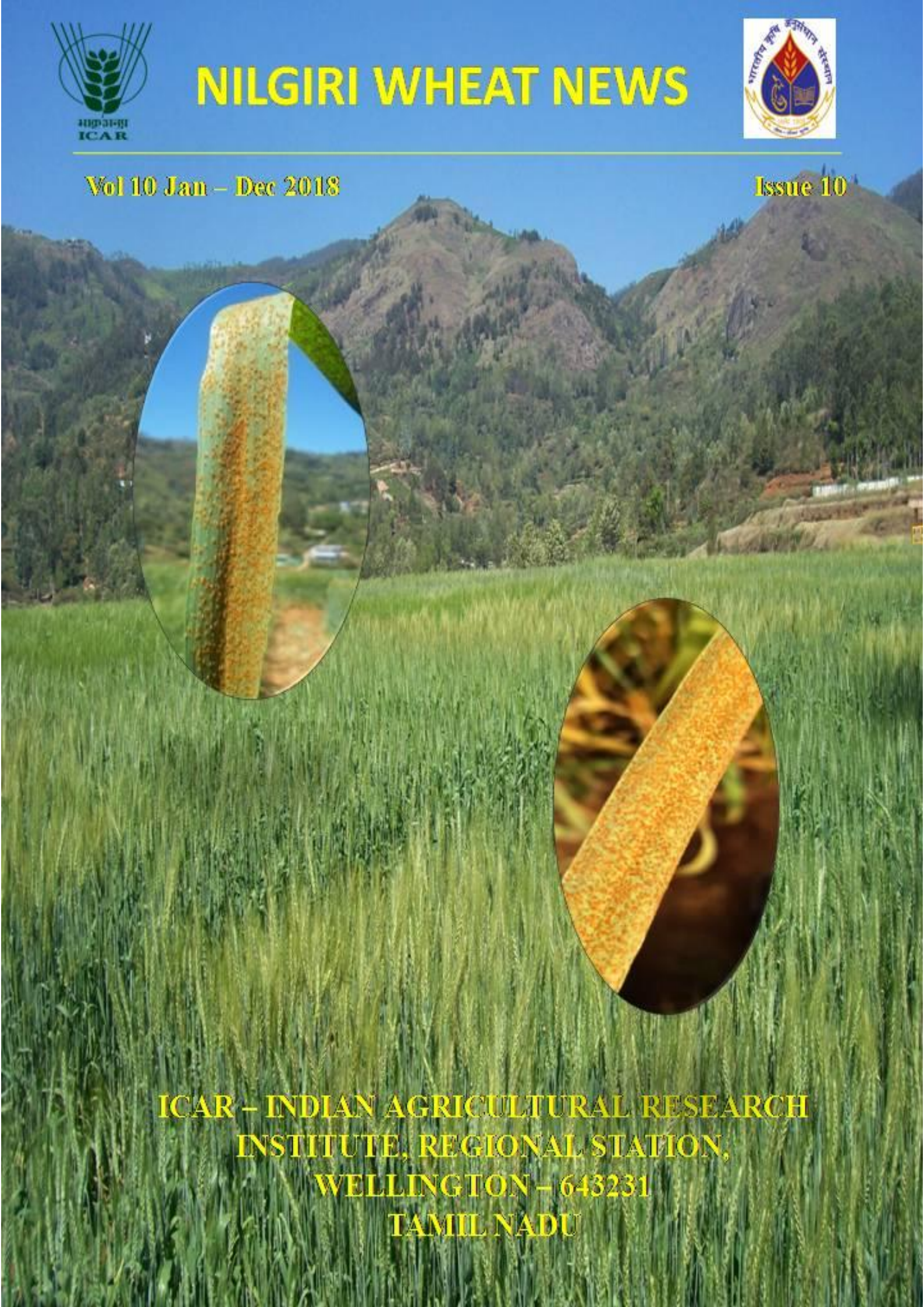
NILGIRI WHEAT NEWS

Vol 10 Jan – Dec 2018

Issue 10



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Gene Stewardship in developing improved Indian bread wheat cultivars and genetic stocks with low terminal disease value-*A compendium –Part-I*:Introgression of leaf rust resistance genes

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Key Words: Leaf rust, Stem Rust, Yellow Rust, Pyramiding, Introgression, MAB, ASR and APR

Introduction

Wheat is one of the most important staple food crop produced in the world and production wise wheat stands next to maize ensuring food and nutrition security of the people around the world. Global wheat production reached to the level of 761 mt – during 2018-19 and further increased to a record production level of 778.6mt during 2021-22(USDA Foreign Agriculture Service) post COVID pandemic. Over the last five decades, the wheat production in India witnessed a moderate growth rate of 2.25% and to reach the production level 106.84mt(revised estimate),harvested from 31.5 mha area during 2020-21. India ranks second in the world wheat production next only to China, with an average productivity of 3.39 t/ha, which is higher than the global average of 3.2 t/ha (Anon,2020). India is the only country cultivating all three commercially grown wheat species in the world and out of the total wheat production 95% is contributed by *Triticum aestivum*, followed by *T.durum*(4%) and *T.dicoccum* (1%). The wheat production in India need to be increased to more than 115mt by 2025to meet the growing demand from the projected population growth and India'sdomestic demand for wheat is expected to exceed 140 million tons by 2050 (Nagarajan 2005). The production constraints of biotic and abiotic stresses are the bottle necks to achieve goal and sustaining the wheat productiontrends arevery crucial to meet the production targets.Among the biotic stresses, rust diseases pose a challenge for wheat production globally with diverse and fast evolving race profiles. In India also, wheat crop is severely damaged by all the three rusts viz., black or stem rust caused by *Puccinia graminis* f. sp. *tritici* Erik. & Henn, brown or leaf rust caused *Puccinia triticina* Erik. and yellow or stripe rust caused by *Puccinia striiformis tritici* West. Under intense epidemic conditions, leaf rust can cause up to 65% yield losses (Chhuneja et al. 2011).

In wheat, based on the phenotypic rust expression and intensity at different growth stages, resistance can be broadly classified as all - stage resistance (ASR) and adult plant resistance (APR). ASR is governed by single major gene and offers race specific resistance (Rosewarne et al. 2013). The effectiveness of such gene can be detected in the early plant growth stages (Wu et al. 2020) and hence this type of resistance is also known as seedling resistance. ASR often fails within few years of deployment due to emergence of virulent variants (Chen 2005). Conversely, APR is effective either at post- seedling or adult plant stage. Generally the disease progress with slow latent period (slow rusting) and is race non-specific. This type of resistance is governed by minor genes having additive effects (Singh et al. 2000) and few also have pleiotropic effect in conferring resistance to multiple diseases (Singh et al. 1998; Herrera-Foessel et al. 2014).

Currently, 80 leaf rust resistant genes have been formally catalogued in wheat (McIntosh et al. 2017). More number of ASR genes from alien sources has been transferred into wheat (Friebe et al. 1996) but not many are utilized in the breeding programs. Short listing effective genes against the occurring pathotypes of rusts in a particular region or an environment, introgressing/pyramiding them to get effective resistance and durability is all about gene stewardship. Many of the identified resistant genes offer ASR and are

effective throughout the crop growth stages of wheat. However the number of APR genes so far identified and reported is very limited which include, ***Lr34+****Yr18+BDV1+Pm38+Sr resistance/Ltn,****Lr46/****Yr29/Pm39/Ltn,****Lr67+*** *Yr46 and Lr68* Stackingor pyramiding two or more effective ASR and APR genes expected to extend the durability and would prevent or delay the breakdown of resistance genes. The host resistance through developing disease resistant varieties by way of stacking effective genes is one of the most effective and eco-friendly methods to combat the rusts and PM (Singh et al. 2020). The host resistances achieved through introgression of rust resistance genes are often overcome by evolving new rust races & its frequency is hastened by changing climate. As the pathogen keeps evolving, stacking resistance genes is being pursued by the wheat breeders the effective way to extend the resistance offered by the genes. Hence we need to evolve the strategies like gene pyramiding of either race specific or race nonspecific genes(APR genes) or combination of both.

Materials and methods

A meticulously planned wheat improvement programme employing typical conventional back-cross method for introgression of effective rust and powdery mildew resistance genes were initiated during late nineteen eighties and introgression of additional effective genes were also done as and when new genes are identified and obtained. The well adapted commercial Indian bread wheat varieties from across all wheat production zones were taken for the programme and similarly over the 3 decades of work newer varieties susceptible to one or more rusts were added as and when it became a commercial/popular variety omitting the older ones. The reference stocks(RIL's) or donors obtained were initially evaluated for its resistance and only the effective leaf rust genes conferring resistance to occurring virulent pathotypes were taken. The effective genes were introgressed initially through conventional back-cross method taking advantage of Wellington climatic conditions where in three crop cycles per annum is possible and also location is considered as natural 'hot spot' for all three rusts and other foliar diseases round the year. Subsequently when advent of genetic markers evolved and made available, the combination of both conventional and MAB approaches were undertaken. Initially number of back-crosses were given was3-9 in developing BC and NIL lines and now addition of MAB we restrict the BC to $BC_{2\ or\ 3}$.

Table-1: The effective leaf rust resistance genes taken at IARI, RS, Wellingtonfor developing rust resistant wheat lines/stocks in the background of commercial Indian bread wheat cultivars, source, reference stocks used as donor and its chromosomal location

Genes taken	Derived Source	Reference stocks used (RIL's)	Chromosomal location
Lr9 (Lr9, has been reported with virulence of race 77-7 (121R127; Nayar et al. 2003)	*Aegilops umbellulata*	Abe	6BL
(**Lr19**+Sr25)+(Sr 36+Pm6) (77-8 race reported from PZ, India during 2008)	*Thinopyrum ponticum*	'Sunstar', 'Cook' and later 'Wheatear'	7DL
Lr24+Sr24 (40-1 race reported from Wellington on Sr24)	*Thinopyrum ponticum*	Tr380-14*7/3Ag#14 Janz, Agent	3DL
Lr26+Sr31+Yr9+Pm8 (77-1 race pathotype reported from Wellington virulent on Lr26)	*Secale cereal (Petkus rye)*	WH 542(Bacanora)	T1BL1RS
Lr28 (77-10 pathotype reported from Wellington virulent on Lr28**)**	*Aegilops speltoides*	CS 2A/2M 4/2	4AL
Lr32	*Ae.squorrosa/T.tauschii*	C86-8/KalyansonaF4/ Thatcher Lr32 later	3DS
Lr34+Yr18+BDV1+ Pm 38+ *Sr resistance/Ltn* (APR-race non-specific)/**Pleiotropic**	*T.aestivum* Terenizo	RL 6058 Webster	7DS
Lr35+Sr39	*Ae.speltoides*	Thatcher+ Lr35	2B
Lr37+Sr38+Yr17	*Ae.ventricosa*	Thatcher*8/VPM 1, RL 6081	2AS
Lr39	*T.tauschii*	KS 92 WGRC 15, EZ 350692	2DS
Lr44	*T.spelta*	EC 381202 (RL 6147)	1BL
Lr45	*Secale cereale(Imperial Rye)*	EC 381203 (RL 6144)	TAS-2R
Lr46/*Yr29/Pm39/Ltn* **Pleiotropic(APR)**	*T.aestivum*	PAVON 76, Dimond Bird	1BL
Lr47	*Aegilops speltoides*	PAVON 76S,	7AS
Lr53/Yr35 (APR)	*T.turgidum-T.dicocoides*	Avocet'S'	6BS
Lr57*+Yr40*	*Ae.geneculata*	TA 5602	5DS(T5DL.5DS-M^{g}S(0.95),
Lr67+ Yr46	*T.aestivum*	RL6077	4DL
Lr68	*T.aestivum*	Parula	7BL

Table-2: Donor parents taken in the back-cross programme(*Triticum aestivum*) and its adult plant response to rust& powdery mildew diseases at Wellington

Stock	Gene(s)	Reaction to			
		Stem rust	Leaf rust	Stripe rust	Powdery mildew
Abe	*Lr9 Sr36*	15R MR	F	40S	1
Sunstar*6/C80-1	*Lr19 Sr25*	10R MR-30R MR	F	F	4
Cook*6/C 80-1	*Lr19 Sr25 Sr36 Pm6*	F	F	F	1
Tr380-14*7/3Ag#14	*Lr24 Sr24*	15R MR	F	5MR	2+
DARF*6/3Ag3/Kite	*Lr24 Sr24 Sr26*	10R MR-20R MR	F	10MS	3
WH 542	*Lr26 Sr31 Yr9 Pm8*	10R MR	80S	F	3
CS 2A/2M 4/2	*Lr28 Sr34 Yr8*	90S	F	F	0-1
C86-8/Kalyansona F4	*Lr32*	70S	F	90S	3
RL 6058	*Lr 34 Yr18 BDV1 Pm38*	F	F	F	0-1
Thatcher+ Lr 35	*Lr35 Sr 39*	F	F	F	2
Thatcher*8/VPM 1,RL 6081	*Lr37 Sr38 Yr17*	20R MR MS	F	15MS	4
KS 92 WGRC 15 EZ 350692	*Lr 39*	40S	F	F	2
EC 381202	*Lr 44*	20S	20S	F	2
EC 381203	*Lr 45*	S	F	S	3
PAVON 76	*Lr 46*		20MS		
PAVON 7 S3	*Lr 47*	F	F	10S	2

Table-3: Recurrent parents (*Triticum aestivum*) taken in the Back-Cross programme and its adult plant response to rusts and powdery mildew diseases at Wellington

Stock	Gene(s)	Reaction to			
		Stem rust	Leaf rust	Stripe rust	Powdery mildew
1. C 306	*Lr34+*	90S	90S	F	3
2. HD 2009		40S	60S	100S	3
3. HD 2285		30MS	100S	30S	3
4. HD 2329		80S	90S	90S	3
5. HD 2402	*Sr2+*	30S	100S	F	3
6. HD 2687	*Sr31 Lr26 Yr9 Pm8*	15R MR	80S	F	3
7.HD 2733	*Sr31 Lr26 Yr9 Pm8*	5MR	80S	F	3
8.HD 2877	*Sr31 Lr26 Yr9 Pm8*	5MR	80S	F	3
9. HI 1077		30MS S	50S	40S	3
10. HS 240	*Sr31 Lr26 Yr9 Pm8*	5R MR	70S	F	3
11. HUW 234		20MS S	100S	F	3
12. J 24		90S	100S	100S	3
13. Kalyansona		80S	90S	90S	3

