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Characterization of Slow Rusting Resistance against *Puccinia striiformis* f. sp. tritici in Candidate and Released Bread Wheat Cultivars of Pakistan

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Abstract

A set of 50 candidate and released bread wheat cultivars of Pakistan were studied to identify slow rusting resistance against yellow rust caused by *Puccinia striiformis* f. sp. tritici (PST). Seedling tests were done under glasshouse conditions at Thiverval-Grignon, INRA, France while slow rusting resistance was evaluated during 2005-07 in field plots using epidemiological variables at Peshawar, Pakistan. Twenty nine cultivars (58%) displayed susceptibility against the two PST races used in the seedling tests. Epidemiological parameters of resistance at the adult-plant stage were significantly ($P<0.01$) different for years, cultivars and cultivar x year interactions over three years. Analyses were done for sequence tagged site (STS) marker, csLV34 at INRA and revealed that 40% of the tested cultivars possessed adult plant resistance gene Yr18 linked allele of 150 bp. Of the 50 cultivars evaluated under field trials, 11 were highly resistant to both PST races as in the seedling tests and 39 showed different levels of slow rusting. Cultivars Bakhtawar-93, Punjab-96, Bahawalpur-95, V-00183 and V-00125 were relatively more stable over 3-years as Final Rust Severity (FRS), Area Under Disease Progress Curve (AUDPC) and Infection Rate (r) values were 74, 81, and 63% less of the susceptible control, Morocco, respectively. These cultivars also possessed the marker linked to Yr18 and could be exploited for the deployment of Yr18 in breeding for slow rusting in wheat. Both FRS and ACI are suitable parameters and can be used for phenotypic selection in a wheat breeding program for slow rusting resistance.

Keywords: csLV34; Leaf tip necrosis; Slow yellow rusting; Wheat; Yr18

Introduction

Of the total worldwide area planted to wheat (215 million ha), nearly half (44%) is in Asia where it is cultivated on 62 million ha in China, India and Pakistan [1]. However, food security and sustainability are the two core issues faced by most of the Asian countries. Historically, wheat rusts have been the most important biotic stresses responsible for unstable production in Asia and other parts of the world. Among the three rusts, greater yield reduction is brought about by yellow rust caused by *Puccinia striiformis* Westend. f. sp. tritici Eriks [2]. Yellow rust remains a major constraint in Asia, threatening 43 million ha of wheat. In Southeast Asia, Pakistan is second in terms of wheat area, but its national average yield is stagnant at around 2 t. ha-1 [3]. Diseases are one of the major production constraints in Pakistan, and 1% loss in production accounts for a loss of 36.6 million US dollars [4]. Yellow rust is a high profile, economically important disease capable of attacking 70% of the wheat area in the country [1] and has caused 13 epidemics [5,6]. Four major yellow rust epidemics were recorded in 1978, 1997-98 and 2005 and caused respective losses of US$244 million, $33 million and $100 million to the Pakistan economy [5].

Although several effective fungicides are available for yellow rust control [7], their use increases production cost and are not considered environment friendly [1]. Growing cultivars with adequate levels of genetic resistance remains the most efficient and environmentally-safe means of controlling yellow rust. It is achieved by deployment of cultivars, with race specific/or race-nonspecific resistance. Race-specific resistance genes provide highly effective protection against the disease depending on a specific recognition event between the host and the pathogen in accordance with gene-for-gene interaction [8] but are not considered durable [9]. Conversely, race-nonspecific resistance in wheat, also referred as slow rusting [10], adult plant resistance and (or) partial resistance [11], is mainly polygenic and more durable [12].

Characteristics of race non-specific resistance in the wheat-Puccinia system include a non-hypersensitive and (or) partial resistance response with variable disease severity levels under field conditions across locations and years [13]. A more durable resistance [14] to rusts involves slow rusting [10], a form of partial resistance in which host genotypes retard or delay rust development [15,16] by various means [17]. Cultivars possessing slow rusting resistance display higher infection type at seedling growth stage [18] and is quantitatively inherited [19,20]. In many cereal-rust pathosystems, quantitative aspects of cultivar resistance have been investigated by disease severity at a certain crop development stage, the area under the disease progress curve (AUDPC) or by apparent infection rate 'r' and average coefficient of infection (ACI) values for adult plant resistance (APR) [11,21-24].

Gene designations and genomic locations of three slow rusting loci have been reported previously [25]. The Lr34/Yr18 complex on chromosome 7DS [26] and the Lr46/Yr29 complex on 1BL [27], express resistance to both leaf (brown) (P. triticina) and yellow rusts.

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The Lr34/Yr18 locus, is of high value due to its contribution towards durable resistance against two rust pathogens [28]. This gene pair also co-segregates with other traits such as leaf tip necrosis, powdery mildew [Blumeria graminis (DC.) E.O. Speer f. sp. tritici Em. Marchal] resistance (Pm 8), and tolerance to Barley yellow dwarf virus (Bdv1) [29-33]. Due to the multiple disease resistance imparted by the Yr18/Lr34 locus, it has become one of the most valuable genic regions for disease resistance in wheat. However, information on slow rusting resistance conferred by Yr18/Lr34 complex in Pakistani wheat is not available. Therefore, this study was conducted to characterize a comprehensive set of 50 Pakistani candidate and released bread wheat cultivars for slow rusting resistance at both phenotypic and genotypic levels and to test the efficiency of different epidemiological parameters in selecting slow rusting genotypes.

Materials and Methods

Plant material

The host material of the current study included a comprehensive set of 17 candidate and 33 released cultivars (hereafter called cultivars) from Pakistan along with two controls i.e. Morocco (field tests) and Jupateco-73 (molecular analyses) presented with their pedigrees in Table 1. Seed of Morocco and other varieties were obtained from the National wheat improvement program, Islamabad, while those of yellow rust near isogenic lines along with Jupateco-73 (Resistant) were provided by Dr. Colin Wellings of Plant Breeding Institute, University of Sydney, Australia.

Seedling tests

Seedling tests were carried out at Thiverval-Grignon, INRA, France. Sets containing the 50 cultivars were sown in standard peat soil in 7 7 cm plastic pots at 12-15 seeds/cultivar. At 12-14 days after sowing, each set was inoculated separately with two PST races i.e. 70E16-v27 and 70E0-v27, predominantly present in the region where field experiments were performed [34] using a fresh spore suspension in mineral oil (Soltrol 170). The plants were incubated in a dew chamber at 100% R H. at 10-12°C for 24 h. Plants were then maintained in controlled growth chambers with a day/night rhythm of 16/8 h, a light intensity of circa 190 µEs-I m-2 (approx. 14 .000 Lux) and a temperature of 18.5°C/15°C. Infection types were recorded 15 days post inoculation using a 0-9 scale [35]. Infection types were classified as Resistant (IT=0-3), Intermediate (IT=4-6) and Susceptible (IT=7- 9).

Field tests at adult plant stages

Field tests were carried out at NIFA Research Farm Peshawar during 2005-07(Y1-Y3) seasons in Pakistan. The same 50 wheat cultivars used in the seedling tests were grown along with the susceptible control, Morocco [6]. Cultivars were grown in four-row plots, each 3 m long with 30 cm between rows. The experiment was arranged in randomized complete block design with three replications. A mixture of Morocco and a yellow rust susceptible local land race [36] was sown around the trial site to facilitate a rust epidemic. In an adjacent plot, a set of Avocet isolines [37] each with one of the resistance genes Yr1, Yr5, Yr6, Yr7, Yr8, Yr9, Yr10, Yr15, Yr17, Yr18, Yr24, Yr26, Yr27, Yr32, YrSp, Jupateco R (Yr18), Jupateco S, Avocet R (YrA) and Avocet S were also raised for three years to provide virulence information for the PST races prevalent in the test (i.e. natural and inoculated). Inoculum of the two PST races used in the seedling tests were inoculated in the field tests by following the procedure described by Roelfs et al. [38] and Khanna et al. [39]. Both spreader lines and test cultivars were inoculated each year uniformly at heading stage in the evening by spraying with a suspension of 100 mg spores (50 mg of each race) per 1-liter sterile distilled water with 2-3 drops of Tween 20 using an ultra-low volume turbo-air sprayer.

