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Evaluation of Wheat Genotypes for Seedling and Adult Plant Resistance to Leaf Rust (*Puccinia triticina*)

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ABSTRACT

Leaf rust, caused by *Puccinia triticina*. is an economically important wheat disease which occurs in all the river basin and plain areas of Nepal. Ninety-nine wheat lines that included most of the released genotypes and new advanced breeding lines were subjected to multi-pathotype evaluation of *P. triticina* both at seedling and adult plant stage at Directorate of Wheat Research (DWR), Shimla, India. Sixteen pathotypes of the pathogen were used for seedling evaluation while the two most predominant pathotypes, 121R63-1 (THTTS) and 21R55 (PHTTL) were used to screen for adult plant resistance (APR). In addition, SSR marker csLV34_Lr34sp was also used for identifying Lr34 gene in these lines. Thirty-five lines were susceptible at seedling and adult plant stages. Different combinations of seven resistance genes, viz *Lr*1, *Lr*10, *Lr*13, *Lr*19, *Lr*23, *Lr*26 and *Lr*34 were characterized by applying the gene matching technique. The most commonly detected gene was *Lr*13 (37% of the tested lines), followed by *Lr*26 (33%), *Lr*10 (32%), *Lr*23 (31%), *Lr*34 (26%), *Lr*1 (11%), and *Lr*19 (1%). Evidence of unidentified seedling resistance genes was also noticed. Thirty one lines showed APR to the two pathotypes among which 11 had susceptible response at seedling stage. Forty four lines showed APR to 121R63-1 with 20 showing susceptibility at seedling stage. Likewise, 57 lines showed APR reaction to pathotype 21R55 while 18 supported susceptible reaction at seedling stage. The information on resistance genes and race-specific adult plant resistance of the tested materials appears useful for future wheat breeding program in Nepal and promotion of varietiesto keep leaf rust below the economic threshold.

Keywords: Genotypes, Leaf rust, Pathotypes, *Puccinia triticina*, Wheat

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is the third most important crop in Nepal after rice and maize. It is occupied an area of 7,40,150 hectares with the total production of 18,56,191 metric tons and the average productivity of 2.50 t/ha [1]. On an average, it covers 22% of the total cultivated area and contributes 17.3% of cereal production in which hilly region represents 44% of the total wheat production. The majority of hills and mountain districts are often food deficit and wheat is a major crop that carries potential to increase food self sufficiency in traditional hill system where significant area remains fallow during the winter [2]. In the last decades, National Wheat Research Program (NWRP) has released high yielding wheat genotypes to cope up with the problem of food deficiency in hilly areas. At present, the wheat varieties adopted widely in the country are NL 297, Annapurna 1, Annapurna 3, BL 1022, Bhrikuti, BL 1135, Annapurna 4, BL 1473 and Gautam. The national report suggests that more than 96% of wheat cultivated area in Nepal is covered by the improved genotypes [2]. Though the area of cultivation of wheat has expanded considerably over last decade, its productivity could not be increased significantly due to several factors. Among them, leaf rust is the major diseases which cause significant yields loss in wheat crops in Terai and some parts of hills.

Leaf rust (*P. triticina*) occurs on major wheat area falling under river basin (<600m) and terai plains of Nepal. This disease was considered to be the number one disease in the late 1960s and early 70s when RR 21, Kalyansona and other local wheat cultivars were prevalent in the country [3]. The predominant pathotype of leaf rust in Nepal has been reported to be of 77-2 followed by 77-4 and 77-5 [4]. In the last couples of years, the leaf rust has been frequently observed in Kathmandu valley and some hilly districts (up to 1600m amsl) and appears to increase year by year [2]. It might be due to genetic vulnerability of varietal spectrum and the changing climatic conditions. Hence, this disease needs more attention for understanding virulence pattern of pathotypes and resistance genes present in the wheat genotypes of Nepal for developing a better breeding strategy. A research study was carried out to identify the virulence pattern of pathotypes and postulation of rust resistance genes with respect to leaf rust in wheat genotypes from Nepal.

2. MATERIALS AND METHODS

2.1. Genotypes used

Ninety-nine bread wheat genotypes were used which included the released varieties of Nepal and promising lines under testing in national trials. These genotypes were tested against different pathotypes of *P. triticina* at Regional station, DWR, Flowerdale, shimla.

2.2. Seedling Resistance Test (SRT) for leaf rust resistance gene/s postulation

A set of reference line known to possess specific *Lr* gene/s commonly occurring in wheat and standard differentials were used to postulate rust resistance gene/s. Five seeds of each genotype were clump/hill planted in an aluminum pan tray filled with sterilized

loam soil. Each tray contained 18 lines and a susceptible check Agra local. Each set of testing material had differentials which acted as a check for the purity of pathotype and behavior of common resistance gene under that set of condition.

