Indian Journal of Agricultural Sciences **95** (4): 371–375, April 2025/Article
https://doi.org/10.56093/ijas.v95i4.154766

Inheritance studies for stripe rust and identification of multiple rust resistant genotypes in bread wheat (*Triticum aestivum*)

VISHNU KUMAR[1]*, P S SHEKHAWAT[2], SUDHEER KUMAR[3], O P GANGWAR[4], B S TYAGI[5], ARUN GUPTA[5], VIKAS GUPTA[5] and G P SINGH[1]

ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana 132 001, India

Received: 07 August 2024; Accepted: 21 January 2025

ABSTRACT

The present study was carried out during winter (*rabi*) season 2021–22 at ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana to ascertain genetic basis of stripe rust (YR) resistance with bread wheat (*Triticum aestivum* L.) resistant stock, IC0640204 and considering seven susceptible genotypes, namely IC261932, IC532880, IC536321, EC609338, Restorer5, Restorer37 and Restorer38 for YR, summer nursery 2022 and 2022–23. IC0640204 conferred YR resistance against 10 pathotypes at the seedling stage, including the prevalent YR races, 110S119 and 238S119. Seven highly susceptible bread wheat genotypes were crossed with IC0640204 during winter (*rabi*), 2021–22 at Karnal and the developed materials were advanced at Dalang Maidan, Himachal Pradesh. During *rabi* 2022–23, the monogenic dominant inheritance (3R:1S) was observed in the F_2 generation at both Karnal and Durgapura locations, indicating presence of a major gene in the genotype IC0640204. Furthermore, agronomically promising genotypes were screened at eight locations each against stripe, leaf and stem rusts under artificial epiphytotic conditions to identify multiple rust resistant genotypes. The genotypes WAP2206, WAP2207 and WAP2208 conferred seedling resistance (0) against the most virulent YR pathotypes, 110S119 and 238S119. The genotype WAP2206 showed resistance against stem (ACI:0.7) and brown rusts (ACI:0.3), while WAP2207 conferred resistance against stripe (ACI:0.6) and leaf rusts (ACI:0.3). The widely grown wheat cultivars such as HD2967, HD3086 and DBW187 have been compromised against these newly evolved YR races. Therefore, IC0640204 has great significance for delivering resistance into the breeding programs. Several deployed genes for leaf and stem rust namely, *Lr9, Lr19, Lr26, Lr28 and Sr24 and Sr25* have been overcome by the virulent pathotypes. The genotypes, WAP2206, WAP2207, WAP2211 and WAP2212 can be deployed for pre-emptive breeding and enriching allelic diversity.

Keywords: Multiple rust resistance, Stripe rust, Wheat

India is the second largest wheat (*Triticum aestivum* L.) producer and recorded an all-time high wheat production of 113.29 million tonnes during the year 2023–24. It contributes 71.5% and 32.5% of the production in South Asia and Asia, respectively and nearly 14.3% of the global wheat production. Worldwide 798.97 million tonnes wheat was produced during 2023 from an acerage of 220.40 million ha with the productivity of 3.68 t/ha (FAOSTAT 2025). In view of the burgeoning population and climate change wheat production needs to be doubled by 2050 (Gimenez *et al.* 2021, Mottaleb *et al.* 2023). Several foliar diseases, including wheat rusts: stripe/yellow-*Pst*, leaf/brown- *Pt* and stem/black- *Pgt* are of economic importance and can cause substantial yield losses.

Effective yellow rust (Yr) gene(s) have been overcome by the pathogen and some of the examples are, *Yr2, Yr9, Yr17* and *Yr27*. These gene(s) were effective against *Pst* and ensured food security worldwide (Hu *et al.* 2023). To date, 87 Yr gene(s) have been catalogued, where 22 gene(s), viz. *Yr11, Yr12, Yr13, Yr14, Yr16, Yr18/Lr34/Sr57/Pm38/Ltn1, Yr29/Lr46/Sr58/Pm39/Ltn2, Yr30/Lr27/Sr2, Yr36, Yr39, Yr46/Lr67/Sr55/Pm46/Ltn3, Yr52, Yr59, Yr62, Yr68, Yr71, Yr75, Yr77, Yr78, Yr79, Yr80* and *Yr82* are documented for conferring partial resistance (McIntosh *et al.* 1995, McIntosh *et al.* 2018, Pakeerathan *et al.* 2019, Huang *et al.* 2024). Khan *et al.* (2024) reported that three APR genes, viz. *Yr18* and *Yr 46* gene complexes and *Yr36* have been cloned and *Yr18* and lineage has been widely utilized.

