J. Farm Sci., 30(2): (157-163) 2017

Phenotypic and molecular analysis of slow leaf rusting in wheat genotypes

K. J. YASHAVANTHA KUMAR AND S. A. DESAI

Department of Genetics and Plant Breeding, College of Agriculture
University of Agricultural Sciences, Dharwad - 580 005, Karnataka, India
E-mail: sumadhwa5@gmail.com

(Received: October, 2016 ; Accepted: June, 2017)

Abstract: The phenotypic evaluation of adult plant leaf rust infection data from the field study among the 102 test cultivars, were categorized as R, MR, MS and S during 2014-15, at AICRP Wheat scheme, UAS Dharwad. The tightly linked molecular markers (STS/SSR) csLV34, Xwmc44, Xcfd71 and csGS respectively for slow leaf rusting APR genes *viz., Lr34, Lr46, Lr67* and *Lr68* were used to characterize the wheat genotypes at molecular level. In the present material *Lr34* observed with the frequency of 7.8%, *Lr*46 with 50%, *Lr68* with 15.7% and *Lr67* the least frequent (3.9%) slow rusting gene. The genotypes with three genes combination found to be more effective than two gene combination. The high levels of resistance in some genotypes without *Lr34* and other APR genes may be caused by various gene combinations not fully characterized herein. The outcome of the investigation emphasizes the utilization of genotypes VL907, Parula and Lerma Rojo, carried multiple minor genes with low AUDPC and low ACI. These genotypes may be utilised in the resistance breeding programme in order to have broad spectrum durable leaf rust resistance.

Key words: Genotype, Marker, Resistance, Slow rusting

Introduction

Wheat has accompanied humans since 3,000 to 4,000 BC. It has evolved in part by nature and in part by human manipulation from its primitive form (Einkorn wheat) into the present main cultivated species; bread wheat (*Triticum aestivum* L.) The world acreage under wheat crop during the year 2013 was 219 million hectares and the production of 715 million tonnes, with an average yield of 3268 kg per hectare (Anon, 2015). It is also the main staple food of India and occupies a central position in agricultural policies. In India wheat was grown over an area of 29.65 million hectares and the production of 95.9 million tonnes with an average productivity of 3153 kg per hectare during the year 2013-14 (Anon, 2015a).

Leaf rust in India is probably the most variable pathogen because of its widespread occurrence. Epidemics of wheat rusts have been avoided in India through monitoring variation, evaluation of advance lines and their deployment based on the pathotype distribution (Nayar *et al.,* 2002). However, frequent emergence of new variants renders a rust resistant variety of wheat susceptible to brown rust (Bhardwaj *et al.,* 2005). Although the timely application of fungicides can provide adequate control but their use adds to production costs and they are environment unfriendly. Thus growing resistant varieties is the most effective, environmentally safe and efficient control strategy for wheat rusts. A large number of varieties have been released in India but genetic diversity at the farmer's field level is very limited, hence their diversification by the introgression and pyramiding of various leaf rust resistance (*Lr*) genes was the first attempt to use the present genetic diversity within hexaploid wheat and its wild relatives against this disease (Schnurbusch *et al.,* 2004).

Two different types of resistance are often described in the literature against specialized fungi that parasitize living cells are race-specific resistance, which is also known as vertical or hypersensitive resistance and race-nonspecific, or horizontal, non-hypersensitive, partial or slow rusting resistance. The application of the concepts of slow rusting resistance and partial resistance has dominated several bread wheat improvement programs, including the program at the International Maize and Wheat Improvement Center (CIMMYT). One way to prolong the effectiveness of these genes is to 'pyramid' or combine several effective race specific genes into a single cultivar. Adult plant resistance (APR) to rust diseases is being used extensively, and sometimes in conjunction with seedling resistances for rust control. As the name suggests, APR cannot be assayed simply at the seedling stage and is most commonly assayed in field nurseries in adult plants. As such, assays can be done only once a year and are dependent on environmental conditions. Two classes of APR to rust have been observed in wheat and are characterized by either hypersensitive or non-hypersensitive response. A partial resistance phenotype and other effective rust resistance genes often present in the background can interfere with the scoring of progeny and hence, the precise genetic mapping of APR genes, so appropriate genetic stocks must be assembled to study these genes (Ellis *et al.,* 2007).

A small group of leaf rust resistance genes are known as "slow rusting APR genes", such as *Lr34, Lr46, Lr67* and *Lr68.* They provide durable and non-specific adult plant resistance but their effect is more reduced than that of race-specific genes (Jelena *et al.,* 2009). Lagudah *et al.* (2006) developed an STS marker, *csLV34* that maps 0.4 cM from Lr34, and was validated in many lines and cultivars from different breeding programs worldwide. Suenaga *et al*. (2003) determined that the microsatellite locus *Xwmc44* is located 5.6-cM proximal to the putative QTL for *Lr46.* An SSR marker *Xwmc 44* produced 242 bp marker band for the presence of *Lr46* and other than this band was considered to be absence of *Lr46.* The *Lr67* gene for adult plant resistance (APR) to leaf rust was identified in the common wheat accession PI250413 and transferred into Thatcher to produce the backcross line RL6077 (Thatcher*6/PI250413). PCR amplification of SSR marker Xcfd71 in RL6077 produced a 214-bp allele from chromosome 4DL (Hiebert *et al.,* 2010). The likely origin of *Lr68* is the Brazilian cultivar Frontana. The dominant STS marker csGS at 1.2 cM from the gene producing 385 bp fragment size has been described as diagnostic to *Lr68*

