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VIRULENCE ANALYSIS OF *PUCCINIA TRITICINIA* CAUSE OF LEAF RUST OF WHEAT

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Abstract

Virulence analysis of wheat provides breeders early information about the behavior of their material. Survey of wheat leaf rust using seedling differentials are very useful in describing virulence variation, geographical distribution of virulence pathotypes, and how leaf rust phenotypes change in response to host selection. Wheat leaf rust samples collected from the different wheat growing areas of Pakistan and single spore culture was multiplied for virulence analysis. Very high virulence frequencies (75-100%) showed by the resistance genes *Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr3ka, Lr3bg, Lr10, Lr11, Lr14a, Lr14b, Lr15, Lr17, Lr18, Lr21, Lr23, Lr25, Lr26, Lr30, Lr32, Lr33, Lr35, Lr37* and *LrB.* Resistance genes *Lr9, Lr19, Lr28* and Lr34 demonstrated their effectiveness of pathogen population of *Puccinia triticina. Lr13, Lr16, Lr24* and *Lr29* falls in 51-75% virulence frequencies range. *Lr36* and *Lr23+* showed the virulence in the range 26-50%. Virulence frequencies at different locations in the country and utilization of this data for wheat improvement is discussed

Introduction

Leaf rust caused by *Puccinia triticina* Eriks & Henn., is a serious production hazard (Mclntosh *et al.,* 1995). Historically rusts have been considered the major biotic production constraints both in Asia and rest of the world (Singh & Rajaram, 1991). Neglecting the other vagaries which lower the production of wheat, leaf rust alone can cause 30-50% yield loss under favorable environmental conditions by growing the susceptible cultivars. History is full of rust epidemics (Boralug, 1968; Bernan, 1994) which indicates the importance of disease. The 1978 leaf rust epidemics in Pakistan caused 10% yield loss (Hassan, 1979). *Puccinia triticina* generally reproduces asexually and has the capability to evolve new virulent forms; hence it is able to attack previously resistant hosts through mutation. It is able to produce large amount of urediospores, which affect in long distance dissemination. Consequently, the wheat breeders have released several wheat varieties but none of the varieties had durable rust resistance for the long years

Plant pathologists have grouped morphologically similar individuals that differ in host range into *formae specialis*, which are defined according to the ability to attack on a particular species. Early in the 20th century, the *formae specialis* was further subdivided into physiological races, which differed in their capacity to attack certain wheat cultivars. Physiological races are characterized by the reactions on a set of host differentials carrying different sources of resistance (Stakman, 1919). Therefore, such a diversified change needs a regular check and monitoring.

Breeding for resistant cultivars represents the most cost effective means of controlling leaf rust. Effective breeding strategies, however, depend on an understanding of genetic variation in pathogen and host as well as epidemiology of the pathogen. For leaf rust, this includes the ability of the pathogen to survive through the non-crop season and the probability of the disease occurring in the different growing areas of Pakistan.

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Selection for virulence by growing cultivars with pathotype specific resistance plays a major role in determining the pathotype population of the pathogen. Virulence pathotype can be characterized based on reaction on host seedling differential set within few days from receipt of the rust collection (Kolmer, 1997). Inoculations of spores from single uridinia onto differential sets of seedling plants minimizing the amount of rust required and also the amount of time and space required to grow the different sets. Survey of wheat leaf rust using seedling differentials have been very useful in describing virulence variation, geographical distribution of virulence pathotypes and how leaf rust phenotypes change in response to host selection (Kolmer, 1992).

In Pakistan, some systemic work was conducted by Aslam (1975) and Hussain, (1979) to identify the virulence factors of *P. recondita*. Hussain *et al*., (1978), identified effective genes on the basis of differential lines. Investigation on the prevalence and distribution of physiologic races of *P. recondita* revealed the presence of 8 different races in Pakistan during 1961 to 1975. However, since then limited information is available hence the present study was taken up to identify the virulence frequencies of leaf rust pathogen by collection of leaf rust disease samples from farmer's fields as well as trap nurseries in different districts of Sindh, Baluchistan, Punjab and NWFP provinces.

Materials and Methods

Rust disease survey: Two surveys were conducted during present course of investigations in Sindh, Punjab, NWFP and Baluchistan. First wheat survey was conducted during 2002-2003 at adult plant stage in 6 districts in Sindh, 11 in Punjab, 4 in NWFP and one in Balochistan. Second wheat survey was conducted during 2003-2004 covering the same key locations except district Khushab (Punjab) as was done during 2002-2003.

