Original Article

EXPLORING DURABLE GENETIC RESISTANCE AGAINST LEAF RUST THROUGH PHENOTYPIC CHARACTERIZATION AND *LR34* LINKED STS MARKER IN WHEAT GERMPLASM

EXPLORANDO A RESISTÊNCIA GENÉTICA DURADOURA CONTRA A FERRUGEM DA FOLHA ATRAVÉS DA CARACTERIZAÇÃO FENOTÍPICA E DO STS MARKER RELACIONADO AO (GENE) LR34 DO GERMOPLASMA DO TRIGO

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ABSTRACT: Present study was aimed to screening the population of 25 wheat genotypes from Baluchistan region of Pakistan along with five commercial cultivars for leaf rust adult plant resistance (APR) through gene postulation using natural inoculation of *Puccinia triticina Erikss* local pathotype. Infection severity was recorded on scale in comparison with susceptible control "Morroco" cultivar. On the basis of phenotypic score, seven accessions and four varieties (Zardana-89, Sariab-92, Zarlashta-99 and Raskoh-05) with AUDPC values up to 20% were characterized as resistant genotypes. Coefficient of infection (CI) score ranged from 0-10 for some accessions and cultivars showing high level of adult plant resistance. Furthermore, bi-allelic STS marker *csLV34* having close linkage with *Lr34* (0.4cM). This marker amplified one gene specific allele of 150bp in 21 genotypes, including 19 accessions and two commercial varieties (Sariab-92 and Zarghoon-79) which confirmed presence of *Lr34* gene conferring adult plant resistance against leaf rust. The rust pathogenicity scale varied for accessions from resistant to moderately susceptible. However, beside *Lr34*, phenotypic gene postulation, in combination with marker assisted selection for leaf rust resistance, has revealed presence of some other unknown resistance genes in local wheat germplasm which signified its use in wheat improvement programs both locally and abroad.

KEYWORDS: Adult Plant Resistance. AUDPC. Cultivars. Marker Assisted Selection. Wheat rust.

INTRODUCTION

Leaf rust is one of the major biotic stresses of wheat (*Triticum aestivum* L.), making difficult its production, worldwide. Leaf rust is probably the most destructive wheat disease due to its repeated occurrence, prolonged presence throughout crop life cycle, pathogenicity, and epidemic nature. It breaks out during anthesis period at the time of grain formation and lasts long to inflict heavy damages to the yield if moderate temperature and high humidity is available (RATTU et al., 2010). The dynamic and rapid adaptation of its causative fungal pathogen (*Puccinia triticina* Erikss) conduce to a constant challenge of genetic resistance of the wheat cultivars, what is typically dependent on the effectiveness of the genes during the whole developmental cycle of the plant (BIANCHIN et al., 2012). To confront the challenge of rust, genetic resistance is the most effective, economical, long-term and environment friendly way instead of using expensive hazardous chemical applications (PINK, 2002; SHAH et al., 2014).

More than 60 leaf rust resistance genes (*Lr*1 to *Lr*68) have been located on 20 of the total of 21 wheat chromosomes working under gene for gene principle to counter avirulence of many isolates of fungal pathogens (MCINTOSH et al., 1995; MUSTAFA et al., 2013). The presence of important loci in host plant conferring resistance based on hypersensitive response (HR) against specific races of the leaf rust pathogen has been found ineffective against ever adapting new pathotypes with increased and variable mechanism of pathogenicity (LAGUDAH, 2009). Thus, it is necessary explore the race non-specific "durable resistance" against rusts in wheat germplasm, which work could spend lot of years. The characterization of *Lr34* gene linked to leaf rust resistance in wheat by DYCK (1987) was major breakthrough; as it was found to be responsible for conferring non-race specific resistance against disease over a long time. Studies

Received: 20/01/16
Accepted: 02/05/16

have confirmed effectiveness of *Lr*34 gene (also known as *Yr*18) against other wheat rusts type and disease i.e. stripe or yellow rusts *(Puccinia striiformis)* (MCINTOSH, 1992; SINGH, 1992), powdery mildew (*Blumeria graminis*) (SPIELMEYER et al., 2005) and stem rust *(Puccinia graminis)* (DYCK, 1992). Therefore, worldwide efforts are on to utilize natural mode of durable rust resistance through modern agriculture approaches by identification and gene pyramiding of rust resistance genes in bread wheat accessions and commercial cultivars (KHAN, 1987; DAKOURI et al., 2013).

Gene postulation has remained preferred method of researchers for systematic evaluation of local wheat and other closely related cereal's genetic stocks to screen large populations for rust resistance genes (SINGH et al., 2001; MIRZA et al., 2000; RATTU et al., 2010; HUSSAIN et al., 2015). However, in recent years, application of molecular markers, i.e., SCAR, SSR, CAPS and STS linked to leaf rust resistance have established their importance as an alternative to traditional phenotype based methods in the field to characterize large populations (SINGH et al., 2001; MAGO et al., 2005; DAKOURI et al., 2013).

