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Inheritance and molecular mapping of leaf rust resistance gene in hexaploid wheat Synthetic 45

Prem Chand Gyani, J. B. Sharma, Vinod*, Niharika Mallik and S. K. Jha

Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012

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Abstract

To contain the infection of newly evolved leaf rust virulence(s), diverse source of germplasm need to be explored for identification of novel genes. In order to exploit wild resources, CIMMYT has developed a series of synthetic hexaploid wheat (SHW) by combining the accessions of D genome donor *Triticum tauschii* and *T. durum*. In the present study, Synthetic 45, was evaluated for effectiveness of its resistance against diverse leaf rust pathotypes. Mode of inheritance of resistance to understand the nature of genetics and molecular mapping to locate its chromosomal position were studied. Characterization of leaf rust resistance in Synthetic 45 by multi-pathotype tests showed a high degree of seedling resistance to 20 diverse pathotypes of leaf rust pathogen and adult plant resistance against two most prevalent pathotypes, 77-5 and 104-2. Inheritance studies showed that resistance in Synthetic 45 was governed by a single recessive gene. Molecular mapping and linkage with microsatellite markers, *Xwmc432* and *Xcfd15* have indicated that the resistance gene is located on short arm of 1D chromosome with 6.1 cM distal to *Xwmc432* and 10.6 cM to *Xcfd15* with 4.6 cM distance among the two markers. The gene identified in Synthetic 45 has been tentatively designated as *LrSyn45*.

Key words: Synthetic Hexaploid Wheat, leaf rust resistance, inheritance, mapping, wheat rust

Introduction

Three wheat rusts (leaf, stem and stripe rusts) continue to pose a serious threat by inflicting yield losses in different parts of world. Among the three, leaf rust caused by *Puccinia triticina* Eriks. is most common and widespread on wheat (*Triticum aestivum* L.) in India (Tomar et al. 2014). Depending upon the severity and duration of infection, the losses may reach up to 50% of the yield. In India, 51 pathotypes of leaf rust have been reported during 1931-2016, out of which 15 new pathotypes have been identified in nearly last 10 years. Many useful resistance genes like *Lr9*, *Lr19* and *Lr28* have been rendered ineffective (Nayar et al. 2003; Bhardwaj et al. 2005, 2010a and 2011). Pathotype 77-5 (121R63-1) is currently the most virulent and frequent in the Indian subcontinent, which knocks down several leaf rust resistance genes like, *Lr1, Lr2a, Lr2b, Lr2c, Lr3a, Lr10, Lr11, Lr14a, Lr14b, Lr15, Lr16, Lr17a, Lr20, Lr23, Lr26, Lr27+Lr31, Lr33, Lr36, Lr38, Lr43* and *Lr44* (Bhardwaj et al. 2010b). Further, most of the native *T. aestivum* leaf rust resistance genes became ineffective in India to the most virulent and prevalent pathotypes of group 77. Only a few leaf rust resistance genes *viz*., *Lr1, Lr3, Lr9, Lr10, Lr13, Lr14a, Lr17, Lr19, Lr23, Lr24, Lr26, Lr28* and *Lr34* have been exploited in the leading Indian wheat varieties (Bhardwaj et al. 2010c). Among the major seedling resistance genes utilized, only *Lr24* is having no known virulence in India, however, virulence is known in other parts of the world (Singh 1991).

In a scenario of rapid virulence development against major genes, searching new sources of resistance particularly from related species of wheat shows immense potential. Goat grass (*Triticum tauschii*), the D-genome donor to bread wheat, a relatively untapped germplasm pool was used for production of synthetic hexaploid wheats (SHWs) at CIMMYT. The SHWs possess potential variability for morpho-agronomic traits as well as resistance/ tolerance to biotic/abiotic stresses (Valkoun et al. 1990; Cox et al. 1994). The Division of Genetics, IARI,

*Corresponding author's e-mail: vinod.genetics@gmail.com
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New Delhi maintains an elite set of 65 SHWs. One of these SHWs, designated as Synthetic 45 was observed to be resistant to leaf rust in field conditions under artificial epiphytotics with most virulent and prevalent pathotype, 77-5(121R63-1) of leaf rust. Therefore, the present investigation was undertaken to study effectiveness of resistance in Synthetic 45 against an array of leaf rust pathotypes, to determine nature and number of gene(s) controlling resistance and to locate the position of resistance gene(s) by linked microsatellite markers.

