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LEAF RUST RESISTANCE IN SEMI DWARF WHEAT CULTIVARS: A CONSPECTUS OF POST GREEN REVOLUTION PERIOD IN PAKISTAN

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Abstract

This study evaluates the genetics of thirty-eight commercial wheat varieties using specific molecular marker for six significant *Lr* genes *(Lr10, Lr13, Lr21, Lr24, Lr26, Lr27* and *Lr31*) revealing the presence of these genes in 18, 16, 0, 0, 16, 27 and 21 varieties respectively. Thirty one commercial wheat varieties bear more than one *Lr* genes. To optimize the observations ABI 3730 capillary array method was used for the detection of required product of specific size with sensitivity in single nucleotide polymorphism. The molecular marker with cM distance, less than 1 showed a valuable prediction for effective genes using conventional Gel electrophoresis image. The STS markers showed efficiency to verify four effective genes (*Lr10, Lr21, Lr24, and Lr27)* in local germplasm with parallel analysis from field trial at Regional Agriculture Institute, Bahawalpur, Pakistan. Specificity of three SSR markers confirmed with sensitive ABI 3730 analysis with fine peak intensities. The data provided showed that the genes (*Lr10, Lr13, Lr26,* and *Lr27-Lr31)* are widely distributed in Pakistan varieties while the absence of *Lr24* and *Lr21,* hence provided a motivation to transfer such widely effective genes to enhance resistance through MAS breeding. Findings showed that the marker assisted selection employing sensitive ABI 3730 analysis of distributed *Lr* genes in Pakistani wheat will help to establish gene pyramiding against leaf rust races and hence a way forward to integrate effective genes in future wheat varieties. The reliability of STS and specific SSR marker under diverse genetic background will also be a futuristic approach.

Introduction

According to the International Grain Council (2011), the total production of 690 million tons was recorded for wheat globally. Over 150 million populations are intimately aware of good harvest of wheat crop (Anon., 2004). *Puccinia recondita* f. sp. *tritici,* a biotrophic fungal species, is the principal pathogen of wheat. Worldwide surveys revealed that this pathogen evolves quickly developing new identifiable races (Roelfs *et al.,* 1992). With 100% infection rate on susceptible varieties, the leaf rust intensity may range from 40-50% as recorded in 1973 (Hassan *et al.,* 1973, Rasheed *et al*., 2012). Shortly after this, in 1978 a major leaf rust epidemic caused 10% yield loss that cost a national loss of US $86 million (Hussain *et al.,* 1980). To improve any species genetically, assessment of genetic diversity is prerequisite (Khatiba *et al.,* 2012). The production of resistance wheat varieties however, is the most economical way to control the disease. Resistant cultivars developed by pyramiding effective *Lr* genes may significantly reduce yield losses caused by fungal pathogen of leaf rust in Pakistan (Khan, 1987).

More than sixty leaf rust resistance genes (*Lr)* have been identified so far and localized on the wheat chromosomes. In addition to the designated resistance genes quantitative trait loci (QTLs) are also present (McIntosh *et al.,* 2008). Leaf rust Resistant genes (*Lr*) have been identified and used to control disease. Nature of most of the genes is race specific or major genes, against which rust pathogens mutate and rendered them ineffective/susceptible. To explore the genetic basis of resistance, the availability of information about genes present in the current varieties is a basic study (Rattu *et al.,* 2010). DNA based markers linked to *Lr* genes may prove useful in overwhelming the limitations of phenotypic gene postulation. According to Gupta *et al.,* (1999) and Francia *et al.,* (2005), the use of molecular markers facilitates an indirect selection of traits that are problematic to phenotype, the pyramiding of genes, the maintenance of recessive alleles in backcrossing pedigrees and also the choice of parents in crossing programs.

