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Full Length Research Paper

Leaf rust (*Puccinia triticina*) resistance genes determination using race differentials and molecular markers in winter – facultative wheat (*Triticum aestivum* L.)

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Abstract

Puccinia triticina, the causal agent of leaf (brown) rust of wheat, causes major yield losses in winter-facultative wheat around the world. The most sustainable approach to leaf rust control is through the use of disease resistant varieties, particularly those that are slow rusting. We evaluated 76 winter-facultative wheat genotypes from the International Winter Wheat Program and 40 Thatcher leaf rust isolines. Identification of the slow rusting phenotypes required prediction of Lr genes in the greenhouse and screening of genotypes against naturally occurring leaf rust epidemics in the field. Various Lr genes: Lr1, Lr3a, Lr10, Lr13, Lr14a, Lr10, Lr16, Lr17, Lr23, Lr24, Lr26, Lr27, Lr31 were determined by comparison to differentials, whilst the presence of Lr34 was determined by molecular markers. A large amount of variation existed for slow rusting amongst the lines tested. Incorporating the sources of Lr genes determined here and in other studies into wheat genotypes might secure future yield and quality of winter – facultative wheat.

Key Words: AUDPC, gene postulation, leaf rust, slow rusting, winter - facultative wheat, Turkey.

INTRODUCTION

Wheat (Triticum aestivum) is one of the primary food crops grown across the globe, and plays an important role in agricultural systems of developing countries. Wheat production is frequently devastated by several diseases fungal diseases.

The three (yellow, leaf, and stem) rusts, the most important wheat fungal diseases are the most destructive wheat pathogens, significantly reducing yield and quality –especially reducing kernel weight (Huerta-Espino 2011)- by restricting photosynthesis on leaves (Loegering 1967; Arslan et al. 2002). Severity of leaf rust increased recently in the West Asia region and also in Central and Eastern Europe (Morgounov et al. 2012). Higher virulence and wider adaptation (Morgounov et al. 2012) make leaf rust control difficult with genetic resistance. Wheat cultivars with race specific resistance genes in the US select for virulent P. triticina races that can overcome the cultivar resistance. P. triticina populations around the world are characterized by high levels of virulence to the most common leaf rust resistance genes in the regional germplasm (Goyeau et al. 2006; Park et al. 2001). The deployment of rust resistance genes is the most economical means of diseases control and is highly recommended in all plant breeding programs (Stubbs et al. 1986; Herrera-Foessel et al. 2011; Rafiei Boroujeni 2011). Slow rusting - +a polygenic, race-non-specific, and

durable resistance type with fewer and smaller uredinia, longer latent periods, resistance at given growth stages, and environment-resistance reaction- has recently become common in breeding programs (Roelfs et al. 1992). Prior to incorporating major and / or minor resistance genes into varieties of economical importance, a breeding program must first identify resistance genes. Many leaf rust resistance (Lr) genes have been identified thus far: Kolmer (1996): 46 Lr genes; Dyck et al. (1966): Lr 13 and Lr12 genes; and McIntosh (1992): Lr1, Lr2, Lr3, Lr13, Lr17, and Lr24 genes, and incorporated into varieties. Molecular marker-based breeding and selection techniques have also been successfully applied for the identification of leaf rust genes: Lr47, Lr51, Lr37 (Shabnam et al. 2010); Lr34 (Vida et al. (2009); and Lr10 (Feuillet et al. 2003).

International Winter Wheat Improvement Program (IWWIP), run jointly by Turkey, International Center for Wheat and Maize (CIMMYT), and International Center for Agricultural Research in Dry Areas (ICARDA), improves winter-facultative wheat germplasm for Central Asia, West Asia, and North Africa. Leaf rust is a threat to wheat production over 35 million hectares in these regions (Braun 1996). To limit the impact of leaf rust on wheat production in the region, there is an urgent need to elucidate the presence of known Lr genes in the IWWIP germplasm. The main aim of this study was to identify Lr resistance genes and understand the level of slow leaf rusting in IWWIP germplasm, using differential lines and molecular markers.

Materials and methods

Plant material

The 75 winter or facultative wheat genotypes (Table 1) a susceptible check (Sabalan), and 40 Thatcher isolines of P. L. Dyck developed at CIMMYT (Table 2) were tested against Lr pathotypes both in the greenhouse and in the field.

Greenhouse evaluations

Fully grown 9-10 day old seedlings of genotypes (Table 1 and Table 2) were inoculated with urediniospores of races: MFB/SP, BBG/BN, CCJ/SP, CBJ/QB, CBJ/QQ, MBJ/SP, TBD/TM, MCJ/QM, MCJ/SP, TNM/JM, TCB/TD, LCJ/BN obtained from CIMMYT. Inoculum concentration was normalized to 2-3 mgml-1 (Long and Kolmer 1989; Sing 1991; Singh and Rajaram 1991) and applied using light weight mineral oil, Soltrol 70 (Philips 66 Company, Oklahoma, USA). Six to eight seedlings, planted at a 5 cm distance, were tested, using 4 sets of boxes, each box containing 38 genotypes. Inoculated plants were kept in a dew chamber for 15 hours at 18-24 0C in the dark, then transferred for 5 hours into 60% humidity. Lastly, the plants were transferred to 15 minutes 60% humidity, followed by 45 minutes normal conditions, were placed into a greenhouse at 23-25 0C (Singh 1992).

Infection types were recorded after 10 days using 0-4 scale (Stakman et al. 1962), where: 0 = no uredinia or other macroscopic signs of infection; ; = no uredinia but hypersensitive or chlorotic flecks; 1 = small uredinia surrounded by necrosis; 2 = small to medium uredinia surrounded by green islands; X = random distribution of variable sized uredinia on single leaf with a pure culture; 3 and 4 = medium to large sized uredinia without chlorosis or necrosis; + = uredinia somewhat larger than normal for the infection type; - = uredinia somewhat smaller than normal for the infection type; c = more chlorosis than normal for the infection type. The presence of Lr genes in the genotypes was postulated by comparing the infection types displayed by each genotype to the infection types of known Lr genes in Thatcher differentials (Singh and Rajaram 1991; Singh et al. 2001).