Sixteen pathotypes of *P. triticina* were used for identifying the leaf rust resistance gene/s. The pathotypes were selected from different avirulence and virulence groups. A small quantity (20 mg) of rust urediospore was suspended in light weight non phytotoxic 4 ml paraffin oil (soltrol), mixed thoroughly and inoculated on the test genotypes and differentials. The inoculated plants were fine sprayed with water and incubated for 48 hours under saturated humid condition in a glass chamber placed under the glass house benches. The glasshouse was kept under recommended temperature (22±2ºC), humidity (60%) and day light, 15000 lux[5] The response of inoculation was recorded after 14 days . The infection type matrices of the test hosts were compared with those of the differential hosts and the resistance gene/s present in the test lines were postulated by using the gene matching technique based on Nayer *et al*., 1991.

2.3. Adult plant resistance (APR) test for leaf rust

Adult plants of all genotpyes were grown as a clump containing 5 seeds in a controlled polythene house, maintained at 24 hr 22±2ºC temperature and light intensity of about 15000 lux for 12 hours. The distance between the clumps in each row was 25 cm. Near isogenic lines (NILs) carrying known APR genes, and leaf rust susceptible wheat cultivar Agra local were also planted for comparing adult plant resistance. Three plantings, 21 day apart, were made. The adult plants were inoculated with two commonly dominated leaf rust pathotypes 77-5 (121R63-1) and 104-2 (21R55) on 43-55 growth stage (boot just visible to one half of the ear emerged) of the modified Zadok's scale [6] Seven-days-old seedlings of each test line grown in aluminum bread pans were also inoculated so that the response of seedlings and adult plants could be compared under same set of condition. The fungal inoculations consisted of spraying a set of seedlings and adult plants with 80 mg uredospores of a *P. triticina* pathotype suspended in 20 ml of soltrol (non toxic mineral oil) using an atomizer. The polythene house was kept with saturated for humidity by using a humidifier. The infection pattern in the adult plants and seedling were recorded 22 days after inoculation. APR was inferred on the basis of the reactions of the seedlings and the adult plants. APR was considered hypersensitive when necrosis was associated with the infection types (; , ;1) and non-hypersensitive when only chlorosis was observed with the infection types (2, 2+).

2.4. Determining Lr34 rust resistance genes using SSR marker

The genotypes were tested for SSR marker genotyping. DNA extractions were performed using DNA Landmarks standard protocol. DNA concentrations were measured using Hoechst dye and the quality of the DNA samples were checked on a 0.8% agarose gel. Once the DNA quality passed the quality control, the DNA samples were then used for PCR amplification with scLV34_Lr34sp SSR rust marker. For detection on the ABI 3730XL platform, fluorescently labelled M13 primers were used in the PCR reactions to anneal to the M13 tails of the forward primers. SSR was screened on tested genotypes using DLM SSR protocol on the ABI 3730XL capillary electrophoresis system. The genotyping results were scored using the Gene Mapper software V4.0. The failed samples were repeated at least once.

3. RESULTS

3.1. Seedling resistance test

A range of infection types (ITs) was displayed by the genotypes and the controls possessing known genes for resistance to *P. triticina*. The genotypes were classified into 5 resistance groups (RGs) based on seedling ITs with sixteen pathotypes of *P. triticina*.

3.1.1. RG1:

This relatively large group was divided into three sub-groups. The sub-group I comprised of 26 genotypes: Aditya, Brikuti, BL 2879, BL 3063, BL 3467, BL 3404, Munal 1, BL 3778, WK 1123, WK 1481, WK 1504, WK 1716, WK 1719, WK 1720, WK 1721, WK 1733, WK 1743, WK 1775, WK 1964, Chonte 1, BL 3960, BL 4095 and BL 4154. Most of the pathotypes (avirulent/virulent to *Lr*13) produced similar pattern on these genotypes. The patterns were similar to that of the control Egret, which carries *Lr*13. Typical of seedlings with *Lr*13, the responses were often associated with chlorosis. The sub-group II included genotypes (Rohini, WK 936, WK 1182, WK 1204, WK 1444, WK 1627, WK 1661, WK 1723, WK 1727, WK 1809, WK 1967 and Danphe 1) and responded very similarly to the sub group I, except that there were clear differences between IT responses to pathotypes 77, 104B and 106 (avirulent for *Lr*13 or *Lr*10) and 77-7, 104-2 and 162-1 (virulent to *Lr*10). The difference in response of genotypes in sub group II to these pathotypes was attributed to *Lr*10, as shown by the control Thatcher* 6/Lee. The genotypes in subgroup II were therefore postulated to carry *Lr*13+*Lr*10 (Table 1).

