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Development of bread wheat (*Triticum aestivum* L.) lines with specific rust resistance genes and their authentication through molecular markers

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Abstract

High yielding cultivars of wheat *viz*. PBW343, UP2338 and WH542 were used to incorporate multiple rust resistance genes against leaf, stem and stripe rusts from winter wheat or agronomically inferior wheat lines carrying known rust resistance genes. Genetic stocks were evaluated for seedling resistance as well as field resistance with prevalent and virulent pathotypes of *Puccinia triticina*, *Puccinia graminis tritici* and *Puccinia striiformis.* With the help of classical tools resistance genes were fixed in the early generation. Incorporation of resistance gene in the genetic stock *viz. Yr5, Yr10, Yr15, YrSP, Lr9, Lr19, Lr24, Lr26, Lr32, Lr39, Lr28, Lr42, Lr45* was confirmed through allelism tests and presence of leaf rust resistance genes *viz.Lr9, Lr19*, *Lr24* and *Lr26* were also authenticated through molecular markers. Rust resistance and utility of genetic stocks are discussed.

Key words: Wheat, leaf rust, stem rust, stripe rust, rust resistance, molecular markers, gene pyramiding

Introduction

Wheat is grown through out India in various diverse agro ecological zones. The production of wheat in India has crossed 70 million tons in the last decade. This increase in productivity is primarily attributed to the cultivation of new high yielding varieties namely, PBW343, WH542, UP2338, RAJ3765, HUW468, HI1077 and others. The enhanced productivity was realized not only because of their high yield potential but also due to better resistance against major diseases. Rusts are the most important diseases of wheat in India. Leaf rust (*Puccinia triticina* Eriks.) has been reported through out the country, stem rust (*Puccinia graminis* pers. f. sp. *tritici* Eriks. & Henn.) assumes more importance in central and peninsular states and stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici*) pose threat mainly in the north western plains zone as it proliferates in cool and moist climate [1]**.** Cultivation of resistant varieties is the most effective, eco-friendly and economically viable method of combating the rust diseases [2]. In the past, several varieties were bred that were resistant to rusts but these became susceptible in due course of time with the occurrence of new virulence [3]. Most of the present day high yielding varieties are moderate to highly susceptible to either leaf rust or stripe rust or both while there is good level of resistance to stem rust. Incorporation of resistance is a continuous process so as to counter the ever evolving rust pathogens that render previously resistant genes ineffective. Molecular markers can be utilized to facilitate authentic incorporation of specific rust resistance genes. An elaborate pre-breeding programme was initiated at the Directorate of Wheat Research, Regional Station, Flowerdale, Shimla to develop agronomically superior wheat lines with specific rust resistance genes whose utility is well established in the targeted region and subsequently several lines with useful resistance genes have been developed. The present paper describes methods used for their development and their utility in wheat breeding in India.

Material and Methods

Experimental sites

The crossing programme was initiated in 1995-96. The first batch of genetic stocks with specific rust resistance genes were generated in 2001. Genetic stocks were evaluated during 2005-2006. The F_1's, back cross (BC_1),

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F_2 and F_3 generations were screened under controlled conditions. The facility of summer nursery, Indian Agricultural Research Institute, Regional Station, Wellington was utilized for generation advancement where the lines were evaluated for leaf rust, stem rust and agronomic traits. A set of 13 selected genetic stocks (Table 3) were evaluated in three locations at Wellington (natural hot spot for leaf and stem rust), Directorate of Wheat Research, Karnal under artificial epiphytotic for leaf and stripe rust and Dalang Maidan (natural epiphytotic for stripe rust). These lines were also screened in the seedling stage at Directorate of Wheat Research, Regional Station, Flowerdale, Shimla under temperature controlled glass house condition. Agronomic traits were evaluated at DWR, Karnal in randomized block design with three replications. A two row plot of 2 meter length represented each entry. Ten plants were tagged randomly from each row for recording observations on average spike length, average plant height and days to maturity. Test weight (1000-grain weight), yield per meter row and rust disease severity were recorded on whole plot basis.

Plant material

Three improved bread wheat cultivars *viz.* PBW343, WH542 and UP2338 were used as recipient parent for the incorporation of specific rust resistance genes. Leaf rust and stem rust resistance genes were transferred from Thatcher+*Lr19*+*Sr25*, Centurk (*Lr10*+*Lr24*+*Sr24*), CS2D2M3/8 (*Lr28*), Thatcher+*Lr32*, KS92WGRC15 (*Lr39*), KS91WGRC11 (*Lr42*), Tc*7/ST-1 (*Lr45*). Stripe rust resistance genes were transferred from winter wheat accessions *viz. Triticum spelta album* (*Yr5*), Moro (*Yr10*), CN25087 (*Yr15*), Spaldings Prolific (*YrSP*).