(Fossel *et al.,* 2012). Better utilization of available genetic resistance resources of wheat to breed for improved rust resistance requires an in depth molecular and phenotypic characterization of its genetic diversity. Therefore, the present investigation was undertaken with an objective of molecular screening of slow leaf rusting APR genes *viz., Lr34, Lr46, Lr67* and *Lr68* and phenotypic analysis slow leaf rusting in 102 wheat genotypes including 100 from Indian and 2 from CIMMYT.

Material and methods

The experiment was undertaken at All India Coordinated Wheat Improvement Project, Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad (UASD) during 2014-15 wheat cropping season. A total of 100 diverse wheat genotypes collected from different parts of the country under the initiative of CRP-NUE project in India and two from CIMMYT were obtained for molecular and phenotypic analysis for slow leaf rusting (Table 1). The tightly linked molecular markers (STS/SSR) for slow leaf rusting APR genes *viz., Lr34, Lr46, Lr67* and *Lr68* were used to characterize the genotypes. Genomic DNA extraction was extracted from fresh leaves using cetyltrimethylammonium bromide (CTAB) method (Allen *et al.,* 2006) with little modifications. Polymerase Chain Reactions (PCR) were performed by using a protocol appropriate for pair of primers. DNA amplification was performed in 20 µl reaction mixture.

The contents of the reaction mixture are given in Table 2. The thermo profile for the PCR reaction of different primer combination was set as shown in Table 3. The PCR products were mixed with 2 ìl of loading dye (0.25% bromophenol blue with 40% sucrose) and were loaded into each well and separated on 2 per cent agarose gel using 1X TAE buffer of pH 8.0 containing ethidium bromide. After electrophoresis gel viewed under UV-transilluminator (JH-Bio). The tightly linked marker for the respective gene of interest specific amplicon size was observed on the agarose gel (Plate 1). The STS marker *csLV34* for *LR34* gene was codominant in nature, amplified 150 bp for the presence and 200 bp for the absence of gene. The SSR marker *Xwmc44* for *Lr46* gene was multiple allelic in nature, amplified 242 bp for the presence and other than 242 bp were considered as absence of gene. The SSR marker *Xcfd71* for was codominant in nature, amplified 216 bp for the presence and 190 bp for the absence of the gene. The STS marker csGS for *Lr68* was dominant in nature, amplified 385 bp PCR product for the presence and no product amplification for the absence of the gene. The marker fragment amplification of respective slow rusting genes after PCR and electrophoresis captured under UV-trans illuminator shown in plate 1.

The set of 102 wheat genotypes were field tested for slow leaf rusting components during 2014-15 cropping season. The field experiment was layed out in augumented design, in a plot size of 0.8 m^2 (1 m length of 4 rows with 0.20 cm between rows). Susceptible checks were planted after every ten genotypes and all around the experimental plots using the universal susceptible varieties like Lal Bahadur, Agra Local, and Local Red. At boot leaf stage of the crop, field was maintained under irrigation and after a day the suspension of mixture of pathotypes of leaf rust was sprayed on the genotypes. Five plants were randomly selected in each plot and tagged.

Infection types and disease severity of leaf rust was recorded at an interval of seven days by following Loegering scale. Average coefficient of infection (ACI) was calculated by multiplying the per cent infection and response value, assigned to each infection type, as per Loegering scale (Joshi *et al.,* 1988). The "Area Under Disease Progress Curve" (AUDPC) was calculated by using the formula suggested by Wilcoxson *et al.* (1975).

Results and discussion

The adult plant leaf rust infection data from the field study during 2014-15 among the 102 test cultivars were categorized as R, MR, MS and S types based on infection types given by Joshi *et al.,* 1988 (Fig. 1). Large number of genetotypes (64 cultivars) displayed 'S' type leaf rust response with diseases severity ranging from 10 to 100 per cent. The genotypes with resistant reaction response 'R' was observed in 13 genotypes, while 'MR' and 'MS' type of leaf rust response was observed in 6 cultivars and 19 cultivars respectively. Thirteen genotypes displayed no incidence of leaf rust while 3 have shown in the class 81 to 100 per cent leaf rust severity. Adult plant leaf rust parameters in the material was ranging from zero to 100S for final leaf rust response, zero to 77.3 for the mean ACI and zero to 1337 for AUDPC.

Frequency distribution of slow leaf rusting APR genes *Lr34,* Lr46, Lr67 and *Lr68* in wheat shown in Fig. 2 of the 102 investigated genotypes, *Lr34* found in 8 genotypes with the presence of 150-bp fragment representing a frequency of 7.8 per cent of cultivars, in a similar experiment Priyamvada *et al.* (2009) found *Lr34* in approximately 20 per cent of the entries. Fifty per cent of the genotypes under investigation were found to have *Lr46* produced the fragment size of 242 bp, indicating the slow rusting gene *Lr46* to be more common than *Lr34, Lr67* and *Lr68* in the present material.