Table 1 Infection types of wheat genotypes in resistance group II and deferential Malakoff, Thatcher* 6/Lee, Egret with sixteen *P. triticina* pathotypes

No.	Genotypes	Sub groups	12-5	12-7	12-8	77	77-1	77—2	77-5	77-7	77-8	77-10	77A-1	104-2	104A	104B	106	162-1	postulated gene(s)
1	Bhrikuti	I	;1	3+	0;	33+	33+	33+	3+	;1	3+	3+	3	3+3+	;	33+	0;	33+	*Lr*13+
2	BL 2879	I	33+	3+	3+	0;	;1	0;	3 , 3+	;1	0;	3+	3	3+	;	0;	0;	3+	*Lr*13+
3	BL 3063	I	0;	3	3+	33+	3	3+	3+	3+	33+	3+	33+	3+ , 2	33+	0;	0;	-	*Lr*13+
4	BL 3467	I	;1	-	3+	0;	-	0;	3+	3+	3+	2	3	3+ , 12	33+	;	;- , 2	-	*Lr*13+
5	Munal 1		3	;1 ;3+	;1	12	;1	3	2, 3-	3	;1	2	33+	12	;1	0	0;	;3	*Lr*13+
6	NL 1007	I	0; 1p 3+	3+	3	3+	3+	3+	3+	0;	0;	3+	;	3+ , ;1	33+	-	0;	3+	*Lr*13+
7	BL 3778	I	3	3+	-	0;	-	33+	3+	2	0;	33+	;1	33+ , 3	;1	0	0;	3-	*Lr*13+
8	WK 1719	I	3+	; , 3	3+	3+	3+	3+	3+	0;	3+	3+	3+	3+ , 3	12	;1	0;	33+	*Lr*13+
9	WK 1720	I	2+	0;	-	3+	0	3+	3+	3+	3+	3+	3+	33+	12	2	0;	3+	*Lr*13+
10	WK 1721	I	3+	X	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	;1	33+	;1	3+	*Lr*13+
11	WK 1733	I	;1	2	0;	3+	33+	33+	3+	;1	33+	3+	33+	3+	2	0		33+	*Lr*13+
12	WK 1743	I	3+	3+	3+	;-	0	0;	3+	;1	33+	;1	0;	3+	;1	0;	;-	3+	*Lr*13+
13	WK 1775	I	3+	3+	;	0;	3+	0;	3+	33+	3+	3-	0;	3+	;	;	0;	3+	*Lr*13+
14	Chonte 1	I	33+	33+	0;	3+	2	33+	33+	;1	2	3+	3	3+, 33+	33+	0	;-	3	*Lr*13+
15	BL 3960	I	0; , 1p 2	-	0;	3	0	1p 3	3+	-	-	33+	-	-	-	0	0;	;1	*Lr*13+
16	BL 4095	I	3	2+		;1	0	33+	3+	3+	0;	33+		3	;1	-	-		*Lr*13+
17	BL 4154	I	0;		0;	33+	0	33+	3+	0;	0;		3-	33+	;-	0	-	X	*Lr*13+
18	BL 3978	I	0;	; , 1p2	2	;-	-	;1	3+	-	-	-	-	-	-	2	0;	3	*Lr*13+
19	Danphe 1	II	0;			2	;	2 , ;	;1 , 3+	;1	;1	2+		; , ;1	;1	0;	0; , 2	0; , 3+	*Lr13*+10+
20	WK 1804	II	33+	33+	3+	0;	0	0; ,1p 3+	3+	3+	0;	2	0;	3+	;-	0	0;	3+	*Lr*13+10+
21	Rohini	II	0;	0;	0;	0;	0;	3+	3+	33+	0;	3	3	3- , ;1	12	0	0;	33+	*Lr*13+10+
22	WK 936	II	;1	3+	0;	0;	0;	;- , 0;	33+	2	3+	2	0;	3+ ,33+	;	0	0;	;3	*Lr*13+10+
23	WK 1204	II	;1	3+	;1	;-	3+	3+	3+	3+	3+	3+	3 ,1p 2	3+ ,33+	;	0	0;	3+	*Lr*13+10+
24	WK 1444	II	0;	3+	0;	;-	33+	3+	3+	;1	2	3+	;1	3- , 2	;-	0	;	3-	*Lr*13+10+
25	WK 1723	II	;	X	0;	;	3+	3+	3+	3+	3+	3+	3-	12 ,33+	;	12	0;	X+	*Lr*13+10+
26	WK 1727	II	;	33+	;	;	3+	3+	3+	-	3+	3+	2	2	;1	0	0;	33+	*Lr*13+10+
27	WK 1909	II			0;	0;	0	3+	3+			;1		3+			0;	3+	*Lr*13+10+
28	WK 1967	II	;1	2	3+	;-		0; , 1p 2	2	;1	0;	;1	3+	2+	;-	2	;-	3-	*Lr*13+10+
29	WK 1701	II	0;	2	;1	0;	-	0;	2 , 3	2	12	2	0;	2 , 3+	;-	0	0;	;	*Lr*13+10
Malakoff	1		0;	0;	0;	3+	3	3+	3+	3+	3+	3+	3+	3+	3+	3+	0;	0;	
Thatcher* 6/Lee	10		;-	X	1	;	3+	33+	3+	3+	3+	3+	;1	3+	;1	;1	;	3+	
Egret	13		;-	3	3	3+	3	3	3+	3+	3	3	;1	3+	3	3+	0;	3+	

3.1.2. RG2:

This group contained a single genotype NL971 with presence of *Lr*10 gene. This gene was confirmed by low IT reaction on pathotypes 12-5, 77 and 106. In addition, it also appears to show the presence of additional gene(s) due to low IT reaction to pathotype virulent to *Lr*10 (Table 2).

