

Characterization of adult plant leaf rust resistance gene *Lr34* in Indian wheat genotypes using an STS marker

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Abstract

Genetic resistance is the safest, economical and most effective method of protecting the crop. Durable sources of leaf rust resistance often express a partial, growth stage- specific phenotype referred to as adult plant resistance. One such effective and durable leaf rust adult plant resistance gene deployed world over has been *Lr34*. Because *Lr34* works mostly in adult plants and in combination with other rust resistance genes (such as, *Lr34/Yr18*, *Lr46/Yr29*, and *Yr30*), it is difficult to determine its presence in wheat genotypes. Therefore, there is need to look an alternate method of detecting this resistance gene into the germplasm. Consequently upon availability of a diagnostic marker for *Lr34*, screening for effective allele of the gene was carried out in Indian wheat genotypes. Among 300 elite wheat lines screened, 60 showed presence of the allele. The resulting genotypes were categorized on the basis of wheat agro climatic zones as well as the centers responsible for developing these genotypes. Amongst the zones, North Western Plains Zone is credited with maximum number of genotypes possessing *Lr34* gene (33.3%) followed by North Eastern Plains Zone (28.3%), Peninsular Zone (13.3%) and Northern Hill Zones (11.6%). Remaining areas, which included Central Zone and Southern Hills Zone showed 13.2% genotypes amongst *Lr34* positives. Considering center wise wheat breeding, PAU, Ludhiana and IARI, New Delhi contributed 16.6% and 11.6% genotypes possessing *Lr34* allele, respectively. Identified genotypes can be utilized for further enhancement of genetic resistance in the respective zones and at the breeding centers to provide much needed durable leaf rust resistance.

Key words: APR, leaf rust, STS markers

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Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop in India, cultivated worldwide with annual production of 94.88 MMT (million metric tons) <http://www.usda.gov/oce/commodity/wasde/latest.pdf> in previous year, which is the second largest wheat producer after China. Leaf (or brown) rust caused by *Puccinia triticina* Erikss. is an important and most widely distributed foliar disease of wheat world over. It has potential of causing significant yield losses in India as it occurs in all the wheat growing areas. The rust accelerates foliar senescence, reducing cumulative light interception. Shifting virulence pattern, evolution of new virulences through migration, mutation, or recombination and selection of existing virulences, is a continuous challenge to breed leaf rust resistant varieties.

Any systematic breeding program aimed at developing cultivars with long lasting resistance depends primarily upon availability of diverse and well characterized effective resistance genes. Linked DNA markers show promise for achieving such combinations; however, a national and regional strategy is necessary to achieve a long- term success. The most promising long- term management strategy is to breed and deploy cultivars carrying durable resistance based on minor, slow rusting

genes with additive effects. CIMMYT studies show that combining 4-5 such genes result in a high level of resistance, comparable to immunity. Traditional genetic and molecular mapping studies have demonstrated high genetic diversity for such minor genes. Significant efforts have gone into identifying closely linked markers that could be used effectively in marker- assisted selection. Targeted incorporation of durable resistance into mega cultivars grown in Asia by using a 'single- bulk breeding scheme' is being pursued at CIMMYT with encouraging result (Singh *et al.*, 2004). The *Lr34* gene interacts with other minor/major genes providing effective or durable leaf rust resistance to cultivars. The slow rusting resistance to leaf rust is common in spring wheat germplasm. At least 10-12 slow rusting genes are involved in the adult plant resistance of CIMMYT wheat and also identified lines, such as Amadina where *Lr34* is absent, but whose level of resistance is high. Therefore, it is believed that durable resistance is feasible even in the absence of *Lr34*. This is the case of variety Pavon 76, where it was identified a new gene *Lr46* for slow rusting on chromosome 1BL (Singh *et al.*, 1998, William *et al.*, 2003). Gene *Lr46* also functions in a similar manner to *Lr34* (Martinez *et al.*, 2001). When susceptible cultivars display 100% leaf rust severity, cultivars with only *Lr34* display approximately 40% severity; cultivars with *Lr34* and one or two additional minor genes display 10-15% severity; and cultivars with *Lr34* and two or three additional genes display 1-5%

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severity (Singh *et al.*, 2004). Both *Lr34* and *Yr18* has long been recognized as carrying the most durable forms of resistance. Other rust resistance genes may be more effective against specific pathotypes of rust, but their effectiveness is eventually overcome by new virulences. Because *Lr34* and *Yr18* are mostly effective in adult plants and in combination with other rust resistance genes, it is difficult for scientists to determine if they are present when a wheat plant displays resistance, or if resistance is caused by other resistance genes.