In the present study, in order to identify potential sources of resistance against leaf rust in previously underutilized genotypes, our goal was evaluated Pakistani commercial cultivars and local accessions of the Baluchistan province (near the proposed center of origin of wheat) for leaf rust resistance gene *Lr*34, through gene postulation under field conditions and employing *Lr*34 linked Sequence Tagged Sites (STS) markers, to further validate the postulation for use in rust resistant cultivar breeding programs.

MATERIALS AND METHODS

A total of 30 genotypes (Table 1) were characterized in the field of Ayub Agricultural Research Institute (AARI) Faisalabad (Pakistan), in November 2010 along with a susceptible control Morocco as previous study suggested this to be the most susceptible to rust among local wheat varieties (Afzal et al., 2008). The plant material included twenty five Pakistani wheat genotypes from Baluchistan province (near the proposed center of origin of wheat) which were obtained from the gene bank of Plant Genetic Resources Institute PGRI, National Agriculture Research Center Islamabad and five wheat varieties, i.e., Zarghoon-79, Zamindar-80, Zardana-89, Sariab-92, Zarlashta-99, and Raskoh-05, which were previously released by Agriculture Research Institute Quetta (ARIQ) Baluchistan over the last years. In the augmented field design, each genotype was planted in a 2m long row with 30 cm distance between the rows. For natural inoculation, mixture of leaf rust (*Puccinia triticina* Erikss) susceptible "Morocco" was sown around the trial as a spreader row to make possible development of rust epidemic throughout the controlled field. The entire compulsory agronomic and crop husbandry practices were carried out during the field experiment.

Table 1. List of Accessions and varieties from Baluchistan (Pakistan) characterized for leaf rust resistant *Lr*34 gene.

S No.	Variety/ Accession	Origin	Source
1	18746	Mastung, Baluchistan	NARC, Islamabad
2	11750	Kalat, Baluchistan	NARC, Islamabad
3	11758	Turbat, Baluchistan	NARC, Islamabad
4	11317	Noshki, Baluchistan	NARC, Islamabad
5	18741	Dhadar, Baluchistan	NARC, Islamabad
6	11224	Loralai, Baluchistan	NARC, Islamabad
7	11285	Pishin, Baluchistan	NARC, Islamabad
8	12120	Kalat, Baluchistan	NARC, Islamabad
9	11318	Noshki, Baluchistan	NARC, Islamabad
10	11756	Panjgur, Baluchistan	NARC, Islamabad
11	11760	Turbat, Baluchistan	NARC, Islamabad
12	11761	Khuzdar, Baluchistan	NARC, Islamabad
13	11286	Pishin, Baluchistan	NARC, Islamabad

14	12114	Kharan, Baluchistan	NARC, Islamabad
15	11240	Sibi, Baluchistan	NARC, Islamabad
16	11241	Sibi, Baluchistan	NARC, Islamabad
17	11225	Loralai, Baluchistan	NARC, Islamabad
18	11160	Mastung, Baluchistan	NARC, Islamabad
19	11153	Quetta, Baluchistan	NARC, Islamabad
20	18771	Quetta, Baluchistan	NARC, Islamabad
21	11226	Loralai, Baluchistan	NARC, Islamabad
22	11762	Khuzdar, Baluchistan	NARC, Islamabad
23	12113	Kharan, Baluchistan	NARC, Islamabad
24	11757	Panjgur, Baluchistan	NARC, Islamabad
25	18742	Dhadar, Baluchistan	NARC, Islamabad
26	Zarghon-79	Baluchistan	ARIQ, Quetta
27	Zardana-89	Baluchistan	ARIQ, Quetta
28	Sariab-92	Baluchistan	ARIQ, Quetta
29	Zarlashta-99	Baluchistan	ARIQ, Quetta
30	Raskoh-05	Baluchistan	ARIQ, Quetta

Disease testing in the field

Infection types (IT) and disease severity data was recorded in the field at three stages: 1) during adult plant phase at Feekes stage 10, when the first spikelet was visible, 2) then at the Feekes 10.5.3 growth stage when pollination was complete and disease development was at its peak as the susceptible control Morocco reached a disease severity of 100S, and 3) at Feekes 11.3 stage of maturity when kernels were hardened. The severity of disease was noted as percentage of the rust infectivity on the plants with reference to the modified Cobb scale (PETERSON et al., 1948) incorporating both percentage of leaf area diseased and the host reaction. To assess host response and infection type a modified scale method of Mcneal et al. (1971) was used. The score on scale for host-pathogen relation ranged up to 8 classes: Immune= no disease, R= resistant, RMR= Resistant-Moderately Resistant, MR= Moderately resistant, MRMS= Moderately resistant-Moderately Susceptible, MS= Moderately Susceptible, MSS= Moderately Susceptible-Susceptible, and S= Susceptible (see table 2). Area under Disease Progress Curve (AUDPC) and relative AUDPC in relation to susceptible control "Morocco" with 100% AUDPC value was calculated using SHANER and FINNEY (1977) formula;

