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Identification of Molecular Markers Linked to Leaf Rust Resistance Genes in Wheat and Their Detection in the Local Near-Isogenic Line

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ABSTRACT

Sixty-five random amplified polymorphic DNA (*RAPD*) *primers were used for the detection of polymorphism among recipient and donor parents and their isogenic lines linked to leaf rust resistance genes*, *Lr*9 *and the resistant gene in kharchia local mutant KLM*4-3*B. Three primers showed polymorphism among recurrent parent*, *donor parent and isogenic lines.*

Keywords*: *RAPD*, *Lr*9, *Isogenic Lines

1. Introduction

With the introduction of semi-dwarf, photoinsensitive, fertilizer responsive and the high yielding varieties of wheat, the wheat production in India has increased from 12 million tonnes in 1966 to 70 million tonnes in the recent years. Wheat exceeds every other grain crop in acreage and production and is, therefore, the most important cereal of the world. 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 [1-5]. Breeding for resistance against leaf rust is an economical, efficient and environmentally safe control measure to reduce these losses. 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. 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 has made it possible to pyramid several genes in single line using marker assisted selection (MAS) and tagging of genes with molecular markers is pre-requisite for MAS.

A number of rust resistance genes, including leaf rust, have been transferred from wild relatives of wheat into cultivated wheats [6]. Most of which could not be exploited because of extensive linkage drag. One of the leaf rust resistance genes, *Lr*9 transferred from *Aegilops umbellulata* [7] and located on chromosome 6BL, has no undesirable effect associated with it [8]. 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.

Keeping this in view the present study was undertaken to identify molecular markers linked with *Lr*9 and KLM4-3B and to pyramid *Lr*9 with rust resistant gene in KLM4-3B, as both the genes provide resistance against most of the leaf rust pathotypes of the Indian subcontinent.

2. Materials and Methods

2.1. Plant Material

Near-isogenic lines carrying the leaf rust resistance genes *Lr*9 and the *Lr* gene of KLM4-3B in the background of

WL711 developed at the School of Biotechnology were used along with the donor and the recurrent parents for identifying RAPD markers linked to the two genes.

2.2. Genomic DNA Isolation

Approximately 5 g fresh weight of young leaves were harvested from plants grown in the field and DNA was extracted as per the method of Dellaporta [9].

2.3. Random Amplified Polymorphic DNA (RAPD) Analysis

RAPD analysis was carried out as described by Williams [10] using 10 base pair (bp) primers from Operon Technologies Inc., Alameda, California. PCR was performed in a reaction volume of 25 µl, containing 2.5 µl of 1 mM dNTPs, 3.2 µl of 15 mM $MgC1_2$, 2.5 µl of 10X buffer containing 10 mM tris-HC1 pH 8.3, 2.0 mM $MgC1_2$, 50 mM KC1, 1.5 µl (20 ng) of single 10-base primer, 1 µl (30 ng) of template DNA and 0.5 µl (1 unit) of Taq polymerase (Stratagene). The reaction mixture was topped with 50 µl of sterilized mineral oil. Template DNA was initially denatured at 94˚C for 5 minutes followed by 45 cycles of PCR amplification under following parameters. One minute denaturation at 94˚C, one minute primer annealing at 36˚C and 2 minutes primer extension at 72˚C further followed by 5 minutes final extension at 72˚C on a Perkin Elmer Cetus Thermal Cycler.

2.4. Gel Electrophoresis and Photography

5 µl loading buffer consisting of 0.5% bromophenol blue, 0.5% xylene cynole FF and 50% glycerol in 1XTAE buffer (0.04M Tris-acetate, 0.00lM EDTA) was added to the PCR amplification products for visualization of gel run. Aliquots of 25 µl of DNA products from the PCR amplification were loaded in 1.5% agarose gel prepared in 1 × TAE buffer at 3 V/cm. Gel was stained with ethidium bromide and photographed under UV light.

