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Phenotypic and Molecular Characterization of Wheat Leaf Rust Resistance Gene *Lr*$_{34}$ in Iranian Wheat Cultivars and Advanced Lines

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ABSTRACT

Lr$_{34}$ is a vital gene in developing resistance to leaf rust, stripe rust, and powdery mildew of wheat. Providing simultaneous resistance to various pathogens has made this gene valuable in breeding for wheat resistance to many diseases. The present study investigates the *csLV*34 marker's capability in diagnosing this locus in130 wheat commercial cultivars and advanced wheat lines from Iran, and assesses the impact of this gene on disease severity in field conditions. To assess the reactions of cultivars and lines which contained *Lr*$_{34}$ under epidemic conditions of leaf rust, these cultivars were cultivated during the 2009 and 2010 cropping season. Of the 130 studied cultivars, 43 contained *Lr*$_{34}$. Cultivars that were selected and studied in stress conditions had the most frequent presence of *Lr*$_{34}$. It can be concluded that this gene plays a vital role in increasing the tolerance of cultivars under stress conditions. *Lr*$_{34}$ seems to cause active transition of materials out of the cell. In addition to being resistant to several important diseases of wheat, *Lr*$_{34}$ can increase tolerance to stresses such as salinity. Considering the calculated value for AUDPC (3% - 440%/d) in cultivars containing *Lr*$_{34}$, it seems that some cultivars contained additional resistance genes. The rate of infection in all cultivars, when presence of *Lr*$_{34}$ was detected through the molecular marker, was lower than in other cultivars. Field results confirmed the results of the analysis using the *csLV*34*b* molecular marker.

Keywords: *Lr*$_{34}$ Gene; AUDPC; Salinity Stress; Leaf Rust; *Puccinia triticina*

1. Introduction

Leaf rust caused by *Puccinia triticina* (*Pt*) is the most common and widely distributed of the three wheat rusts. Although losses from leaf rust are usually less damaging than those from stem rust and stripe rust, because of its regular and widespread occurrence, the global leaf rust damages are greater than the other two rust [1,2]. Wheat leaf rust is present in all wheat growing areas in Iran. In general, leaf rust is the second most important disease of wheat in Iran but in southern areas leaf rust is the most important disease of wheat [3]. The *Pt* population in Iran is extremely dynamic and a large number of races were found in a recent study of pathogenic variability of *Pt* population in Iran [4]. Improving the resistance of wheat cultivars to this disease is a preventive strategy with the greatest effect on reducing its damage. Rust resistance genes in wheat can be categorized into two groups: seedling resistance genes and adult plant resistance (APR) genes. Seedling resistance genes appear in both the seedling and adult plant stages and can be recognized; as a result, they show resistance in all phenotypic stages. Seedling resistance genes often lead to a hyper sensitive reaction (cell death-HR) or to lignifications of the cell membrane [5]. Adult plant gene resistance acts non-specifically on race pathogens in the adult plant stage, and cultivars containing these genes are susceptible at seedling stage and have various levels of comparative resistance to disease at the adult plant stage [6]. This type of resistance is called race non-specific gene resistance since there is no relationship between host genes and pathogen genes. Additionally, it provides resistance to all pathogen isolates. Compared with susceptible plants, *Lr*$_{34}$ resistance has a longer period of infection with fewer and smaller uredinial pustules at two weeks after infection [6,7].

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More than 70 leaf rust resistance genes have been identified, most of which are involved in race-specific category and follow the boom and bust cycle due to the high pathogenic variability of *Pt* population. Among the race non-specific genes, the Lr_{34}/Yr_{18} complex [7], Lr_{46} [8] and Lr_{67} [9] are the most commonly used genes in global wheat. The Lr_{34} gene was initially introduced as an APR gene in cultivar "Frontana" [10], this gene encoding ATP Binding Cassette (ABC) transporter[7], with the locus of the gene on the short arm of wheat chromosome 7D [11].

With few exceptions, the race specific genes are associated with a very short durability. There is increasing interests in identification and development of race-non-specific slow rusting genes which have been shown to be more durable than race-specific genes [12,13]. So far leaf rust pathogens have not been reported as virulent on Lr_{34} [7,14]. Lr_{34} is genetically linked with the stripe rust adult-plant resistance gene Yr_{18}, morphological marker leaf tip necrosis (*Ltn*1) [15], and an adult-plant powdery mildew resistance gene (*Pm*38) [16,17]. Tolerance to Barley yellow dwarf virus (*Bdv*1) in Lr_{34} carrying cultivars is also reported [18]. The incorporation of leaf rust resistance gene Lr_{34} into "Thatcher" background is known to enhance stem rust resistance [10,11]. These simultaneous resistances to several pathogens have made the Lr_{34}/Yr_{18} locus one of the most valuable gene regions for disease resistance breeding in wheat. Leaf tip necrosis (*Ltn*1) has been used as a phenotypic marker for field selection of slow-rusting resistance conferred by Lr_{34}/Yr_{18} [15] by national and international breeding programs, but because of variable expression of *Ltn*1 under different environmental conditions and in different genetic background [18], the leaf tip necrosis could not be used as a reliable and diagnostic marker.