In Pakistani environment the *Lr10, Lr13,* L*r26* and *Lr27-Lr31* are widely distributed (Rattu *et al.,* 2010; Singh & Gupta, 1991). As most of our germplasm is of CIMMYT origin containing these most dominant genes and have been postulated in our most of common commercial varieties (Mirza *et al.,* 2000). For most of leaf rust resistant genes many efficient markers have been described from the last fifteen years. The traditional approach is time-consuming and labor intensive for transferring *Lr* genes from wheat related species or pyramiding genes in elite breeding lines. In segregating populations it is complicated by the need to perform inoculation tests on plants, also demanding the application of specific races. To meet the future threats, we need to convert our breeding priorities according to the continued change in environment (Iqbal *et al.,* 2012). However more specific and reliable molecular markers, particularly *STS, SCAR and CAPS* markers have been developed only for twenty one of these genes viz. *Lr1, Lr10, Lr13, Lr16, Lr19, Lr20, Lr21, Lr24, Lr25, Lr26, Lr28, Lr29, Lr35, Lr34, Lr37, Lr39, Lr46, Lr47, Lr50, Lr51* and *LrW* (Chelkowski *et al.,* 2003; Cherukuri *et al.,* 2003, 2005; Blaszczyk *et al.,* 2004; Prabhu *et al.,* 2004; Gupta *et al.,* 2005, 2006; Helguera *et al.,* 2005; Hiebert *et al.,* 2005; Mago *et al.,* 2005; Obert *et al.,* 2005; http://maswheat.ucdavis.edu). In marker assisted selection (MAS) the detection of molecular markers linked to the *Lr* genes is a basic application of markers. The degree of linkage between a marker and a trait, the effectiveness of a marker in different genetic backgrounds and a high reproducibility and reliability of a marker across laboratories is necessary for a successful application of

DNA markers in breeding programs (Gupta *et al.,* 1999, Akbar *et al*., 2011).

The objective of present work was to screen leaf rust resistant genes in thirty-four wheat cultivars released after 1980 in Punjab including three from Kyber pukhtoonkhwan and one from Sindh, Pakistan. In current study we performed the marker-assisted selection of effective genes for leaf rust using specific STS and SSR markers. The information provided by this research will be used to develop pyramiding plan against rapidly mutating pathogen.

Materials and Methods

Plant material: Seeds of wheat cultivars used in this study were obtained from the plant genetics laboratory, Department of Plant Sciences, Quaid-i-Azam University, Islamabad while the recently released varieties were obtained from RARI, Bahawalpur, Pakistan. The near isogenic lines were obtained from CIMMYT Mexico. We have also used American lines as positive control, provided by USDA Genotyping laboratory, Kansas State University, USA.

PCR amplification of wheat genomic DNA with specific primers: Genomic DNA was extracted from seven days old seedling leaves with slight modifications in CTAB method described by Suman *et al*., (1999). Polymerase chain reaction (PCR) was performed in 12µL reaction volume containing: 3.0µl (30ng/µl) of genomic DNA, 2.0µl 10 × PCR buffer (500mM KCL, 100mM Tris-HCl-pH 8.8, 0.8% Nonidet P40), 2µl of 25mM MgCl2, 0.8µl 5.0mM dNTPs (Fermentas), 0.2µl of 10.0µM of tailed primer, 0.3µl 10.0µM reverse primer, 0.1µl of 10µM Dye M13 primer and 11.30µl PCR H_2O, 0.3µl (5 U/µl) Taq DNA Polymerase (Fermentas). Each PCR was repeated at least twice. The specific PCR primers were used to verify *STS* and S*SR* markers for *Lr* genes in 38 wheat cultivars are listed in (Table 1). Primers were synthesized by *e-oligos*, Genelink (NY, USA). PCR amplification was performed in a DNA Engine peltier thermal cycler 200 (BIO- RAD, Mexico). The cycle conditions for each primer set are listed in (Table 2). After amplification specific PCR products were resolved on 1.4% agarose gel. Bands were visualized with UV light in a Gel documentation apparatus (BIO-RAD laboratories, Milan, Italy). ABI 3730 capillary array analysis was performed in USDA genotyping lab Kansas State University, USA.

Table 1. Specific PCR primers used to verify STS, SCAR, CAPS markers for leaf rust resistance genes.

