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Full Length Research Paper

Identification of leaf rust resistant gene *Lr10* in Pakistani wheat germplasm

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Leaf (brown) rust is the major disease of wheat in Pakistan and other countries. The disease is more effectively controlled when several rust resistance genes are pyramided into a single line. Molecular survey was conducted to screen 25 Pakistan wheat germplasm for the presence of leaf rust resistance gene *Lr10* using specific STS primer. The survey revealed that out of the 25 germplasm/lines grown in Hazara University Botanic garden, 18 genotypes were observed with *Lr10* gene, while seven genotypes did not show the presence of *Lr10* gene. The identification of *Lr10* in Pakistan wheat germplasm will help in accelerating the breeding program in future, including the pyramiding of different resistant genes in wheat varieties.

Key words: Wheat, leaf rust, *Lr10*, molecular markers.

INTRODUCTION

Wheat plays a central role in Pakistan's food economy, both in terms of production and consumption. In Pakistani, it is grown on about 18,00,000 hectares. In 2009, wheat production in Pakistan was 24 million tons (MINFAL, 2009). Gap in production and consumption is escalating due to ever increasing population. Wheat production is also decreasing due to the attack of certain diseases like rusts, smuts, powdery mildews, etc. Rust diseases of wheat are among the oldest plant diseases known to man. Leaf rust is the most destructive and devastating disease due to its time of appearance, nature of attack, regular occurrence and prolonged growing season that is prevalent for its development in the wheat growing areas of the world (Khan et al., 1997). Since the discovery of rust, numerous studies have been conducted on the life cycles of rust pathogens and their management. Due to airborne nature of the disease, use of chemicals is neither economical nor feasible on a large scale. The only economic and practical control of rust diseases can be achieved through genetic resistance (Pathan and Park, 2006; McIntosh, 1988).

Breeding for durable resistance against leaf rust disease in wheat is based on the combination of different leaf rust (*Lr*) resistance genes in one cultivar. The selection of genotypes containing several leaf rust resistance genes using infected leaf rust isolates with defined avirulence genes is very time consuming. Over the last 20 years, DNA marker techniques have been developed which helps in the selection of plants on the basis of genotype so that environmental variables may not affect the selection of plants for a particular trait (Xu et al., 2005). The development of molecular markers for specific leaf rust genes allows the detection of these genes independently of the phenotype. Molecular markers can be used in marker-assisted selection for an efficient combination of genes in the pyramiding strategy to create a more durable resistance (Feuillet et al., 1995).

The leaf rust resistance gene *Lr10* originates from the wheat gene pool. *Lr10* is a single-copy gene on chromosome 1AS. It confers enhanced resistance to leaf rust.

This study was carried out for the molecular screening of the presence of *Lr10* gene in the selected wheat varieties and their use in gene pyramiding, using specific STS marker.

MATERIALS AND METHODS

25 varieties of common wheat collected from different region of Pakistan were included in the study. The list of all these varieties is

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Table 1. Rust rating scale.

S/N	Rust percentage (%)	Rating	Host reaction
1	0	9	Very highly resistant (VHR)
2	0-5	8	Highly resistant (HR)
3	5-10	7	Resistant (R)
4	10-15	6	Moderately resistant (MR)
5	15-25	5	Moderately susceptible (MS)
6	25-40	4	Moderately susceptible to susceptible (MSS)
7	40-70	3	Susceptible (S)
8	70-90	2	Highly susceptible (HS)
9	90-100	1	Very highly susceptible (VHS)

Table 2. Leaf rust severity (%) on the 25 studied wheat varieties.

