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Research Article

Effectiveness of *Thinopyrum ponticum*-derived wheat leaf rust resistance gene, *Lr24* in India - a revisit

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Abstract

Leaf or brown rust is one of the most devastating rust diseases in wheat. Development and deployment of resistance wheat cultivars is considered as the most effective, economic and sustainable method of protecting against the yield losses due to leaf rust. Though several alien resistance genes have been reported and utilized, *Thinopyrum ponticum* derived leaf rust resistance gene *Lr24/Sr24* has been proved to be effective for nearly three decades in India. The effectiveness of this gene was validated recently at seedling and adult plant stage. In the sixteen (16) backcrossed inbred lines (BILs) showed resistance reaction to the existing pathotypes. Furthermore, the presence of *Lr24* gene was confirmed in BILs through molecular markers. The results showed that *Lr24* gene conferred all stage resistance to the existing pathotypes of *P. triticina* f.sp. *tritici*. This emphasizes the importance of *Lr24* gene in Indian wheat breeding program and its prolonged protection of wheat crop from leaf rust in India.

Keywords: *Triticum aestivum*, Leaf rust, *Puccinia triticina, Lr24*, All stage resistance (ASR), Back crossed inbred lines (BIL's)

1. Introduction

Leaf or brown rust caused by *Puccinia triticina* Eriks. (*Pt*) is one of the most serious diseases targeting wheat worldwide. *P. triticina* is an obligate parasite that can easily survive in areas with mild temperatures and moist conditions (Figueroa *et al.*, 2017). The upper surface of the leaves is occupied with infectious orange-brown urediniospores (Wegulo and Emmanuel, 2012). These spores can travel hundreds of miles by wind thereby resulting in an endemic outbreak (Bolton *et al.*, 2008). The pathogen exhibits wider adaptability and hence can cause losses upto 70% under favourable climatic conditions (Figueroa *et al.*, 2017). Constant monitoring of leaf rust is crucial in order to keep track of any fluctuation in the pathogen population.

In India, changes in the pathogen population have been so far recorded for 49 pathotypes of *P. triticina* (Tomar *et al.* 2014). Among them, *P. triticina* race 77-1, 77-5, 77-9 and 104-2 are the most virulent pathotypes with more frequency in India (Prasad *et al.*, 2020). The most effective and economical approach to control this disease is deployment of leaf rust resistance genes.

Until now nearly 80 leaf rust resistance genes have been reported in wheat (McIntosh *et al.*, 2017). Of which many of them have been transferred from alien sources such as *Aegilops umbellulata*(*Lr9*), *Thinopyrum ponticum (Agropyron elongatum)* (*Lr19, Lr24*, and *Lr29*), *Aegilops speltoides* (*Lr28, Lr35, Lr36, Lr47* and *Lr51*), *Aegilops ventricosa* (*Lr37*) and

Triticum tauschii (*Lr21*, *Lr22a, Lr32, Lr39, Lr41, Lr42*, and *Lr43*) (MdAktar-Uz-Zaman *et al.*, 2017).

Lr24 derived from *Thinopyrum ponticum* (Podpera) Lu & Wong (syn. *Agropyron elongatum* (Host) Beauv.) is one of the important genes providing resistance to leaf rust for almost three decades in India (Tomar *et al.*, 2014). The spontaneous translocation occurred between the alien donor and chromosome 3D of bread wheat (Gough and Merkle, 1971). *Lr24* is also reported to be closely associated with the stem rust resistance gene *Sr24* (Mago *et al.*, 2005) and also with red grain colour. Later efforts were made to develop stocks with white seeded germplasm (McIntosh, 1995). Since 1993, *Lr24* has been effectively deployed in most Indian released varieties.

Previously, virulence for the resistance gene *Lr24* has been reported in North America (Browder, 1973), Canada (Kolmer, 1991), South America (Singh, 1991), South Africa (Pretorius *et al.*, 1990), Eastern Australia (Park *et al.*, 2002), Nepal (Mishra *et al.*, 2001) and Pakistan (Fayyaz *et al.*, 2008). But, in India, until now, the resistance gene *Lr24* provided all stage resistance (ASR) to all the occurring leaf rust pathotypes of *P. triticina* Eriks. (*Pt*) (Tomar *et al.*, 2014).

In this study, 16 backcross inbred lines (BILs) which were in the background of fifteen Indian wheat varieties were subjected to multi-race seedling tests, field tests and molecular marker detection for two seasons (2019,2020) and its efficacy over the period of years against the prevalent pathotype of *P. triticina* is being reported.