Rust severity and leaf tip necrosis were recorded on flag leaves after flowering when severity on cv. Morocco reached at least 50%. Severity estimates were based on modified Cobb's Scale [40], while host response to infection was scored according to [41,43] and converted to a Coefficient of Infection as described by [24]. Infection rate 'r' was estimated for the whole epidemic period following the 50% severity rating on Morocco (Vanderplank, 1968). The AUDPC was calculated using the equation adopted by [43] based on 3-4 severity ratings with 7-10-day intervals. The presence or absence of leaf tip necrosis on flag leaves was recorded thrice each year at the same time as rust severity and host response [44]. Relative values of FRS, AUDPC and 'r' for each variety were then analyzed by considering values of cv. Morocco as 100%.

Molecular analyses for Yr18

Seeds of the 50 wheat cultivars along with positive control, cv. Jupateco-73 (Resistant) were sprouted in pre-labeled Petri dishes. DNA was isolated from one week old seedlings as described by Imtiaz et al., [43]. Briefly, leaf tissue was freeze dried, homogenized to a fine powder, allowed to thaw, and suspended in 1 ml of CTAB DNA isolation buffer. After thorough mixing, 1 ml chloroform/isoamylalcohol was added, and samples were kept on a shaker for 20 min. The samples were then centrifuged for 20 min, and the top aqueous layer of each sample was transferred to a new Eppendorf tube. One ml isopropanol was added to precipitate the DNA which was then suspended in TE buffer. PCR amplification was performed in 10 µl reaction using csLV34F5'GTTG GTTAAGACTGGTGATGG3' and csLV34R5'TGCTTGCTATTGC TGAATAGT3' primers (Lagudah et al., 2006). The reaction mixture contained 1.0 µl (10 picomol) each of reverse and forward primers, 1.0-µl (2 mM) dNTPs, 1.0 µl 10X PCR buffer, 0.1-µl (5 unit/ µl) Taq-polymerase, 2.0 µl DNA (60-70 ng template DNA) and 4.9 µl d-H_2O. PCR was performed in an Eppendorf Mastercycler® Gradient, at 94°C for 3 min, followed by 45 cycles of 15 sec at 94°C, 15 sec at 58°C, 15 sec at 72°C, and a final extension step of 10 min at 72°C. PCR products were resolved by electrophoresis in 1.2% agarose gel and visualized under UV light following staining with ethidium bromide (500 µl/l).

Statistical analyses

Phenotypic data of disease parameters over three years were statistically analyzed using combined analyses of variance appropriate for randomized complete block design using statistical software R. Genotypic means were compared using Tukey's honestly significant difference (HSD) test. The stability structures of four disease parameters over three years were analyzed individually through Principal Component Analysis (PCA) on cultivar by environment (year) interaction based on additive main effects and multiplicative interaction effects (AMMI) model using the revised version of Gest software [45,46].

Results

Results of seedling and adult plant tests of 50 cultivars are presented in Tables 1 and 2 respectively. Variability in seedling or all stage resistance was observed where more than half of the cultivars were found susceptible to both races used in seedling test. In field trials, highly significant ($P<0.01$) differences were found for cultivars, years and cultivar-year interactions for FRS, AUDPC, r and final ACI. FRS of susceptible control, Morocco was 54-81% (Table 2), indicating that an

Code	Cultivars	Pedigree	PST races		LTN	csLV34 marker
			70E16v27	70E0v27		
Group 1 (Seedling susceptible)						
1	Pirsabak-91	KVZ//CNO/CHR/3/0N/4/KAL/BB	S	S	3	Absent
2	Tandojam-83	TZPP/PL/7C	S	S	4	Absent
3	Pak-81	KVZ/BUHO/S//KAL/BB	S	S	4	Absent
4	WL-711	S308/CHRIS//KAL	S	S	3	Absent
5	Kohistan-97	V-1562//CHRC 'S'/HORK/3/KUFRA/4/CARP 'S'/BJY 'S'	S	S	3	Absent
6	Sind-81	NORTENO/MEXIPAK	S	S	7	Present
7	Maxi-Pak	KVZ/BUHO//KAL/BB	S	S	3	Absent
8	Sarsabz	PITIC-62/FROND//MEXIPAK/3/PITIC-62/MAZOE-79-75-76	S	S	Absent	Absent
9	V-01180	PB96/V87094/MH97	S	S	6	Present
10	Sariab-92	BB/GLL//CARP/3/PVN	S	S	4	Absent
11	Zargoon-79	CC/1N1A/3/TOB/CFN//BB/4/7C	S	S	3	Absent
12	Kaghan-93	TTR/JUN	S	S	Absent	Absent
13	Zardana-89	CNO 'S'67-8156 XTOB-66-CNO 67//NOV-66/11-12300/LR6408156-PVN-76	S	S	4	Absent
14	Nowshehra-96	?	S	S	4	Absent
15	V-00183	LU 26/HD2179//*2 INQ 91	S	S	6	Present
16	Faisalabad-83	FURY//KALYANSONA/BLUEBIRD	S	S	6	Present
17	Punjab-96	SA42*2/4CC/INIA//BB/3/INIA/HD832	S	S	6	Present
18	99B4012	PTS/3/TOB/LEN//BB/HD832-5//ON/5/G.V.ALD 'S'/HPO 'S'	S	S	3	Absent
19	V-99022	INQ 91/3/PB81//F3.71/TRM	S	S	7	Present
20	99B2237	SPARROW/INIA/V-7394/WL 711/3/BAU 'S'	S	S	4	Absent
21	Bakhtawar-93	JUP/BJY 'S'//URES	S	S	7	Present
22	91BT010-84	?	S	S	3	Absent
23	Wafaq-2001	OPATA/RAYON//KAUZ	S	S	3	Absent
24	93T347	TTR'S'/SKA//WL711/3/CHI'S'	S	S	7	Present
25	CT-00231	SNI/TRAP#1/BAV 92	S	S	7	Present
26	99B2278	PND88//BB 'S'/TOB66	S	S	7	Present
27	Parwaz-94	V.5648/PRL	S	S	3	Absent
28	7_03	CHAM 4//URES/BOW 'S'	S	S	3	Absent
29	Kohsar-95	PSN/BOW	S	S	Absent	Present
Group 2 (Seedling resistant)						
30	Kohinoor-83	ORE-FI-158/FUNDULEA//MXFN(SIB)/2*TIBA/3/COCORAQUE-75	R	R	6	Present
31	SN-122	KAUS/TRAP//KAUZ	R	R	3	Absent
32	Rohtas-90	1N1A66/AG.D1//1N166/3/GEN81	R	R	3	Absent
33	V-00125	BUBLBUL//F3.71/TRM/3/CROW	R	R	6	Present
34	Bahawalpur-95	AVRORA/UP-301//GALLO/SUPER-X/3/(SIB)PEWEE/4/ (SIB)MAYA-74//PEWEE MAIPO(SIB)/	R	R	7	Present
35	Diamond	CHIL/2*PASTOR	R	R	7	Present
36	Soughat-90	?	R	R	Absent	Absent
37	Suleman-96	F6.74/BUN//SIS/3/VEE#7	R	R	7	Present
38	SARC-5	?	R	R	6	Present
39	Faisalabad-85	MAYA/MON//KVZ/TRM	R	R	4	Present
40	SD-66	CHIL/ALD//PVN/YECOORA 70	R	R	2	Absent
Group 3 (Seedling resistant/susceptible)						
41	Kirin-95	?	R	S	3	Absent
42	Shaheen-94	?	R	S	Absent	Absent
43	Inqilab-91	WL711/CROW 'S'	R	S	3	Absent
44	Pasban-90	1N1A/A.DISTT//1N1A/3/GEN81	H	R	4	Absent
45	Blue Silver	1154 -388/NA/3/YT54/NIOB/LR64	R	S	3	Absent
46	Watan-94	Lu26/HD2179	R	S	Absent	Absent
47	Chakwal-86	FLN/ACC//ANA75	I	S	3	Absent
48	Shahkar-95	WL711//F3.71/TRM	R(H)	S(H)	Absent	Present
49	RWM-9313	VEE # 5 'S'/SARA//SOGHAT 90	S	R	8	Present
50	NRDW-1	CHAM-3//2*CHEN/ALTAR-84	I	R	4	Absent
51	Jupateco-R(Control)	II-12300//LERMA-ROJO-64/II-8156/3/NORTENO-67	-	-	7	Present
52	Morocco (Control)		S	S	Absent	Absent