Pathogen material

The F_1's, BC_1, BC_2, BC_3 and F_2 generations were tested either with *P. triticina* (*Ptr*) Pathotype (pt.) 21R55 or *P. striiformis* (*Pst*) pt. 46S119. The F_3, F_4, F_5, F_6 and F_7 generations were tested with *Ptr* pt. 21R55, *P. graminis* (*Pgr*) pt. 62G29-1, and *Pst* pt. 46S119. The genetic stocks were tested with *Ptr* pts. 21R55, 121R63-1 and 121R127, *Pgr* pts. 62G29 and 62G29-1, and *Pst* pts. 46S119 and 78S84 at the seedling stage. At Karnal, plants were artificially inoculated with stripe rust pt.46S119 and mixture of leaf rust pathotypes 121R63-1 and 21R55. The avirulence-virulence (against important resistance genes) of the pathotypes used in the study are as follows: *Ptr* pt. 121R63-1 avirulent on *Lr9, 19, 24, 28, 32, 39, 42, 45* and virulent on *Lr 23, 26; Ptr* pt. 21R55 avirulent on *Lr9, 19, 24, 28, , 32, 39, , 42, 45,* virulent on *Lr23, 26*; *Ptr* pt. 121R127 avirulent on *Lr19, 24, 28, 32, 39, 41, 42, 45* and virulent on *Lr 9, 23,26, Pgr* pt. 62G29 avirulent on *Sr 24, 25, 31* virulent on *Sr5, 11 Pgr* pt. 62G29-1 avirulent on *Sr24, 25, 31,* virulent on *Sr5, 9e, 11*; *Pst* pt. 46S119 avirulent on *Yr5,10, 15,27,SP,Su,CV* virulent on *Yr 2, 3, 9; Pst* pt. 78S84 avirulent *Yr5,10,15,25,SP,CV* on virulent on *Yr2, 9, 27.*

Seedling resistance test

The seedlings were raised in small plastic pots or aluminum pans. In each lot of testing material susceptible line and other control lines were kept as checks. Fully expanded primary leaves were inoculated with uredospores suspended in non-phytotoxic isoparaffinic oil (soltrol 170, Philips Chevron). Inoculation of leaf, stem and stripe rusts were carried out in temperature controlled glass house at 20-22°C, 22-24°C and 14-16°C, respectively. The inoculated seedlings were kept in a humid glass chamber for 48 hours. Infection types (IT's) were recorded 14 days after inoculation and classified according to [4]. IT's 0; (naught fleck), ; (fleck) and ;2 (fleck two) were classified as the resistant reactions whereas IT's 3 (three) and 3+ (three plus) were designated as susceptible reactions.

Field resistance test

Terminal disease severity scores were recorded for the assessment of field resistance. Rust severity was recorded as per modified Cobb's scale [5] for all three rusts. At Karnal, plants were artificially inoculated with stripe rust pathotype 46S119 and mixture of leaf rust pathotypes 121R63-1 and 21R55.

Molecular markers

Molecular markers of *Lr9*, *Lr19*, *Lr24* and *Lr26* developed by [6-9] were utilized for authenticating the incorporation of rust resistance genes in the genetic stocks. The PCR conditions of the molecular markers are given in Table 1.

Selection scheme

The improved cultivars were crossed with the donor lines carrying rust resistance genes. All F_1's, BC_1, F_2 and F_3 generations were screened under temperature controlled glass house condition at seedling stage. The F_3 lines (100-500 depending on the cross) were screened simultaneously with *Puccinia triticina* pt. 21R55, *Puccinia graminis* pt. 62G29-1 and *Puccinia striiformis* pt. 46S119 and only the lines that were homozygous resistant to all or concerned rusts were

Table 1. PCR profiles for *Lr9, Lr19, Lr24* and *Lr26* genes

Genes	Components	Cycles	Reference
Lr9	2mMMgCl$_2$, 100µMdNTP, 40Nm primer, 0.5U Taq Polymerase, 50ng DNA	1x 94^{0}C 6' 40 x 94^{0}C 1'; 62^{0}C 1'; 72^{0}C 2' 1x 72^{0}C 4'	Schachermayr *et al.*, 1994
Lr19	2mMMgCl$_2$, 0.2µMdNTP, 12.5p moles primer, 0.6U Taq Polymerase, 50ng DNA	1x 94^{0}C 4' 30 x 94^{0}C 0.5'; 60^{0}C 0.5'; 72^{0}C 0.5' 1x 72^{0}C5'	Prins *et al.*, 2001
Sr24	2mMMgCl$_2$, 200µMdNTP, 0.6µM primer, 1.0U Taq Polymerase, 50ng DNA	1x 94^{0}C 0.5' 38 x 94^{0}C 1.5'; 68^{0}C 2'; 72^{0}C 2' 1x 72^{0}C 5'	Mago *et al.*, 2005
Lr26 (Rye	2mMMgCl$_2$, 0.2µMdNTP, 0.1µM primer, 0.5U Taq Polymerase, 50ng DNA Chromatin)	1x 94^{0}C 0.25' 45 x 94^{0}C 1'; 55^{0}C 2'; 72^{0}C 1' 1x 72^{0}C 5'	Fransis *et al.*, 1995

advanced to next generation. For all crosses 100-150 F_3 seeds were obtained per family and 25-30 individuals were tested in each family. All plants of selected F_3 families were planted at Wellington so as to increase the probability of desirable segregants. The F_5 and F_7 generations were also screened at Wellington for field resistance. Finally, genetic stocks (in F_8 generation) along with checks were screened under controlled glass house condition, field condition with artificial epiphytotic and natural hot spots.