Field evaluations

Previously vernalized genotypes (Davidson 1985; Akın 1992) were planted in a randomized complete block design with two replications at CIMMYT (El Batan, Mexico), on May 22nd, 2007. Plots consisted of two 1 – meter rows were seeded at a distance of 15 by 70 cm. Susceptible spreaders were planted at every 20 rows. The average rainfall during the season was 460.9 mm, the minimum temperature 1.49 oC in December and the maximum 28.27 oC in April. Two predominant pathotypes, MCJ/SP and MBJ/SP, were first sprayed on spreaders and then, on genotypes. Inoculum applied was 1 grl-1 in water, with a drop of glycerin (Stubbs et al. 1986). Leaf rust severity and response were recorded 5 times on flag leaves at 7-8 day intervals, starting with the appearance of first symptoms during the shooting stage. Severity estimations were scored using the modified Cobb scale (Peterson et al. 1948) and growth stages using the Zadoks Scale (Zadoks 1974). The response to infection was scored as: R = resistant, smaller uredia surrounded by necrotic tissues; MR = moderately resistant, smaller uredia surrounded by necrotic tissues; MS = moderately susceptible, moderate sized uredia without necrotic tissues; S = susceptible, large sized uredia without necrotic tissues.

The Area under the Disease Progress Curve (AUDPC) was calculated for each genotype for the leaf rust scores in Excel. The formula was:

∑_(n=1)^(n=n)▒〖(Days between two readings)*((First reading + Second reading)/2)〗

Table 1. International Winter Wheat Improvement Program genotypes used in this study, with cross/cultivar name and cross numbers

No	Cross / Cultivar	Cross No	No	Cross / Cultivar	Cross No
1	DMN//SUT/AG(ES86-7)/3/OPATA/4/TX71A1039..	CIT88061T	39	YE2453//PPBB68/CHRC	TCI950019
2	LAGOS-11	CIT88129T	40	F1502-W9-01//KS82W409/STP	TCI950220
3	PMF/MAYA//YACO/3/CO693591/CTK	CIT90095T	41	HATUSHA/2*ID800994.W/VEE	TCI952049
4	HUAPEI76/MNCH//YE2453	CIT935216	42	CA8055/4/ROMTAST/BON/3/DIBO//SU92/CI13645/5..	TCI951084
5	RSK/CA8055//CHAM6	CIT922189	43	YE2453//PPBB68/CHRC	TCI950019
6	KVZ/HB2009/5/CNN/KHARKOV//KC66/3/SKP35/4/VEE	ICWH87046	44	AGRI/BJY//VEE/3/KRC66	TCI951025
7	MEX COMP3/4/F134.71/NAC/6/LOM11/SON64/4/PJ/..	CIT922470	45	YMH/HYS//VPM/MOS4-2-16-1-7/3/ST35i/4/..	TCI950068
8	PYN//TAM101/AMI/3/KRC66/SERI	CIT94072	46	TRK13 RESEL//TRAP#1/BOW	CMSW90M375
9	ES14/SITTA//AGRI/NAC	CIT937193	47	BONITO-36	SWM17702
10	ES14/SITTA//AGRI/NAC	CIT937193	48	BONITO-44	SWM17702
11	DOGU88//SST102/MKUZI/3/CA8055	CIT937011	49	KALYOZ-20	CMSW91M00018S
12	85ZHONG33/ZLATOSTRUI//PLK70/LIRA	CIT937069	50	8023.16.1.1/KAUZ	CMSW92WM00378S
13	UT1556.68/VEE9//AK702/3/UNKN	CIT925145	51	FRTL//AGRI/NAC	CMSW93WM0071
14	VORONA/OPATA//PYN/BAU	TCI951324	52	CO72.3839/TI-R//FASAN/3/CO72.3839/TI-R	CMWW91M00034T
15	JUP/4/CLLF/3/II14-53/ODIN//CI134431/SEL6425/..	TCI951414	53	BATERA//KEA/TOW/3/TAM200	CMWW91M00040T
16	90ZHONG657//BAU/KAUZ	TCI951462	54	OK81306//ANB/BUC/3/[SAULESKU 43]	CMWW91M00096T
17	SAVALAN//KRC66/SERI/5/JUP/4/CLLF/3/II14-53 ..	TCI952093	55	SHARK-4	CMSW90M128
18	SAVALAN//KRC66/SERI/5/JUP/4/CLLF/3/II14-53 ..	TCI952093	56	SKAUZ/HATUSHA	CMSW93WM0034
19	AU/3/MINN//HK/38MA/4/YMH/34A/5/CT/GGT/6/PYN..	TCI952137	57	PONY/OPATA//PSN/BOW	CMSW94WM00846
20	KS82W422/SWM754308//KS831182/KS82W422/3/...	TCI952142	58	PNR2548/STAR1	SWM940476
21	F4141-W-1-1/PASTOR//PYN/BAU	TCI952274	59	WIT910555/3/VPM/MOS83-11-4-8/PEW	WIE921116*
22	F1502-W9-01//KS82W409/STP	TCI950220	60	ID800994.W/MO88	CMWS92Y00272S
23	89ZHONG 108/5/NOR/6720//YMH/3/ZZ/4/PJ/HN4//GLL	TCI950804	61	TAM106 RESEL/TX69D4819/6/WRM/4/FN/3*TH..	CMWW90M113
24	ATAY/89ZHONG2	TCI950001	62	TORIK-16	SWM5069
25	KS82W409/SPN//TAM106/TX78V3630	TCI951385	63	TX71A1039.V1*3/AMI//BUC/CHRC	CMSW89Y234
26	MV17/3/CROC_1/AE.SQUARROSA (205)//KAUZ	TCI951412	64	PYN/BAU	SWM15182
27	MV17/3/CROC_1/AE.SQUARROSA (205)//KAUZ	TCI951412	65	TAST/SPRW//ZAR/3/CHUM18//JUP/BJY	CMSW94WM00868
28	AU/3/MINN//HK/38MA/4/YMH/ERA/5/PMF//CNO/GLL..	TCI951429	66	F900K/PRINIA	CMSW94WM00884
29	BATAVIA//TAMEX/OPATA/3/ID800994.W/VEE	TCI952163	67	AKULA/5/GOV/AZ//MUS/3/DODO/4/BOW	CMSW94WM00939
30	PIOPIO/ATTILA/4/YMH/TOB//MCD/3/LIRA	TCI952252	68	AKULA/5/GOV/AZ//MUS/3/DODO/4/BOW	CMSW94WM00939
31	ORE.F1.158/FDL//BLO/3/SHI4414/CROW/4/MNCH..	TCI950778	69	ASP/7C//MIDA/WRM/3/CHOIX	CMSW95WM00094S
32	AGRI/BJY//VEE/3/KS82142/CUPE	TCI951027	70	94.43591/CHOIX	CMSW95WM00559S
33	HATUSHA/OMID/3/AGRI/BJY//VEE	TCI952361	71	PONY/OPATA//PSN/BOW	CMSW94WM00846
34	MV17/FANDANGO	TCI950567	72	AGRI/NAC(ES91-17)//ATTILA	CMSW94WM00923
35	F130-L-1-12*2/MILAN	TCI952271	73	ASP/7C//MIDA/WRM/3/CHOIX	CMSW95WM00094S
36	JUP/4/CLLF/3/II14.53/ODIN//CI13431/5/IL-75-2534	TCI950143	74	94.43591/CHOIX	CMSW95WM00559S
37	SAVALAN/GRK//PYN/BAU	TCI952089	75	AGRI/BJY//VEE/3/PRINIA	CMSW94WM00828
38	BATAVIA//TAMEX/OPATA/3/ID800994.W/VEE	TCI952163	76	SABALAN (Susceptible check)	