LTN: leaf tip necrosis

Table 1: Code, pedigree, seedling resistance, leaf tip necrosis (LTN) frequency and Yr18 data of tested cultivars.

Code	Cultivars	FRS (%)				AUDPC				r				ACI (%)			
		Y1	Y2	Y3	Mean	Y1	Y2	Y3	Mean	Y1	Y2	Y3	Mean	Y1	Y2	Y3	Mean
Group 1 (seedling susceptible)																	
1	Pirsabak-91	57	87	63	69 (98)	642	1807	753	1067 (75)	0.07	0.07	0.22	0.12 (63)	41	43	37	40
2	Tandojam-83	80	73	67	73 (104)	922	1272	771	988 (69)	0.10	0.12	0.19	0.14 (74)	53	47	33	44
3	Pak-81	67	87	60	71 (101)	828	1538	478	948 (66)	0.03	0.18	0.17	0.13 (68)	33	52	30	38
4	WL-711	80	73	57	70 (99)	922	1283	551	919 (64)	0.10	0.10	0.21	0.14 (74)	53	55	32	47
5	Kohistan-97	80	73	50	68 (97)	922	1168	655	915 (64)	0.10	0.13	0.09	0.11 (58)	34	37	25	32
6	Sind-81	67	67	32	55 (78)	793	1397	356	849 (59)	0.06	0.05	0.12	0.08 (42)	33	27	16	25
7	Maxi-Pak	60	70	47	59 (84)	653	1365	500	839 (59)	0.06	0.08	0.18	0.11 (58)	36	35	23	31
8	Sarsabz	60	70	33	54 (77)	595	1228	524	782 (55)	0.10	0.12	0.05	0.09 (47)	30	43	17	30
9	V-01180	33	44	42	40 (57)	624	1308	321	751 (53)	0.05	0.08	0.05	0.06 (32)	25	33	15	24
10	Sariab-92	60	70	15	48 (68)	700	1071	270	680 (48)	0.06	0.11	0.08	0.08 (42)	19	35	5	20
11	Zargoon	30	70	27	42 (60)	233	1378	317	643 (45)	0.09	0.07	0.10	0.09 (47)	13	35	13	20
12	Kaghan-93	70	20	18	36 (51)	805	840	187	611 (43)	0.07	0.15	0.06	0.10 (53)	24	23	12	20
13	Zardana-89	50	47	22	39 (55)	490	888	379	586 (41)	0.11	0.07	0.16	0.12 (63)	31	20	11	21
14	Nowshehra-96	40	60	27	42 (60)	389	1080	268	579 (41)	0.13	0.11	0.05	0.10 (47)	15	30	11	19
15	V-00183	37	45	39	40 (57)	502	840	388	576 (40)	0.11	0.08	0.08	0.09 (47)	33	27	13	24
16	Faisalabad-83	77	43	10	43 (61)*	863	605	209	559 (39)*	0.11	0.13	0.08	0.10 (47)	32	22	4	19
17	Punjab-96	53	47	23	41 (58)	560	720	398	559 (39)	0.08	0.13	0.03	0.08 (42)	27	23	6	19
18	99B4012	30	35	36	34 (48)	169	1163	247	526 (37)	0.09	0.13	0.09	0.10 (47)	9	35	13	19
19	V-99022	39	45	37	40 (57)	677	429	398	501 (35)	0.09	0.09	0.05	0.08 (42)	32	21	10	21
20	99B2237	48	58	54	54 (77)	688	574	88	450 (32)	0.05	0.13	0.00	0.06 (32)	28	22	2	17
21	Bakhtawar-93	37	37	5	26 (37)	385	585	67	346 (24)	0.06	0.12	0.08	0.09 (47)	18	13	2	11
22	91BT010-84	36	44	39	40 (57)	455	361	101	306 (21)	0.08	0.08	0.07	0.08 (42)	18	9	7	11
23	Wafaq-2001	33	43	40	39 (55)	548	208	43	267 (19)	0.06	0.09	0.00	0.05 (26)	22	8	0	10
24	93T347	34	39	36	36 (51)	455	214	24	231 (16)	0.08	0.11	0.00	0.06 (32)	22	7	0	9
25	CT-00231	34	41	41	39 (55)	327	116	17	153 (11)	0.02	0.07	0.03	0.04 (21)	20	5	0	8
26	99B2278	33	37	36	35 (50)	0	340	11	117 (8)	0.00	0.10	0.00	0.03 (16)	0	13	0	4
27	Parwaz-94	20	33	2	18 (26)	93	227	17	112 (8)	0.12	0.13	0.03	0.09 (47)	5	8	0	5
28	7_03	34	44	39	39 (55)	152	150	11	104 (7)	0.02	0.04	0.00	0.02 (11)	5	5	0	3
29	Kohsar-93	0	30	3	11 (16)	0	23	33	19 (1)	0.00	0.03	0.04	0.03 (16)	0	1	8	3
Group 2 (seedling resistant)																	
30	Kohinoor-83	47	73	33	51 (72)	560	1440	313	771 (54)	0.04	0.09	0.15	0.09 (47)	27	37	11	25
31	SN-122	29	45	43	39 (55)	368	983	418	590 (41)	0.07	0.18	0.13	0.12 (63)	15	35	8	19
32	Rohtas-90	60	40	23	41 (58)	572	663	453	563 (39)	0.12	0.12	0.13	0.12 (63)	30	18	12	20
33	V-00125	36	40	37	38 (54)	607	901	218	575 (40)	0.08	0.11	0.11	0.10 (47)	24	28	11	21
34	Bahawalpur-95	27	23	3	18 (26)	193	582	38	271 (19)	0.09	0.08	0.03	0.07 (37)	12	10	6	9
35	Diamond	39	50	42	44 (62)	443	316	51	270 (19)	0.10	0.09	0.01	0.06 (32)	17	12	0	10
36	Soughat	15	17	8	13 (18)	298	238	122	219 (15)	0.14	0.10	0.05	0.10 (53)	5	8	1	5
37	Suleman-96	40	17	0	19 (27)	362	252	12	208 (15)	0.10	0.07	0.04	0.07 (37)	18	4	0	7
38	SARC-5	39	47	43	43 (61)	123	318	173	204 (14)	0.11	0.11	0.05	0.09 (47)	5	11	5	7
39	Faisalabad-85	30	17	5	17 (24)	280	220	67	189 (13)	0.10	0.09	0.04	0.08 (42)	15	8	3	9
40	SD-66	34	43	42	40 (57)	0	220	83	101 (7)	0.00	0.09	0.09	0.06 (32)	0	8	1	3
Group 3 (Seedling resistant/susceptible)																	
41	Kirin-95	63	83	53	67 (95)	642	1378	654	891 (62)	0.09	0.12	0.15	0.12 (63)	28	53	27	36
42	Shaheen-94	53	67	32	51 (72)	537	1061	650	749 (52)	0.08	0.10	0.06	0.08 (42)	18	28	16	21
43	Inqilab-91	60	67	33	53 (75)	624	1223	335	727 (51)	0.12	0.08	0.12	0.11 (58)	21	36	17	24
44	Pasban	47	60	30	46 (65)	467	1225	312	668 (47)	0.09	0.05	0.12	0.09 (47)	23	30	13	22
45	Blue Silver	57	53	40	50 (71)	566	873	423	621 (43)	0.10	0.08	0.17	0.12 (63)	23	27	20	23
46	Watan-94	67	20	3	30 (43)	712	333	66	370 (26)	0.16	0.07	0.04	0.09 (47)	38	8	0	15
47	Chakwal-86	20	33	7	20 (28)	268	383	175	276 (19)	0.01	0.16	0.07	0.08 (42)	18	17	2	12
48	Shahkar-95	57	12	3	24 (34)	531	84	39	218 (15)	0.14	0.11	0.03	0.09 (47)	28	5	3	12
49	RWM-9313	34	42	37	38 (54)	280	172	22	158 (11)	0.30	0.12	0.00	0.14 (74)	20	14	0	11
50	NRDW-1	40	51	46	46 (65)	280	8	11	100 (7)	0.08	0.03	0.00	0.04 (21)	14	0	0	5
51	Morocco (Susceptible Check)	81	77	54	70 (100)	1256	1933	844	1344 (100)	0,28	0,14	0,14	0.19 (100)	100	97	49	82
Year Means	46.16	49.2	31.7		497	764.4	272		0.08	0.09	0.07		23.72	24.07	10.78		