Materials and methods

Development of populations for inheritance and mapping

Leaf rust resistant Synthetic 45 (68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. squarrosa*), was evaluated against diverse pathotypes of leaf rust to identify resistance gene(s) and its mapping on a specific chromosome. Highly susceptible genotypes, Agra Local and Kharchia Local were used as contrasting parents for development of segregating populations involving Synthetic 45. Pure inoculum of 20 pathotypes of leaf rust pathogen *P. triticina* Eriks. used in the study for multipathotype testing was obtained from IIWBR, Regional Station, Flowerdale, Shimla. The most virulent pathotype, 77-5 was selected for inheritance studies.

Resistant parent Synthetic 45 was crossed with susceptible parents, Agra Local and Kharchia Local during *rabi* 2014-15 at IARI, New Delhi. The F_1 plants were raised during summer 2015 at IARI Regional Station, Wellington, The Nilgiris, Tamil Nadu to obtain F_2 seeds for genetic analysis. The F_1 hybrids were backcrossed to susceptible parents to obtain BC_1F_1 generations in off-season (summer nursery) at Dalang maidan, Lahaul Spiti, Himachal Pradesh. The F_1, F_2 and BC_1F_1 generations were raised and tested along with parents at seedling stage under glass house conditions at IARI, New Delhi during *rabi* 2015-16.

Multipathotype testing and screening of populations against leaf rust

For multipathotype testing, seedlings of resistant parent Synthetic 45, susceptible parents Agra Local and Kharchia Local were raised in 10 cm pots, while for inheritance studies P_1, P_2, F_1, F_2 and BC_1F_1 seedlings were raised in the rectangular trays (28cm ×10cm× 7.5cm). Ten days old seedlings were inoculated with 20 pathotypes separately by spray as suspension of rust uredospores in water along with a drop of Tween 20 (Polysorbate 20). Inoculated pots were kept in humidity chamber for 48 hours before shifting to glass house benches (Joshi et al. 1988). Infection types were recorded after 12 days of inoculation following 0-4 scale classification as per Stakman et al. (1962). The infection type 0, ;, 1 and 2 were classified as resistant reaction while infection types 3 and 4 were classified as susceptible.

Genetic analysis

For inheritance studies, the F_1, F_2 and BC_1F_1 generations along with parental lines were tested at seedling stage against pathotype 77-5 of leaf rust pathogen. Chi-square test was applied to observed F_2 phenotypes to determine goodness of fit for a ratio according to Panse and Sukhatme (1967) and to compare the observed ratio with that expected for Mendelian segregation for leaf rust resistance gene. To verify the F_2 results and proposed hypothesis BC_1F_1 generation was genetically analysed.

Genomic DNA isolation and PCR amplification

All the tested F_2 seedlings of the cross Synthetic 45 × Agra Local were transplanted in field for molecular mapping. Tender and fresh leaves were collected from 40-45 days old plants and DNA was isolated following CTAB method (Murray and Thompson, 1980). The RNA was digested and removed by treating the samples with DNase free RNase (10 mg/ml) denatured at 70^{o}C. The purified DNA was quantified on 0.8% agarose gel by loading 1μl of DNA samples, along with known quantity of λ uncut DNA (100ng, 200 ng). The quantified DNA was diluted with TE buffer to a working concentration of approximately 25ng/μl. The PCR for SSR marker analyses were performed with 10 μl reaction volume in the 96-well PCR plates with thermal seal in thermal cycler (model Master cycler pro S, Eppendorf) at temperature profile of 94^{0}C for 4 min for initial denaturation, followed by 45 cycles having Ist step at 94^{o}C for 1 min, IInd step at specific annealing temperature for 1 min and IIIrd step at 72^{o}C for 1 min, with a final extension after 45th cycle at 72^{o}C for 10 min. The amplified PCR products were subsequently resolved on 3.5% metaphor gel in 1X TBE buffer and visualized under UV trans-illuminator in a gel documentation system (Gel Documentation System, Syngene).