Development and application of molecular markers for Lr_{34}/Yr_{18} have been an important objective in marker-assisted selection in breeding for durable leaf rust resistance. Application of previously developed markers, such as gwm295 and gwm1220 [19,20], has shown their limited use in breeding application due to their low diagnostic capability in precise detection of Lr_{34} in different genetic backgrounds. During the last few years significant progress has been made in the development of more closely linked markers for Lr_{34}/Yr_{18} complex trait such as SWM10 and *csLV*34 [21]. More recently, Kolmer *et al.* (2008) [18] confirmed robustness of a tightly linked *csLV*34 marker with Lr_{34}/Yr_{18} across a wide range of global wheat germplasm and its utility in wheat breeding. In present study seedling and adult-plant assessment of resistance to leaf rust coupled with application of the tightly linked marker *csLV*34 with Lr_{34}/Yr_{18} were used in the characterization of adult-plant resistance of some of the Iranian bread and durum wheat genotypes to leaf rust.

2. Materials and Methods

2.1. Plant Materials

There were 130 commercial wheat cultivars and advanced lines of hexaploid and tetraploid wheat from Iran (**Table 1**). Seeds of test genotypes were obtained from department of cereal research at Seed and Plant Improvement Institute Research (SPII), Karaj, Iran. A set of Thatcher near isogenic lines (TcNILs) were used both at seedling and adult-plant assessments. Seeds of TcNILs were kindly provided by International Maize and Wheat Improvement Center (CIMMYT). The universal leaf rust susceptible cultivar "Thatcher" and a local susceptible cultivar "Bolani" were used in seedling and adult plant tests.

2.2. Seedling Test

Assessment of seedling resistance was carried out at cereal rust pathology laboratory at SPII. The 130 test genotypes and leaf rust Thatcher near isogenic lines were used in seedling assessment against a local *Pt* isolate. Eight to 10 seeds of each genotype were planted in a 9 cm diameter pot filled with potting mix at two replications. The seedlings were grown in a rust-free greenhouse at 20˚C and 16 hours light. At two leaf stage, seedlings were inoculated with the local leaf rust isolate collected from the field trial site at Khuzestan Agricultural Research Station, in south of Iran. This isolate used in seedling tests and field inoculations. Urediniospores stored at −80˚C were first heat shocked at 42˚C for 5 minutes and then mixed with Talcum powder (1:4). Seedlings were inoculated with the talc-spore mixture using a small duster. Inoculated plants were placed overnight in a humid chamber at 17˚C ± 2˚C and dark condition. After the incubation period, plants were placed in a greenhouse with 20˚C ± 2˚C and 16 hrs supplementary light. Seedling infection types were recorded 12 days post-inoculation using; (fleck) and 0 to 4 scale [22]. Infection types; and 0 to 3 were considered resistant reactions, while infection types higher than 3 were considered as susceptible.

2.3. Field Experiments

In order to evaluate adult-plant resistance of test genotypes and TcNILs, a field trial was carried out under mist irrigation system at Khuzestan Agricultural Research Center in 2009. Each genotype was planted as two 1-m row plot and 30 cm space. To facilitate inoculum build-up and uniform dissemination of infection, the susceptible cultivar Bolani was planted perpendicular to the rows of entries. Bolani was also planted at each 10 plot intervals. Disease epidemic was created by artificial inocula-

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tion of the local leaf rust isolate collected from the same site in 2008. Preserved urediniospores were first multiplied on susceptible cultivar Bolani under greenhouse conditions following the above described procedure. Freshly collected urediniospores were mixed with talcum powder and inoculation was carried over entries after misting irrigation at late afternoons by atomizer backpack duster. First inoculation was started at 20 January 2010 when plants were at tillering stage and it was repeated four times at fortnight intervals. Disease severities (0% - 100%) were recorded according to the Modified Cobb's scale [23] and the adult-plant reactions were recorded for the major infection types R (resistant), MR (moderately resistant), MS (moderately susceptible) and S (susceptible) according to Roelfs *et al.* (1992) [24]. Field scoring started from early onset of uniform infections in Bloani with 10 days intervals. Data on the disease severities and infection types were used in calculation of coefficient of infection (CI) for each individual score [24].The area under the disease progress curve (AUDPC) [25] was then calculated as follows using the CIs for three disease scores:

$$\mathrm{AUDPC} = \sum_{i=1}^{N_i-1}\left[\frac{y_i + y_{i+1}}{2}\left(t_{i+1} - t_i\right)\right]$$

with:

i—index for scoring date;

y_i—Coefficient of leaf rust infection at scoring date i;

t_i—scoring date i expressed in days after scoring date 1;

n—total number of scoring dates in the trial.

2.4. DNA Extraction and PCR Analysis

For extraction of genomic DNA, 100 mg of harvested leaves from 14 days old seedlings of each tested genotype was ground to fine powder on liquid nitrogen. The fine powder was immediately transferred into a 2 ml tubes and the small scale DNA extraction protocol was followed as described in CIMMYT applied molecular protocol [26]. PCR reaction was performed in 20 µl for the *CsLV*34 marker following published protocol [18,21] in a PTC 100 Thermocycler (MJ Research, Waltham, MA). The PCR product was separated on 1.5% agarose gel containing TBE 0.5× buffer. Digital molecular imager UV (Gel DocTM XR Bio rad Universal Hood II) was used for visualization and documentation of banding patterns.

3. Results

3.1. Molecular Marker Screening

The presence of a 150-bp band is diagnostic of the Lr_{34} gene, indicating the presence of Lr_{34} in cultivars and lines carrying the gene. This band belongs to the *csLV*34*b* allele, which is associated with Lr_{34}. Another longer band (229-bp) is produced and belongs to the *csLV*34*a* allele, which is associated with the absence of Lr_{34}; *i.e*. when the cultivar does not contain Lr_{34}, and then the longer band is produced. However, in cultivars that are heterozygous both alleles are present and so both bands are generated simultaneously.