Molecular evaluations

DNA extraction was carried out with the CTAB method of Saghai-Maroof et al. (1984) modified according to CIMMYT (International Wheat and Maize Improvement Center)'s manual of laboratory protocols (http://www.cimmyt.cgiar.org/ABC/Protocols/manualABC.html). Twenty seeds per genotype were grown in the greenhouse for two weeks, then bulks of young leaves from 10 plants per genotype were harvested for DNA extraction. PCR-reactions were carried out with Applied Biosystem 9700 PCR systems. Each 10 □l reaction mixture contained 50 ng templates DNA, 250 nM of each primer, 200 □M dNTPs, 200 mM MgCl2, 1□ PCR buffer and 2.0 U of Taq-polymerase. PCR was carried out with the following standard temperature profile: 30 cycles with a 2 min denaturing step at 94°C, 1 min annealing temperatures between 54 and 62°C depending on the different primer combinations, and 2 min extension at 72°C. The 1 min time spread of the standard profile cycle was modified in some cases to fully optimize amplification conditions.

Amplification products were separated by electrophoresis in 2.5% agarose gels and visualized by means of 0.5mg/ML ethidium bromide and UV light.

RESULTS

Greenhouse evaluations

Seedlings of Thatcher near isogenic lines with Lr3ka, Lr16, Lr21, Lr29, Lr30, and Lr32 resistance genes had low or medium infections against to all 12 pathotypes (Table 2). Comparisons between cultivars / crosses and Thatcher lines indicated that these genes were absent in winter-facultative genotypes tested, as they showed higher infection scores, against all of the pathotypes.

Table 2: Differential isolines used in this study, along with their reactions in the greenhouse and the field