14. Lok-1	*Lr34, Sr2+*	70S	80S	80S	3
15. NI 5439	*Lr34*	90S	90S	100S	3
16. PBW 226		20S	90S	F	3
17. Sonalika	*Sr2+*	60S	80S	60S	3
18. UP 262		50S	50S	50S	3
19.VL 421		60S	90S	80S	3
20. WH 147		90S	90S	90S	3
21. WH 542	*Sr31 Lr26 Yr9 Pm8*	10R MR	80S	F	3
22. WL 711		100S	100S	90S	3
23. HI 977		F	60S	40S	2
24. HP 1205		60SS	80SS	90S	3
25. PBN 51	*Sr31 Lr26 Yr9 Pm8*	20MR	40S	S	2
26. PBW 343	*Sr31+, Yr24*	20MR	60S	5S	3
25. Raj 3077		5MR	60SS	60SS	1
26. HW 3070	*Lr24+, Sr31 Lr26 Yr9 Pm8*	F	F	5S	2
27.GW 273					
28.Lalbahadur		20S	80S	20S	
29.NIAW 34	*Sr2+*				
30.UP 2425					
31.HD 2189	*Sr2+*	20S	60S	20S	3
32.PBW 502	*Sr31 Lr26 Yr9 Pm8*	20MR	40S	5S	3
33. UP 2338		20MR	60S	F	2

*** Genetic markers available in public domain. Details on genetic markers can be accessed through *maswheat.ucdavis.edu/***

Initial efforts in introgression of single effective genes into commercial Indian bread wheat cultivars

Leaf rust genes

Aegilops umbellulata*-derived*Lr9

Initially the rust resistance gene *Lr9* was transferred to Chinese spring wheat-translocation 47 became known as 'Transfer' (Sears, 1956). The gene *Lr9* is located on 6B of the chromosomal arm showing low infection type of 0; and occasionally 1+ and has low environmental variability. However virulence for *Lr9* was found in USA in 1971 four years after its use in soft red winter wheat (Shaner et al., 1972). The source stock Abe and Arthur 71 were used for transferring *Lr9* into commercial cultivars of India at IARI, Regional Station, Wellington (Tomar and Menon, 1998). Initially the gene *Lr9* conferred seedling resistance to 13 Indian races of leaf rust (Sawhney *et al.,* 1977) and has consistently displayed immune reaction to leaf in adult plant stage at Wellington for over 25 years (Tomar and Menon, 1998). At IARI, RS, Wellington the authors had developed near isogenic &BC lines carrying *Lr9* in the back-ground of 13well adapted Indian commercial bread wheat cultivars given in table such as HW 2067 (HD 2009), HW 2054(HD 2285), HW 2055(HD 2329), HW 2070(HS 240), HW 2056(HUW 234), HW 2053(Kalyansona), HW 2052(LOK-1), HW 2060(NI 5439), HW 2057(PBW 226), HW 2051(Sonalika), HW 2058(WH 147), HW 2059(WH 542).

Table-4: Details on donor, back-crossed derivatives, genes it carried and adult plant response to rusts and powdery mildew under natural epiphytotic conditions(Field response)

Sl. No	Donor/Backcrossed lines/ Recurrent cultivars		Genes it carried	Adult plant Response to (under field conditions)				Response to 77-7 new leaf rust pathotype
				Stem rust	Leaf rust	Stripe rust	Powdery mildew (0-9 scale)	
	Abe	Christened as	*Lr9*	15R MR	F	40S	4	40S
1.	HD 2009*3/Abe	HW 2067	*Lr9*	40S	F	100S	4	40S
	HD 2009			40S	60S	100S	3	60S
2.	HD 2285*6/Abe	HW 2054	*Lr9*	30MS	F	30S	4	80S
	HD 2285			30MS	100S	30S	3	100S
3.	HD 2329*6/Abe	HW 2055	*Lr9*	80S	F	80S	4	60S
	HD 2329			80S	90S	80S	3	100S
4.	HD 2402*6/Abe		*Lr9*	30S	F	F	3	60S
	HD 2402			30S	100S	F	3	80S
5.	HS 240*3/Abe	HW 2070	*Sr31 Lr9 Lr26 Yr9 Pm8*	5R MR	F	F	4	60S
	HS240		*Sr31 Lr26 Yr9 Pm8*	5R MR	70S	F	3	70S
6.	HUW 234*6/Abe	HW 2056	*Lr9*	20MS S	F	F	4	80S
	HUW 234			20MS S	100S	F	3	100S
7.	Kalyansona*6/Abe	HW 2053	*Lr9*	80S	F	90S	4	80S
	Kalyansona			80S	80S	90S	3	80S
8.	Lok-1*6/Abe	HW 2052	*Lr9*	70S	F	90S	4	60S
	Lok-1			70S	80S	80S	3	80S
9.	NI 5439*6/Abe	HW 2060	*Lr9*	90S	F	100S	4	60S
	NI 5439			90S	90S	100S	3	90S
10.	PBW 226*6/Abe	HW 2057	*Lr9*	20S	F	F	4	60S
	PBW 226			20S	90S	F	3	80S
11.	Sonalika*7/Abe	HW 2051	*Lr9*	60S	F	60S	4	60S
	Sonalika			60S	80S	60S	3	80S
12.	WH 147*6/Abe	HW 2058	*Lr9*	90S	F	90S	4	70S
	WH 147			90S	90S	90S	3	80S
13.	WH 542*6/Abe	HW 2059	*Sr31 Lr9 Lr26 Yr9 Pm8*	15R MR	F	F	4	60S
	WH 542		*Sr31 Lr26 Yr9 Pm8*	10R MR	80S	F	3	80S

However, even before its deployment for commercial use, Tomar & Menon 1998 detected virulence for *Lr9* in September 1998 on the Indian bread wheat cultivars Sonalika*7/Abe(Sonali-HW 2051) and HUW 234*5/Abe (HW 2056), both carrying the gene *Lr9*. The virulence was further confirmed on other Indian common wheats with *Lr9* as well as on Transfer, Thatcher*6/Transfer (RL6010), addition line (CS 6U), Abe, Arthur 71 and Oasis (Nayar *et al.*, 2003) and the pathotype has been designated as 77-7. **As per the author's observation, the alien segment with *Lr9* is associated with an enhanced susceptibility to powdery mildew in the backcross derivatives.**

Thinopyrum ponticum*-derived*Lr19/Sr25

The linked genes *Lr19/Sr25* located in the chromosome 7D transferred from *Thinopyrum ponticum* (10x) in the winter wheat Agrus which could not be utilized in wheat breeding because of their undesirable linkages with yellow flour pigmentation which was not commercially acceptable. Agatha a line resulting in the translocation stock T4 when subjected to irradiation carried *Lr19*/*Sr25* devoid of yellow pigmentation (Sharma and Knott, 1966) and subsequent independent transfers of *Lr19/Sr25* Chinese spring were used worldwide to exploit this gene(Knott1980 and 1984). In one of the lines, Agatha-235, the transferred from*Thinopyrum ponticum* carried only leaf rust resistance gene, presumably *Sr25* was lost along with the gene for yellow pigmentation (Friebe *et al.,* 1994 and 1996). However, the authors initially has used lines Sunstar*6/C 80-1 in white seeded background with highly reduced pigmentation as a donor parent in the backcross programme to introgress *Lr19/Sr25* into the susceptible Indian cultivars and developed twenty(20) NIL's or BC lines carrying *Lr19/Sr25***(Table-5)**but later the gene source Sunstar*6/C 80-1 and 20 introgressed lines were confirmed to carry *Lr24/Sr24* and not *Lr19/Sr25* (Prabhu *et al.,* 1998). Out of these HW 2044 and HW 2045 were released as cultivars in India.

Further the authors have used the Australian cultivar 'Cook' as donor parent which carry *Lr19/Sr25*, *Sr36/Pm6* and later 'Wheatear'. A wide spectrum of virulent pathotypes of leaf rust prevalent in USA, Canada, Australia, India and other countries on *Lr19* (Agatha). A Virulent race of *P.recondita* (CBJ/QQ)for *Lr19* was detected in in Mexico (Huerta-Espino and Singh, 1994) from the cultivar Oasis 86. Authors have developed additional 36 back-crossed lines **(Table-5A)**carrying *Lr19* using 'Cook' as donor and later it was molecularly confirmed to carry either *Lr19/Sr25* alone or *Lr19/Sr25* and *Sr*36/*Pm6*(Sivasamy *et al.,* 2009) although yield reductions associated with alien genetic transfers have been reported in some cases in hexaploid wheat cultivars in Sweden (Sunann) and Mexico (Oasis 86) (McIntosh *et al.*, 1995). However, Singh *et al.,* 2006 reported enhanced yield in the wheat derivatives carry *Lr19*/*Sr25* gene complex. However the authors have observed enhanced yield in the derived lines carrying Lr19/Sr25 with further yield enhancement when Sr36/Pm6 was present(Attributed to additional resistance to powdery mildew). The authors also observed theenhanced susceptibility to *Pm* in the constituted lines which carry *Lr19/Sr25.*

Table-5&5A: Details on donor, back-crossed derivatives, adult plant response to rusts and powdery mildew under natural epiphytotic conditions(Field response) for the lines introgressed with *Lr19/Sr25*

Sl. No	Donor/Backcrossed lines/ Recurrent cultivars		Genes it carried	Adult plant Response to (under field conditions)			
				Stem rust	Leaf rust	Stripe rust	Powdery mildew (0-9 scale)
1	C306*7//Sunstar*6/C80-1	HW 2083	*Sr25 Lr19*	30MR MS	F	F	4
	C 306			90S	90S	F	3
2	HD2009*4//Sunstar*6/C80-1	HW 2079	*Sr25 Lr19*	5R MR MS	F	100S	4
	HD 2009			40S	60S	100S	3
3	HD2285*6//Sunstar*6/C80-1	HW 2049	*Sr25 Lr19*	15R MR	F	30S	4
	HD 2285			30MS	100S	30S	3
4	HD2329*7//Sunstar*6/C80-1	HW 2046	*Sr25 Lr19*	20R MR MS	F	90S	4
	HD 2329			80S	90S	90S	3
5	HD2402*5//Sunstar*6/C80-1	HW 2045	*Sr25 Lr19*	TR MR	F	F	4
	HD 2402			30S	100S	F	3
6	HD 2687*5//Sunstar* 6 /C80-1	HW 2078	*Sr25 Lr19 Sr31 Lr26 Yr9 Pm8*	15R MR	F	F	3
	HD 2687		*Sr31 Lr26 Yr9 Pm8*	15R MR	80S	F	3
7	HI1077*5//Sunstar*6/C80-1	HW 2048	*Sr25 Lr19*	20R	F	40MS	4
	HI 1077			30MS S	50S	40MS	3
8	HP 1205*5 //Sunstar* 6/C80-1	HW 2085	*Sr25 Lr19*	60SS	F	90S	3
	HP 1205			60SS	80SS	90S	3
9	HS240*3//Sunstar*6/C80-1	HW 2080	*Sr25 Sr31 Lr19 Lr26 Yr9 Pm8*	TR MR	F	F	4
	HS 240		*Sr31 Lr26 Yr9 Pm8*	5R MR	70S	F	3
10	HUW234*6//Sunstar*6/C80-1	HW 2043	*Sr25 Lr19*	TR MR	F	F	4
	HUW 234			20MS S	100S	F	3
11	J24*4//Sunstar*6/C80-1	HW 2084	*Sr25 Lr19*	40MS S	F	100S	4
	J 24			90S	100S	100S	3
12	Kalyansona*6// Sunstar*6/C80-1	HW 2081	*Sr25 Lr19*	20R MR MS S	F	90S	4
	Kalyansona			80S	80S	90S	3
13	Lok-1*7//Sunstar*6 /C80-1	HW 2041	*Sr25 Lr19*	5R MR MS S	F	80S	4
	Lok-1			70S	80S	80S	3
14	MACS 2496*7// Sunstar* 6 /C80-1	HW 2086	*Sr25 Lr19*				
	MACS 2496						
15	NI5439*6//Sunstar*6/C80-1	HW 2082	*Sr25 Lr19*	30MR MS S	F	100S	4
	NI 5439			90S	90S	100S	3
16	PBW226*5//Sunstar*6/C80-1	HW 2044	*Sr25 Lr19*	TR MR	F	F	4
	PBW 226			20S	90S	F	3
17	Sonalika*6//Sunstar*6/C80-1	HW2050	*Sr25 Lr19*	15R MR	F	60S	4
	Sonalika			60S	80S	60S	3
18	UP 262*6//Sunstar* 6/C80-1	HW 2087	*Sr25 Lr19*	50S	F	50S	3
	UP 262			50S	50S	50S	3
19	WH147*7//Sunstar*6/C80-1	HW 2042	*Sr25 Lr19*	30R MR MS S	F	90S	S
	WH 147			90S	90S	90S	3
20	WH542*6//Sunstar*6/C80-1	HW 2047	*Sr25 Sr31 Lr19 Lr26 Yr9 Pm8*	TR MR	F	F	4
	WH 542		*Sr31 Lr26 Yr9 Pm8*	10R MR	80S	F	3
Table-5A							
	Cook*6/C 80-1		***Sr25 Sr36 Lr19 Pm6***	**F**	**F**	**F**	**1**
21	C 306*2//Cook*6/C 80-1	HW 3601	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	C 306			90S	90S	F	3
22	GW 273* 2//Cook* 6/C 80-1	HW 3602	*Sr25 Sr36 Lr19*	F	F		1