Table 2 Infection types on wheat genotypes in resistance group II and deferential Thatcher* 6/Lee with *P. triticina* pathotypes

No.	Genotypes	12-5	12-7	12—8	77	77-1	77--2	77-5	77-7	77-8	77-10	77A-1	104-2	104A	104B	106	62-1	postulated gene (s)
1	NL-971	0;	0;	0;	0;	0	33+	33+	3+	2	3+	3+	;1	-	0	0;	33+	*Lr*10+
hatcher*6/Lee	10	;-	X	1	;	;1	33+	3+	3+	3+	3+	3+	;-	;1	;1	3	3	

3.1.3. RG3:

In this group, genotype Chewink 1 was present which showed low IT reaction with all the tested *P. triticina* pathotypes except 77-8. Hence, clearly indicated the presence of *Lr*19 (Table 3).

Table 3 Infection types on wheat genotypes in resistance group III and deferential HD 2189 with *P. triticina* pathotypes

No.	Genotypes	12-5	12-7	12—8	77	77-1	77—2	77-5	77-7	77-8	77-10	77A-1	104-2	104A	104B	106	62-1	postulated gene (s)
1	Chewink 1	0;	0;	0;	;-	0;	0;	;-	0;	3+	0;	0;	;	;-	0	0;	;	*Lr*19
	*Lr*19	0;	0;	0;	0;	0;	0;	;	;	3+	;	;	;	;;	;	;	;	

3.1.4. RG4:

This group was divided into two sub-groups. The sub-group I comprised of 11 genotypes (Kanti, WK 1639, WK 1654, WK 1686, WK 1776, WK 1797, WK 1804, WK 1974, WK 1996, BL 3978 and BL 4171). The pathotypes 77-2, 77-8 and 77A-1 (avirulent for *Lr*26) showed low ITs (0; to ;), whereas pathotypes 77-5 and 77-7 (virulent for *Lr*26) showed high IT reactions. The patterns were similar to that of the control Beno, which carries *Lr*26. The sub-group II included 4 genotypes (WK 1997, WK 1998, BL 4131 and RR 21) and responded very similarly to sub group 1, except that there were clear differences between IT responses to pathotypes 12-5, 12-8 and 77-1 (virulent to *Lr*26 and avirulent for *Lr*10). The difference in response of genotypes in sub group II to these pathotypes was attributed to *Lr*10, as shown by the control Thatcher* 6/Lee. The genotypes in subgroup II were therefore postulated to have genes *Lr*26 and *Lr*10 (Table 4).

Table 4 Infection types of wheat genotypes in resistance group IV and deferential Thatcher* 6/Lee, Benno with *P. triticina* pathotypes

S.N.	Genotypes	Sub groups	12-5	12-7	12-8	77	77-1	77--2	77-5	77-7	77-8	77-10	77A-1	104-2	104A	104B	106	62-1	postulated gene (s)
1	Kanti	I	3+	3+	0;	;-	;	0;	3-	3+	0;	;1	0;	3	;-	0	0;	33+	*Lr*26+
2	WK 1797	I	;1	;1	0;	0;	3+	0;	3+	2+	0;	2	0;	33+ , ;1	;-	0	0;	;	*Lr*26
3	WK 1974	I	33+	33+		;-	3	;-	3+	3+	0;	2	0;	33+	;1	0	;-	12+	*Lr*26+
4	WK 1996	I	3+	X+3	0;	0;	3	0;	3=	3+	0;	3	0;	3+	;	0	0;	33+	*Lr*26+
5	Kiskardee 1	I	0;	0;	0;	;-	0;	;-	0; , ;	0;		;	0;	0;	;-	0	0;	0;	*Lr*26+
6	BL 4171	I	2	;1	-	0;	0	0;	3+	-	-	-	-	-	-	0	0;	;1	*Lr*26+
7	WK 1997	II	0;	3	0;	0;	-	0;	3+	3+	0;	3	0;	3+	;-	0	0;	;	*Lr*26+10+
8	WK 1998	II	;1	33+	-	0;	-	0;	; , 12	3+	0;	;1	0;	3+	0;	0	0;	0;	*Lr*26+10+
9	BL 4131	II	2		0;	0;	0	0;	3+					0;		0	0;	33+	*Lr*26+10+
10	WK 1776	II	0;	33+	33+	;-	2	0;	3+	3+	0;	;1, 1p 3	0;	3+	;	0	0;	2	*Lr*26+10+
Thatcher* 6/Lee	10		;-	X	1				3+	3+	3+	3+	;1	;-	;1	;1	0;	3	
Benno	26		3+	3+	3+	0;	3+	0;	3+	3+	0;	3+	0;	3+	0;	0;	0;	3+	