During 1987–88, YR race 47S102 was prevalent and later new pathotypes, viz. 46S102, 46S119 and 78S84 were reported (Gangwar *et al.* 2016). Of late, new virulent pathotypes, 110S119 and 238S119 evolved for which IC0640204 possesses fair resistance. *Agropyron* segment

[1]ICAR-National Bureau of Plant Genetic Resources, New Delhi; [2]Rajasthan Agricultural Research Institute, Durgapura, Jaipur, Rajasthan; [3]ICAR-Indian Institute of Pulse Research, Bikaner, Rajasthan; [4]ICAR-Indian Institute of Wheat and Barley Research, Regional Station, Shimla, Himachal Pradesh; [5]ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana.
*Corresponding author email: vishnupbg@gmail.com

carrying *Lr24/*Sr24 provided effective resistance against leaf (Lr) and stem (Sr) rusts and *Sr31* in a combination of *Sr2*, *Sr24*, *Sr5*, and *Sr8* ensured sustained wheat production (Bhardwaj *et al.* 2019a). Similarly, Lr gene(s), namely *Lr9, Lr13, Lr24, Lr25, Lr28, Lr32, Lr34, Lr39, Lr45* and *Lr47* need further enrichment. Therefore, genetic basis of YR resistance in IC0640204 was studied and multiple rust resistant novel sources were identified.

MATERIALS AND METHODS

The present study was carried out during winter (*rabi*) season of 2021–22 at ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana. The study was carried out with bread wheat resistant stock, IC0640204 and considering seven susceptible genotypes, namely IC261932, IC532880, IC536321, EC609338, Restorer5, Restorer37 and Restorer38 for YR during 2021–22, summer nursery 2022 and 2022–23 (Supplementary Table 1) at ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana. The seedling resistance test (SRT) against *Pst* pathotypes (Table 1) was conducted at ICAR-Indian Institute of Wheat and Barley Research, Regional Station, Shimla, Himachal Pradesh. The SRT was carried out following standard protocols and further as described in Pradhan *et al.* (2020).

The SRT infection types (IT: resistant or susceptible) were recorded following Stakman *et al.* (1962). ITs between 0–2 were recorded as resistant and ITs of 3–3+ were counted susceptible. IT, 3–3+ was categorized when 3 and 3+ pustules were present together. During 2021–22, seven crosses, namely C-1 (IC261932 (SRB-79)/IC0640204), C-2 ((IC532880 (WIC-447)/IC640204)), C-3 ((IC536321 (NC-59610)/IC640204)), C-4 ((EC609338 (07A37)/IC640204)), C-5 (RES5/IC640204), C-6 (RES 37/IC640204) and C-7 (RES 38/IC640204) were attempted. After keeping some seeds, the F_1s of all the crosses were advanced during summer nursery, 2022 at Dalang Maidan, Himachal Pradesh. The obtained F_2s from summer nursery 2022 along with remnant F_1s and parents were grown and screened at Karnal (L1) and Durgapura (L2) locations during *rabi*, 2022–23 against the mixture of most virulent *Pst* pathotypes, including 110S119 and 238S119.

Table 1 YR, LR and SR pathotypes used for SRT

YR	LR	SR
46S119	12–5 (29R45 = FHTPM)	11 (79G31 = RRTSF)
111S68	77–1 (109R63 = THTTB)	21A-2 (75G5 = CCTJC)
6S0	77–5 (121R63–1 = THTTM)	117–6 (37G19 = KRCSC)
P (46S103)	77–9 (121R60–1 = MHTKL)	40A (62G29 = PTHSC)
238S119	104–2 (21R55 = PHTTL)	122 (7G11= RRJQC)
14S64		
78S84		
110S119		
T (47S103)		
110S84		

SRT, Seedling resistance test; YR, Yellow rust; LR, Leaf rust; SR, Stem rust.