The more frequent presence of *Lr46* in Indian wheat germplasm is mainly due to the use of cultivars such as PBW343, Attila *etc*. in our wheat breeding programs (Sivasamy *et al.,* 2014). *Lr67* marker (*Xcfd71*) fragment 214 bp was observed to be the least frequent (3.9%) slow rusting gene while, *Lr34* (7.8%), *Lr46* (50%) and *Lr68* (15.7%) were found more frequently in the present material. The more frequent presence of *Lr46* in Indian wheat germplasm is mainly due to the use of cultivars such as *PBW343*,

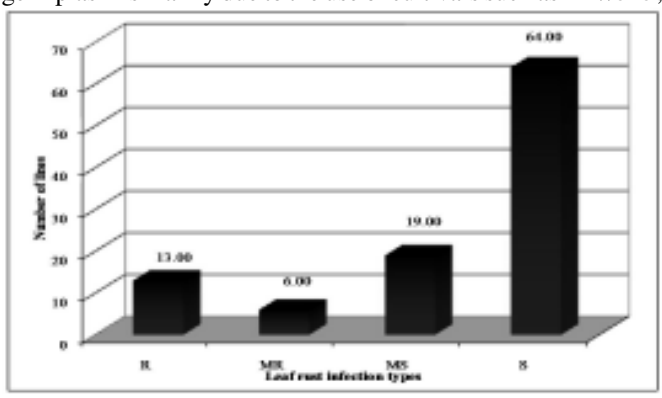
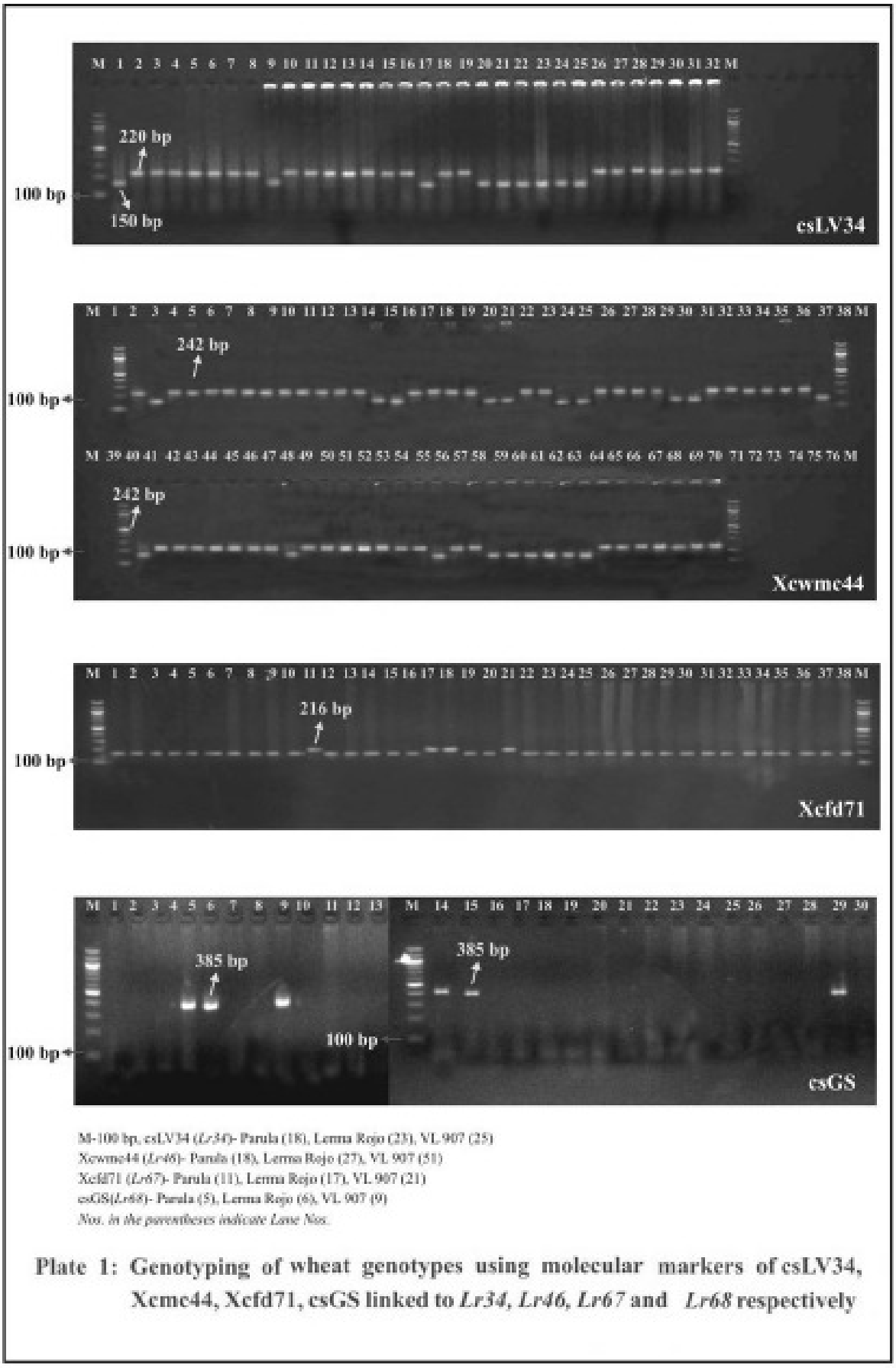


