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Phenotypic Screening and Molecular characterization for Leaf Rust Resistance Gene *Lr*9 in Wheat

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ABSTRACT: Field screening during both the experimental years of 2014-15 and 2015-16 has revealed that four varieties such as HW 2021, HP 1731, WH 896 and UP2565 were found as no infection of leaf rust at all, so they were considered as immune to disease. While five varieties such as WL1562, Chakwal 86, Rawal87, WH 1105, C306 were identified with disease severity ranged from 20 to 60% severity of susceptible response, so these varieties were identified as highly susceptible to leaf rust. The other remaining 15 varieties were recorded as disease severity ranged from TR to maximum of 10% of resistant response, so these varieties were considered as resistant to leaf rust. The result of AUDPC value has revealed that the maximum value of 880.25 was recorded in WH1105 followed by WL1562 with 540.4 values so they were considered as highly susceptible varieties. The maximum rate of infection was recorded in C306 with 0.382 followed by HD2189 and UP2572 with 0.249 values. Confirmation of presence of *Lr9* gene was exhibited with the amplification product size of 550bp with single fragment in 4 varieties such as HD2189, UP2572, UP2748 and WH896 and resistant check (UP2425) by SCAR marker SCS5.

Keywords: Leaf rust, SCAR marker, *Lr9*, resistant, AUDPC.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second most important cereal and food crop of India in World after China in terms of areas, production and consumption. India is the second largest producer of wheat in the world after China. In India during 2020-21, wheat is grown over an area of 31.76 mha with a landmark of total production of 109.52 mt and the total average national productivity of 3464 kg/ha (ICAR-IIWBR, 2021). Despite the fact that several diseases and pests are known to impair wheat grain yield potential and quality, among them**,** leaf or brown rust of wheat, caused by *Puccinia triticina* Erisk. is the most predominantly confined disease in all the wheat growing zone of India (Bhardwaj *et al.,* 2006). Since the pathogen inoculums are abundant in both the North and South regions of India, it is well dispersed among the three wheat rusts (Joshi, 1975). Wheat leaf rust epidemics were reported in various years, including 1786, 1827, 1832, 1894, 1897, 1947, 1948, 1972, and 1973 (Nagaranjan and Joshi, 1975). Again, the Sonalika leaf rust epidemic in Uttar Pradesh and a part of Bihar, India, resulted in 1 million tonnes of loss (Joshi, 1975). Wheat rust research in India began in 1992 with the identification of the first pathotype, and it was later documented in 1931 (Mehta, 1940). The protection of wheat against rust infections caused by *Puccinia* spp. is particularly important for Georgia as reported by Natsarishvili *et al.* (2016).

The maximum yield losses due to leaf rust were reported as 30-40% mostly by reduction in 1000 grain weight (Rao, 1989). In India, leaf rust caused yield losses in the range of 0.8 to 1 million tonnes in the Northwest region during 1971 and 1973 (Joshi, 1975). Yield loss of 5-10% was reported in Uttar Pradesh in 1986 due to leaf rust infection in wheat (Byerlee and Moya 1993). Leaf rust infection in wheat has been linked to a drop in the number of kernels per head and a decrease in kernel weight (Kolmer *et al.*, 2005). Leaf rust has been expected to harm 80% of wheat

production in India (21.6 million hectares) in ideal conditions (Singh *et al*., 2004). It has the potential to cause yield losses of up to 50% and because it occurs more frequently and widely, it results in greater total annual losses of wheat production worldwide (Huerta-Espino *et al*., 2011). However, for the management of leaf rust, the most useful and economically effective measure is the utilization of resistant cultivars. The development of new cultivars with improved genetic resistance has a great impact in reducing production costs and also risks of environmental pollution due to heavy use of fungicide against wheat rusts (Dholakia *et al*., 2013). For the successful implementation of resistant sources against leaf rust, effective field screening of wheat lines is an important task. For screening of partial resistance of genotypes, field based assessment can be done by using various measures such as final rust severity (FRS), area under disease progress curve (AUDPC), and coefficient of infection (CI) (Pathan and Park, 2006). The survey and surveillance, identification of pathotypes, understanding the epidemiology of rust pathogens, and identification of novel sources of rust resistance in wheat were all important components of the wheat rust research (Pal *et al.*, 1952).