Postulation of rust resistance genes

Rust resistance genes were postulated on the basis of host-pathogen interaction, pedigree, genetic linkage, test of allelism using lines having known genes and molecular markers. The pedigree and resistance to specific pathotypes indicated presence of *Lr9, Lr19, Lr24, Lr28, Lr32, Lr39, Lr42, Lr45, Yr5*, Y*r10, Yr15, YrSP, YrCD, Sr31* or combination of these genes in the genetic stocks. The genes *Lr19/Sr25*, *Lr24/Sr24* and *Lr26/Sr31/ Yr9* are completely linked, therefore, postulation of either of these genes confirmed the presence of other linked genes. The reaction of Tc+*Lr9*, Tc+*Lr19,* Tc+*Lr26 Lr28*, *Lr39*, *Yr5*, *Yr10*, *Yr15*, *Yr* gene from Cappelle Desprez and *YrSP* against avirulent pathotypes was 0; whereas the IT's of Tc+*Lr24, Lr42, Lr45* was ;1 while Tc+*Lr32* gave ;12. Resistance genes were postulated according to [10]. The gene combinations, namely, *Lr19+Lr9+Lr24+Lr26* could not be distinguished on the basis of seedling IT's. These combinations were confirmed through molecular markers.

Results and discussion

Appropriate selection procedure enabled fixation of specific rust resistance genes in early generation (Table 2). Agronomic traits were selected in subsequent generations. Genetic stocks were evaluated for rust resistance at different locations (Tables 3 and 4). Field resistance of genetic stocks indicated that two lines namely, FLW21 and FLW29 were resistant to all the rusts, seven lines namely, FLW15, FLW18, FLW20, FLW24, FLW25, FLW26 and FLW27 had resistance to leaf rust as well as stem rusts whereas four lines namely, FLW10, FLW13, FLW16 and FLW17 were resistant to stripe rust and stem rust (Table 3). Resistance of these lines was confirmed through multi-pathotype seedling resistance tests under temperature controlled glass house conditions (Table 4).

Genetic stocks resistant to all rusts

FLW21 was free from leaf rust, stem rust and stripe rust at all locations. Seedling resistance test corroborated observations of field resistance. At seedling stage it was highly resistant (0; to ;1 IT's) to leaf rust pathotypes, moderately resistant to stem rust pathotypes and immune to stripe rust pathotypes. Resistance of FLW21 was derived from Centurk, reported to carry *Lr24/Sr24* [11] and CN25087, carrying *Yr15*. Test of allelism confirmed presence of *Lr24/Sr24* and *Yr15* in FLW21 (Table 5). Molecular marker analysis corroborated results of test of allelism (Fig. 1). Additional *Sr* genes are expected in this stock because it showed resistance to *Pgr* pt. 62G29-1 which is virulent on *Sr24*. Resistance to pt. 62G29-1 is likely to be due to presence of *Sr31*. Molecular marker and test of allelism confirmed presence of *Lr26/Sr31* (Table 5 and Fig. 3). FLW21 had test weight (1000-grain weight) 37.7g, average plant height was 91.9cm and matured in about 122 days. This stock is an important source of resistance to all rusts in India.

Table 2. Rust resistance in F_2 and F_3 generations

Cross	F_2				Homozygous resistant F_3 families						Name of the selected advanced lines
	Leaf rust 21R55		Stripe rust 62G29-1		Leaf rust	Stem rust	Stripe rust	Leaf rust	Stripe rust	All	
	R	S	R	S							
WH542/Moro (*Yr10*)	-	-	234	69	-	53	71	0	13	0	FLW10
WH542/CN25087 (*Yr15*)	-	-	63	22	-	0	14	0	0	0	FLW13
PBW343/Tc+*Lr32*	211	56	-	-	67	45	49	11	14	4	FLW15
UP2338/*Triticm spelta album* (*Yr5*)	-	-	205	54	-	51	68	0	12	0	FLW16
WH542/Spaldings Prolific	-	-	227	58	-	53	80	0	10	0	FLW17
PBW343/KS92WGRC15 (*Lr39*)	258	70	-	-	82	68	65	15	15	4	FLW18
PBW343/Tc*Lr19//*FLW6 *(Lr9+24)*	383	8	-	-	186	154	0		0	0	FLW20
UP2338/Centurk// UP2338/*Yr15*(CN25087)	302	94	-	-	101	76	71	21	18	5	FLW21
PBW343/ Tc+*Lr19*	226	71	-	-	69	95	53	27	23	7	FLW24
PBW343/ CS2D2M3/8 (*Lr28*)	233	72	-	-	78	52	55	15	16	4	FLW25
PBW343/KS91WGRC11 (*Lr42*)	201	61	-	-	63	57	50	12	11	3	FLW26
PBW343/Tc*7/ST-1 (*Lr45*)	243	75	-	-	77	56	62	13	14	3	FLW27
PBW343/CD// PBW343/FLW7	437	154	-	-	141	103	28	29	26	6	FLW29