			Pm6				
	GW 273						
23	HD 2009*3//Cook*6/ C 80-1	HW 3603	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	HD 2009			40S	60S	100S	3
24	HD 2189*3//Cook*6/ C 80-1	HW 3604	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	HD 2189						
25	HD 2285*3//Cook*6/ C 80-1	HW 3605	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	HD 2285			30MS	100S	30S	3
26	HD 2329*3//Cook*6 /C 80-1	HW 3606	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	HD 2329			80S	90S	80S	3
27	HD 2402*3//Cook*6/ C 80-1	HW 3607	*Sr25 Sr31 Sr36 Lr19 Lr26 Yr9 Pm6 Pm8*	F	F	F	1
	HD 2402			30S	100S	F	3
28	HD 2687*3//Cook*6 / C 80-1	HW 3608	*Sr25 Sr31 Sr36 Lr19 Lr26 Yr9 Pm6 Pm8*	F	F	F	1
	HD 2687		*Sr31 Lr26 Yr9 Pm8*	10R MR	80S	F	3
29	HD 2733*3//Cook*6 / C 80-1	HW 3609	*Sr25 Sr36 Lr19 Pm6*	F	F		1
	HD 2733						
30	HD 2877*3//Cook*6 / C 80-1	HW 3610	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	HD 2877				80S		
31	HI 977*3//Cook*6 / C 80-1	HW 3611	*Sr25 Sr36 Lr19 Pm6*	F	F		1
	HI 977						
32	HI 1077*3//Cook*6 / C 80-1	HW 3612	*Sr25 Sr36 Lr19 Pm6*	F	F		1
	HI 1077			30MS S	50S	40MS	3
33	HP 1205*3//Cook*6 / C 80-1	HW 3613	*Sr25 Sr36 Lr19 Pm6*	F	F		1
	HP 1205						
34	HS 240*3//Cook*6 / C 80-1	HW 3614	*Sr31 Lr26 Yr9 Pm8 +Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	HS 240		*Sr31 Lr26 Yr9 Pm8*	5R MR	70S	F	3
35	HUW 234*3//Cook* 6 /C 80-1	HW 3615	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	HUW 234			20MS S	100S	F	3
36	J 24*3//Cook*6/C 80-1	HW 3616	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	J 24			90S	100S	100S	3
37	Kalyansona*3//Cook*6/C 80-1	HW 3617	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	Kalyansona			80S	80S	90S	3
38	Lal Bahadur*3// Cook*6/C 80-1	HW 3618	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	Lal Bahadur				80S		3
39	Lok-1*3//Cook*6/C 80-1	HW 3619	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	Lok-1			70S	80S	80S	3
40	MACS 2496*3//Cook *6/C 80-1	HW 3620	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	MACS 2496				90S		3
41	NI 5439*3//Cook*6/ C 80-1	HW 3621	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	NI 5439			90S	90S	100S	3
42	NI 5439*3//Cook*6/ C 80-1	HW 3621A	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	NI 5439			90S	90S	100S	3
43	NIAW34*3//Cook*6/ C 80-1	HW 3622	*Sr25 Sr36 Lr19*	F	F		1

			Pm6				
	NIAW 34						
44	PBN 51*3//Cook*6 /C 80-1	HW 3623	*Sr25 Sr36 Lr19 Pm6*				
	PBN 51						
45	PBW 226*3//Cook*6 /C 80-1	HW 3624	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	PBW 226			20S	90S	F	3
46	PBW 343*3//Cook*6 /C 80-1	HW 3625	*Sr25 Sr36 Lr19 Pm6*	F	F		1
	PBW 343				80S		3
47	PBW 502*3//Cook*6 /C 80-1	HW 3626	*Sr25 Sr36 Lr19 Pm6*	F	F		1
	PBW 502						3
48	Raj 3077*3//Cook*6 /C 80-1	HW 3627	*Sr25 Sr36 Lr19 Pm6*	F	F		1
	Raj 3077						3
49	Raj 3077*3//Cook*6 /C 80-1	HW 3627 A	*Sr25 Sr36 Lr19 Pm6*	F	F		1
	Raj 3077						3
50	UP 262*3//Cook*6/C 80-1	HW 3628	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	UP 262			50S	50S	50S	3
51	UP 2338*3//Cook* 6/C 80-1	HW 3629	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	UP 2338			70S	80S	80S	3
52	UP 2425*3//Cook* 6/C 80-1	HW 3630	*Sr25 Sr36 Lr19 Pm6*	F	F		1
	UP 2425						
53	WH 147*3//Cook*6 /C 80-1	HW 3631	*Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	WH 147			90S	90S	90S	3
54	WH 542*3//Cook*6 /C 80-1	HW 3632	*Sr31 Lr26 Yr9 Pm8 +Sr25 Sr36 Lr19 Pm6*	F	F	F	1
	WH 542		*Sr31 Lr26 Yr9 Pm8*	10R MR	80S	F	3
55	Yr10*3//Cook*6 /C 80-1	HW 3633	*Sr25 Sr36 Lr19 Pm6, yr10*	F	F	F	1
	Yr10			10R MR	80S	F	3
56	Yr 15*3//Cook*6 /C 80-1	HW 3634	*Sr25 Sr36 Lr19 Pm6, Yr15*	F	F	F	1
	Yr 15				90S	F	3

Thinopyrum ponticum – derived
Lr24/Sr24

The leaf rust resistance gene *Lr24/Sr24* was originally detected in a bread wheat line possessing spontaneous translocation involving chromosome 3D in agent in the stock Agent derived from *Agropyron elongatum* (*Syn=Thinopyrum ponticum*) selected from across of this line cv. Triumph is the origin (Gough and Merkle, 1971). The gene present in the genotype Agent is a spontaneous wheat- translocation involving 3 Ag and 3DL chromosomes. In Agent, *Lr24* is tightly linked with stem rust resistance gene *Sr24* and it was reported to with red seed colour (McIntosh *et al.,* 1977). The initial impediment to use of *Lr24/Sr24* from Agent was in association with red grain colour but RA McIntosh and M. Partridge were able to recover white-seeded recombinants. One of the white seeded recombinant lines *Tr 380-4**7/3 Ag#14* proved to be a valuable genetic stock for wheat improvement, was used to develop eighteen (18) BC lines of popular Indian cultivars HW 2002 HW 2002A, HW 2003, HW 2004, HW2007, HW 2008, HW 2011, HW 2012, HW

2015, HW 2016, HW 2017, HW 2018, HW 2019, HW 2071, HW 2020, HW 2022, & HW 2072, HW 2073 all carrying*Lr24/Sr24* at Wellington

However pathotypes virulent on *Lr24* have been reported from North America (Browder, 1973), Canada (Kolmer, 1991), South America (Singh, 1991) and South Africa (Pretorius *et al.*, 1990) but this gene is highly effective in Australia and India. However, *Lr24* still continues to be highly effective in seeding as well as in adult stage to Indian pathotypes of *P.recondita* and virulence for *Lr24* occur in low frequencies in most geographical areas(Huerta-Espino,1992). Sivasamy et.al(2017) further confirmed that *Lr24* leaf rust gene continued to offer high levels of resistance all the occurring pathotypes in India, although the linked stem rust resistance gene *Sr24* is not effective bread wheat cultivars carrying *Lr24/Sr24* are widely grown in Australia, North America and South Africa. From this back-cross programme HW 2004 (Amar), HW 2045 (Kausambi), HD 2833 (Tripti), HD 2888 (Pusa Wheat-105)COW(W)-1and HW 5207(Pusa Navagiri) all carrying Lr24/Sr24 gene complexes has been released for commercial cultivation under different wheat cultivating zones in India. Other wheat cultivars viz., DL784-3 (Vidisha), DL788-2 (Vaishali), , HD 2781 (Aditya), HI 1500 (Amrita), MP4010, Raj4037, HD2851 (Pusa Vishesh), HI 1531, AKAW3722 (Vimal), AKAW4627 all carrying *Lr24/Sr24* have been released over the years in India for commercial cultivation. The deployment of this effective gene complex in Indian released cultivars for the last more than a decade, widely across India played pivotal role in checkmating the brown rust (Tomar.*et.al,* 2014).. The isolates of *P. graminis tritici*virulent to *Sr24* appeared in South Africa (Le Roux and Rijkenburg, 1987) and in India (pathotype 40-1) (Bhardwaj *et al.*, 1990) although the donor *TR 380-14*7/3Ag#14* exhibited a high level of adult plant resistance to stem rust indicating the presence of some additional factors for resistance.

Lr26/Sr31/Yr9/Pm8

The *Secale cereale*-derived linked gene *Lr26/Sr31/Yr9/Pm8* is a spontaneous wheat-rye substitution which was identified by Kattermann (1937) in which the wheat chromosome 1B was replaced by rye chromosome 1R (1BL.1R#1S). Occurrence of 1B (1BL.1RS) are1R (1B) were reported by Mettin *et al.,* 1973 and Zeller, 1973. However, some wheat consists of both substitution and translocation biotypes (Zeller, 1973) were also reported and *Lr26* is completely linked with *Sr31*, *Yr9* and *Pm8*. The linkage of *Yr9* for stripe rust resistance and *Pm8* for powdery mildew resistance was reported by (Bartos *et al.*, 1973). Rajaram *et al.,* 1983., reported the significant heterotic effect on grain yield of this translocation. However, genotypes carrying this translocation has the the linkage drag of poor grain quality, because doughs made from these tend to be sticky with over mixing and earlier reports of tight linkage to red grain. The global importance of 1RS in wheat programmes has been well documented. Depending on the wheat genotype into which the translocation has been introduced, 1RS may also directly increased yield (Villareal *et al.*, 1997). This disease resistance gene complex had a major impact on global wheat production is evident from worldwide exploitation for its yield increase both in many winter and spring wheat cultivars. However after the Ug99 pathotype became virulent on *Sr31* worldwide efforts are on to diversify the genetic basis of resistance; nevertheless one can't ignore the yield advantage associated with this gene complex. Although more than 60% of

Indian wheat cultivars carrying this gene complex are complementing wheat yields in India, unintentionally the Indian cultivars carry additional gene complexes like *Sr2+, Sr22, Sr14, Lr34+, Lr46+, Lr67+* gene complexes minimises the direct threat from Ug99 stem rust race or Lr26 virulent race occurring in India.

Lr28

McIntosh *et al.* 1982 reported that the *Ae. speltoides*-derived gene located in 4AL mapped at 53cM from the centromere. This gene was incidentally transferred while attempts were made to transfer the stripe rust resistance gene (*Yr8*) from *Aegilops comosa* var. *comosa* to wheat, a high pairing line of *Aegilops speltoides* was used by Riley *et al.,* (1968a) to induce homoeologous recombination. The gene Lr28 shows infection type 0; sometimes 1+to 2+ especially with South American isolates (Huerta-Espino,1992). The authours used the line CS *2A/2M#4/2* in their BC programme which was identified to have a gene for leaf rust resistance, *Lr28* derived from *Ae. speltoides* (McIntosh *et al*., 1982). The segment carrying *Lr28* is most likely to be derived from the short arm of *Ae. speltoides* chromosome 7S#2 during homoeologous recombination resulting in the translocate in chromsome T4AS. 4AL-7S#2 (Friebe *et al.,* 1996).

The gene *Lr28* confers a high degree of resistance in seedling as well as at adult plant stage to all the leaf rust pathotypes prevailing in India. It is also widely effective in Australia, South Asia and Europe. However, most *P.recondita* isolates in North America are virulent to *Lr28*. No detrimental effects are associated with the presence of *Lr28* but the durability of resistance is likely to be low (McIntosh *et al.*, 1995). Of late a pathotype virulent on *Lr28* has been reported from Wellington in India (Bhardwaj *et al.,* 2010b), although it was not reported earlier from Indian sub-continent. The cultivar Sunland was registered in New South Wales, Australia as prime with hard texture of seed in 1992. In India, the authors of the compendium have also transferred *Lr28* into sixteen Indian cultivars susceptible to leaf rust and developed BC lines *viz*., HW 2034 (C 306), HW 2064 (HD 2009), HW 2038 (HD 2285), HW 2037 (HD 2329), HW 2065 (HD 2402), HW 2066 (HD 2687), HW 2063 (HS 240), HW 2039 (HUW 234), HW 2036 (J 24), HW 2061 (Kalyansona), HW 2032 (LOK-1), HW 2035 (NI 5439), HW 2040 (PBW 226), HW 2031 (Sonalika), HW 2033 (WH 147) and HW 2062 (WH 542) and out of these HW 2034 has been released in India as cultivar as MACS 6145. Two backcross lines, HD 2329*7/CS 2A/2M#4/2 and WH147*7/CS 2A/2M#4/2 did not show any yield difference under rust free conditions as compared to recurrent parents HD 2329 and WH 147, respectively. Some of the backcross lines carrying the gene *Lr28* showed fast rusting to stem rust and reduced susceptibility to Powdery mildew as compared to their recurrent parents under natural condition at Wellington indicating the phenomenon of fast rusting to stem rust which appears to be associated with the resistance imparted by *Lr28* (Tomar and Menon, 1999).

Lr32

Kerber 1987 and 1988 confirmed the location of this gene at 3DS which was derived from *Aegilops squarrosa (=T.tauchii)* whichhas been found effective in adult plant stage at Wellington as well as in seedling stage with low infection type to the prevalent pathotypes of leaf rust with low environmental variability. Sawhney and Sharma (1990) reported that *Lr32* produces mainly infection type 2 against different pathotypes of leaf rust

in seedling stage. Huerta-Espino (1992) and Kerber (1987) also recorded low infection type (; to 2+) on the lines carrying *Lr32* as well as on donor *Ae.squarrosa*. Pathogenic variability for *Lr32* has not been reported except an isolate from Bulgaria and Turkey. Commercially this gene has not been exploited much, however, the authors have transferred *Lr32* initially using C86-8/Kalyansona F4 in the background of fourteen Indian bread wheat cultivars susceptible to leaf rust namely HW 4001 (C 306), HW 4002 (HD 2285), HW 4003 (HD 2329), HW 4015 (HD 2687), HW 4013 (HS 240), HW 4004 (HUW 234), HW 4005 (Kalyansona), HW 4006 (LOK-1), HW 4007 (NI 5439), HW 4008 (PBW 226), HW 4009 (Sonalika), HW 4012 (UP 262), HW 4010 (WH 147), HW 4011 (WH 542) and later Prabhu *et al.*, 1998 confirmed molecularly that all these lines including the donor C86-8/KalyansonaF4 carried only *Lr28* and hence the authors used the donor Thatcher *Lr32* later to transfer this gene into commercial cultivars. The backcross derivatives with *Lr32* also showed fast rusting to stem rust and reduced susceptibility to Pm as compared to their recurrent parents (Tomar and Menon, 1999) similar to that of *Lr28*.