Fig 1. Phenotypic distribution of the leaf rust infection types in wheat



M-100 bp, csLV34 (*Lr34*)- Parula (18), Lerma Rojo (23), VL 907 (25)
Xcwmc44 (*Lr46*)- Parula (18), Lerma Rojo (27), VL 907 (51)
Xcfd71 (*Lr67*)- Parula (11), Lerma Rojo (17), VL 907 (21)
csGS(*Lr68*)- Parula (5), Lerma Rojo (6), VL 907 (9)
Nos. in the parentheses indicate Lane Nos.

Plate 1: Genotyping of wheat genotypes using molecular markers of csLV34, Xcmc44, Xcfd71, csGS linked to *Lr34, Lr46, Lr67* and *Lr68* respectively

Table 1. Indian wheat genotypes and two from CIMMYT used for tracking of slow leaf rusting minor genes

Sl. No.	Genotype	Pedigree	Sl. No.	Genotype	Pedigree
1	GW322	PBW 173/GW 196	52	KRL19	PBW255/KRL1-4
2	Parula	FKN/3/2*Frontana//KENYA 350 AD.9C.2/ Gabo 55/4/Bluebird/Chanate	53	PBW222	NP890/HD2160
3	RL6077	THATCHER*6/PI-250413	54	HI1544	HD 2402/HW 3007
4	NIAW1415	GW 9506/PRL//PRL	55	HD2643	VEE'S'/HD2407//HD 2329
5	HD2932	KAUZ/STAR//HD2643	56	DL803-3	HUW 202/K 7537/MUTANT OF HD2160
6	NI917	C591*CHARTER (EX-73)	57	MP1202	POCIS/3/KAUZ82.BOW//KAUZ
7	HD2189	HD 1963/HD 1931	58	HW2045	HD 2402 *6/SUNSTAR *6/C-80-1
8	NP890	GAZA (DR)/2*C 281	59	HPW155	BT 2549/FATH
9	DWR195	BONMARA -105-7	60	WR544	KALYANSONA/HD1999//HD2204/DW38
10	RAJ1482	NAPO-TOB 'S78156/KAL-BB	61	HD2864	DL 509-2/DL 377-8
11	NIAW34	CNO79/PRL"S"	62	RAJ3765	HD 2402/VL639
12	MACS2846	CPAN 6079/MACS 2340.	63	RAJ3777	RAJ3160/HD2449
13	NP839	GB-AUS/N14	64	K53	SEL.LOCAL OF JHANSI
14	Raj4037	DL788-2/RAJ3717	65	Kenphad39	ENPHAD 25'S'
15	K7410	K812'S'/KALYANSONA	66	K68	NP773/K13
16	UP301	LR.*SON.64	67	DWR162	KVZ/BUHO//KAL/BB
17	DBW39	ATTILA/HUI	68	HW517	BB-CC/CIANO'S'*//NO66-PI62
18	WH542	JUP/BJY"S"//URES	69	NP818	DO/E518//SPP/NP114/3/WIS245'S'
19	VL401	PENJAMO (TRIGOENANO) SELECTION	70	IWP72	E 5606/2* KS
20	K8027	NP875/4/N10B/Y53//Y50/3/KT54B/5/2*K852	71	GW366	DL 802-3/GW 232
21	VZ804	CPAN 3018/CPAN 3004//PBW 65	72	Lal Bahadur	S 54723 *RS 31-1 ML 293 BB*KAL2ML319CNO-KAL*CD1 (KAL-INIA*INIA-BB) ML328BB-KAL2ML408 RON-CHA* KAL-NOR67 ML 414 TOB-INIA*KAL
22	VL829	IBWSN 149/CPAN 2099	73	UP262	S 308/BJ 66
23	Kharchia65	KHARCHIA LOCAL/EG 953	74	K9006	CPAN1687/HD2204
24	MACS6222	HD 2189*2//MACS 2496	75	HS365	HS 207/SONALIKA
25	KRL1-4	KHARCHIA65/WL711	76	HYB633	EB76/E176
26	HP1102	8156 (B)/NAD63	77	WH711	ALD'S'HUAC//HD2285/3/HFW-17
27	HD2833	PBW226/HW1042//HD 2285	78	HUW 206	KAVKAZ/BUHO//KALYANSONA/BLUE BIRD
28	UP2425	HD 2320/UP 2263	79	K9107	K 8101/K 68
29	AKW1071	VEE'S7 3FLN/ACC//ANA	80	NP100	MNWH/NP 22
30	NP824	"WIS 245'S""/NP 165//NP 770/3/C 518/NP 165"	81	WH1021	NYOT95/SONAK
31	GW1139	MACS2340/IWP5070	82	HD1982	YT54/N10B//HD845
32	UP115	(NP887* E4870) UP302	83	RAJ1972	HD2195/HD2160
33	HS1138-6-4	E4870/SONALIKA	84	NP799	NP 792 'S'
34	SafedLerma	Y50//N10B/3/LR52/3*LR	85	Lerma Rojo	Y50/N 10B//L 52/3/2*LR
35	HUW468	CPAN-1962/TON I//LIRA'S'/PRL'S'	86	PBW502	W 485/PBW 343//RAJ 1482
36	Sonalika	1154-388/AN/3/YT54/N10B/LR64	87	NP111	MUTANT OF NP 4
37	HD2824	PTO-1/CNO 79/PRL/GAA/3/HD1951	88	RAJ4083	PBW 343/UP 2442//WR 258/UP 2425
38	HUW55	E 4870/HD 1982//INIA 66/HD 2189	89	NP792	WIS 245SIB/NP 165
39	NP101	MNWH/NP 22	90	J405	CNO/I NIA66//BB/3/CNO//PI/GLL
40	NP718	NP 710'S	91	MACS2971	KTR 5*2/NP 200
41	NP846	NP760/RIONEGRO	92	Narbada4	GB-AVS/N14/3/PW5//TH/NP165
42	HD2135	H41-3 (HD1962* (E4870*K 65))	93	HD2329	HD1962/E 4870/3/K 65/5/HD1553/4/UP262
43	HD1941	E 5477 * S64	94	HD2285	249/HD2150//HD2186
44	HD1949	YT54/N10B//NP 852	95	AKW381	S-308/NI5439
45	MPO1259	H41-3 (HD1962* (E4870*K 65))	96	HI784	NAPO/TOB'S'/3/8156//KAL/BB
46	VL404	KT/BAGE//FN/GU/3/ST 464 (DR)/P174106 (DR)	97	K0307	K 8321/UP 2003
47	HI617	SELECTION FROM C 306	98	GW173	TW275/7/6/1/LOK-1
48	WL711	ALD'S'HUAC//HD2285/3/HFW-17	99	PBW396	CNO67/MFD//MON"S"/3/SERI
49	HS240	AU/KAL-BB//WOP'S7 PAVON'S'	100	Kenphad25	K 58F (L.1/N14)
50	VL907	DYBR 1982-83/842 ABVD 50/VW 9365//PBW 343	101	UAS 304	SERIICEP80120//KAUZ/PBW343
51	UP2526	HD 2009/SKA//HD 2329	102	PBW343	ND/VG 7944//KAL/BB3YACO S/4/VEE# 5S

