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IDENTIFICATION OF LEAF RUST GENES IN WHEAT VARIETIES THROUGH STS MARKERS

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Abstract

Leaf rust caused by *Puccinia triticinia* is the most destructive disease which reduces wheat production. Thirty-eight wheat varieties were screened for four leaf rust resistance genes *viz. Lr10, Lr26, Lr34* and *Lr47* through STS markers. Among them *Lr10* gene showed its presence in 16 genotypes, *Lr26* in 8, both *Lr34* and *Lr47* in 10 each. Variety Anza+2ns showed promising results by the presence of three *Lr* genes (*Lr10*, *Lr26* and *Lr47*) surely recommended for crop improvement and/or direct utility at farmers field. Chakwal-97, Indus-79, Marwat-Y-01, Mumal-2002, Khyber-83, Pasban-90, Pari-73, Lr-268, and Satluge-86 showed 2 genes presence while 23 with single gene and 5 without any response. A diagnostic band of size 310, 258, 1000 and 380-450 bps was amplified showing presence of *Lr10, Lr34, Lr26* and *Lr47*. Marker STS are suggested for large scale germplasm screening during marker assisted breeding.

Introduction

Wheat is an important staple food crop of the human beings and its by-products are used for the livestock. It is cultivated almost throughout the world while in Pakistan it is grown on about 18,00,000 hectares and during 2009 its production was recorded as 24 million tons (Anonymous 2009). Common bread wheat (*Triticum aestivum* L.) belonging to family Poaceae, genomically it is an allohexaploid, AABBDD, having 2n = 6x - 42 chromosomes. Various rust attacks on this crop have been reported, among these leaf rust is one of the most destructive diseases and decreases the yield up to 30% (Hussain *et al*. 2011). Leaf rust caused by the fungus *Puccinia triticina* (formerly *P. recondita* f. sp. *tritici*), is one of the most important foliar diseases of this crop (Bulos *et al*. 2006). It causes cardinal yield decreases in susceptible cultivars, mainly in the years with a high infection pressure of the pathogen. Additionally, it reduces wheat yields in susceptible varieties and appears every year with disease severity depending on weather conditions (Pasquini *et al*. 2003).

Moreover, due to airborne nature of the disease, use of chemicals is neither economical nor feasible on a large scale. According to Pathan and Park (2006), the only economic and practical control of rust diseases can be achieved through genetic resistance. Over 100 genes are resistant to the following rust fungi: *Puccinia recondita* f. sp. *tritici*, (47 *Lr* - leaf rust genes), *P. striiformis* (18 *Yr* - yellow rust genes) and *P. graminis* f. sp. *tritici* (41 *Sr* - stripe rust genes) have been identified in wheat and its wild relatives (Chelokwski and Stepien 2001). The 'pyramiding strategy' as to say the incorporation of more than one resistance gene to the same or different pathogens in a single genotype, could aid the breeder to maintain resistance any longer (Nocente *et al*. 2007). In recent years, molecular markers get popularity to use in the identification of *Lr* genes in wheat genotypes.

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The leaf rust resistance gene *Lr10* originates from the wheat gene pool and present on 1AS chromosome of wheat. *Lr10* itself is not a very effective gene; however, it is suggested to play a role in leaf rust resistance in combination with other *Lr* genes (Hussain *et al.* 2011). Further, the *Lr34* gene has been widely used in wheat breeding programmes because of its durable resistance to leaf rust and its association with *Yr18*, a stripe rust resistance gene. It was first reported as a modifier of adult plant resistance in the cultivar Frontana and this gene was also found in a number of wheat cultivars present around the world (Malik *et al.* 2007) and was later mapped to chromosome arm 7DS (Roder *et al*. 1998). Rye (*Secale cereale* L.) is the source of the *Lr26* gene which is present on short arm of chromosome 1 (1RS) (Rajaram *et al.* 1983). Mago *et al*. (2002) for the first time developed markers for *Lr26* and made it possible to study the *Lr26* gene in wheat genome.

The leaf rust resistance gene *Lr47* is located within a segment of *Triticum speltoides* Taush. Chromosome 7S transferred to the chromosome 7A of hexaploid wheat into an interstitial translocation 20-30 cM long (Dubcovsky *et al*. 1998). Primers using for the amplification of *Lr26* and *Lr47* genes were described by Mago *et al*. (2002) and Helguera *et al*. (2005). Present study was conducted to identify four *Lr* genes *viz.*, *Lr10*, *Lr26*, *Lr34* and *Lr47* in 38 wheat varieties.