However, the discovery of molecular markers for resistance genes has able to speed up molecular assisted selection and the pyramiding of important genes in breeding programmes, resulting in a more valuable background in less time and at a lower cost (Babu *et al*., 2004). Therefore, the use of molecular markers makes it easier to discover resistance genes in segregating populations at the DNA level and incorporate them into current high yield cultivars. As a result, molecular markers can be employed as a selection tool, and they are critical for identifying loci carrying leaf rust resistance genes and ensuring their proper usage in resistance breeding. So, considering the importance of identifying promising resistant sources for the management of leaf rust of wheat in field condition, the present investigation was aimed to identify the promising source of resistant varieties against leaf rust of wheat in field condition along with their conformity for the detection of *Lr9* gene by SCAR marker at genetic level.

MATERIALS AND METHODS

A. Field screening

Screening of wheat varieties were conducted during *Rabi* seasons of 2014-2015 and 2015-16 at Norman E. Borlaug Crop Research Centre, G.B. Pant University of Agriculture & Technology, Pantnagar, in order to identify their response against leaf rust of wheat. The materials used in the study consist of 24 varieties *viz*. WL1562, PBW660, HD2160, HD2189, HP1731, Rawal87, Gourab, WL711, RAJ3765, HW2021, C306, Chakwal86, UP2628, UP2526, UP2855, WH1105, DPBW621-50, UP2572, UP2748, UP2565, UP2844, UP2785, UP2865 and WH896. The epiphytotics condition for maintaining of high disease pressure during the crop seasons was carried out by artificial inoculation of urediospores suspension in the infector lines which are sown in the border row. For this purpose, twice inoculation (first at seedling and second at booting stage of crop) was performed in the susceptible varieties (Agra local, A-9-30-1 and LWH) sown in the border lines as an infector's lines by spraying of inoculum suspension having urediospores mixtures of predominant pathotypes of particular location of pantnagar *viz.* 12-2, 77-2, 77-5 and 104-2 of leaf rust of wheat. The powder urediospores of all the pathotypes were mixed in a container and diluted in water followed by adding two drops of tween 20. These particular pathotypes were procured from Regional Research Station, ICAR, Indian Institute of Wheat & Barley Research, Flowerdale, Shimla. The first emergence of symptoms *i.e.* development of leaf dot pustules on the leaves were examined critically. After successfully development of disease of near about 60 per cent of susceptible response in infector's line, recording of disease severity of leaf rust was started as per cent of infection from the individual line of variety according to Modified Cobb's scale as stated by Peterson *et al.* (1948). The disease severity was determined by visual observations, below 5 per cent severity, the intervals used were Trace to 2 per cent. Usually 5 per cent interval was used from 5 to 20 per cent severity and 10 per cent intervals between 20-100 per cent. The data were recorded for sixth times at seven days interval till the plant get adult plant stage. Then, coefficient of infection (CI) was calculated by multiplying severity score with constant values of response type *viz*. 0.2, 0.4, 0.6, 0.8, 1 for R, MR, X, MS and S respectively shown in table 1. After that, the area under the disease progress curve (AUDPC), which is a useful measure for determining variety resistance, was computed for all wheat varieties by using following formula:

$$\text{AUDPC} = \frac{1}{2}\sum_{i-1}^{n}\left\{\left(X_i + X_{i+1}\right)\times t_i\right\}$$

where, X_i and X_{i+1} are severities on date i and date $i+1$, respectively; t_i is the number of days in between date i and date $i+1$; n is the number of observation recorded.

Further, another disease progress parameter *i.e*. rate of infection (r) as a function of time was estimated to determine the ability of different wheat varieties against the development of leaf rust infection at adult plant stage under field conditions. It was calculated from different rust score recorded after 7 days interval and it was estimated by using the following formula as mention by Vander Plank (1963).

$$r = \frac{2.3}{t}\left(\log\frac{X_2}{1-X_2} - \log\frac{X_1}{1-X_1}\right)$$

where, X_1 = disease severity at date t_1, X_2 = disease severity at date t_2, t = days interval between two dates.