2. Material and Methods

2.1 Plant and Fungal material

Sixteen BILs were developed by introgressing *Lr24* gene through backcross breeding method using Tr380-14*7/3/Ag#14 and DarfKite (white seeded germplasm) as donor parents in the background of eighteen Indian wheat varieties from 1990-2000, continuously maintained and evaluated at ICAR- Indian Agricultural Research Institute (IARI), Regional Station, Wellington, Tamil Nadu, India located at ($11^0$22'47.5''N; $76^0$46'26.1''E; altitude 1850 AMSL).

As *Lr24/Sr24* genes are tightly linked dominant genes, six to seven backcrosses were made with the respective recurrent parents within a short span of time by raising three experimental crops a year, under natural epiphytotic conditions. The genotypes were evaluated phenotypically and genotypes resembling the respective recurrent parents were selected and selfed for five subsequent generations. The final constituted BILs carrying *Lr24* along with the corresponding recurrent parents and donor (listed in Table 1& 2) were used for this study.

Table 1: Phenotypic validation of Seedling (SRT) and Adult plant resistance response of *Lr24/Sr24* in NILs/back crossed lines, recurrent parent and donors against leaf rust pathotypes during 2019

S. No	Variety	Pedigree/details	SRT Score (IARI, RS, Wellington mixed races)	SRT Score (IIWBR, Shimla)						Field Score (IARI, RS, Wellington)
				12-5	77-2	77-5	77-9	104-2	106	
1	**HW 2001A**	**Sonalika*7//Tr380-14**	**;**	**-**	**;**	**2**	**2**	**2**	**;-**	**0**
2	Sonalika	Recurrent Parent	3+	3+	3+	3+	3+	3+	0;	100S
3	**HW 2002**	**K.sona*6//Tr380-14**	**0**	-	;-	;	-	;	0;	**0**
4	Kalyansona	Recurrent Parent	3+	-	3+	3+	3+	3+	0;	100S
5	**HW 2003**	NI 5439*7//Tr380-14	0	;	;-	;	0;	;-	0;	**0**
6	NI 5439	Recurrent Parent	2+	-	-	3+	;-	-	-	80S
7	**HW 2004**	**C306*6//Tr380-14**	**0**	**0;**	**-**	**-**	**1**	**-**	**-**	**0**
8	C 306	Recurrent Parent	3+	1	3+	2	3+	3+	0;	60S
9	**HW 2006**	**Lok-1*6//Tr380-14**	**0**	;	1	-	-	;	0;	**0**
10	LOK 1	Recurrent Parent	3+	-	-	-	-	-	-	100S
11	**HW 2007**	**HD2329*6//Tr380-14**	**0**	-	2	-	-	-	-	**0**
12	HD 2329	Recurrent Parent	3+	0;	-	0;	3+	0;	0;	80S
13	**HW 2008**	**HD2285*6//Tr380-14**	**0**	-	-	-	-	-	0;	**0**
14	HD 2285	Recurrent Parent	3+	-	-	-	-	-	-	80S
15	**HW 2014**	**Wl 711*6//Tr380-14**	**0**	;	;-	;-	;	;-	-	**0**

16	WL 711	Recurrent Parent	3+	-	-	-	-	3+	0;	100S
17	**HW 2015**	**HUW 234*6//Tr380-14**	**;**	0;	-	;-	;-	0;	0;	**0**
18	HUW 234	Recurrent Parent	3+	;	3+	3	3+	3+	0;	100S
19	**HW 2016**	**PBW 226*6//Tr380-14**	**0**	0;	;	;-	;-	;	0;	**0**
20	PBW 226	Recurrent Parent	3	;	-	3+	3+	;	0;	80S
21	**HW 2017**	**HD 2402*6//Tr380-14**	**0**	0;	;-	0;	;-	;-	;-	**0**
22	HD 2402	Recurrent Parent	3+	;	-	-	-	-	0;	80S
23	**HW 2018**	**HI 1077*6//Tr380-14**	**0**	0;	;-	0;	;-	;	0;	**0**
24	HI 1077	Recurrent Parent	3	0;	3+	;	3+	-	0;	80S
25	**HW 2019**	WH 542*6//Tr380-14	;	0;	0;	;-	;-	;-	0;	**0**
26	WH 542	Recurrent Parent	3	3	0;	3+	3+	3+	0;	80S
27	**HW 2020**	**HS 240*6//Tr380-14**	**0**	**0;**	**;-**	**0;**	**0;**	**0;**	**0;**	**0**
28	HS 240	Recurrent Parent	3+	0;	0;	2	3+	3+	0;	100S
29	**HW 2022**	**WH 147*6//Tr380-14**	0;	;-	;-	0;	;	2	;1	0
30	WH 147	Recurrent Parent	3+	1-2	3+	3+	3+	3-3+	0;	80S
31	DarfKite (Resistant check)	*Lr24/Sr24*	0	0;	0;	0;	-	0;	0;	0

Table 2: Phenotypic validation of Seedling (SRT) and Adult plant resistance response of *Lr24/Sr24* in NILs/back crossed lines, recurrent parent and donors against predominant leaf and stem rust pathotypes during 2020.