Materials and Methods

Thirty eight wheat varieties obtained from National Gene Bank, Plant Genetic Resources Program, NARC, Islamabad were subjected to PCR-STS markers studies (Table 1). Genomic DNA was isolated from fresh leaves by following the protocol of Weining and Langridge (1992). Fresh leaves were harvested from each variety and immediately placed in liquid nitrogen. The leaf material was crushed with the help of needle, then 500 µl DNA extraction buffer was added. In the second step, 500 µl of phenol: chloroform: isoamyl alcohol (25 : 24 : 1) was added and centrifuged at 13200 rpm for 5 min. Aqueous phase (supernatant) was transferred into a fresh tube and 50 µl 3M Sodium acetate (pH 4.8) and 500 µl isopropanol were added and mixed thoroughly. Tubes were again centrifuged at 13200 rpm for 5 min., this time supernatant was discarded and the DNA pellet was washed with 70% ethanol. After washing and air drying DNA pellet was dissolved in TE buffer. Genomic DNA was then treated with 1 µl RNAse A for 24 hrs to remove RNA. Samples were then stored at 4^{o}C in refrigerator. Extracted DNA was examined on 1% agarose gel to check quality and quantity of DNA.

PCR amplification was performed according to the conditions described by Devos and Gale (1992) on Applied Biosystems (USA). Cocktail for the PCR was as follows; distilled water 6.7 µl, 100mM $MgCl_2$ 2.5µl, 0.75 µl of each forward and reverse specific STS primers (10µM), template DNA 01 µl, 10X PCR buffer 1.5 µl, 10mM dNTPs mix 1.2 µl, and 5 unit of Taq DNA polymerase 0.6 µl, with a total volume of 15 µl. Amplification reaction for Lr10 gene is 94^0 -5 min, 35 cycles (94^0 -30 s, 55^0 - 30s, 72^0 - 1 min), 72^0 - 3min and 4^0 - hold. Primer used for amplification of *Lr10* gene was with a forward (5'-GTGTAATGCATGCAGGTTCC-3') and a reverse base sequence (5'-AGGTGTGAGTGAGTTATGTT-3'), while for *Lr34* the reaction involved is 95^0 -3 min, 30 cycles (94^0 -30 s, 61^0 - 60s, 72^0 - 70 s), 72^0 - 10 min and 4^0 – hold and the primer used for Lr34 gene is with a forward (5'-GTGAAGCAGACCCACAACAC-3') and a reverse base sequence (5'-GACGGCTGCGACGTAGAG-3') (Roder *et al.* 1998). Amplification reaction for *Lr26* gene is 94^0 -3 min, 30 cycles (94^0 -30 s, 56^0 - 60s, 72^0 - 70 s), 25^0 - 60 s and 4^0 - hold. The primer used for identification of *Lr26* gene was with a forward (5'-CTCTGT GGATAGTTACTTGATCGA-3') and a reverse (5'-CCTAGAACATGCATGGCTGT TACA-3') base sequence (Mago *et al*. 2002). While for *Lr47* the reaction involved is 95^0 -3 min, 40 cycles (94^0 - 30s, 55^0 - 30s, 72^0 - 30s), 72^0 - 7 min and 4^0 – hold and the primer used for *Lr47* gene is with

a forward (5'-GCTGATGACCCTGACCGGT-3' and a reverse (5'-GGGCAGGCGTTT ATTCC AG-3') base sequence (Helguera *et al*. 2005). The PCR products were then run on electrophoresis apparatus along 3% agarose gel with the addition of ethidium bromide as staining dye. The bands were photographed and visualized under UV light using Uvitech gel documentation system (Model BTS-20M). Data were scored on the basis of presence or absence of respective bands linked with leaf rust resistance genes. DNA ladder of 100 bps purchased from Fermentas was used as standard marker for comparison.

Table 1. The presence (+) or absence (-) of the genes in 38 wheat varieties.