3. Results and Discussion

In the present investigation, to identify molecular markers linked with *Lr*9 and KLM4-3B and to pyramid *Lr*9 with rust resistant gene in KLM4-3B, RAPD markers were tried on near-isogenic lines carrying the leaf rust resistance genes *Lr*9 and the *Lr* gene of KLM4-3B in the background of WL711 along the recipient and donor parents of the isogenic lines for the two genes in WL711. The results pertaining to these studies are presented here.

3.1. RAPD Analysis

65 random primers were tried to study polymorphism among recurrent and donor parents and their isogenic line (**Table 1**). Out of 65 primers tested, 42 primers gave amplification (64.62%). Total number of loci amplified with 42 primers tested in WL711 , KLM4-3B, isogenic line of *Lr* KLM4-3B, Thatcher + *Lr*9 and isogenic line of *Lr*9 were 148, 145, 140, 126 and 126, respectively. Limited polymorphism was detected among WL 711, Thatcher + *Lr*9, WL 711 + *Lr*9, *Lr* KLM4-3B, WL 711 + *Lr* KLM4-3B when genomic DNA was amplified with the RAPD primers.

Table 1. Number of RAPD loci in recurrent parent, donor parent and isogenic lines.

Primer Primer	Sequence of the primer 5' to 3'	Number of RAPD loci				
		WL 711	KLM 4-3B	WL711 + KLM4-3B	Thatcher + *Lr*9	WL711 + *Lr*9
OPA-02	TGCCGAGCTC	3	3	3	3	3
OPA-14	TCTGTGCTGG	2	1	1	1	1
OPA-15	TTCCGAACCC	2	2	2	2	2
OPA-16	AGCCAGCGAA	2	2	2	3	3
OPA-18	AGGTGACCGT	3	3	3	2	2
OPA-19	CAAACGTCGG	2	3	2	1	1
OPB-05	TGCGCCCTTC	5	5	5	5	5
OPB-06	TGCTCTGCCC	3	3	3	3	3
OPB-15	GGAGGGTGTT	6	3	3	3	3
OPB-17	AGGGAACGAG	5	3	3	3	3
OPC-02	GTGAGGCGTC	2	2	2	2	2
OPC-04	CCGCATCTAC	-	2	-	-	-
OPC-16	CACACTCCAG	2	3	5	5	3
OPC-20	ACTTCGCCAC	5	5	5	3	5
OPD-l2	CACCGTATCC	3	1	3	3	4
OPD-l4	CTTCCCCAAG	2	2	2	-	-

Primer Primer	Sequence of the primer 5' to 3'	Number of RAPD loci				
		WL 711	KLM 4-3B	WL711 + KLM4-3B	Thatcher + *Lr*9	WL711 + *Lr*9
OPE-10	CACCAGGTGA	1	-	-	-	-
OPE-20	AACGGTGACC	4	4	2	4	4
OPG-13	CTCTCCGCCA	3	3	3	3	3
OPG-l4	GGATGAGACC	4	4	4	4	4
OPG-l7	ACGACCBACA	2	2	2	2	2
OPJ-01	CCCGGCATAA	6	6	6	6	6
OPJ-05	CTCCATGGGG	1	1	1	-	-
OPJ-10	AAGCCCGAGG	7	6	6	7	7
OPJ-12	GTCCCGTGGT	2	2	2	2	2
OPK-10	GTGCAACGTG	10	10	10	10	10
OPK-13	GGTTGTACCC	1	1	1	1	1
OPK-15	CTCCTGCCAA	3	-	-	-	-
OPK-16	GAGCGTCGAA	3	3	3	3	3
OPO-10	TCAGAGCGCC	4	4	4	3	3
OPO-11	GACAGGAGGT	1	1	1	1	1
OPP-01	GTAGCACTCC	6	6	6	6	6
OPP-05	CCCCGGTAAC	4	4	4	-	-
OPP-06	GTGGGCTGAC	4	4	4	2	2
OPP-10	TCCCGCCTAC	5	5	5	3	3
OPP-19	GGCTTGGCCT	6	6	6	6	6
OPZ-12	TCAACGGGAC	3	3	3	3	3
OPZ-15	CAGGGCTTTC	1	1	1	1	1
OPZ-17	CCTTCCCACT	5	5	5	5	5
OPBA-06	ATATTTGGCC	4	4	4	3	3
OPBA-12	GCCCTTAGCA	3	3	3	2	2
OPBA-14	GCAATGGGAT	3	3	3	3	3
Total Number of Loci	**148**	**145**	**140**	**126**	**126**	