3.1.5. RG5:

This group was relatively large and was divided in to 5 subgroups. The sub group I comprised of 6 genotypes showed high IT reaction on pathotypes 12-5, 12-7, 77-2, 77-7, 77-10, 104-2 and 104B, whereas low IT (0; to ;1) response on 12-8, 77, 77-1, 77A-1, 77-8, 104A, 106 and 162-1. This indicated that these genotypes had *Lr*23. In sub group II, the genotypes carried an additional gene *Lr*10 associated with *Lr*23. The pathotypes 12-7, 77-2, 77-5 and 104-2 showed high ITs (virulent for both *Lr*23 and *Lr*10). The sub group III had nine genotypes showed high IT reaction (virulent for both *Lr*23 and *Lr*26). Similarly, low IT reaction on pathotypes 104B and 77-2 (virulent for *Lr*23 and avirulent for *Lr*26) and 12-8, 77-1 and 184-2 (virulent to *Lr*26 and *Lr*23) indicated that these genotypes possessed both *Lr*23 and *Lr*26. The sub group IV contained eight genotypes and their reaction was very similar to the sub-group III. However, low IT (0;) was observed for the pathotypes 12-5, 12-7, 12-8 and 162-1 due to the presence of *Lr*1. Hence, this group showed the presence of *Lr*23, *Lr*26 and *Lr*1+ genes. Likewise, the sub-group V had twelve genotypes. They were also quite similar with sub-group III and IV except low IT response for 77-7. The sub-group V possessed *Lr*10 in addition to *Lr*1, *Lr*23 and *Lr*26 (Table 5).

Table 5 Infection types on wheat genotypes in resistance group VII and deferential Malakoff, Thatcher* 6/Lee, IWP 94, Benno