Sl. No.	Variety name	Lr genes presence or absence				Sl. No.	Variety name	Lr genes presence or absence			
		Lr10	*Lr26*	*Lr34*	*Lr47*			*Lr10*	*Lr26*	*Lr34*	*Lr47*
1	1036+Lr51+Yr	-	+	-	-	20	Pasban-90	+	+	-	-
2	Anza+2ns	+	+	-	+	21	Potohar-93	-	-	+	-
3	Anmol-91	+	-	-	-	22	Punjab	+	-	-	-
4	AS-2002	+	-	-	- +	23	Punjab-76	+	-		- -
5	Bahawalpur-79	-	-	-	+	24	Rawal-87	-	-	+	-
6	Bakhtawar-94	-	-	-	-	25	Satluge-86	+	-	+	-
7	Chakawal-86,	-	-	-	+	26	Shalimar-88	-	-	+	+
8	Chakwal-97	+	-	-	-	27	SH-2003	-	-	-	+
9	Indus-79	+	+	-	+	28	Suleman-96	-	-	-	-
10	Iqbal-2000	-	-	-	- +	29	Soghat-90	+	-	-	- +
11	Khyber-83	+	-	+	-	30	Tandojam-83	-	+	-	-
12	Khaghan-93	-	-	-	-	31	Uqab-2000	-	-	-	-
13	LR-268	+	+	-	-	32	Wardak-85	-	-	+	-
14	Lr51+Yr	-	-	-	-	33	Wadanak-95	-	-	+	-
15	Marwat-Y-01	+	+	-	-	34	Wadanak-98	-	-	+	-
16	Mumal-2002	+	-	+	+ -	35	Yr	-	-	-	- -
17	Nuri-70	+	-	-		36	YR-5	-	-	-	
18	Pak-81	-	-	-		37	ZA-77	-	-	-	
19	Pari-73	+	-	+		38	Zamindar-80	-	+	-	

Remarks: *Lr10* present in 16 varieties, *Lr26* in 08 varieties, *Lr34* in 10 varieties and Lr47 in 10 varieties. .

Results and Discussion

Although wheat is infected by different pathogens causing various diseases, rust is one of them and some time it becomes epidemic in many parts of the world. It is believed that, in wheat, certain gene combinations give better and long lasting resistance to rust diseases than given by any of the genes individually (Hussain *et al*. 2011). The cultivars developed following pyramiding strategy with various *Lr* genes might reduce the yield loss due to these pathogens. A diagnostic band of size 310 and 258 bps was amplified showing the presence of *Lr10* and *Lr34* gene, respectively (Fig. 1). The molecular screening showed that out of 38 varieties, the marker for *Lr10*

was identified as a fragment of 310 bps in 16 varieties namely: Anmol-91, AS-2002, Anza+2ns, Chakwal-97, Indus-79, Khyber-83, LR-268, Marwat-Y-01, Mumal-2002, Nuri-70, Pari-73, Punjab-76, Punjab, Pasban-90, Soghat-90, and Satluge-86. This findings are also supported by Weining and Langridge (1992). On the other hand, the band for *Lr34* showed its presence in 10 varieties *viz.*, Indus-79, Khyber-83, Mumal-2002, Pari-73 and Potohar-93, Rawal-87, Satluge-86, Wadanak-98, Wadanak-95, and Wardak-85. (Fig. 2). More or less similar results were reported by Malik *et al.* (2007) and Baber *et al*. (2010).

Additionally, a diagnostic band of 1000 and 380-450 bps was amplified showing the presence of *Lr26* and *Lr47* genes, respectively (Figs 3 and 4). The molecular screening shows that out of 38 varieties, the marker for *Lr26* gene amplified a fragment of 1000 bps in eight varieties namely: 1036+Lr51+Yr, Anza+2ns, Indus-79, LR-268, Marwat-Y-01, Pasban-90, Tandojam-83 and Zamindar-80. Urbanovich *et al*. (2006) carried out the molecular screening of 68 wheat varieties and found the *Lr26* gene in 11 varieties. In case of *Lr47*, the band was found in 10 varieties namely, Anza+2ns, Bahawalpur-79, Bakhtawar-94, Chakawal-86, Iqbal-2000, Khaghan-93, Pak-81, Suleman-96, SH-2003, and Uqab-2000. Four wheat varieties *viz*. Chakawal-86, Lr51+Yr, YR-5 and ZA-77 were found not to possess any of the four *Lr* genes.

