas

MS - Moderately susceptible. Medium uremia with no necrosis but possibly some distinct chlorosis

S - Susceptible: Large uredia and little or no chlorosis are present. Readings of severity and reaction are recorded together with severity first. For example:

TR - Trace severity of resistant type infection

10MR - 10 percent severity of a moderately resistant type infection 50S - 50 percent severity of a susceptible type infection.

The coefficientof Infection (CI) for both rusts has been calculated in the manner used in CIMMYT andIRN (USDA) asfollows:

The coefficient of infection (CI) was calculated by multiplying the response value by the intensity of infection by percent. The average Coefficient of Infection (ACI) was derived from the sum of the CI values of each entry divided by the number of locations.

After some modifications, a rating scale for disease resistance was adopted by PARC in 1982 for use with cereal rusts (Aslam, 1982), based on the scale by Doling (1965) for selecting wheat varieties to powdery mildew and later adopted by ARC of Great Britain for the farmers. The '0' to '9' scale, previously designated as Resistance Index (R.I), has been re-designated as RRI (Relative et al.). RRI is calculated on a 0 to 9 scale, where 0 denotes most susceptible, and nine is highly resistant.

The RRIis calculatedaccording to the following formula.

Lines that, through yield testing, show high yield stability even under high infection conditions increase their Index rating by "1".

The selected brown rust-tolerant wheat genotypes were hybridized during 2022-23. The experimental material consisted of nine wheat genotypes and six wheat lines viz. 9479, 9486, 9515, 9519, 9520, and 9521 (Female parents) and three testers viz. Punjab-11, Aas-11, Chakwal-50 (Male parents). These wheat genotypes were crossed in line \times tester fashion during the crop season in 2022-23 in the experimental area of the Department of Plant Breeding and Genetics, University of Sarghoda. The F_1 Seeds and nine parents were planted in the field using a randomized complete block design with three replications during the third week of November 2023. Each replication consisted of nine parents and 18 F1 crosses. Plant-to-plant and row-torow distances were 10 cm and 25 cm, respectively. Two seeds per hole were sown with the dibbler's help, which was later thinned to one seedling per hole after germination. The experiment was conducted under normal conditions from sowing to maturity following everyday production technology. At maturity, five well-guarded plants from each line were selected to record the data for plant height, number of tillers per plant, flag leaf area, peduncle length, spike length, number of spikelets per spike, number of grain per spike, 1000 grain weight, grain yield per plant and spike density. The data was subjected to analysis of variance to calculate significant differences among crosses and parents (Steel *et al*., 1997). Specific and general combining ability effects were estimated through combining ability analysis using the method proposed by Kempthorne (1957).

Result and Discussion

The most suitable and environmentally benign method of controlling losses brought on by leaf rust is through

genotypic resistance. The results of the wheat variety screenings conducted in 2021–2022 against leaf rust varied. Six genotypes indicated tolerance against brown leaf rust out of 100. During this time, three cultivars, Punjab 11, Aas 11, and Chakwal 50, were susceptible. Other types, however, had conflicting results. At the same time, other varieties showed a mixed response. The development of disease and sporulation is significantly influenced by temperature (Kolmer JA, 2005). The genotypes indicated tolerance to brown leaf rust, as indicated in Table 1. However, environmental variables are also crucial in the development of disease. After analyzing three years of data, it is determined that temperature, humidity, and rainfall enhance the likelihood of contracting leaf rust (Schnurbusch, 2019). Rainfall and humidity have a positive link with leaf rust, but temperature has a negative correlation. Low temperatures (18–25°C) with high humidity are ideal for spore growth. A temperature above 80% and rainfall between 10 mm and more strongly encourages disease (Huerta-Espino *et al*., 2011). To assess the combining ability and transfer disease, hybridization of tolerant genes in high-yielding genotypes was performed in line with the tester mating design. The resultant 18 F1 progenies were planted in 2023-24. The analysis of variance results, presented in Table 2, explains that there were highly significant genotypic differences present for all the ten characters, viz., flag leaf area, plant height, peduncle length, spike length, tillers per plant, spikelets per spike, grains per spike, 1000 grain weight grain yield per plant and spike density studied among F¹ progenies and the parental lines. Interpretation of line \times tester mating design analysis revealed significant differences between parents, crosses, interaction parents vs crosses, testers, lines, and interaction lines \times testers for yield and its related traits. General combining ability and specific combining ability analysis are presented in table-4 and table-5. The adverse GCA effects for plant height were observed for line 9486 (-3.34) and among testers in Punjab-11(-0.44) and Chakwal-50 (- 2.18). The adverse SCA effects were observed for 9520 \times Chakwal-50 (-2.31), 9519 \times Aas-11 (-1.35), and 9486 \times Chakwal-50 (-1.35). Among lines and testers, 9520 and Chakwal-50 had the highest and most positive GCA effects (0.53, 0.51) for productive tillers per plant, respectively. For tillers per plant, the cross combination, $9515 \times$ Chakwal-50, had the highest and most positive SCA effects (0.61), followed by $9486 \times PB-11$ (0.45). The positive and highest GCA effect for flag leaf area is in line 9486 and tester Ass-11 (5.20, 2.22 cm²), respectively. The cross combination 9486 \times Chakwal-50 (6.93) had the highest positive SCA effects for the flag leaf area. All the genotypes were significant for peduncle length. Among lines, 9486 had the highest positive GCA effects (2.04), and among testers, Aas-11 had the highest positive GCA effects (1.55) for peduncle length. The line 9486 and tester Aas-11 were good general combiners for peduncle length. Among lines 9479 (0.45) and among testers, Aas-11 (0.39) had positive and highest GCA effects for spike length. Among crosses, 9479 \times Chakwal-50 had the highest and positive SCA effects (0.81), followed by $9486 \times$ Chakwal-50 (0.48), $9519 \times$ Aas-11 (0.43) for spike length. Among lines, 9521 had the highest and positive GCA effects (0.87) , and among testers, Chakwal-50 had the highest and positive GCA effects (0.84) for spikelets per spike. Among crosses, the cross $9515 \times$ Chakwal-50 had the highest and positive SCA effects (1.02)