Lr34/Sr57/Yr18/BDV1/Pm38/Ltn

The gene *Lr34* derived from varieties Terenzio showed a moderate level of susceptibility to leaf rust (30-60 MS) at adult plant stage. Sawhney and Sharma (1990) reported that *Lr34* mainly exhibited an infection type 3 to many pathotypes of leaf rust in the seedling. Several researchers have, however, reported that *Lr34* interacted favourably with *Lr13* (Roelfs, 1988) and also with *Lr33* and *LrT3* (Samborski and Dyck, 1982) to confer durable resistance to leaf rust. The transfer of *Lr34* an APR gene which is associated with *Sr57, Yr18*, BDV1 and leaf tip necrosis(*Ltn*) genes as peliotropic association if pyramided with major or minor gene(s) expected to give durable rust resistance (Singh and Rajaram. 1992) but alone observed to be ineffective at Wellington, while wheat varieties, Chris, Frontana and La Prevision all carrying the genes *Lr13* and *Lr34* exhibited moderate susceptibility with the exception of Era showing resistance to leaf rust. The study indicated that there is little or no interaction between *Lr34* and *Lr13*. The stocks RL 6058, Webster and Diamondbird carry *Lr34* which are used at Wellington to introgress and pyramid the gene with other APR genes *Lr46, Lr67* and *Lr68* with the objective of developing durable rust resistant wheat varieties.

Lr35

The gene Lr35 located at 2B linked to a gene for seedling resistance to stem rust gene Sr39 (Kerber and Dyck, 1990). The adult plant resistance gene *Lr35* was transferred by homoeologous recombination from *Aegilops speltoides* chromosome 2S#2 to wheat chromosome 2B (Kerber and Dyck, 1990). The line RL 6082 (TC*6/RL 5711) carrying *Lr35* which is linked to *Sr39* showed moderate susceptibility with low intensity (30 MR, MS) to leaf rust and high degree resistance to stem rust at Wellington. Seedling tests of line carrying the gene *Lr35* produced an infection type 3 (Sawhney *et al.*, 1994) with selected pathotypes of leaf rust. They explained that *Lr35* imparts races non-specific adult plant resistance which is likely to be durable. The gene *Lr35* has not been used for cultivar improvement (McIntosh *et al.*, 1995). Although *Lr35* reportedto have yield penalty in the lines introgressed with this gene when compared to recurrent parent, in our experiences careful selection of transgressive segregants facilitates selecting of lines without having any yield penalty. The gene *Lr35* linked to *Sr39* is effective against stem

rust races occurring in India and Ug99 which can be well exploited.

Lr37/Sr38/Yr17

The gene *Lr37* is derived from *Aegilops ventricosa* (Bariana and McIntosh, 1994) and is present in a French winter wheat VPM1 which has been selected for resistance to strawbreaker disease or eye spot disease caused by *Pseudocercosporella herpotrichoides* located on chromosome 7D. The gene *Lr37* was found effective against the virulent Indian pathotypes of *P. recondita* in adult plant stage at Wellington. This gene is located on the short arm of chromsome 2A and is linked with the genes *Sr38* and *Yr17* (Bariana and McIntosh, 1993) both providing moderate degree of resistance to stem rust and stripe rust, respectively, Kloppers and Pretorius (1995) found that the gene *Lr37* conferred slow rusting resistance as they observed fewer uredia smaller uredinium with a low rate of uredinial appearance. In India, the authors have incorporated the gene *Lr37* in sixteen commercial Indian bread wheat cultivars susceptible to leaf rust viz.,. HW 4022 (HD 2285), HW 4023 (HD 2329), HW 4033 (HD 2687) HW 4032 (HS 240), HW 4024 (HUW 234), HW 4025 (Kalyansona), HW 4026 (Lok-1), HW 4034 (MACS 2496), HW 4027 (NI 5439), HW 4035 (PBN 51), HW 4028 (PBW 226), HW 4029 (Sonalika), HW 4030 (WH 147) HW 4031 (WH 542), HW 4036 (HI 1077) and HW 4037(RAJ 3077) effecting five back-crosses. The expressions of resistance in the seedling stage with leaf rust pathotypes 77-5, 77-6 and 77-7 greatly vary (0; 1n to x =) (V.C. Sinha, Personal communication) in different genetic backgrounds. In the adult plant stage, the resistance of *Lr37* was of high magnitude in HD 2285, Kalyansona, NI 5439 and WH 147 backgrounds, while the same level of resistance could not be combined in the backcross derivatives of HD 2329, Lok-1 and Sonalika and it was observed that effectiveness of this gene is highly influenced by temperature and showed susceptible reaction at > 25^{o}C day temperature. More over the linked yellow rust resistance gene *Yr17* showed susceptible reaction in certain genetic back-ground particularly in Kalyansona back-ground. The lines carrying *Lr37* seem to be associated with any enhanced susceptibility to Nilgiris flora of *Erysiphe graminis tritici* (Menon and Tomar, 2001) (Score 4) as compared to the recurrent parents (Score 3). Enhanced susceptibility to powdery mildew is also evident on the donor line compared to Thatcher and its derivatives. These linked genes have been used in Australian wheat cultivars, Sunbird, Sun state and Trident (McIntosh *et al.*, 1995). *Lr37* is usually expressed as an adult plant resistance gene.

Lr39

A leaf rust resistance gene designated *Lr39* was transferred from *Azeglio's Tauscher* accession TA 1675 to the wheat cultivar Wichita and released as the wheat germplasm KS86WGRC02 (Gill *et al.,* 1988). The gene had been located on the short arm of chromosome 2D by geocentric analysis and the seedlings showed infection type of 0 to ;.

The genetic mapping of leaf rust resistance in KS89WGRC10 on chromosome 2DS, along with similar race specificity observed for *Lr39* and *Lr41* suggests that these two genes are the same. This is also supported by results of our alleles studies with line WX93D246R-1 that also has *Lr39*. To date, *Lr39* has been transferred to wheat from at least four accessions of *Age. Tauscher* of diverse geographic origin as well as an accession of *Ae. cylindrical*. The closely linked wheat microsatellite marker *Xgdm35* should

be useful for marker-assisted selection for *Lr39* (*Lr41*).

At Wellington, the donor KS92WGRC15 is being used and it is conferring high degree of resistance with infection of 0 to ; in the seedling. Introgression of this effective gene is in progress at Wellington and authours successfully transferred this gene into commercial Indian cultivar HD 2285 (HW 3904) and it is being evaluated under the common varietal trial(CVT) of IARI.

Lr44

Triticum spelta- derived gene *Lr44* located on the 1BL transferred to RL 6147(EC 381202) exhibited moderate resistance to leaf rust at Wellington with the field reaction of 20S, 20S, F and 2 for stem, leaf, stripe and Pm respectively. This is being exploited at Wellington to impart yellow rust resistance by pyramiding with other leaf and stem rust genes.

Lr45

Mukade *et al.,* (1970) using X-rays transferred leaf rust resistance from *Secale cereale* cv. Petkus to wheat. The leaf rust resistance gene designated as *Lr45* has been located on the wheat-rye translocation chromosome T2AS-2R#3S. 2R#3L (Friebe *et al.*, 1996). Over last nine years of evaluation against leaf rust at Wellington indicated that the gene is effective against prevailing leaf rust pathotypes in the Nilgiris. The gene conferred high degree of leaf rust resistance against all the prevailing leaf rust pathotypes. The authors have successfully transferred this gene into 30 commercial bread wheat cultivars of India and developed mapping populations and from these materials Bhojaraja (2012) mapped the *Secale cereal*e - derived gene *Lr45* on 2A and developed PCR based co-dominant markers, for the first time. The tightly linked phenotypic marker *pink colour of the awns and margin of glumes* which get well expressed during dough stage under cooler weather conditions was well exploited by authors initially to transfer this gene in the absence of genetic marker and developed back-cross derivatives HW 4501 (C306), HW 4502 (GW273), HW 4503 (HD 2189), HW 4504 (HD2285), HW 4505 (HD 2329), HW 4506 (HD 2402), HW 4507 (HD2687), HW 4508 (HD 2733), HW 4509 (HD 2877), HW 4510 (HI 977), HW 4511 (HI 1077), HW 4512 (HP 1205), HW 4513 (HS 240), HW4514 (HUW 234), HW 4515 (J 24), HW 4516 (Kalyansona), HW 4517 (Lalbahadur), HW 4518 (Lok-1), HW 4519(MACS 2496), HW 4520 (NI 5439), HW4521 (NIAW 34), HW 4522 (PBN 51), HW4523 (PBW 226), HW4524 (PBW 343), HW4525 (PBW 502), HW 4526 (Raj 3077), HW 4527 (UP 2338), HW 4528 (UP 2425), HW 4529 (WH 147) and HW 4530 (WH 542) which carry *Lr45*. Further these lines were pyramided with *Triticum timopheevii*-derived stem rust resistance gene *Sr36/Pm6* to develop wheat lines conferring resistance to leaf, stem rusts and powdery mildew, another interesting observations were, in all the *Lr45* introgressed lines the ear length increased with increased grain weight and lower most spike sterility observed in some recurrent parent restored as fertile ones.

Lr46

Leaf rust resistance gene *Lr46* is a slow rusting gene. This gene do not provide the host plant with complete immunity against a set of leaf rust (*Puccinia triticina*) races; instead they can delay the infection process or reduce the development of symptoms caused by a wider range of leaf rust races on adult plants.

Singh *et al.,* first described *Lr46* in 1998 in cultivar Pavon 76, and located on chromosome 1B. Martinez *et al.,* (2001) showed that the latency period of infected adult plants was significantly lower in plants carrying *Lr46* compared to the controls without the gene. *Lr46* was also responsible for an increase in the fraction of early aborted fungal colonies. The type of resistance conferred by *Lr46* is similar to that of *Lr34*, although with a smaller effect.

William *et al.,* (2003) found that *Lr46* was tightly linked or pleiotropic to a stripe rust resistance gene designated *Yr29.* The tight linkage of a slow rusting gene to a stripe rust resistance gene was also found for the pair *Lr34/Yr18*. The authors effectively used this gene complex in gene pyramiding in developing durable rust resistance wheat varieties in select Indian commercial wheat cultivars HD 2733, UP 2338, PBW 343 and HD 2687 at Wellington

Lr47

The leaf rust resistance gene *Lr47* confers resistance to a wide spectrum of leaf rust strains at Wellington. This gene was transferred from chromosome 7S of *Triticum speltoides* to chromosome 7A of *Triticum aestivum*. Pavon 76 a donor carrying this gene is used for transferring this useful gene and the marker *Xabc465* is effectively used in the MAB programme.

Table-4: Lr47 introgressed lines at Wellington

Back-crossed line/Recurrent parent	Name assinged	Gene complex it carries
C306*2//Pavon 7S 3	HW 4701	Lr 47
C 306		
GW 273*2//Pavon 7S 3	HW 4702	Lr 47
GW 273		
HD 2189*2//Pavon 7S 3	HW 4703	Lr 47
HD 2189		
HD 2285*2//Pavon 7S 3	HW 4704	Lr 47
HD 2285		
HD 2329*2//Pavon 7S 3	HW 4705	Lr 47
HD 2329		
HD 2402 *2//Pavon 7S 3	HW 4706	Lr 47
HD 2402		
HD 2687*2//Pavon 7S 3	HW 4707	Sr 31 Lr 26 Yr 9 Pm 8 + Lr 47
HD 2687		Sr31 Lr26 Yr9 Pm8
HD 2733*2//Pavon 7S 3	HW 4708	Lr 47
HD 2733		
HD 2877*2//Pavon 7S 3	HW 4709	Lr 47
HD 2877		
HI 977*2//Pavon 7S 3	HW 4710	Lr 47
HI 977		
HI 1077*2//Pavon 7S 3	HW 4711	Lr 47
HI 1077		
HP 1205*2//RL 6144 // Cook*6/C 80-1	HW 4712	Lr 47
HP 1205		
HS 240*2//Pavon 7S 3	HW 4713	Sr 31 Lr 26 Yr 9 Pm 8 + Lr 47
HS 240		Sr31 Lr26 Yr9 Pm8

HUW 234*2//Pavon 7S 3	HW 4714	Lr 47
HUW 234		
J 24*2//Pavon 7S 3	HW 4715	Lr 47
J24		
Kalyasona*2//Pavon 7S 3	HW 4716	Lr 47
Kalyansona		
LalBahadur*2//Pavon 7S 3	HW 4717	Lr 47
LalBahadur		
Lok 1*2//Pavon 7S 3	HW 4718	Lr 47
Lok-1		
MACS 2496*2//Pavon 7S 3	HW 4719	Lr 47
MACS 2496		
NI 5439*2//Pavon 7S 3	HW 4720	Lr 47
NI 5439		
NIAW 34*2//Pavon 7S 3	HW 4721	Lr 47
NIAW 34		
PBN 51*2//Pavon 7S 3	HW 4722	Lr 47
PBN 51		
PBW 226*2//Pavon 7S 3	HW 4723	Lr 47
PBW 226		
PBW 343*2//Pavon 7S 3	HW 4724	Lr 47
PBW 343		
PBW 502*2//Pavon 7S 3	HW 4725	Lr 47
PBW 502		
Raj 3077*2//Pavon 7S 3	HW 4726	Lr 47
Raj 3077		
Raj 3077*2//Pavon 7S 3	HW 4726 A	Lr 47
Raj 3077		
UP 2338*2//Pavon 7S 3	HW 4727	Lr 47
UP2338		
UP 2425*2//Pavon 7S 3	HW 4728	Lr 47
UP 2425		
WH 147*2//Pavon 7S 3	HW 4729	Lr 47
WH 147		
WH 542*2//Pavon 7S 3	HW 4730	Sr 31 Lr 26 Yr 9 Pm 8 + Lr 47
WH 542		Sr31 Lr26 Yr9 Pm8
Yr 10*2//Pavon 7S 3	HW 4731	Lr 47
Yr 10		

Lr53

The rust resistance genes *Lr53* and *Yr35*, transferred to common wheat from *Triticum dicoccoides,* were reported previously to be completely linked on chromosome 6B. The Australian line AUS 91388 carrying *Lr53/Yr35*was used at Wellington to transfer this gene. The gene showed infection type 0 to 1 in seedling and MS response in the adult plant stage. However the authors observed fast rusting for stem rust in the donor and the derived lines. Table

Lr57

The wheat *(Triticum aestivum L.)-Aegilops geniculata* introgression T5DL.5DS-5MgS (0.95), with stripe rust resistance gene *Yr40* and leaf rust resistance gene *Lr57*, is an effective source of resistance against most isolates of the rust pathogen in Kansas and India.