No	Genes	Pedigre	Tester lines	Chromosom	Linkage	Source for tester	Origin	Low Infection Type		Explanation
								Greenhouse	Field	
1	***Lr1***	TC*6/CENTENATRIO	RL6003	5DL		Centenario	*Triticum aestivum L.*	0;	I	
2	***Lr2a***	TC*6/WEBSTER	RL6016	2DS		Webster	*Triticum aestivum L.*	0; -;1	I,MR	
3	***Lr2b***	TC*6/CARINA	RL6019	2DS		Carina	*Triticum aestivum L.*	;1 -;1+	R,MR	
4	***Lr2c***	TC*6/LOROS	RL 6047	2DS		Brevit	*Triticum aestivum L.*	;IN -23	MR-R	
5	***Lr3***	TC*6/DEMOCRAT	RL6002	6BL	*Sr11*	Democrat	*Triticum aestivum L.*	;C -23	R,MR	
6	***Lr3Bg***	BAGE/8*TC	RL 6042	6BL	*Sr11*	Bage	*Triticum aestivum L.*	;C - 23	MR-MS	
7	***Lr3Ka***	TC*6/ANIVERSARIO	RL6007	6BL	*Sr11*	Klein Aniversario	*Triticum aestivum L.*	;C -12C	MR-MS	
8	***Lr9***	TRANSFER/6*TC	RL6010	6BL		Transfer	*Aegilops umbellulata*	0; -;1	I	
9	***Lr10***	TC*6/EXCHANGE	RL6004	IAS		Exchange	*Triticum aestivum L.*	; -,2	R-MS	
10	***Lr11***	HUSSAR (W976)	RL6053	2A		Hussar	*Triticum aestivum L.*	2	MR	
11	***Lr12***	EXCHANGE/6*TC	RL6011	4A		Exchange	*Triticum aestivum L.*		;12-	Adult plant resistance
12	***Lr13***	MANITOU	MANITOU	2BS	*Ne2m, Lr23*	Frontana	*Triticum aestivum L.*		;1	Adult plant resistance
13	***Lr14a***	SELKIRK/6*TC	RL6013	7BL		Selkirk	*Yaroslav emmer*	X	MS	
14	***Lr14b***	TC*6/MARIA ESCOBAR	RL6006	7BL		Maria Escobar	*Triticum aestivum L.*	X	MS	
15	***Lr15***	TC*6/KENYA1483	RL6052	2DS	*Lr2, Sr6*	W1483	*Triticum aestivum L.*	;C	R	
16	***Lr16***	TC*6/EXCHANGE	RL6005	2BS	*Sr23*	Exchange	*Triticum aestivum L.*	,1 N	MS-MR	
17	***Lr17***	KLEIN LUCERO/6*TC	RL6008	2AS	*Lr37, Sr38, Yr17*	Klein Lucero	*Triticum aestivum L.*	;1+,0;	MR-MS	
18	***Lr18***	TC*7/AFRICA 43	RL6009	5BL		Africa 43	*T. timopheevi*	2+2-	MS	
19	***Lr19***	TC*7/TR	RL6040	7DL	*Sr25*	*Thinopyrum ponticum*	*Thinopyrum ponticum*	0;	R	
20	***Lr20***	THEW (W203)	THEW	7AL	*Pm1, S15, Sr22*	Timmo	*Triticum aestivum L.*	0;	R	
21	***Lr21***	TC*6/RL5406	RL6043	1DL		*T. tauschii*	*T. tauschii*	0,,12-	I	
22	***Lr22a***	TC*6/RL5404	RL6044	2DS	*Tg, W2*	*T. tauschii*	*T. tauschii*	-	MR	Adult plant resistance
23	***Lr22b***	THATCHER	Thatcher	2DS	*Tg, W2*	Thatcher	*Triticum aestivum L.*	-	R	Adult plant resistance
24	***Lr23***	LEE 310/6*TC	RL 6012	2BS	*Lr13, Sr9*	Gabo	*Durum wheat*	1;, 23	MR,MS	
25	***Lr24***	TC*6/AGENT	RL6064	3DL	*Sr24*	Agent	*Thinopyrum ponticum*	0;	R	
26	***Lr25***	TRANSEC (AWNED)	TRANSEC	4AB	*Pm7*	Transec	*Secale cereale*	;N	I	
27	***Lr26***	TC*6/ST-1-25	RL6078	1BL-1RS	*Sr31, Yr9*	St-1-25	*Secale cereale*	0;, ;1	MR	
28	***Lr10, Lr27+Lr31***	GATCHER (W3201)	GATCHER	3BS	*Sr2*	Gatcher	*Triticum aestivum L.*	X-	MR	*Complementary with Lr31*
29	***Lr28***	CS2D-2M	RL6079	4BL		C-77-1	*A. speltoides*	0;	I	
30	***Lr29***	TC*6/CS7AG#11	RL6080	7DS		CS7D-Ag#11	*Thinopyrum ponticum*	;1 N	R	
31	***Lr30***	TC*6/TERENZ10	RL6049	4BL		Terenzio	*Triticum aestivum L.*	123	R	
32	***Lr32***	TCLR32	RL5497-1	3D		*T. tauschii*	*T. tauschii*	;1+	MR	
33	***Lr33***	TC*6/PI58548	RL6057	1BL	*Lr26*	PI58458	*Triticum aestivum L.*	1	MR	
34	***Lr34***	TC*6/PI58548	RL6058	7D	*Yr18, Bdv1*	PI58548	*Triticum aestivum L.*	12C	MR-MS	
35	***Lr35***	RL5711	RL5711	2B	*Sr32?*	*A. speltoides*	*A. speltoides*	-	?	Adult plant resistance and linkage with Sr
36	***Lr36***	E84018	E84018	6BS		*A. speltoides*	*A. speltoides*	01N	?	
37	***Lr37***	TC*6/VPM	RL6081	2AS	*Sr38, Yr17*	VPM	*A. ventricosa*	12Y	I	
38	***Lr B***	TC*6//CARINA	RL6051			Brevit				
39	***Lr13***	WL711	WL711							
40	***Lr27+31***	BAVIACORA	BAVIACORA							

Table 3. Genes postulated in the 76 genotypes tested, against 12 different leaf rust (Puccinia triticina) pathotypes and and molecular markers. Line numbers are the same as in Table 1. Races tested are listed above the columns, the presence/absence of the Lr34 marker is indicated in the rightmost column. Postulated genes are based on comparison to differential lines