S/N	Variety	Leaf rust (% age)	Host reaction
1	SANDAL	21.223BCD	MS
2	C-228	22.223BCD	MS
3	C-250	28.333ABC	MSS
4	WL-711	40.113A	S
5	BARANI 83	21.330BCD	MS
6	C-273	29.443AB	MSS
7	LYP-73	19.777BCD	MS
8	C-591	14.667D	MR
9	PAK-81	28.330ABC	MSS
10	SIND-81	19.443BCD	MS
11	CHENAB-79	22.777ABCD	MS
12	PUNJAB-76	19.887BCD	MS
13	ZARGHON-79	14.443D	MR
14	KOHINOOR-83	18.890BCD	MS
15	B-SILVER	28.333ABC	MS
16	LU-26	20.220BCD	MS
17	FBD-08	18.887BCD	MS
18	BAHAWALPUR-79	15.000CD	MR
19	LASANI-08	19.440BCD	MS
20	KIRAN	30.000AB	MSS
21	SUSSI	31.110AB	MSS
22	FAKHRI SARHAD	9.443D	R
23	MARAJ 08	9.890D	R
24	TANDOJAN-83	18.333BCD	MS
25	SHAHKAR-95	12.553D	MR

given in Table 2.

Leaf rust resistance screening

All the genotypes were screened for leaf rust resistance. Leaf rust severity was estimated visually as the percentage of leaf area affected using the slightly modified integrated scale (1 to 9) for rust evaluation (Goel and Saini, 2001) and the international unified leaf rust scale (Johnston and Browder, 1964) as shown in Table 1. The data were then statistically analyzed and LSD test was performed using computer package MStatC.

DNA isolation

Small scale DNA isolation protocol (Weining and Langridge, 1992) was used to isolate DNA from the leaves of the plants. About 10 cm long fresh leaves of each variety were collected and immediately put into liquid nitrogen. The leaf material was crushed with the help of knitting needle and 500 µl DNA extraction buffer was added. After that, 500 µl of phenol : chloroform : iso amyl alcohol (in the ratio of 25:24:1) was then added and centrifuged at 13200 rpm for 5

Table 3. List of 25 wheat germplasm showing presence and absence of *Lr10* gene.

S/N	Variety	Lr10
1	SANDAL	-
2	C-228	-
3	C-250	+
4	WL-711	-
5	BARANI 83	+
6	C-273	-
7	LYP-73	+
8	C-591	+
9	PAK-81	+
10	SIND-81	+
11	CHENAB-79	-
12	PUNJAB-76	+
13	ZARGHON-79	+
14	KOHINOOR-83	+
15	B-SILVER	-
16	LU-26	+
17	FBD-08	+
18	BAHAWALPUR-79	+
19	LASANI-08	-
20	KIRAN	+
21	SUSSI	+
22	FAKHRI SARHAD	+
23	MARAJ 08	+
24	TANDOJAN-83	+
25	SHAHKAR-95	+

min. Aqueous phase was transferred into a fresh tube, 50 µl 3 M sodium acetate (pH = 4.8) and 500 µl isopropanol was added and mixed gently. Tubes were again centrifuged at 13200 rpm for 5 min, supernatant was discarded and the DNA pellet was washed with 70% ethanol. Genomic DNA was then treated with 1 µl RNAs A for 24 h to remove RNA. The DNA was analyzed on 1% agarose gel to check the quality and quantity of DNA.

PCR amplification of *Lr10* gene

PCR reactions were assembled using standard protocol (Devos and Gale, 1992). The master mix formulation for the PCR reaction was: distilled water 6.5 µl, 100 mM $MgCl_2$ 2.20 µl, 0.6 µl of each forward and reverse Specific STS primers (10 µM), template DNA 02 µl, 10 x PCR buffer 1.5 µl, 10 mM dNTPs mix 01 µl and 5 U of Taq DNA Polymerase 0.6 µl, with a total volume of 15 µl. Amplification reaction involved an initial denaturation step at 94°C for 3 min followed by 30 cycles each consisting of denaturation step at 94°C for 45 s, annealing step at 57°C for 45 s and a polymerization step at 72°C for 30 s followed by extension step at 72°C for 10 min and a final hold at 4°C. The STS marker used for the identification of *Lr10* gene was with a forward (5'-GTGTAATGCATGCAGGTTCC-3') and reverse (5'-AGGTGTGAGT GAGTTATGTT-3') base sequence (Stepien et al., 2003). All amplification reactions were performed using the Gene Amp PCR system 2700 (Applied Biosystem). The PCR products were electrophoresed on 1% agarose gel with ethidium bromide staining and visualized under UV light using Uvitech gel documentation system. Data were scored from good quality photographs of each amplification reaction for the presence or absence of the leaf rust resistance gene *Lr10* linked DNA fragments and were compared with the band size described by Stepien et al. (2003).