During the wheat survey, samples of leaves having speculating pustules of leaf rust were collected and brought to the Crop Diseases Research Programme (CDRP), Murree for multiplication of inoculum under controlled conditions in glass house for further studies.

The rust infected leaves with sporulating pustules ranged from 80 to 100 samples were collected from farmer's fields in Thatta, Hyderabad, Khairpur, Nawabshah, Larkana, Sukkur, Bahawalpur, Muzaffargarh, Jhang, Sargodha, Hafizabad, Sialkot, Gujranwala, Sheikhupura, Gujrat, Islamabad, Faisalabad, Nowshehra, Peshawar, Charsadda, Mardan and Naseerabad. Leaf rust disease samples were also collected from the CDRP disease nurseries planted at Nuclear Institute of Agriculture (NIA), Tandojam; Regional Agricultural Research Institute (RARI), Bahawalpur; University of Agriculture, Faisalabad (UAF); Pulses Research Station (PRS), Sialkot; Cereal Crops Research Institute (CCRI), Pirsabak (Nowshehra) and Nuclear Institute for Food and Agriculture (NIFA), Peshawar.

The diseased leaf samples were pressed in the folds of newspaper and placed in an envelope for transportation to CDRP, Murree glass-house. These samples were stored in refrigerator at 4^{o}C till further analysis.

Multiplication and preservation of *Puccinia triticina* inoculum: The inoculum was increased and maintained on universally susceptible variety Morocco. Seeds of Morocco were sown in 11 x 11cm plastic pots and placed at room temperature till germination. Upon germination these seedlings were then shifted to glass house where the conditions

were maintained at 25-30^{o}C day and 19-21^{o}C night temperature. When the seedlings of Morocco reached at two leaf stage, there were treated with Maleic hydrazide (5 mg with 50 ml of water per pot) (Singh, 1991) and inoculated with spores from the disease leaf samples collected and stored earlier. Four pots of Morocco with approximately 5 plants per pot were inoculated at two leaf stage with the inoculum in the form of urediospores from individual rust sample by applying on the dorsal and ventral side of the leaf with the help of moistened finger and thumb. The inoculated seedlings were incubated in dew chamber for 24 hours at 18-20^{o}C temperature with 90% humidity. The seedlings were then transferred to glass house where temperature was maintained at 18-24^{o}C day and 19-21^{o}C night. Eight to 10 days after inoculation, pustules of leaf rust appeared on the leaves of Morocco from which inoculum was collected on the 14th day with the help of mechanical cyclone collector in a zero size capsule and preserved in vacuum glass vial which was later transferred in a refrigerator till further use. For each isolate and multiplication of culture a separate collector was used.

Single spore culture of *Puccinia triticina*: Eight to 9 days old Morocco seedlings were inoculated by spraying the urediospores previously increased suspended in light parafine mineral oil (70 ether: 30 oil) the plants were allowed to dry for 1 hour, placed in dew chamber overnight at 18-20^{o}C and transferred to greenhouse where temperature was maintained at 18-24^{o}C day and 19-21^{o}C at night. Seven day after inoculation, the leaves were trimmed with scissors so that a single uredium remained on the trimmed upper edge of the leaves and could be preserved with purity. Fourteen days after inoculation, urediospores were collected using a vacuum powered cyclone spore collector. Single pustule inoculum was increased on densely cultivated Morocco grown in trays by the method described above.

Virulence analysis of *Puccinia triticina*: A set consisting of 39 near-isogenic Thatcher lines were used for virulence analysis as differentials (Table 1). The near-isogenic lines were planted in plastic tray measuring 28 x 34 x 6 cm containing soil, silt, and compost (2:1:1). Seeds were sown at uniform depth and distance. Eight to nine day old seedlings of Thatcher near-isogenic lines were inoculated with single pustule inoculum from different leaf rust samples by the method described above.

Leaf rust disease assessment: The infection types for all the isogenic lines were recorded after 12 days on appearance of pustules on near-isogenic lines, the infection types for all the near-isogenic lines were recorded using standard disease scoring scale 0-4 (Stakman *et al*., 1962) presented in Table 1. The virulence patterns on differential sets were assessed on the basis of low infection types produced by each line in response to infection (infection type 0, 1 and 2 represented avirulent while 3 and 4 represent virulent) (Stakman *et al*., 1962).