Of the130 investigated genotypes, 87 lacked Lr_{34}; shown by the presence of the 229-bpband of allele *csLV*34*a*. There were 43 genotypes with Lr_{34}, shown by the presence of the 150-bp band. These 43genotypes were divided into two groups: 26 homozygous cultivars with the 150-bp band and17 genotypes with both the 229- and 150-bp bands. Of the 17 hetero zygous genotypes, 15 had unique phenotype markers. In these 15 genotypes, there produced PCR product possessed a three-band pattern consisting *csLV*34*a* and *csLV*34*b* alleles, both of which were accompanied by an additional band with a higher molecular weight (280 bp). In the study of global wheat cultivars Kolmer *et al.* (2008) observed the three-band pattern in heterozygous cultivars; however, most heterozygous cultivars had a two-band pattern and cultivars with a three-band pattern were less frequent. Moreover, the positive control sample in the present study was the 150-bp band; and "Thatcher", susceptible "Bolani", and all susceptible cultivars only produced the 229-bp band and the negative control sample (water) had no bands (**Figure 1**).

There was a high frequency of *csLV*34 allele (150 bp), which is associated with Lr_{34} in the assessed Iranian lines and cultivars. Of the 130 studied genotypes, 43 contained the diagnostic Lr_{34} allele, representing a frequency of 33% (**Table 1**) as follows:

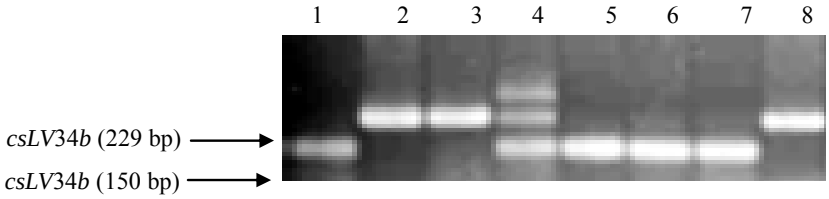

Figure 1. Polymerase chain reaction amplification products from wheat cultivars using *csLV*34 marker. 1. Thatcher +Lr_{34} (Lr_{34}); 2. Falat (Lr_{34}); 3. Ghods (Lr_{34}); 4. Star (heterozygote); 5. Bam (Lr_{34}); 6. Dez (Lr_{34}); 7. Aflak (Lr_{34}); 8. Chamran (Lr_{34}).

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Table 1. Verification of Lr_{34} by applied molecular marker *csLV*34 and comparison of leaf rust infection in Iranian commercial wheat cultivars and advanced lines in adult and seeding plant.

Entry No	Wheat variety	Pedigree		Final field score	Seedling infection type	AUDPC	*csLV*34
1	Chamran	Attila,(CM85836-50Y-OM-OY-3M-OY)	Bread Wheat	10MS	2	44	a
2	Falat	Kvz/Buho"s"//Kal/Bb= Seri82	Bread Wheat	5MS	0	22	a
3	Maron	Avd*Pchu((28mt54A*N10-Brv21-1c/Kt54B)Nar59,1093))7c	Bread Wheat	30MS	2	290	a
4	Navid	(Kirkpinar 79) 63-112/66-2*7C	Bread Wheat	40MS	3	340	a
5	Hirmand	Byt/4/Jar//Cfn//Sr70/3/Jup"s"	Bread Wheat	5MS	;	23	b
6	Alvand	1-27-6275/CF1770	Bread Wheat	60MS	3	580	a
7	Alamoot	KVZ/Ti71/3/Maya"s"//Bb/Inia/4/Kj2/5/Anza/3/Pi/Ndr//Hys	Bread Wheat	30MS	N , 1	220	a
8	Mahdavi	Ti/Pch/5/Mt48/3/Wt*//Nar59/Tota63/4/Mus	Bread Wheat	50MS	2	560	a
9	Zarin	PK15841	Bread Wheat	5MS	; , 1	23	a
10	Darab 2	Maya"s"/Nac	Bread Wheat	10MS	; , 1	44	b
Check	Susceptible control	Bolany	Bread Wheat	80S		1120	a
11	Chanab	Chanab	Bread Wheat	40MS	3	414	a
12	Tajan	Bow"s"/Nkt"s"(CM67428-GM-LR-5M-3R-LB-Y)	Bread Wheat	20MS	N , 1	90	b
13	Atrak	Bow"s"/Nkt"s"(CM67428-GM-LR-5M-3R-LB-Y)	Bread Wheat	30MS	0	282	b
14	Nicknejad	F13471/Crow"s"	Bread Wheat	30MS	3-	129	b
15	Kavir	Stm/3/Kal//V534/Jit716	Bread Wheat	30MS	2 , 1	202	a
16	Shirodi	Attila,(CM85836-4Y-OM-OY-8M-OY-opz)	Bread Wheat	R	0	4	a
17	Marvdasht	HD2172/Bloudan//Azadi	Bread Wheat	5MS	0	24	a
18	Pishtaz	Alvand//Aldan/Ias58	Bread Wheat	20MS	2	242	a
19	Shiraz	Gv/D630//Ald"s"/3/Azd	Bread Wheat	50S	3	510	a
20	Dez	Kauz*2/Opta//Kauz	Bread Wheat	10MR	0	41	b
Check	Susceptible control	Bolany	Bread Wheat	90S		1410	a
21	Hamon	Falat/Roshan	Bread Wheat	80S	3+	660	a
22	Toos	"Spn/Mcd//Cama/3/Nzr"	Bread Wheat	50S	3	572	a
23	Shahriar	KVZ/Ti71/3/Maya"s"//Bb/Inia/4/Kj2/5/Anza/3/Pi/Ndr//Hys	Bread Wheat	50MS	2	250	a
24	Ghods	Rsh/5/Wt/4/Nor10/K54*2//Fn/3/Ptr/6/Omid//Kal/Bb	Bread Wheat	60S	2	630	a