RESULTS AND DISCUSSION

The wheat cultivars are attacked by various types of rust pathogens. They become susceptible to rusts due to their narrow genetic base for resistance and the rapid rate of evolution of the pathogen. Yield losses due to rusts had been reported in many wheat producing countries in most years and periodic epidemics during the last century resulted in famine situation in many parts of the world. Allan et al. (1963) and Ali et al. (2007) have also reported the existence of association between rust infection and grain yield losses. To date, more than 45 stem rust resistance Sr (genes) (McIntosh et al., 2003) and nearly 58 leaf rust resistance genes have been identified against different races of this fungus. Leaf rust resistance genes are designated as *Lr1* through *Lr58* (McIntosh et al., 2005; Kuraparthy et al., 2007). Therefore, it is necessary to search for new sources for resistance. Genetic resistance is one of the important methods to control

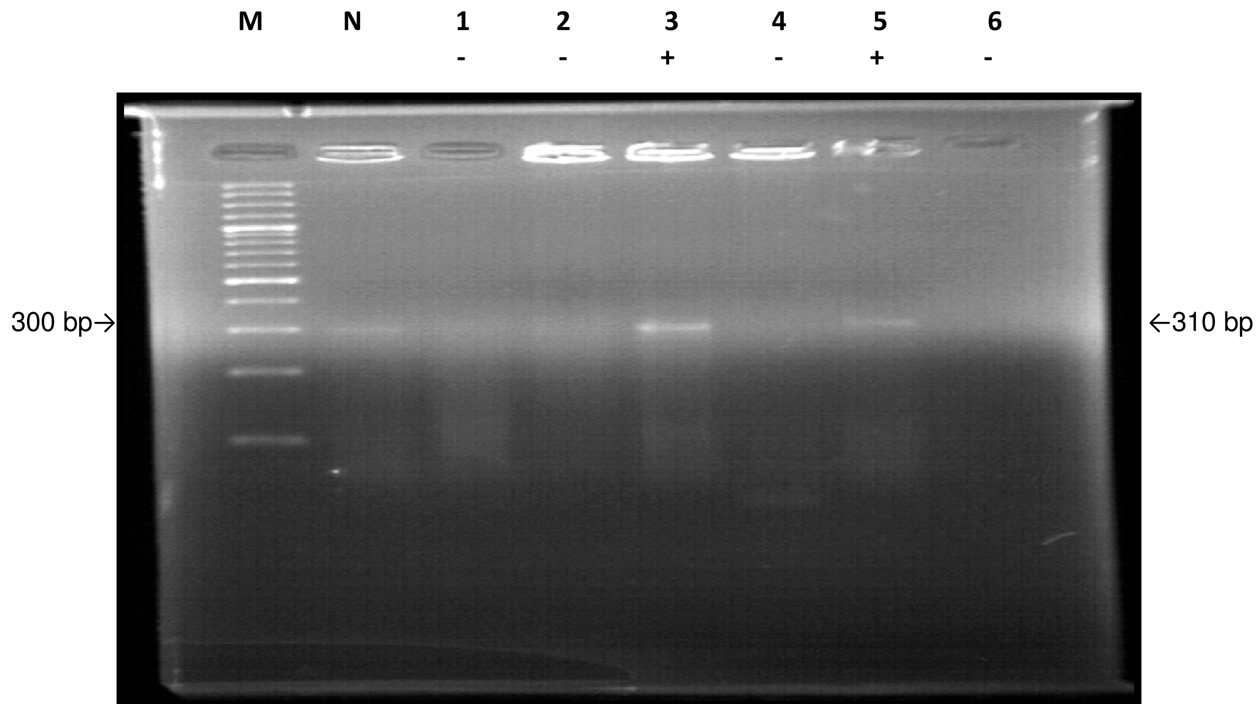

Figure 1. PCR amplification showing the presence and absence of *Lr10* gene in wheat germplasm. + Sign represent presence of gene whereas - sign indicates absence of *Lr10* gene. M = 100 bp size marker.