S. No.	Wheat Lines	Pedigree/details	SRT Score (IARI, RS, Wellington) mixed races leaf rust pathotypes*	SRT Score IIWBR, Flowerdale Shimla Leaf rust pathotypes			SRT Score IIWBR, Flowerdale Shimla Stem rust pathotypes			Adult plant response under natural epiphytotic conditions at IARI, RS, Wellington
				77-5	77-9	104-2	15-1	40-1	40A	
1	**HW 2002**	**K.sona *6//Tr380-14**	**0**	**;**	**;**	**0;**	**2-**	**3-**	**2-**	**0**
2	**HW 2002A**	**K.sona *6//Tr380-14**	**0**	**;**	**;**	**0;**	**2=**	**3-**	**2-**	**0**
3	Kalyansona	Recurrent Parent	3+	3+	3+	3+	2-	3+	3+	100S
4	**HW 2003**	**NI5439(*7//Tr380-14**	**0;**	**;**	**;**	**;**	**;**	**2=**	**;1**	**0**
5	NI 5439	Recurrent Parent	2+	;	3+	0;	2=	3+	3+	80S
6	**HW 2004**	**C306*6//Tr380-14**	**0**	**12**	**;**	**;**	**;**	**3-**	**2-**	**0**
7	C 306	Recurrent Parent	3+	3+	3+	3+	3+	3+	3+	80S
8	**HW2007**	**HD 2329*6//Tr380-14**	**0**	**;1**	**;1**	**;1**	**0;**	**2=**	**2-**	**0**
9	HD 2329	Recurrent Parent	3+	3+	;1	3+	3+	33+	3+	100S
10	**HW 2008**	**HD 2285*6//Tr380-14**	**0;**	**;1**	**;**	**;**	**;**	**2=**	**2-**	**0**
11	HD 2285	Recurrent Parent	3+	3+	;1	3+	;1	2=	3+	80S
12	**HW 2010**	**J24*6//Tr380-14**)	**0;**	**;**	**;**	**;**	**;**	**2=**	**2-**	**0**
13	J24	Recurrent Parent	3+	3+	3+	3+	3+	33+	3+	100S
14	**HW 2011**	**HD2009*6//Tr380-14**	**0**	**0;**	**;1**	**0;**	**2-**	**2=**	**12-**	**0**
15	HD 2009	Recurrent Parent	2+	3+	3+	3+	2-	3-	2-	80S
16	**HW 2012**	**UP 262*6//Tr380-14**	**0;**	**;**	**;**	**;**	**0;**	**2=**	**2-**	**0**
17	UP 262	Recurrent Parent	2+	3+	;1	3+	2-	33+	3	80S
18	**HW 2015**	**HUW234*6//Tr380-14**	**;**	**;1**	**;**	**0;**	**2-**	**2-**	**2-**	**0**
19	HUW 234	Recurrent Parent	3+	3+	3+	3+	2=	2=	3+	100S
20	**HW 2016**	**PBW226*6//Tr380-14**	**0**	**;**	**;**	**;**	**0;**	**2=**	**2-**	**0**
21	PBW 226	Recurrent Parent	3	;1	12	3+	0;	0;	;	80S
22	**HW 2017**	**HD2402(*Lr24/Sr24*)**	**0;**	**;**	**;**	**;**	**;**	**2=**	**2**	**0**
23	HD 2402	Recurrent Parent	3+	3+	;1	3+	0;	2=	2	80S

24	**HW 2018**	**HI1077*6//Tr380-14**	**0**	**0;**	**;-**	**0;**	**;**	**2=**	**2-**	**0**
25	HI 1077	Recurrent Parent	3	3+	3+	3+	;	2=	2	80S
26	**HW 2019**	**WH 542*6//Tr380-14**	**;**	**;1**	**0;**	**0;**	**0;**	**2=**	**12**	**0**
27	WH542	Recurrent Parent	3+	3+	;1	3	0;	2=	;	80S
28	**HW 2020**	**HS240*6//Tr380-14**	**0**	**;1**	**;1**	**;-**	**2-**	**2=**	**2-**	**0**
29	HS 240	Recurrent Parent	3+	0;	;-	0;	2=	0;	2-	80S
30	**HW 2022**	**WH147*6//Tr380-14**	**0;**	**;-**	**;-**	**0;**	**;**	**2**	**;1**	**0**
31	WH 147	Recurrent Parent	3+	3+	3+	3+	0;	3+	2-	100S
32	Agent	*Lr24/Sr24* (Resistant check)	-	**;**	**;**	**;**	**2=**	**3+**	**2-**	**0**
33	*Tr380-14*	*Lr24/Sr24*	0;	0;	0;	0;	0;	2-	2	0