The wheat variety WL 711 carrying *Lr57* developed at PAU, Ludhiana was used for transferring the gene *Lr57* into commercial bread wheat cultivars of India.

The gene *Lr57* if effective against prevalent pathotypes of leaf rust in India can be well exploited.

Lr67

The **Lr67** gene for adult plant resistance (APR) to leaf rust was identified in the common wheat accession PI250413 (Dyck and Samborski, 1979) and transferred into Thatcher to produce the backcross line RL6077 (Thatcher*6/PI250413). **Lr67** is phenotypically similar to **Lr34** because it could also be associated with resistance to stem rust (Dyck *et al.,* 1994) and stripe rust (Singh,1992), although **Lr67** confers a lower level of leaf rust resistance than that induced by **Lr34** (Hiebert *et al.,* 2010) and they observed leaf tip necrosis, which is associated with **Lr34** and **Lr46**, was also recorded in segregants carrying **Lr67.** Combinations of **Lr34**, **Lr46,** and **Lr67** represent an attractive option to breeders for durable multi-pathogen resistance to leaf rust, stripe rust, stem rust, and powdery mildew. Comparison of RL6077 with Thatcher and RL6106 (Thatcher + *Lr34*) over 4 years of field testing showed that both **Lr34** and **Lr67** conditioned improved resistance compared with Thatcher, with **Lr34** conferring a higher level of resistance compared with **Lr67**. Also, field trials showed no significant effect of the APR gene on average yield and other agronomic traits, such as height, maturity, lodging and kernel weight. Similarly, end-use quality traits (whole wheat, particle size and SDS sedimentation) did not differ significantly for both lines. This gene is being exploited by pyramiding with other APR genes *Lr34* and *Lr46* for developing durable rust wheat varieties in select commercial bread wheat cultivars at Wellington.

Interestingly many released Indian wheat cultivars carried the APR genes *Lr34+, Lr46+ and Lr67*+ gene complexes. Our observations at Wellington (India) are that the linked APR genes *Lr46/Yr29* present in Pavon76 and Diamond Bird produced 10MR-20MS and 10S reactions at adult plant stage to leaf and stripe rusts, respectively. Similarly, RL6077 carrying *Lr67/Yr46* exhibited 30 MRMS reaction to leaf rust and 20S to stripe rust. The genotype Parula carrying *Lr34, Lr46* and *Lr68* showed 20 MR to leaf, 20S to stripe and 40MSS to stem rust against prevailing pathotypes in the Nilgiris. Sivasamy *et al*. (2013) identified 36 Indian germplasm lines of which five carried *Lr46*, another five possessed *Lr67* and seven lines carried the combination of *Lr34* and *Lr67*. The genes *Lr46* and *Lr67* have not yet been postulated among Indian cultivars. Many breeding programs are using these genes in developing cultivars with slow rusting resistance for all three rusts.

Lr68

Lr68 is an adult plant resistance (APR) conferring slow rusting resistance to wheat leaf rust caused by **Puccinia triticina**. This gene, formerly designated **LrP**, was first described in CIMMYT's spring bread wheat Parula (FKN/3/2*Frontana//KENYA 350 AD.9C.2/Gabo 55/4/Bluebird/Chanate). Parula is a line developed at CIMMYT in 1981 that already had *Lr34* and *Lr46* (William *et al.,* 1997, 2007, Herrera-Foessel *et al.,* 2009). The likely origin of **Lr68** is the Brazilian cultivar Frontana (Herrera-Foessel *et al.,* 2012). Lillemo *et al.,* (2011) tested the effect of **Lr68**, **Lr34** and **Lr46** on leaf rust in an F_6 recombinant inbred line population derived from a Avocet-*YrA* x Parula cross in nine field environments in Mexico, Brazil, Argentina, Uruguay and Chile. The authors were able to confirm additive effects of **Lr68** with the other two slow rusting genes at each site, and also showed that in sites in Argentina and Uruguay, **Lr68** showed a stronger effect than **Lr34**.

Herrera-Fossel *et al.,* (2012) showed in field tests in Mexico that **Lr68**-carrying lines had lower severities to leaf

rust infection with ***P. triticina*** races MCJ/SP and MBJ/SP than the susceptible checks. For most of the tests, the effect of **Lr68** was smaller than those of **Lr34**, **Lr46** and ***Lr67*****,** and the combined effect of **Lr34**, **Lr46** and **Lr68** in Parula resulted in near immunity. The line Parula carrying these gene complexes are currently used at Wellington to develop durable rust resistant wheat varieties which are in advance stage of constituting. In the MAB programme at Wellington STS marker, csLV34, *Xgwm295* for *Lr34*, *Xwmc44* for *Lr46*, *Xcfd71-4D* and *Xcfd23-4D* for *Lr67* were used to pyramid the genes.

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In vitro pollen germination protocol for triticale pollen (X Triticosecale Wittmack)

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An *in vitro* pollen germination medium(PGM) for Triticale has been reported for the first time. Triticale pollen is very difficult to germinate on artificial medium. Pollen viability and pollen vigor of triticale lines may be tested within 10-15 minutes using this protocol. PGM used for triticale consisted of 19% maltose, 15% poly ethylene glycol 4000, 60 mgl^{-1} boric acid, 20 mgl^{-1} calcium nitrate, 80 mgl^{-1} potassium nitrate , 160 mgl^{-1} magnesium sulphate and 1% agar. This PGM may be altered slightly to suit to other genotypes. This complex medium contains many inorganic salts but in low concentration. During the flowering period, a pollen sterility of less than 10% was recorded. Triticale pollen was viable more than 1.45h which is much longer time as compared to wheat. This tool would be useful in triticale breeding especially in wide hybridization

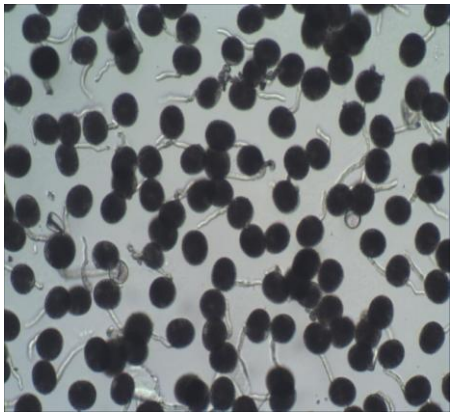

Figure: Photomicrograph showing triticale pollen germination in PGM consists of 19% maltose+ 15% PEG 6000 +, 60mgl^{-1} boric acid+ 20 mgl^{-1} calcium nitrate, 80 mgl^{-1} potassium nitrate + 160 mgl^{-1} magnesium sulphate + 1% agar 500 mgl^{-1}

Stacking Effective All Stage Resistance(ASR) and Adult Plant Resistance(APR) Rust Genes in Wheat Cultivars for Multiple Disease Resistance

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Abstract

Rusts continue to be the major diseases in wheat causing significant yield losses. Host resistance is the most economical and sustainable approach to manage rust diseases. In this study, an effective alien leaf rust resistance gene *Lr45*-derived from *Secale cereale L.* and linked stem rust and powdery mildew resistance gene *Sr36/Pm6*-derived from *Triticum timopheevii* were successfully pyramided and validated into well adapted Indian wheat cultivars which were already carrying APR stem rust gene *Sr2/Lr27/Yr30*, through marker assisted backcross selection (MABC). The recurrent parents were crossed with donor wheat lines RL 6144(*Lr45*) and Cook (*Sr36*) simultaneously. Three efficient microsatellite markers linked with these genes $G372_{185}$(*Lr45)*, *Stm773-2(Sr36)* and *Xgwm533(Sr2)* were used to confirm the introgression of the genes. The constituted lines carrying pyramided genes in each background with better agronomic traits of their recurrent parents can be used as resistance stocks for wheat improvement programs in the development of new wheat cultivars.

Keywords: *T. aestivum*, *Puccinia,* Pink awns, PBC, MABC

Introduction

Wheat (*Triticum aestivum* L.) is the most widely grown crop across the globe and production wise stands second to maize and the world wheat production has exceeded 700 million tons in the past few years(World Agri production 2022). India is the second largest producer of wheat in the world contributing about 16% to the total wheat production (Singh et al. 2017). The annual wheat production during 2020-21 crop seasons is recorded to be 109.52 million tons (Statista 2021). However, sustaining the wheat production is crucial in a country like India, where the domestic demand for wheat production is expected to exceed 140 million tons by 2050 (Nagarajan 2005).

In India, the major constraints to wheat production are environmental stresses and biotic stresses. Among the biotic stresses, rusts (leaf, stem and stripe rust) are the major concern which causes significant yield losses. Of the three rusts, leaf or brown rust caused by *Puccinia triticina* Eriks is one of the most disruptive diseases affecting wheat crop worldwide due to its widespread occurrence (Bhardwaj et al. 2021). In India, various leaf rust pathotypes are prevalent in all the wheat growing zones (Bhardwaj et al.

2019). Under intense epidemic conditions, leaf rust can cause up to 65% yield losses (Chhuneja et al. 2011). Stem rust caused by *Puccinia graminis* f.sp.*tritici* is another major constraint to the global wheat production. In India, the Central and Peninsular zones under wheat cultivation are vulnerable to stem rust (Mallick et al. 2022). Losses due to stem rust epidemics have ranged between 10 - 50% in the past years (Roelfs et al. 1992). But the recent emergence and spread of Ug99 race and its variants has reported to cause heavy damage upto100% (Beard et al. 2006). Stripe rust, commonly referred as yellow rust is caused by *Puccinia striiformis*West. f. sp.*tritici* Eriks. E. Henn. It is a destructive disease that can also cause 100% yield losses (Chen 2005). Stripe rust is mostlyfavored by cool and moist weather conditions (Chen et al. 2014) causing a serious problem in cooler parts of Northern India (Kaur et al. 2020).

Besides rusts, powdery mildew (PM) caused by *Blumeria graminis* f. sp. *tritici* (Bgt) is an emerging disease causing considerable yield. The estimated yield loss of PM is up to 35% (Sharma et al. 1996). In India, North Western Plain zone, Northern Hill zone and Southern Hill zone are more prone to PM (Singh et al. 2009). Recently, the frequency of PM incidence has increased in several wheat-growing regions of the world, including India (Vikas et al. 2020).

In wheat, based on the phenotypic rust expression and intensity at different growth stages, resistance can be broadly classified as all - stage resistance (ASR) and adult plant resistance (APR) (Bowei et al. 2022). ASR is governed by single major gene and offers race specific resistance (Rosewarne et al. 2013). The effectiveness of such gene can be detected in the early plant growth stages (Wu et al. 2020) and hence this type of resistance is also known as seedling resistance. ASR often fails within few years of deployment due to emergence of virulent variants (Chen 2005). Conversely, APR is effective either at post- seedling or adult plant stage. Generally the disease progress with slow latent period (slow rusting) and is race non-specific. This type of resistance is governed by minor genes having additive effects (Singh et al. 2000) and few also have pleiotropic effect in conferring resistance to multiple diseases (Singh et al. 1998; Herrera-Foessel et al. 2014).

Developing disease resistant varieties by stacking effective genes is one of the most effective and eco-friendly methods to combat the rusts and PM (Singh et al. 2020). As the pathogen keeps evolving, stacking resistance genes is being pursued by the wheat breeders to extend the resistance offered by the genes.

Currently, 80 leaf rust resistant genes, 60 genes for stem rust resistance and 70 genes for stripe rust resistance have been formally catalogued in wheat (McIntosh et al. 2017). In case of PM, more than 100 formally designated powdery mildew (*Pm*) resistance genes have been identified (Wu et al. 2021). Many of the identified resistant genes offer ASR and are effective throughout the crop growth stages of wheat. However the number of APR genes so far identified and reported is very limited.Stackingor pyramiding two or more effective ASR and APR genes expected to extend the durability and would prevent or delay the breakdown of resistance genes (Vikas et al. 2021).

Wild relatives of wheat offer a huge number of gene pool for rust resistance (Narang et al. 2019). More number of ASR genes from alien sources has been transferred into wheat (Friebe et al. 1996) but not many are utilized in the breeding programs. *Triticum* and *Aegilops* species are the main gene pools of domesticated wheat (Pour-Aboughadareh et al. 2021). Additionally, Rye (*Secale cereale L.*) belonging to the tertiary gene pool is one of the important donors of disease resistance to wheat (Naik et al. 2015). The 1BL.1RS wheat – rye translocation carrying the resistance genes

Sr31/Lr26/Yr9/Pm8 from Petkus rye has contributed significantly to world wheat production. This translocation is no longer effective and occurrences of virulent pathotypes for *Sr31/Lr26/Yr9/Pm8* are reported across the globe in many continents. However stem rust resistance gene *Sr31+* is still continued to be effective in the Indian sub-continent (Prasad et al. 2022).

Similar translocation T2AS-2RS.2RL from Petkus rye has also not gained much importance. This translocation was postulated to carry a new leaf rust resistance gene and was designated as *Lr45* (Naik et al. 2015). A distinct morphological trait the 'pink awns or glumes' is tightly linked to this gene (Sivasamy et al. 2010) that appears during the early flowering period (anthesis) and then gradually disappears as the maturity progresses and it was observed that its expression differs across varied environments. This phenotypical trait is an added advantage for the visual screening of lines carrying *Lr45* gene and cost effective. Thus far, the ASR gene *Lr45* is reported to be effective both in the seedling and adult plant stage against the occurring leaf rust pathotypes worldwide (Zhang et al. 2006). However, the effective leaf rust resistance gene *Lr45* has been less deployed in the wheat breeding programs.

Triticum timopheevii (secondary gene pool) derived stem rust resistance gene *Sr36* is a widely deployed ASR gene. The stem rust gene *Sr36* was originally transferred to wheat chromosome 2B (Gyarfas 1978; McIntosh and Luig 1973). *Sr36* has been commercially deployed in several wheat breeding program worldwide (Sai Prasad et al. 2014). However, the TTKS virulence (Ug99) to *Sr31* was later followed by virulence to *Sr36* with a variant TTTSK (Jin et al. 2009). Hitherto it is effective to all the prevalent stem rust races including the lineages of the Ug99 except TTTSK (Chemayek et al. 2017). *Sr36* is also reported to be tightly linked to effective powdery mildew resistance gene *Pm6* (Jorgensen and Jensen 1973). In India, the linked gene *Sr36/Pm6* confers effective resistance to stem and powdery mildew (Sivasamy et al. 2017).