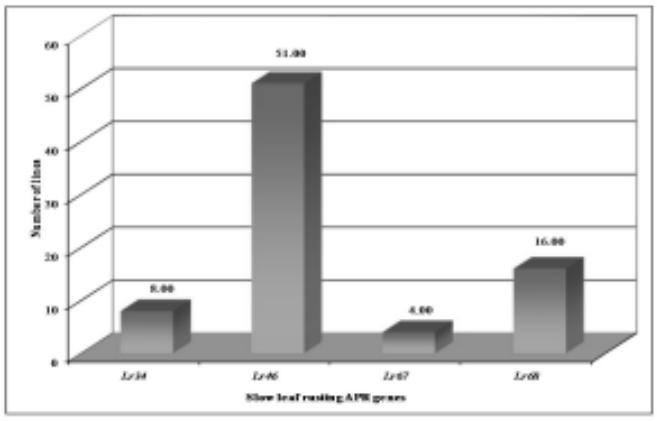


Fig 2. Distribution slow leaf rusting APR genes *Lr34, Lr46, Lr67* and *Lr68* in wheat

Attila etc. in our wheat breeding programs (Sivasamy *et al.*, 2014). The results of our study indicated that low frequency of important effective slow rusting gene *Lr34* in the tested material, and hence it is important to use the lines possessing *Lr34,* an 'undefeated gene', in the future breeding program to obtain durable leaf rust resistance. The bread *wheat v*ariety HD 2189, a slow rusting genotype has been in cultivation for 3 decades in India, which carried both *Lr34* and *Lr46*. Many of the modern day varieties have the parentage of HD 2189, got an advantage of the slow rusting genes residing in it. Some of the such important varieties are MACS 6222 carried the *Lr34* gene, a highly promising variety at PZ and HUW 55 variety under cultivation at NEPZ carried the *Lr46* gene (Table. 4).

Table 2. The contents of the PCR reaction mixture

Reaction components	Concentration	Volume (µl)
Sterile distilled water	-	13.8
Standard taq reaction buffer	(+$MgCl_2$)	10 x 2
each dNTP	2.5 mM	1
Forward primer	10 pico moles	1
Reverse primer	10 pico moles	1
Taq polymerase	1 units/µl	0.2
DNA templet	50 ng/µl	2
Total		20

leaf rust severity with three APR gene combination *Lr34, Lr46* and *Lr68.* The genotypes with three gene combination exhibited AUDPC value ranging between 22.40 and 32.60 (Table 4). This clearly indicates the genotypes with three genes combination found to be more effective than two gene combination. The Indian cultivar, HD2189 with AUDPC of 648.89 carries *Lr34* and *Lr46* genes but was susceptible to leaf rust and shows 40S under natural field condition under different races of leaf rust pathogen. This study indicates that the gene combination present in the background of variety HD2189 is relatively less effective.