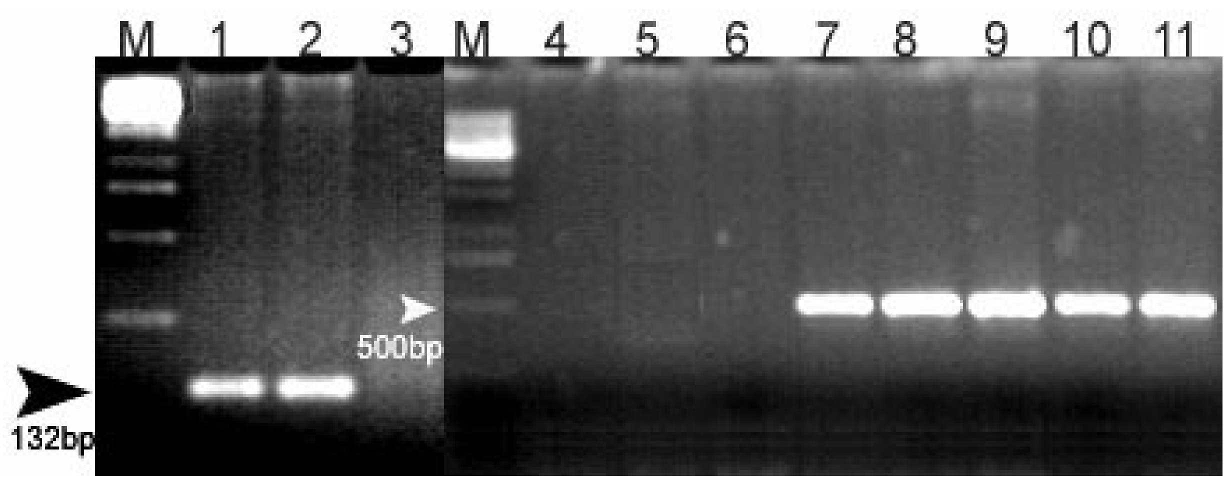

Fig. 1. **Lanes 1-3: *Lr19* marker, 132 bp fragment [7] : 1-FLW20, 2-FLW24, 3-PBW343; Lanes 4-11: *Lr24* marker, 500 bp fragment [8]; 4-PBW343, 5-UP2338, 6-HP1633,7-HP1776, 8-FLW2, 9-FLW6, 10-FLW20, 11-FLW21**

Table 3. Evaluation of genetic stocks for field resistance at multi-locations

	Leaf rust			Stem rust			Stripe rust		
	K(F)	W(F)	D(F)	K(F)	W(F)	D(F)	K(F)	W(F)	D(F)
Agra Local	100S	100S	60S	R	80S	10S	80S	20S	80S
Cappelle Desprez	100S	80S	60S	R	40S	R	R	R	R
Centurk (*Lr24*)	R	R	R	R	R	R	80S	10S	80S
China84-40022	80S	100S	60S	R	R	R	R	R	R
HP1633 (*Lr9*)	R	R	R	R	80S	R	80S	20S	80S
HP1776 (*Lr24*)	R	R	R	R	10MR	R	60S	10S	80S
Tc+*Lr19*	R	R	R	R	R	R	80S	20S	80S
CS2D2M3/8 (*Lr28*)	R	R	R	R	80S	R	60S	20S	80S
Tc+*Lr32*	R	R	R	R	60S	R	80S	20S	80S
KS86WGRC02 (*Lr39*)	R	R	R	R	60S	R	80S	20S	80S
KS91WGRC11 (*Lr42*)	R	R	R	R	80S	R	80S	20S	80S
Tc*7/ST-1 (*Lr45*)	R	R	R	R	80S	R	60S	20S	80S
Moro (*Yr10*)	80S	80S	60S	R	80S	R	R	R	R
PBW343	60S	60S	40S	R	R	R	R	R	R
Spaldings Prolific (*YrSP*)	100S	100S	60S	R	80S	10S	R	R	R
Triticm spelta album (*Yr5*)	80S	100S	60S	R	80S	R	R	R	R
UP2338	80S	100S	40S	R	R	R	60S	R	80S
WH542	80S	80S	40S	R	R	R	40S	R	40S
CN25087 (*Yr15*)	100S	100S	60S	R	80S	10S	R	R	R
FLW10 (WH542/ Moro)	30S	40S	20S	R	80S	R	R	R	R
FLW13 (WH542/*Yr15*CN25087)	80S	80S	60S	R	80S	R	R	R	R
FLW15 (PBW343/Tc+*Lr32*)	R	R	R	R	R	R	R	R	R
FLW16 (UP2338/*Triticum spelta album*)	80S	100S	40S	R	R	R	R	R	R
FLW17 (WH542/Spaldings Prolific)	100S	100S	40S	R	R	R	R	R	R
FLW18 (PBW343/ KS86WGRC02(*Lr39*)	R	R	R	R	R	R	R	R	R
FLW20 (PBW343/Tc+ *Lr19*//FLW6 *Lr9+24*)	R	R	R	R	R	R	60S	10S	80S
FLW21 (UP2338/Centurk//*Yr15*)	R	R	R	R	R	R	R	R	R
FLW24 (PBW343/Tc+*Lr19*)	R	R	R	R	R	R	R	R	R
FLW25 (PBW343/ CS2D2M3/8(*Lr28*)	R	R	R	R	R	R	R	R	R
FLW26 (PBW343/ KS91WGRC11(*Lr42*)	R	R	R	R	R	R	R	R	R
FLW27 (PBW343/ Tc*7/ST-1 (*Lr45*)	R	R	R	R	R	R	R	R	R
FLW29 [PBW343/CD// PBW343/FLW7(*Lr28*)]	R	R	R	R	R	R	R	R	R

K(F)-Field resistance at Karnal; W(F)-Field resistance at Wellington; D(F)-Field resistance at Dalang Maidan
R = Resistant; S = Susceptible

