Identification and validation of molecular markers for rust resistance in wheat genotypes for marker assisted breeding in India

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Abstract

Rust disease is caused by fungus that wreaks havocon aerial parts of the wheat crop and has been identified as one of the most destructive diseases in wheat in almost all parts of India. This disease may potentially reduce wheat yields up to 20% globally. Validation of linked marker genes for various biotic and abiotic characters suggested that the linked genes can be used in marker assisted breeding programs. In the present experiment, phenotypic screening against different pathotypes of leaf and stripe rust at Shimla, Himachal Pradesh was carried out and it has been found that FLW30 shows resistance against nine rust pathotypes i.e. four pathotypes for stripe rust and five pathotypes for leaf rust.

Three resistance genes including leaf rust resistance (Lr28) and stripe rust resistance (Yr9 and Yr15) were validated in different donor parents i.e. FLW29, FLW30 and PBW343 and two Gpc-B1 improved lines of HUW234 and HUW468 (iHUW234 and iHUW468) as recipient parents. On validation, it has been found that FLW30 contained all three rust resistance genes, whereas PBW 343 possessed Lr28 and Yr15 genes and FLW29 has only Yr9gene in their background. Therefore, the identified wheat genotypes could be used as potential donor for leaf and stripe rust resistance in marker-assisted breeding.

Keyword: *Triticum aestivum*, rust resistance, molecular marker, marker assisted selection, rust pathotypes.

Introduction

Wheat is one of the most important cereal crops in the world because it provides 20% of total energy in the human diet including carbohydrates (60-80%) and proteins (8–15%) and thus plays an important role in global food security[6]. After maize, wheat is the second-most significant crop in the world and it provides food for about 36% of the world's population[41]. In terms of both output volume and crop acreage, wheat is the second-largest crop in the world. In the year 2024–2025, global wheat production was approximately 793million metric tonnes, with an increase of about five million tonnes compared to that of 2023–2024[44]. India produced 117.5million tonnes of wheat grain in 2024-25[21].

Rust is a major disease in wheat that reduces wheat production significantly. Rust infection reduced yields by 10% to 30% worldwide[34]. In northern India, stripe rust is referred to as "Piliya" (yellow) and it is most prevalent in the foothills of Himachal Pradesh. Punjab, parts of Haryana, the plains of Jammu and Kashmir and the Tarai region of Uttarakhand[33].The disease is an excellent air traveller, capable of spreading over long distances in favourable weather conditions[3,4]. In the temperature range of 10˚C to 30˚C, *Puccinia triticina* Eriks causes leaf or brown rust on wheat leaf blades and leaf sheaths[3]. About 83*R* genes showing resistance against leaf rust in wheat have been identified to understand the mechanisms of rust resistance and about ten *Lr* genes have been cloned viz. *Lr1*, *Lr9*, *Lr10*, *Lr13*, *Lr14a*, *Lr21*, *Lr22a*, *Lr34*, *Lr*42 and *Lr67*[14,16,49].

The *P. striiformis* f.sp. *tritici* causes yellow or stripe rust on wheat and showed virulence in temperate regions of the world[4]. Stripe rust is a common wheat disease in the early spring, winter, or at high elevations. Stripe rust infection can occur at temperatures as low as 0°C and as high as 11°C to 23°C[11]. More than 80 stripe rust resistance genes in wheat have been mapped and ten *R* genes have been cloned[15,20].

Most of the epidemiology research has been done in Europe, reviewed by Zadoks and Bouwman[50] and Rapilly[29]. The use of disease resistance-associated molecular markers allows for the early identification of resistant genotypes[25,28]. Furthermore, by testing for the presence of multiple molecular markers (multiplexing), several resistance genes can be tracked at the same time and the markers can be used at an early developmental stage[8]. Genetic resistance is the most reliable form of resistance due to its efficiency, durability and cost effectiveness[12,38,39,47,48]. The development of molecular markers for resistance genes and their application in breeding could speed up gene pyramiding into valuable backgrounds and reduce costs[2]. Gene-specific flanking markers have been validated in parental genotypes and are used for resistance gene introgression or pyramiding[5,36].

