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VIRULENCE PHENOTYPING OF LEAF RUST (*PUCCINIA TRITICINA*) ISOLATES FROM SOUTHERN PAKISTAN

[a]Abdul W. Channa, [a]Hadi Bux, [b]Mahboob A. Sial, [c]Ghulam H. Jatoi, [d]Raj Kumar
[a] *Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan.*
[b] *Plant Genetics Division, Nuclear Institute of Agriculture (NIA), Tandojam, Pakistan.*
[c] *Department of Agriculture, Mir Chakar Khan Rind University, Sibi, Balochistan, Pakistan.*
[d] *Department of Botany Adamjee Government Science College, Karachi, Sindh, Pakistan.*

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ABSTRACT

Collections of *Puccinia triticina* were made from farmers' fields of five different agro-ecological locations (Sakrand, Tandojam, Larkana, Sanghar and Badin) of Sindh province, Pakistan from 2015 and 2016, to identify the virulence variation. Single uredinial isolates were investigated for virulence phenotyping on 24 near isogenic (Thatcher wheat) lines which differ for single *Lr* resistance genes. Spores from two locations (Sakrand and Tandojam) were not viable and could not be revived and only urediniospores of three locations (Larkana, Sanghar and Badin) were revived. None of the pathotypes had virulence to Thatcher wheat lines with leaf rust resistance genes *Lr23* and *Lr42*. However, *Lr24, LrB, Lr10, Lr14b* and *Lr20* genes exhibited susceptibility response i.e. (HITs 3 & 4) with all tested pathotypes. Based on virulence, ten virulence phenotypes (MSCTNS, RTSTNS, RKTRGS, PNDQDS, JDBQGJ, MDPSDS, RTPTPS, MNPSDS, MJLTGS and MSPTDS) were identified among the ten isolates, designated with six-letter code. Two phenotypes RTSTNS & RTPTPS exhibited broad spectrum, both were virulent to nineteen resistance genes of leaf rust while pathotype JDBQGJ had narrow spectrum as compared to all other tested, with virulence to just eight resistance genes of leaf rust. Among the locations virulence variability of leaf rust was also recorded. Most of identified races were virulent to more than one of leaf rust resistance genes. Resistance genes (*Lr42* and *Lr23*) identified as effective can be exploited to achieve leaf rust resistance in wheat. Further, the study provides virulence profile of the area may help to manage the leaf rust pathogen.

Corresponding Author: Abdul W. Channa
Email: awabdulwajid440@gmail.com

INTRODUCTION

Puccinia triticina causative agent of brown rust disease is one of the most destructive and widespread rust pathogens worldwide (Kolmer, 2005; Hovmøller *et al.*, 2010; Ellis *et al.*, 2014) causing significant yield (a severe epidemic of leaf rust resulted a nationwide 10% loss) reductions including grain quality (Sumíková and Hanzalova, 2010). World production of wheat was recorded 760 million tons while wheat sown on total area of approx. 215 million hectares (FAO-S, 2020) whereas in Pakistan, during (2019-20) wheat growing season, wheat was sown on land area of 8.825 million hectares (Pakistan Economic Survey, 2020). Wheat leaf rust can be serious in continental regions the Great Plains of North America, in warm to hot summer temperatures of South Asia, coastal regions of humid conditions of Turkey, South America,

Central Asia, Russia, Canada, Southern, eastern, central Europe, China, Uruguay and South Africa (Kolmer *et al.*, 2013; Kolmer and Hughes, 2013; Morgounov *et al.*, 2007; Morgounov *et al.*, 2011; Hanzalova and Bartoš, 2014; Liu and Chen, 2012). High variation was found in populations of fungal pathogen globally, with huge numbers of virulence phenotypes or races in addition to a high variability of molecular variation (Ordoñez and Kolmer, 2009). Leaf rust prevails in Pakistan, on an annual basis throughout the wheat-producing areas (Saari and Prescott, 1985; Yamin *et al.*, 2021) most regular in the central and southern regions of the country and causing frequent yield reductions. Pathogen can survive on wheat during the summer in western region mountains and then disseminates to the wheat-producing areas of Indus basin in Punjab and Sindh provinces (Nagarajan and Joshi, 1985).