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25	Sistan	Bank"s"/Vee"s"	Bread Wheat	30MS	3	202	b
26	Bam	Vee"s"/Nac//1-66-22	Bread Wheat	30MS	2	202	b
27	Neishabour	1-63-31/3/12300/Tob//cno/sx	Bread Wheat	30MS	3-	200	b
28	Bahar	Bloyka	Bread Wheat	10MS	; , 1	42	a
29	Moghan 1	(LR-N10B)*An3E	Bread Wheat	30MS	3	282	b
30	Moghan 2	chotiLerma	Bread Wheat	30MS	2 , 1	200	b
Check	Susceptible control	Bolany	Bread Wheat	70S		1010	a
31	Moghan 3	Luan/3/V763.23/V879.C8//Pvn/4/ Picus/5/Opata	Bread Wheat	20MS	;	120	a
32	Darya	SHAU/Chil	Bread Wheat	20MS	1 , 2	400	a
33	Yavarous	Yavaros79	Durum wheat	5MR	1	10	MIS
34	Zagros	CN,79/7/2*Seri82	Bread Wheat	R	1++	3	b
35	Arta	Arta	Bread Wheat	20MS	;	161	a
36	Sepahan	Azd/5/L2453/1347/4/Kal//Bb/Kal/3/ Au//Y50E/Kal*3	Bread Wheat	20MS	N , 1	160	b
37	Star	Star"s"	Bread Wheat	20MS	2+	120	b
38	Dena	Tarro3	Durum wheat	R	;	3	MIS
39	Pishgham	Bkt/90-Zhong87	Bread Wheat	70MS	3	530	a
40	Sabalan	908*FnA12)*1-32-4382	Bread Wheat	50MS	3	620	a
Check	Susceptible control	Bolany	Bread Wheat	90S		1310	a
41	Sivand	Kaus"s"/Azd	Bread Wheat	10MR	2	40	a
42	Omid	Omid	Bread Wheat	50MS	3	540	a
43	Shapasand	Shapasand	Bread Wheat	90S	3	1130	a
44	Karaj 1	(200H*Vfn)Rsh	Bread Wheat	80S	3	1040	a
45	Karaj 2	(Fa*Th-Mt)Omid	Bread Wheat	70S	3	990	a
46	Karaj 3	(Drc*Mxp/Son64*Tzpp-Y54)Nai60	Bread Wheat	60MS	3	580	a
47	Rasool	Veery"s"=Kvz/Buho "s"//Kal/BB	Bread Wheat	40MS	;	440	b
48	Tabasea	Tabasea	Bread Wheat	80S	3	1040	a
49	Adl	Adl	Bread Wheat	50S	3	710	a

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50	Inia	Inia	Bread Wheat	40MS	1	402	b
Check	Susceptible control	Bolany	Bread Wheat	100S		1280	a
51	Golestan	Alondra"s"	Bread Wheat	20MS	2	161	a
52	Alborz	Fn-Md*K117a/Cofn2(Son64-k1.Rend/ Cno"s"LR642-SON64)CM-2182	Bread Wheat	R	N , 1	81	a
53	Kaveh	Fta-P1	Bread Wheat	50S	3	590	a
54	SorkhTokhm	SorkhTokhm	Bread Wheat	80S	3	1160	a
55	Azar 2	Azar 2	Bread Wheat	40MS	2	482	a
56	Morvaread	ilan/shaw7	Bread Wheat	R	;	3	a
57	Gaspard	Gaspard	Bread Wheat	10MS	1+	46	a
58	Gascogen	Gascogen	Bread Wheat	20MS	2+	122	a
59	Sayvan	Sayvan	Bread Wheat	40MS	2	490	a
60	MV-17	MV-17	Bread Wheat	R	;	3	b
Check	Susceptible control	Bolany	Bread Wheat	70S		1150	a
61	Karkheh	Shwa/Mald//Aaz	Durum wheat	R	1	3	MIS
62	Arya	Stork	Durum wheat	20MS	2	160	MIS
63	N-88-3	MERUA//TURACO/CHIL/3/TAJAN	Bread Wheat	30MS	2	160	a
64	N-86-4	MILAN CM75118//KA CM 75118/ K1/3/TAJAN (DH)	Bread Wheat	20MR	;	80	a
65	N-86-6	VORONA/CNO79//KAUZ/3/MILAN	Bread Wheat	R	0	3	a
66	N-86-11	CMH82A.1294/2*KAUZ//MUNIA/ CHTO/3/MILAN	Bread Wheat	R	0	3	a
67	N-87-4	BAV92/PRINIA//TAM200/PRL	Bread Wheat	R	0	3	a
68	N-87-6	JIMAI36/3/3/OASIS/SKAUZ// 4*BCN/4/89ZHONG2	Bread Wheat	10MR	0	40	a
69	N-87-9	SUNSU/PBW343	Bread Wheat	R	0	3	a
70	N-87-13	PF74354//LD/ALD/4/2*BR12*2/3/ JUP//PAR214*6/FB6631/5/ SW89-5124*2/FASAN/6/TILH	Bread Wheat	R	0	3	a
Check	Susceptible control	Bolany	Bread Wheat	80S		1060	a
71	N-87-16	NANJING2149/KAUZ/4/JUP/ALD"S"// KIT"S"/3/VEE"S"/5/SHA 7// HAHN"S"*2/PRL"S"	Bread Wheat	30MS	3	160	a
72	C-87-14	SHA 7//HAHN"S"*2/PRL "S"/3/ATRAK	Bread Wheat	10MR	0	62	a
73	C-83-7	Alvand//Ns732/Her	Bread Wheat	40MS	2	402	a