Gene pyramiding studies in wheat have been conducted against a variety of diseases[5,6,43] and it remains a very useful and efficient method for improving resistance in the context

of popular wheat genotypes. Two popular wheat cultivars, HUW234 and HUW488, were improved with grain protein content (*Gpc-B1*) (> 3ppm) by using a marker-assisted selection (MAS) approach[22]. These improved genotypes, designated as improved HUW234 (iHUW234) and improved HUW468 (iHUW468), lacked potential resistance against leaf and stripe rust. In this study, we validate the molecular markers linked with leaf and stripe rust resistance in various genotypes that could be used as donor lines to improve iHUW234 and iHUW468 to increase their rust resistance.

Material and Methods

Plant materials and phenotyping for rust infection: FLW30 and FLW29 wheat varieties were obtained from ICAR-IIWBR, Regional Station Flowerdale, Shimla and PBW343 was obtained from PAU, Ludhiana. These varieties were used as donor parents for the validation of leaf rust (*Lr28*) and stripe rust (*Yr9* and *Yr15).* The seed materials were raised in the *Rabi* season of 2016-17 at Agricultural Research Farm, BHU, Varanasi for linked gene validation with the help of specific primers and then in the off-season of 2017 at ICAR-IIWBR, Regional Station, Dalang Maidan, Himachal Pradesh for phenotypic selection against leaf and stripe rust infection. These parental lines were also screened at the same time with different pathotypes in epiphytotic conditions at ICAR-IARI, Regional Station Shimla, Himachal Pradesh.

The disease screening was done as per modified Cobb's scale of 0–100%[26]. The scale was developed to determine the proportion of the area affected by stripe or leaf infection by rust pustules. The rust severity percentages were determined individually for leaf and stripe rust[26]. The infection type (IT) was noted as part of host response assessment[31]. The final disease severity score for each individuals was determined by multiplying their IT estimation by the appropriate numerical value with Tr-0.1, R-0.2, MR-0.4, M-0.6, MS-0.8 and S-1.0. The average coefficient of infection (ACI) was calculated by averaging the scores for each genotype[31].

DNA extractions and PCR Protocol: A modified CTAB method was used to extract DNA from 21-day-old wheat seedlings[32]. The extracted DNA was kept at –20°C until it was needed. The molecular markers were chosen from published data that are closely linked to these specific genes, *Gpc-B1*, *Lr28*, *Yr9* and *Yr15* (Table 1). The PCR was performed in 15µL volume containing 1µLof 100 ng DNA template, 1.5µL of 10x PCR buffer containing 500mM KCl and Tris-HCl (pH 8.4, MBI Fermentas, Germany), 0.2µL of 10Mm dNTP (MBI Fermentas, Germany), 1.0µL forward and1.0µL reverse primer (Metabion, Germany), 0.2µL of $MgCl_2$ (MBI Fermentas, Germany), 9.9µL of double distilled water and 0.2 µL(5U/µL) *Taq polymerase* enzyme (MBI Fermentas, Germany).

The reaction was run in a thermal cycler (Bio-Rad mycyclerTM Thermal cycler and Eppendorf AG 22331 Hamburg) with the PCR condition: 94°C initial denaturation for 4 minutes, 94°C denaturation for 1minutes, 55°C annealing for 1minutes, 72°C for 1 minute and then final extension for 10 minutes. The details of the primers are given in table 1. After performing PCR, the amplified products were separated on agarose gel (2.0-2.5%), which was made by mixing agarose (6.0-7.5 gm) to 300 ml TAE buffer (1X) in a flask (1000 ml capacity) and agarose was boiled carefully till it completely melted and finally ethidium bromide 9µl was added to the gel.

Finally, 2 µl of 10X loading dye (MBI, fermentas) was added in each DNA sample and this mixture was loaded in the agarose gel. The amplified fragments were examined using UV light and gel photographed was obtained using Gel documentation system (UVP, GelDoc-It®Imager). All the wheat genotypes were screened to determine the presence or absence of resistance genes linked markers.