Lr Genes	Marker set	Sequence of primer	References
Lr10	F1.2245 Lr10-6/r2	GTG TAA TGC ATG CAG GTT CC AGG TGT GAG TGA GTT ATG TT	Schachermayr *et al.,* 1997
Lr13	Xgwm630-2B	GTG CCT GTG CCA TCG TC CGA AAG TAA CAG CGC AGT GA	Seyfarth *et al.,* 2000
Lr21	Lr21L Lr21R	CGC TTT TAC CGA GAT TGG TC TCT GGT ATC TCA CGA AGC CTT	http://maswheat.ucdavis.edu/protocols/Lr21/index.htm
Lr24	J09/1 J09/2	TCT AGT CTG TAC ATG GGG GC TGG CAC ATG AAC TCC AT CG	Schachermayr *et al.,* 1995
Lr26	P6M12-P	GTACTAGTATCCAGAGGTCACAAG CAGACAAACAGAGTACGGGC	Mago *et al.,* 2005
Lr27	Xgwm389	ATC ATG TCG ATC TCC TTG ACG TGC CAT GCA CAT TAG CAG AT	Spielmeyer *et al.,* 2003
Lr31	Xgwm251	CAA CTG GTT GCT ACA CAA GCA GGG ATG TCT GTT CCA TCT TAG	Singh & Bowden, 2010

Table 2. Thermocycle temperature profiles for all primer sets used in the present study.

Genes	PCR cycle condition
Lr10	94°C – 3 min.; 35 cycles (94°C – 45 s.; 57°C – 45 s; 72°C – 30 s); 72°C – 3 min
Lr13	Touch Down PCR*
Lr21	Touch Down PCR*
Lr24	94°C–4 min.; 40 cycles (92°C – 1 min.; 60°C – 1 min.; 72°C – 2 min.); 72°C – 5 min.
Lr26	Touch Down PCR*
Lr27	Touch Down PCR*
Lr31	Touch Down PCR*

* 1. 95°C, 5 min, 2. 96°C, 1 min, 3. 68°C, 3 min, -2.0°C/cycle, 4. 72°C, 1 min, 5. Goto step 2, 4 more times, 6. 96°C, 1 min, 7. 58°C, 2 min, -2.0°C/cycle, 8. 72°C, 1 min, 9. Goto step 6, 4 more times, 10. 96°C, 20 sec, 11. 50°C, 20 sec, 12. 72°C, 30 sec, 13. Goto step 10, 39 more times, 14. 72°C, 5 min, 15. 4°C, 5

Cluster analysis: Only discrete, reproducible, well-resolved fragments were scored, and the data were analyzed using the MVSP 3.1 (Multivariate Statistical Package) program (Kovach, 1999). The MVSP software package version 3.1 was used to calculate Jaccard's (1908) similarity coefficients and a dendrogram was constructed using the neighbor-joining algorithm.

Results and Discussion

We studied the molecular marker assisted screening of Pakistani wheat varieties especially from wheat growing area of Punjab province with reference to locally and worldwide distributed leaf rust resistant genes. This study revealed that the screening of genes *(Lr10, Lr13, Lr21, Lr24, Lr26* and *Lr27- Lr31)* is important to evaluate the genetic information because the observations exhibited that the combination of *Lr27-Lr31* with *Lr26* is significant under the response in field trials. In local conditions these genes are widely distributed as reported by Fayyaz *et al*., 2008; Rattu *et al.,* 2010; Mirza *et al.,* 2000. While in some cases the effectiveness of these genes to pathogen races is partial as observed by Mirza *et al*., 2000. The screening of respective genes according to their presence in Pakistani wheat varieties is summarized in Table. 3.

Table 3. Identification of Leaf rust resistant genes by using the specific STS and SSR markers in wheat commercial lines of Pakistan.