The high levels of resistance in some genotypes without *Lr34* may be caused by various gene combinations not fully characterized herein. Besides *Lr34*, several APR genes have been reported including *Lr12, Lr13, Lr22a, Lr22b, Lr35, Lr37, Lr46, Lr67* and *Lr68.* Sivasamy *et al.* (2014) discussed in his research paper that, *Lr13* which originated from South America germplasm, was commonly found in wheat germplasm worldwide. Despite being defeated, *Lr13* in combination with other APR genes may provide an acceptable level of field resistance. It was reported in six-monthly newsletter Mehtaensis (Anon, 2015b), GW322 carried

Table 3. The thermo profile for the PCR reaction

Steps	*Lr34 (csLV34)*			*Lr46 (Xwmc44)*			*L67 (XcfD71)*			*Lr68 (csGS)*		
	Temperature (°C)	Time in seconds	Cycles	Temperature (°C)	Time in seconds	Cycles	Temperature (°C)	Time in seconds	Cycles	Temperature (°C)	Time in seconds	Cycles
Initial denaturation	94	180	1	94	180	1	94	180	1	94	180	1
Denaturation	94	60	36	94	60	36	94	60	36	94	60	36
Annealing	55	60		61	45		60	45		58	50	
Extension	72	60		72	45		72	45		72	50	
Final extension	72	7 min	1	72	7 min	1	72	7 min	1	72	7 min	1
Hold	4	Forever		4	Forever		4	Forever		4	Forever	

Three gene combinations was observed in three genotypes, Parula and Lerma Rojo carried *Lr34, Lr46* and *Lr68,* while *VL907* carried *Lr34, Lr46* and *Lr67.* Eleven genotypes were observed to have a combination of two of the four genes under investigation. None of the genotypes was found to carry all the four genes *viz., Lr34, Lr46, Lr67* and *Lr68.* HD2189 and UP2526 carried the combination of *Lr34* and *Lr46,* GW1139 and Sujata (HI 617) with the combination of *Lr46* and *Lr67* and six genotypes *viz.,* MACS2846, NP 718, NP 818, NP 846, K 9006 and UP262 were carried the gene combination of *Lr34* and *Lr68.* All these two gene combinations have shown AUDPC value ranging from 63.88 to 544 except UP2526 was found to be immune. The genotypes with visible leaf rust infection were considered to be lacking major effective resistant genes for the prevailing pathotypes, among which, VL907 with three APR gene combination of *Lr34, Lr46* and *Lr67* recorded lowest final leaf rust severity score of 5 percent, followed by Parula and Lerma Rojo of 10 per cent final *Lr13* and also reviewed by Tomar *et al.* (2014). This may be the possible reason that, GW322 confirmed for *Lr46* in our study had shown almost resistant phenotype with low AUDPC (208.00) and ACI (12), despite carrying ineffective *Lr13*, but since it can interact positively with *Lr46. Lr34 i*s also known to interact with seedling or major genes (Sivasamy *et al.,* 2014).

Conclusion: In thist study, molecular markers were utilized to validate *Lr* genes in different 102 wheat genotypes. Validated markers can be easily utilized in marker assisted selection (MAS) for the early generation of selection of desirable plants to accumulate more number of slow rusting genes, which would enhance the resistance of the genotypes/lines and provide durable resistance. The outcome of the investigation emphasizes the utilization of genotypes, VL907, Parula and Lerma Rojo, which carried multiple minor genes with low AUDPC and ACI. These genotypes may be utilized in the resistance breeding prgramme in order to have broad spectrum durable leaf rust resistance.

Table 4. List of wheat cultivars assessed for leaf rust parameters and slow leaf rusting APR genes *Lr34, Lr46, Lr67 and Lr68*