NO	MFB/SP	BBG/BN	CCJ/SP	CBJ/QB	CBJ/QQ	MBJ/SP	TBD/TM	MCJ/QM	MCJ/SP	TNM/JM	TCB/TD	LCJ/BN	Postulated Lr genes	
1	3+	3+	X	;1	3	3+	3+	3+	;1	3+	;	3	10,+	Lr34
2	3+	3+	3	3	3	3+	3+	3+	1P 3+	4	3+	3+	None	CSLV34
3	23C	3+	3	;1-	3	3+	22+	3	1P 3+	3+	;	3C3	10,+	Lr34
4	; 3+ 2p 4p	; X 1p 3p	3+	;1-	3+	3+	4	3+	2P 3+	X	;	3+	10,14a, +	
5	3C3	;	3+	;1	0; 3C3 2p4p	3+	3C3	12	4	;12	23C	;	3,10,23	Lr34
6	;	0;	23C	;1-	0;	1+	0;	;1	3	1 3 1p 1p	;1	1+	16,26	Lr34
7	;	0;	3+	12	;12	3+	12	;1 1+3C 1L 2L	4	;1-	;	3	17,23	Lr34
8	3+	;1	X+	X	X-	3	X+	3+	4	4	;12	X	27+31	
9	3+	X	3+	3	3+	miss	3+	3+	3C3	3+	3+	4	14a	Lr34
10	3	1X	3+	X	X 3+ 2p 4p	3+	3+	3+	4	3+	X 3	3+	27+31	
11	3+	;1-	;1-	;	;1-	;1	;	;1-	;1-	X	;	;1-	24,26	Lr34
12	3+	;1	;1-	;	;	;	;	;	;1	X	;1-	;1	24,26	NA
13	3	X	3+	;	3	3+	3	3	3+	3	;	X+	10,13	
14	23C	0;	12	1	0;	1+3C	;	3+	23C 3+ 6p3p	;12	3	3+	26,+	
15	;1-	;1-	3	;1-	1	3+	;	1	4	;1	;1-	4	17,23	
16	23C	0;	3	;1	0;	12	;	1	4	;12	;1-	1+	26,+	
17	3+	X-	3	;	;12	3+	3+	1+	4	3+	;	3	10,14a,23	
18	3	X	3	;	;12	3+	3+	1+	4	3+	;	3	10,14a,23	
19	;1-	;	3	;	X	3+	4	4	4	;1	0;	2+3C	10,17, +	
20	3+	1	3+	3+	3+	3+	4	4	4	4	4	X	13	
21	23C	3+4	3+	;	X+	3+	22+	4	4	3	0;	3+	10, +	Lr34
22	12	X	3+	23C	12	3+	22+	23C	4	12	22+	23C	+	Lr34
23	3+	0;	;	0;	0;	3+	3+	3+	4	12	3+	0;	1,3,+	
24	;	0;	0;	0;	0;	;1	;1-	;1-	;1	;1-	;	;1-	1,10,16	
25	22+	X+	3+	3+	12	3	2	3	4	2+3C	22+	22+	+	
26	12	0;	23C	;1	0;	12	0;	3+	3+	;1	2+3	2+3	26,+	
27	0;	0;	;	0;	0;	0;	0;	0;	;	;1-	0;	;1-	Res to all	Lr34
28	;1-	0;	23C	;1	0;	12	0;	23C	3+	;1-	;12	3	26,+	Lr34
29	12	0;	3+	;	0;	12	;	3+	4	;1	0;	3+	10,26,+	Lr34
30	23C	X	3+	23C	X	3+	3+	3+	4	3+	22+	12	13,+	
31	3C3	;	3+	12	3+	3+	3+	3+	3+	12	4	;	3,+	
32	3+	;1	3+	;	;	3+	3 ; 3p1p	;1-	4	3	4	X+	13,23	Lr34
33	3+	0;	0;	0;	0;	3+	22+	1	4	3C3	4	3+	1,23	
34	3+	X	3+	3+	3+	3+	4	4	4	3C3	4	;	13,+	
35	3C3	3	3	3	3+	3+	4	3+	3+	3+	3+	3	+	Lr34
36	;1-	;1	1+	;1-	;1-	1+	1	1	1+3C	;1	;1-	1	16	
37	23C	X+	3	3	3	3	3-3	3	3+	3C3	3	3	14a,+	Lr34
38	3+	0;	0;	0;	0;	3+	3	3+	3+	3+	0;	X	1,10,13	Lr34
39	23C	;1	3	3	23C	3+	23C	3C3	3+	3	3C3	22+	+	
40	1	1	3+	3	12	3	12	22+	3+	12	22+	12	+	
41	;	0;	0;	0;	0;	;12	0;	; 23C 3p3p	1+2	;1	;	1+	17,26,+	Lr34
42	23C	X	3C3	;1-	12	3	3-3	;1	3+	2+3	3+	3+	14a,23,+	Lr34
43	23C	;1	3	;1-	3	3	3	3	3+	3	0;	1+3C	10,13	
44	;	X	;1	;1-	;1	3+	3C3	;1-	X	23C	0;	3	+	Lr34
45	;1	0;	;	;1-	0;	;1	;	23C	;	12	12	;	+	Lr34
46	;	;1-	;	;	;	;12	;	;1	1	;	0;	;1-	Res to all	Lr34
47	3	X	3+	;1-	;1	3	3	;1	4	3+	4	X+	13,23	

Table 3 Contd.

48	3+	X	3+	;1-	;1-	3	3C3	;1	4	3+	3+	X	13,23	Lr34
49	12	3+	23	12	3	23C	12 23C 1L 2L	23C	33C	2- 3 1L 2L	12	3+	+	Lr34
50	2	0;	3	1	0;	12	;	3C3	4	X	3	3+	26, +(MFB)	
51	12	0;	0;	;	0;	3-3	23C	3+	3C3	12	0;	3	1,10, +	NA
52	2+3	;	;	;	;	;1-	;	;	;1-	1	;1-	;	24,26,+	
53	1	;	;	0;	0;	;	;	;	;1-	1+	0;	;	16,24	
54	;1-	;1-	1	1	;1-	1+	1	1	1+	1+	;1	1	16	
55	3	;1	;1	;1	;	3+	3+	3+	4	4	X	X+	+	
56	;1-	0;	0;	0;	0;	1	;	;1	1	1	1-	1-	1,16,26	
57	3+	0;	3	0;	0;	3+ 12 6p1p	0;	3+	3+	12	0;	X	10,13(hetero),26(hetero)	NA
58	;	0;	;	0;	0;	;	;	3C3	;1-	1+	0;	;	16,17,26	Lr34
59	23C	0;	0;	0;	;	3+	23C	23C	;12	X	23C	1	1,3+	
60	12	0;	3	;	0;	;1	0;	3+	4	;12	;	3+	10,26, +(MFB)	Lr34
61	3-3	;	3	3+	3	3	3	3+	3+	3	3+	0;	3	
62	;1-	;	3+	;	X	3	3+	1	4	;1	;	3+	10,17,23	
63	3C3	0;	0;	0;	0;	0;	0;	0;	;	X	0;	0;	24,+	
64	3	;	;1-	;	;	;1-	;	;1	;1-	X	;1-	;1-	24,26	
65	1	0;	0;	0;	0;	1	;	1	;	1	;	0;	Res to all	
66	3C3	1	23C	22+	23C	3+	3+	12	2+ 3+ 1L2L	12	3	0;	3,+	
67	23C	0;	;	;	23C	3+	22+	3	;	12	0;	0;	+	Lr34
68	;	;	;	0;	;	1+	;1	;1-	;	1	0;	0;	+	
69	;12	0;	23C	0;	0;	;1	0;	23C	3	;1-	0;	3	10,17,26,+	Lr34
70	;1	0;	3	12	0;	12	;	3	4	;1	2 3 3p3p	0;	3,26,+	
71	12	0;	3+	0;	0;	12	;	23C	4	12	0;	X+	10,13,26,+	Lr34
72	3	0;	0;	0;	0;	3+	22+	;1	4	1+3C	4	3C3	1,23,+	
73	23C	0;	3C3	1	0;	23C	;	3+	3C3	;1-	3+	0;	3,26	
74	;1	0;	2	;1-	0;	12	;	12	23C	;1-	3C3 1 5p2p	0;	3,17,26,+	
75	23C	X	3C3	;1-	;1	3	3-3	;1	3+	2+3	4	4	14a,23,+	Lr34
76	3+	3+	3+	3+	3+	3+	3	4	4	1+3C	3+	4	+	