Rubiales and Niks (1995) studied the infection process and indicated that slow rusting resistance due to gene *Lr34* was based on a reduced rate of haustorium formation in early stages of infection, in association with relatively little or no cell necrosis. Observations indicate a different mechanism for *Lr34* - based slow rusting compared with hypersensitivity, which is associated with the action of race-specific resistance. In cases where response of plant to infection is not sufficient to infer presence of the resistance gene(s), DNA markers are more accurate in determining such gene(s). Consequent upon availability of a diagnostic marker for *Lr34*, marker assisted screening for effective allele of the gene was carried out in Indian wheat genotypes.

Materials and methods

A set of 300 wheat genotypes mainly consisting of advanced varietal test entries and the popular cultivars of India (list available with the authors which can be supplied on demand) were subjected to identification of *Lr34* gene (effective allele). The genotypes were procured from the Germplasm Resource Unit of the DWR, Karnal.

Lagudah *et al.*, (2006) reported a marker *csLV34*, which generates fragment of size 150bp in *Lr34* carrying genotypes and 229bp in non -*Lr34* carrying genotypes. The reported DNA marker *csLV34* with primer sequence forward (5'GTTGGTTAAGACTGGTGATGG3') and reverse (5'TGCTTGCT ATTGCTGAATAGT3') were got synthesized from M/s Bangalore Genei to screen for the presence of *Lr34* /*Yr18* gene in selected wheat genotypes. Genomic DNA of genotypes was extracted using liquid Nitrogen with CTAB method (Doyle *et al.*, 1987), amplified by reported *csLV34* STS marker.

Polymerase chain reaction (PCR) was performed in 25µl of 10X PCR buffer, 2.0 µl of dNTPs (2.5mM each dNTPs) & 1 µl each of forward & reverse primers (100 pmol/ µl) and 100 ng of DNA in a S 1000™ Thermal cycler – Biorad. Primer annealing temperature was 55°C. Products of STS primers used were resolved on 2.5% high resolution agarose (Bangalore Genei) with 1X TAE buffer and visualized by ethidium bromide staining.

Results and discussion

Lr34 is a durable rust resistance gene utilized in several countries (Singh *et al.*, 1991). It is also a non- hypersensitive adult plant resistance gene which has been characterized at molecular level and for which a diagnostic, co-dominant marker is available (Lagudah *et al.*, 2006). Besides limited number of hypersensitive seedling resistance genes (*Lr24*, *Lr28*, and *Lr35*) there are few durable ones (*Lr34*, *Lr46*, *Lr67*) effective in India. Adult plant resistance gene for leaf rust such as *Lr34* requires special skills in selection because of confounding effects of other background genes. The utility of MAS becomes quite imperative in such cases.

To ascertain the Indian wheat genotypes possessing *Lr34* gene, 300 genotypes mainly consisting of advanced varietal test entries of the last decade and the popular cultivars of India, were screened with *csLV34* STS marker (Lagudah *et al.*, 2006). The result shows presence of the gene in 60 genotypes. The genotypes with *Lr34* have been depicted in Table 1 along with their pedigree details, respective adaptive zone and centers at which these were developed.

As wheat improvement program under the umbrella of All India Coordinated Wheat and Barley Improvement Program is carried out for six agro climatic zones, the genotypes with *Lr34* have been categorized on the basis of the zones for which they were recommended for evaluation / cultivation. This carries importance since a varietal development program prefers to use genotypes adapted to the respective zones in their crossing block. Amongst the zones, North Western Plains Zone is credited with maximum number of genotypes possessing *Lr34* gene (33.3%) followed by North Eastern Plains Zone (28.3%), Peninsular Zone (13.3%) and Northern Hill Zones (11.6%). Rest others, which included Central Zone and Southern Hills Zone showed 13.2 per cent genotypes amongst *Lr34* positives.

All major wheat breeding centers are involved in developing lines possessing rust resistance. Therefore categorization of findings (genotypes with *Lr34* gene) has been also done on the basis of centers developing these genotypes. Amongst the centers, PAU, Ludhiana gets maximum proportions of genotypes i.e. 16.6 per cent with *Lr34* to its credit followed by IARI, New Delhi with 11.6 per cent. Rest others centers jointly own 71 per cent of the genotypes with *Lr34* as depicted in Figure 1.