Some genes of agronomic importance, however, have been tagged with RAPD markers in tomato [11,12], in rice [13-15], and in wheat [16-19]. The three primers (OPA-19, OPD-12, OPJ-10) were polymorphic between WL711 and other stocks. One RAPD locus amplified with primer OPA-19 was observed in Thatcher + *Lr*9 and its isogenic lines. The amplification product was absent in WL711 (**Figure 1**). This indicated association of this RAPD marker with *Lr*9.

Similarly, with RAPD primer OPD-12, one locus was specifically amplified in KLM4-3B and the isogenic line carrying *Lr* KLM4-3B. The amplification product was absent in WL711 (**Figure 2**).

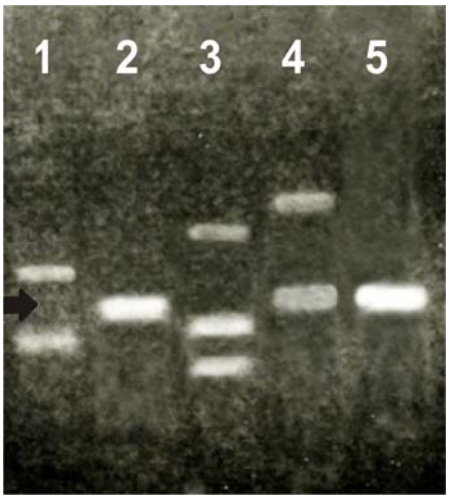

Figure 1. Genomic DNA amplification with primer OPA-19. Lane1: WL711; lane2: *Lr*9; lane3: KLM4-3B; lane4: WL711 + KLM4-3B; lane5: WL711 + *Lr*9.

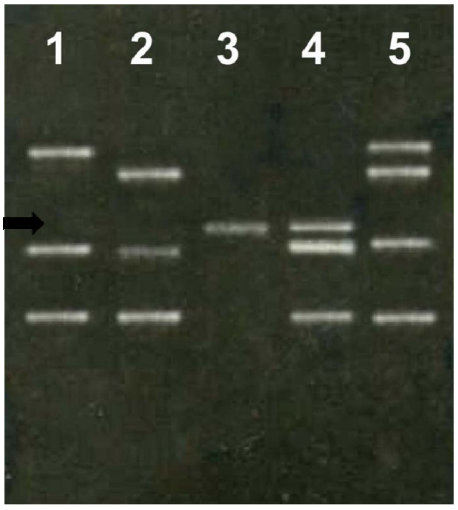

Figure 2. Genomic DNA amplification with primer OPD-12. Lane1: WL711; lane2: *Lr*9; lane3: KLM4-3B; lane4: Wl711 + KLM4-3B; lane5: WL711 + *Lr*9.

Amplification of a distinct RAPD loci associated with *Lr*9 with RAPD, primer OPA-19 and its absence in donor and isogenic lines containing *Lr*KLM4-3B vs RAPD primer OPD-12 which amplified a distinct loci associated with *Lr*KLM4-3B and absent in lines with *Lr*9 further indicate that *Lr*9 and *Lr*KLM4-3B are non-allelic.

Bread wheat has a narrow genetic base. This was also shown by limited polymorphism for many molecular markers [20,21]. This limited polymorphism along with higher ploidy level and high repetitive DNA content has impeded genetic linkage mapping in wheat. RAPD markers behave as dominant markers because polymorphism is detected as the presence/absence of bands. RAPD markers provide a quick and cost effective method for generating genetic maps and analyzing population. MAS could be useful in the development of highly resistant germplasm based on new combinations of *Lr* genes are *Lr*53 [22], *Lr*56 [23], *Lr*57 [24], *Lr*59 [25], *Lr*62 [26], *Lr*63 and *Lr*66 [27].

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