Some other single and multigenic combinations, however, (Table 3) were identified in the genotypes: Lr1 (in 8 genotypes), Lr3a (7 genotypes), Lr10 (17 genotypes), Lr13 (12 genotypes), Lr14a (7 genotypes), Lr10 (1 genotype), Lr16 (7 genotypes), Lr17 (8 genotypes), Lr23 (12 genotypes), Lr24 (6 genotypes), Lr26 (3 genotypes), Lr27(2 genotypes), Lr31 (2 genotypes). Genotype 2 had high infection types against to all 12 races, and appeared to carry no resistance genes, while genotypes 27, 46 and 65 were resistant to all races. Full-sib genotypes, 67–68, with some unknown genes and 47 - 48 with Lr 13 and Lr 23 genes, had similar infection types to all pathotypes.

Genotype 24 had low infection to all races, and was postulated to carry Lr1, Lr16 and Lr26. The seven genotypes completely resistant to BBG/BN, LCJ/BN (phenotype of ;, 0 and 1) all carried Lr3 gene alone or in combination, as RL 6002. Genotype 5 had Lr 3 and Lr10

Table 4. Infection severity, infection type, and % AUDPC of 76 genotypes against to MBJ/SP, MCJ/SP pathotypes

No	Infection severity	Infection type	AUDPC %		Infection severity	Infection type	AUDPC %
				No			
6	0		0	**40**	10	MS	10
26	0		0	**5**	20	MSS	11
27	0		0	**4**	10	MR	11
44	0		0	**8**	20	MS	12
53	0		0	**3**	20	MS-MR	13
58	0		0	**17**	35	MS	13
59	0		0	**71**	20	MS	13
63	0		0	**43**	15	MR	14
64	0		0	**49**	15	MS	14
69	0		0	**1**	25	MS	16
52	0		0	**37**	40	MS-S	17
12	1	MS-MR	0	**25**	35	MS-S	17
11	1	MS-MR	0	**73**	25	MS	19
45	5	MR	1	**9**	30	MS-S	19
35	5	MS	1	**18**	50	MS	19
46	1	R	1	**66**	20	MS	20
24	10	MR	2	**42**	20	MS	21
61	10	MS	3	**38**	20	MS	21
30	10	MR	3	**29**	40	MS-S	21
14	10	MS	3	**23**	50	MS-S	21
41	15	MS	3	**36**	30	MS	25
74	10	MS	4	**10**	55	MS-S	25
47	10	S	4	**39**	35	MS	29
21	10	MR	4	**2**	40	MS	35
60	10	MR	4	**57**	70	S	47
54	10	MS-MR	4	**20**	85	S	48
56	5	MS	5	**7**	75	MS-S	50
32	15	MS	6	**31**	80	S	56
51	10	MR	6	**13**	70	MS-S	58
50	10	MS	6	**62**	80	S	67
68	10	MS-MR	7	**70**	85	S	69
28	10	MS-MR	7	**15**	80	S	74
48	20	MS-S	7	**72**	90	S	80
19	20	MS-MR	8	**55**	85	S	82
34	35	MS-S	8	**65**	90	S	95
16	10	MS	8	**33**	90	S	96
22	10	MS	9	**76**	100	S	100
					$LSD_{0.05}$		**7.24**

together, similar to RL6004; and most likely Lr23. Genotype 74, on the other hand, most likely had Lr16 in addition to Lr1 and Lr10.

Lr10 was either alone or in combination with Lr1, Lr13, Lr14a, Lr17, Lr23 and Lr26 in 17 genotypes (Table 3). RL6004 carried Lr10, and had higher infection type against all pathotypes except CBJ/QB and TCB/TD, CBJ/QB and TCB/TD. Genotypes 1, 3, and 21 had some unidentified genes besides Lr10 as they had low infection types against CBJ/QB, TCB/TD, and some other

Table 5. Grouping wheat genotypes by seedling infection type and field reaction severity against to leaf rust (Puccinia triticina) MBJ/SP and MCJ/SP pathotypes. The numbers presented show the number of lines in each category.