Table 1: Modified Cobb's scale for recording rust of wheat (Peterson *et al*., 1948).

Reaction type	Response value	Category	Visible symptoms
0	0.0	Immune	No visible infection
R	0.2	Resistance	Necrotic areas with or without uredia
MR	0.4	Moderately resistance	Necrotic areas with small uredia
X	0.6	Intermediate	Variable sized uredia with necrosis or chlorosis and fully susceptible
MS	0.8	Moderately susceptible	Medium sized uredia with no necrosis but some chlorosis
S	1.0	Susceptible	Large sized uredia with no necrosis and chlorosis

B. Molecular work

The DNA from 24 wheat vareities, along with one resistant (UP2425) and one susceptible check (Agra Local) were extracted from the young seedling leaves by using CTAB method given by Doyle and Doyle (1990). The PCR amplification was carried out for 35 cycle with the primer sequence of 5'-TGCGCCCTTCAAAGGAAG-3'R for forward and 5'-TGCGCCCTTCTGAACTGTAT-3' of reverse of SCAR marker, SCS5 linked with *Lr9* gene. The denaturation temperature of 94^{o}C for 5 min followed by annealing temperature of 55^{o}C for 1 min and extension temperature of 72^{o}C for 7 min. Then, a 2.5 per cent agarose gel was generated by dissolving an adequate amount of agarose in 0.5X TBE solution and electrophoresis was performed in 0.5X TBE buffer at 50V for 4 hours. Ethidium bromide solution was used to stain the gel. The gel picture was seen in a gel documentation system after de-staining in de-ionized water (Gel Doc).

RESULT AND DISCUSSION

The result of field screening during both the experimental years has revealed that four varieties such as HW 2021, HP 1731, WH 896 and UP2565 were found as no infection of leaf rust at all, so they were considered as immune to disease (Table 2). While five varieties such as WL1562, Chakwal 86, Rawal87, WH 1105, C306 were identified with disease severity ranged from 20 to 60 per cent severity of susceptible response, so these varieties were identified as highly susceptible to leaf rust of wheat in field conditions. The other remaining 15 varieties were recorded as disease severity ranged from TR to maximum of 10 per cent of resistant response, so these varieties were considered as resistant to leaf rust (Table 2).

The result of AUDPC value has revealed that the maximum value of 880.25 was recorded in WH1105 followed by WL1562 with 540.4 values (Table 3), so they were considered as highly susceptible varieties while others remaining 22 varieties were recorded with AUDPC value ranged from 0 to 285 values were under the category of resistant. The maximum rate of infection was recorded in C306 with 0.382 followed by HD2189 and UP2572 with 0.249 values. The r value ranged from 0.049 to 0.161 was recorded in the maximum varieties which indicated that they were having good response of resistance against disease development.

The result of molecular characterization has indicated that the confirmation of presence of *Lr9* gene was exhibited with the amplification product size of 550bp with single fragment in 4 vareities such as HD2189, UP2572, UP2748 and WH896 and resistant check (UP2425) by SCAR marker SCS5. While 20 vareities and susceptible check (Agra Local) show absence of *Lr9* gene with no amplification (Fig. 1).

According to Khan *et al.* (2002), a severity of up to 40% was indicative of moderately resistant to moderately susceptible plants and hence they were regarded phenotypically resistant to stripe rust. Draz *et al.* (2015) examined 49 wheat genotypes for leaf rust resistance and discovered that 10 varieties had a high level of adult plant resistance with a resistance reaction severity of less than 20%. Similarly, based on the reaction of their field data, Anwar *et al.* (2019) identified 7 lines as resistant to leaf rust. Kumar *et al.* (2019) screened 6319 germplasm based on disease severity and average coefficient of infection of leaf rust under epiphytotic field conditions over ten multilocations in India for two years and found that 190 germplasms, namely 31, 42, 53, 32, and 32 germplasms, showed immune, resistant, moderately resistant, moderately susceptible, and susceptible reactions to leaf rust of wheat, respectively.