Stem rust APR gene *Sr2*, provides broad – spectrum protection against stem rust. It is located on the short arm of wheat chromosome 3B (Hare and McIntosh 1979) introgressed from Yaroslav (*Triticum turgidum* var. *dicoccum*) by McFadden (1930). Additionally, *Sr2* is reported to be linked/pleiotropic to leaf rust resistance gene *Lr27* and stripe rust resistance gene *Yr30*. Hare and McIntosh (1979) identified a close association between *Sr2* and pseudo-black chaff (PBC) - a dark pigmentation around the stem internodes and glumes post anthesis (Kuspira and Unrau 1958). PBC as a phenotypical marker facilitates the selection of *Sr2* introgressed lines in breeding programs but its expression differ across varied environment and genotypes (Singh et al. 2008).

Developing wheat cultivars stacked with multiple resistance genes through conventional breeding method is time consuming. Availability of specific linked microsatellite markers to the resistance genes makes the success of the wheat breeding program easy and swift. Marker assisted backcross (MABC) breeding is a recent approach to transfer multiple genes for resistance effectively. The recurrent parents chosen in this study were released during the post green revolution period. These varieties had best adaptability to the changing environmental scenario and easy combining ability. Advancement of new virulent races renders these varieties susceptible. Thus combining ASR and APR genes in such cultivars would lead to the development of resistant stocks with better adaptability. With this objective, stacking multiple resistance genes for rusts and PM in adapted wheat cultivars was initiated. The present study reports the pyramiding of ASR leaf rust gene *Lr45* and stem rust gene *Sr36/Pm6* with APR stem

rust gene *Sr2/Lr27/Yr30* through MABC in the background of eight well adapted Indian bread wheat varieties.

Materials and Methods

Plant material &breeding scheme

Secale cereale L. derived leaf rust resistance gene *Lr45* was introgressed from a near-isogenic line (NIL) of Thatcher (Thatcher*7/ST-1 = RL6144) (Naik et al. 2015) in the back ground of eight well adapted Indian bread wheat varieties viz., HD 2329, HD2402, LOK-1, MACS 2496, NIAW34, RAJ 3077, PBW 343 and PBW 502 which were already carrying stem rust APR gene *Sr2/Lr27/Yr30/Pbc*, but susceptible to rusts and PM diseases. Similarly, an Australian line Cook (Cook*6/C80-1) (Chemayek et al. 2017), reported to carry *Triticum timopheevi* derived gene *Sr36/Pm6* was used as a donor to transfer *Sr36* gene into the same background. These genes were introgressed into the wheat varieties by adapting backcross breeding approach assisted by linked molecular markers at ICAR-Indian Agricultural Research Institute (IARI), Regional Station, Wellington, The Nilgiris through two parallel backcrossing schemes. Proper agronomical practices were followed for raising the crop in each generation.

The crosses were initiated during Rabi, 2016/17 and F1s carrying both the genes individually were further inter-crossed in order to pyramid both the genes *Lr45* and *Sr36*. The resulting F1s were then backcrossed with respective recurrent parents up to three generations to raise BC3F1 populations to recover the genome.The backcross progeny with the target genes were selected based on phenotype and MABC in each generation. From BC3 onwards the marker selected homozygous plants were selfed for six generations to obtain homozygosity of the alleles received from the donor. Scheme followed for the pyramiding of genes is given in Figure1. Stable pyramided lines constituted at BC3F6 in the background of HD 2329, HD2402, LOK-1, MACS 2496, NIAW34, RAJ 3077, PBW 343 and PBW 502 were christened as HW 3641, HW 3642, HW 3654, HW 3655, HW 3657, HW 3662, HW 3660 and HW 3661 respectively

Marker assisted backcross selection breeding

Molecular markers

Previously validated gene specific microsatellite markers viz.$G372_{185}$ linked to *Lr45*, *Stm773-2* linked to *Sr36* and *Xgwm533* linked to *Sr2* were used for the foreground selection of the genes in the study. The details of the markers used in the present study are given in Table1.

DNA extraction and PCR amplification

DNA was extracted from 14-20 days old seedlings following modified CTAB method (Doyle and Doyle, 1990). The isolated DNA was quantified on 0.8% agarose gel. The DNA was diluted in Millique water to adjust the final concentration of the DNA to 25ng/µl.

PCR reactions was carried out in 20µl reaction containing 25-50ng of template DNA, 0.2µM of each forward and reverse primer, Dream Taq Hot Start Green PCR mix (Thermo Fischer Scientific) and nuclease free water. The PCR reaction was carried out in an Applied Biosystem thermocycler (Veriti) with the following thermal profile: initial denaturation step of 94 ºC for 4 min, followedby 35 cycles of 94 ºC for 1 min (denaturation),60 ºC ($G372_{185}$) and55ºC (*Stm773-2*)for 1 min (primer annealing) and 72 ºC for1 min (primer extension), with a final extension of72 ºC for 10 min. For *Xgwm533*: initial denaturation at 95 ºC for 3 min, followedby 35 cycles of 94 ºC for 30 sec (denaturation), 60 ºCfor 30 sec (primer annealing) and 72 ºC for 45 sec (primer extension), with a final extension of72 ºC for 10 min.The PCR products were resolved in 3% agarose gel and visualized under the gel documentation unit (Syngene, Gene Genius bio imaging system, UK).

Morphological selection of the backcross progenies based on visual traits

As mentioned earlier, the leaf rust resistance gene *Lr45* and stem rust APR gene *Sr2* are tightly linked to the phenotypical markers viz., pink awns or glume and PBC respectively that was used for the visual screening of the lines introgressed with these genes pre(*Lr45*) and post(*Sr2*+) anthesis. Likewise, stem rust resistance gene *Sr36* is tightly linked to powdery mildew resistance gene *Pm6*. As powdery mildew appears early during crop growth season, powdery mildew resistant plants are obliquely confirmed to have acquired the stem rust resistance gene *Sr36*. The phenotypic selection based on linked visual traits was further confirmed simultaneously through MABC for the presence of the pyramided genes since its expressions are environmental or temperature sensitive

Phenotypic evaluation of the backcross progenies for leaf and stem rusts

In addition to the morphological markers, phenotypic selection was carried out in each generation to select resistant plants for leaf, stem rusts and powdery mildew under natural epiphytotic conditions. As Wellington, being a natural 'hotspot' for rusts and powdery mildew allows natural selection of resistant lines. Gene pyramided plants were space planted in one meter row of five lines each with the line spacing of 23cm between the rows. Infector or spreader rows containing a mixture of highly susceptible lines were sown around the population and also in the interval of every 20 rows. Additionally the rust spores were sprayed at regular intervals of 15 days as a suspension in water with a drop of Tween 20 (0.75µl/ml) to ensure early disease appearance and adequate disease pressure.

The field response to leaf and stem rusts were recorded at adult plant stage (=Z80) (Zadoks scale) (Zadoks et al. 1974) forselected plants carrying targeted *Lr* and *Sr* genes as the percent of leaf and stem area covered with uredinio spores according to modified Cobb scale(Peterson et al. 1948) by which severity of rust is recorded on 0–100scale combined with the type of infection response (Loegering 1959, Joshi et al. 1982) and the scoring as follows: R (resistant; necrotic areas with or without uredia present); MR (moderately resistant; small uredia present and surrounded by necrotic areas); MS (moderately susceptible; medium uredia without necrosis but with or without chlorosis) and S (susceptible; large uredia without necrosis and chlorosis).Scores up to 20S is considered resistant. The severity of PM was scoredfollowing a 0-9 scale (Sheng and Duan 1991). The scoring is as follows: 0: free from infection, 1: a few isolated lesions on only the lowest leaves, 3: light infections on the lower third of the plant with the lower most leaves infected at moderate to severe levels, 5: severe infection on lower leaves with light to moderate infection on the middle leaves, 7: severe infections on both lower and middle leaves with some infection on the flag leaf as well, and 9: severe infection on all leaves with spikes infected as well. Scale of 0-3 is considered as resistant.

Stable pyramided lines were also tested in the seedling stage for leaf and stem rust resistance under artificial greenhouse conditions at ICAR-Indian Institute of Wheat and Barley research (IIWBR), Regional Station, Flowerdale, Shimla. The seeds were sown in trays and about 10 days old seedlings were inoculated with six pathotypes of leaf rust viz.,12-5 (29R45), 77-1 (109R63), 77-5 (121R63-1),77-8 (253R31), 77-9 (121R61-1) and 104-2 (21R55) and four predominant pathotypes of stem rust viz., 11(79G31), 40A (62G29), 40-2 (58G13-3) and 117-6 (37G19). Infection types (ITs) on the seedlingswere recorded 14 days post inoculation using Stakman scale (Stakman et al. 1962). The Infection types (ITs) 3,3+ were considered susceptible, whereas lower ITs ('0', '1','2' and 'X') were considered as resistant.

Agronomic Evaluation of the advanced lines

Phenotypically and molecularly confirmed individual plants carrying the combination of the resistance genes *Lr45*, *Sr36* and *Sr2* were selected and bulks of BC3F5/F6 generation were sown along with respective recurrent parents to evaluate the agronomic performance. Ten resistant plants were selected from each background and data on plant height (PH) (cm), number of productive tillers per plants (NPT), spike length (SPL) (cm), grain number per spike (GNS) and thousand grain weight (TGW) (gm) were recorded.

Results

Foreground selection of Lr45, Sr36 and Sr2

Marker assisted backcross selection was performed from the F1 generation onwards. Molecular marker analysis using the linked microsatellite marker $G372_{185}$ to *Lr45* gene revealed a single 185bp allele in the homozygous lines carrying the gene among the backcross population (Figure2). Being a co-dominant marker, it additionally revealed a 127bp allele or a null allele in the lines devoid of the gene and both the alleles in the heterozygous lines.

For stem rust resistance gene *Sr36/Pm6,* the STM (Sequence tagged microsatellite) marker, *Stm773-2*developed a 155bp amplicon in the pyramided lines homozygous for the presence of the gene. While a 195bp allele was amplified in the lines homozygous for the absence of this gene.Both the alleles were amplified in the heterozygous lines (Figure 3).

Microsatellite marker (*Xgwm533*) linked to *Sr2* amplified a 120bp allele in the lines positive for the gene. Being a co-dominant marker, a 150 bp allele or null allele was amplified in the lines without the gene (Figure 4).

The donors, RL6144 (*Lr45*) and Cook (*Sr36*) along with Kingbird (*Sr2*) were used as positive controls to authenticate the molecular marker results. Amplification of 185bpfor*Lr45* ($G372_{185}$), 155bp for *Sr36* (*Stm773-2*) and 120bp for *Sr2* (*Xgwm533*) was visualized in the respective donors/positive control. In both the cases, either a negative allele or a null allele amplified in the recurrent parents except for *Sr2* (*Xgwm533*) as all the recurrent parents earlier carried *Sr2* gene. The marker results are presented in Table 2. The backcrossed lines positive for presence of targeted genes were selected in each generation and were subjected to backcrosses and further forwarded to the succeeding generations.

Phenotypic evaluation and screening of the backcross population

Phenotypic evaluation in the field was carried out from the F1 onwards through BC3F6 stage alongwith the recurrent parents. The advance lines stacked with leaf and stem rust resistance genes *Lr45, Sr36/Pm6* and *Sr2+* were completely resistant with immune response to both leaf and stem rusts. Among the selected recurrent parents the cultivars PBW 343, PBW 502 and MACS 2496 were susceptible only to leaf rust (20S-40S) and resistant to stem rust as they were known to carry the stem rust resistance gene *Sr31+.*Whereas, a field score of 20S-80S were recorded in the other recurrent parents (HD 2329, HD 2402, Lok-1, RAJ 3077 and NIAW 34) showing susceptibility for both stem rust and leaf rusts. Furthermore, the presence of tightly linked PM resistance gene *Pm6* to *Sr36* provided high level of PM resistance in the gene stacked lines with a score of '0-1' and otherwise all the recurrent parents were highly susceptible to PM.

The seedlings of the pyramided lines carrying the rust resistance gene showed a resistant infection response of 0-1 for the tested leaf and stem rust pathotypes. A susceptible infection type, 'IT' 33+ was recorded in the seedlings of the susceptible recurrent parents for both leaf and stem rust races. The rust and PM responses of the pyramided lines, donor

and recurrent parents are given in Table 2. In addition to phenotypic screening, the morphological traits such as pink awns/glume linked to *Lr45* and PBC linked to *Sr2*+ were used for further visual confirmation of the presence of the respective genes (Figure 5).

Agronomic performance of the pyramided lines

Different agronomic traits (PH, NPT, SPL, GNS and TGW) of both the parents and pyramided lines at BC3F6 were assessed during two crop seasons of 2020-21 at Wellington (both winter and summer seasons). Based on the resistance to leaf and stem rust at the phenotypic and molecular level, ten individual plants from each respective background were selected. The individual lines were raised and bulked and were evaluated in comparison with the respective recurrent parentsfor agronomic traits. The mean data on agronomic traits are presented in Table 3. The gene pyramided lines had agronomically superior traits in comparison to their respective recurrent parent. The traits such as PH, NPT, SPL, GNS and TGW were comparable with the respective recurrent parent. In particular, there was a slight increase in spike length with lax ear that significantly increasedthe TGW in all the populations except in the background of NIAW 34. From the data, it was observed that all the gene stacked lines carrying *Lr45, Sr36/Pm6* and *Sr2*showedbetter TGW than that of the recurrent parent.

Discussions

Rusts and PM has caused considerable losses to wheat production worldwide and in India. Enhancing the genetic resistance of adapted or elite wheat cultivars to rusts and PM is highly decisive approach to tackle the rapid momentum of pathogen evolution. The resistance offered by single ASR or major gene has inherent risk of being overcome by the newly evolving rust pathotypes. Pyramiding of multiple effective ASR genes coupled with APR rust genes in the background of adapted cultivars is considered to be an effective strategy to enhance the resistance and its durability (Jin et al. 2022).In the process of stacking resistance genes, the synergistic effect of the stacked genes has been reported to increase the life of each gene (Klymiuk et al. 2018; Mundt 2018).Two important APR genes, *Sr2*+ and *Lr34*+ in combination with other ASR and APR genes have been widely deployed in wheat breeding programs providing long lasting resistance to stem and leaf rusts (Ellis et al. 2014, Huerta-Espino et al. 2020).