No.	Genotypes	Sub group	12-5	12-7	12--8	77	77-1	77-2	77-5	77-7	77-8	77-10	77A-1	104-2	104A	104B	106	62-1	postulated gene (s)
1	Becard 1	I	2	X	0;	;1	0;	33+	; 1p 3+	;	0;	33+	;	3+ , ;1	-	33+	;	X	*Lr*23+
2	BL 3989	I	33+	3+	;1			33+	;1 , 2	3+	0;	;	0;	3+	;-	33+	0;	;-	*Lr*23+
3	BL 4088	I	-	2+	;1	2	0	33+	3+	3+	12	33+	Y	33+	;1	0	0;	-	*Lr*23+
4	BL 4110	I	33+	3+	0;	0;	0	33+	3+	-	-	0;	-	33+	-	0	0;	3+	*Lr*23+
5	Achyut	II	;1	3+	;	;	;1	33+	3+	3+	;1	0;	;	3+	;1	2+	0; , 2	X , 3+	*Lr*23+10+
6	BL 1473	II	0;	3+	0;	;1	0;	33+	3-	3+	;	33+	;	33+	;	0;	0;	;1	*Lr*23+10+
7	BL 4118	II	2	3+	;	2	-	33+	3+	-	-	33+	-	-	-	0	0;	X	*Lr*23+10+
8	Pasang Lhamu	III	3+	33+	0;	0;	-	;-	3	;-	;	;1	0;	2, 1p3+	;-	0	0;	0;	*Lr*26+23+
9	WK 1970	III	2	2	2	-	;	;-	12	3-	0;	;1	0;	3 , 3+	0;	-	0;	;1	*Lr*26+23+
10	BL 4009	III	3	0;	;1,1p 3	;-	0	0;	3+	2+	0;	;1,1p 3	0;	3+	;-	0	0;	;	*Lr*26+23+
11	BL 4040	III	;1	2	;1	0;	0	0;	3+	3+	0;	2	-	3+	;	0	0;	;1	*Lr*26+23+
12	BL 4085	III	-	-	;1	-	-	;-	3+	3+	-	;1	-	3+	0;	-	0;	-	*Lr*26+23
13	BL 4086	III	2	;1	0;	0;	-	0;	3+	3+	-	;1	-	3+	0;	0	0;	;1	*Lr*26+23+
14	BL 3472	IV	3+	0;	0;	0;	0;	0;	3+	;1 , 3+	0;	2	0;	3+	;- , 2	0;	0;	0;	*Lr*26+23+1+
15	BL 3840	IV	0;	0;	0;	0;	0;	0;	0;	33+	0;	;-	;-	3+	;-	0	0;	0;	*Lr*26+23+1+
16	WK 1973	IV	;-	-	;-	;-	-	;-	3	3	;-	;1	0;	3-	;1	0	;-	0;	*Lr*26+23+1
17	WK 2000	IV	0;	;-	0;	0;	2	;-	3+	2	;-	;1	0;	;1	0;	0	-	0;	*Lr*26+23+1+
18	WK 2005	IV	0;	0;	0;	0;	0	;-	2	;1	0;	;1	0;	3+	;-	0	0;	;- *Sr*2	*Lr*26+23+1+
19	WK 2007	IV	0;	0;	0;	0;	3	0;	;1 , 2	;1	0;	;1	0;	2 , 33+	;-	0	0;	0;	*Lr*26+23+1+
20	BL 3945	IV	0;	-	0;	;-	-	0;	3+	-	0;	3	0;	-	;-	0	0;	0;	*Lr*26+23+1+
21	BL 3503	V	;1	3-	0;	0;	;1	0; , ;1	;1	0;	0;	;	0;	3+	;-	0;	0;	2+	*Lr*26+23+10+
22	BL 3623	V	; , 1p 2+	33+	0;	0;	0;	0;	3 , 3+	;	0;	;1	0;	; ,1p 3+	;-	0;	0;	0;	*Lr*26+23+10+
23	BL 3629	V	12	3+	;1	;-	;	0;	3	;1	0;	;1	0;	3+ , 12	;-	0;	0;	0;	*Lr*26+23+10+
24	Gautam	V	0;	;	0;	0;	0;	0;	3	0;	0;	;1	0;	X+3	;-	0;	0;	-	*Lr*26+23+10+
25	BL 3555	V	0;	33+	0;	;-	;	;- , 0;	33+	-	0;	2	-	3	-	0	0;	;1	*Lr*23+26+10+
26	BL 3819	V	;1	3-	0;	0;	;1	0;	3+	2	0;	;1	0;	0; , ;1	;-	0	0;	;	*Lr*26+23+10+
27	WK 1968	V	;	3	; 1p 3+	;-	2	;-	12	;-	;-	;1	;-	2	;	0	;-	;1	*Lr*26+23+10+
28	BL 4061	V	0;	3+	;1	0;	0	1p 0;	3+	3	0;	2	-	3+	;-	0	0;	-	*Lr*26+23+10+
29	BL 4123	V	2	3+	12	0;	0	0;	3+	-	-	;1	-	0; 1p3+	-	0;	0;	;1	*Lr*26+23+10+
30	RR 21	V	2	33+	;	-	0	0;	3	-	-	-	-	0;	-	-	;-	;	*Lr*26+23+10+
Malakoff	1		0;	0;	0;	0;	3	3+	3+	3+	3+	3+	3+	3+	3+	3+	0;	0;	
Thatcher* 6/Lee	10		;-	3+	1	;	;1	33+	3+	3+	3+	3+	;1	;-	;1	;1	;	3	

IWP 94	23		3+	3+	1	0;	;1	3+	3+	3+	;1	3+	0;	3+	;	3+	1	;
Benno	26		3+	3+	3+	0;	3+	0;	3+	3+	0;	3+	0;	3+	0;	0;	0;	3+

3.2. Adult plant resistance test

Wheat genotypes lacking seedling resistance genes or possessing genes for which virulence(s) were available were subjected to adult plant resistance tests in polyhouse. These genotypes were classified into three groups on the basis of disease response at seedling and adult plant stages.

3.2.1. Group A1:

This relatively large group comprised of 44 wheat genotypes that showed susceptible phenotypes at both seedling and adult plant stages in the glasshouse and polyhouse. This group had also included the genotypes that did not show additional APR reaction over pathotypes(s) virulent for the respective seedling resistance genes. Wheat genotypes that possessed gene *Lr*13 and *Lr*26 were susceptible to the tested pathotypes. WK 1803, WK 1961, WK 1970 and WK 2007 were comparatively more susceptible to the two pathotypes.

3.2.2. Group A2:

This group comprised of 22 lines postulated to carry seedling resistance but also exhibited moderate to high levels of APR with pathotypes virulent at seedling stage. The disease response was extremely low at the adult stage (Table 6). The level of disease on WK 1627 was marginally higher that the remaining cultivars of the group. The genotypes WK 1663, Chonte 1, BL 4183, NL 1067 and WK 1964 showed highly resistance response than others.