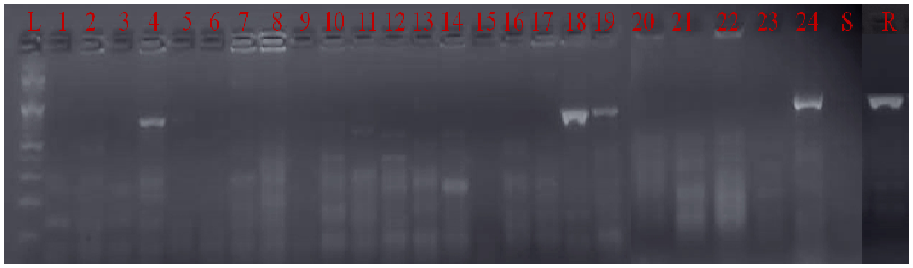
Among 66 varieties of wheat, Leonardo *et al*. (2011) has detected *Lr9* gene in 8 varieties with the amplification size of 550bp. Likewise, Zamanianfard *et al*. (2015) also assessed molecular diversity of 25 durum wheat genotypes by using 11 inter simple sequence repeat primers and reported that 108 fragments, with 83 bands having 77% of polymorphism. According to our both phenotypic and molecular findings, level of resistance and durability in a wheat cultivar could ideally be increased with effective screening in field condition along with confirmation of resistance genes. However, traditional methods for identifying race nonspecific resistance for adult plant resistance genes are rather difficult to use. As a result, the use of molecular markers provides a more reliable technique for identifying adult plant resistance genes at the DNA level in segregating populations and incorporating them into existing high yield cultivars. As a result, molecular markers can be employed as a selection tool, and they are critical for identifying loci containing adult plant resistance genes for leaf rust and ensuring their proper usage in breeding for long-lasting adult plant resistance. Backcross breeding for wheat rust resistance using marker assisted selection has become an important feature of Indian wheat breeding initiatives to improve rust resistance in promising wheat lines and cultivars (Bhardwaj, 2012).

Table 2: Disease severity and Coefficient of infection (CI) of wheat varieties.

Variety	Final rust severity score		Coefficient of infection (CI)		Mean
	2014-15	2015-16	2014-15	2015-16	
WL 1562	60S	40S	20.03	14.03	17.03
HD 2189	20S	10R	5.17	2.03	3.60
RAJ 3765	TR	TR	0.33	0.67	0.50
PBW 660	5R	20R	0.53	1.33	0.93
Chakwal 86	20S	20S	7.83	4.40	6.11
Rawal 87	10S	40S	3.40	14.17	8.78
Gourab	5S	5R	1.70	2.00	1.85
WL 711	10R	40S	4.17	7.50	5.83
HD 2160	20R	40S	5.00	12.00	8.51
HW 2021	0	0	0.00	0.00	0.00
C 306	10S	10S	1.70	3.37	2.53
HP 1731	0	0	0.00	0.00	0.00
WH 1105	40S	60S	20.17	30.17	25.17
DPBW 621-50	5R	10S	1.17	3.50	2.33
WH 896	0	0	0.00	0.00	0.00
UP 2628	TR	TR	0.23	0.37	0.3
UP 2526	0	0	0.00	0.00	0.00
UP 2855	TR	TR	0.13	0.53	0.33
UP 2865	0	5R	0.00	1.07	0.53
UP 2572	10R	10R	0.70	0.37	0.53
UP 2748	0	10R	0.00	0.20	0.11
UP 2565	0	0	0.00	0.00	0.00
UP 2844	5R	TR	2.33	0.53	1.43
UP 2785	TR	5MR	0.03	0.10	0.06

Table 3: AUDPC and rate of infection of wheat varieties.