Unintentionally, *Sr2+* has been widely deployed in Indian wheat breeding programs in combination with *Sr9*, *Sr11* and *Sr31* since several decades (Malik et al. 2013, Tomar et al. 2014). A number of varieties incorporated with *Sr2* in addition to other resistance genes have been released for central and southern wheat growing zones of India (Prashar et al. 2008). All the recurrent parents used in the present study were carrying *Sr2+* gene (Bhardwaj, 2011). Further the presence of APR gene *Sr2+* was confirmed through phenotypic (PBC) and genetic marker (*Xgwm533*) in the stacked lines. Haile and Roder (2013) recommended the use of APR gene *Sr2/Lr27/Yr30* in breeding programs in combination with other stem rust resistance genes to enhance the resistance to stem rusts. The APR gene *Sr2+* in combination with other resistance genes is effective against Ug99 lineage (Alabushev et al. 2019). Phenotyping the resistance offered by *Sr2* is difficult to score in field conditions as it confers partial resistance (Spielmeyer et al. 2003). Thus, the microsatellite marker, *Xgwm533* linked to *Sr2* accelerated the selection of lines carrying this gene and also the tightly linked phenotypical marker PBC. Several studies have been reported on the genetic association between PBC (phenotypical marker) and *Sr2*. The varying expression of PBC is dependent on the genotype and environment (Singh et al. 2008). Still, PBC can be used as a phenotypical marker for the screening of *Sr2*+ carrying lines. Additionally, the linked/pleiotropic gene

Lr27 and *Yr30* is reported to reduce the level of mean leaf rust severity by 17.2% and stripe rust severity by 7.2% in most of the CIMMYT lines (Malik et al. 2013).

Similarly, one of the recurrent parents, HD 2329 was postulated to carry *Lr34*+ gene that supplemented the resistance offered by *Lr45* in the gene stacked lines in this background. Also, the effectiveness of *Sr31*+ to the Indian stem rust pathotypes in the background of MACS 2496, PBW 343 and PBW 502 complimented the resistance provided by *Sr36/Pm6*and *Sr2*+. The other commonly present resistance genes in the recurrent parents were *Lr13+, Lr10+, Lr23+, Lr1+, Sr8b+, Sr9b+, Sr11+,Yr2+, Yr18+, Yr2KS+* and *Yr27*+ (Table 4). Most of these genes have become in-effective in the past. However, the accumulated residual effect of the defeated genes generally termed as "ghost effect" (Sharma et al. 2021) also added fairly towards resistance when stacked with other effective ASR and APR resistance genes.

Stacking several disease resistant genes in a single varietal background through phenotyping is practically difficult. Molecular markers that are tightly linked with specific resistant genes and their easy access aids in successful marker assisted selection. MABC is one of the most promising approaches for the incorporation and stacking of resistant genes while retaining the essential characteristics of the recurrent parent (Collard et al. 2008).

Several genes from rye (*Secale cereale L.)* have been transferred to wheat either as translocations or substitutions. The earliest and widely used source is 1R from Petkus rye (Crespo-Herrera et al. 2017). The well-known 1BL/1RS translocation carrying the genes, *Sr31+Lr26+Yr9+Pm8* has contributed significantly to the world wheat production and several hundreds of cultivars possessing this gene loci were released during the mid-1990s. Three of the recurrent parents (PBW 343, PBW 502 and MACS 2496) deployed in this study also carried *Sr31+.*

Nevertheless, effective leaf rust resistant gene *Lr45* derived from rye (Mukade et al. 1970) confers effective all stage resistance (ASR) to leaf rust but has not been well utilized. Also the less availability of molecular markers makes the utilization of *Lr45* limited in marker assisted selection (Naik et al. 2015). Earlier, Zhang et al (2006) developed an AFLP (Amplified fragment length polymorphism) marker for the detection of *Lr45* gene and later a SCAR (Sequence characterized amplified region) marker was developed for the same by Fein et al (2009). Of late, Naik et al (2015) reported a highly polymorphic, co-dominant microsatellite marker ($G372_{185}$) that supported the efficient utilization of *Lr45*gene in the wheat improvement program against leaf rusts. Previously, the efficiency and specificity of this marker to *Lr45* was validated in Thatcher based NILs and it was reported that two distinct alleles relating to the presence and absence of this gene was sufficient to identify the lines carrying this gene (Crespo-Herrera et al. 2017).In addition to molecular confirmation, the close linkage between *Lr45* and pink glume or awns is an extra advantage in the phenotyping of this gene.This trait expresses during early anthesis and then gradually disappears as the crop matures. The expression of pink awns/glumes linked to *Lr45* varied according to the environmental conditions and it was observed that the trait was more expressed under lower temperature conditions.

Stem rust resistance gene *Sr36* originally transferred from *T.timopheevii* has been well utilized in the wheat breeding program in the recent years. Though virulence has been reported to this gene (Jin et al. 2009), it is still effective in the Indian sub-continent (Sai Prasad et al. 2014) and it continues to be well exploited in combination with other minor or major genes (Jin et al. 2009).The tight linkage between *Pm6* and *Sr36* effectively

controlled powdery mildew severity in the gene stacked lines. Recently, *Sr36/Pm6* has been effectively pyramided with other stem rust resistance genes *Sr24/Lr24* and *Sr26* (Vikas et al 2021).The marker analysis with tightly linked microsatellite marker *Stm773-2*clearly distinguished between homozygous resistant and homozygous susceptible lines.The co-dominant nature of this marker assisted in easy identification of heterozygotes (Tsilo et al. 2008).

Our results show that leaf and stem rust resistance genes *Lr45, Sr36/Pm6* and *Sr2+*were successfully stacked assisted by linked molecular markers for foreground selection in the background of eight adapted cultivars which provided resistance to both leaf and stem rusts and were furthermore resistant to PM.

The main components contributing to wheat yield are SPL, GNS and TGW (Zheng et al. 2020). The backcross derived lines stacked with *Lr45, Sr36* and *Sr2* had agronomically superior traits such as increased NPT, SPLand TGW in comparison to the recurrent parents. The pyramided lines in all the background had increased TGW in comparison to their recurrent parents except in the background of NIAW 34. In certain backgrounds viz., Raj 3077, PBW 343 and PBW 502, there was an increase in GNS and SPL. Normally in the wheat varieties, PBW 343 and PBW 502, the lower most 2-3 spikelet remain sterile depending on environmental conditions. It was observed that the fertility of the lower most spikelets were also recovered after the introgression of *Lr45* gene and which increased the GNS. Generally, 'bucketing effect' of the growth habit combined with lax ear in the most derived lines carrying *Lr45* was observed. However rigorous selection in large segregating population benefitted in an ideal and desirable ideotype devoid of 'Bucketing'. The lax and increased spike length (SPL) of the ear head could have been possibly contributed to increase in TGW. The homeologous pairing between wheat and rye has allowed the introduction of desirable agronomic traits in wheat from rye together with resistance to rusts as was witnessed in the 1B/1RL translocation (Zhou et al. 2007). Similarly, the 2R translocation carrying *Lr45* gene enhanced the agronomic performance of the derived lines.The QTLs linked to these trait needs to be evaluated further. The MABC derived lines were resistant to leaf and stem rusts and in addition were also resistant to PM due to the presence of *Pm6* gene that inherits together with *Sr36*.Resistance to PM also suppressed the severity of leaf blight that too added to the better agronomic performance of these lines.Eventually, grain yield combined with end use quality is the key factor for the success of wheat breeding. Thus these gene stacked lines requires further field and quality testing.

Conclusion

The combination of ASR and APR gene has found to have more significant effects. The developedgene stacked lineswith *Lr45*, *Sr36* and *Sr2* could be a possible source of new resistant variety or could be suggested as potential germplasm resistant to leaf rust, stem rust and PM. The co-dominant markers $G372_{185,}$ *Stm773-2* and *Xgwm533* allows efficient detection of the genes in homozygous state and would serve as an important tool in the rapid transfer of these genes into adapted wheat cultivars.

Conflict of Interest

Authors declare that they have no conflict of interest.

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Table 1: Details of the microsatellite markers linked to the rust resistance genes in wheat

S.No.	Marker	Sequence of primer	Tm (°C)	Linked Gene	Reference
1.	*$G372_{185}$-F*	AATAGAGCCCTGGGACTGGG	60	*Lr45*	Naik et al (2015)
	$G372_{185}$-R	CTCTTGAAACACAAAGCACA			
2.	*stm773-F*	ATGGTTTGTTGTGTTGTGTGTAGG	55	*Sr36/Pm6*	Tsilo et al(2008)
	stm773-R	AAACGCCCCAACCACCTCTCTC			
3.	*Xgwm533-F*	AAGGCGAATCAAACGGAATA	60	*Sr2/Lr27/Yr30*	Spielmeyer et al (2003)
	Xgwm533-R	GTTGCTTTAGGGGAAAAGCC			

Table 2: Disease response in the seedling and adult plant stage and marker analysis data of the donor, recurrent parent and the pyramided lines

S.No.	Parent/ Cross	Seedling response leaf rust pathotypes						Seedling response stem rust pathotype				Adult plant response			Marker Confirmation		
		12-5	77-1	77-5	77-8	77-9	104-2	11	40A	40-2	117-6	Leaf rust	Stem rust	PM	*Lr45* (G372185)	*Sr36/Pm6* (stm773)	*Sr2* (Xgwm533)
1.	HD 2329	;	;-	3+	3+	3+	;1	2=	2C	;-	;-	80S	60S	6	-	-	+
2.	HW 3641	;-	0;	;-	0;	;-	;	0;	0;	;	;-	F	F	0	+	+	+
3.	HD2402	;-	;1	33+	;-	3+	3+	12	12	0;	;-	40S	20S	6	-	-	+
4.	HW 3642	;-	0;	0;	;-	;-	;	0;	;-	0;	;	F	F	0	+	+	+
5.	LOK-1	33+	3+	3+	3+	3+	3+	3+	3+	0;	3+	60S	60S	7	-	-	+
6.	HW 3654	;	0;	;1	;	;-	;1	12	0;	;-	0;	F	F	0	+	+	+
7.	MACS2496	22+	;12	3+	0;	;-	3+	2=	;-	0;	2=	20S	F	6	-	-	+
8.	HW 3655	0;	;-	0;	0;	;-	;-	;-	;-	0;	0;	F	F	0	+	+	+
9.	NIAW 34	23	3+	X+ 3	;-	3+	3+	;-	12	2=	;-	40S	20S	6	-	-	+
10.	HW 3657	;-	;-	0;	;-	;-	;	0;	0;	;-	0;	F	F	0	+	+	+
11.	RAJ 3077	;-	;1	23	;-	3+	33+	;-	;-	;	;-	80S	20S	7	-	-	+
12.	HW 3662	0;	;-	0;	;-	;-	;-	2+	12	0;	;-	F	F	0	+	+	+
13.	PBW 343	;-	3+	3+	0;	3+	3+	2=	0;	;	;	40S	F	7	-	-	+
14.	HW 3660	;-	;-	0;	;-	;-	;	0;	0;	0;	0;	F	F	0	+	+	+
15.	PBW 502	33+	3+	3+	0;	3	3+	;-	;-	;-	;	40S	F	7	-	-	+
16.	HW 3661	;-	;-	0;	0;	;-	;-	;-	2=	0;	;-	F	F	0	+	+	+
17.	RL6144 (*Lr45*)	0;	;-	0;	;	;-	;-	0;	3+	0;	0;	F	60S	4	+	-	+
18.	COOK (*Sr36/Pm6*)	3+	-	0;	3+	3+	3+	;-	2-	1	;-	80S	F	0	-	+	-

*F-Free; S-Susceptible

Table 3: Mean and Standard Deviation (SD) of agronomically significanttraits of the recurrent parents and gene stacked lines

S.No.	Parent/Cross	PH (cm)	NPT	SPL (cm)	GNS	TGW (g)
1.	HD 2329	70 ±2.07	8 ±0.54	9 ±0.25	51 ±3.42	26 ±0.83
2.	HW 3641	**80 ±1.65***	**10 ±2.60***	**9 ±0.13**	**48 ±2.12***	**40 ±0.54***
3.	HD2402	82 ±1.14	11 ±0.54	9 ±0.26	55 ±2.68	34 ±1.14
4.	HW 3642	**84 ±1.51***	**12 ±1.67**	**12 ±0.54**	**56 ±1.64***	**39 ±0.89***
5.	LOK-1	89 ±0.65	7 ±1.09	9 ±0.2	45 ±2.12	32 ±1.22
6.	HW 3654	**75 ±2.75***	**10 ±1.47***	**10 ±1.03***	**51 ±3.99***	**34 ±2.45**
7.	MACS2496	89 ±0.68	8 ±0.89	11.2 ±0.22	65 ±3.87	32 ±0.54
8.	HW 3655	**90 ±1.03***	**9 ±0.83**	**11.2 ±0.56**	**64 ±2.50**	**42 ±0.45****
9.	NIAW 34	87 ±0.83	8 ±0.54	8.5 ±0.08	57 ±2.12	34 ±1.14
10.	HW 3657	**76 ±2.96***	**12 ±1.64***	**9 ±0.08**	**55 ±1.64**	**31 ±1.09****
11.	RAJ 3077	83 ±2.12	9 ±0.89	7.7 ±0.17	45 ±2.12	24 ±1.14
12.	HW 3662	**82 ±1.51**	**14 ±1.30***	**10.2 ±0.79***	**49 ±1.64***	**32 ±0.70***
13.	PBW 343	83 ±2.38	12 ±1.09	8.6 ±0.11	54 ±2.68	28 ±2.07
14.	HW 3660	**80 ±2.02**	**14 ±0.70***	**9.9 ±0.37***	**61 ±2.68***	**30 ±0.54***
15.	PBW 502	74 ±1.14	9 ±0.54	9.8 ±0.20	51 ±2.12	26 ±1.92
16.	HW 3661	**82 ±1.17***	**13 ±2.12***	**10.6 ±0.65***	**56 ±1.64***	**32 ±0.70***

***** Recurrent parent and gene stacked line have significant difference ($P < 0.05$),** Recurrent parent and gene stacked line have extremely significant difference

#PH- Plant height, NPT - Number of productive tillers per plants, SPL- spike length, GNS- Grain number per spike and TGW-Thousand grain weight