The susceptible infector rows were inoculated at Zadok GS 20 (seedling stage) with YR uredospores and frequent sprays were made on the genetic materials. One extra irrigation was applied for creating a congenial micro-climate for better disease severity. YR reactions were recorded at Zadok GS 60–69 (early-late flowering), and the modified Cobb scale (Peterson *et al.* 1948) was adopted. The different ITs were recorded as per the standard procedure given by Roelfs *et al.* (1992) on the R (resistant), MR (moderately resistant), MS (moderately susceptible) and S (susceptible) scales. For field screening at the adult plant stage (APS), the R + MR and MS + S scales were merged together for the F_2 individuals. The chi-square test (χ^2) was applied to test the obtained and expected frequencies for the goodness of fit in MS Excel. Additionally, six agronomical superior bread wheat genotypes (Supplementary Table 1) were selected out of 100 exotics (CIMMYT trials) genotypes and were further screened against major pathotypes of *Pst, pt* and *Pgt* at ICAR-Indian Institute of Wheat and Barley Research, Regional Station, Shimla, Himachal Pradesh for SRT analysis following standard procedures (Pradhan *et al.* 2020, Prasad *et al.* 2022). The details of the pathotypes are presented in Table 1. For YR APS scores, the genotypes were screened under epiphytotic conditions at eight stations, namely Durgapura, Gurdaspur, Hisar, Ludhiana, Karnal, Dhaulakuan, Jammu and Malan. The SR evaluation was carried out at Pune, Mahabaleshwar, Vijapur, Dharwar, Indore, Niphad, Powarkheda and Wellington. The LR pathotypes vary from north to south India hence the screening was done separately at Ludhiana, Durgapura, Ayodhya, Sabour, Kanpur, Karnal and Delhi in north India. Whereas, the centres for screening against LR south Indian pathotypes were the same as SR. The heatmap and biplot were generated using R version 4.1.1.

RESULTS AND DISCUSSION

Rusts are economic diseases in wheat and especially temperature variations in the face of climate change has favoured wheat pathotypes to evolve more rapidly. To date, 87 yellow rust gene(s) have been catalogued, however under prevailing Indian situation most of the wheat varieties are susceptible to the yellow rust (Yao *et al.* 2025). The available sources conferring resistance against the rusts are limited, low yielding and transfer of these gene(s) is broadly associated with linkage drags. The bread wheat genotype IC0640204 is high yielding, resistant against wide range of YR pathotypes and possess drought tolerant. The reactions obtained in seedling resistance test (SRT), adult plant stage (APS) field reactions and inheritance studies in combination of seven highly susceptible wheat genotypes are discussed.

SRT for stripe rust in IC0640204: The genotypes IC261932, IC532880 and EC609338 showed susceptibility (3+) against all the tested pathotypes of *Pst* at the seedling stage (Table 2). The genotype, IC536321 was recorded with susceptible reactions against the *Pst* races, viz. P, 238S119, 78S84 and 110S119 (>3). The restorer genotypes, namely Res 5, 37 and 38 were found susceptible against the *Pst* pathotypes, 238S119 and 110S119. The resistant stock, IC0640204 showed nearly immune type of reactions (0; and ;-) against all the tested pathotypes, including the prevalent and virulent races, 110S119 and 238S119. This confirmed the presence of an avirulent *R* gene against all the tested pathotypes, namely 46S119, P, 111S68, 6S0, 238S119, 14S64, 78S84, 110S119, T and 110S84. Bouvet *et al.* (2022) described that such all stage effective gene(s) expressed at the seedling stage are characterised by a hypersensitive response and extends resistance throughout the growth of the wheat plant.

The rest of the seven parents showed susceptible reactions to the prevalent races 110S119 and 238S119. Haider *et al.* (2023) reported that 110S119 and 238S119 are recently evolved aggressive stripe rust races having virulence for several stripe rust gene(s), including *YrA, YrSU, Yr2, Yr3, Yr4, Yr6, Yr7, Yr8, Yr9, Yr17, Yr19, Yr21, Yr22, Yr23, Yr25* and *Yr27.* Gangwar *et al.* (2019) also discussed that the *Pst* pathotypes, 238S119 and 110S119 are more aggressive and Riebesel 47/51 (*Yr2, Yr9*) and Hobbit (*Yr14*) have been overcome by these races. The mega bread wheat cultivars like HD2967 and DBW187 are susceptible at farmers fields indicating that these races are competitive and warranted for identification of new resistant sources. Huang *et al.* (2024) summarized that wide spread cultivation of few wheat varieties can lead to the emergence and dissemination of new virulent strains of *Pst*, and discovery of novel resistant sources is utmost and continuously required.