Area Under Disease Progress Curve (AUDPC) data of the genotypes possessing *Lr34* gene was compiled and presented in form of a bar diagram as proportion to the most susceptible check (genotype with highest AUDPC value) as given in Figure 2. Genotypes showing resistance (indicated using green bar) prominently appear indicating most of the genotypes possessing high proportion of leaf rust resistance. As the rust pathotypic flora of Northern

and Southern India are quite distinct in terms of their virulence, Figure 2 depicts the relative performance of the genotypes against population of *P. triticina* in Southern and Northern India, respectively.

About 63% of the genotypes showed high level of resistance towards pathotypes of both South as well as North India. This means the *Lr34* gene in these genotypes played significant and effective role. However, presence of other resistance genes in the background genes enhancing

the level of resistance along with *Lr34* gene could not be ruled out. Table 1 provides the additional information about the other leaf rust resistance genes in the genotypes as identified through host-pathogen interaction.

The information presented herewith will be quite helpful in utilization of these identified genotypes for further enhancement of genetic resistance in the respective zones and at the breeding centers.

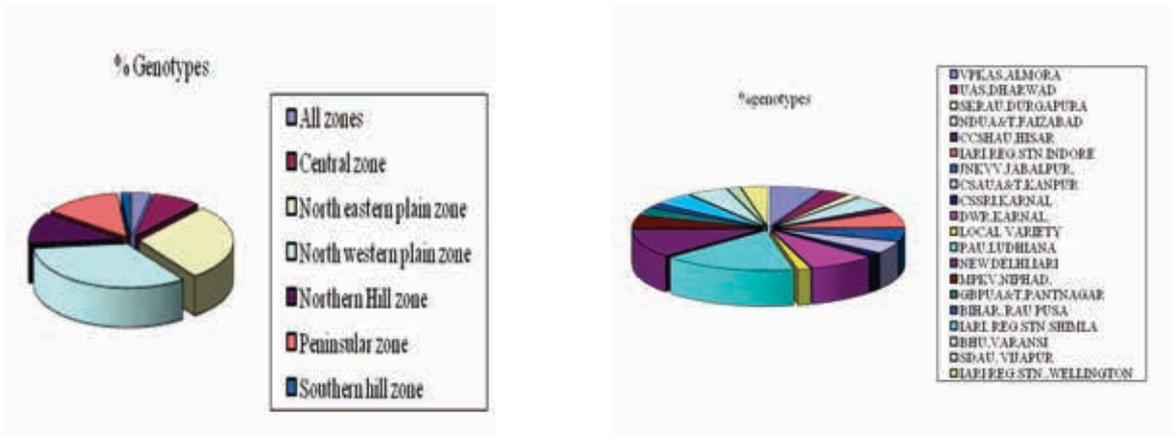


Fig 1. Distribution of *Lr34* gene carrying wheat genotypes based on (a) agro-ecological zone and (b) wheat breeding centers