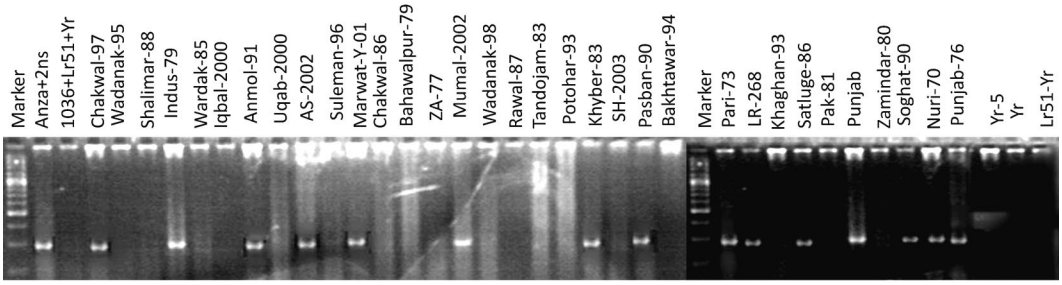

Fig. 1. PCR amplification of 38 varieties for *Lr 10* gene with 310 bp size. M (DNA ladder as Marker of 100 bps).

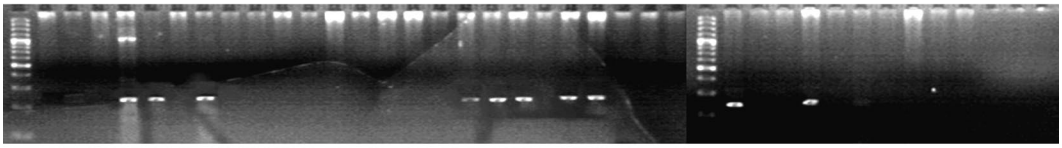

Fig. 2. PCR amplification of 38 varieties for *Lr 34* gene with 258 bp size. M (DNA ladder as Marker of 100 bps).

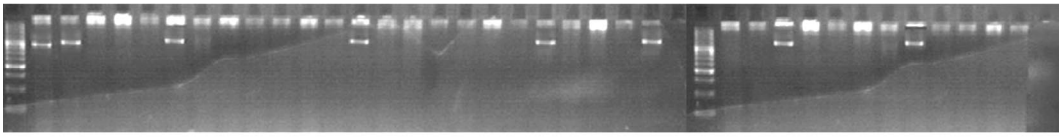

Fig. 3. PCR amplification of 38 varieties for *Lr 26* gene with 1000 bp size. M (DNA ladder as Marker of 100 bps).

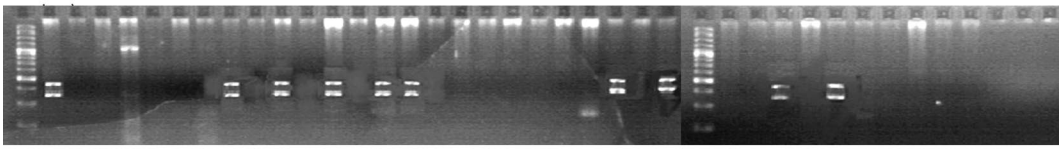

Fig. 4. PCR amplification of 38 varieties for *Lr 47* gene with 380-450 bp size. M (DNA laddeer as Marker for 100 bps).

Presence of two *Lr* genes in the same genotype were found in eight varieties. Among them Khyber-83, Mumal-2002, Pari-73 and Satluge-86 were found to be resistant for both of the *Lr10 and Lr34* genes; Indus-79, Marwat-Y-01 and Pasban-90 for both *Lr10* and *Lr26* genes and

Chakwal-97 for both the *Lr10* and *Lr47* genes, comprehend higher resistance. Only the wheat variety Anza+2ns was found possess three *Lr* genes viz. *Lr10*, *Lr26* and *Lr47* in the same genotype. This showed the more resistance level as compared to all other genotypes screened tin the present study. These genotypes may be recommended for further exploitation in hybridization and breeding programms.

Tyryshkin and Tyryshkina (2003) stated that growing resistant varieties is cheapest, profitable and ecofriendly method and the incorporation of genetic resistance to the pathogen into adapted germplasm is a major goal in wheat breeding programs. It helps in revenue generation as to avoid costly fungicides, thus reducing environmental contamination risks and decreasing production costs. Hussain *et al.* (2011) supported the idea that in wheat certain gene combinations give better and long lasting resistance to rust diseases than individual gene. The cultivars developed following pyramiding strategy of various *Lr* genes containing varieties certainly helpful in reducing yield loss. The present findings will be helpful for wheat breeders to choice appropriate *Lr* genes alone or in combination and to accelerate crop improvement programs for pyramiding these genes in the diversified gene pool. This will expected to be ended in the outcome that high yield disease free wheat grain be available for increasing population.