Result and Discussion

Lr12, Lr22a, and *Lr22b* genes for leaf rust resistance displayed consistently high infection type to all isolates at seedling stage consequently these genes are known to confer low reaction only in adult plants, therefore, virulence status in the pathogen for these genes could not be determined (Singh, 1991).

Table 1. Host series with known *Lr* genes used for leaf rust virulence study in glasshouse with low seedling infection types 0-4 rating scale.

S. No.	Lines	Genes	Low infection types seedling
1.	THATCHER	Lr 22B	APR
2.	RL6003	Lr 1	0;
3.	RL6016	Lr2A	0;,;1
4.	RL6019	Lr2B	;1,;1+
5.	RL6047	Lr2C	;IN,2
6.	RL6002	Lr3	;C,2
7.	RL6007	Lr3KA	;C,12C
8.	RL6042	Lr3BG	;C,23
9.	RL6010	Lr9	0;
10.	RL6004	Lr10	;,2
11.	W976	Lr 11	; ,1,2,3-,Y
12.	RL6011	Lr 12	APR
13.	MANITUOU	Lr 13	X
14.	RL6013	Lr 14A	X
15.	RL6006	Lr 14B	X
16.	RL6052	Lr 15	;C
17.	RL6005	Lr 16	,1 N
18.	RL6008	Lr 17	;1+,0;
19.	RL6009	Lr 18	2+2-
20.	RL6040	Lr 19	0;
21.	W203	Lr 20	0
22.	RL6043	Lr 21	0,,12+
23.	RL6044	Lr 22A	APR
24.	RL6012	Lr 23	1;,23
25.	RL6064	Lr 24	0, ;1
26.	TC*?/TRANSEC	Lr 25	0;, ;1, 2+
27.	RL6078	Lr 26	0;,1
28.	W3201	Lr 10, 27+31	X
29.	CS2D-2M	Lr 28	0;
30.	RL6080	Lr 29	;1 N
31.	RL6049	Lr 30	123
32.	RL5497	Lr 32	;1+
33.	RL6057	Lr 33	1
34.	RL6058	Lr 34	12C
35.	RL5711	Lr 35	_
36.	E84018	Lr 36	0; 1+
37.	RL6081	Lr 37	12Y
38.	RL6051	Lr B	2, ;
39.	GAZA (W277)	Lr 23, +	1; ,23

During 2002-2003 very high virulence frequencies (75-100 %) of leaf rust have been found on the resistance genes *Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr3ka, Lr3bg, Lr10, Lr11, Lr14a, Lr14b, Lr15, Lr18, Lr20, Lr21, Lr23, Lr25, Lr26, gatcher, Lr30, Lr32, Lr33, Lr35, Lr37,* and *LrB.* Resistance genes *Lr9, Lr19, Lr28,* and *Lr34* demonstrated their effectiveness of pathogen population of *P. triticina. Lr13, Lr16, Lr17, Lr24,* and *Lr29* falls in 51-75 percent virulence frequencies range. *Lr36* and *Lr23+* showed virulence in the range 26-50%.

During 2003-2004, very high virulence frequencies (75-100%) was also showed by the resistance genes Lr1, *Lr2a, Lr2b, Lr2c, Lr3, Lr3ka, Lr3bg, Lr10, Lr11, Lr14a, Lr14b, Lr15, Lr17, Lr18, Lr21, Lr23, Lr25, Lr26, Lr30, Lr32, Lr33, Lr35, Lr37,* and *LrB.* Resistance genes *Lr9, Lr19, Lr28,* and *Lr34* demonstrated their effectiveness of pathogen population of *P. triticina*. *Lr13, Lr16, Lr24,* and *Lr29* falls in 51-75% virulence frequencies range. *Lr36* and *Lr23+* showed the virulence in the range 26-50%.