S.No	Varieties	Lr10	Lr13	Lr21	Lr24	Lr26	Lr27	Lr31	Rust response from field in 2010
									Brown rust
1.	PAK 81	-	-	-	-	+	-	+	5MS
2.	PUNJAB 81	+	-	-	-	-	+	-	5R
3.	BARANI-83	-	-	-	-	-	-	-	0
4.	FSD 83	+	+	-	-	-	-	+	30S
5.	KOHINOOR 83	-	-	-	-	-	-	-	TR
6.	KAGHAN 83 (K.P)	+	-	-	-	-	+	+	TR
7.	FSD 85	-	+	-	-	+	-	+	TR
8.	WADANAK 85	-	-	-	-	+	-	+	5MR
9.	CHAKWAL 86	+	-	-	-	-	+	-	5R
10.	KHYBER 87 (K.P)	-	+	-	-	-	-	-	5MRMS
11.	PUNJNAND 88	-	-	-	-	+	+	-	60S
12.	ROHTAS 90	+	+	-	-	+	+	+	10MSS
13.	PASBAN 90	-	+	-	-	+	+		10MR
14.	INQILAB 91	+	+	-	-	+	+	+	0
15.	BAKHTAWAR 93 (K.P)	-	-	-	-	-	-	+	0
16.	PARWAZ 94	+	-	-	-	+	+	-	TR
17.	KOHSAR 95	+	+	-	-	+	+	+	20MSS
18.	SHAHKAR 95	-	+	-	-	+	+	+	TR
19.	PUNJAB 96	-	-	-	-	+	-	+	10MS
20.	TATARA (K.P)	+	-	-	-	+	+	+	5MR
21.	MH 97	-	+	-	-	-	-	-	20S
22.	KOHISTAN 97	-	+	-	-	-	+	+	0
23.	DURUM 97	-	-	-	-	+	+	+	0
24.	CHENAB 2000	-	+	-	-	+	+	+	10S
25.	AUQAB 2000	+	-	-	-	-	+	-	TR
26.	IQBAL 2000	-	+		-	-	+	+	TR
27.	WAFAQ 2002	+	-	-	-	-	+	-	TMR
28.	BHAKKAR 2002	+	+	-	-	-	+	-	0
29.	MOOMAL 2002 (Sindh)	-	-	-	-	-	+	-	10MS
30.	GA 2002	+	-	-	-	-	+	-	TR
31.	SH 2002	-	-	-	-	-	-	-	5MR
32.	IMDAD 2005	+	-	-	-	+	+	+	0
33.	SHAFAQ 2006	+	+	-	-	-	+	-	TR
34.	FAREED 2006	+	-		-	-	+	-	0
35.	SEHER 2006	+	-	-	-	-	+	+	0
36.	LASANI 2008	-	+	-	-	-	+	+	5MSS
37.	MIRAJ 2008	-	-	-	-	+	+	+	0
38.	AAS	+	+	-	-	-	+	+	5MSS
39.	THACHER/ MOROCCO	-	-	-	-	-	-	-	100S
40.	Positive control	+	+	+	+	+	+	+	

+ = Positive
- = Negative

Lr10: According to information generated through conventional methods by Rattu *et al.,* (2010), the *Lr10* gene is present in 36% Pakistani wheat cultivars selected in this study. The available data showed that *Lr10* is a common gene in local wheat varieties as observed in our work. Schachermayr *et al.,* (1997) reported a marker set (*F1 2245* and *Lr10-6/r*^{2}), amplified a product size of 310 bp in eighteen selected commercial lines. The specific product of marker visualized by gel electrophoresis and ABI capillary array methods (Fig. 1). Baber *et al.,* (2010) reported wide distribution with 91% to explore the *Lr10* genetic sources using the same molecular marker. Field data indicated that the *Lr10* has partially resistant nature against pathogen but its efficiency is high by observing in combination with *Lr27-Lr31* as described in cluster analysis in Fig. 3. Reported work of Wisniewska *et al.,* (2003) verify our observation that the *Lr10* gene may be effective with other resistance genes. So in future the pyramiding of this gene with other genes using the Marker Assisted Selection (Svetlana *et al.,* 2003) will be helpful to enhance the immunity of Pakistan wheat.