*Predominant leaf rust races occurring at Wellington are 77-1, 77-5, 77-9 and less frequent ones are 12-4, 12-8, 20, 77-6, 104-1, 162 & 1R31(*Mehtaensis 40 (2) July 20, IIWBR, Shimla*)

DNA isolation and molecular validation of Lr24 gene

The leaf samples were collected from the BILs, recurrent parents and donor from 15 days old seedlings and their DNA was isolated using CTAB method (Murray and Thompson, 1980). Two markers, *Sr*24#12 (Mago *et al.*, 2005) and $SCS73_{719}$ (Prabhu *et al.*, 2004) used to molecularly validate the NILs for the presence of *Lr*24 gene along with the recurrent parents and donor parent Tr380-14*7/3/Ag#14 and Darf kite.

The DNA samples were amplified with STS marker *Sr*24#12 and SCAR marker $SCS73_{719}$. The PCR reactions were carried out with 2X Dream Taq PCR master mix (Thermo Fisher Scientific) and 0.4pm forward and reverse primers. For *Sr*24#12, initial denaturation was kept at 94°C for 5mins, and 35 cycles of 94°C for 30s, 55°C for 30s and 72°C for 1min, and the final extension at 72°C for 10min and for $SCS73_{719}$ same condition with annealing temperature of 51°C was carried out. The PCR products were resolved with 1.2% agarose gel and documented with gel documentation system (Syngene, Gene Genius Match GGM/D2/F2-1).

2.2 Seedling Resistance Test

The seedling response test (SRT) of molecularly validated 16 BIL's (Lr24) and their corresponding recurrent parents were done with 6 different leaf rust pathotypes viz., 12-5, 77-2, 77-5, 77-9, 104-2 and 106 during 2019 and again 16 different sets of BIL's against three predominantly occurring leaf rust pathotypes 77-5, 77-9 and 104-2 and stem rust pathotypes 15-1, 40-1 and 40A during 2020 at ICAR, Indian Institute of Wheat and Barley Research (IIWBR), Flowerdale, Shimla located at a latitude of 31.088 and longitude 77.186, with an altitude of 2000 m AMSL. Lines were inoculated on 14 day old seedlings with a suspension containing 10 mg spores of leaf rust pathotype using an atomizer and incubated in humid chambers with diffused light at 20–22 °C for 48 hours. After 48 hours they were kept at glass house at 22°C and maintained for symptom development. Symptoms appeared ten days after inoculation and seedling reactions were recorded. Infection response was determined based on the host response to leaf rust using Stakman scale (0-4) (Stakman *et al.,* 1962). Infection types of 0 to 2 were considered as resistant and infection types of 3 to 3+ and more were considered as susceptible.

2.3 Adult plant resistance

The near isogenic lines with their recurrent parents were sown in field at ICAR-IARI, Regional Station, Wellington, Tamil Nadu with spreader rows around the field. Wellington is a natural hotspot for rusts and it survives here though around the year crop cycles, self sown plants and green bridge maintained at the station provides regular supply of rust inoculum to the breeding materials. Rust inoculum was sprayed using aqueous suspension of viable uredospores of prevailing rust pathotypes at two different plant seedling stages, first and fifth leaf (12 and 16 Zadoks scale) (Zadoks et al., 1974). Recommended cultivation practices were followed for raising the crop (Singh et al., 2006). The spreader rows were ensured with maximum susceptibility up to 100S.The adult plant reaction in the field conditions was recorded as per modified Cobb's scale (Peterson *et al.,* 1948) for two consecutive seasons (Kharif 2019 and Rabi 2019-20) as follows: 0- No visible infection, R- Resistant: necrotic areas with or without minute uredia, MR- Moderately resistant: small uredia, surrounded by necrotic areas, MX- Intermediate: variable sized uredia, some with necrosis or chlorosis, MS- Moderately

susceptible: medium uredia with no chlorosis present, S-Susceptible: large uredia, no necrosis or chlorosis.

3. Results and Discussion

Among the several leaf rust resistant genes deployed in wheat, *Lr24* is one of the most effective genes conferring high level of resistance to *P.triticina* in India (Tomar *et al.*, 2014). In the recent past, efforts have been made to understand seedling and adult plant resistance provided by *Lr24* gene through transcriptome analysis (Manjunatha, 2015).