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74	C-83-8	130L1.11//F35.70/Mo73/4/Ymh/Tob //Mcd/3/Lira	Bread Wheat	10MS	2	122	b
75	C-84-8	Mihan = BKT/90Zhong87	Bread Wheat	50MS	0	560	a
76	C-85-6	Mv17/Zrn	Bread Wheat	30MS	0	122	b
77	C-85-3	Ghk"S"/Bow"S"//90Zong87/3/Shiroodi	Bread Wheat	30MS	0	202	a
78	C-86-3	Bloudan/3/Bb/7C*2//Y50E/3*Kal/4/Mv17	Bread Wheat	40MS	2	420	a
79	C-86-5	Yan7578.128//Chil/2*Star	Bread Wheat	40MS	3	340	b
80	C-86-6	Yan7578.128//Chil/2*Star	Bread Wheat	40MS	3	242	a
Check	Susceptible control	Bolany	Bread Wheat	80S		1020	a
81	M-85-6	Seri 82//Shuha"S"/4/Rbs/Anza/3/Kvz/ Hys//Ymg/Tob	Bread Wheat	10MS	2++	122	b
82	M-85-7	Seri82//Shuha"S"/4/Rbs/Anza/3/Kvz/ Hys//Ymg/Tob	Bread Wheat	R	3	4	b
83	M-85-15	Mv22-77//Stephon/3/Mon"s"/Lmu"s"// Falke/4/Zarin	Bread Wheat	50S	3	690	a
84	M-85-16	PASTOR/3/VORONA/CNO79//KAUZ	Bread Wheat	R	;	4	a
85	M-85-16	PASTOR/3/VORONA/CNO79//KAUZ	Bread Wheat	R	;	3	a
86	M-86-3	Gaspard/3/Jup/Bjy//Kauz/4/Kayson/Glenson	Bread Wheat	5MR	0	19	a
87	M-86-5	Alvd//Aldan/Ias*2/3/Gaspard	Bread Wheat	40MS	2+	241	a
88	M-86-7	Alvd//Aldan/Ias/3/Druchamps/4/kauz/Stm	Bread Wheat	30MS	1	201	a
89	M-86-9	Owl 85256-*3OH-*O-*EOH/Mv17/ 3/Alvd//Aldan/Ias	Bread Wheat	40MS	3	401	a
90	M-87-18	BABAX/LR42//BABAX	Bread Wheat	R	;	3	a
Check	Susceptible control	Bolany	Bread Wheat	60S		960	a
91	Aflak	S-80-18	Bread Wheat	5MR	3	12	b
92	S-83-3	Attila 50Y//Attila/Bcn	Bread Wheat	R	0	3	a
93	S-83-4	F60314.76/MRL//CNO79/3/KA/NAC/ 4/STAR	Bread Wheat	R	0	3	b
94	S-84-14	PASTOR/3/KAUZ*2/OPATA//KAUZ	Bread Wheat	10MS	;	48	b
95	S-85-19	INQALAB91*2/KUKUN	Bread Wheat	R	;	3	a
96	S-87-2	VEE/PJN//2*KAUZ/3/PASTOR	Bread Wheat	5MR	0	12	a
97	S-87-8	KAUZ*2/BOW//KAUZ/3/BABAX	Bread Wheat	10MR	3	22	a
98	S-87-12	PASTOR/3/VORONA/CNO79//KAUZ	Bread Wheat	5MR	;	12	a