$AUDPC= \sum[(0.5)\ (Y_{i+1}+Y_i)\ (T_{i+1}+T_i)]$

where Y_i= disease severity at *i*th date and T= days of the assessment. Genotypes were classified as Resistant, moderately resistant moderately susceptible, moderately susceptible and susceptible having AUDPC range of 0-20%, 21-40%, 41-60% and above 60% respectively.

Also Coefficient of Infection (CI) was calculated by combining disease severity and host response data as defined by Pathan and Park (2006).

Table 2. McNeal et al. (1971) rating scale for Host response and infection type

Host Response	Infection type	Disease Symptom
Immune	0	No uredinia
Resistant	1	No uredina but presence of necrotic/chlorotic spots
Resistant-Moderately- Resistant	2	Small uredina and green leaves spots with necrotic/chlorotic border
Moderately- Resistant	3	Randomly distributed uredina on single leaf
Moderately resistant-Moderately Susceptible	4	Orderly distributed variable sized uredina and larger ones at leaf tip

Moderately Susceptible	5	Orderly distributed variable sized uredina and larger ones at leaf base
Moderately Susceptible-Susceptible	6	Medium-sized uredina associated with chlorosis
Susceptible	7	Large uredina without chlorosis

Molecular analysis

DNA was extracted from fresh leaves of 2 weeks old seedlings using modified CTAB method (HAMEED et al., 2004). *Lr*34 specific STS marker c*sLV34* (Gene link NY, USA) as reported by LAGUDAH et al. (2006) was employed to confirm presence or absence of the gene in 30 genotypes including genotypes and cultivars along with negative control "Morocco". PCR reaction was carried out in a 20µl aliquot containing 80-90ng of template DNA, 2.5µl Mg-free 10X PCR Buffer (Fermentas), 0.5µl (5unit/µl) of *Taq* DNA polymerase (Fermentas), 25mM of $MgCl_2$, 2.5mM dNTPs (Sigma Chemical Co., St. Louis, MO), and 0.4µM of each forward and reverse primer. PCR was performed in Biorad machine (DNA Engine ALS-1296, USA) which was programmed for 45 consecutive cycles each one consisting of 2 minutes at 94°C, 3 min at 55°C annealing, 2 min at 72°C followed by a 10 min extension step at 72°C. PCR product was resolved on 2% agarose gel along with molecular size standard and visualized under Gel doc apparatus.

Data analysis

For field data, frequency distribution was performed upon disease severity scoring data and genotypes were classified accordingly using MS Excel 2007. Likewise for STS banding pattern, all amplified bands were considered dominant markers (present=1, absent=0) and their binary data was used to generate unpaired group arithmetic mean method (UPGMA) based dendrogram using NTSys PC version 2.1 (Exeter software Inc. USA).

RESULTS

Among 25 wheat accessions, only two genotypes (011286 and 011225) were moderately resistant to moderately susceptible (MRMS), followed by one (018771) moderately susceptible to susceptible (MS-S), and one (011762) moderately susceptible (MS), while 21 genotypes were susceptible (S). Among five wheat varieties, three (Zarghoon-79, Sariab-92, Raskoh-05) were moderately susceptible (MS), and two (Zardana-89, Zarlashta-99) were susceptible (S) (See table 3 for summary of disease phenotyping).

AUDPC value

Based on AUDPC values, the genotypes were classified into four distinct groups (Figure 1).

Table 3. The Infection Type, AUDPC, coefficient of infection, and rAUDPC for leaf rust in selected Pakistan wheat accessions and varieties to leaf rust.

Sr. No	Accessions/ variety	Leaf rust field observations			AUDPC	rAUDPC	Coefficient of Infection
		1st	2nd	3rd			
1	18746	20 S	25 S	30 S	637.5	38.63	25
2	11750	20 S	25 S	30 S	637.5	38.63	25
3	11758	10 S	15 S	20 S	387.5	23.48	15
4	11317	10 S	15 S	20 S	387.5	23.48	15
5	18741	5 S	10 S	20 S	300	18.18	11.67
6	11224	30 S	40 S	40 S	950	57.57	36.67
7	11285	30 S	35 S	40 S	887.5	53.78	35
8	12120	30 S	35 S	40 S	887.5	53.78	35
9	11318	20 S	25 S	30 S	637.5	38.63	25