Seedling resistance test carried out at ICAR- IIWBR, RS, Flowerdale, Shimla for the 16 BIL's carrying *Lr24* against 6 different pathotypes of leaf rust (12-5, 77-2, 77-5, 77-9, 104-2 and 106) showed resistance reaction. The infection type developed by the BIL's and the corresponding recurrent parents at the seedling stage under greenhouse conditions was recorded and is presented in Table-1and Table-2. The recurrent parents showed susceptible reaction with the infection score ranging from 2 to 3+ against different pathotypes during 2019 and 2020. The donor screened against leaf rust pathotypes exhibited high level of seedling resistance with infection type (IT) '0'.The BILs showed resistant response ranging from 0 to 2 indicating the presence of the gene. The results revealed and confirmed that *Lr24* gene had seedling resistant response to the occurring leaf rust pathotypes in India.

Wellington is an important hotspot for rusts in the southern and peninsular India. The weather conditions in the hills are very congenial for host and pathogens and the rusts are highly destructive throughout the year. A wide spectrum of stem and leaf rust pathotypes are prevalent in this hills (Bahadur 1986; Nayar *et al.*, 1988). The results of the seedling resistance test done at ICAR- IARI, RS, Wellington using mixed pathotypes collected from field was in concordance with the results of at ICAR- IIWBR, RS, Flowerdale, Shimla.

Field screening was carried out at IARI, Regional Station, Wellington, The Nilgiris, Tamil Nadu under natural and artificially created epiphytotic conditions at Wellington for two successive seasons (2019 and 2020). The field reactions of the recurrent parents showed susceptible response with severity ranging from 80-100S, while the BILs and donor parents carrying *Lr24* showed resistant response (0). The scores were tabulated in Table 1&2. This is in agreement with the report stating that *Lr24* is continuing to be effective against the occuring leaf rust pathotypes prevailing in India (Bhardwaj *et al.*, 2021).

These lines were also evaluated against the predominant pathotypes of *P.graminis.* f. sp. *tritici* such as 15-1, 40-1 and 40-A during 2020. The *Lr24* linked stem rust gene *Sr24* recorded susceptible infection types IT 3+ particularly to the race 40-1 for which virulence has been reported in India (Bhardwaj *et al.*, 1990).

Furthermore, DNA isolated from the 16 BIL's and their corresponding recurrent parents were subjected to PCR analysis using the gene specific markers viz., *Sr*24#12 (STS marker) and $SCS73_{719}$ (SCAR marker) during 2019. All the lines carrying *Lr24/Sr24* gene(s) amplified a 500bp positive band for *Sr*24#12 (Fig. 1A and 1B) and 650bp positive band for $SCS73_{719}$ (Fig. 2A and 2B). Whereas, no amplification was seen in the corresponding recurrent parents for both the markers thereby indicating the absence of gene. Further re-confirmation was done during 2020 and all the BILs were amplified with 500bp positive band for *Sr*24#12 (Fig.3 & 4). Molecular validation of *Lr24* gene using two different markers (*Sr24#12* and *SCS73719*) showed that all the 16 BILs carried *Lr24* gene. It confirms that the meticulously planned conventional breeding approaches followed at IARI Regional Station, Wellington to develop the backcross inbred lines were efficient, systematic and successful.

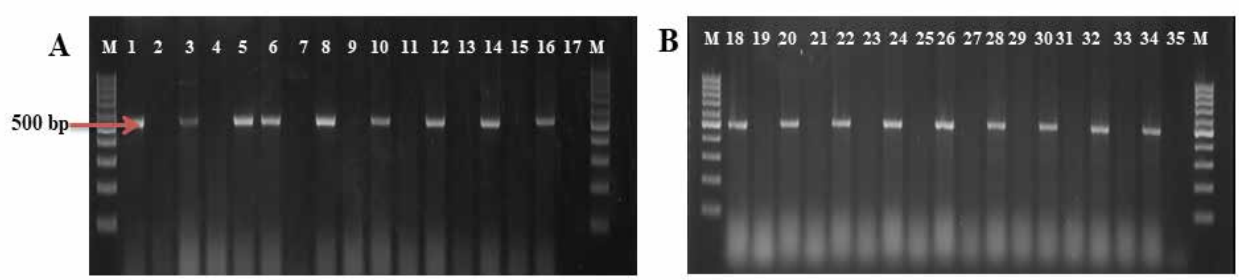

Fig 1: Molecular validation of *Lr24* gene in NIL's with *Sr24*#12marker (2019)