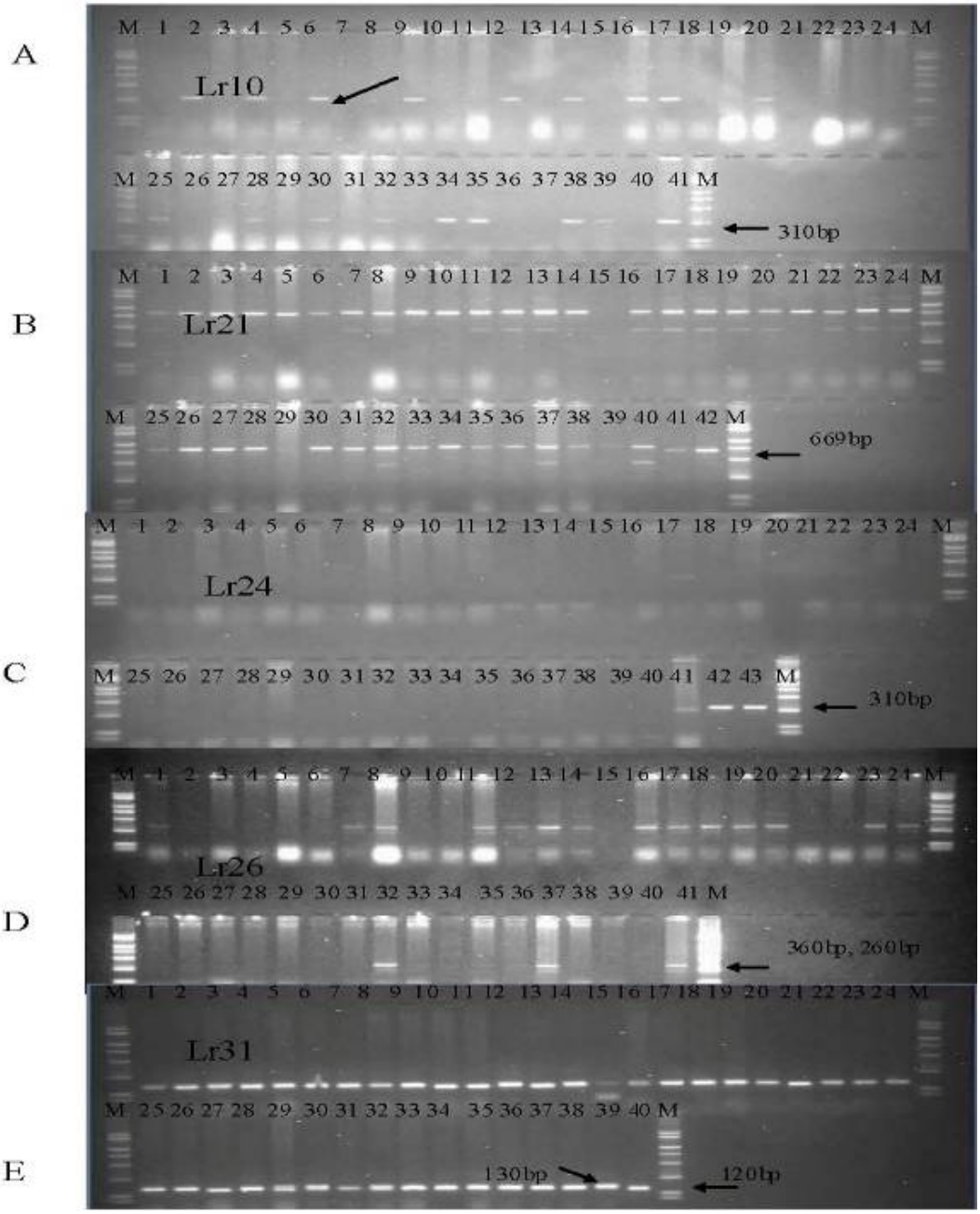

Fig. 1. Amplification products of STS markers specific to *Lr10, Lr21, Lr24, Lr26 and Lr31* gene. Arrows indicate required product obtained through polymerase chain reaction. A. Lane: 40- Thatcher, Lane: 41- ThLr10 B. Lane: 40-Thatcher, Lane: 41- WGRC02, Lane: 42- WGRC07 C. Lane: 39- Morocco, Lane: 40- Thatcher, Lane: 41- Hitch, Lane: 42, 43- LcSr24Ag D. Lane: 40-Thatcher, Lane: 41- ThLr26 E. Lane: 39-Thacher, Lane: 40- Inqilab, M: 1 Kb plus DNA Ladder.