10	11756	20 S	25 S	30 S	637.5	38.63	19
11	11760	30 S	35 S	40 S	887.5	53.78	35
12	11761	20 S	25S	30 S	637.5	38.63	25
13	11286	10MRMS	20MRMS	30MRMS	525	31.81	10
14	12114	20 S	25 S	30 S	637.5	38.63	25
15	11240	5 S	10 S	10 S	225	13.63	8.83
16	11241	5 S	10 S	15 S	362.5	21.96	10
17	11225	10MRMS	10MRMS	20MRMS	325	19.69	6.66
18	11160	20 S	30 S	30 S	475	28.78	26.67
19	11153	5 S	10 S	10 S	225 S	13.63	8.33
20	18771	20MSS	25 MSS	25MSS	600	36.36	17.49
21	11226	20 S	25 S	30 S	637.5	38.63	25
22	11762	20 MS	30 MS	30 MS	700	42.42	20
23	12113	10 S	15 S	25 S	425	25.75	16.67
24	11757	20 S	25 S	30 S	637.5	38.63	25
25	18742	5 S	10 S	20 S	300	18.18	16.67
26	Zarghon-79	10 MS	15 MS	35 MS	425	25.75	15
27	Zardana-89	10 S	15 S	20 S	687.5	41.66	15
28	Sariab-92	5 MS	10 MS	15 MS	262.5	15.9	7.5
29	Zarlashta-99	0	10S	20 S	275	16.66	10
30	Raskoh-05	5MS	10 MS	20 MS	300	18.18	8.75
31	Morocco	70S	80 S	100 S	1650 S	100	

S=Susceptible, MS=Moderately Susceptible, MSS=Moderately Susceptible-Susceptible, MRMS=Moderately Resistant-Moderately Susceptible.

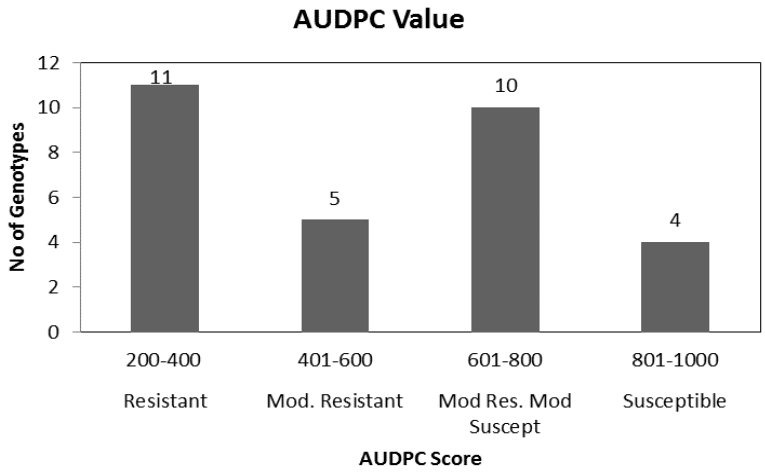

Figure 1. Distribution of genotypes into different classes for leaf rust resistance and AUDPC score.

The first group of resistant (R) genotypes comprised of eight accessions (011758, 011317, 018741, 011240, 011241, 011225, 011153, and 018742), and three varieties (Sariab-92, Zarlashta-99, and Raskoh-05) with AUDPC values up to 400. While five genotypes (011286, 011160, Zarghon-79, 018771, 012113) showing AUDPC values from 401 to 600 were Moderately Resistant (MR). A third group comprised of ten genotypes (018746, 011750, 011318, 011756, 011761, 012114, 011226, 011762, 011757, and Zardana-89) with AUDPC values from 601 to 800, and the remaining group four (13.3%) genotypes (011224, 011285, 012120, and 011760)

showing AUDPC above 800 as compared to the reference lines were susceptible.

For relative AUDPC, susceptible check "Morocco" was used as reference line. On the basis of disease severity score in comparison with susceptible check, all the genotypes were classified into 3 groups. Fourteen genotypes (011758, 011317, 018741, 011240, 011241, 011225, 011160, 011153, 012113, 018742, Zarghon-79, Sariab-92, Zarlashta-99, and Raskoh-05) were characterized as resistant, showing rAUDPC values up to 30% and were placed in the first group. The second group was comprised of 12 genotypes, including 018746, 011750, 011318, 011756, 011761, 011286, 012114, 018771, 011226, 011762, 011757, Zardana-89, having rAUDPC values from 31 to 50% and were classified as moderately resistant to moderately susceptible. A third group was comprised of four susceptible genotypes (011224, 011285, 012120, and 011760), with rAUDPC values above 50% (table 3).

Coefficient of infection

PATHAN and PARK (2006) procedure was used to classify all individuals into three classes based on their CI score, i.e., 0-10 as high, 11-20 as moderate, and above 21as low level of adult plant resistance (Figure 2).