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99	S-87-18	CBRD-3/STORK X DICOCCOIDES	Bread Wheat	5MR	;	12	a
100	S-87-20	OASIS/SKAUZ//4*BCN/3/2*PASTOR	Bread Wheat	30MS	;	122	b
Check	Susceptible control	Bolany	Bread Wheat	70S		1070	a
101	S-87-21	520- BABAX/LR42//BABAX*2/ 3/VIVITSI	Bread Wheat	R	0	3	a
102	DM-79-2	PORTO-7	Durum wheat	5MR	0	16	MIS
103	DM-81-6	PLATA-1/SNM//PLATA-9	Durum wheat	R	;	3	MIS
104	DM-82-6	SOOTY-9/RASCON-37	Durum wheat	R	;	3	MIS
105	DM-83-10	AUK/GUIL//GREEN	Durum wheat	R	;	3	MIS
106	DM-84-3	RASCON-37/BEJAH-7	Durum wheat	R	;	4	MIS
107	DM-85-10	RASCON-37/BEJAH-7	Durum wheat	5MR	;	12	MIS
108	WS-85-10	PRL/2*PASTOR	Bread Wheat	10MR	3	22	a
109	WS-85-15	PBW343*2/KONK	Bread Wheat	R	0	3	b
110	WS-86-5	Shi#4414/Crow"S"//Azd	Bread Wheat	R	0	3	a
Check	Susceptible control	Bolany	Bread Wheat	80S		1080	a
111	WS-86-8	SW89.5181/KAUZ	Bread Wheat	R	0	25	b
112	WS-86-11	MUNIA/3/RUFF/FGO//YAV79/4/ PASTOR	Bread Wheat	20MS	;	83	a
113	WS-86-12	PJN/BOW//OPATA*2/3/CROC_1/ AE.SQUARROSA (224)//OPATA	Bread Wheat	10MS	0	42	b
114	WS-86-13	VORONA/CNO79//KAUZ/3/MILAN	Bread Wheat	5MS	0	22	a
115	WS-86-14	KAUZ/PASTOR	Bread Wheat	20MS	0	82	b
116	MS-85-15	Ombu/Alamo//Mahooti/3/1-66-22	Bread Wheat	30MS	0	124	b
117	MS-85-12	Ombu/Alamo//Alvd/3/Kauz/Stm	Bread Wheat	30MS	1	160	b
118	MS-84-13	GF-gy54/Attila	Bread Wheat	40MS	3	242	b
119	MS-85-17	Sakha 8/Darab#2//1-66-22	Bread Wheat	30MS	3	204	b
120	MS-84-16	Gkzombor/Zrn	Bread Wheat	30MR	3	300	a
Check	Susceptible control	Bolany	Bread Wheat	80S		1300	a
121	SS-85-6	1-66-22/3/GUP/BGY//kauz	Bread Wheat	30MS	3	202	b
122	SS-85-10	OMBU/ALAMO//ALVD/3/1-66-22	Bread Wheat	30MS	3	202	b

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Continued

123	SS-85-11	OMBU/ALAMO//MAHOOTI/3/1-66-22	Bread Wheat	30MS	;	202	b
124	SS-85-14	SAKHA 8/DARAB#2//1-66-22	Bread Wheat	30MS	;	282	b
125	SS-85-20	OMBU/ALAMO//KAV/3/PASOR/ SORKHTOKHM..	Bread Wheat	30MS	0	202	b
126	DW-79-5	LAGOST-2	Durum wheat	20MS	;	89	b
127	DW-81-18	SORA/2*PLATA12	Durum wheat	20MS	;	85	b
128	DW-84-5	GREEN_14//YAV_10/AUK	Durum wheat	R	;	6	MIS
129	Bezostaya	Bezostaya	Bread Wheat		3		b
130	Veerynak	Veerynak	Bread Wheat		2		a
Check	Susceptible control	Bolany	Bread Wheat	100S	3+	1280	a
Check	Positive control	Thatcher (Lr_{34})	Bread Wheat	30MS	3-	300	b

Among commercial Iranian cultivars, 18 of 64 cultivars contained *csLV*34*b*, indicating presence of Lr_{34}; only "Hirmand" and "Bam" which were the result of national crossing program, which had origin in international germplasm.

None of the nine advanced lines bread wheat for the warm and humid climate of northern Iran carried Lr_{34}, *i.e*. they contained *csLV*34*a*. Among the nine advanced lines bread wheat for cold climates, three had Lr_{34}; among the 11 advanced lines bread wheat for the warm climate of the south, five carried Lr_{34}.

From nine lines bread wheat for mild climates, two had the 150-bp band indicating the presence of Lr_{34}.

However, cultivars studied in environmental stress conditions and selected to assess their tolerance of these conditions were totally different as follows. Among the eight chosen advanced lines bread wheat for humid stress conditions, four had Lr_{34} (50%). Of the five selected advanced lines bread wheat for saline conditions located in mild climate areas, four contained Lr_{34}; among five advanced lines bread wheat for saline conditions in the warm climate of the south, all five carried Lr_{34}, *i.e*. 90% of advanced cultivars bread wheat to tolerate salinity contained Lr_{34}. It seems that cultivars carrying Lr_{34} could better tolerate stress conditions, and most cultivars previously introduced for areas with salinity stress in mild climates such as "Hirmand", "Sistan", "Neishabour", "Nicknejad", "Tajan", and "Darab2" contained Lr_{34}. "Inia" is a cultivar resistant to salinity in experiments and also contained Lr_{34}. "Star" is a late maturity cultivar, and is presently widely cultivated in Khuzestan Province. In evaluation of salinity resistance of wheat cultivars in laboratory and field conditions, the percentage of germination and seedling establishment of "Star" under saline conditions was good relative superiority. "Bam" was recently introduced for mild climate areas with soil and water salinity stress, and contains Lr_{34} [27]. Cultivars that were not resistant to environmental stress such as salinity lacked Lr_{34}, e.g. lines bread wheat for the northern climate or cultivars "Darya", "Golestan", "Alborz", "Kaveh", and "Bahar" (**Table 1**).

In the present research, 13 genotypes of durum wheat were also investigated, four cultivars and seven lines of which did not produce any bands to confirm the presence or absence of Lr_{34}. A separate experiment for these cultivars was repeatedly conducted with positive and negative controls and a similar result was obtained. Absence of a reproduced band or piece in durum wheat was likely due to the lack of the D genome since Lr_{34} is located on the small arm of chromosome 7 of genome D and primers should be placed on this part to be reproduced. Because of the absence of this genome in tetraploid cultivars (e.g. durum wheat), this piece was not reproduced in these cultivars.