Parental polymorphism and Bulked Segregant Analysis (BSA)

The parental polymorphism survey was carried out between Synthetic 45 and Agra Local using 807 SSR markers covering all the 21 chromosomes of the three (A, B and D) genomes of wheat. The polymorphic markers identified by parental polymorphism were used in BSA as described by Michelmore et al. (1991) to identify putatively linked SSR markers. The resistant and susceptible bulks were constituted by pooling equal amount of DNA from 10 resistant and 10 susceptible F_2 plants, respectively.

Linkage analysis and map construction

The markers distinguishing two bulks and parents were considered as putatively linked to leaf rust resistance gene in Synthetic 45. Such putative markers identified in BSA were used for genotyping of F_2 population and linkage analysis was done using MAPMAKER version 3.0 to construct linkage map (Lander et al. 1987) with a minimal LOD score of 3.0 and a maximal genetic distance of 30.0 cM. The genetic distance was calculated in cM using the mapping function of Kosambi (1944).

Results and discussion

Effectiveness of leaf rust resistance in Synthetic 45

At seedling stage Synthetic 45 expressed high levels of resistance (Fig. 1) against all the 20 pathotypes with infection type (IT) ranging from ';' to '1' (Table 1). In contrast, Agra Local and Kharchia Local were susceptible exhibiting ITs '33^+' to '3^+' against all the 20 pathotypes considered in the study. The host-pathogen interaction between Synthetic 45 and 20 pathotypes revealed that leaf rust resistance of Synthetic 45 is broad spectrum having effectiveness against diverse pathotypes of leaf rust pathogen (*P. triticina)*, including the two most predominant pathotypes *viz.*, 77-5 and 104-2 in the natural pathogen population in India. Analysis based on avirulence/virulence formula of 20 pathotypes used in the study and actual infection types produced on Synthetic 45 by host pathogen interaction, indicated that the leaf rust resistance in Synthetic 45 could be a new source of resistance or one among the designated *Lr* gene(s) not present in the differential set commonly used in to identify new pathotypes of leaf rust pathogen.

At adult plant stage also Synthetic 45 exhibited highly resistant response of 5R (5% severity of rust

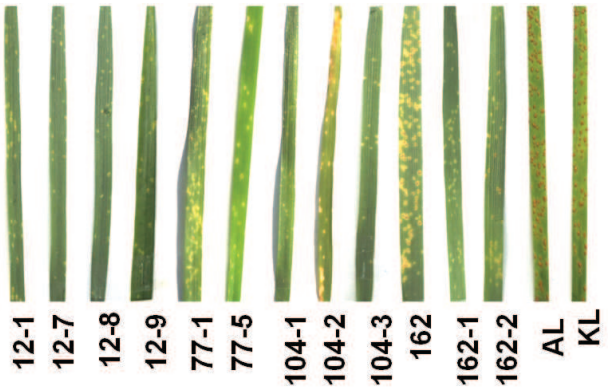

Fig. 1. Disease reaction of Synthetic45 against leaf rust pathogen

Table 1. Infection types on Synthetic 45, Agra Local and Kharchia Local against 20 pathotypes of *Puccinia triticina* when tested at seedling stage of plant growth at mean temperature range 20-28°C