Although in Pakistan, susceptible alternative hosts for pathogen are not identified; hence for dispersal and survival, it is dependent on the clonal urediniospore phase from year to year. Pathogen can cause yield reductions up to 40% in cultivars with susceptibility through reducing grains per spike and declining kernel weight (Khan *et al.*, 2013). According to level of resistance or susceptibility, leaf rust infection ranged between 2.0% and 41% losses in grain weight of wheat cultivars due to infection of leaf rust (Bajwa *et al.*, 1986), whereas in Egypt yield losses could reach up to 50% (Abdel *et al.*, 1980). To prevent future yield losses, the ongoing advancement of resistant cultivars (Channa, 2021) requires information of the detection of new races and changing virulence patterns of rust fungus. For the recognition of resistance genes, incessant modeling of forecast, rigorous and frequent monitoring should be created in country. Uredinial stage is distinguishing character of leaf rust and diameter of uredinia is about 1.5 mm which round to ovoid, erumpent, orange to brown colored. It creates orange-brown sub-globoid urediniospores approximately 20 microm in diameter and having echinulate walls, up to 8-germ pores dispersed in thick (Bolton, 2008). Wheat leaf rust surveillance utilizing seedling differentials is very informative in describing geographical distribution of virulence pathotypes of *P. triticina*, their virulence variation and how phenotypes modify in response to selection of host. It was noticed while differentiating virulence/avirulence structure of the leaf rust pathogen population, near isogenic lines were greatly effective. These lines were utilized for particular rust resistance genes in previously published virulence studies (Kolmer and Liu, 2000) to facilitate determining relative frequency of pathotypes and virulence phenotypes (McIntosh *et al.*, 1995). Substantial number of wheat genotypes can be evaluated as seedling with distinct virulence phenotypes of pathogen and the ITs in contrast with wheat differential lines that vary for recognized *Lr* resistance genes (Hubbard *et al.*, 2015). Based on ITs on a differentials set, numerous strains of each formae speciale of rust species were initially recognized as races while further distinguished into pathotypes (Bhardwaj, 2012).

For race analysis, differential lines or varieties with known genes are considered as a fundamental and important constituent. These differential lines facilitate to recognize the genetics of interactions of host-parasite (McCallum *et al.*, 2016). Based on gene-for-gene specificity, resistance genes of leaf rust can be postulated in the wheat varieties by confirming which differential lines have low infection types to the similar virulence phenotypes as the wheat varieties. Particular knowledge can be provided with respect to individual genes by utilizing these differential lines, in the existing pathogen population. Hence, wheat improvement programs are guided through virulence pattern to design required genes to target in particular wheat producing regions. In the present research study, *P. triticina* collections from Pakistan were distinguished for virulence utilizing a standard set of differential hosts. For development of wheat cultivars having resistance against leaf rust, the surveys have been effective resource for long-term population biology studies of pathogen. Isolates obtained from surveys of virulence can be utilized for evaluating the genetic differentiation of *Puccinia triticina* genotype using technique of molecular markers. Objectives of the virulence investigation were to recognize predominant virulence phenotypes in the leading wheat producing regions, to identify the virulence divergence of the pathogen, to investigate the intensity and distribution of new phenotypes and describe if varieties of wheat with key resistance genes of leaf rust have had a discriminating impact on the pathogen population. Keeping the above goals, present studies has been carried out to understand virulence variation of leaf rust isolates from southern parts of Pakistan.

MATERIAL AND METHODS

Rust disease survey and collection of leaf rust

diseased leaf samples

Annual field surveys were conducted regularly across wheat producing regions in five distinct agro-ecological districts of Sindh Province, Pakistan at adult-plant stage (from late January to early April) during 2014-15 to 2015-16 cropping season. A collection comprised of flag leaves with uredinial infections collected from commercial wheat fields viz. Sakrand, Tandojam, Badin, Sanghar and Larkana Sindh province, Pakistan (Table 1). The disease severity of host cultivars (Sairab-92, PUNJ-86, Nowshera-96, DWR-97, Shalimar-88, Khyber-87, Punjab-85, Kohinoor-83, Sarhad-82, Punjab-81, Zarghoon-79, Dirik, LYP-73, PARI-73, SA-72, Chenab-70, Up-262, Sandal, Barani-70, Barani-83, C-228, SKD-1, Sassui, Pirsbk-04, Pirsbk-08, Mairaj-08, AAS-11, NIA Amber, Pavon and Khirman) was recorded visually on whole plants as the plant response (infection types) and percentage of plant tissue affected using modified Cobb's scoring scale of rust disease under natural field environment (Peterson *et al.*, 1948). Spores from two locations viz. Sakrand and Tandojam were not viable and could not be revived and only urediniospores of three locations (Larkana, Sanghar and Badin) were revived. Location, collector's information, date of collection, severity, cultivar, growth stage of the crop and any other relevant information was recorded for each sample collected. Diseased leaves were dispatched in glassine bags for pathogenicity tests to the Department of Plant Pathology, University of Minnesota, Saint Paul campus, USA, where these were dried at temperature of room and then located in a refrigerator at 4 °C until treated for virulence differentiation.