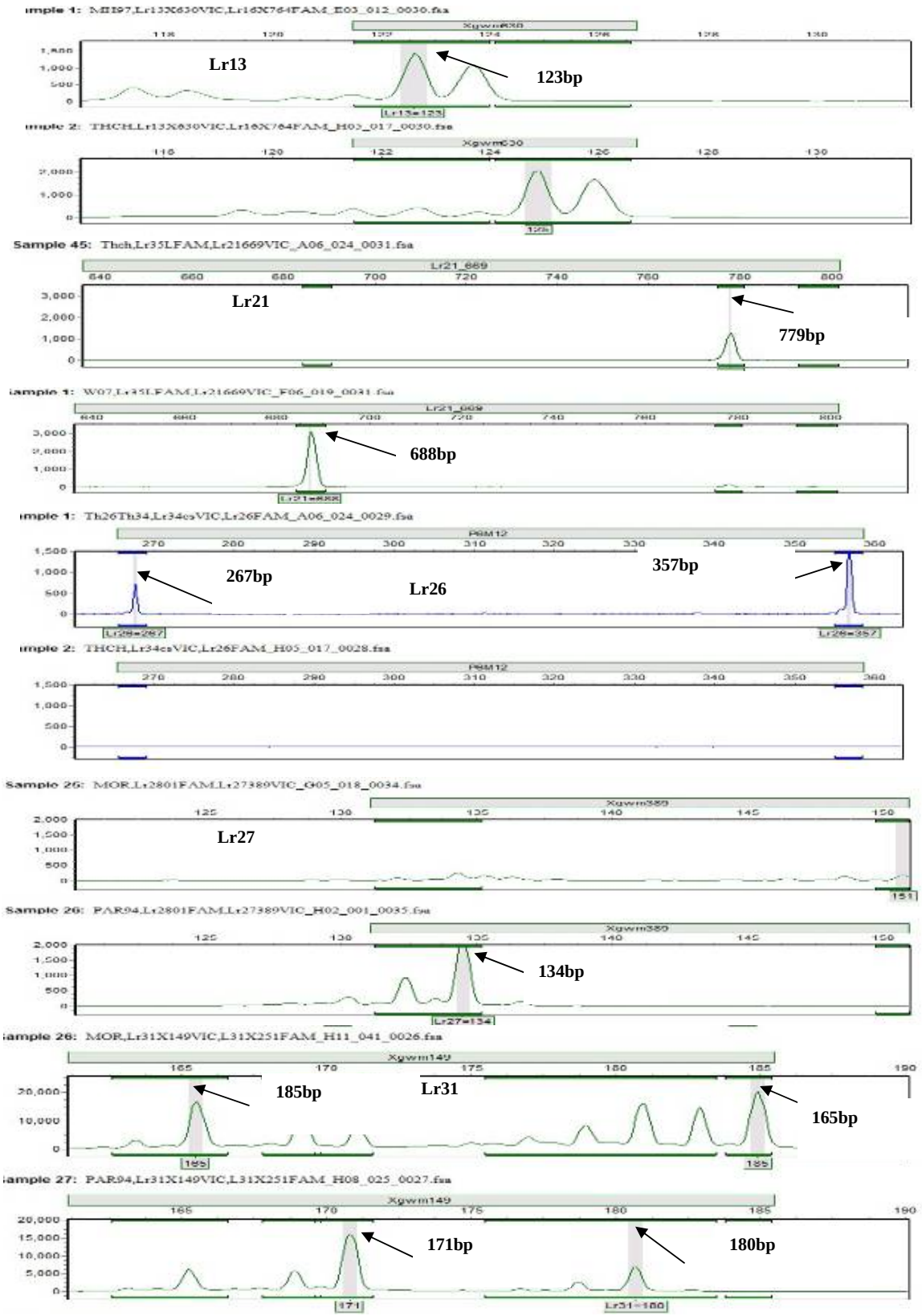

Fig. 2. ABI electrophorogram of simple sequence repeat (SSR) and sequence tagged site (STS) markers, represent the specific peaks. Arrows differentiated required size of gene linked markers and presence of other close alleles from susceptible and resistant wheat varieties with respect to these genes.