LSD for years (P<0.01) 3.4 1.4 0.01 0.27
LSD for varieties (P< 0.01) 9.8 4.0 0.04 0.78
LSD for interaction (P<0.01) 24.0 9.7 0.09 1.92

*=Values in the parenthesis are relative values of the susceptible control-Morocco for each variable

Table 2: Final rust severity (FRS), area under disease progress curve (AUDPC), mean infection rate (r) and average coefficients of infection for three years (i.e. Y1–Y3) of 51 bread wheat varieties along with susceptible control following infection *Puccinia striiformis f. sp. tritici*

acceptable epidemic pressure was established over the three-year field experiment. Despite inoculation, rust development was influenced by the yearly weather pattern. Mean rust severity during 2004-05, 2005-06 and 2006-07 was 46%, 49% and 31% respectively. No significant difference was observed in the mean rust severity of 2004-05 and 2005-06. The final rust severities among the 50 cultivars varied between 0-87% (Table 2). Across years, correlations among the four parameters were positive (0.80 to 0.98) and highly significant (P<0.01). Cultivars were classified into three groups on the basis of seedling response to the two PST races (Table 1). Group 1 was susceptible to both races, group 2 was resistant to both races and group 3 had variable responses to the two at seedling stage.

Group 1

Twenty nine (58%) cultivars (code 1 to 29) were susceptible at the seedling stage to both races (Table 1) and expressed different levels of slow rusting in field tests. Cultivars from this group had 16-104%, 1-75% and 16-74% values of FRS, AUDPC and r respectively when compared with the susceptible control Morocco. Over year values of FRS were statistically similar in five (code 1 to 5), three (code 6 to 8), nine (code 9 to 17) and five (code 22 to 26) cultivars of group 1. The least FRS of 11% was recorded in cultivar with code 29 which was followed by code 27 (18%) and code 21 (26%) respectively. The pattern of AUDPC over years was not consistent with FRS among group 1 cultivars. The highest and lowest mean AUDPC values of 1067 and 19 were estimated for code 1 and code 29 cultivars respectively. Both code 16 and 17 cultivars had the same AUDPC value of 559. Five varieties (code 9, 15, 18, 19, and 20) had AUDPC values of 751, 576, 526, 501 and 450 respectively. AUDPC values < 350 were estimated for code 21 to 28 varieties. Maximum value of r (0.14) was estimated for both code 2 and 4 cultivars which was statistically non-significant from five cultivars belonging to code 1, 3, 5, 7 and 13. No significant difference was observed in r values of sixteen cultivars belonging to code 6, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, and 24. The lowest values of r were recorded in four cultivars (code 25, 26, 28 and 29) which were statistically non-significant among themselves. The ACI values of group 1 cultivars over years varied between 3-47 (Table 2).

Nine replications (3 each year) were used to evaluate the presence or absence of LTN symptoms (Table 1). Ten cultivars in this group expressed LTN in six or more of the replications and were therefore considered positive for LTN while in the remaining cultivars it was less frequent and was not observed in three cultivars. Molecular analyses with marker csLV34 amplified two alleles one of 150 bp size tightly linked with resistance gene Yr18 and another 230 bp size, not associated with resistance (Figure 1). Ten cultivars with codes 6, 9, 15, 16, 17, 19, 21, 24, 25 and 26 carried marker allele of 150 bp indicative of the presence of Yr18 in these cultivars. Only code 29 cultivar did not express LTN, but it did have the marker for Yr18. AMMI analysis extracted values of the genotype x year interaction for principal component axis scores (PC-1 and PC-2) along with their % contribution in respect of the varieties under test over three years are graphically presented in four biplots (Figures 2a-2d). Group 1 varieties (i.e. code 1 to 29) for the four parameters in AMMI biplots indicated their sensitivity to the environmental interaction due to their occurrence away from the center of the biplot over three years except (code-5, 15, 17 and 21) which demonstrated an overall relative stability pattern due to their position near the origin.

Group 2

Eleven cultivars (22%) with code 30 to 40 of group 2 were resistant

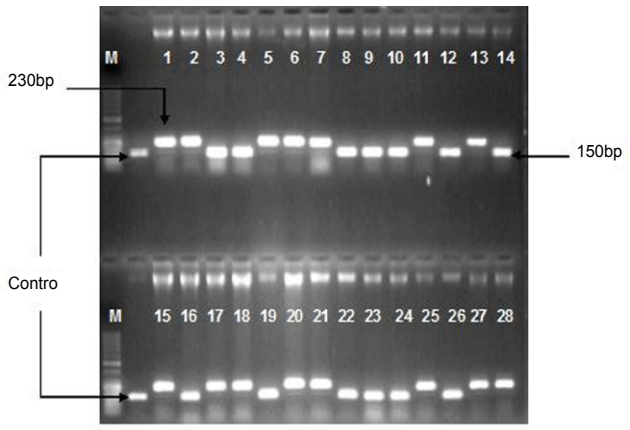

Figure 1: csLV34 PCR amplification products of 28 varieties along with positive control; resistant varieties 3. Bahawalpur-95; 4. Suleman-96; 8. Kohsar-93; 9. Punjab-96; 10. Shahkar-95; 12. Faisalabad-85; 14. Bakhtawar-93; 16 Sind-81; 19 CT-00231; 22 V-01180; 23 RWM-9313; 24 SARC-5; 26 93T347, susceptible varieties 1. Shaheen-94; 2. Zardana-89; 5. Kaghan-93; 6. Rohtas-90; 7. Inqilab-91; 11. Watan-94; 13. Soughat-90; 15 Pasban-90; 17 Kohistan-97; 18 SD-66; 20.Nowshara-96; 21.SN-122; 25.7_3; 27.91BT010-84; 28.99B2237. M stands for size standard.