Variety	AUDPC			r (Rate of infection)		
	2014-15	2015-16	Mean	2014-15	2015-16	Mean
WL 1562	631.41	449.4	540.4	0.315	0.207	0.261
HD 2189	147.01	57.4	102.2	0.229	0.269	0.249
RAJ 3765	10.51	24.5	17.57	0.000	0.000	0.00
PBW 660	15.44	42.0	28.74	0.166	0.000	0.083
Chakwal 86	255.51	114.8	185.15	0.092	0.230	0.161
Rawal 87	107.81	455.0	281.4	0.143	0.121	0.132
Gourab	53.92	66.5	60.2	0.000	0.079	0.039
WL 711	140.0	175.0	157.5	0.053	0.363	0.208
HD 2160	140.0	364.0	252	0.000	0.150	0.075
HW 2021	0.00	0.00	0.00	0.000	0.000	0.00
C 306	36.41	106.4	71.41	0.574	0.191	0.382
HP 1731	0.00	0.00	0.00	0.000	0.000	0.00
WH 1105	707.01	1053.51	880.25	0.150	0.143	0.146
DPBW 621-50	31.51	112.0	71.75	0.118	0.114	0.116
WH 896	0.00	0.00	0.00	0.000	0.000	0.00
UP 2628	6.31	11.91	9.12	0.116	0.116	0.116
UP 2526	0.00	0.00	0.00	0.000	0.000	0.00
UP 2855	4.91	15.41	10.15	0.000	0.166	0.083
UP 2865	0	30.81	15.41	0.000	0.145	0.072
UP 2572	22.41	8.41	15.41	0.166	0.332	0.249
UP 2748	0.00	4.92	2.45	0.000	0.231	0.11
UP 2565	0.00	0.00	0.00	0.000	0.000	0.00
UP 2844	84.01	15.41	49.72	0.034	0.166	0.10
UP 2785	0.72	2.81	1.75	0.000	0.099	0.049



*(L- 100bp ladder, S-Agra Local, R-UP2425, 1- WL 1562, 2-PBW660, 3- HD2160, 4- HD2189, 5- HP 1731, 6- Rawal 87, 7- Gourab, 8- WL711, 9- RAJ 3765, 10- HW2021, 11- C306, 12- Chakwal86, 13- UP2628, 14- UP2526, 15- UP2855, 16- WH1105, 17- DPBW621-50, 18- UP2572, 19- UP2748, 20- UP2565, 21- UP2844, 22- UP2785, 23- UP2865, 24- WH896)

Fig. 1. Amplification profile among wheat varieties by SCAR marker SCS5.

Furthermore, combining numerous seedling resistance genes, such as Lr9, Lr16, Lr19, Lr25, and Lr29, with multiple effective APR genes, such as Lr34, Lr42, and Lr46, is expected to offer long-term resistance to leaf rust (Vida *et al*., 2009). In line with the findings of Leonardo *et al*. (2011), who used STS, SCAR, and RAPD markers to detect a similar trend for the mining of multiple Lr genes, including Lr9, Lr24, Lr25, Lr29, Lr35, and Lr37. As a result, molecular markers can identify genetic variety as well as molecular characterization (Song *et al.*, 2003).

CONCLUSION

From the above findings it can be concluded that four varieties *viz.* HD2189, UP2572, UP2748 and WH896 were identified as promising varieties showing great resistant at both phenotypic and genotypic level linked with *Lr9* gene against leaf rust of wheat. Moreover, some wheat varieties were showing resistant to leaf rust in field conditions but not detected with presence of *Lr9* gene which may be due to presence of some other *Lr* genes. Thus, proper field evaluation of resistant vareities in high disease pressure along with the application of molecular marker could be an important challenging area in identifying resistant sources of varieties for enhancing rust resistance programme to combat wheat rust.

FUTURE SCOPE

Leaf rust of wheat has been successfully managed by breeding for rust resistance genes since, genetic resistance is being a cost effective and environmentally friendly way to combat rust infections in field conditions. However, producing high level and long lasting resistant types is a difficult process and time consuming task which requires knowledge of the pathogen's pathogenicity structure as well as a resistance sources for breeding purposes, so the application of molecular markers linked with specific *Lr* genes could be an important challenging and future prospects for identifying more durable form of resistance against leaf rust of wheat.

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Conflict of Interest. None.

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