many pathogenic epidemics. Resistance based on single major gene is often considered short-lived due to the genetic shifts or the emergence of new virulence in the pathogen population in response to selection imposed by the host. It is believed that, in wheat, certain gene combinations give better and long lasting resistance to rust diseases than any of the genes individually (Dyck and Samborski, 1982). Thus, consistent with global trends, resistant cultivars developed by pyramiding effective *Lr* genes have significantly reduced losses caused by rusts in Pakistan as well (Khan, 1987).

Molecular marker offers an effective way of assessing the resistance of a set of genotypes. Various researchers screened certain wheat cultivars using SSR, STS and other such markers. Amplification of DNA using *Lr10* specific primers has been described as a way to detect *Lr10* gene and the amplified fragments corresponded to leaf rust resistance (Stepien et al., 2003). In this study, *Lr10* specific STS primer was used to detect the presence of *Lr10* gene. The screening of 25 wheat genotypes for *Lr10* gene provides information about presence and absence of resistant gene *Lr10* in Pakistani wheat germplasm and varieties.

Leaf rust resistance

The rust severity and LSD data of the 25 local wheat genotypes is shown in Table 2. A considerably high disease pressure was recorded at the testing site as maximum rust severity up to 40% was recorded for variety WL-711, followed by Sussi (31%). Fkhri Sarhad and Maraj-08 were found with the lowest rust severity (9.44 and 9.89%, respectively). According to the prescribed 1 to 9 rating scale, the tested varieties were grouped into nine groups. The results based on rust resistance showed that variety WL-711 was found to be susceptible (rating 3), varieties C-250, C-273, Pak-81, B-Silver, Kiran and Sussi were moderately susceptible to susceptible (rating 4), Sandal, C-228, Barani 83, Lyp-73, Sind-81, Chenab-79, Punjab-76, Kohinoor-83, LU-26, Fbd-08, Lasani-08 and Tandojan-83 were moderately susceptible (rating 5), C-591, Zarghon-79, Bahawalpur-79 and Shahkar-95 were moderately resistant (rating 6), while Fakhri Sarhad and Maraj-08 were resistant (rating 7).

Molecular studies

A diagnostic band of 310 bp was amplified showing the presence of *Lr10* gene (Figures 1 to 4). The polymorphic survey revealed that out of the 25 varieties, the marker for *Lr10* was identified as a fragment of 310 bp in 18 varieties namely: C-250, Barani 83, LYP-73, C-591, Pak-81, Sind-81, Punjab-76, Zarghon-79, Kohinoor-83, LU-26, Fbd-08, Bahawalpur-79, Kiran, Sussi, Fakhri Sarhad, Maraj-08, tandojan-83 and Shahkar-95, while seven