S.No.	Pathotype designation		Synthetic 45	Agra local	Kharchia local
	Old	New			
1	12A	5R13	;1^-	3^+	3^+
2	12-1	5R37	;1í	33^+	33^+
3	12-7	93R45	;1^-	33^+	3^+
4	12-8	49R45	;$1^=$	3^+	33^+
5	12-9	93R37	;$1^=$	3^+	33^+
6	77-1	109R63	;1^-	33^+	33^+
7	77-2	109R31-1	;$1^=$	3^+	3^+
8	77-5	121R63-1	;1^-	3^+	3^+
9	77-8	253R31	;1^-	3^+	33^+
10	77-10	377R60-1	1^-	33^+	33^+
11	104-1	21R31-1	;$1^=$	3^+	3^+
12	104-2	21R55	;$1^{=N}$	3^+	33^+
13	104-3	21R63	;1í	3^+	3^+
14	104-4	21R57	;1=	3^+	33^+
15	107-1	45R35	;	3^+	33^+
16	108-1	57R27	;	33^+	3^+
17	162	93R7	1^-1	3^+	3^+
18	162A	93R15	1^-	33^+	33^+
19	162-1	93R47	;$1^=$	33^+	3^+
20	162-2	93R39	;1^-	3^+	33^+

with resistant pustules) against pathotype 77-5 and TR (Trace i.e. <1% severity with resistant pustules) against pathotype 104-2 during 2 years of testing. In comparison, check Agra Local produced susceptible response of 80S (80% severity of rust with susceptible pustules) to pathotype 77-5, and 70S to pathotype 104-2, while Kharchia Local produced susceptible response of 90S to pathotype 77-5 and 70-80S to pathotype 104-2 (Table 2). Results further suggest that leaf rust resistance of Synthetic 45 is also highly effective at adult stage of plant growth against the most prevalent pathotypes (77-5 and 104-2) in India. The reaction on F_{1s}, Synthetic 45 × Agra Local and Synthetic 45 x Kharchia Local produced susceptible ITs '33^{+}', '3^{+}', respectively (Fig. 1) indicating that resistance in Synthetic 45 is recessive in nature (Table 3).

Table 2. Adult plant response of Synthetic 45, Agra Local and Kharchia Local against leaf rust (*Puccinia triticina)* pathotypes 77-5 and 104-2 in field conditions during 2014-15 and 2015-16

Pathotype	Year	Parental line		
		Synthetic 45	Agra local	Kharchia local
77-5	2014-15	5R	80S	90S
	2015-16	5R	80S	90S
104-2	2014-15	TR	70S	80S
	2015-16	TR	70S	70S

The two F_2 populations derived from the crosses, Synthetic 45 × Agra Local (266 plants) and Synthetic 45 × Kharchia Local (135 plants) tested against pathotype 77-5 segregated into 1 resistant: 3 susceptible ratio with good fit (χ^2=0.1253, $P_{1d.f.}$ =0.7234 and χ^2=0.0617, $P_{1d.f.}$=0.8038, respectively) suggesting monogenic recessive genetic control of resistance. In addition, the 47 plants of BC_1F_1 population generated by Synthetic 45/Agra Local// Synthetic 45 and 42 plants of BC_1F_1 population generated by Synthetic 45/Kharchia Local//Synthetic 45 fitted well in test cross ratio of 1R:1S (χ^2=0.1915, $P_{1d.f.}$=0.6617 and χ^2=0.3809, $P_{1d.f.}$=0.5371 respectively) confirming the F_2 results of functioning of single recessive gene. BC_1F_1 populations derived from backcrossing of F_{1s} with susceptible parent, Synthetic 45/Agra Local//Agra Local (157 plants) and Synthetic 45/Kharchia Local//Kharchia Local (92 plants) did not segregate into resistant and susceptible phenotypes but all seedlings tested showed susceptibility to pathotype 77-5, as expected. The genetic analysis of both the populations demonstrated and supported that the resistance in Synthetic 45 is governed by a single recessive gene. The resistance gene identified in Synthetic 45 has been putatively named as '*LrSyn45*'.