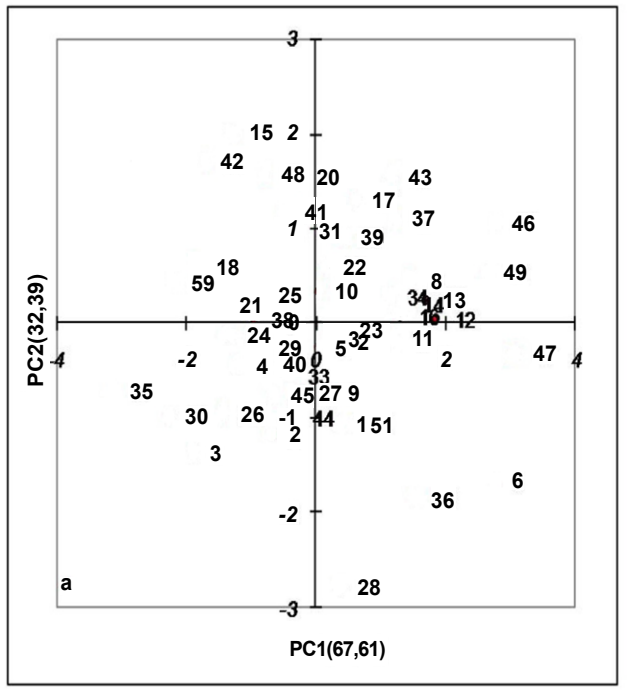

Figure 2a: Additive main effects and multiplicative interaction (AMMI) biplot showing relative stability of fifty varieties (code 1 to code 50) along with susceptible control (51) over three years for final rust severity (FRS) with respective PC1 and PC2 scores.

at seedling stage to both PST races (Table 1) while in field tests, FRS, AUDPC and r values of these cultivars were 28-72%, 7-54% and 32-63% less than the susceptible control Morocco (Table 2). The highest mean FRS over year was recorded in cultivar possessing code 30 (51%), followed by code 35 (44%), code 38 (43%), code 32 (41%), code 20 (40%), code 31 (39%) and code 33 (38%) which were statistically non-significant among themselves. Statistically similar albeit lowest

mean FRS was recorded in four cultivars having code 34, 36, 37 and 39. Cultivar with code 30 had the highest mean AUDPC value of 771 over year which was followed by code 31 and 33 cultivars respectively. AUDPC values <300 were recorded for the remaining seven cultivars. The highest r value of 0.12 was recorded for each of the code 31 and 32 cultivars which were statistically non-significant from both code 33 and 36 cultivars. Lowest r value of 0.06 was recorded for code 35 and 40 cultivars which was statistically similar with the remaining cultivars of this group (Table 2). Mean ACI values of group 2 cultivars over year fluctuated between 3-25 (Table 2). LTN expression was observed 6-7 times in six cultivars of group 2 while it was less frequently displayed by code 39 cultivar on four occasions and all these seven cultivars carried Yr18 associated allele of 150 bp (Table 1 and Figure 1) which was absent in the remaining cultivars. Group 2 cultivars coded 33, and 34 (V-00125 and Bahawalpur-95) were stable over three years for all the parameters due to their closeness to the origin in the AMMI biplots while the remaining cultivars coded 30, 31, 32, 35-40 were relatively unstable (Figures 2a-2d).

Group 3

Of the 50 cultivars, ten (20%) were resistant, susceptible, intermediate or heterogeneous in response to one of the two PST races (Table 1). FRS, AUDPC and r values of group 3 cultivars were 5-72%, 38-93%, and 37-79% less than the susceptible control Morocco (Table 2). Maximum and minimum FRS of 67% and 20% was observed in code 41 and 47 cultivars respectively. Non-significant differences in the FRS were observed in five cultivars having code 42, 43, 44, 45 and 50 while all these had around 30% less disease than cv. Morocco. Cultivar with code 50 had the least AUDPC value of 100 while its maximum value reached 891 in code 41 cultivar which was followed by code 42 (749), code 43 (727), code 44 (668), code 45 (621), code 46 (370), code 47 (276), code 48 (218) and code 49 cultivars. The highest and lowest r value of 0.14 and 0.04 were recorded for code 49 and code 50 cultivars respectively. Non-significant difference of r values were recorded for six cultivars belonging to code 41, 43, 44, 45, 46 and 48 which had 40-50% less r values than the susceptible control Morocco (Table 2).

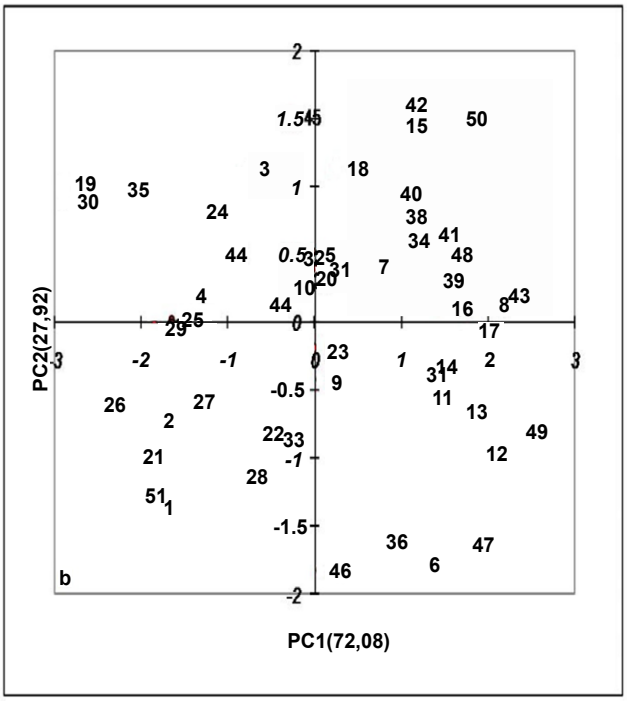

Figure 2b: Additive main effects and multiplicative interaction (AMMI) biplot showing relative stability of fifty varieties (code 1 to code 50) along with susceptible control (51) over three years for Area Under Disease Progress Curve (AUDPC) with respective PC1 and PC2 scores.

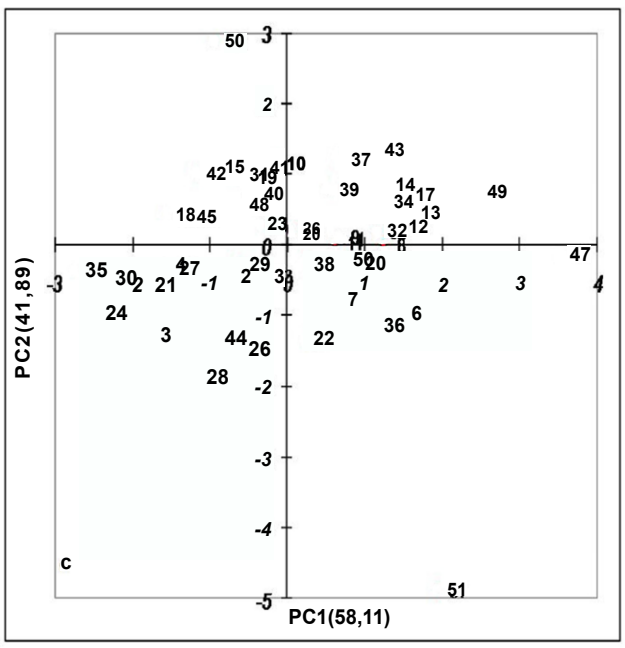

Figure 2c: Additive main effects and multiplicative interaction (AMMI) biplot showing relative stability of fifty varieties (code 1 to code 50) along with susceptible control (51) over three years for Average Coefficient of Infection (ACI) with respective PC1 and PC2 scores.