for the number of spikelets per spike, followed by 9486 \times Aas-11 (0.76), $9520 \times PB$ -11 (0.67), $9521 \times As$ -11 (0.76). Among female parents, 9521 had the highest and most positive GCA effects (2.35), and among males, Chakwal-50 and Aas-11 had positive GCA effects (0.36, 0.05) for grains per spike, respectively. The highest SCA effects were observed for $9479 \times$ Aas-11 (3.64) for grains per spike. The line 9486 and tester Chakwal-50 had the highest and most positive GCA effects (2.63, 1.91), respectively, for 1000 grain weight. Among crosses, the cross $9479 \times AAS-11$ had the highest and most positive SCA effects (11.58), followed by $9520 \times PB-11$ (5.44) and $9515 \times PB-11(4.70)$ for 1000grain weight. For grain yield, two female and one male parent show a positive GCA value, as shown in Table 4. Line 9521 had the highest and most positive GCA effects (0.06), and among testers, Chakwal-50 had the highest GCA effects (0.06) for spike density. The highest and positive SCA effects were observed for $9515 \times$ Chakwal-50 (0.13), followed by $9486 \times PB-11$ (0.10) for spike density. Wheat breeders' primary objective is developing wheat varieties with improved yield-related characteristics. Availability of genetically based variation is a pre-requisite for the selection of new cultivars. Present wheat material was deliberated to generate information on GC and SC for yield and yield-related traits. The assessment of genetic components of variation revealed that in the inheritance of studied traits, non-additive gene effects were predominant. As short-stature plants desired, a negative value is best for plant height. The results are similar reported by Kumar and Kerkhi (2015), Majeed et al. (2011), and Malik *et al*. (1988). Productive tillers per plant are a significant yield

Figure 1: Brown leaf rust on wheat

component. These findings conform with those of Saeed et al. (2001), Majeed et al. (2011), Yadav and Sirohi (2011), and Saeed *et al.* (2016). Flag leaf area had a positive association with grain yield. These outcomes are by the results of Awan *et al*. (2005), Moosavi *et al*. (2005), Saeed *et al*. (2001), and Rehman et al. (2013). The spike length is an essential trait, as a longer spike length produces more yields. These results are by Dhadhal *et al*. (2008), Baloch *et al*. (2011), Ajmal *et al.* (2004), Faisal *et al.* (2005), and Guo et al. (2018). Spikelets per spike contribute positively towards grain yield. The more spikelets per spike, the greater will be the yield. These findings conform with the results reported by Saeed *et al*. (2005), Malik *et al*. (2005), Chowdhry *et al*. (2007), Ajmal *et al.* (2004), Faisal *et al.* (2005) and Bibi *et al.* (2013). Grains per spike are also an essential factor for enhanced grain yield. These findings match with the results of Saeed et al. (2001), Singh et al. (2002), Hassan et al. (2007), Milano (2008), Khan et al. (2007), and Philipp *et al.* (2018). The results of Nazir *et al*. (2005) differ from these findings. 1000-grain weight is also an essential characteristic, positively contributing towards grain yield, our primary objective. Similar results are reported by Majeed *et al*. (2011), Singh *et al.* (2002), Ajmal *et al.* (2004), and Mecha *et al.* (2017). The positive value of spike density is essential for enhanced yield as it is related to more spike length and spikelets per spike. These findings were quite close to the results of Awan *et al*. (2005), Hassan *et al.* (2007), Saeed *et al.* (2005), Iqbal and Khan (2006), Mahpara *et al.* (2008) and Pesaraklu *et al.* (2016) for spike density.