Identification of molecular marker(s) linked to the leaf rust resistance in Synthetic 45

Out of the 807 SSR markers, 115 showed

Table 3. Infection types on parental lines, F_1, F_2 and BC_1F_1 of crosses involving Synthetic 45, Agra Local and Kharchia Local against pathotype 77-5 of *P. triticina* at seedling stage at mean temperature range 20-28^{0}C

Line/population	No. seedlings			Expected ratio	χ^2	df	P-Value
	Resistant (IT ;$1^{=}$ to 1^{+})	Susceptible (IT 3 to 3^{+})	Total	(R:S)			
Synthetic 45 (Syn. 45)	12	0	12				
Agra Local	0	10	10				
Kharchia Local	0	11	11				
Syn.45/AL-F_1	0	14	14				
Syn.45/KL-F_1	0	10	10				
Syn.45/AL-F_2	69	197	266	1:3	0.1253	1	0.7234
Syn.45/KL-F_2	35	100	135	1:3	0.0617	1	0.8038
Syn.45/AL//Syn.45-BC_1F_1	22	25	47	1:1	0.1915	1	0.6617
Syn.45/KL//Syn.45-BC_1F_1	19	23	42	1:1	0.3809	1	0.5371
Syn.45/AL//AL-BC_1F_1	0	157	157	0:1			
Syn.45/KL//KL-BC_1F_1	0	92	92	0:1			

polymorphism between the two parental lines, Synthetic 45 and Agra Local. The polymorphic markers were used for Bulked Segregant Analysis (BSA). Out of 266 transplanted F_2 plants, only 134 F_2 plants survived and were considered for DNA isolation and molecular mapping of leaf rust resistance gene. BSA performed with 115 polymorphic SSR markers on contrasting bulks along with parents showed two SSR markers, *Xwmc432* and *Xcfd15* located on short arm of chromosome 1D as co-segregating with leaf resistance gene in Synthetic 45 and were recognized as putatively linked (Fig. 2). The F_2 population was

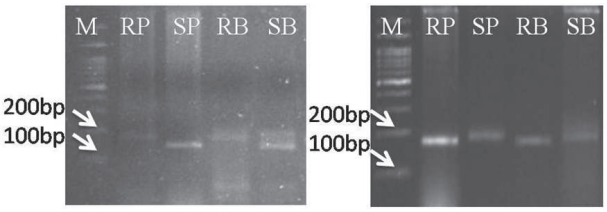

Fig. 2. Bulked Segregant Analysis by SSR markers *Xwmc432* and *Xcfd15* located on short arm of chromosome 1D

genotyped using both the co-segregating SSR markers, *Xwmc432* and *Xcfd15*. The calculated value of Chi-square tests for SSR marker, *Xwmc432* ($\chi^2_{(1:2:1)}$ =2.999, P_{2df}=0.2231) and *Xcfd15* ($\chi^2_{(1:2:1)}$ =0.7313, P_{2df}=0.6937) individually fit well in the expected genetic ratios in F_2 populations. The F_2 phenotypic classes (resistant and susceptible) were used for genetic linkage analysis. Linkage was detected between the *LrSyn45* and the SSR marker *Xwmc432* (χ^2_L=136.6817, P_{2df}<0.001) (Table 4). Similarly, the linkage was detected between *LrSyn45* and SSR marker *Xcfd15* (χ^2_L=101.8559, P_{2df}<0.001) (Table 5).

Table 4. Joint segregation of co-dominant SSR marker *Xwmc432* with F_2 phenotypes from the cross Synthetic 45 x Agra Local

F_2 Phenotype	*Xwmc432*				$\chi2_{(1:3)}$	P-Value
	A	H	B	Total		
Resistant (rr)	37	1	3	41	2.2388	1df,0.1346
Susceptible (R-)	2	56	35	93		
Total	39	57	38	134		
χ^2 (1:2:1) = 2.9999, 2df, P = 0.2231						
χ^2(Compounded) 3:6:3:1:2:1= 141.9204, 5df, P<0.001						
χ^2 difference (Linkage) = 136.6817, 2df, P<0.001						

A=Homozygous for Synthetic 45 allele, B=Homozygous for Agra Local allele, H= Heterozygous

Table 5. Joint segregation of co-dominant SSR marker *Xwmc432* with F_2 phenotypes from the cross Synthetic 45 x Agra Local