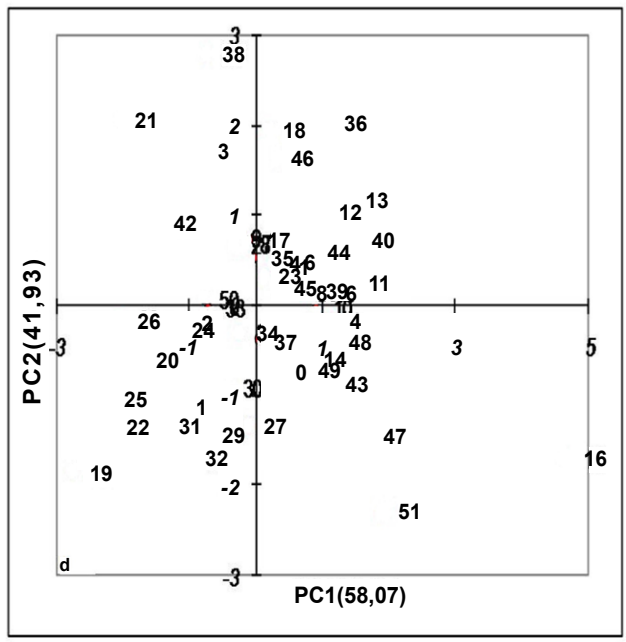

Figure 2d: Additive main effects and multiplicative interaction (AMMI) biplot showing relative stability of fifty varieties (code 1 to code 50) along with susceptible control (51) over three years for infection rate (r) with respective PC1 and PC2 scores.

The means of ACI over year varied between 5-36. Only one cultivar (code 49) expressed LTN with the highest frequency (i.e.8) while it was absent in three (code 42, 46 and 48) cultivars. LTN was displayed with a frequency of 3-4 in the remaining cultivars. Yr18 linked allele of 150 bp was amplified by code 48 and code 49 cultivars (Figure 1) while the non Yr18 associated allele of 230 bp was detected in the remaining cultivars of this group. Stability pattern of group 3 cultivars with codes from 41 to 50 are shown in AMMI biplots (Figures 2a-2d) where code 44 and code 45 were found relatively more stable for FRS, AUDPC and ACI.

Discussion

In the present study, 50 genotypes were studied under field conditions, 29 were susceptible, and 11 were resistant at seedling stage, whereas, 10 were either resistant or susceptible to one of the races at seedling stage. Disease pressure, as assessed by FRS of Morocco, was sufficient in all seasons, however, the yearly mean of 2006-07 (Y3) for FRS was significantly lower compared to previous two years. This decrease in FRS could be due to rainfall which was more during March and April in 2006-07 (259 mm) when compared with 2005-06 (49 mm) and 2004-05 (112 mm) and this may be one of the major reasons for lower FRS since extended or heavy rain showers might have exhausted available spore stocks and thus inhibited further sporulation process for hours. Similar findings were also observed in rain simulation and field experiments [47,48]. Furthermore, increase in yellow rust severity is a function of increase in number of infection and the growth of infections [22] which appeared to be affected by 2006-07 weather conditions. Disease developed on almost all tested cultivars in each year presenting evidence that seedling resistance was partly effective under field conditions as these cultivars displayed significantly low FRS than cv.Morocco except six cultivars having codes 1-5 and 7. Additionally, presence of significant disease severity levels on Avocet isolines with resistance genes i.e. Yr1 (70%), Yr6 (100%), Yr7 (100%), Yr8 (20%), Yr9 (80%), Yr17 (60%), Yr18 (40%), Yr27 (100%), Jupateco-R (60%), Jupateco-S (80%), Avocet-R (80%) and Avocet-S (100%) at the experimental site is indicative of the fact that some diversity either in the number of genes involved and or the size of their effect may be responsible for this type of resistance [12].

The mean AUDPC value of the 2005-06 growth season was the highest which was significantly different from the other two seasons. AUDPC values of 31 cultivars (code 1-15, 17, 18, 21, 30-34, 38, 40-45 and 47) and Morocco were maximum during 2005-06 when compared with 2004-05 and 2006-07 seasons. One of the factors responsible for this high AUDPC values during 2005-06 was that rust epidemic started one week earlier than the other two seasons. Leaf area was 50% rusted in cv. Morocco on 17th March in 2006 while a similar disease level was recorded on 24th and 22nd March in 2005 and 2007, respectively. Cultivars having 70-90% and 50-70% less AUDPC of cv. Morocco were regarded better and medium slow rusting, respectively. Cultivars falling in these two ranges of relative AUDPC belonged to all three groups presented in Table 2. Apparent infection rate of all cultivars (except code 6, 28, 29, 44 and 50) were either equivalent or higher than cv. Morocco during 2005-06 due to the fact that disease scoring was made when rust severity was >50% on cv. Morocco. Therefore actual r-value for cv. Morocco may even have been more. Some of the cultivars belonging to group 1 (code 3, 5, 8, 9, 10, 12, 16, 17, 18, 20, 21, 23, 24, 25, 26, 27 and 28), group 2 (code 31) and group 3 (code 42 and 47) had higher infection rate values during 2005-06. This could be attributed to start of earlier epidemic this year. Higher mean r-values than Morocco were recorded for code 3, code 12 (group 1), code 31 (group 2) and code 47 (group 3). Except code 47 cultivar, r-values were high in those cultivars where rust development was comparatively rapid such as code 3, 12 and 31 while it was low in slow rusting cultivars. A similar trend has been reported in early blight of potatoes (Holley et al., 1983). Response of the tested cultivars to the matching pathotypes were displayed by the disease intensity under field conditions and were thus included for calculating ACI values which are based on FRS and infection types. Both these sub-factors are influenced by environment. However, high (upto 20 ACI) and stable field resistance assessed by this parameter was displayed by group1 (code 21, 22, 25-28 and 29), group 2 (code 34-40) and group 3 (code 47, 49 and 50) cultivars consistently each year.

Cultivars, years and cultivars x year's interactions displayed highly significant difference for slow rusting parameters. Resistance was expressed well in the present study in all three years, the cultivar x year interaction variance for the four disease parameters were small compared to the large genotype variance. This stable expression of resistance is also reported in wheat [49] and barley [50] leaf rust systems. Similarly Keller et al. [51] found only a small impact of cultivar x environment interactions for quantitative powdery mildew resistance. Furthermore, across year's association between all parameters were positively correlated with highly significant values. Similar findings have been reported previously [21]. Two types of statistical models were used in the present study. ANOVA, which is an additive model and is effective in partitioning the total sum of squares into the genotype main effects and environments effect and GEI (genotype x environment interaction) which does not provide insight into GEI structure [52]. To determine stability of underlying interaction component (parameters and years), AMMI model was used which separates the additive variance and then applies Principal Component Analyses (PCA) to the interaction portion to extract a new set of co-ordinate axis which explains the interaction pattern. Cultivars, which occurred close to the center on the biplot, tended to have similar expression of resistance in three years while genotypes away from the center may either differ in resistance stability or showed a different pattern of response over the years. In the present study, AMMI model identified and displayed the overall relatively stable cultivars belonging to group 1 (code 5, 15, 17 and 21), group 2 (code 33 and 34) and group 3 (code 44 and 45). The AMMI model has been successfully utilized to analyze the G × E interactions and identify stable resistant host genotypes for broom rapes (Orobanche sp.) resistance in faba beans [53], blast resistance in rice [54], net blotch resistance in barley [55] and late blight resistance in potato [56].