Apart from SRT, all the parental genotypes were recorded for field reactions at the APS for consecutive two years. The seven parents selected for genetic studies showed field reactions of 100S against YR. The APS reactions again confirmed the prevalence of the rust races 110S119 and 238S119 and avirulence of these races against the *R* gene present in the resistant stock IC0640204 as this showed complete resistance at adult plant stage.

Inheritance study: F_1 progenies of all the seven crosses showed complete resistance indicating the dominant nature of inheritance in the resistant parent. The heatmap of Karnal (L_1) and Durgapura (L_2) revealed that the crosses, C-1 and C-5 were segregated with maximum plant count for resistant plants (Fig. 1A). It was observed that the susceptible classes were lower than the resistant plant at both the locations. This indicated that the frequency skewed towards more resistance class is associated with the complete dominant gene present in the resistant stock IC0640204. Zhang *et al.* (2024) reported contrary findings, while studied stripe rust recessive gene, tentatively designated as *YrZ15–1949*. Wang *et al.* (2022) and Yao *et al.* (2025) reported that developing plant resistant cultivars is the best approach particularly by combining effective all-stage resistance (ASR) gene(s) to achieve high-level of resistance. Presently, only few major gene(s), namely *Yr5, Yr15, Yr64* and *Yr65* are effective

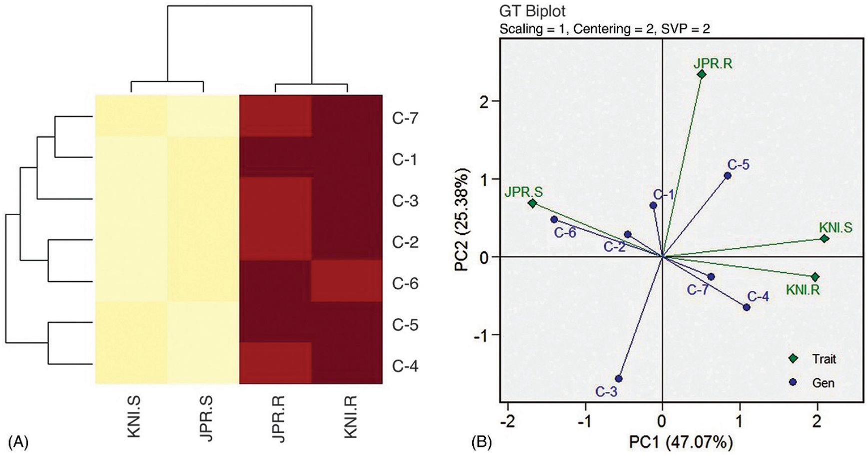

Fig. 1 (A) Heatmap (B) biplot of resistant and susceptible plants in F2 progenies.

Table 2 Seedling resistance test (SRT) reactions against wheat rusts

Genotypes	46S119	P	111S68	6S0	238S119	14S64	78S84	110S119	T	110S84
Susceptible genotypes										
IC261932	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
IC532880	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
IC536321	1	3+	0;	0;	3+	;	3-	33+	0;	0;
EC609338	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Res5	0;	;	0;	0;	3+	0;	0;	3+	0;	3+
Res37	0;	3+	0;	0;	3+	0;	;-	3+	3+	2-
Res38	;-	;	0;	0;	3+	0;	;	3+	0;	3+
Resistant donor										
IC0640204	0;	0;	0;	0;	;-	0;	0;	0;	0;	0;
Mega variety										
DBW187	;	3	0;	0;	3+	0;	;	33+	0;	;-

Table 3 Adult plant stage reactions based on multi-location evaluation of wheat genotypes

Genotype	Stem rust		Leaf rust (S)		Leaf rust (N)		Yellow rust	
	HS	ACI	HS	ACI	HS	ACI	HS	ACI
WAP2206	10MR	0.7	10R	0.3	20S	2.9	20MS	4.9
WAP2207	10S	2.5	10S	1.5	5MR	0.3	5S	0.6
WAP2208	20S	5.6	20S	8.3	20S	5.7	10S	2.5
WAP2210	10S	5.1	20S	5.9	20S	7.9	40MS	10.9
WAP2211	20S	9.1	10S	1.8	10MR	0.6	20MS	6.6
WAP2212	20S	7.8	R	0	15S	5.7	20S	7.5
Infector	100S	75.0	80S	75.0	100S	78.6	80S	75.0

ACI, Average coefficient of infection; HS, Highest score; MR, Moderately resistant; S, Susceptible; R, Resistant; MS, Moderately susceptible.

against the *Pst* populations worldwide and the YR source IC0640204 can be a potential donor for YR resistance, especially under Indian conditions (Chen *et al.* 2021).