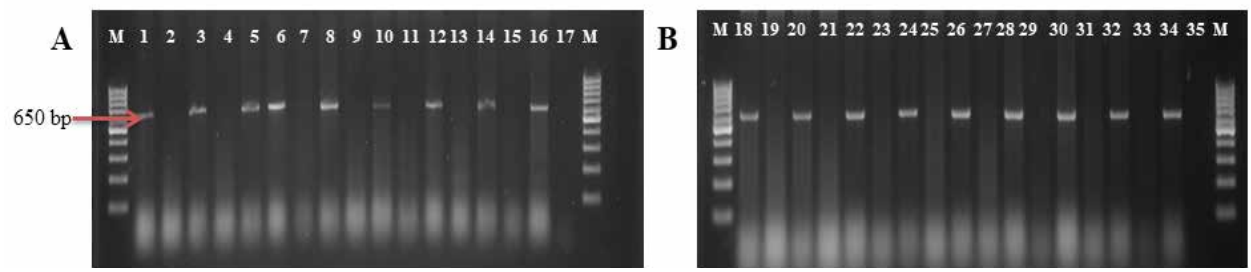

Fig 2: Molecular validation of *Lr24* gene in 16 NIL's with SCS73719marker(2019)

Fig. 1 and Fig. 2: M- 100bp Ladder, 1- Darfkite (Positive control), 2- Sonalika (RP), 3- HW 2001A, 4- Kalyansona(RP), 5- HW 2002, 6-HW 2002A, 7- NI5439(RP), 8- HW 2003, 9- C306(RP), 10- HW 2004, 11- WH147(RP), 12- HW 2005, 13- LOK1(RP), 14- HW 2006, 15- HD2329(RP), 16- HW 2007, 17- NTC, 18- Darfkite, 19- HD 2285(RP), 20- HW 2008, 21- WL711(RP), 22- HW 2014, 23- HUW234(RP), 24- HW 2015, 25- PBW226(RP), 26- HW 2016, 27- HD2402(RP), 28- HW 2017, 29- HI1077(RP), 30- HW 2018, 31- WH542(RP), 32- HW 2019, 33- HS 240(RP), 34- HW 2020, 35- NTC

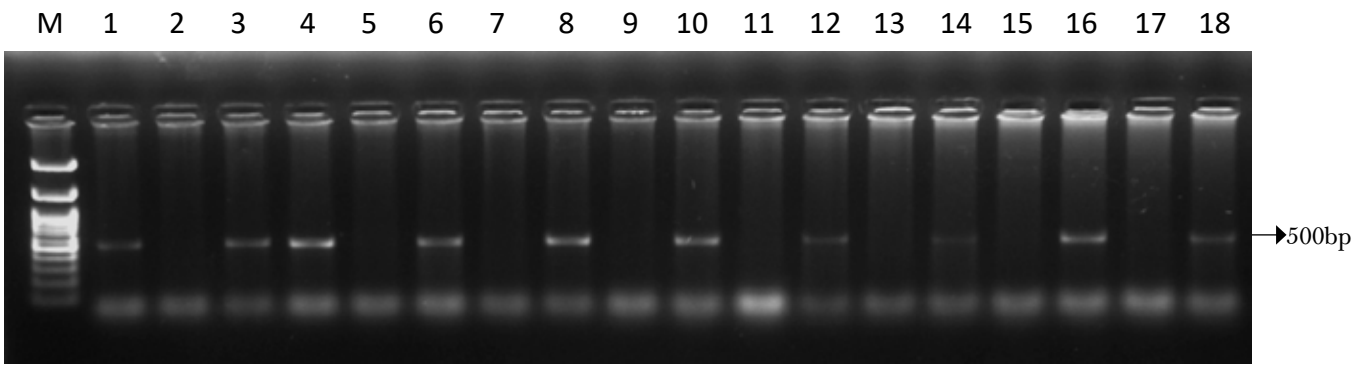

Fig 3: Molecular validation of *Lr24* gene in NIL's with *Sr24#*12marker re-confirmed during 2020

M-100bp Ladder, 1.Tr380-4 (Donor/Positive control), 2. Kalyansona (RP), 3.HW 2002 (Kalyansona * *Lr24/Sr24*), 4. HW 2002A (Kalyansona * *Lr24/Sr24*), 5. NI 5439(RP), 6. HW 2003 (NI 5439 * *Lr24/Sr24*), 7. C 306 (RP), 8.HW 2004 (C 306 * *Lr24/Sr24*), 9.HD 2329(RP), 10.HW 2007 (HD 2329* *Lr24/Sr24),* 11.HD 2285 (RP), 12.HW 2008 (HD 2285 * *Lr24/Sr24*), 13.J24 (RP), 14. HW 2010 (J24**Lr24/Sr24*)