Table 4. Multi-pathotype seedling test for resistance in the genetic stocks

Parents	Pathotypes							
	Leaf rust				Stem rust		Stripe rust	
	77-5	77-7	77-8	104-2	40A	40-1	46S 119	78S 84
Agra Local	3+	3+	3+	3+	3+	3+	3+	3+
Cappelle Desprez	3+	3+	3+	3+	3+	3	;2	0;
Centurk *(Lr24/Sr24)*	;1	;1	;1	;1	3	2-	3+	3+
HI1077	3+	3+	3+	3+	3+	3+	3+	3+
HUW234	3+	3+	3+	3+	3+	3+	3+	3+
Tc+*Lr19*	0;	0;	3+	0;	2-	2-	3+	3+
(CS2D2M3/8) *Lr28*	0;	0;	0;	0;	3+	3+	3C	23C
Tc+*Lr32*	;12	;12	;12	;12	3+	3+	3+	3+
KS86WGRC02)(*Lr39*)	0;	0;	0;	0;	3+	3+	3+	3+
KS91WGRC11(*Lr42*)	;1	;1	;1	;1	3+	3+	3+	3+
Tc*7/ST-1(*Lr45*)	;1	;1	;1	;1	3+	3+	3+	3+
Moro (*Yr10*)	3+	3+	3+	3+	3+	3+	0;	0;
PBW343	3+	3+	3+	3+	2-	2-	;2	3+
Spaldings Prolific (*YrSP*)	3+	3+	3+	3+	3+	3+	0;	0;
Triticm spelta album (*Yr5*)	3+	3+	3+	3+	3+	3+	0;	0;
UP2338	3+	3+	3+	3+	2-	2-	3+	3+
WH542	3+	3+	3+	3+	2-	2-	3C	3+
CN25087 (*Yr15*)	3+	3+	3+	3+	3+	3+	0;	0;
FLW10 (WH542/ Moro *Yr10*)	3+	3+	3+	3+	2-	2-	0;	0;
FLW13 (WH542/*Yr15*CN25087)	3+	3+	3+	3+	3+	3+	0;	0;
FLW15 (PBW343/Tc+*Lr32*)	;1	;1	;1	;1	2-	2-	;2	3+
FLW16 (UP2338/TSA *Yr5*)	3+	3+	3+	3+	2-	2-	0;	0;
FLW17 (WH542/Spaldings Prolific)	3+	3+	3+	3+	2-	2-	;	0;
FLW18 (PBW343/ KS86WGRC02 *Lr39*)	0;	0;	0;	0;	2-	2-	;2	3+
FLW20 (PBW343/Tc+*Lr19*// FLW6 *Lr9+24*)	0;	0;	0;	0;	2-	2-	3+	3+
FLW21 (UP2338/Centurk//*Yr15*)	;1	;1	;1	;1	2-	2-	0;	0;
FLW24 (PBW343/Tc+*Lr19*)	0;	0;	0;	0;	2-	2-	;2	3+
FLW25 (PBW343/ CS2D2M3/8 (*Lr28*)	0;	0;	0;	0;	2-	2-	;2	3+
FLW26 (PBW343/KS91WGRC11(*Lr42*)	;1	;1	;1	;1	2-	2-	;2	3+
FLW27 (PBW343/ Tc*7/ST-1 (*Lr45*)	;1	;1	;1	;1	2-	2-	;2	3+
FLW29[PBW343/CD// PBW343/FLW7 (*Lr28*)]	0;	0;	0;	0;	2-	2-	0;	0;

FLW29 was free from leaf rust, stem rust and stripe rust at all locations except at Wellington where it gave MR reaction to stripe rust. At seedling stage, it was immune to brown and yellow rust pathotypes and moderately resistant to black rust pathotypes. On the basis of multi-pathotype seedling resistance tests and field resistance at three locations, this stock was expected to carry *Lr28* from FLW7, stripe rust resistance genes from Cappelle Desprez (CD) and *Sr31* from PBW343. Test of allelism confirmed the presence of *Lr28* and *Sr31* in FLW29 (Table 5). FLW29 showed less seedling infection types than CD. *Yr3*, *Yr4* and *Yr16* (the durable stripe rust resistance gene) are reported in CD [12], but presence of these genes could not be

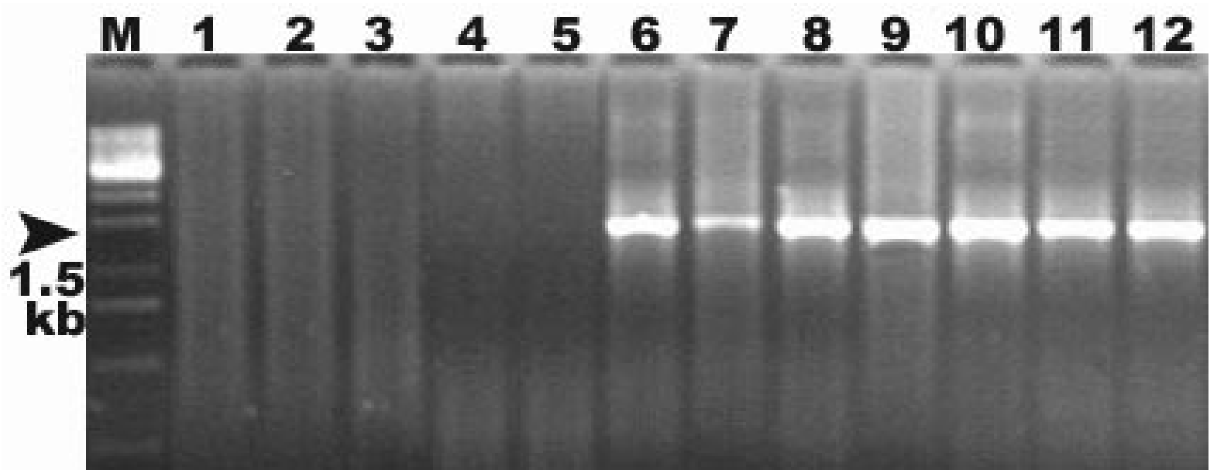

Fig 2. Lanes 1-12: 1BL.1RS marker, 1.5kb fragment (Fransis *et al.* 1995) : 1-BlueboyII, 2-Moro, 3-Mega, 4-TSA, 5-Spaldings prolific, 6-PBW343, 7-FLW2, 8-FLW3, 9-FLW10, 10-FLW12, 11-FLW15, 12-FLW16