3.2. Phenotypic Characterization

About 44 cultivars gave R or MR reactions in field assessment, among which only eight carried Lr_{34}. Most cultivars (35 genotypes) contained Lr_{34} or were MS. The estimated AUDPC for cultivars carrying Lr_{34} was within 3% - 440%/day, indicating that some cultivars may carry

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some other resistance genes as well as Lr_{34}. This was confirmed by further studies conducted with other markers of race-specific genes on the same cultivars (unpublished data). In wheat cultivars with a combination of resistance genes genetic infection type with the highest resistance conceals the impact of the type with lower infection; therefore, these cultivars that contain race-specific resistance genes in addition to Lr_{34}, an infection type of R or MR is seen instead of MS resistance type and the presence of Lr_{34} is masked by other main genes. Accordingly, methods such as molecular methods which can easily identify this gene are important. The phenotypic method used currently relies on *Ltn*1 and makes it very difficult to recognize Lr_{34} from the visual phenotype of leaves since *Ltn*1 does not express equally in different environments. This method requires a lot of experience and the results are not always correct.

AUDPC for the examined cultivars in the present study was within 3% - 1410%/day. AUDPC of control susceptible "Bolani", which was repeated 13 times among the field-grown cultivars (was planted at the end of the experiment and after each plot of 10 cultivars as susceptible control), was calculated to be 960% - 1410%/d, this difference in AUDPC is the result of environment.

AUDPC of other cultivars, except for that of the susceptible control (Check), was 3% - 1160%/day. The lower AUDPC belonged to cultivars that were resistant due to their effective race-specific resistance genes and were discussed previously. The results showed that AUDPC of 500% - 800%/day indicated a susceptible cultivar and AUDPC > 800 indicated a too susceptible one.

In this experiment AUDPC < 500 was regarded as acceptable resistance because about two months after an epidemic of the disease and at the time of maximum flag leaf efficiency in photosynthesis and grain filling, the maximum infection remained at 40 MS. The highest AUDPC for genotypes containing Lr_{34} was for "Rasool", "Inia", and line C-86-5 with values 440%, 402%, and 340%/day, respectively. Most lines and cultivars containing this gene had AUDPC of about 200%/day and "Thatcher" had 300%/day. Accordingly, AUDPC of 250 - 500 was considered as semi-susceptible or relatively resistant; AUDPC of 150 - 250 was considered semi-resistant, and AUDPC < 150 was considered resistant.

The flag leaf plays a crucial role in grain filling. The surface of this leaf in susceptible cultivars can be rapidly covered with leaf rust pustules at the time of grain filling. As a result, the entire surface can be infected and so harms its function. However, cultivars containing Lr_{34} are resistant to rapid development of the pathogen and delay it. The flag leaf of such cultivars is more capable of grain filling and incurs less damage.

In epidemics, leaf rusts do much damage to flag leaves; therefore, assessing resistance to leaf and yellow rust at the adult plant stage is very important in improvement programs. Most assessment of resistance to leaf rust is done on the flag leaf because severity of the disease on leaves reflects the primary growth of the pathogen and damage to the plant [24].

An obvious advantage of presence of Lr_{34}/Yr_{18} in cultivars is the absence of high intensities of infection at the end of the wheat growing season. However, cultivars that do not contain this gene can be highly infected by leaf rust during the whole growing season. Cultivars with race-specific genes, which are widely used in cultivars, are expected not to show long-term resistance for pathotypes that are virulent on the Lr_9 resistance gene. These were previously discussed by Kolmer [28]. However, if resistance of a race-specific gene is broken, Lr_{34} prevents rapid epidemics of the disease and major damage. In all cultivars in which the presence of Lr_{34} was shown by molecular marker, infection rate was less than in cultivars not containing this gene (**Table 1**). Field results confirmed the analysis results concerning the *csLV*34*b* marker (**Table 1**).

Lr_{34} is believed to be dominant. The results clearly showed gradual rust resistance in heterozygous cultivars and with no difference for cultivars homozygous for this gene.

Cultivars not containing Lr_{34} included 87 genotypes, divided into four groups according to resistance and susceptibility in field and greenhouse as follows.

The first group of seven genotypes was susceptible in the seedling stage and resistant in the adult plant stage: N-87-16, C-86-6, M-86-9, S-87-8, WS-85-10, and MS-84-16. These genotypes are crucial since they carry a gene or genes of adult plant stage resistance other than Lr_{34}. Markers are needed to verify and identify the presence of these genes.

The second group included 57 genotypes resistant in both adult plant and seedling stages. This group contained race-specific resistance genes to the utilized genes. These genotypes may contain non-specific race resistance genes other than Lr_{34} that are masked by the effect of specific resistance genes.

The third group included 17 cultivars which were susceptible in both seedling and adult plant stages. This group lacked adult plant stage genes and effective specific race genes to the applied isolate.

In the fourth group, five cultivars were resistant or immune in the seedling stage but susceptible in the adult plant stage. This showed that these cultivars lacked adult plant stage resistance genes; however, they were influenced by pathogen races other than those present in the seedling stage in the field. At the time of collecting spores and testing them in greenhouse condition, this

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race did not exist or was just part of the field's pathogenic population. The population or race which could infect these cultivars was not present in the population gathered and used in the greenhouse, or alternatively their frequency was low. Thus, they did not have the opportunity to appear under greenhouse conditions but could have greater effect in the field due to the longer time available.

Cultivars carrying Lr_{34} were categorized into three groups, based on their reaction to leaf rust in field and greenhouse. Cultivars in the first group included 33 genotypes that were resistant in both seedling stage and adult plant stages, indicating that they contained effective race-specific genes other than Lr_{34}.