Non-race specific slow rusting genes for yellow rust include Yr18, Yr29, Yr30, Yr36, Yrns-B1, and YrA1 to YrA8 [8] and majority of these are expressed at high temperature. As a major source and contributor to durable adult plant (slow rusting) resistance, Yr18 carrying cultivars have been grown over considerable acreage for more than 50 years [23,57]. Leaf tip necrosis (LTN), a morphological trait, shows complete linkage or pleiotropism with Yr18 and Lr34 gene [28,29] and could be used as a marker to identify wheat lines carrying these genes. LTN trait can be observed in adult plant stage and its expression was considered positive for seventeen cultivars belonging to all the three groups (except code 29, 39 and 48) although less frequent or absent in some varieties. Phenotypes based on LTN have been used in this study; however, its expression can be obscured by genetic background [58] and variable influences of environments [59]. A recently developed molecular marker, csLV34 linked to Yr18 [60], supported these findings in demonstrating that five cultivars (code 15, 17, 21, 33 and 34) possessed slow rusting resistance a characteristic also carring Yr18 linked allele. Two unstable cultivars having code 29 and 48 belong to group 1 and 3

respectively lacked LTN phenotype and also had Yr18 linked allele of 150 bp. Leaf tip necrosis and molecular marker data of code 29, 39 and 48 varieties did not support each other in this study. Singh et al. [58] reported that wheat lines not showing LTN under some environmental conditions may still carry Lr34. Furthermore, combination of optimal moisture for plant development and cool night temperatures after heading stage may be required for consistent expression of leaf tip necrosis [44]. LTN was detected 3-4 times in 25 cultivars belonging to all three groups in which Yr18 associated allele of 150 bp was not amplified. A similar situation has been reported previously [44] where some wheat lines postulated to have lacked Lr34 expressed leaf tip necrosis in a few replications.

Phenotypically stable cultivars with non-Yr18 associated allele of 230 bp may possess other post-seedling resistance genes reported by McIntosh et al. [61] either singly or in combination which needs to be substantiated. A combination of the simple molecular genotyping tool provided by the csLV34 marker together with the knowledge of well-established cultivars with and without Yr18 enabled the most likely origin of this important gene by analyzing parentages of the tested cultivars. It is likely that Yr18 may have descended in 20 varieties from CIMMYT germplasm i.e. Norteno-67, Tobari-66, Lerma Rajo-64/Tezanos Pintos Precoz, Ciano 67, Jupateco 73 and Torim 73 which were present in their backgrounds [62].

Slow rusting cultivars appeared to retain this character even with shift in races of the pathogen over a period of years [63]. Selection for slow rusting under greenhouse may not be an ideal choice since controlled conditions do not fully represent the environment in the field conditions [64]. Field selection of slow rusting trait, preferably by low AUDPC and terminal ratings along with ACI is feasible in situations, such as Pakistan, where greenhouse facilities are inadequate [64-66]. Cultivars diagnosed to carry Yr18 using csLV34 marker with slow rusting characteristics in this study should be further confirmed by Yr18 specific markers [67] and the material be diversified by accumulating 4-5 slow rusting genes to achieve near immunity [2] prior to deployment as a control strategy in the region for combating yellow rust problem.

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References

1. Singh RP, William HM, Huerta-Espino J, Rosewarne G (2005) Wheat rust in Asia: meeting the challenges with old and new technologies. Proceedings of the 4th international crop science congress, Brisbane, Australia, 26 Sep-1 Oct 2004.
2. Singh RP, Nelson JC, Sorrells ME (2000) Mapping *Yr28* and other genes for resistance to stripe rust in wheat. Crop Sci 40: 1148-1155.
3. Anonymous (2006) Ministry of Food, Agriculture and Livestock (Economic Wing), Islamabad. 289 pp.
4. Anonymous (2007) Vision 2030, Government of Pakistan, Islamabad 119 pp.
5. Duveiller E, Singh RP, Nicol JM (2007) The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. Euphytica 157: 417-430.
6. Afzal SN, Haque, MI, Ahmedani MS, Bashir S, Rattu AR (2008) Impact of stripe rust on kernel weight of wheat varieties sown in rainfed areas of Pakistan. Pak J Bot 40: 923-929.
7. Chen XM (2005) Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat. Can J Plant Pathol 27: 314-337.
8. Flor HH (1956) The complementary genic systems in flax and flax rust. Adv Genet 8: 29-54.
9. Boyd LA (2005) Centenary review: Can Robigus defeat an old enemy?–Yellow rust of wheat. Journal of Agricultural Science 143: 1-11.
10. Caldwell RM (1968) Breeding for general and/or specific plant disease resistance. in: Finlay KW, Shephard KW (eds) Proceedings International Wheat Genetics Symposium, 3rd edn. Australian Academy of Sciences, Canberra: 263-272.
11. Parlevliet JE (1979) Components of resistance that reduce the rate of epidemic development. Annu Rev Phytopathol 17: 203-222.
12. Herrera-Foessel SA, Singh RP, Huerta-Espino J, Crossa J, Djurle AJ, et al. (2007) Evaluation of slow rusting resistance components to leaf rust in CIMMYT durum wheats. Euphytica 155: 361-369.
13. Rubiales D, Niks RE (1995) Characterization of *Lr34*, a major gene conferring non-hypersensitive resistance to wheat leaf rust. Plant Dis 79: 1208-1212.
14. Johnson R (1988) Durable resistance to yellow (stripe) rust in wheat and its implications in plant breeding. Pages 63-75 in: Simmonds NW, Rajaram S(eds) Breeding strategies for resistance to the rusts of wheat CIMMYT. Mexico D.F.
15. Clifford BC (1972) The histology of race non-specific resistance to Puccinia hordei Otth in barley. Pages 75-78 in Proceedings of the 5th European and Mediterranean Cereal Rusts Conference, Prague, Czechoslovakia. European Mediterranean Cereal Rusts Foundation.
16. Wilcoxson RD (1981) Genetics of slow rusting in cereals. Stakman-Craigie Symposium on Rust Diseases. Phytopathology 71: 989-993.
17. Kolmer JA (1996) Genetics of resistance to wheat leaf rust. Annu Rev Phytopathol 34: 435-455.
18. Singh RP, Huerta-Espino J, William M (2001) Slow rusting genes based resistance to leaf and yellow rusts in wheat: Genetics and breeding at CIMMYT. Pages 103-108 in: Proceedings of 10th Assembly of the Wheat Breeding Society of Australia.
19. Navabi A, Singh RP, Tewari JP, Briggs KG (2003) Genetic analysis of adult-plant resistance to leaf rust in five spring wheat genotypes. Plant Dis 87: 1522-1529.
20. Navabi A, Singh RP, Tewari JP, Briggs KG (2004) Inheritance of high levels of adult-plant resistance to stripe rust in five spring wheat genotypes. Crop Sci 44: 1156-1162.
21. Steffenson BJ, Webster RK (1992) Quantitative resistance to *Pyrenophora teres* f. sp. teres in barley. Phytopathology 82: 407-411.
22. Broers LHM, Cuesta-Subia X, Lopez-Atilano RM (1996) Field assessment of quantitative resistance to yellow rust in ten spring bread wheat cultivars. Euphytica 90: 9-16.
23. Shaner G (1996) Breeding for partial resistance in oats to rusts. Pages 307-313 in: Scoles G, Rossnagel B, Fairbairn, C (eds) Proc V Int. Oat Conf. and Int. Barley Genetics Symp.
24. Pathan AK, Park RF (2006) Evaluation of seedling and adult plant resistance to leaf rust in European wheat cultivars. Euphytica 149: 327-342.
25. Rosewarne GM, Singh RP, Huerta-Espino, J, William HM, Bouchet S, et al. (2006) Leaf tip necrosis, molecular markers and b1-proteasome subunits associated with the slow rusting resistance genes *Lr46/Yr29*. Theor Appl Genet 112: 500-508.
26. Suenaga K, Singh RP, Huerta-Espino J, William HM (2003) Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93: 881-890.
27. William M, Singh RP, Huerta-Espino J, Islas SO, Hoisington D (2003) Molecular marker mapping of leaf rust resistance gene *Lr46* and its association with stripe rust resistance gene *Yr29* in wheat. Phytopathology 93: 153-159.
28. Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, et al. (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323: 1360-1363.
29. Singh RP (1992) Association between gene Lr34 for leaf rust resistance and leaf tip necrosis in wheat. Crop Sci 32: 874-878.
30. Singh RP (1992) Genetic association of leaf rust resistance gene Lr34 with adult plant resistance to stripe rust in bread wheat. Phytopathology 82: 835-838.
31. McIntosh RA (1992) Close genetic linkage of genes conferring adult plant resistance to leaf rust and stripe rust in wheat. Plant Pathology 41: 523-527.