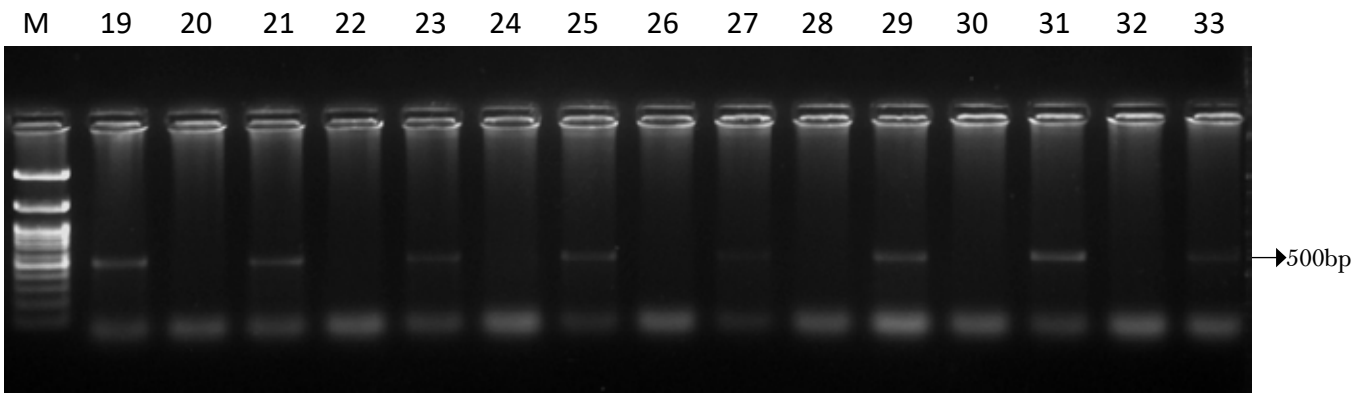

Fig 4: Molecular validation of *Lr24* gene in NIL's with *Sr24#*12marker re-confirmed during 2020

M- 100bp ladder, 19. Tr380-4 (Donor/Positive control), 20.HUW 234(RP), 21.HW 2015(HUW 234 * *Lr24/Sr24*), 22.PBW 226(RP), 23.HW 2016 (PBW 226**Lr24/Sr24*), 24.HD 2402(RP), 25.HW 2017 (HD 2402**Lr24/Sr24*), 26. HI 1077(RP), 27. HW 2018 (HI 1077**Lr24/Sr24*), 28.WH 542(RP), 29.HW 2019(WH 542 * *Lr24/Sr24*), 30.HS 240(RP), 31.HW 2020 (HS 240**Lr24/Sr24*), 32.WH 147(RP), 33. HW 2022 (WH 147**Lr24/Sr24*)

(# RP-Recurrent Parent used as negative control; NTC- Non template control)

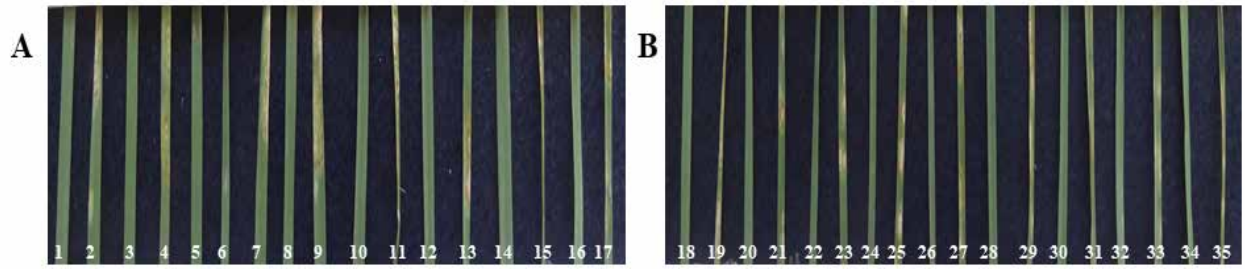

Fig 3: Seedling resistance pattern in NIL's

A: 1- Darfkite, 2- Sonalika, 3- HW 2002, 4- Kalyansona, 5- HW 2002, 6- HW 2002A, 7- NI5439, 8- HW 2003, 9- C306, 10- HW 2004, 11- WH147, 12- HW 2005, 13- LOK1, 14- HW 2006, 15- HD2329, 16- HW 2007, 17- Agralocal; **B:** 18- Drafkite, 19- HD2285, 20- HW 2008, 21- WL711, 22- HW 2014, 23- HUW234, 24- HW 2015, 25- PBW226, 26- HW 2016, 27- HD2402, 28- HW 2017, 29- HI1077, 30- HW 2018, 31- WH542, 32- HW 2019, 33- HS240, 34- HW2020, 35- WH147.