Table.1 List of leaf rust isolates collected from different wheat growing regions of Sindh, Pakistan.

S. No	Pathotypes	Country	Year of collection	Location	Host
1	PK16-10SG	Pakistan	2016	Tando Adam, Sanghar	Wheat
2	PK16-11SG	Pakistan	2016	Tando Adam, Sanghar	Wheat
3	PK16-12SG	Pakistan	2016	Tando Adam, Sanghar	Wheat
4	PK16-18SG	Pakistan	2016	Tando Adam, Sanghar	Wheat
5	PK16-21SG	Pakistan	2016	Tando Adam, Sanghar	Wheat
6	PK16-14BD	Pakistan	2016	Matli, Badin	Wheat
7	PK16-18BD	Pakistan	2016	Matli, Badin	Wheat
8	PK16-19BD	Pakistan	2016	Matli, Badin	Wheat
9	PK16-15LK	Pakistan	2016	Naudero, Larkana	Wheat
10	PK16-17LK	Pakistan	2016	Naudero, Larkana	Wheat
11	PK16-12TJ	Pakistan	2016	NIA, TandoJam	Wheat
12	PK16-23TJ	Pakistan	2016	NIA, TandoJam	Wheat
13	PK16-13SK	Pakistan	2016	WRI,Sakrand	Wheat
14	PK16-24SK	Pakistan	2016	WRI,Sakrand	Wheat

NIA= Nuclear Institute of Agriculture, Tandojam; WRI= Wheat research Institute, Sakrand

Plant material and experimental location

A set comprising of 24 Thatcher near-isogenic lines (McIntosh *et al.*, 1995) with known resistance genes of leaf rust were used for virulence analysis. In total, 10 isolates were evaluated at the plant growth facility greenhouse located at University of Minnesota, Saint Paul campus, MN (USA).

Plant growth conditions

The genotypes were planted in peat pots (7 x 5 x 9 cm; l x w x h) filled with a 50:50 mix of steam sterilized field soil: Sunshine MVP potting mix (gypsum, vermiculite, dolomitic limestone, nutrient charge and Canadian sphagnum peat moss) (Sun Gro Horticulture, Quincy, Michigan) and set inside plastic trays, each holding 16 pots. Sowing of seeds was conducted at uniform depth and distance.

Identification of virulence phenotypes

Leaf samples of rust disease were placed on moist filter paper in a petri dish which that was kept at temperatures 12-19^{o}C overnight. Fresh spores produced on the leaf pieces were used in inoculation for spore increase.

a) Isolation and multiplication of single-pustule

Urediniospores from individual collection were utilized to inoculate seven-day-old seedlings of the universal susceptible variety Morocco that does not contain any

recognized *Lr* resistance (Roelfs, 1992) gene that have been handled with a maleic hydrazide solution 30 ml (1 g dissolved in three liters of water) per pot to increase production of spore. Incubation (16 hours for developing disease for plants under the darkness at 22-25°C), green house environment, and assessment of ITs for sets of differential lines were as reported by (Kolmer and Hughes, 2016). After 12 to 15 days, two to three seedlings with single uredinium were saved per collection and individually protected with cellophane bags (145×235 mm) and tied up at the base with a rubber band to prevent cross contamination (Fetch Jr and Dunsmore, 2004). When single uredinia started to produce secondary rings, approximately 9-14 days after inoculation, urediniospores were collected from individual uredinia directly into gelatin capsules of zero size which firmly fitted with a cyclone spore collector to a vacuum line. Separate collection of urediniospores was taken from 2-3 single uredinia each collection. This spore increasing practice was incessant until adequate spores were created for inoculation of the differential host set.

b) Inoculation of differential host of wheat leaf rust

Differential sets were sown in the greenhouse of the MAES/MDA Plant Growth Facilities (PGF) East 1907 Dudley Avenue St. Paul, MN, 55108 and evaluated for rust infection types. High-pressure sodium lamps were provided as natural daylight from 0800 to 2400 h. In addition, 0.25 ml of oil was mixed with urediniospores of the single-uredinial isolates in the zero capsules of gel & directly inoculated by atomization onto 7- to eight-day old host series of the differentials (4-6 plants each row) of near- isogenic lines of Thatcher wheat with single resistance genes *Lr1*, *2a, 2c, 3, 3ka, 9, 10, 11, 14a, 16, 17, 18, 21, 24, 26, 28, 30, 39, 42, B, 3bg, 14b, 20* and *Lr23*.

