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Allelic Relationship between *Lr*9 and the Leaf Rust Resistance Gene in Kharchia Local Mutant of Wheat

Navjot Kaur Dhillon[1*], Harcharan Singh Dhaliwal[2]

[1]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India; [2] Akal School of Biotechnology, Baru Sahib, Himachal Pradesh, India.
Email: *dhillon.navjot@gmail.com

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ABSTRACT

*To confirm allelic relationship between Lr*9 *and the leaf rust resistance gene in KLM*4-3*B*, *genetics of resistance was studied using crosses* (*WL*711 + *Lr*9) × *WL*711 *and* (WL711 + *LrKLM*4-3*B*) × *WL*711. *The* F_2 *populations in cross* (*WL*711 + *Lr*9) × *WL*711 *and* (*WL*711 + *LrKLM*4-3*B*) × *WL*711 *segregated in ratio of* 3:1 *for disease reaction at seedling stage against pathotype* 77-5 *of leaf rust. This suggests that rust resistance in these stocks are under the control of single dominant genes. Further*, *to study allelic relationship between Lr*9 *and LrKLM*4-3*B*, F_2 *population of the cross* (*WL*711 + *LrKLM*4-3*B*) × (*WL*711 + *Lr*9) *was studied. A segregation ratio of* 15:1 *implies that the two genes Lr*9 *and LrKLM*4-3*B are non-allelic genes.*

***Keywords*:** *Lr*9, *Isogenic Lines*, *Non-Allelic Genes*

1. Introduction

It is imperative to stabilize the wheat production by reducing the losses due to various diseases including leaf rust, stem rust, yellow rust, Karnal bunt etc. Among the diseases, leaf rust caused by *Puccinia recondita* Roberage ex. Desmaz f.sp. *tritici* is one of the most important and devastating foliar diseases of wheat which cause significant yield losses all over the world [1-8]. In all regions in which wheat is grown, rusts have caused periodic severe epidemics [9]. The rust accelerates foliage senescence reducing cumulative light interception of the crop which leads to reduced dry matter production [10]. Breeding for resistance against leaf rust is an economical, efficient and environmentally safe control measure to reduce these losses [11]. Development of disease resistant varieties is one of the most economical methods of control of diseases like leaf rust. However, growing of rust resistant varieties having single gene for resistance results in rapid evolution of virulent biotypes of the pathogen, thereby making the resistance gene ineffective and the variety susceptible to rust. One of the ways to develop varieties with durable rust resistance is to pyramid the genes for resistance in a single variety [12]. It is difficult to pyramid two or more disease resistance genes through conventional means, particularly where the resistance genes in question are effective against all the prevalent pathotypes. However, recent advances in molecular biology have made it possible to pyramid several genes in single line using marker assisted selection (MAS). Tagging of genes with molecular markers is pre-requisite for MAS [13].

A number of rust resistance genes, including those for leaf rust resistance, have been transferred from wild relatives of wheat into cultivated wheats [14,15]. In India, from the analyses of 2630 samples collected from 17 states, one union territory and Nepal from 2005 to 2008, 31 races were identified among which eight were new [16]. Most of these could not, however be exploited commercially because of extensive linkage drag. One of the leaf rust resistance genes, *Lr*9 transferred from *Aegilops umbellulata* [17] and located on chromosome 6BL, has no undesirable effect associated with it [18]. This gene is effective against all the races of leaf rust currently prevalent in Northern India. Similarly, another leaf rust resistance gene identified in (Kharchia local mutant KLM4-3B) is also effective against all the prevalent leaf rust pathotypes in Northern India. In the absence of virulence, capable of differentiating *Lr*9 and KLM4-3B in the In-

dian subcontinent KLM4-3B has been suspected to be *Lr*9 rather than an induced mutant of Kharchia local [19].

High density molecular maps have been constructed in several crops including rice, maize, tomato and *Triticum* [20,21] and a number of genes of economic importance have been tagged with series of molecular markers [22, 23]. Molecular markers closely linked to the genes for rust resistance can be used not only for establishing allelic relationships among resistant sources but also for their pyramiding using marker assisted selection (MAS).

2. Material and Method

2.1. Disease Reaction Studies

Single spore culture of *P recondita* f.sp. *tritici* variants 77-5 (maintained on Agra local) were used for identification of F_2 seedling resistance genes.

2.2. Raising of Seedlings

Seeds of the parents (WL 711, KLM4-3B and Thatcher + Lr9) were sown along with F_2 populations of the three crosses in bread boxes containing a mixture of farmyard manure and sandy loam soil in equal proportions. Agra local was also sown as susceptible check. The seedlings were raised in glass house maintained at a temperature of 25˚C ± 1˚C. Relative humidity above 80 per cent was maintained by using desert cooler. The bread boxes were watered every day to maintain vigour of the seedlings.

First leaf of the seven day old seedling was inoculated with homogeneous mixture of appropriate rust culture and talc, keeping inoculum density of 6 - 10 urediospores per microscopic field at a magnification of 100x under light microscope. After inoculation the seedlings were incubated at 100 percent relative humidity for 16 hours. These seedlings were then transferred to growth chambers.

2.3. Scoring the Infection Types

Fourteen days after inoculation, the infection type on the seedlings was scored using a modification of the scale given by Stakman [24]. The seedlings showing .infection type 0, 1, 2 and X were classified as resistant, whereas those with infection types 3 to 4 were classified as susceptible.