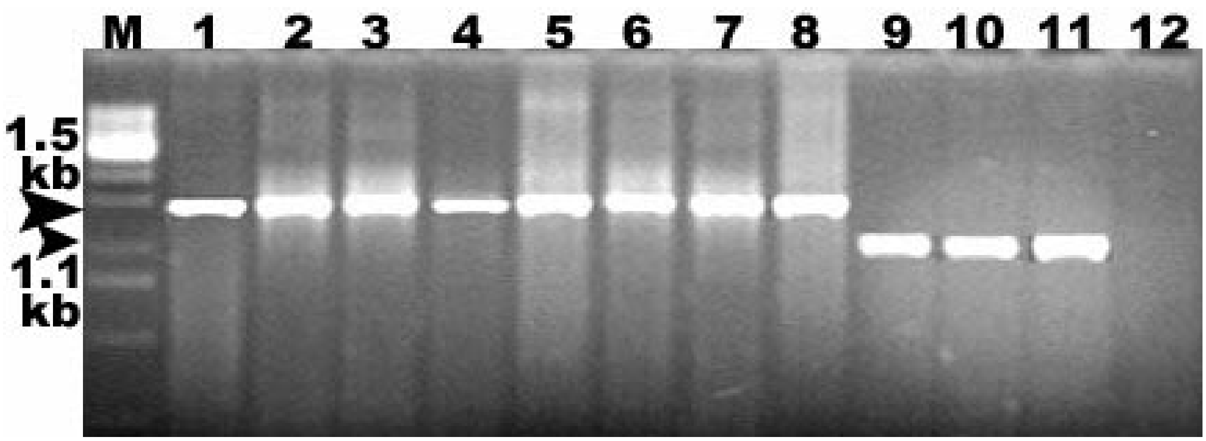

Fig 3. Lanes 1-8: 1BL.1RS marker, 1.5kb fragment (Fransis *et al.* 1995): 1-FLW17, 2-FLW18, 3-FLW20, 4-FLW21, 5-FLW24, 6-FLW25-, 7-FLW26, 8-FLW27; Lanes 9-12: *Lr9* marker, 1.1 kb fragment (Schachermayr *et al.* 1994); 9-FLW6, 10-FLW20, HP1633, 11-HP1776

ascertained in FLW29. It is short in stature (90.7 cm) with 38.9g test weight and matures in about 119 days.

Genetic stocks resistant to leaf rust and stem rust

FLW15 was free from leaf rust, stem rust and stripe rust at all locations in the field. At seedling stage it was highly resistant (; to ;1$^+$ IT's) to leaf rust pathotypes, moderately resistant to stem and stripe rust pathotypes except pt. 78S84 of *Puccinia striiformis*. Resistance to leaf rust was due to *Lr32* (derived fromTc+*Lr32*). Presence of *Lr32* was indicated from the seedling resistance test and pedigree. Test of allelism conclusively proved *Lr32* in this stock (Table 5). Infection types against stem rust pathotypes at seedling stage indicated presence of *Sr31.* Presence of *Sr31/Lr26/Yr9* was authenticated through molecular marker as well as test of allelism. Field resistance and seedling infection types of FLW15 and PBW343 were similar against stripe rust pathotypes and hence it should carry *Yr27* (from PBW343). Test of allelism confirmed presence of *Yr27* in FLW15. It has 40.6g test weight, average plant height was 93.8cm and matured in about 120 days.

FLW18 was free from leaf rust and moderately resistant to stem rust pathotypes at seedling stage whereas it showed complete field resistance to all rusts at all locations (Tables 3 and 4). It's seedling and adult plant resistance against stripe rust was similar to PBW343 and, hence, it is likely to carry *Yr27*. Test of allelism confirmed *Lr39, Sr31/Lr26 and Yr27* (Table 5) and molecular marker authenticated presence of

1BL.1RS in this stock. FLW18 has test weight of 37.8g, average plant height was 87.2 cm and matured in about 124 days.

FLW20 was free from leaf and stem rusts at all locations under field condition. At seedling stage, it showed 0; IT's to leaf rust pathotypes and moderate reaction to stem rust pathotypes. This stock was derived from the cross PBW343 (*Lr26+Yr27*)/ *Lr19* // FLW6 (*Lr9+Lr24*) and hence, it stood a chance to carry multiple rust resistance genes. Test of allelism and molecular data conclusively proved presence of several rust resistance genes, namely, *Lr9, Lr19, Lr24* and *Lr26* in FLW20 (Table 5). It has 41.1g test weight, average plant height was 88.6 cm and matured in about 118 days.