Table 6 Adult-plant infection types and disease responses on genotypes in Group A3 with two Puccinia tritici pathotypes in the polyhouse

Genotypes	Postulated *Lr* gene (s)	Pathotypes (Seedling stage)		Pathotypes (Adult plant stage)	
		77-5(121R63-1)	104-2 (21R55)	77-5(121R63-1)	104-2 (21R55)
Becard-1	*Lr*23+	;	3	;R	0R
BL 3503	*Lr*26+23+10+	;	;	10R	10MR
BL 3629	*Lr*26+23+10+	3	3	10R	10R
Gautam	*Lr*26+23+10	3	3	10R	0R
WK 1504	*Lr*13+	12	2+	20MR	0R
WK 1627	*Lr*13+10+	3-	X	20MR	20MR
WK 1719	*Lr*13+	3+	3	10R	10R
WK 1797	*Lr*26+	3	3	20MR	TR
WK 1964	*Lr*13+	;1	3	0R	TR
WK 1967	*Lr*13+10+	;1	2+	10MR	10MR
WK 1968	*Lr*26+23+10+	12	2+	20MR	5MR
Kiskardee 1	*Lr*26+R+	0;	0;	0R	0MR
Chewink 1	*Lr*19	0;	0;	0R	0MR
Chyakhura 1	R	0;	0;	0R	0MR
NL 1053	-	;	;1	0R	10R
NL 1067	-	;	;1	0R	0R

3.2.3. Group A3:

Ten genotypes (BL 3467, WK 1639, WK 1654, WK 1661, WK 1672, WK 1710, WK 1713, BL4078, BL 4095 and BL 4176) were susceptible at seedling stage against the two pathotypes but were moderately to highly resistant at adult plant stage. WK 1710, WK 1713 and BL 4078 possessed APR, however *Lr* genes could not be inferred. The genotypes WK 1713 and BL 4078 showed marginally higher ITs than others (Table 7).

Table 7 Adult-plant infection types and disease responses on genotypes in Group A4 with two *Puccinia tritici* pathotypes in the polyhouse

Genotypes	Postulated *Lr* gene (s)	Pathotypes (Seedling stage)		Pathotypes (Adult plant stage)	
		77-5(121R63-1)	104-2 (21R55)	77-5(121R63-1)	104-2 (21R55)
BL 3467	*Lr*13+	3+	3+	5R	0R
WK 1639	*Lr*26+	3+	3+	10R	10R
WK 1654	*Lr*26+	3+	3+	10R	10R
WK 1661	*Lr*13+10+	3+	3+	TR	TR
WK 1672	*Lr*26+10+	3+	3+	10MR	10MR
WK 1710	-	3+	3+	10R	10R
WK 1713	-	3+	3+	20MR	20MR
BL 4078	-	3+	3+	20MR	40MR
BL 4095	*Lr*13+	3+	3+	0R	10MR
BL 4176	*Lr*13+1	3+	X	10R	20MR

The genotyping of the samples with SSR marker was successful with an average success rate of 100% polymerase chain reaction (PCR) fragment were amplified from the tested wheat genotype with specific gene. The result showed that the marker amplified on presence of Lr34 gene in 26% of tested lines. The amplification of markers with respect to presence or absence of gene was represented in electropherograms.

4. DISCUSSION

The present study identified seven known genes for seedling resistance to leaf rust either singly or in combination in a range of wheat genotypes grown in Nepal and the south Asia. The frequency of gene occurrence was highest for *Lr*13 (37% of genotypes), followed by *Lr*26 (33%), *Lr*10 (32%), *Lr*23 (31%), *Lr* 34 (26%), *Lr*1 (11%) and *Lr*19 (1%). The gene *Lr*13 was the most commonly postulated gene in the genotypes tested. On the global level also this is probably the most widely distributed gene for resistance to leaf rust pathotypes [7]. The gene *Lr 13* is considered to have originated from South American wheat genotypes Frontana, Frondoso and Fronteria [8]. This gene is ineffective in South Asia where severe leaf rust has occurred in the last century. Although not effective against some pathotypes of *P. triticina* in South Asia, *Lr*13 is still considered important because two-gene combination involving *Lr*13 continue to give excellent leaf rust protection [8]. In addition, *Lr*13 interacts positively with various genes [9] despite reports of the high levels of susceptibility of some wheat with *Lr*13 in South Asia. According to [10], *Lr*13 is known to provide durable types of resistance and therefore could play important role for additional resistance in wheat.

The resistance gene *Lr*26 was present in 41 wheat genotypes either singly or in combination. The gene *Lr*26 is completely linked with *Sr*31 and *Yr*9 and is located on wheat-rye 1B-1R translocation [11]. The gene *Lr*26 is also associated with the powdery mildew resistance gene *Pm*8, but is not always expressed in wheat due to the presence of a suppressor in the wheat genome [12]. The pathotypes 121R63-1 and 21R55 are virulent on *Lr*26 [10]. This resistance nature should be very useful for further breeding purpose because these two pathotypes are the most dominant in south Asia and getting expanded in south Asia.