Biplot depiction is a unique and user friendly graphical method to evaluate the trait and genotypes easily (Hnizil *et al.* 2024). Here, the biplot captured 72.45% of the total variations and indicated that all the crosses were varying for resistant and susceptible combinations at L_1 and L_2 (Fig. 1B). Khan *et al.* (2025a) and Khan *et al.* (2025b) studied the genetic parameters with yellow rust resistance implementing biplot method and enhanced the understanding of genetic resistance mechanisms by capturing wide range of genetic variability. The segregation ratio analyses of different crosses (R × S) to reveal the inheritance mechanism in the resistant stock IC0640204 are presented in the Supplementary Table 2 (Supplementary Table 2). For C-1, 236 and 243 plants were categorized as resistant at L_1 and L_2, whereas, 62 and 65 plants were grouped with susceptible reactions (Supplementary Table 2). These frequencies were nearly close to the expected ratio of 3:1 with a non-significant test of goodness of fit (Supplementary Table 2). The segregation ratio of 3(R):1(S) and skewed frequency towards resistance clearly indicated monogenic dominant inheritance in the resistant stock, IC0640204 at both Karnal and Durgapura locations.

Wheat genotypes with combined rust resistance

SRT of multiple rust resistance genotypes: Three genotypes, viz. WAP2206, WAP2207 and WAP2208 showed resistance against all the considered stripe rust races including, the pre-dominant races, 110S119 and 238S119 (Supplementary Table 3). Whereas, the genotype WAP2212 showed resistance against *Pst* pathotypes, 110S84, 46S119 and 47S103 but found susceptible against 110S119 and 238S119. WAP2207 conferred resistance against the *Pt* races, 12–5, 77–1, 77–9 and 104–2 (Supplementary Table 3). The genotype WAP2212 showed resistance to susceptible reactions for 77–5 and 104–2. Two genotypes, WAP2206 and WAP2207 showed resistance against the tested pathotypes of *Pgt* namely, 11, 21A-2, 117–6, 40A and 122.

Adult plant scores: WAP2207 showed the lowest average coefficient of infection (ACI) of 0.6 (HS:5S), followed by WAP2208 (ACI: 2.5). The genotype WAP2207 was recorded with zero (0) APS stripe rust reactions at seven locations, whereas, the stripe rust severity of 5S was only reported from Hisar centre (Table 3). The genotypes, WAP2206, WAP2207, WAP2211 and WAP2212 showed low ACI against LR.

For SR the genotype WAP2206 showed resistance with the ACI of 0.7 (HS-10MR), followed by WAP2207 (ACI-2.5). Rehman *et al.* (2024) emphasized that wheat rusts pose serious threat to the global food security hence, identification and development of combined novel and effective multiple rust resistance sources is a pragmatic approach to speed-up breeding cycles. For leaf rust, the races are varying from north to south India due to the weather parameters, evolution pressure and other factors. Bhardwaj *et al.* (2019a and 2019b) reported that the pathotype 121R63 of brown rust remained prevalent for more than 20 years, and the pathotype 121R60–1 has gained momentum after 2016. Similarly, the stem rust pathotype 62G29 predominated for a long time and the race 79G31 is now aggressive (Prasad *et al.* 2018). Prasad *et al.* (2022) and Jain *et al.* (2013) reported that black rust genes *Sr2, Sr5, Sr6, Sr7a, Sr7b, Sr8a, Sr8b, Sr9b, Sr9e, Sr11, Sr12, Sr13, Sr17, Sr21, Sr24, Sr25, Sr30,* and *Sr31* are present in Indian wheat varieties. Out of these, *Sr2, Sr11* and *Sr31* are more common in bread wheat, while *Sr7b, Sr9e, Sr11,* and *Sr13* are frequent in durum varieties. Several deployed genes for leaf and stem rust namely, *Lr9, Lr19, Lr26, Lr28* and *Sr24* and *Sr25* have been overcome by the virulent pathotypes (Bhardwaj *et al.* 2019a).

In conclusion, the multiple rust resistant genotypes, WAP2206, WAP2207, WAP2211 and WAP2212 can be utilized for pre-emptive breeding and enriching diversity against the wheat rusts. The stripe rust resistant genetic stock IC0640204 conferred complete resistance against wide range of stripe rust pathotypes, including prevalent pathotypes, 110S119 and 238S119.

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