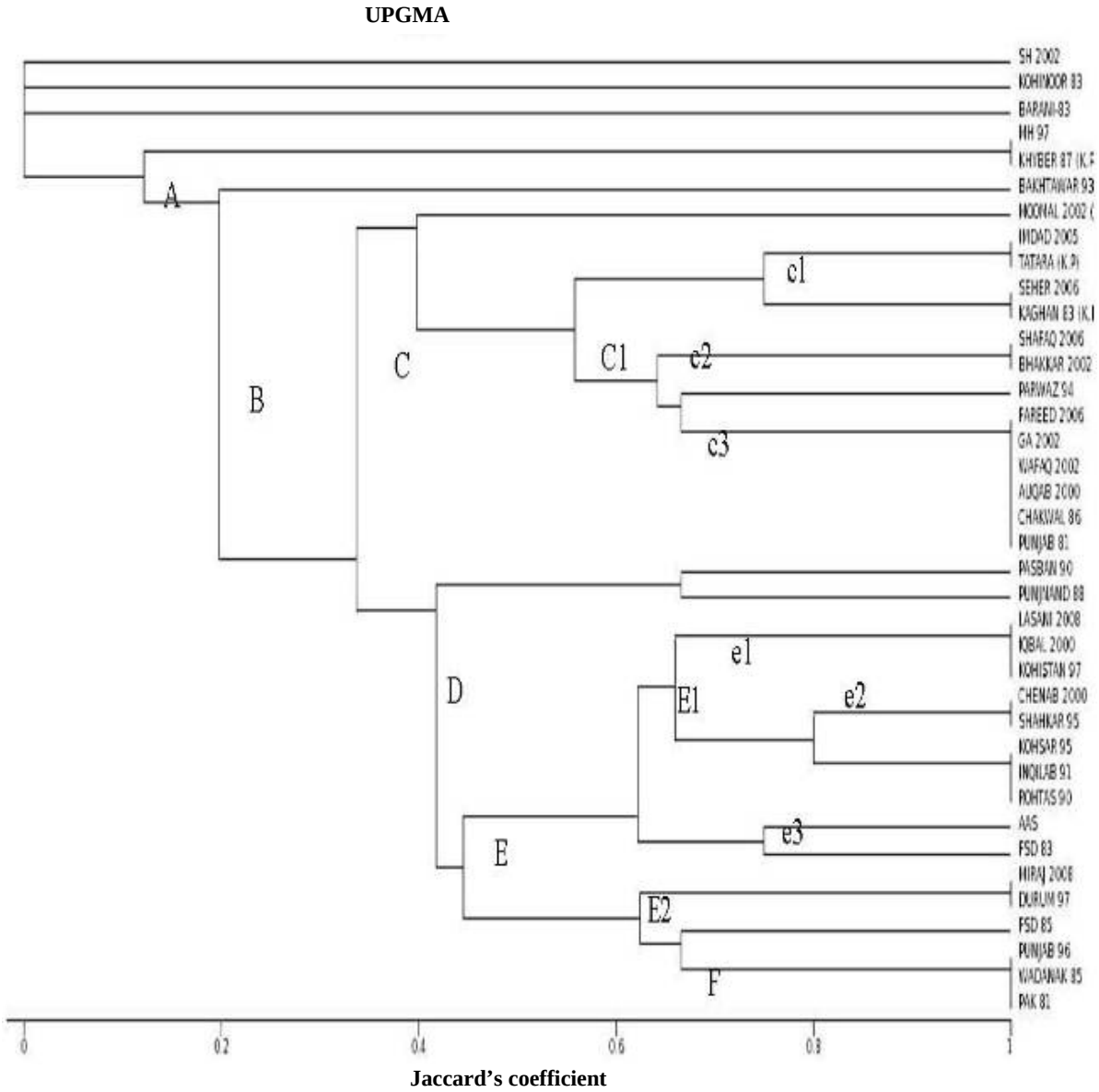

Fig. 3. Dendrogram of molecular data using Jaccard's similarity coefficients under UPGMA.

Lr13: SSR marker *(Xgwm630-2B)* is used to screen the *Lr13* gene from local germplasm as efficiency of this marker is already reported by the work of Seyfarth *et al.,* 2000. This PCR-based microsatellite marker *Xgwm630* shows a closer linkage, represents a potentially better marker for the screening of resistant gene *Lr13*. Nevertheless, the genetic distance (10 cM) is still quite big and integration of *Xgwm630* into MAS seems not feasible yet. According to Mohan *et al.,* (1997) molecular markers should co-segregate or be linked with less than 1 cM with the resistant gene that is successful for application in MAS. Our screening reports showed that the *Lr13* is a widely distributed gene (16 out of 38 varieties) with partially resistant nature. Same observation were also discussed in previous work of Rattu *et al.,* 2010; Mirza *et al.,* 2000; Ahmed *et al.,* 2000. *Lr13* is an adult plant stage resistant gene that shows the durability in combination with *Lr34* (Roelfs, 1988) however as a single gene *Lr13* is no longer effective in most wheat-growing areas (McIntosh *et al.,* 1995). The product of *Xgwm*630 was observed through ABI 3730 analysis to distinguish the specific band (Fig. 2).