F_2 Phenotype	*Xcfd15*				$\chi2_{(1:3)}$	P-Value
	A	H	B	Total		
Resistant (rr)	33	7	1	41	2.2388	1df,0.1346
Susceptible (R-)	4	60	29	93		
Total	37	67	30	134		
χ^2 (1:2:1) = 0.7313, 2df, P = 0.6937						
χ^2(Compounded) 3:6:3:1:2:1= 104.8258, 5df, P<0.001						
χ^2 difference (Linkage) = 101.8559, 2df, P<0.001						

A=Homozygous for Synthetic 45 allele, B=Homozygous for Agra Local allele, H= Heterozygous

Linkage (χ^2_L=189.4333, P_{4df}<0.001) was also detected between the two SSR markers, *Xwmc432* and *Xcfd15*.

The χ^2 linkage value between *LrSyn45* and the marker *Xwmc432* is higher than the χ^2 linkage value between *LrSyn45* and the marker *Xcfd15* suggesting that *LrSyn45* is closer to the marker *Xwmc432* as compared to *Xcfd15.* The highest χ^2 linkage value between the two SSR markers showed that two markers are at the closest distance in comparison to the distances between *LrSyn45* and the two markers. This analysis indicated the sequence of gene and the markers as *LrSyn45-Xwmc432-Xcfd15*. The result of linkage analysis using software MAPMAKER version 3.0 (Lander et al. 1987) for construction of linkage map suggested that the leaf rust resistance gene in *LrSyn.45* is located at 6.1 cM distal to the marker *Xwmc432* and 10.6 cM distal to another marker *Xcfd15* (Fig. 3). The genetic distance between the two markers

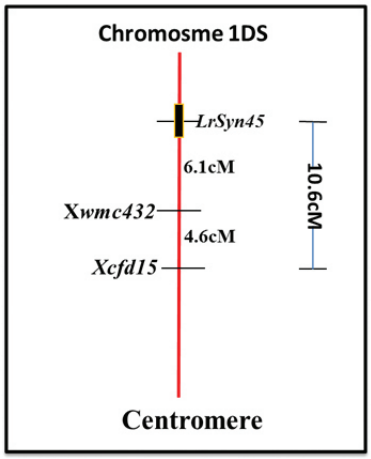

Fig. 3. Linkage map of leaf rust resistance gene *LrSyn45*

was 4.6 cM. Linkage map also suggested the sequence of gene and the markers as *LrSyn45-Xwmc432-Xcfd15*.

Among the known leaf rust resistance gene, three genes *viz*., *Lr21*, *Lr42* and *Lr60* have been reported to be located on short arm of chromosome 1D. *LrSyn45* was considered different from *Lr21,* because *Lr21* is ineffective to Indian pathotypes 12, 12B, 77, 77A-1, 104B and 162 of leaf rust pathogen (Kumar et al. 1988), whereas in our study, Synthetic 45 showed resistance to a common pathotype 162 and other pathotypes of race groups 12, 77 and 104. Regarding the possibility of *LrSyn45* being the *Lr42* is not clear because of different reports on the nature of inheritance of *Lr42 viz*., dominant (Czembor et al. 2008), partial dominant (Cox et al. 1994) and recessive (Liu et al. 2013). However, the inheritance of proposed resistance gene, *LrSyn45* is recessive in the present study. Another leaf rust resistance gene *Lr60* (*LrW2*), also reported to be located on chromosome1DS is dominant in nature (Hiebert et al. 2008). The uniqueness of *LrSyn*45 in synthetic 45 suggests the identified resistance is presumably diverse. However, for further confirmation of the uniqueness of the gene, the test of allelism with *Lr42* and *Lr60* and fine mapping of *LrSyn45* is required.

Authors' contribution

Conceptualization of research (JBS, PCG, V, SKJ); Designing of the experiments (PCG, JBS, V, SKJ); Contribution of experimental materials (JBS); Execution of field/lab experiments and data collection (PCG); Analysis of data and interpretation (PCG, JBS, V, NM, SKJ); Preparation of manuscript (PCG, JBS, V, NM, SKJ)

Declaration

The authors declare no conflict of interest.

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