Sr. No	Genotype	Disease	ACI	RRI
	9479	5R		o
	9486	5R	26.4	7.08
	9515	5R	27	7.01
	9519	5MRMS	8.6	8.05
	9520	5MRMS	24	7.34
o	9521	5MRMS	25.2	7.21
	Punjab-11	100S	64	1.92
	Aas- 11	80S	31.8	5.48
q	Chakwal-50	90S	37.8	4.82

Table 1: Response of Candidate lines to brown leaf rust along with their Average Coefficient Infection and Relative Resistance Index during 2021-22

Table 2: Mean Square ANOVA Of RCBD Design For Yield And Yield Related Traits Studied In Wheat.

Table 3: Mean Squares ANOVA Of Line × Tester Analysis For Yield And Yield Related Traits Studied In Wheat.

Parents	Plant	Number of	Peduncle	Spike	Flag leaf	Spikelets	No. of grains	1000 -grain	Grain vield	Spike
	height	tillers per plant	length (cm)	length	area (cm^2)	per spike	per spike	weight (g)	per plant	density
	(cm)			(cm)					(g)	
Lines (female)										
9479	$2.55*$	$0.11*$	0.12	$0.45*$	$0.50*$	$0.78*$	$0.94*$	1.39*	$-1.03*$	0.004
9486	$-3.34*$	$-0.87*$	$2.04*$	$-1.07*$	$5.20*$	$-1.27*$	$-1.74*$	$2.63*$	-0.73	$0.029*$
9515	$-1.49*$	0.05	-0.65	$0.33*$	0.19	$0.33*$	$-1.16*$	-0.13	-0.53	-0.009
9519	$-0.74*$	$0.42*$	-1.53	0.11	$-2.77*$	$-0.60*$	$-1.96*$	$-0.81*$	$1.58*$	$-0.06*$
9520	$2.42*$	$0.53*$	0.11	0.15	$-1.17*$	-0.11	$1.59*$	$-1.15*$	$1.03*$	$-0.03*$
9521	0.60	$-0.25*$	-0.09	0.03	$-1.94*$	$0.87*$	$2.35*$	$-1.93*$	-0.31	$0.06*$
S.E. of GCA for lines	0.18	0.06	1.63	0.17	0.244	0.15	0.33	0.310	0.74	0.02
Testers (males)										
Punjab-11	$-0.44*$	0.03	0.88	$-0.38*$	$0.50*$	$-0.76*$	$-0.41*$	$-2.75*$	$-0.92*$	-0.01
Aas- 11	$2.62*$	$-0.53*$	$1.55*$	$0.39*$	$2.22*$	-0.09	0.05	$0.84*$	$-1.04*$	$-0.05*$
Chakwal-50	$-2.18*$	$0.51*$	$-2.43*$	-0.01	$-2.72*$	$0.84*$	$0.36*$	$1.91*$	$1.96*$	$0.06*$
S.E. of GCA for	0.13	0.04	1.15	0.12	0.17	0.11	0.23	0.22	0.52	0.01
testers										

Table 4: General combining ability effects of lines and testers for yield and yield-related traits in wheat

Table 5: Specific Combining Ability Effects Of Crosses For Yield And Yield Related Traits In Wheat

Parents	Plant height	Number of	Peduncle	Spike	Flag leaf	Spikelets per	No. of grains	1000 -grain	Grain vield	Spike
	(cm)	tiller per	length (cm)	length	area $(cm2)$	spike	per spike	weight (g)	per plant (g)	density
		plant		(cm)						
Lines (female)										
9479	106.80	7.167	25.00	12.66	21.17	20.87	54.60	50.73	16.80	1.63
9486	108.00	6.467	24.93	10.60	24.77	17.27	51.80	48.47	14.23	1.633
9515	116.27	13.133	31.46	13.33	18.77	19.80	55.40	58.27	20.87	1.500
9519	116.60	12.467	32.80	12.667	22.60	18.07	56.73	53.47	17.10	1.433
9520	116.60	12.667	30.80	12.53	21.07	18.20	58.67	53.00	18.64	1.467
9521	117.00	7.800	35.60	13.03	20.27	19.93	58.80	51.98	15.87	1.533
Testers (males)										
Punjab-11	100.50	8.600	37.000	12.933	21.23	19.33	62.47	51.87	25.03	1.467
Aas- 11	110.07	5.067	35.400	13.46	22.53	20.07	62.27	49.93	18.37	1.467
Chakwal-50	112.20	6.733	29.400	10.20	19.85	21.53	59.00	44.80	16.67	2.133

Table 6: Mean Values of Lines and Testers For Different Yield And Its Related Traits In Wheat.

Table 7: Mean Values Of Crosses For Different Yield and Related Traits In Wheat.

Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript. **Ethics approval and consent to participate**

Approved by the department concerned.

Consent for publication

Approved **Funding**

Not applicable

Conflict of interest

The authors declared the absence of a conflict of interest.

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