32. Spielmeyer W, McIntosh RA, Kolmer J, Lagudah ES (2005) Powdery mildew resistance and *Lr34/Yr18* genes for durable resistance to leaf and stripe rust co-segregate at a locus on the short arm of chromosome 7D of wheat. Theor Applied Genet 111: 731-735.

33. Liang SS, Suenaga K, He ZH, Wang ZL, Liu HY, et al. (2006) Quantitative trait loci mapping for adult-plant resistance to powdery mildew in bread wheat. Phytopathology 96: 784-789.

34. Bahri B (2008) Adaptation et Structuration spatiale des populations méditerranéennes de rouille jaune du blé (*Puccinia striiformis* f. sp. *tritici*). Mémoire de Thèse pour l'obtention du titre de Doctorat en Science du Végétal par l'Université d'Orsay Paris Sud 11: 246.

35. McNeal F, Konzak C, Smith E, Tate W, Russell T (1971) A uniform system for recording and processing cereal research data. US Agric Res Serv 42: 34-121.

36. Ehsan UH, Kirmani MAS, Khan MA, Niaz M (2003) Screening of wheat varieties to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in the field. Asian Journal of Plant Sciences 2: 613-5.

37. Johnson R, Yahyaoui A, Wellings C (2002) Meeting the challenges of yellow rust in cereal crops. Proceedings of the First Regional Conference on Yellow Rust in the Central and West Asia and North Africa Region, Karaj, Iran: 280.

38. Roelfs AP, Singh RP, Saari EE (1992) Rust Diseases of Wheat: Concepts and Methods of Disease Management. Mexico, D.F.: CIMMYT. 81pages.

39. Khanna R, Bansal UK, Saini RG (2005) Genetics of durable resistance to leaf rust and stripe rust of an Indian wheat cultivar HD2009. J Appl Genet 46: 259-263.

40. Peterson, RF, Campbell AB, Hannah AE (1948) A diagrammatic scale for rust intensity on leaves and stems of cereals. Can J Res. 26: 496-500.

41. Vanderplank JE (1968) Disease Resistance in Plants. New York: Academic: 206.

42. Singh RP (1993) Genetic association of gene Bdv1 for tolerance to Barley Yellow Dwarf Virus with genes Lr34 and Yr18 for adult plant resistance to rusts in bread wheat. Plant Dis 77: 1103-1106.

43. Pandey HN, Menon TCM, Rao MV (1989) A single formula for calculating area under disease progress curve. Rachis 2: 38-39.

44. Wamishe YA, Milus EA (2004) Genes for adult plant resistance to leaf rust in soft red winter wheat. Plant Dis 88: 1107-1114.

45. Imtiaz M, Ogbonnaya FC, Oman J, van Ginkel M (2008) Characterization of quantitative trait loci controlling genetic variation for preharvest sprouting in synthetic backcross-derived wheat lines. Genetics 178: 1725-1736.

46. Ukai Y, Nesumi H, Takano Y (1996) GEST: A package of computer programs for the statistical analyses of genotype X environment interaction and stability. Breeding Science 46: 73-79.

47. Pady SM, Kramer CL, Kathak vk, Morgan FL, Bhatti MA (1965) periodicity in airborne cereal rust urediospores. Phytopathology 55: 132-134.

48. Rapilly F, Fournet F, Skajennikoff M (1970) Études sur l'épidémiologie et la biologie de la rouille jaune du blé Puccinia striiformis Westend. Ann Phytopathol 2: 5-31.

49. Broers LHM, Parlevliet JE (1989) Environmental stability of partial resistance in spring wheat to wheat leaf rust. Euphytica 44: 241-245.

50. Parlevliet JE, Broers LHM, Jacobs T (1988) Comparison of partial resistance in wheat and barley to leaf rust. Pages 21-23 in: Proc. 7th Eur and Medi Cereal Rusts Conf., Vienna, Austria.

51. Keller M, Keller B, Schachermayr G, Winzeler M, Schmid JE, et al. (1999) Quantitative trait loci for resistance against powdery mildew in a segregating wheat x spelt population. Theor Appl Genet 98: 903-912.

52. Mahalingam L, Mahendran S, Babu RC, Atlin G (2006) AMMI analysis for stability of grain yield in rice (Oryza sativa L.). International Journal of Botany 2: 104-106.

53. Flores F, Moreno MT, Martinez A, Cubero JI (1996) Genotype-environment interaction in faba bean: comparison of AMMI and principal coordinate models. Field Crops Res 47: 117-127.

54. Abamu FJ, Akinsola EA, Alluri K (1998) Applying the AMMI models to understand genotype-by-environment (GE) interactions in rice reaction to blast disease in Africa. Int J Pest Management 44: 239-245.

55. Robinson J, Jalli M (1999) Sensitivity of resistance to net blotch in barley. J Phytopathol 147: 235.

56. Forbes GA, Chacon MG, Kirk HG, Huarte MA, Damme MV, et al. (2005) Stability of resistance to Phytophthora infestans in potato: an international evaluation. Plant Pathology 54: 364-372.

57. Singh RP, Rajaram S (1994) Genetics of adult plant resistance to stripe rust in ten spring bread wheats. Euphytica 72: 1-7.

58. Singh RP, Chen WQ, He ZH (1999) Leaf rust resistance of spring, facultative, and winter wheat cultivars from China. Plant Dis 83: 644-651.

59. Dyck PL (1991) Genetics of adult plant leaf rust resistance and leaf tip necrosis in wheat. Crop Sci 32: 874-878.

60. Lagudah ES, McFadden H, Singh RP, Huerta-Espino J, Bariana HS, et al. (2006) Molecular genetic characterization of the Lr34/Yr18 slow rusting resistance gene region in wheat. Theor Appl Genet 114: 21-30.

61. McIntosh RA, Wellings CR, Park RF (1995) 'Wheat Rusts: An Atlas of Resistance Genes.' (CSIRO Publications: Victoria, Australia).

62. Kolmer JA, Singh RP, Garvin DF, Viccars L, William HM, et al. (2008) Analysis of the Lr34/Yr18 rust resistance region in wheat germplasm. Crop Sci 48: 1841-1852.

63. Ohm HW, Shaner GE (1976) Three components of slow leaf rusting at different growth stages in wheat. Phytopathology 66: 1356-1360.

64. Singh RP, Payne TS, Rajaram S (1991) Characterization of variability and relationships among components of partial resistance to leaf rust in CIMMYT bread wheats. Theor Appl Genet 82: 674-680.

65. Broers LHM (1989) Partial resistance to wheat leaf rust in 18 spring wheat cultivars. Euphytica 44: 247-258.

66. Singh D, Park RF, McIntosh RA (2007) Characterization of wheat leaf rust resistance gene Lr34 in Australian wheats using components of resistance and the linked molecular marker csLV34. Australian Journal of Agricultural Research 58: 1106-1114.

67. Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, et al. (2009) Gene-specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance to multiple fungal pathogens. Theor Appl Genet 119: 889-898.