FLW24 and FLW25 were highly resistant to leaf, stem and stripe rusts in field evaluation at all locations. At seedling stage, both were immune to leaf rust, moderately resistant to stem rust pathotypes and resistant to pt. 46S119 of stripe rust. Field resistance and seedling resistance test indicated presence of *Lr19/ Lr28*, *Sr31* and *Yr27* in these stocks. Test of allelism conclusively proved *Lr19* and *Lr28* in FLW24 and FLW25, respectively (Table 5). Molecular marker confirmed *Sr31* in both the stocks. Absence of susceptible seedlings against stripe rust pst. 46S119 in F_2 of the crosses of FLW24/PBW343 and FLW25/

Table 5. Test of allelism for specific resistance genes in the genetic stocks

Cross	Pathotype	Number of seedlings		P
		R	S	
FLW10/Av+*Yr10*	*Pst* 46S119	285	0	<0.001
FLW13/Av+*Yr15*	*Pst* 46S119	273	0	<0.001
FLW15/Tc+*Lr32*	*Ptr* 121R63-1	224	0	<0.001
FLW15/ Tc+*Lr26*	*Pgr* 40-1	251	0	<0.001
FLW18/ *Lr39*	121R63-1	201	0	<0.001
FLW18/ PBW343	*Pst* 46S119	236	0	<0.001
FLW16/Av+*Yr5*	*Pst* 46S119	209	0	<0.001
FLW17/Av+*YrSP*	*Pst* 46S119	229	0	<0.001
FLW20/Tc+*Lr9*	*Ptr* 121R63-1	278	0	<0.001
FLW20/Tc+*Lr19*	*Ptr* 121R63-1	237	0	<0.001
FLW20/Agent (*Lr24*)	*Ptr* 121R63-1	249	0	<0.001
FLW20/Tc+*Lr26*	*Ptr* 77-2	411	0	<0.001
FLW21/Agent	*Ptr* 121R63-1	254	0	<0.001
FLW21/Av*Yr15*	*Pst* 46S119	218	0	<0.001
FLW21/Tc+*Lr26*	*Ptr* 77-2	365	0	<0.001
FLW24/Tc+*Lr19*	*Ptr* 121R63-1	255	0	<0.001
FLW24/PBW343	*Pst* 46S119	276	0	<0.001
FLW25/ CS2D2M3/8 (*Lr28*)	*Ptr* 121R63-1	242	0	<0.001
FLW25/PBW343	*Pst* 46S119	285	0	<0.001
FLW26/ KS91WGRC11 (*Lr42*)	*Ptr* 121R63-1	216	0	<0.001
FLW26/PBW343	*Pst* 46S119	301	0	<0.001
FLW26/Tc+*Lr26*	*Pgr* 40-1	316	0	<0.001
FLW27/Tc*7/ST-1 (*Lr45*)	*Ptr* 121R63-1	294	0	<0.001
FLW27/PBW343	*Pst* 46S119	226	0	<0.001
FLW27/PBW343	*Pgr* 40-1	279	0	<0.001
FLW29/Tc+*Lr26*	*Pgr* 40-1	221	0	<0.001
FLW29/CS2D2M3/8(*Lr28*)	*Ptr* 121R63-1	305	0	<0.001

Ptr = *Puccinia triticina*; Pst = *Puccinia striiformis*; Pgr = *Puccinia graminis*; R =Resistant and S = Susceptible.

Table 6. Summary of wheat lines with specific rust resistance genes

Lines	Parentage	Characteristic features Resistant (R), susceptible (S)	Genes present	Pl. H (cm)	Mat. (days)	TW (g)	Y/MR (g)
FLW10	WH542/Moro	R - stripe and stem rusts	*Lr26+Sr31+Yr9+Yr10*	74.0	118	39.7	114.1
FLW13	WH542/CN25087(*Yr15*)	R - stripe and stem rusts	*Lr34+Sr2+Yr15+Yr18*	105.3	116	42.4	131.8
FLW15	PBW343/Tc+*Lr32*	R - leaf and stem rusts	*Lr26+Lr32+Sr31+Yr9+ Yr27*	93.8	120	40.6	133.4
FLW16	UP2338/*Triticm spelta album*	R - stripe and stem rusts	*Lr26+Sr31+Yr9+Yr5*	77.1	119	40.3	132.7
FLW17	WH542/Spaldings Prolific	R - stripe and stem rusts	*Lr26+Sr31+Yr9+YrSP*	76.5	120	37.9	130.5
FLW18	PBW343/*Lr39*(KS92WGRC15)	R - stripe and stem rusts	Lr26+Lr39+Sr31, *Yr9, Yr27*	87.2	124	37.8	128.6
FLW20	PBW343/Tc+*Lr19*//FLW6	R - stripe and stem rusts	*Lr9+Lr19+Lr24+Lr26+Sr24+Sr25+Sr31+Yr9*	88.6	118	41.1	155.4
FLW21	UP2338/Centurk//UP2338/*Yr15*	R - all rusts	*Lr26+Lr24+Sr24+Sr31+ Yr9+Yr15*	91.9	122	37.7	149.8
FLW24	PBW343*3/ Tc+*Lr19*	R - leaf and stem rusts	*Lr19+Lr26+Sr25+Sr31+ Yr9+Yr27*	87.3	118	41..5	120.1
FLW25	PBW343*3/ CS2D2M3/8(*Lr28*)	R - leaf and stem rusts	*Lr28+Lr26+Sr31+Yr9+Yr27*	86.9	119	39.7	143.5
FLW26	PBW343*3/KS91WGRC11(*Lr42*)	R - leaf and stem rusts	*Lr42+Lr26+Sr31+Yr9+Yr27*	89.5	120	38.6	128.7
FLW27	PBW343*3/ *Lr45*	R - leaf and stem rusts	*Lr45+Lr26+Sr31+Yr9+Yr27*	91.6	121	38.5	126.4
FLW29	PBW343/CD//PBW343/FLW7	R - all rusts	*Lr28+Lr26+Sr31+Yr9+Yr* genes from CD	90.7	119	38.9	132.4
	Centurk	R - leaf rust	*Lr24+Sr24*	97.4	154	27.3	58.6
	Moro (*Yr10*)	R - stripe rust	*Yr10*	51.7	160	27.8	57.1
	CN25087 (*Yr15*)	R - stripe rust	*Yr15*	79.2	113	43.3	101.3
	Triticum spelta album (*Yr5*)	R - stripe rust	*Yr5*	119.5	154	41.6	66.4
	Spaldings Prolific	R - stripe rust	*YrSP*	57.6	161	28.1	55.2
	KS92WGRC15 (*Lr39*)	R - leaf rust	*Lr39*	113.5	155	28.6	56.4
	Tc+*Lr19*	R - brown and black rusts	*Lr19+Sr25*	124.1	142	26.7	68.2
	CS2D2M3/8 (*Lr28*)	R - leaf rust	*Lr28*	119.3	143	26.4	51.0
	KS91WGRC11 (*Lr42*)	R - leaf rust	*Lr42*	114.3	145	27.8	58.8
	Tc*7/ST-1 (*Lr45*)	R - leaf rust	*Lr45*	115.5	144	28.3	59.5
	PBW343	S - to leaf & stripe rusts	*Lr26+Sr31+Yr9+Yr27*	92.3	124	42.3	161.3
	UP2338	S - leaf & stripe rusts	*Lr26+Sr31+Yr9*	89.1	129	46.2	140.7
	WH542	S - leaf & stripe rusts	*Lr26+Sr31+Yr9*	88.6	121	39.4	138.8
			SE at 5%	3.21	1.37	1.46	11.35