The gene *Lr*1 was postulated in combination with other resistance genes, *Lr*13, *Lr*23 and *Lr*26. Individually, *Lr*1 was not effective against virulence pathotypes of the leaf rust [13][14]. The gene is therefore regarded as being of use to some extent in combination with other genes such as *Lr*13 in Australia [8]. In Chykhura 1, *Lr*19 was detected. This gene is tightly linked to stem rust resistance gene *Sr*25. The gene *Sr*25 is effective against many virulence pathotypes of stem rust [15]. This suggest that the *Lr*19 could be used effectively in breeding program for developing durable type of resistant genotypes for both leaf and stem rusts. The gene *Lr*10 was postulated in combination with *Lr*13, *Lr*23 and *Lr*26 in only one genotype NL 971. This gene was not found effective on its own or in gene combination in Australia (Singh *et al.*, 2001) but it is still observed perceived as a genetic source for leaf rust resistance in India [15].

Of the total 99 genotypes of wheat, 67 exhibited APR reaction. In many genotypes, *Lr*13 might have conferred APR reaction in combination with *Lr*19, whereas in others *Lr*11, *Lr*12, *Lr*17, *Lr*21, *Lr*22a, *Lr*22b or unknown genes could have contributed. The cultivars Achyut, Danphe-1, Pasang Lhamu, BL 3467, BL 3778, BL 4078, BL 4086, BL 4154, BL 4171, BL4176, WK 1204, WK 1719, WK 1792, and WK 1974 showed the evidence of APR gene(s) against 77-5 (121R63-1) since they were susceptible at the seedling stage. Similarly, Aditya, BL 2879, BL 3063, BL 3467, BL 3472, BL 3840, BL 3978, BL 3989, BL 4078, BL 4095, BL 4110, BL 4176, Gautam, WK 1444, WK 1710, WK 1713, WK 1804, and Chonte 1 also showed the presence of APR against 104-2 (21R55). However, some lines BL 3467

(*Lr*13+), BL 4078, BL 4176 (*Lr*13 + *Lr*1+), WK 1639 (*Lr*26), WK 1654 (*Lr*26+), WK 1661 (*Lr*26+ *Lr*10+), WK 1710, WK 1713 and WK 1672 (*Lr*26+ *Lr*10+) possessed APR gene(s) against both pathotypes

The results indicated that the genotypes with the best leaf rust resistance showed a combination of seedling and APR genes. Some of the tested genotypes BL-1473, BL-3063, BL-3468, BL-3978, BL-4095, BL-4118, BL-4154, NL-1073, Pasang Lhamu, WK1628, WK1710, WK1909, WK1970, WK1998 and Chonte 1) showed APR to leaf rust that indicated the presence of slow rusting gene *Lr*34. The gene *Lr*34 shows slow rusting characters manifested by smaller and fewer pustules at the flag leaf stage in the initial phase of the leaf rust epidemic. Wheat lines that have *Lr*34 plus effective seedling resistance genes often perform higher levels of leaf rust resistance than lines that have only *Lr*34 or seedling resistance genes. The 28 lines (BL 1444, BL 1473, BL 3063, BL 3467, BL 3472, BL 3553, BL 3623, BL 3945, BL 3960, BL 3989, BL 4078, BL 4086, BL 4091, BL 4110, BL 4118, BL 4123, BL 4131, BL 4154, Gautam, Kiskardee, WK 1204, WK 1444, WK 1961, WK 1970, WK 1996, WK 1998, WK 2000, and WK 2007) also showed the presence of *Lr*34 gene in SSR primer sequence csLV34_Lr34sp_test.

Two pathotypes (121R63-1 and 21R55) used in the study are virulent on *Lr*1, *Lr*3, *Lr*10, *Lr*13, *Lr*23 and *Lr*26 [16]. Hence, resistance shown by the lines possessing these genes for these two could be due to presence of other resistance genes. These pathotypes are also frequently detected from different wheat growing area from the plains (Terai) to hilly regions of Nepal. Therefore, the genotypes found resistant to the race-specific pathotype assume significance for the breeding programs targeting durable resistance genotypes in near future. Varietal diversification and deployment of different resistant genes [17] in different environments are needed for better management of year round epidemic of rust pathotypes and also to sustain yield for longer periods of time. This is important for a country like Nepal which has varied climatic conditions across subtropical plains to the temperate high hill areas. The combination of seedling resistance and adult plant resistance has potential to keeps the initial population of pathotypes at low level in the environment.

5. CONCLUSION

There are seven different genes such as *Lr*13, *Lr*26, *Lr*10, *Lr*23, *Lr*34, *Lr*1, and *Lr*19 identified from the wheat lines of the country. The resistance genes postulated in the present study could be helpful for breeders and pathologist in strategic planning of the wheat breeding program and reducing the avoidable loss caused by wheat rusts. The change of pathogen diversity may be caused by selection pressure due to change in wheat genotypes grown in the country. Most of the wheat lines base on two or three genes which makes wheat vulnerable to leaf rust pathogen. Hence, continuous efforts are needed to broaden the genetic base in the cultivated wheat genotypes.

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