Results and Discussion

Wheat crop faces serious threats against rust diseases, especially leaf rust and stripe rust, which severely reduce yield in major wheat-growing regions including India[35]. The longevity of race-specific resistance genes is seriously threatened by the fast development of disease virulence; even if host resistance remains the most economical and sustainable control approach. In the present study, five wheat cultivars of Indian origin including three donors (FLW29, FLW30 and PBW343) and two recipient parents (iHUW234 and iHUW468) were used to identify and to validate leaf (*Lr28*) and stripe (*Yr9* and *Yr15*) rust-resistant genes.

Table 1
List of molecular markers, their sequence and chromosome location associated with leaf and stripe rust resistant genes and grain protein content gene

Gene/QTL	Marker	Location	Sequence	Size (bp)
Lr28	STS	4AL	5'CCC GGC ATA AGT CTA TGG TT3' 5' CAA TGA ATG AGA TAC GTG AA3'	378[24]
Yr9	WMS582	1BS	5'AAGCACTACGAAAATATGAC3' 5' TCTTAAGGGGTGTTATCATA 3'	251[46]
Yr15	Xgwm413	1BS	5' TGCTTGTCTAGATTGCTTGGG 3' 5' GATCGTCTCGTCCTTGGCA 3'	95[30]
Gpc-B1	Xucw108	6BS	5' AGCCAGGGATAGAGGAGGAA3' 5' AGCTGTGAGCTGGTGTCCTT3'	217[42]

In wheat-growing regions of Asia, leaf rust resistance (*Lr*) genes such as *Lr9*, *Lr19*, *Lr24*, *Lr28* and *Lr32* provide complete protection against leaf rust pathotypes[40].

Monosomic analysis and telocentric mapping were used to determine the chromosome location of *Lr28 (*a leaf rust resistance gene of alien origin, viz., *Aegilops speltoides*), which found 39 centimorgans (cM) away from the centromere on wheat chromosome 4AL[19]. The *Yr9* gene, which protects the wheat crop against stripe rust, was discovered in China in a landrace known as CYR29. The gene *Yr9*, located on chromosome 1BS, is linked to the leaf and stem rust resistance genes *Lr26* and *Sr31*[18]. A dominant gene *Yr15*, derived from *T. dicoccoides*[23], provides protection against stripe rust, present on chromosome arm 1BS to a 2.1cM interval on the proximal side by marker Xgwm413[17], which is extremely useful. It is widely used gene that confers resistance against stripe rust and is found in many Indian cultivars.

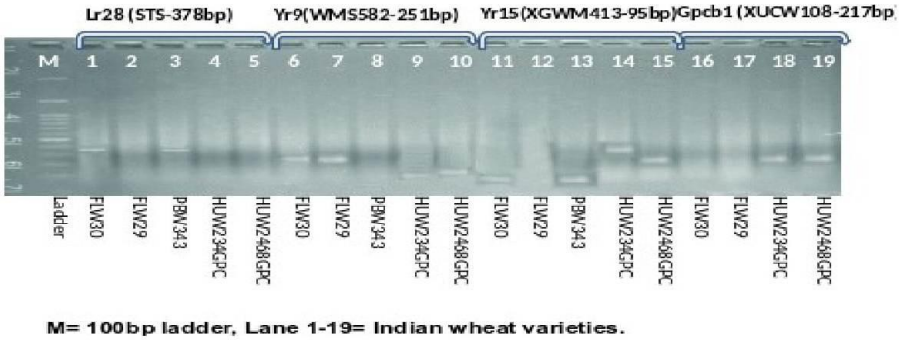
All five wheat genotypes, FLW30, FLW29, PBW343, iHUW234 and iHUW468, were used to validate using gene-specific markers (Table1). Three different genes, *Lr28*, *Yr9* and *Yr15*, were validated in different donors i.e. FLW29, FLW30 and PBW343 and were found to be absent in recipient parents (iHUW234 and iHUW468). In the FLW30 wheat genotype, the *Lr-28* linked gene marker was amplified with band size of 378bp, the *Yr-9* linked gene SSR marker Wms582 was amplified with band size of 251bp, the *Yr-15* linked SSR marker Xgwm413 with band size of 95bp and the *Gpc-B1* linked SSR marker Xucw108 could not be amplified, showing its absence in FLW30. Hence, the wheat cultivar FLW30 has all the rust resistance genes i.e. *Lr28, Yr9 and Yr15* in its background.