Sl. No.	Genotypes	Final response	ACI	AUDPC	Lr34 (csLV34)	Lr46 (xwmc44)	Lr67 (Xcfd71)	Lr68 (csGS)	Sl. No.	Genotypes	Final Response	ACI	AUDPC	Lr34 (csLV34)	Lr46 (xwmc44)	Lr67 (Xcfd71)	Lr68 (csGS)
1	AKW 1071	60S	22.07	308.80	-	-	+	-	52	MACS 6222	0	0.00	0.00	+	-	-	-
2	AKW 381	50S	34.67	568.00	-	+	-	-	53	MP 1202	20S	8.00	96.00	-	+	-	-
3	DBW 39	10MS	3.31	40.96	-	-	-	-	54	MPO 1259	5MS	1.87	22.40	-	-	-	-
4	DL 803-3	20S	20.67	344.00	-	-	+	-	55	NARBADA 4	60S	44.00	696.00	-	+	-	-
5	DWR 162	40S	31.07	548.80	-	+	-	-	56	NI 917	20MR	3.47	41.60	-	+	-	-
6	DWR 195	60S	54.67	896.00	-	-	-	-	57	NIAW 34	TMS	0.91	10.88	-	-	+	-
7	GW 1139	10MS	3.97	63.68	-	+	-	+	58	NP 100	30S	22.67	376.00	-	+	-	-
8	GW 173	0	0.08	1.28	-	-	-	-	59	NP 101	20S	14.40	228.80	-	-	-	-
9	GW 366	10S	2.93	38.40	-	+	-	-	60	NP 111	40S	56.00	912.00	-	-	-	-
10	HD 1941	40S	17.33	248.00	-	+	-	-	61	NP 718	20S	12.07	188.80	-	+	+	-
11	HD 1949	20S	10.00	160.00	-	+	-	-	62	NP 792	60S	50.67	816.00	-	-	-	-
12	HD 1982	5S	6.00	96.00	-	+	-	-	63	NP 799	60S	46.00	760.00	-	+	-	-
13	HD 2135	5MS	0.91	10.88	-	-	+	-	64	NP 818	40S	21.27	316.00	-	+	-	-
14	HD 2285	10S	14.00	224.00	-	-	-	-	65	NP 824	20S	10.00	168.00	-	-	-	-
15	HD 2329	0	0.00	0.00	-	-	-	-	66	NP 839	40S	20.67	304.00	-	-	+	-
16	HD 2643	10S	4.92	62.72	-	+	-	-	67	NP 846	60S	33.00	540.00	-	+	+	-
17	HD 2824	60S	23.20	316.80	-	+	-	-	68	NP 890	5MS	4.80	76.80	-	+	-	-
18	HD 2833	0	0.00	0.00	-	+	-	-	69	PBW 222	10S	11.47	193.60	-	+	-	-
19	HD 2864	40S	18.37	240.32	-	-	-	-	70	PBW 396	20MS	9.89	176.32	-	-	-	-
20	HD 2932	5MS	0.91	10.88	-	-	+	-	71	PBW 502	40S	22.27	369.60	-	+	-	-
21	HI 1544	40MS	17.60	294.40	-	-	-	-	72	RAJ 1482	40S	42.67	720.00	-	-	-	-
22	HI 784	TS	0.40	7.20	-	+	-	-	73	RAJ 1972	0	2.29	40.32	-	-	-	-
23	HP 1102	5MR	2.80	44.80	-	+	-	-	74	RAJ 3765	0	0.75	10.88	-	-	-	-
24	HPW 155	0	0.00	0.00	-	+	-	-	75	RAJ 3777	10MS	4.21	56.32	-	-	-	-
25	HS 1138-6-4	80S	77.33	1256.00	-	-	-	-	76	Raj 4037	0	0.00	0.00	-	+	-	-
26	HS 240	5MS	0.69	8.32	-	+	-	-	77	RAJ 4083	0;	0.00	0.00	-	+	-	-
27	HS 365	0;	0.00	0.00	-	+	-	-	78	SAFED LERMA	60S	52.67	856.00	-	+	-	-
28	HUW 206	60S	40.00	632.00	-	-	-	-	79	SONALIKA	40S	40.00	672.00	-	-	-	-
29	HUW 468	5MR	3.01	49.28	-	-	-	-	80	Sujata (HI 617)	10S	8.00	124.80	-	+	-	+
30	HUW 55	10MS	6.53	113.60	-	+	-	-	81	UP 115	20S	13.44	241.28	-	+	-	-
31	HW 2045	5MS	5.23	88.32	-	-	-	-	82	UP 2425	20S	21.33	352.00	-	-	+	-
32	HW 517	60S	42.67	704.00	-	+	-	-	83	UP 2526	0	0.00	0.00	+	+	-	-
33	HYB 633	40S	18.67	284.00	-	-	-	-	84	UP 262	30S	33.33	544.00	+	-	+	-
34	IWP 72	5MS	5.23	88.32	-	-	-	-	85	UP 301	10S	3.67	44.00	-	+	-	-
35	J 405	20S	13.67	220.00	-	+	-	-	86	VL 401	10MR	2.51	44.48	-	+	-	-
36	K 0307	TMR	0.67	8.00	-	-	-	-	87	VL 404	20S	21.33	352.00	-	-	-	-
37	K 53	40S	36.67	600.00	-	-	-	-	88	VL 804	5S	1.12	13.44	-	-	-	-

Contd....

Sl. No.	Genotypes	Final response	ACI	AUDPC	Lr34 (csLV34)	Lr46 (xwmc44)	Lr67 (Xcfd71)	Lr68 (csGS)	Sl. No.	Genotypes	Final Response	ACI	AUDPC	Lr34 (csLV34)	Lr46 (xwmc44)	Lr67 (Xcfd71)	Lr68 (csGS)
38	K 68	60S	28.40	452.80	-	+	-	-	89	VL 829	0	3.47	48.00	-	+	-	-
39	K 7410	40S	36.67	600.00	-	+	-	-	90	VL 907	5MS	1.87	22.40	+	+	-	+
40	K 8027	10MS	4.29	57.60	-	-	+	-	91	WH 1021	10S	7.07	99.20	-	-	-	-
41	K 9006	40S	16.80	242.40	-	+	+	-	92	WH 542	10S	8.00	124.80	-	-	-	-
42	K 9107	40S	15.25	211.84	-	-	-	-	93	WH 711	40S	24.72	400.64	-	-	+	-
43	KENPHAD 25	40S	36.67	600.00	-	-	-	-	94	WL 711	5S	8.80	151.20	-	+	-	-
44	KENPHAD 39	80S	72.67	1192.00	-	-	-	-	95	WR 544	20S	14.80	240.00	-	+	-	-
45	KHARCHIA 65	30S	40.67	656.00	-	-	-	-	96	Agra local	100S	60.37	1337.80	-	-	-	-
46	KRL 1-4	5MR	1.87	33.60	-	+	-	-	97	GW322	20S	12.00	208.00	-	+	-	-
47	KRL 19	40S	36.67	600.00	-	+	-	-	98	PBW343	30S	22.22	405.20	-	-	-	-
48	LAL BAHADUR	100S	60.67	984.00	-	-	-	-	99	UAS 304	30S	22.22	405.33	-	-	-	-
49	Lermo Rojo	10MS	1.65	19.84	+	+	-	+	100	Parula	10MS	2.66	32.00	+	+	-	+
50	MACS 2846	20S	9.33	144.00	-	+	+	-	101	RL6077	40S	11.48	201.78	-	-	+	-
51	MACS 2971	0;	0.00	0.00	+	-	-	-	102	HD2189	40S	28.59	648.89	+	+	-	-