References

Anonymous 2009. Agriculture Statistics of Pakistan. Ministry of Food, Agriculture and Live Stock, Islamabad, Pakistan. pp. 53-54.

Babar MA Mashhadi F Mehvish A Zahra AN Waheed R Hasnain A Rahman S Hussain N Ali M Khaliq I Aziz A 2010. Identification of rust resistance genes *Lr10* and *Sr9a* in Pakistani wheat germplasm using PCR based molecular markers. Afr. J. Biotech. **9**(8): 1144-1150.

Bulos M Echarte M Sala C 2006. Occurrence of the rust resistance gene *Lr37* from *Aegilops ventricosa* in Argentine cultivars of wheat. Elect. J. Biotech. **9**(5). ISSN: 0717-3458.

Chelokwski J Stepien L 2001. Molecular markers for leaf rust resistance genes in wheat. J. Appl. Genet. **42**(2): 117-126.

Devos KM Gale MD 1992. The use of randomly amplified polymorphic DNA markers in wheat. Theor. Appl. Genet. **101**: 107-118.

Dubcovsky J Echaide M Antonelli EF Lukaszewski AJ 1998. Molecular characterization of two *Triticum speltoides* interstitial translocations carrying leaf rust and green bug resistance genes. Crop Sci. **38**(6): 1655-1660.

Helguera M Vanzetti L Soria M Khan IA Kolmer J Dubcovsky J 2005. PCR Markers for *Triticum speltoides* leaf rust resistance Gene *Lr51* and their use to develop isogenic hard red spring wheat lines. Crop Sci. **45**:728-734.

Hussain W Inamullah Ahmad H Iqbal MS Abbasi FM Rabnawaz Ahmad W Liaqat S Hussain 2011. Identification of leaf rust resistant gene LR10 in Pakistani wheat germplasm. Afri. J. Biotech. **10**(43): 8578-8584.

Mago R Spielmeyer W Lawrence GL 2002. Identification and mapping of molecular markers linked to leaf rust resistance genes located on chromosome 1RS of Rye using wheat-Rye translocation lines. Theor. Appl. Genet. **104**: 1317-1324.

Malik T Iqbal AA Chowdhry MA Kashif M Rahman S 2007. DNA marker for leaf rust disease in wheat. Pak. J. Bot. **39**(1): 239-243.

Nocente F Gazza L Pasquini M 2007. Evaluation of leaf rust resistance genes *Lr1, Lr9, Lr24, Lr47* and their introgression into common wheat cultivars by marker-assisted selection. Euphytica **155**: 329-336.

Pasquini M Pancaldi D Casulli F 2003. Genetic variation in Italian populations of *Puccinia recondita* f. sp. *tritici* from 1990 to 2001. J. Genet. Breed. **57**: 191-200.

Pathan AK Park RF 2006. Evaluation of seedling and adult plant resistance to leaf rust in European wheat cultivars. Euphytica **149**: 327-342.

Rajaram S Mann CHE Ortiz-Ferrara G Kazi AM 1983. Adaptation, stability and high yield potential of certain 1B/1R CIMMYT Wheat, in Proc. 6th Int Wheat Genet. Symp., Sakomoto S (Ed), Kyoto, Japan. pp. 613-621.

Roder MS Korzun V Wendehake K Plaschke J Tixier MH Leroy P Ganal MW 1998. A microsatellite map of wheat. Genetics **194**: 2007-2023.

Tyryshkin LG Tyryshkina NA 2003. Resistance to diseases in wheat collection samples and somaclonal variants. Czech J. Genet. Plant Breed. **39**(1): 21-23.

Urbanovich OY Malyshev SV Dolmatovich TV Kartel NA 2006. Identification of leaf rust resistance genes in wheat (*Triticum aestivum* L.) cultivars using molecular markers. Genetika **42**(5): 675-683.

Weining S- Langridge P 1992. Identification and mapping of polymorphism in cereals based on polymerase chain reaction. Theor. Appl. Genet. **82**: 209-216.

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