The wheat cultivar FLW29 possesses only the *Yr-9* linked SSR marker Wms582. The rest of the rust resistant genes, *Lr28* and *Yr15*, linked with marker Xgwm413 and the *Gpc-B1* gene linked with marker Xucw108 respectively, were absent in FLW29. The wheat cultivar PBW343 has an*Lr28* gene amplified with a 378bp band and an *Yr15* linked SSR marker Xgwm413 amplify on a 95bp band, but no *Yr9* rust resistant gene and grain protein content gene *Gpc-B1*. Rust resistant gene markers *Lr28* STS, Wms582 and Xgwm413 for *Lr-28*, *Yr-9* and *Yr-15* respectively, are absent in iHUW234 and iHUW468. Only the *Gpc-B1* (grain protein content) gene was present in iHUW234 and iHUW468 (Table 2) (Figure 1).

The disease severity was measured at the adult plant stage in all five wheat varieties for leaf and stripe rust using the modified Cobb's method[26] at ICAR-IIWBR, Dalang Maidan, Keylong, Himachal Pradesh. The phenotypic disease screening suggested that FLW30 was rust-free (O, 0%), but FLW29, iHUW234 and iHUW468 showed susceptibility (S, 75%) to leaf and stripe rust and PBW343 showed moderate resistance (MR, 15%) (Figure 2). Seedling tests with the rust pathotypes were also conducted at the ICAR-IIWBR, Regional Station, Flowerdale, Shimla. The gene combinations that are effective in conferring resistance against the prevalent pathotypes of two rusts include leaf rust (*Lr28*) and stripe rust (*Yr9* and *Yr15*). However, in recent years, new virulent races of some of these genes have emerged[27]. The 12, 77 and 104 leaf rust groups are among the most important new races.

Table 2
The presence or absence of grain protein content gene (*Gpc-B1*) and three rust resistance genes (*Lr28, Yr9* and *Yr15*) based on molecular markers in wheat genotypes

Genotype/Lines	*Gpc-B1*(Xucw-108) 217bp	*Lr28* (STS) 378bp	*Yr9* (Wms582) 251bp	*Yr15* (Xgwm413) 95bp
HUW 234 (*gpc-B1*)	+	-	-	-
HUW468 (*gpc-B1*)	+	-	-	-
FLW30	-	+	+	+
FLW29	-	-	+	-
PBW 343	-	+	-	+



M= 100bp ladder, Lane 1-19= Indian wheat varieties.

Figure 1: Validation of molecular markers in donor and recipient parents for the genes *Lr28, Yr9, Yr 15* and *Gpc-B1* in five Indian wheat varieties

The most predominant pathotypes among these races were 77[7]. The five leaf rust and four stripe rust pathotypes used in this experiment, namely 12-5, 77-8, 77-9, 77-5, 104-2 and 238S119, 110S119, 47S103 and 78S84, respectively, were found to be resistant to FLW30, whereas other genotypes showed different combinations, indicating that they are both susceptible and resistant to these pathotypes (Table 3).The two wheat genotypes (iHUW234 and iHUW468) were found to be susceptible to leaf rust pathotypes 12-5, 77-8, 77-9, 77-5 and 104-2, including the susceptible check Agra local. The FLW29 was resistant to leaf rust pathotypes 77-8 and 104-2 while FLW30 and PBW343 were resistant to pathotypes 12-5, 77-8, 77-9 and 77-5 except 104-2 pathotypes susceptible to PBW343. The remaining genotypes were found to be susceptible.