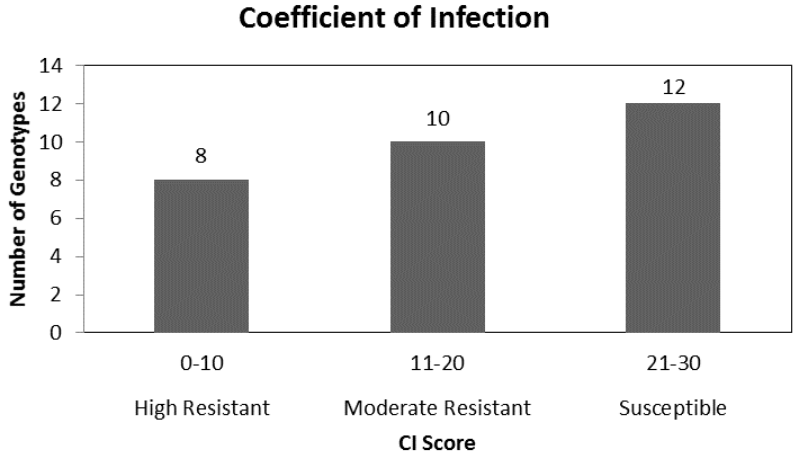

Figure 2. Classification of germplasm based on response to leaf rust infection.

Eight wheat accessions and varieties (011286, 011240, 011241, 011225, 011153, Sariab-92, Zarlashta-99, and Raskoh-05) have shown high level of adult plant resistance. Ten genotypes (011758, 011317, 018741, 011756, 018771, 011762, 012113, 018742, Zarghon-79, and Zardana-89) showed a moderate level of adult plant resistance. Twelve accessions (18746, 011750, 011224, 011285, 012120, 011318, 011760, 011761, 012114, 011160, 011226, and 011757) have shown CI above 21and hence were low in adult plant resistance (Table 3).

3.3. Molecular Characterization of germplasm using STS marker linked to *Lr*34

The marker STS *csLV34* (LAGUDAH et al. 2006) used to screen *Lr*34 gene revealed polymorphism in wheat accessions. *CsLV34* amplified one gene specific allele of 150 bp in 21 genotypes including 19 accessions from Baluchistan region (018746, 011750, 011758, 011317, 011224, 012120, 011318, 011760, 012114, 011240, 011241, 011225, 011160, 011153, 018771, 011226, 011762, 012113, and 011757) and two commercial varieties (Zarghon-79 and Sariab-92) which confirmed the presence of *Lr*34 gene. A variant sequence of 100 bp was amplified in remaining accessions (018741, 011285, 011756, 011761, 012761, and 018742) and cultivars Zardana-89, Zarlashta-99, and Raskoh-05, which is not necessarily linked to *Lr*34. Therefore, the presence of that gene among these genotypes was not confirmed (Figure 3). The comparison between field and morphological data revealed no consistency, because some genotypes without *Lr34* genes were found to carry some level of genetic resistance (Table 4).

Cluster analysis distributed 9 genotypes with *Lr34* marker in group I while remaining 21 accessions and varieties who failed to amplify 150bp marker clustered in group II (Figure 4