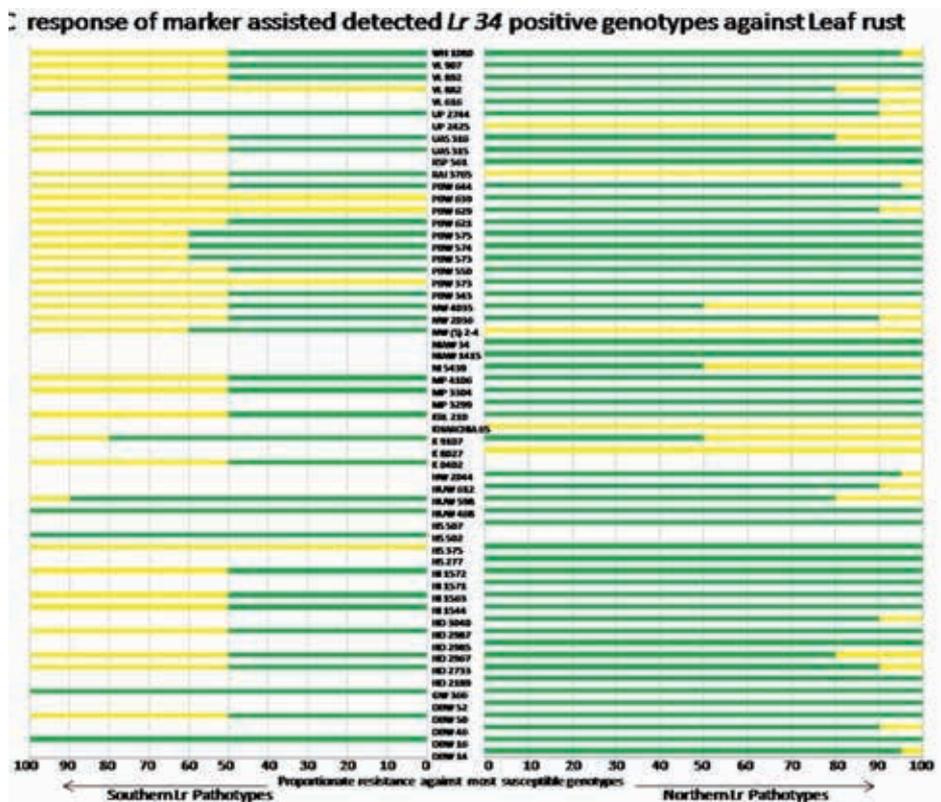


Fig 2. Response of genotypes found possessing effective alleles of *Lr34* towards pathotypic races of Northern and Southern wheat agro-ecological zones compared to the susceptible check

Table 1. Other *Lr* genes postulated in genotypes found possessing *Lr34* effective allele through marker assisted screening

S. No.	Genotype	Pedigree	Zone wise distribution	Centre wise distribution	<i>Lr</i> gene postulated
1	DBW 14	RAJ3765/PBW343	North eastern plain zone	Karnal, DWR	23
2	DBW 16	RAJ3765/WR484//HUW468	North eastern plain zone	Karnal, DWR	13,10
3	DBW 46	PBW343/INQ21	North eastern plain zone	Karnal, DWR	13
4	DBW 50	KAUZ//ALTAR84/AOS/3/MILAN/ KAUZ/4/HUITES	North western plain zone	Karnal, DWR	26
5	DBW 52	CAL/NH//H567.71/3/SERI/4/CAL/ NH//H567.71/5/2*KAUZ/6/PASTOR	North eastern plain zone	Karnal, DWR	26
6	GW 366*	DL802-3/GW232	Central zone	Vijapur, SDAU	-
7	HD 2009	LR64A/NAI60	North western plain zone	New Delhi, IARI	13
8	HD 2189*	HD1963/HD1931	Peninsular zone	New Delhi, IARI	13
9	HD 2733*	ATTILA/3/TUI/CARC//CHEN/ CHTO/4/ATTILA	North eastern plain zone	New Delhi, IARI	26
10	HD 2967	ALD/CUC//URES/HD216 OM/HD 2278	North western plain zone	New Delhi, IARI	13
11	HD 2985	PBW 343/PASTOR	North western plain zone	New Delhi, IARI	-
12	HD 2987	HI1011/HD2348//MENDOS// IWP72/DL153-2	North western plain zone	New Delhi, IARI	-
13	HD 3040	WR1027/HD2851	Peninsular zone	New Delhi, IARI	-
14	HI 1544	HINDI62/BOBWHITE/CPAN2099	Central zone	Indore, IARI.REG. STN	-
15	HI 1563	MACS2496*2/MC10	North eastern plain zone	Indore, IARI.REG. STN	-
16	HI 1571	RAJ3777/WLT277//HW2045	Peninsular zone	Indore, IARI.REG. STN	-
17	HI 1572	SKAUZ*2/FCT	Central zone	Indore, IARI.REG. STN	-
18	HS 277*	KUZ/CGN	Northern hill zone	Shimla, IARI, REG. STN.	26
19	HS 375	BB/G 11/CJ71/3/TA EST//KAL/BB CNO 79/4/CS/TH.CN//CH.CEN/3/	Northern hill zone	Wellington, IARI Reg. STN.	26
20	HS 502	ALD/PVN/5/GISVZ	Northern hill zone	Shimla, IARI, Reg. STN.	13
21	HS 507	KAUZ/MYNA/VUL//BUC/FLK/4/ MILAN	Northern hill zone	Shimla, IARI, Reg. STN.	26,1
22	HUW 468*	CPAN-1962/TONICHI-81/LIRA/ PARULA[2800]	North eastern plain zone	Varanasi, BHU	13
23	HUW 598*	WH581/HUW395//RAJ3765	North eastern plain zone	Varanasi, BHU	-
24	HUW 612	HUW202/HUW300//HUW395	North eastern plain zone	Varanasi, BHU	13
25	HW 2044	PBW226*5//SUNSTAR*6/C80-1	Southern hill zone	Wellington, IARI Reg. STN.	24
26	K 0402	HP1731/UP2425	North eastern plain zone	Kanpur, CSAUA&T	13
27	K 8027	HD1696/2*K852	North eastern plain zone	Kanpur, CSAUA&T	13
28	K 9107	K8101/K68	North eastern plain zone	Kanpur, CSAUA&T	13
29	KHARCHIA ⁶⁵	Selection from local variety	All zones	Local Variety	-
30	KRL 210	PBW65/2*PASTOR	All zones	Karnal, CSSRI	13,10
31	MP 3299	DOVE/BUC/DL788-2	Peninsular zone	Jabalpur, JNKVV	-
32	MP 3304	GW322/J485	Central zone	Jabalpur, JNKVV	-
33	MP 4106	CHIBIA/PRL11/CM65531	Central zone	Jabalpur, JNKVV	-
34	NI 5439*	NI8883/MP1055	Peninsular zone	Niphad, MPKV	-
35	NIAW 1415	GW9506/PRL//PRL	Peninsular zone	Niphad, MPKV	26
36	NIAW 34*	CNO79/PRL"S"	Peninsular zone	Niphad, MPKV	26
37	NW (S) 2-4*	NS732/NER//KAUZ	North eastern plain zone	Faizabad, NDU&T	-
38	NW 2036*	BOW/CROWN/BUC/PVN	North eastern plain zone	Faizabad, NDU&T	26,23
39	NW 4035	RAJ3785/DBW09	North eastern plain zone	Faizabad, NDU&T	23,10