	Field reaction severity												
Seedling infection type	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
0;, ;	4	-	-	-	1	-	-	-	-	-	-	-	-
;1, 1+	4	9	1	-	-	1	-	-	-	-	-	-	-
2, 2+	1	5	1	-			-	-	-	-	-	-	-
X+, 2+3c, 3c, 3c3		1	-		2			-	-	-	-	-	-
3, 3+, 4		8	8	4	11	2	1	3	2	3	-	2	2

pathotypes. Genotypes 13, 38, 43, 57, and 71 with Lr10 and Lr13 had low infection for CBJ/QB, TCB/TD because of Lr10 and for BBG/BN and LCJ/BN because of Lr13.

Genotypes 4, 17, and 18 with (X) against BBG/BN were postulated to have Lr10 and Lr14a together. Genotypes 19, 62, and 69 carried Lr10 and Lr17 genes because of low infection types against MFB/SP, BBG/BN, and TNM/JM pathotypes, similar to RL6008.

Genotype 62 was resistant to CBJ/QB, CBJ/QQ, MCJ/SP, and TNM/JM pathotypes, and most likely carries Lr23 beside Lr10, because of the similarity to the RL6012 reaction.

Genotype 69 had Lr10 and Lr17 together with Lr26 because of low infection types against BBG/BN, CBJ/QB, CBJ/QQ, MBJ/SP, TBD/TM, and TNM/JM pathotypes, similar to RL6078. Genotypes 29 and 60, because of their reactions to all 12 leaf rust pathotypes, were assumed to carry some unknown genes besides Lr10 and Lr26.

Lr13 existed in 12 genotypes alone or in combinaton with Lr10, Lr23, and Lr26 (Table 2). Genotypes with Lr13 gene had low or medium (0; and X+) infection types when challenged with BBG/BN and LCJ/BN, similar to Manitou and WL711. Genotype 20, with low infection types against to all races except BBG/BN and LCJ/BN, was assumed to have Lr13 gene. Genotypes 30 and 34, with their medium infection types to some races, except BBG/BN and LCJ/BN, had some unknown additive genes besides Lr13. Genotypes 32, 47, and 48 carrying Lr13 and Lr23 in combination had low infection types against to BBG/BN, LCJ/BN, CBJ/QB, and MCJ/QM. Four genotypes with Lr13 and Lr10 showed low infection types to BBG/BN and LCJ/BN due to Lr13, and to CBJ/QB and TCB/TD because of Lr10.

Four genotypes had Lr14a alone or in combination as shown by low or medium infection types (;1 or X) against to BBG/BN pathotype, similar to RL6013. Genotype 4 had Lr14a and Lr10 because of its a virulence to BBG/BN, CBJ/QB, TCB/TB and some some other genes because of its reaction to TNM/JM.

Five genotypes had Lr16 alone or in combination. All races were avirulent against all genotypes with Lr16 (Table 2) similar to RL6005. Genotype 36, with (;1 or 1+) infection types, was assumed to have Lr16 alone. Genotypes 24 and 56, showed low infection types against BBG/BN, CCJ/SP, CBJ/QB, and CBJ/QQ had Lr16 and Lr1 together, similar to RL6003. Moreover, genotype 56 had Lr1 and Lr16 besides Lr26; genotype 53, Lr16 and Lr24; genotype 6, Lr16 and Lr26.

Eight genotypes had Lr17 in combination (Table 2). Genotypes with Lr17 had low infection types to MFB/SP, BBG/BN, TNM/JM, and TCB/TD as RL6088, but higher to other 8 pathotypes. McIntosh et al. (1995) reported that low infection types in Lr17 genotypes varied, 1, 2, X, X+3. Genotypes 7 and 15 had Lr17 and Lr23 in combination. These two genotypes had low infection types for CBJ/QB, CBJ/QQ, TBN/TM, and MCJ/QM probably because of Lr23. Genotype 91 had Lr17 and Lr26 in combination.

Ten genotypes with Lr23 most likely had known or unknown genes in combination. All ten genotypes with Lr23 had low infection types to CBJ/QB, CBJ/QQ, MCJ/QM, similar to RL6012 (Table 2). Some genotypes had Lr23 in various combinations with Lr1, Lr3, Lr10, Lr14a, Lr17, and Lr26.

Lr26 alone or in combination with Lr1, Lr3, Lr10, Lr13, Lr16, Lr17, Lr23, and some unknown genes existed in 14 genotypes (Table 3). All genotypes with Lr26 had low or medium infection types to BBG/BN, CBJ/QB, CBJ/QQ, MBJ/SP, TBD/TM, and TNM/JM, similar to RL6078. Genotypes 14, 16, 26, 28, and 50 had some unknown Lr genes as well.

Molecular Evaluations

Screening the 76 genotypes with the Lr34 marker revealed that this resistance gene existed in the following: 1, 5, 6, 7, 9, 11, 21, 22, 26, 27, 28, 29, 32, 35, 37, 38, 41, 42, 44, 45, 46, 48, 49, 58, 60, 67, 69, 71, and 74 (Table 3).

Field evaluations

Final disease ratings in the field and AUDPC% (of susceptible check, Sabalan) of genotypes, against to MBJ/SP and MCJ/SP pathotypes are presented in Table 4. The largest AUDPC and field disease rating was for 2240 and 100S infection for Sabalan.