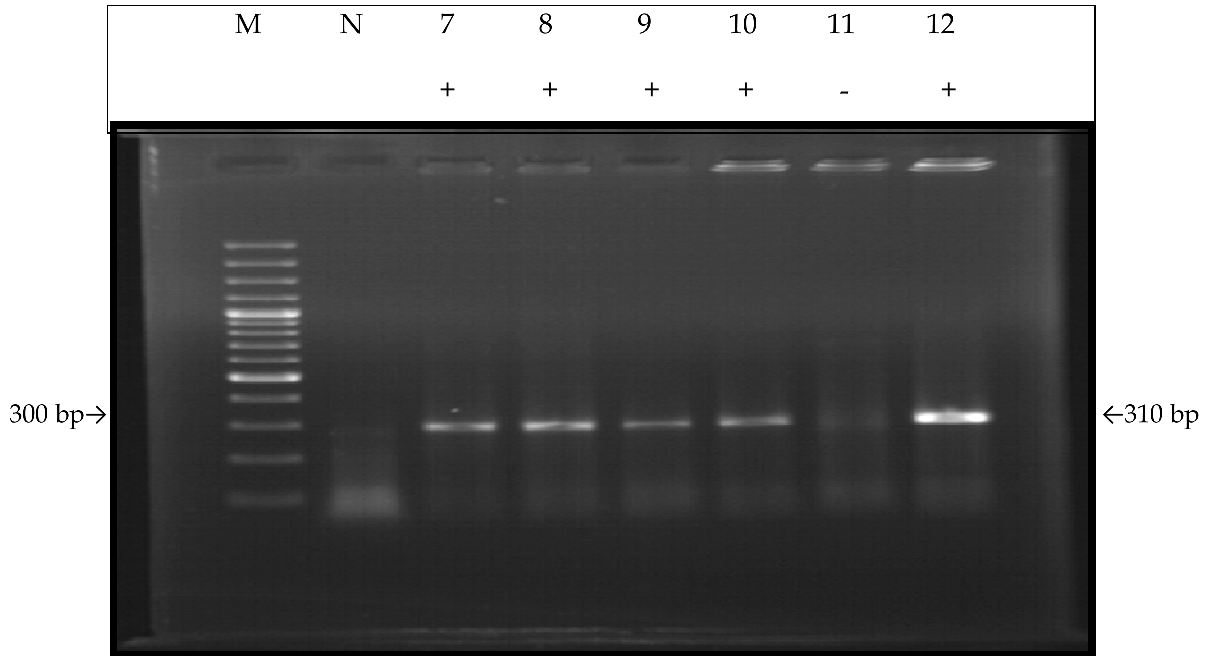

Figure 2. PCR amplification showing the presence and absence of *Lr10* gene in wheat germplasm. + Sign represent presence of gene whereas - sign indicates absence of *Lr10* gene. M = 100 bp size marker.

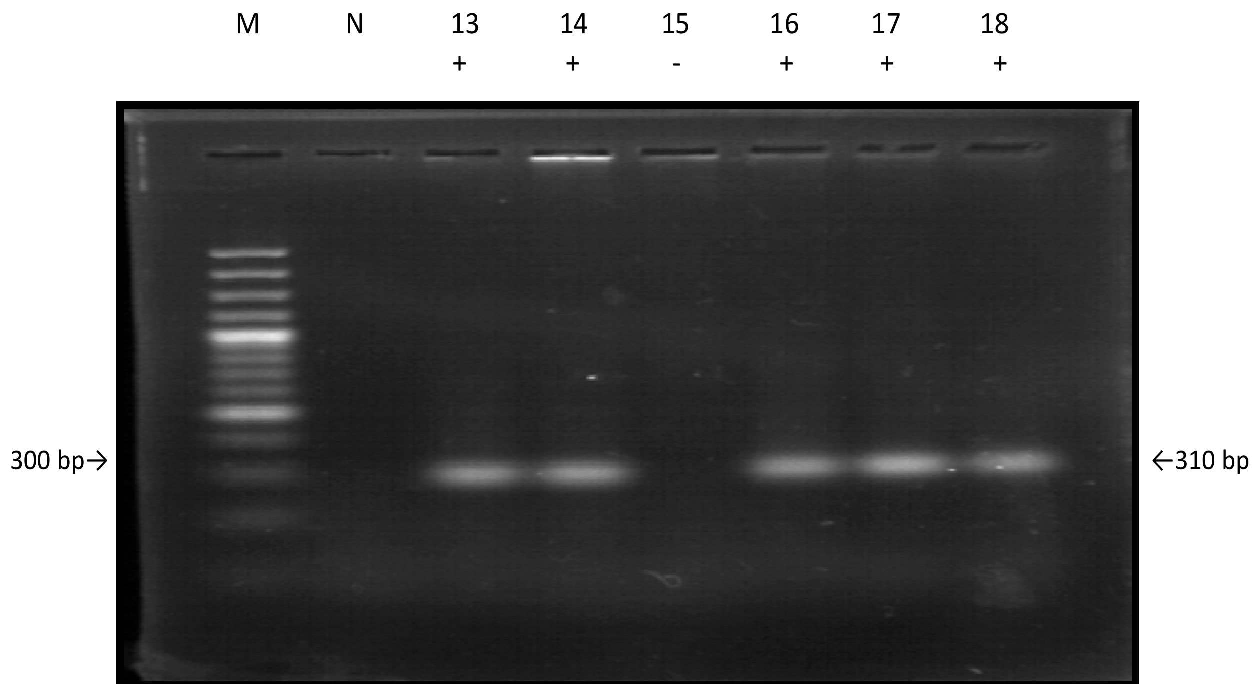

Figure 3. PCR amplification showing the presence and absence of *Lr10* gene in wheat germplasm. + Sign represent presence of gene whereas, - sign indicates absence of *Lr10* gene. M = 100 bp size marker.