S. No.	Genotype	Pedigree	Zone wise distribution	Centre wise distribution	Lr gene postulated
40	PBW 343*	ND/VG1944//KAL//BB/3/ YACO'S/4/VEE#5'S	North western plain zone	Ludhiana, PAU	26
41	PBW 373*	ND/VG1944//KAL//BB/3/ YACO'S/4/VEE#5'S	North western plain zone	Ludhiana, PAU	26
42	PBW 550*	WH594/RAJ3856//W485	North western plain zone	Ludhiana, PAU	26
43	PBW 573*	WH594/RAJ3814	North western plain zone	Ludhiana, PAU	26
44	PBW 574*	HD2643//CS/Ae.sq	North western plain zone	Ludhiana, PAU	26
45	PBW 575*	PBW343/HUW235	North western plain zone	Ludhiana, PAU	-
46	PBW 621	KAUZ/ALTAR84/AOS/3/MILAN/ KAUZ/4/HUITES	North western plain zone	Ludhiana, PAU	10
47	PBW 629	CHIL/ALD//JUP/COC/3/PVN/4/ GEN/5/KAUZ/CHO//BUC	North western plain zone	Ludhiana, PAU	26
48	PBW 639	HW2019/PBW49	North western plain zone	Ludhiana, PAU	13
49	PBW 644	PBW175/HD2643	North western plain zone	Ludhiana, PAU	23,1
50	RAJ 3765*	HD2402/VL 639	North western plain zone	Durgapura, SKRAU	13,10
51	RSP 561	HD2637/Ae. crassa// HD2687	North eastern plain zone	Rau Pusa, BIHAR	26,23
52	UAS 315	DWR163/DHARWAD DRY//DWR 225	North western plain zone	Dharwad,UAS	26
53	UAS 316	PASTOR/ DHARWAD DRY/ DWR39	Peninsular zone	Dharwad,UAS	-
54	UP 2425*	HD2320/UP2263	North western plain zone	Pantnagar,GBPUA&T	26,23,1
55	UP 2744	CPAN4073/HW1085	North western plain zone	Pantnagar,GBPUA&T	26
56	VL 616*	SONALIKA/CPAN-1507[793][1441]; SONALIKA/P-46[1324][2848]	Northern hill zone	Almora,VPKAS	3
57	VL 882*	CPAN3031/PMF/MAYA/YACO	Northern hill zone	Almora,VPKAS	26,1
58	VL 892*	WH542/PBW246	Northern hill zone	Almora,VPKAS	13,10
59	VL 907	DYBR 1982-83/842 ABVD 50/VW 9365//PBW 343	North eastern plain zone	Almora,VPKAS	26,23,10
60	WH 1080	21STSAWSN151	North western plain zone	Hisar, CCSHAU	13

The asterisked marked (*),- Genotypes are revalidated reconfirmed using Lr34 marker which are already mentioned possessing effective allele (after Priyamvada et al., (2009)

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