Though virulence to *Lr24* has been reported worldwide, however, *Lr24* still continues to be highly effective in seedling as well as in adult stage to Indian pathotypes of *P.triticina* and virulence for *Lr24* occur in low frequencies in most geographical areas(Huerta-Espino,1992) and Australia (Prasad *et al.*, 2018). Depending on the various climatic zones in India, the following leaf rust resistance genes *Lr9, Lr19, Lr24* and *Lr34* have been strongly recommended for the management of leaf rust pathotypes (Bahadur *et al.*, 1994).

Though *Lr24* provides all stage resistance to the prevailing Indian pathotypes of *P.triticina*, relying on monogene culture has a disadvantage of being overcome by new evolving leaf rust pathotypes. Thus, stacking of single effective genes with other resistant race specific or APR genes might confer long term resistance to leaf rust. Similar works of pyramiding *Lr24+Lr28* (Kumar *et al.*, 2017) and *Lr24+Lr19* (Singh *et al.*, 2017) have been reported.

'Agent' was the first wheat carrying the *Thinopyrum* derived segment with leaf and stem rust resistance gene, *Lr24/Sr24* respectively (Smith *et al.*, 1968). In India the first bread wheat variety DL 784-3 (Vidisha) carrying *Lr24/Sr24* was released in 1993. Since then more than 16 varieties carrying *Lr24/Sr24* have been released and are continuing to be in cultivation in India. The following varieties viz., DL784-3, HW 2004, DL788-2, HW 2045, HD 2781, HI 1500, MP4010, Raj4037, HD2851, HD 2833, HI 1531, COW(W)-1, HD 2888, AKAW3722, AKAW4627 and HW 5207(Pusa Navagiri) all carrying *Lr24/Sr24* have been released for commercial cultivation occupying a total of 15 million ha over a period of 20 years (Tomar *et al.*,2014). The adult plant scoring for leaf rust in the Indian varieties released to different wheat cultivating zones carrying specific leaf rust resistance gene(s) *Lr24/Sr24* is given in **Table 3.** This clearly indicates the effective resistance conferred by the gene. Furthermore, the release of more number of varieties with *Lr24* evidently remarks that the alien translocation 3Ag#3DL does not impair on yield (Singh *et al.*, 2007).

Table 3: Adult plant scoring for leaf rust in the released varieties carrying specific leaf rust resistance gene(s) *Lr24/Sr24* at ICAR-IARI, Regional Station, Wellington

S.No.	Name of the variety	Year and Zone for which released	Adult plant response under natural epiphytotic conditions at IARI, RS, Wellington	
			Leaf Rust	Stem Rust
	DL 784-3 (Vidisha)	1993, NEPZ	0	0
	HW 2004 (Amar)	1995, CZ	0	10MR
	DL 788-2 (Vaishali)	1996, CZ	0	20MS
	HW 2045 (Kaushambi)	2002, NEPZ	0	20MR
	HD 2781 (Aditya)	2002, PZ	0	20MS
	HI 1500 (Amrita)	2002,CZ	0	20M

MP 4010	2002, CZ	0	20MS
RAJ 4037	2003, PZ	0	20MR
HD 2851 (Pusa Vishesh)	2003, DELHI	0	10MS
HD 2833 (Trpti)	2004, PZ	0	10M
NW 1067	2004, UP	0	20MS
HI 1531	2005, CZ	0	20MR
COW(W)1	2005, TN	0	20MS
HD 2888 (Pusa wheat)	2005, NEPZ	0	20MR
AKAW 3722	2005, VIDARBHA	0	20MS
AKAW 4627	2011, PZ	0	20MS
HW 5216 (Pusa Thenmalai)	2012,SHZ	0	0

The deployment of this effective gene complex in Indian released cultivars for more than a decade played pivotal role in checkmating the *P.triticina* pathotypes prevalent in India (Tomar *et al.,* 2014). From this study it is confirmed that *Lr24* gene continued to provide resistance both in seedling as well as adult plant stage against all the occurring leaf rust pathotypes in India for more than three decades.

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Author's contribution

Conceptualization of research (MS); Designing of the experiments (MS, JP, VKV, CM); Contribution of experimental materials (MS); Execution of field/lab experiments and data collection (SCB, RN, SP, AS, SV, JP); Analysis of data and interpretation (MS, RN, SP, JP, VKV); Preparation of the manuscript (MS, RN, SP, JP, VKV).

Declaration

The authors declare no conflict of interest.

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