genotypes; Sandal, C-228, WL-711, C-273, Chenab-79, B-Silver and Lasani-08 did not show the presence of *Lr10* gene (Figures 1 to 4). Similar results were obtained by Baber et al. (2010) and Malik et al. (2007), while screening Pakistani wheat germplasm for leaf rust resistance using PCR based molecular markers.

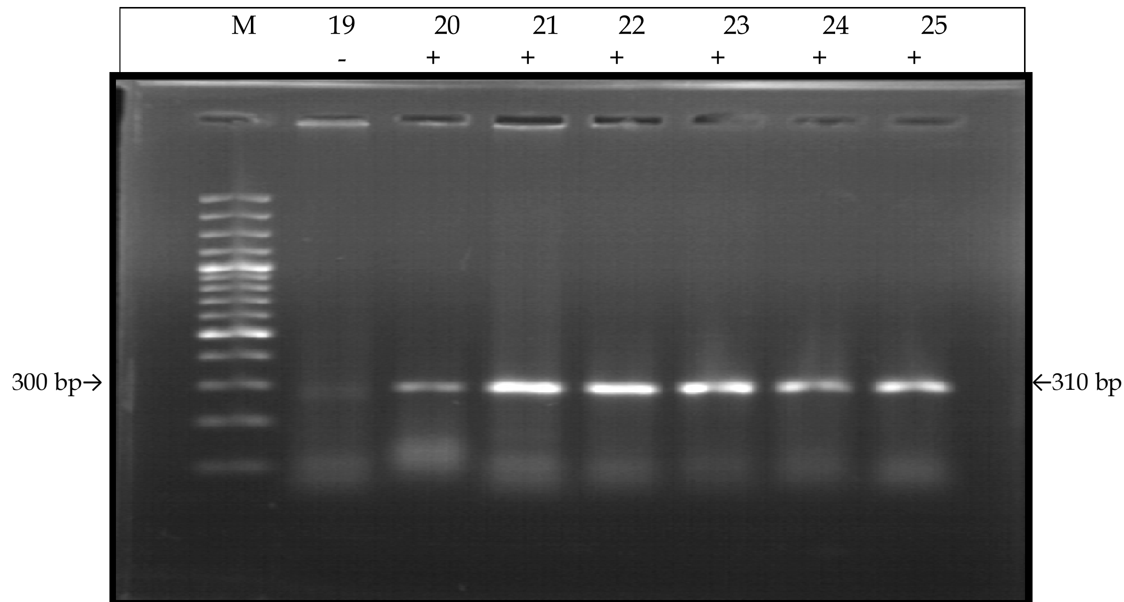

Figure 4. PCR amplification showing the presence and absence of *Lr10* gene in wheat germplasm. + Sign represent presence of gene whereas - sign indicates absence of *Lr10* gene. M = 100 bp size marker.

The *Lr10* gene was amplified from the DNA samples of resistant genotypes like Fakhri Sarhad, Maraj 08 and Shahkar 95 and was absent in the susceptible genotypes, WL-711 and C-273. The genotypes C-591, Zarghon-79, Bahawalpur-79 and Shahkar-95 were also found to have *Lr10* gene and were moderately resistant to leaf rust. B-Silver, Sandal, C-228, Chenab 79 and Lasani 08 were lacking *Lr10* gene and were found to be moderately susceptible. The identification of *Lr10* in Pakistan wheat germplasm will help in accelerating the breeding program in future, including pyramiding of different wheat resistant genes in wheat cultivars.

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