Lr21: An STS marker on the electronic lab manual of the Wheat Genetics Resource Center at Kansas State University, USA was used to amplify a specific band of 669bp for positive germplasm WGRC02 and WGRC07 using Gel electrophoresis and ABI analysis, while no amplification was detected from Pakistani wheat varieties (Figs. 1&2). Previous observation discussed by Mirza *et al.,* 2000 verifies the least frequent presence of *Lr21*. The integration of *Lr21* in future breeding program therefore will be an effective approach to develop resistance because the gene is effective against *Puccinia triticina* (Kolmer, 1994).

Lr24: Gultyaeva *et al.,* (2002) reported that *Lr24* gene is highly effective in Europe and Russia. In the hard red winter wheat of the southern and central Great Plains region of North America, gene *Lr24* has been used in cultivars grown in the last 20 years (McVey & Long, 1993). In our research, a set of marker *J09* is used to screen the *Lr24* gene. The STS marker *J09* showed the band size of 310bp in positive cultivar Hitch and LcSr24Ag but none of selected varieties bearing this gene (Fig. 1). The efficiency of this marker is already published by Schachemayr *et al.,* 1995 & Prabhu *et al.,* 2004. The rare distribution of that gene in Pakistan is significant to introduce new varieties under its most effective nature in wheat growing areas globally.

Lr26: Lr26 is the most predominant gene in Pakistani wheat varieties (Rattu *et al.,* 2010 & Mirza *et al.,* 2000) and it is present singly or in combination. Zeller & Hasm, (1983) revealed that this gene is present in many winter and spring wheat carrying IRS chromosome. Nayyar *et al.,* (1991) reported that virulence for this gene has emerged in most wheat growing area in Indian subcontinent. PCR based marker *P6M12*-P is used in current study (Mago *et al.,* 2005) to distinguish the specific band size with sensitive peak visualization of ABI capillary array technique as Sun *et al.,* 2010 (Fig. 2).

Lr27-Lr31: Singh & McIntosh, (1984) found that *Lr27* and *Lr31* in the cultivar Gatcher conferred resistance only when present together. The resistance of these genes is in complementary condition as selected germplasm; in fifteen cases the *Lr27-Lr31* complimentary condition is present. Crossa *et al.,* (2007) reported a closely linked SSR marker *(Xgwm389)* to screen the *Lr27* while Singh & Bowden, 2010 described a SSR marker with 0.9 cM distance from *Sr2* that is closely linked or identical to *Lr31* (Fig. 2).

In cluster analysis group C was further sub divided into sub groups, as the Fig. 3 indicated subgroups consist of genotypes having *Lr10, Lr26, Lr27* and *Lr31* gene combination in large number of varieties. According to our field trial in 2010 the resistance of this complementary condition is significant while the observation of Mirza *et al.,* 2000 & Rattu *et al.,* 2006 indicated the susceptible nature with these genes gives an alarming situation because the response of these genes can fluctuate under different mutant races. It is necessary to develop pyramiding pattern for the combination of these with other durable resistant genes like *Lr34* to increase the duration of resistance to develop a major and minor gene pyramiding.

Conclusion

This study explores that the marker assisted selection for *Lr* genes is an efficient tool to screen the effective leaf rust resistant genes from wheat lines by avoiding the time consuming and laborious methods. The comparative study with previous data showed that STS maker may potentially use in molecular breeding under their specificity for linked genes. In local environment mostly genes have lost the resistance potential against leaf rust pathogen while in combination with other gene they can enhance the response. The pyramiding of *Lr27-Lr31* with *Lr26* may be effective against leaf rust. On the other hand the combination of *Lr26* with a minor gene like *Lr34* will be significant. The integration of rare genes *(Lr24, Lr21)* is necessary to increase the gene opportunity in local wheat genome. We also need to facilitate more advance techniques like ABI 3730 to visualize the specific peak with sensitivity of single nucleotide polymorphism. Pyramiding of genes will be innovative approach to develop resistance against mutant races of leaf rust in future.

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