The virulence frequency in the provinces has been presented in the Table 3. The high virulence frequency on *Lr1, Lr3, Lr10, Lr13, Lr16* and *Lr26* is probably related to the frequency of these resistance genes in the varieties cultivated in Pakistan (Hussain *et al*., 1979). Gene postulation in commercial varieties revealed the presence of these genes in our commercial varieties (Mirza *et al*., 2000). *Lr10* which is present in our cultivars Auqab-2000, Punjab-96, Inqilab-91, Margalla-99, Chakwal-86, Anmol-91, Soghat-90, and Zarghoon have high frequencies during the study period indicates that the lines possessing this gene should be carefully selected for breeding. *Lr13* is the gene of adult plant resistance genes present in the cultivars Parwaz-94, MH-97, Wafaq-2001, Haider-200, Zarlashta and Sariab-92. The percent virulence frequency of this gene has increased from 2002-03 to 2003-04. *Lr13* is the temperature sensitive gene at seedling and is genetically linked with *Lr23* (Mclntosh, 1995). *Lr26* which is the most dominant gene is loosing its effectiveness. The alien source of disease resistance has had a major impact on global wheat production as indicated its presence in many winter and spring wheat and is genetically linked with *Yr9*, *Sr31* and *Pm8* (Mclntosh, 1995). *Lr10, 27+31* the gene present in our most popular commercial variety Inqilab-91. The parents of the Inqilab-91 in its pedigree have Crow and WL-711, which are slow ruster and have adult plant resistance. The percent virulence frequency of this gene is very high. This may cause a serious problem by growing Inquilab-91 on large area in the country.

Lr34 which is considered the gene for slow rusting and due to its wide effectiveness as a source of resistance under field condition and its interactive effects (German & Kolmer, 1992), it has been selected in many breeding program directed at leaf rust resistance and is genetically linked with *Yr18* and leaf rust tip necrosis (Mclntosh, 1995). This gene is widely used in the CIMMYT wheat program for its effectiveness for durable resistance. *Lr34* when alone is ineffective but when in combination with other genes give excellent results.

Because of its widespread, *Lr9* has not been widely deployed despite its wide spread effectiveness (Mclntosh *et al*., 1995) and this gene was postulated in only one cultivar Marvi-2000 (Rattu, 2006) which is planted in the Sindh province. *Lr9* is located on chromosome 6B. Virulence for *Lr9* was found in USA in 1971, four years after its use in soft red winter wheats (Shanner *et al*., 1972). Its virulence then was also observed in Brazil and Argentina. Huerta Espino (1992) found virulence in isolates from Italy, Burundi, and Pakistan though the virulence frequency was very low.

*Lr*19 is located in chromosome 7AL. Despite the acceptable protection level of *Lr*19 and lack of virulence in Pakistan, the limited use of this gene is attributed to yellow flour pigment. A mutant with lighter yellow colour was later on produced (Knott, 1980), which was further improved by recombining various wheat lines that encompass *Lr*19, Bdv2 and had white flour colour (Singh *et al*., 2001).

Table 2. Virulence of isolates and frequency (%) of *Puccinia triticinia* on 39 near-isogenic lines.

Lr Genes	2002-2003		2003-2004	
	Virulent isolates (Nos.)	Virulent isolates (%)	Virulent isolates (Nos.)	Virulent isolates (%)
Lr1	**176**	**99**	**134**	**97**
Lr2a	162	91	120	87
Lr2b	157	94	130	94
Lr2c	174	98	133	98
Lr3	**174**	**98**	**138**	**100**
Lr3ka	162	91	119	86
Lr3bg	166	93	126	91
Lr9	0	0	0	0
Lr10	**178**	**100**	**130**	**94**
Lr11	174	98	131	95
Lr13	**112**	**63**	**98**	**71**
Lr14a	162	91	123	89
Lr14b	174	98	130	94
Lr15	153	86	105	76
Lr16	**93**	**52**	**84**	**61**
Lr17	125	70	114	83
Lr18	173	97	130	94
Lr19	0	0	0	0
Lr20	157	88	90	65
Lr21	166	93	109	79
Lr23	151	85	110	80
Lr24	130	73	101	73
Lr25	162	91	117	85
Lr26	**167**	**94**	**113**	**82**
Lr10, 27+31	144	81	97	70
Lr28	2	1	4	3
Lr29	107	60	102	74
Lr30	153	86	123	89
Lr32	158	89	109	79
Lr33	166	93	126	91
Lr34	21	12	30	22
Lr35	171	96	134	97
Lr36	68	38	59	43
Lr37	164	92	119	86
LrB	167	94	132	96
Lr23,+	69	39	63	46
Total isolates	**178**	**-**	**138**	**-**

Table 3. Percent virulence of *Puccinia triticina* on near-isogenic lines in different provinces of Pakistan during 2003 and 2004.