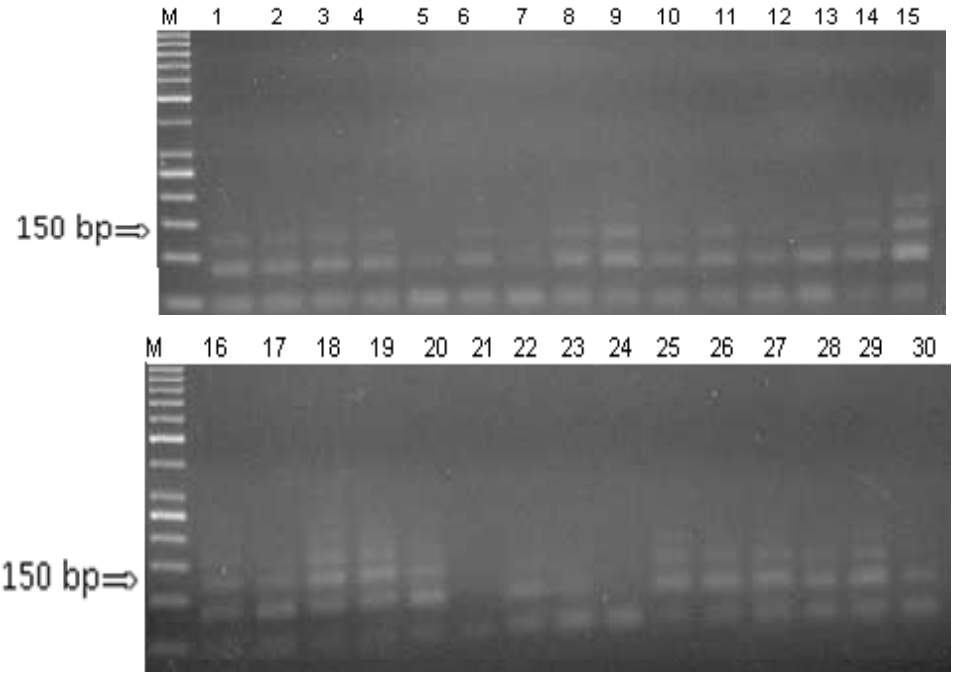

Figure 3. PCR based analysis of leaf rust resistant genes through STS markers;
From 1 to 30: 018746, 011750, 011758, 011317, 018741, 011224, 011285, 012120, 011318, 011756, 011760, 011761, 011286, 012114, 011240, 011241, 011225, 011160, 011153, Zarghon-79, Zardana-89, Sariab-92, Zarlashta-99, Raskoh-05, 018771, 011226, 011762, 012113, 011757, 018742. M=marker

Table 4. A comparison between molecular marker *csLV*34 data, field observation and Coefficient of Infection.

Sr. No	Accessions	*Lr34* Gene Status	Field observation	CI value
1	18746	+	S	25
2	11750	+	S	25
3	11758	+	MR	15
4	11317	+	MR	15
5	18741	-	MR	11.67
6	11224	+	S	36.67
7	11285	-	S	35
8	12120	+	S	35
9	11318	+	S	25
10	11756	-	MR	19
11	11760	+	S	35
12	11761	-	S	25
13	11286	-	MR	10
14	12114	+	S	25
15	11240	+	R	8.83
16	11241	+	R	10
17	11225	+	R	6.66
18	11160	+	S	26.67
19	11153	+	R	8.33
20	18771	+	MR	17.49

21	11226	+	S	25
22	11762	+	MR	20
23	12113	+	MR	16.67
24	11757	+	S	25
25	18742	-	MR	16.67
26	Zarghon-79	+	MR	15
27	Zardana-89	-	MR	15
28	Sariab-92	+	R	7.5
29	Zarlashta-99	-	R	10
30	Raskoh-05	-	R	8.75

S=Susceptible, R= Resistant, MR= Moderately Resistant.

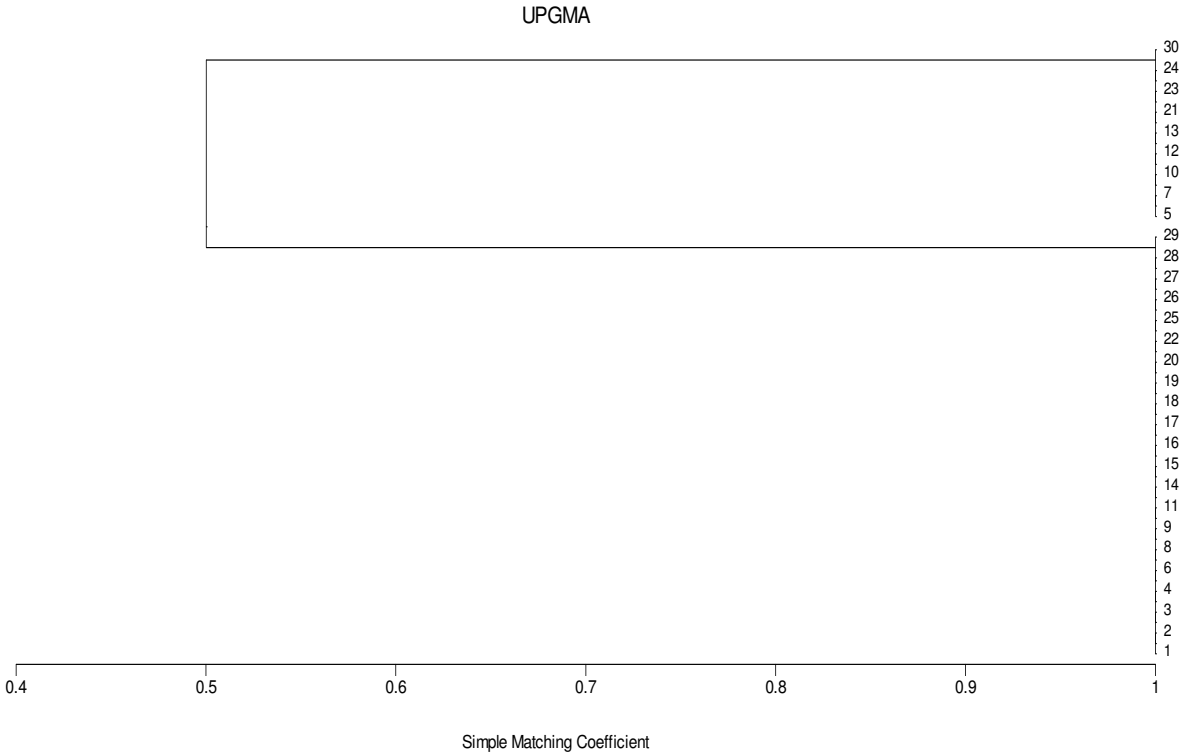

Figure 4. UPGMA based dendrogram showing two distinct groups with *Lr34* presence and absence.