Table 4: Resistance genes present in the recurrent arents and gene stacked lines based on multi-pathotype testing and molecular marker confirmation

#Multi-pathotype testing -Bhardwaj (2011)

S.No.	Parent /Cross	Pedigree	Leaf rust genes	Stem rust genes	Yellow rust genes	PM genes
1.	HD 2329	HD1962/E4870/3/K 65/5/HD1553/4/UP262	*13+,10+, 34+,27+*	*2+,8b+, 9b+,11+,57+*	*2+, 18+*	*38+,30+*
2.	**HW 3641**	**HD2329*3/RL 6144//HD 2329*3/Cook**	***45, 27+***	***36+, 2+***	***30+***	***6+***
3.	HD2402	HD2177//CNO67/BB/3/ HD2160/4/HD2236	*Lr23+, 27+*	*2+,8b+, 9b+,11+*	*2KS+*	-
4.	**HW 3642**	**HD2329*3/RL 6144//HD 2329*3/Cook**	***45, 27+***	***36+, 2+***	***30+***	***6+***
5.	LOK-1	S308 / S331	*13+, 27+*	*2+, 9b+, 11+*	*2KS+, 30+*	-
6.	**HW 3654**	**HD 2329*3/RL 6144//HD 2329*3/Cook**	***45, 27+***	***36+, 2+***	***30+***	***6+***
7.	MACS2496	SERI"S"	*27+, 26+, 23+, 1+*	*2+, 31+*	*9+, 30+*	-
8.	**HW 3655**	**HD 2329*3/RL 6144//HD 2329*3/Cook**	***45, 27+***	***36+, 2+***	***30+***	***6+***
9.	NIAW 34	CNO 79/PRL "S"	*13+, 34+, 27+*	*2+,11+, 57+*	*18+, 30+*	*38+*
10.	**HW 3657**	**HD 2329*3/RL 6144//HD 2329*3/Cook**	***45, 27+***	***36+, Sr2+***	***30+***	***6+***
11.	RAJ 307	HD 2267/RAJ 1482/5/BB/INIA66'S'/N APO	*23+, 27+*	*2+*	*2+, 30+*	-
12.	**HW 3662**	**HD 2329*3/RL 6144//HD 2329*3/Cook**	***45, 27+***	***36+, 2+***	***30+***	***6+***
13.	PBW 343	ND/VG9144//KAL/BB/3/ YCO"S" /4/VEE#S "S"	*26+, 27+*	*2+, 5+, 31+*	*9+, 27+, 30+*	
14.	**HW 3660**	**HD 2329*3/RL 6144//HD 2329*3/Cook**	***45, 27+***	***36+, 2+***	***30+***	***6+***
15.	PBW 502	W 485 / PBW 343 // RAJ 1482	*26+, 27+*	*2+, 5+, 31+*	*9+, 30+*	-
16.	**HW 3661**	**HD 2329*3/RL 6144//HD 2329*3/Cook**	***45, 27+***	***36+, 2+***	***30+***	***6+***
17.	RL6144	Tc x *Secale cereale*	*45*	-	-	-
18.	COOK	Timgalen/Condor sib//Condor	-	*36+*	-	*6+*

Figure 1: Scheme of stacking leaf rust resistance gene *Lr45*, stem rust resistance gene *Sr36/Pm6* and stem rust APR gene *Sr2* into elite wheat cultivars through MABC

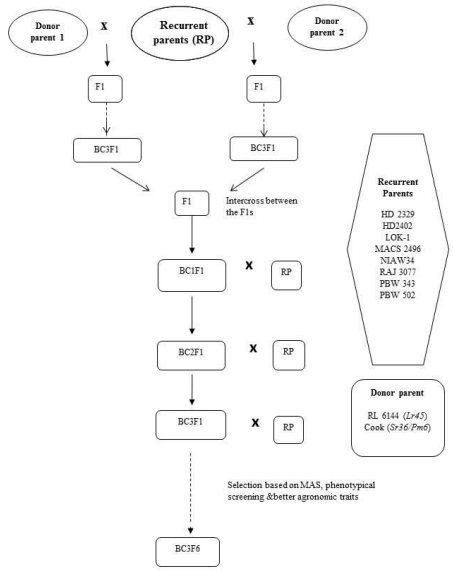
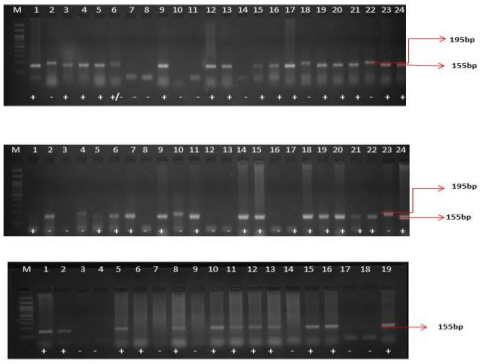


Figure 3 : Molecular confirmation of leaf rust resistance gene *Sr36/Pm6 using* marker *stm773* in donor, recurrent parent and pyramided lines

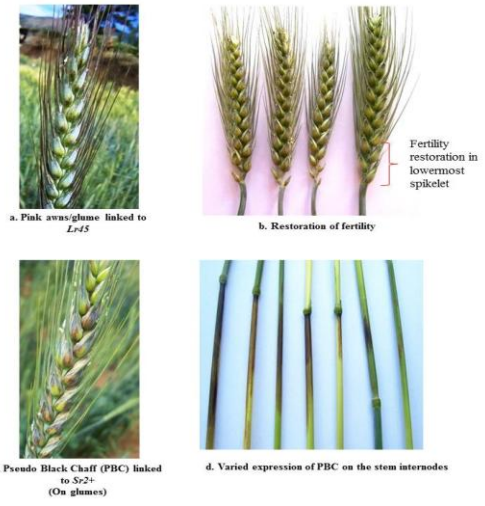


Lane1: M-Marker (100bp), 1-Cook (Positive control), 2- HD 2329, 3-9 : HW 3641, 10: HD 2402, 11-17 : HW 3642, 18 – Lok-1, 19-24 : HW 3654

Lane 2: M-Marker (100bp), 1- MACS 2496, 2-7: HW 3655, 8-NIAW 34, 9-15: HW 3657, 16- Raj 3077, 17-23: HW 3662, 24-Cook (Positive control).

Lane 3: M- Marker (100bp), 1-8 HW 3660, 9- PBW 343, 10- 17: HW 3661, 18- PBW 502, 19- Cook (Positive control)

Figure5 : Morphological traits linked to the rust resistance genes



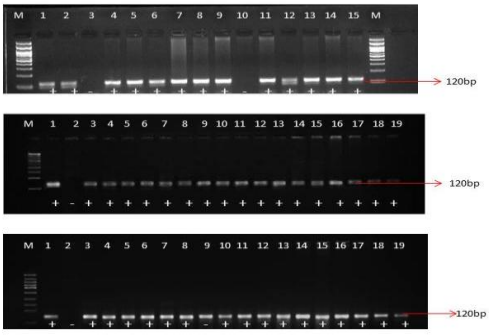
a. Pink awns/glume linked to *Lr45*

b. Restoration of fertility

c. Pseudo Black Chaff (PBC) linked to *Sr2*+ (On glumes)

d. Varied expression of PBC on the stem internodes

Figure 4 : Molecular confirmation of stem rust APR gene *Sr2* using marker *Xgwm533* in the recurrent parents and pyramided lines BC3F4 & BC3F6

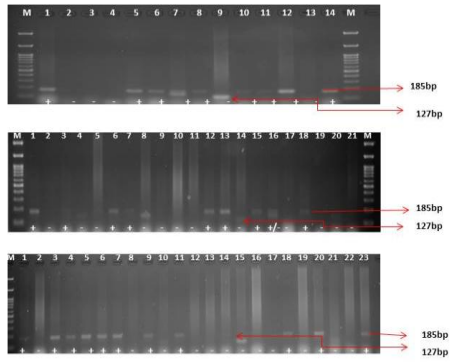


Lane 1: M – Marker, 1-2 Kingbird (Positive control), 3- Agralocal (Negative control), 4- HD2329, 5-8: HW 3641, 9-HD 2402, 10-15: HW 3642.

Lane 2: M-Marker, 1 Kingbird (Positive control), 2- Agralocal (Negative control), 3- LOK-1, 4-8 HW 3654, 9- MACS 2496, 10-13- HW 3655, 14-NIAW 34, 15- 19 – HW 3657.

Lane 3: M- Marker, 1-Kingbird (Positive control), 2- Agralocal (Negative control), 3- Raj 3077, 4-7 HW 3662, *8*- PBW 343, 9-14- HW 3660, 15-PBW 502, 16–19 HW 3661.

Figure 2 : Molecular confirmation of leaf rust resistance gene *Lr45* using marker *G372$_{185}$* in donor, recurrent parent and pyramided lines BC3F4 & BC3F6



Lane 1: M – Marker, 1- RL 6144(Positive control), 2- Agralocal (Negative control), 3- HD2329, 4-8: HW 3641, 9-HD 2402, 10-14: HW 3642

Lane 2: M-Marker, 1- RL 6144(Positive control), 2- Agralocal (Negative control), 3-7 HW 3654, 8-Lok-1, 9-MACS 2496, 10-16: HW 3655, 17-NIAW 34, 18-21: HW 3657

Lane 3: M- Marker, 1- RL 6144(Positive control), 2- Agralocal (Negative control), 3- 7: HW 3662, 8-Raj 3077, 9-14 HW 3660, 15-PBW 343, 16- PBW 502, 17-23 – HW 3661

Diversity in field reactions of rusts in Indian wheat varieties at hot spot location (Wellington)

P. Nallathambi, C. Umamaheswari, Rajesh K Meena, R. Selva Kumar, N. Preeti, and Kiran Gaikwad

The leaf and stem rusts are endemic diseases of wheat in Nilgiri hills. Therefore, large number of *Triticum* species is critically evaluated to find out the reaction pattern and resistant sources. In continuation of our regular activities on recurrent evaluations, a

total of 394 commercially released varieties were evaluated against the prevailing pathotypes of *Puccinia triticina* and *P. graminis* f.sp. *tritici* at our hot spot location (Wellington) under field conditions. Overall results demonstrated a distinct diversity in susceptible and resistant reactions between the varieties and growing seasons. Both leaf and stem reactions were variable among the commercially released varieties. Some of varieties such as DDK 1001, DL 784-3 (VAISHALI), DWR 185, HI 8498 (MALAV SHAKTI),HS 207,HS 295,HS 375 (HIMGIRI),HUW 510,HW 2004 (AMAR), HW 2045 (KAUSHAMBI),PBW 396 and TL 2942 were identified as completely free from both stem and leaf rust infections or field resistant during summer (off-seasons) as well as winter (main) seasons. However, about 16% out of 394 varieties namely Arpa (cg 5011), A 90, A 206, C 306, Chhoti Lerma, CPAN 1796, CPAN 3004, C 281, C 285, C 518, C 286, DWR 16, DBW 17, DBW 16, DWL 5023, DL 788-2, DL 153-2, DL 803-3, DPW 621-50, GW 503, GW 1, GW 190, GW 496, GW 322, GW 273, GABO, WG 357, HPW 42, HPW 184, HS 240, HP 1761, HD 2833, HD 2824, HD 2864, HS 420, HI 8381, HP 1493, HI 385, HB 208, HP 1761, HD 2833, HD 2610, HW 1095, HW 657, HS 524 (40S), JOB 666, TAWA 267 (40S), Type 11, JWS 17, JNK 4W 184, K 0307, KRL 210, NP 884, PDW 215, RAJ 3765, Raj 821, Ridley, UP 2584, UAS 415, VL 832, VL 616, WH 1021, WH 147 and WH 542 which were free from rusts during summer season had expressed susceptible reactions during the main(Rabi) seasons. Results also clearly indicated the influence of the epidemiological factors particularly maximum mean (16.8) and minimum mean (10.2oC) temperatures and leaf wetness (127.4mm) in addition to 80.0% RH have favored for the better perpetuation of inoculums, subsequent micro-cyclic infections, establishment and expression of both rusts severity. These weather parameters required to be congenial in addition to potential inoculums of urediopores. In addition to these test varieties, more number of *Tritucum* lines are being evaluated at our hot spot location (hot spot) to find out the diseases reaction profile over the periods and identify durable resistance types.

First report of *Meloidogyne hapla*infestation on *Parthenium hysterophorus* from India

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Parthenium hysterophorus is one of the noxious invasive weeds in India. It was observed even at an altitude of 1800 MSL in the Nilgiris, Tamil Nadu. During our recent survey (2021)targeting the"*Susceptibility of weed species to Root-knot nematodes (RKN), Meloidogyne spp.*", at ICAR-IARI, Regional Station, Wellington, we came across several Parthenium roots infested with RKN. Due to the economic importance of RKN-parthenium association, a separate systemic survey was initiated to identify the extent of RKN infestation in partheniumat IARI farm (Wellington). The results showed >50% incidence of

RKN infestation in parthenium in certain fields. The female nematodes from the roots were teased out from the roots and were identified as *Meloidogyne hapla* and *M. incognita* through perineal patternstudies. Further, observation of galled roots under the microscope showed the presence of healthy egg massesand juveniles of RKN. Healthy infective juveniles of RKN were observed, when the infested roots (1cm root bits) were placed for extraction using modified Baermann method. Hence for the first time, we are reporting the ability of *Meloidogyne hapla*to infest, establish and reproduce on *Parthenium hysterophorus*. We further reiterate the need for designing a novel and sustainable nematode management strategiesto minimize the establishment of RKN *via* weed hosts.

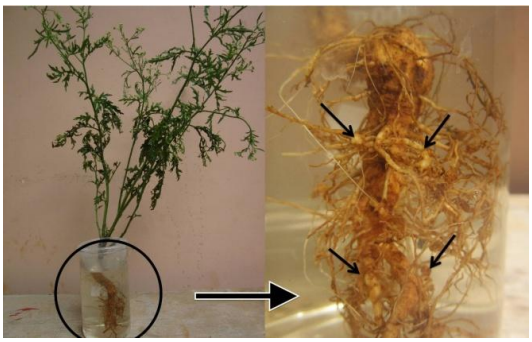

Figure 1: Parthenium root infested with* Meloidogyne *spp. (*M. hapla *and* M. incognita*)