Isogenic lines	2002-2003					2003-2004				
	Sindh	Punjab	NWFP	Balochistan	Average	Sindh	Punjab	NWFP	Balochistan	Average
Lr 22B	APR	APR	APR	APR	APR	APR	APR	APR	APR	APR
Lr 1	100	98	100	100	**100**	92	96	100	100	**97**
Lr2A	98	82	83	100	**91**	89	87	71	100	**87**
Lr2B	100	84	92	100	**94**	97	90	88	100	**94**
Lr2C	100	92	100	100	**98**	97	95	100	100	**98**
Lr3	100	91	100	100	**98**	100	99	100	100	**100**
Lr3KA	100	90	75	100	**91**	76	92	75	100	**86**
Lr3BG	96	87	88	100	**93**	95	92	88	90	**91**
Lr9	0	0	0	0	**0**	0	0	0	0	**0**
Lr10	100	100	100	100	**100**	97	79	100	100	**94**
Lr 11	99	95	100	100	**98**	90	90	100	100	**95**
Lr 12	APR	APR	APR	APR	APR	APR	APR	APR	APR	APR
Lr 13	89	57	25	80	**63**	73	86	46	80	**71**
Lr 14A	92	94	100	80	**91**	82	92	100	80	**89**
Lr 14B	99	93	100	100	**97**	94	92	100	90	**94**
Lr 15	94	91	58	100	**86**	75	86	63	80	**76**
Lr 16	45	45	29	90	**52**	51	77	25	90	**61**
Lr 17	92	72	17	100	**70**	97	98	38	100	**83**
Lr 18	100	88	100	100	**97**	94	80	100	100	**94**
Lr 19	14	0	0	0	**3**	0	0	0	0	**0**
Lr 20	94	87	69	100	**87**	76	75	29	80	**75**
Lr 21	93	80	100	100	**93**	53	81	100	80	**79**
Lr 22A	APR	APR	APR	APR	APR	APR	APR	APR	APR	APR
Lr 23	85	77	96	80	**84**	73	69	96	80	**80**
Lr 24	65	66	92	70	**73**	48	85	79	80	**73**
Lr 25	99	76	88	100	**91**	96	78	67	100	**85**
Lr 26	99	95	100	80	**93**	73	88	88	80	**82**
Lr 10, 27+31	85	77	81	80	**81**	83	68	50	80	**70**
Lr 28	0	0	0	0	**0**	0	0	0	0	**0**
Lr 29	40	67	42	90	**60**	60	81	63	90	**74**
Lr 30	89	81	75	100	**86**	94	75	88	100	**89**
Lr 32	95	80	100	80	**89**	96	87	42	90	**79**
Lr 33	99	73	100	100	**93**	97	76	92	100	**91**
Lr 34	13	11	4	20	**12**	13	22	42	10	**22**
Lr 35	96	92	96	100	**96**	92	97	100	100	**97**
Lr 36	26	48	38	40	**38**	44	61	25	40	**43**
Lr 37	89	79	100	100	**92**	84	68	100	90	**86**
Lr B	83	92	100	100	**94**	90	92	100	100	**96**
Lr 23, +	31	44	61	20	**39**	40	50	54	40	**46**

*Lr*28 is located on 4AL and the effectiveness of these three genes may be due to the absence of the host.

The overall wheat rust frequency suggests similar genetic background of cultivars due to the fact that frequency of all the genes at different locations was same. As most of our germplasm is of CIMMYT origin containing most dominant genes *Lr1, Lr3, Lr10, Lr13, Lr16, Lr23, Lr26* and Gatcher gene and these genes have been postulated in our most of common commercial varieties (Mirza *et al*., 2000).

Frequent and rigorous monitoring and continuous modeling of forecast should be established in the country for the identification of genes for resistance with concurrent knowledge of the changes occurring in the population of *P. triticina*. Survey of wheat leaf rust using seedling differentials are very useful in describing virulence variation, geographical distribution of virulence pathotypes, and how leaf rust phenotypes change in response to host selection. This activity should be executed in wheat growing seasons by comprehensive survey and planting of wheat rust trap nurseries at hot spots. This will ultimately provide timely warning to wheat breeders about the change in virulence.

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