DISCUSSION

Leaf rust, a common wheat disease caused by *Puccinia triticina* Eriks & Henn, is a serious production hazard (MCINTOSH et al., 1995). Incorporation of more than one gene to cultivars for durable leaf rust resistance has remained the focus of the breeders to cope with the dynamic nature of the pathogen (ROELFS, 1988). To address this issue, gene postulation as well as molecular marker approach is being utilized for enhancing rust resistance mainly through identification of durable rust resistance gene and pyramiding different seedling and adult plant resistance genes. Considering this, wheat genotypes postulated to carry *Lr34* gene were screened with microsatellites and STS marker (*csLV*34) which were found to be a useful as molecular marker and *Lr34* gene were reported to be linked closely (LAGUDAH et al., 2006).

The current study was conducted to characterize selected wheat genotypes (5 varieties and 25 accessions) from Pakistan (Baluchistan) for race-nonspecific resistance to leaf rust through gene postulation and molecular characterization for durable rust resistance *Lr34* gene. Rattu et al. (2010)

postulated host material of Pakistani wheat for *Lr* family of leaf rust resistance genes including *Lr34* and had confirmed its presence in local genetic stocks. Previous study of FAYYAZ et al. (2008) has also examined 39 isogenic wheat lines and 12 commercial cultivars from Pakistan at different locations and observed virulence of *Lr34* at Karachi and Nawabshah. In present investigation, the field testing revealed that 40% (12 genotypes) of germplasm studied was moderately resistant to moderately susceptible at adult plant stage having moderate values of rAUDPC (31 to 50 %) and low values of CI (0%–10%) and are potential donors for durable resistance against leaf rust. Similarly, the classification of wheat varieties based on values of rAUDPC was made by KHAN et al. (2002) describing groups from susceptible to fully rust resistant individuals. ALI et al. (2009) also used AUDPC and coefficient of infection (CI) approach to study partial resistance against yellow rust in wheat and observed multiple level of disease resistance from highly susceptible to partial resistance. We used the same methodology for disease severity determination and observed more than half of genotypes to be susceptible to virulence of leaf rust pathogen. The higher rate of occurrence of rust disease in wheat was also confirmed by Ali et al. (2009) as they found 90% of the wheat lines used in their study as susceptible. Cultivars possessing slow rusting illustrated lower rAUDPC at adult stage have race-nonspecific resistance as also described by Sandoval-Islas et al. (1998) and Singh et al. (2005). Because the durable resistance, like slow rusting and High-Temperature Adult Plant resistance is polygenic (at least 2-3 controlling genes) as also described by Dehghani and Moghaddam (2004), therefore it remains successful for longer time, even if the pathogen under goes mutations. Hence, by our findings, lines showing low frequency of disease severity with lower AUDPC values could be considered as slow rusting lines carrying durable rust resistance against *Puccinia triticina* Eriks and could be utilized in breeding programs.