Pl. H = Plant height; Mat. = maturity; TW = 1000-grain weight; Y/MR = Yield per meter row

PBW343 confirmed *Yr27* in both stocks. FLW24 has medium spikes and test weight 41.5g, average plant height was 87.3 cm and matured in about 118 days. FLW25 has test weight of 39.7g, average plant height was 86.9 cm and matured in about 119 days.

Resistance pattern of FLW26 and FLW27 were similar at adult plant as well as seedling stages. Field resistance and seedling resistance test indicated presence of *Lr42/Lr45*, *Sr31* and *Yr27* in these stocks which were conclusively proved through either test of allelism or molecular data (Table 5 and Fig. 3). FLW26 has test weight of 38.6g, average plant height was 89.5 cm and matured in about 120 days. FLW27 has test weight of 38.5g, average plant height was 91.6 cm and matured in about 121 days.

Stocks resistant to stripe and stem rusts

FLW10 was immune to stripe rust at adult plant and seedling stages at all the locations and against all the pathotypes, respectively and it showed moderate field and seedling resistance against black rust. Based on seedling resistance test and pedigree, *Sr31* and *Yr10* was postulated in FLW10. Molecular marker authenticated *Sr31* (Fig. 1) whereas test of allelism confirmed *Yr10* (Table 5). FLW10 has small spikes and test weight 39.7g, average plant height was 74 cm and matured in about 118 days.

FLW13 was free from stripe rust at all locations and in seedling test it was immune to all pathotypes of stripe rust. It was moderately resistant to stem rust at seedling and adult plant stages. Seedling resistance test and pedigree indicated *Yr15* in this stock. It was confirmed through test of allelism (Table 5). FLW13 has test weight of 42.4g, average plant height was 105.3 cm and matured in about 116 days.

FLW16 was free from stripe rust at all the locations and immune at seedling stage to all the pathotypes. It was moderately resistant to stem rust pathotypes in the seedling and field evaluations. *Sr31* and *Yr5* were confirmed in this stock through molecular marker and or test of allelism. FLW16 has test weight of 40.3g, average plant height was 77.1 cm and matured in about 119 days.

FLW17 was free from stripe rust at adult plant and seedling stages at all locations and against all the pathotypes, respectively and it showed moderate field and seedling resistance against stem rust. Based on seedling resistance test and pedigree, *Sr31* and *YrSP* were postulated in FLW17. Molecular marker authenticated *Sr31* (Fig. 3) whereas test of allelism confirmed *YrSP* (Table 5). FLW17 has medium spikes and test weight 37.9g, average plant height was 76.5 cm and matured in about 120 days.

Specific rust resistance genes were fixed in early generation which enabled selection for agronomic traits in F_4 onwards. The stocks resistant to leaf and stem rust can be utilized in the breeding programme if the second parent of the cross carries stripe rust resistance gene. Similarly, stock resistant to stripe and stem rusts will be useful if other parents of the intended cross carry leaf rust resistance genes. FLW20 and FLW24 carrying *Sr25* provides resistance to pt. Ug99 [13] of stem rust and, hence, can be utilized in anticipatory breeding against pt. Ug99. FLW20 has the unique distinction of having multiple and effective leaf rust and stem rust resistance genes. Depending on the need these genetic stocks can be utilized in breeding programmes for incorporation of rust resistance.

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