Further, disease reaction of F_2 population of three different crosses at adult stage were scored by using Modified Cobb Scale by Peterson [25].

2.4. Statistical Analysis

Simple Chi-square (χ^2) test was applied to fit appropriate genetic ratios in F_2 generation obtained from the three crosses (WL 711 + *Lr*9) × WL 711, (WL 711 + *Lr*KLM4-3B) × WL 711 and (WL 711 + *Lr*9) × (WL 711 + *Lr*KLM4-3B). Chi-square value was calculated using the following formula:

$$\chi^2_{(n-1)\text{d.f.}} = \sum \frac{(\text{O}-\text{E})^2}{\text{E}}$$

where,

n = Number of phenotypic classes.

d.f. = Degree of freedom.

O = Number of observed plants in a phenotypic class.

E = Number of expected plants in a phenotypic class.

3. Results and Discussion

In the present investigation, to confirm the allelic relationship between the leaf rust genes *Lr*9 and the resistant gene in KLM4-3B, F_2 generations of three crosses (WL 711 + *Lr*9) × WL 711, (WL 711 + *Lr*KLM4-3B) × WL 711 and (WL 711 + *Lr*9) × (WL 711 + *Lr*KLM4-3B) were studied. The results pertaining to these studies are presented here.

Results of the cross of isogenic line of the leaf rust resistant gene of KLM4-3B (*Lr*KLM4-3B) with the recurrent parent, WL711 are presented in **Tables 1** and **2**. Out of 122 F_2 plants, 86 were resistant and 36 susceptible to leaf rust pathotype 77-5. The infection type observed on resistant plants were 0, 0, 1, 1^+, 2, 2^+. The segregation of F_2 plants showed a good fit to 3:1 ratio ($\chi^2 = 1.32$). This indicated that the *Lr* KLM4-3B is dominant.

In the second cross of isogenic lines of *Lr*9 with the recurrent parent, out of 126 F_2 plants 89 plants were resistant and 37 were susceptible to the leaf rust pathotype 77-5 [**Table 1**]. The ratio of resistant to susceptible plants did not differ significantly from 3:1 ($\chi^2 = 1.28$). This indicated that *Lr*9 also behaves as dominant gene to pathotypes 77-5.

In the cross between isogenic lines carrying *Lr*9 and *LrKLM*4-3*B*, out of 101 F_2 plants, 91 showed resistant reaction and 10 were susceptible to pathotype 77-5. This did not differ significantly from 15 resistant: 1 susceptible ratio (**Table 1**). This suggested that the two leaf rust resistant genes, *Lr*9 and *Lr*KLM4-3B, are non-allelic. The earlier studies have also shown that these two leaf rust genes are non-allelic [19]. *Lr*9 is an alien gene on chromosome 6BL translocation from *Aegilops umbellata* [17], whereas *Lr*KLM4-3B was identified to be resistant to leaf rust [26]. Preliminary work carried out at the School of Biotechnology, Punjab Agricultural University has shown that the *Lr*KLM4-3B is not a mutant gene as claimed earlier [26] but is associated with translocation involving chromosome 2BL (Dhaliwal and Harjit Singh, Pers.Commu.). These observations further support that these two genes are non-allelic.

4. Conclusions

Genetics of resistance studied of F2 population using

Table 1. F_2 segregation for reaction to leaf rust pathotype 77-5 in three different crosses.

Sr. No.	Crosses	Total no. of plants	Observed number of plants					Expected ratio	X^2 (Cal.)	Probability (P)
			Resistant (O-2$^+$)	3$^+$	4$^-$	4	Total			
1.	(WL711 + *Lr* KLM4-3B) × WL711)	122	86	14	4	18	36	3:1	1.32^{NS}	0.10 - 0.25
2.	(WL711 + *Lr*9 × WL711)	126	89	16	2	19	37	3:1	1.28^{NS}	0.10 - 0.25
3.	(WL711 – *Lr* KLM4-3B) × (WL711 + *Lr*9)	101	91	5	2	3	10	15:1	2.29^{NS}	0.05 - 0.10

Table 2. Adult stage disease reaction of F_2 population.

Seedling reaction	(WL 711 + *Lr*KLM4-3B) × WL711		(WL711 + *Lr*9) × WL711		(WL711 – *Lr* KLM4-3B) × (WL711+ *Lr*9)	
	Adult reaction	Number of plants	Adult reaction	Number of plants	Adult reaction	Number of plants
0, 0;, ;,	0	75	0	79	0	86
1, 1$^+$, 2, 2$^+$	TS	5	TS	4	TS	1
(R)	5S	9	5S	6	5S	4
	10S	4	10S	4	10S	5
3$^+$, 4	40S	4	40S	7	40S	3
(S)	60S	26	60S	23	60S	2
	80S	2	80S	3	80S	-

crosses (WL711 +*Lr*9) × WL711 and (WL711 + *LrKLM*4-3*B*) × WL711 segregated in ratio of 3:1 for reaction to pathotype 77-5 of leaf rust. This suggested that rust resistance in these stocks is under the control, of single dominant genes. Further, to study allelic relationship between *Lr*9 and *LrKLM*4-3*B*, F_2 population of the cross (WL711 +*LrKLM*4-3*B*) × (WL711 +*Lr*9) was studied. A segregation ratio of 15:1 demonstrate that the two genes *Lr*9 and *Lr*KLM4-3B are two different non-allelic genes.

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