Thirteen genotypes had 0 last field reading and 0% AUDPC%, 20 genotypes with 5 last field reading and 1-7% AUDPC and 9 genotypes 10 last field reading and 8 – 12% AUDPC. These were assumed resistant. 19

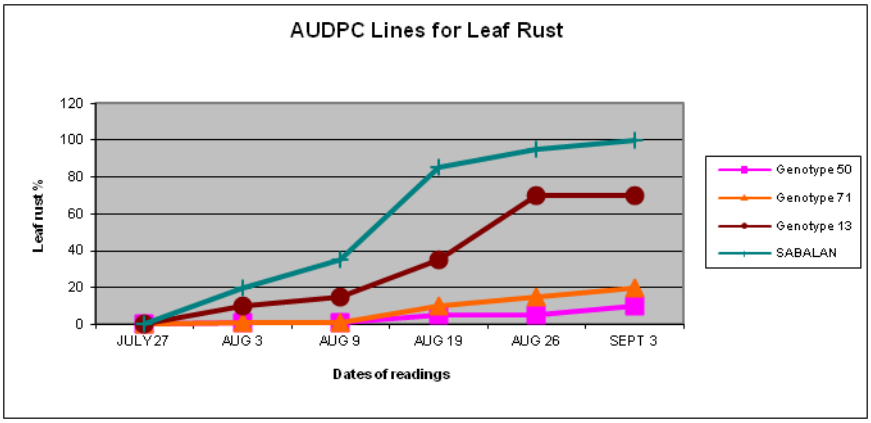

Figure 1. AUDPC lines of some IWWIP genotypes.

genotypes, with 15, 20, and 30 last field readings and 13 – 29% AUDPCs were moderately resistant. Six genotypes with, last field readings of 40, 50, and 60 and 35 – 58% AUDPC were moderately susceptible. Eight genotypes with more than 70 last field readings and 67 – 100% AUDPC were susceptible.

Slow rusting

Slow rusting or partial resistance (Caldwell 1968; Parlevliet 1975) is a type of durable resistance, where rust infection develops slowly on slow rusting plants, which do not develop disease due to a longer latent period or fewer/smaller uredinias (Singh et al. 1991; Kolmer 1996). Seventy six genotypes studied had various levels of infection types to both or one of MBJ/SP and MCJ/SP in the greenhouse or in the field (Table 5).

Four seedlings had low infection scores of around 0 field reaction, while and one seedling showed 20. Four seedlings with; 1, 1+ had 0 in the field, 9 did 5, 1 did 10, and 1 did 30. All genotypes in this group exhibited race-specific resistance. Similarly, one seedling 2, 2+ infection type 0 and 5 did 5, and 1 did 10 in the field. These were also race-specific resistant ones. One genotype with 0 and 2 genotypes with 20 infection types in the field had X+, 2+3c, 3c, and 3c3 in the seedling and were postulated to have also race-specific resistance genes. These 3 genotypes had the last reaction field reactions of 10MS - 30MSS and were considered slow rusted, since they revealed higher infection in the seedling but lower in the adult stage (Singh et al. 1991; Singh et al. 1998).

Seedlings with 3, 3+, and 4 susceptible reactions for any or both of MBJ/SP and MCJ/SP pathotypes had a field reaction of 5-10 percent infection. Those genotypes are predicted to carry race-specific adult plant resistance. Similarly, seedlings with high 3, 3+, and 4 infection type in the greenhouse had 20-35 5- 60 MR, MS or S field infection types.

The AUDPCs of most of genotypes studied here were smaller (Figure 1) than that of Sabalan. AUDPCs ranged between 0-2240 (Sabalan = 2240). Five genotypes had high susceptibility to leaf rust in the field. They had 80-100 AUDPC and 80-100S for the last leaf rust readings. They were postulated to have no adult plant resistance genes. Slow rusting was clearly indicated in some winter – facultative genotypes for leaf rust.

DISCUSSION

Resistance breeding with defined genes for slow rusting is a feasible way to overcome losses by leaf rust. This approach is also an environmental friendly one. The genes identified here in the genotypes and discussed below will serve for this purpose as well.

While Lr3ka, Lr16, Lr21, Lr29, Lr30, and Lr32 resistance genes were absent in winter-facultative genotypes, Lr1 (in 8 genotypes), Lr3a (7), Lr10 (17), Lr13 (12), Lr14a (7), Lr10 (1), Lr16 (7), Lr17 (8), Lr23 (12), Lr24 (6), Lr26 (3), Lr27 (2), Lr31 (2) were shown by differentials to be prevalent. Lr34 was also found to be present in many genotypes. Furthermore, a larger variation in genotypes for slow rusting existed. Some genes we identified here were similar to those in the USA, Mexico, China, and Japan, while some not (Singh 1992; Singh 1999; Kolmer 2003; Singh et al. 2001). Potentially novel sources of leaf rust resistance genes could be very useful in breeding future winter wheat cultivars (Singh 1992; Singh 1999; Kolmer 2003; Singh et al. 2001), therefore should continue to be investigated.

Leaf rust resistance genes must have originated from some old cultivars: Chinese Spring, Frondoso, Frontiera for Lr 13 (Caldwell et al. 1957; Roefs 1988), Knox (Caldwell et al. 1954) for Lr12 and Lr34, which were later most likely utilized as resistant parents to improve Atlas 66, Atlas 50, Coastal, and Coker 47 – 27 (Caldwell et al. 1957). Other sources of leaf rust resistance were

probably sought for resistance, some of which are most likely represented in this germplasm, as well as in cultivars of other regions. Incorporation of various genes with different genetic backgrounds assured, different types of resistance, the most preferred being the durable slow rusting type. Thirty four resistant cultivars identified in this study, with 0 – 7 AUDPC% and 20 final disease rating, indicated a very high level of resistance. That slow infection of wheat plants in slow rusting or partial resistance (Caldwell 1968; Parlevliet 1975), while it permits disease develop, but by limiting the loss due to leaf rust, assures a higher yielding, better quality crop, because of longer latent period or fewer - smaller uredinas (Singh et al. 1991; Kolmer 1996).

Our results indicated that 1) some resistance genes were effective, others not; 2) both seedling and/or field resistance existed in the cultivars; 3) slow rusting, determined by AUDPC% over the most susceptible cultivar, was clear and indicated a good genetic background in IWWIP; 5) searching and / or incorporation novel resistance genes from other sources identified here, into winter – facultative wheat genotypes might stop future leaf rust damages in winter wheat growing areas of the world, including Central Asia, West Asia, and North Africa, where IWWIP targets.

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