(+) - Presence of gene, (-) - Absence of gene

References

Allen, G. C., Flores-Vergara, M. A., Krasynanski, S., Kumar, S. and Thompson, W. F., (2006), A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nat. Protocols* 1: 2320–2325. pmid:17406474.

Anonymous, (2015), Production Crops. Food and Agriculture Organization of the United Nations, Statistics division. http: //faostat3. fao. org/ download/Q/QC/E downloaded : 2015-07-2.

Anonymous, (2015a), Agricultural Statistics at a Glance 2014, Directorate of Economics and Statistics, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India 2015, LXXII (2): 2.

Anonymous, (2015b), Pathotype distribution of Puccinia species, Directorate of Wheat Research, Regional Station, Shimla, Himachal Pradesh, India. *Mehtaensis,* 35 (2): 3-6.

Bhardwaj, S. C., Prashar, M., Kumar, S., Jain, S. K. and Datta, D., (2005), *Lr*19 resistance in wheat becomes susceptible to *P. triticina* in India. *Plant Disease,* 89: 1360.

Ellis, J. G., Rohit, M., Raja, K., Peter, N. D., Helen, M., Greg, L., Wolfgang, S. and Laguda, E., (2007), Wheat rust resistance research at CSIRO. *Australian J. Agric. Res.,* 58 : 507–511.

Foessel, H. S. A., Singh, R. P., Huerta-Espino, J., Rosewarne, G. M., Periyannan, S. K., Viccar, L., Calvo-Salazar, V., Lan, C. and Lagudah, E. S., (2012), *Lr*68: a new gene conferring slow rusting resistance to leaf rust in wheat. *Theor. Appl. Genet.,* 124: 1475-1486.

Hiebert, C. W., Thomas, J. B., McCallum, B. D. and Somers, D. J., (2010), Genetic mapping of the wheat leaf rust resistance gene *Lr*60 (*Lr*W2). *Crop Sci.,* 48 : 1020–1026.

Jelena, B., Boskovim, M. and Prijin, E., 2009, Accumulations of genes for durable resistance to wheat leaf rust pathogen, *J. Agric. Sci.,* 53(3): 163-172.

Joshi, L. M., Singh, D. V. and Srivastava, K. D., (1988), Manual of Wheat Disease, Malhotra Publishing House, New Delhi, p. 75.

Lagudah, E. S., McFadden, H., Singh, R. P., Huerta-Espino, J., Bariana, H. S. and Spielmeyer, W., (2006), Molecular genetic characterization of the Lr34 / Yr18 slow rusting resistance gene region in wheat. *Theor. Appl. Genet.,* 114: 21-30.

Nayar, S. K., Bhardwaj, S. C. and Jain, S. K., (2002), Fungal diseases of wheat-rusts. In: Diseases of field crops. Gupta V. K., Paul Y. S. (Eds) Indus Publishing Company, New Delhi, India. pp. 88

Priyamvada, Tiwari, R., Saharan, M. S., Chatrath, R., Priyanka, S. and Mishra, B., (2009), STS marker based tracking of slow rusting *Lr*34 gene in Indian wheat genotypes. *Indian J. Biotechnol.,* 8: 207-213.

Schnurbusch, T., Bossolini, E., Messner, M. and Keller, B., (2004), Tagging and validation of a major quantitative trait locus for leaf resistance and leaf tip necrosis in winter wheat cultivar Forno. Phytopathology, 94: 1036-1041.

Sivasamy, M., Aparna, M., Kumar, J., Jayaprakash, P. and Vikas, V. K., (2014), Phenotypic and molecular confirmation of durable adult plant leaf rust resistance (APR) genes *Lr*34+, *Lr*46+ and *Lr*67+ linked to leaf tip necrosis (LTN) in select registered Indian wheat (*T. aestivum*) genetic stocks. *Cereal Res. Commun.,* 42: 262-273.

Suenaga, K., Singh, R. P., Huerta-Espino, J. and William, H. M., (2003), Microsatellite markers for genes *Lr*34/*Yr*18 and other Quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. *Phytopathology,* 93: 881-890.

Wilcoxson, R. D., Skovmand, B. and Atif, A. H., (1975), Evaluation of wheat cultivars for ability to retard development of stem rust. *Ann. Appl. Biol.,* 80: 275-281.