For its relative facility, specificity and efficiency, many authors have employed PCR-based DNA markers to verify presence of leaf rust resistance in wheat (CHERUKURI et al., 2003; CHERUKURI et al., 2005; PRABHU et al., 2004; OBERT et al., 2005; LAGUDAH et al., 2006; DAKOURI et al., 2013; MUSTAFA et al., 2013). Also, it requires no laborious means or to wait for particular plant stage to observe time bound gene expression controlling trait of interest, i.e., adult plant resistance. Therefore, for further justification of leaf rust resistance estimation, *Lr*34 linked sequence tagged sites (STS) molecular marker (*csLV*34) were used to mark the presence or absence of the gene in accessions and cultivars. The marker gave successful amplification in twenty one (70%) out of thirty genotypes in the form of 150bp amplicon which is considered to be linked to durable resistance due to close linkage (0.4 cM) between this locus and *Lr*34 (Lagudah et al., 2006). A relatively smaller size amplicon of less than 100 bp was amplified in remaining 9 genotypes which is considered a marker for susceptible allele as LAGUDAH et al. (2006) revealed that a known fragment of 79bp (insertion in intron) is found to be linked to leaf rust susceptibility in bread wheat. Therefore, these wheat lines were found to be devoid of *Lr*34 gene. However, when molecular data was compared with field study, we observed three sets of observations. First type included those genotypes whose molecular data for *Lr*34 presence corresponded well with the field data, i.e., performance of genotype in the field against virulence of *Puccinia triticina* Erikss local pathotype(s). The genotypes that showed the presence of 150bp band for *Lr*34 exhibited moderately resistant to moderately susceptibility as also described by SHAH et al. (2010) and PRIYUMVADA et al. (2009). Similarly, the genotypes which failed to show the presence of 150bp band for *Lr*34 gene and were susceptible to leaf rust in the field. Hence, the absence of this gene as revealed by marker data corresponded well with the expression data in the field as also mentioned by LAGUDHA et al. (2006). In the second category of observation both the data sets did not match with each other, i.e., some of the genotypes showed the presence of *Lr*34 in the molecular analysis but in the field they remained susceptible to leaf rust. This contrast in the experimental and field results may be due to random mutations, suppression or deletion (AWAN et al., 2007), or evolution of new pathotype could also be the possible reason of inability of the wheat lines to cope with the avirulences (DAKOURI et al., 2013). Similarly, some of the genotypes did not reveal the presence of *Lr*34 locus when screened through molecular marker, although in the field those lines exhibited moderate resistance to leaf rust. This type of discrepancies have been reported in the recent past as Mcintosh (1992) postulated *Lr*34 in Popular wheat cultivars "Cappelle Desprez" on the basis of observed genetic association of leaf and stripe rust resistance. But, in a stark contrast, Lagudah et al. (2009) observed the cultivar to be devoid of the *Lr*34 using resistance gene specific marker. Besides, presence of other rust

resistant gene(s) could also be the possible reason of plant's resistance against disease. That was recently demonstrated by Spielmeyer et al. (2013). They confirmed a presence of new leaf rust resistant gene (*Lr*67) which is almost similar in many characteristics to *Lr*34. Also, could be that PCR failure to amplify the particular band during amplification may be another probability of inconsistency between field and molecular marker data, as reported by Mustafa et al. (2013). Determining the presence of *Lr*34 in current cultivars can be helpful to predict the field resistance and durability of these cultivars and to aid decisions in selecting parents for future breeding and development of new and improved cultivars with increased leaf rust resistance.

CONCLUSION

Phenotypic gene postulation as well as application of molecular markers linked to *Lr*34 has confirmed presence of one or more genes in Baluchistan's accessions conferring natural resistance against leaf rust. The resistant genotypes should be used as a contributory source of adult plant resistance in wheat improvement programs.

ACKNOWLEDGEMENTS

We acknowledge the contribution of Gene bank of PGRI, NARC Islamabad-Pakistan for provided accession seeds and relative information, and to Ayub Agricultural Research Institute Faisalabad-Pakistan for providing field facilities to conduct this experiment.

RESUMO: O presente estudo teve como objetivo a triagem da população de 25 genótipos de trigo do Baluchistão, região do Paquistão, juntamente com cinco cultivares comerciais para o estudo da resistência à ferrugem da folha em plantas adultas (leaf rust adult plant resistance, APR, em inglês) através da postulação gênica usando a inoculação natural do patótipo local da *Puccinia triticina Erikks.* A gravidade da infecção foi registrada na escala em comparação ao cultivar de controle suscetível "Morroco". Com base na pontuação fenotípica, sete acessões e quatro variedades (Zardana-89, Sariab-92, Zarlashta-99 and Raskoh-05) com valores de AUDPC (area under the disease progress curve, em inglês) até 20% foram caracterizados como genótipos resistentes. A pontuação do coeficiente de infecção (CI) variou no intervalo de 0-10 para algumas acessões e cultivares evidenciando uma elevada resistência nas plantas adultas. Além disso, o STS marker para o csLV34 bi-alélico demonstrou uma ligação estreita com o Lr34 (0.4cM). Este marcador amplificou um alelo específico do gene do 150bp em 21 genótipos, incluindo 19 acessões e duas variedades comerciais (Sariab-92 and Zarghoon-79) o que confirmou a presença do gene Lr34 conferindo resistência às plantas adultas contra a ferrugem da folha. A escala de patogenicidade da ferrugem para as acessões de resistente a moderadamente suscetível. Contudo, além do Lr34, a postulação gênica fenotípica, em combinação com a seleção auxiliada (ou assistida) por marcadores para a resistência da ferrugem da folha, revelou a presença de outros genes resistentes desconhecidos no germoplasma do trigo local o que justifica a sua utilização em programas de melhoramento do trigo tanto a nível local quanto a nível internacional.

PALAVRAS-CHAVE: Resistência em plantas adultas. AUDPC. Cultivares. Seleção auxiliada por marcadores. Ferrugem do trigo.

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