The second category included two groups. The first group contains delight cultivars susceptible in the seedling stage and semi-resistant (MR) or semi-susceptible (MS) in the adult plant stage. This is characteristic of Lr_{34} and these cultivars apparently carried only Lr_{34}. The second group was "Aflak" and the line M-85-7. They were totally susceptible in the seedling stage and completely resistant in the adult plant stage. It seems that these cultivars lack the effective race-specific resistance gene to the utilized isolate. However, their high resistance in field conditions indicated that they contained a gene or genes of adult plant stage resistance other than Lr_{34}. This makes them unique and they require further investigation.

4. Discussion

In the present study, in cultivars of Iranian origin Lr_{34} was only present in cultivars and lines linked with the very old 22-66-1 lines of ill-defined pedigree. Almost all other Iranian cultivars containing this gene originated from international germplasm, especially from CYMMIT. Lr_{34} was also present in cultivars of international germplasm. Introducing cultivars of CYMMIT origin into Iran has increased the frequency of Lr_{34} in Iranian cultivars. Lr_{34} has received much attention in recent years, since this gene is present in high frequency in CIMMYT bread wheat germplasm and derived cultivars with CIMMYT origin [29].

Examination of 123 local cultivars (landraces) of Iran by Kolmer *et al.* [18] showed that only three cultivars (2.4%) had Lr_{34}. Also, in other parts of the world, the *csLV*34*b* allele did not exist in most local cultivars and had a low frequency, compared with the general frequency in improved wheat cultivars. The incongruity of *csLV*34*b* occurring among improved and local cultivars may be directly or indirectly caused by improvement trials to combine Lr_{34}/Yr_{18} into new cultivars. Among international cultivars, those from CYMMIT showed high frequency (30%) of *csLV*34*b*.

The mentioned result was verified by assessing the infection, analysis of molecular markers, and data gathered through pedigree for presence of Lr_{34}. Of 130 cultivars, which had Lr_{34} according to pedigree, specific bands were produced in only 43. Due to the high sensitivity of this marker in detecting the Lr_{34} gene allele, having pure seeds from the desired cultivars, and not mixing with other cultivars are critical to providing reliable results. Studies have shown that due to probable mistakes in data in pedigrees, it is crucial to apply specific molecular markers to confirm the presence of resistant genes against leaf rust in wheat cultivars. Many researchers have concluded that molecular markers are better for this prediction than pedigree data [30,31].

In the present study, cultivars selected and investigated in stress conditions had the highest percentage presence of Lr_{34} and it seems that this gene was effective in increasing the tolerance of cultivars in environmental stress conditions. All chosen lines and cultivars of the warm and humid climate in the north of Iran lacked Lr_{34}, and these cultivars were selected in environmental conditions without stresses such as drought, heat, cold, and salinity. The warm and dry climate of the south of Iran, followed by lines bread wheat lines for cold climates, had the highest frequency of Lr_{34}.

Lr_{34} belongs to the super family of ABC transporters that produce proteins connected to the plasma membrane, which plays an important role in transferring materials in and out of the membrane. ABC transporters can transport a wide range of materials that can be cytotoxic, including ions, so that they transport macromolecules against the diffusion gradient on both sides of the cell membrane [32,33]. Drug transporters were primarily recognized in cancer cells which were resistant to drugs. These transporters carry the consumed drugs out of cancer cells and make the cells resistant to drugs. This mechanism was also discovered in drug-resistant fungi such that, in the resistant mutant fungi, gene expression or drug transporter genes and accordingly related proteins greatly increased. Consequently, by discharging more and lowering fungicide concentrations below the fatality threshold in fungal cells this causes resistance to fungicides. In addition to fungicide disposal, drug transporters can pass mycotoxin discharge of other fungi, natural antimicrobial compounds of other organisms, and plant defense compounds out of the cell and cause resistance in fungi [34].

It seems that ABC transporters are one effective factor in resistance to salinity in plants. This system is probably active in cultivars resistant to salinity that contain ABC transporters, and extra salt ions are actively pumped out of the cells. As a result, transporters enable salinity tolerance in various cultivars or help the process of identi-

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fying salinity ions and prevent them entering the cytosol. Accordingly, these cultivars are probably resistant to salinity. This hypothesis was also strengthened by assessing durum cultivars, which lack the D genome and are more susceptible to salinity than wheat cultivars. Therefore, Lr_{34}—in addition to providing resistance to leaf rust, yellow rust, powdery mildew, and barley yellow dwarf virus in wheat—plays an important role in improving tolerance to environmental stresses such as salinity. Perhaps one mechanism of Lr_{34} in providing relative resistance to leaf rust agent pathogen is removing toxins, metabolites, or other harmful substances discharged by pathogens into host cells. As an example, virulent factors, which are discharged by haustoria of pathogens and are vital for aiding pathogen growth in the host cell, are pumped out of the cell by these transporters. This makes the pathogen grow slowly compared to hosts which lack this gene. Additionally, the phenotype of fewer and smaller pustules, and a longer latent period, would consequently appear since these substances lower the growth of the out-of-cell pathogen and reduce its density in the environment. This material is harmful to plant cells and its removal causes the plant tolerance to increase with no negative impact on plant physiological functions. Pumping harmful substances out of plant cells actually increase the plant's tolerance to biotic and abiotic stresses. A more accurate assessment of the relationship between this gene and the tolerance to environmental stresses such as salinity needs a further and more complete investigation.

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