Except for FLW30, which exhibits resistance, all four genotypes of wheat (FLW29, PBW343, iHUW234 and iHUW468) were found to be susceptible to the pathotypes 238S119 of stripe rust, including the susceptible check Agra local. The pst pathotype 110S119 was resistant to two lines, FLW30 and PBW343. The pst pathotype 47S103 was resistant to three lines, FLW30, FLW29 and PBW343 and the pst pathotype 78S84 was resistant to two lines, FLW30 and FLW29. The phenotypic screening of all pathotypes of stripe and leaf rust is presented in table 3. Thus, FLW30 was the only genotype that showed resistance to all nine pathotypes of leaf and stripe rust and could be used as a potential donor parent in marker-assisted breeding.

It is very difficult for a pathogen to overcome a combination of race-specific and non-race-specific resistant genes. The HUW234 carries *Lr50*, *Lr34* and *Lr13* genes but is susceptible to leaf rust and shows 100S susceptibility under natural field conditions at ICAR-IARI, regional station Wellington, Tamilnadu (India) with a mixture of different races of leaf rust pathogen. According to this research, the gene combination found in this variety was ineffective. The *LR34*, *Lr46* and *Lr50* genes are present in HUW468 and confer 80S susceptibility[45]. Introgression of multiple rust resistance genes using MAS has improved the echelon of resistance in Indian wheat cultivars[14].

As a result, the sources of resistance and validated markers discovered in this study can be used in a marker-assisted breeding program for multiple genes stacking. Molecular markers that are closely linked to the genes, can aid in the selection of lines that carry one or more resistance genes at an early stage of growth[1,35,37].

Table 3
Summaries of the results of phenotypic screening of five genotypes and a check cv. Agra local against different pathotypes of the leaf and stripe rusts under glass-house conditions

Wheat rust	Pathotypes	Distribution of wheat growing zone of India	Status	HUW 234	HUW 468	FLW 30	FLW 29	PBW 343	Agra local
Stripe rust	238S119	NWPZ and NHZ	Predominant	S	S	R	S	S	S
	110S119		Predominant	S	S	R	S	R	S
	47S103		High virulence	S	S	R	R	R	S
	78S84		Predominant	S	S	R	R	S	S
Leaf rust	12-5	Present in uniformly in all zone	Predominant	S	S	R	S	R	S
	77-8		Predominant	S	S	R	R	R	S
	77-9		Predominant	S	S	R	S	R	S
	77-5		Predominant	S	S	R	S	R	S
	104-2		High virulence	S	S	R	R	S	S

Figure 2: Phenotypic screening for leaf and stripe rust diseases of wheat genotypes under natural condition of rust hotspot location at ICAR-IIWBR Dalang maidan, Keylong Himachal Pradesh with Modified Cobb's method[26]

Table 4
FLW 30 was used as donor parent and quality improved varieties HUW234 and HUW468 were used as recipient's parent

S.N.	Wheat variety Name	Targeted genes for pyramiding
1.	FLW30	*Lr*28, *Yr*9, *Yr*15
2.	iHUW234	HUW234+*Gpc-B1*+*Lr24*+HGW (HGW=High Grain Weight)
3.	iHUW468	HUW468+*Gpc-B1*+*Lr24*+HGW (HGW=High Grain Weight)

The present experiment suggested that the wheat variety FLW30 could be used as a donor parent to improve iHUW234 and iHUW468 (Table 4) for leaf rust (*Lr28*) and stripe rust (*Yr9* and *Yr15)* resistance genes. These wheat genotypes can be used in marker-assisted breeding for rust resistance and as potential donors for leaf rust and stripe rust resistance. The two mega wheat varieties, iHUW234 and iHUW468, can be improved through these validated genes (*Lr28*, *Yr9* and *Yr15*) by using gene pyramiding.

Acknowledgement

Department of Biotechnology, Government of India, is duly acknowledged for financial support to conduct present investigation. Authors would like to acknowledge Regional Station, ICAR-IIWBR Dalang Maidan and Flowerdale, Shimla (H.P.) and ICAR-IARI Wellington (T.N.) for off-season trial facilities and rust screening under field and epiphytotic conditions.

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(Received 16th September 2025, accepted 17th October 2025)