c) Virulence phenotyping

Standard disease scoring scale 0- 4 was used for recording data on the infection types (ITs) for all the differential sets after 12 days of inoculation/ on appearance of pustules as described by (DL and Kolmer, 1989) and presented in (Table 2). (IT0 refers to uredinia absent; IT; equals hypersensitive flecks with necrosis without uredinia; IT1 defines small uredinia, with necrosis or chlorosis; IT2 terms uredinia size of small to medium, often enclosed with necrosis or chlorosis; IT3 indicates uredinia of medium-sized without necrosis or chlorosis; and IT4= uredinia without necrosis or chlorosis). The virulence patterns on near isogenic lines were evaluated based on low infection types generated by each line in response to infection (IT)=(0, 0; (fleck), 1, 1+, 2 and 2+ represented avirulent while 3-, 3+ and 4 represent virulent) adopted by (DL and Kolmer, 1989).

Table 2. Virulence/avirulence pattern of Pakistan wheat leaf rust pathotypes detected at seedlings of U.S near isogenic lines (NILs).

Pathotypes	Virulence	Avirulence
MSCTNS	1, 3a, 9, 16, 24, 30, B, 10, 14, 18, 21, 41, 3bg,14b, 20	2a, 2c, 26, 3ka, 11, 17, 28, 42, 23
RTSTNS	1, 2a, 3a, 9, 16, 24, 26, 3ka, 11, 17, B, 10, 14a, 18, 21, 41, 3bg, 14b, 20	2c, 30, 28, 42, 23
RKTRGS	1, 2a, 3a, 16, 24, 26, 3ka, 11, 17, 30, B, 10, 18, 28, 3bg, 14b, 20	2c, 9, 14a, 21, 41, 42, 23
PNDQDS	1, 2c, 3a, 9, 24, 17, B, 10, 41, 3bg, 14b, 20	2a, 26, 3ka, 11, 30, 14a, 18, 21, 28, 42, 23
JDBQGJ	2a, 2c, 24, B, 10, 28, 14b, 20	1, 3a, 9, 16, 26, 3ka, 11, 17, 30, 14a, 18, 21, 41, 42, 3bg, 23
MDPSDS	1, 3a, 24, 3ka, 17, 30, B, 10, 14a, 41, 3bg, 14b, 20	2a, 2c, 9, 16, 26, 11, 18, 21, 28, 42, 23
RTPTNS	1, 2a, 3a, 9, 16, 24, 26, 3ka, 17, 30, B, 10, 14a, 18, 21, 41, 14b, 20	2c, 11, 28, 42, 23
MNPSDS	1, 3a, 9, 24, 3ka, 17, 30, B, 10, 14a, 41, 3bg, 14b, 20	2a, 2c, 16, 26, 11, 18, 21, 28, 42, 23
MJLTGS	1, 3a, 16, 24, 3ka, B, 10,14a, 18, 28, 3bg,14b, 20	2a, 2c, 9, 26, 11, 17, 30, 21, 41, 42, 23
MSPTDS	1, 3a, 9,16, 24, 3ka, 17, 30, B, 10, 14a, 18, 41, 3bg, 14b, 20	2a, 2c, 26, 11, 21, 28, 42, 23

Virulence/avirulence formulae according to US differentials in Table. NILs= near isogenic lines

d) Race Designation

Race analysis was based on reaction of inoculated known near isogenic lines. A six letter code based on the original code recommended for pathogen (DL and

Kolmer, 1989) explains the low or high (ITs) of individually isolate to the 24 lines of differential. Single letter links to the (ITs) of 4 lines of differentials. The Thatcher lines with genes *Lr1, Lr2a, Lr2c* and *Lr3* were the four lines in the first set of differentials; lines with genes *Lr9, Lr16, Lr24* and *Lr26* were the second set; lines with genes *Lr3ka, Lr11, Lr17* and *Lr30* were the third set; lines with genes *LrB, Lr10, Lr14a* and *Lr18* were the fourth set; lines with genes *Lr21, Lr28, Lr39* and *Lr42* were the fifth set and *Lr3bg, Lr14b, Lr20* and *Lr23* were the sixth set of differentials (Supplementary Table 1).

To describe the low or high infection type of each rust isolate to the 24 North American differential host rows, individual letter corresponded to the four differentials infection types (Supplementary Table 1). For example, (LITs) on the 4 hosts in a set was categorized with the 'B' letter, whereas (HIT) on the 4 hosts was characterized with a 'T' letter. Hence, if an isolate produced low infection type (resistant reaction) on the 24 differential hosts, the race was assigned with a six letter race code 'BBBBBB'. Likewise, if an isolate that produced a HIT (susceptible reaction) on the 24 wheat differential hosts have a race code 'TTTTTT'. When feasible, isolates with phenotypes that were documented only once were investigated a second time to validate their phenotype.

RESULTS

Virulence composition of pathotypes diversified significantly among locations (Table 3). *Puccinia triticina* pathotypes from Badin and Larkana locations were virulent to genes *Lr2c and Lr11*. Data recording showed that virulence for *Lr2a, Lr2c, Lr26, Lr11, Lr18, Lr21, Lr42* and *Lr23* was not recognized from pathotypes collected from Larkana only. Virulence for *Lr23* and *Lr42* was not observed from any of the pathotypes. However, pathotypes from all the locations were virulent to leaf rust resistance genes i.e. *Lr1, Lr3, Lr9, Lr16, Lr24, Lr3ka, Lr17, Lr30, LrB, Lr10, Lr14a, Lr18, Lr41, Lr3bg, Lr14b* and *Lr20* (Table 4).

Similar virulence responses were recorded through evaluation of leaf rust field nurseries at all locations as most of isogenic lines containing leaf rust resistance genes i.e. *Lr1, Lr3, Lr16, Lr24, Lr3ka, Lr17, Lr30, Lr10, Lr14a, Lr18, Lr14b* and *Lr20* identified ineffective to all pathotypes at seedling stage, also showed susceptible reactions at all locations under natural field conditions. While *Lr9, Lr19* and *Lr28* were found effective at all tested locations.

Lines with genes *Lr9* and *Lr28* which were resistant at field conditions were recorded with similar results at seedling stage as *Lr28* had high infection types (3+HITs) with just three pathotypes from 2 locations i.e. RKTRGS, JDBQGJ (Sanghar) and MJLTGS (Larkana). No virulence for *Lr28* was recorded pathotype from Badin. While *Lr9* had low infection types (0, ;1, ;2, 2+LITs) with just four pathotypes i.e. RKTRGS, JDBQGJ (Sanghar), MDPSDS (Badin) and MJLTGS (Larkana) (Table 5).

Virulence data showed that seedlings of 24 Thatcher near-isogenic lines having leaf rust resistance genes *Lr42* and *Lr23* continuously displayed low infection types (resistance response) with all pathotypes rated 0, ;1 and 2+ when inoculated with 10 different pathotypes. *Lr24, LrB, Lr10, Lr14b* and *Lr20* exhibited susceptibility response i.e. (HITs) 3 and 4 with all pathotypes (Table 5). Results also revealed that *Lr2a, Lr2c, Lr30, Lr18 and Lr28* had intermediate ITs with some other pathotypes. *Lr1, Lr3a*and *Lr3bg* had high infection types except one pathotype. *Lr21* displayed all low (ITs 0; 1, 2) with all except three pathotypes with high infection types (HIT 3+).

Table 3. Virulence phenotypes of *Puccinia triticina* identified by virulence to 24 wheat isogenic lines with single leaf rust resistance genes from Sindh province, Pakistan.

Locations	Number of Isolates	Number of pathotypes
Sanghar	5	5
Badin	3	3
Larkana	2	2
Total	10	10

Total number of virulence phenotypes were identified from 10 isolates of different locations

Table 4. Virulence pattern of *Puccinia triticina* collected from different wheat growing regions/ locations of Sindh province, Pakistan.

	Sanghar	Badin	Larkana
Lr1	+	+	+
Lr2a	+	+	-
Lr2c	+	-	-
Lr3a	+	+	+
Lr9	+	+	+
Lr16	+	+	+
Lr24	+	+	+
Lr26	+	+	-
Lr3ka	+	+	+
Lr11	+	-	-
Lr17	+	+	+
Lr30	+	+	+
LrB	+	+	+
Lr10	+	+	+
Lr14a	+	+	+
Lr18	+	+	+
Lr21	+	+	-
Lr28	+	-	+
Lr41	+	+	+
Lr42	-	-	-
Lr3bg	+	+	+
Lr14b	+	+	+
Lr20	+	+	+
Lr23	-	-	-

Based on virulence data, 10 virulence phenotypes were characterized among the ten isolates investigated for virulence. These ten virulence phenotypes viz., MSCTNS, RTSTNS, RKTRGS, PNDQDS, JDBQGJ, MDPSDS, RTPTPS, MNPSDS, MJLTGS and MSPTDS were designated with race code (Supplementary Table 2-11) utilizing North American leaf rust differential set (Long and Kolmer, 1989) were collected from different locations i.e. Badin, Sanghar and Larkana Sindh province, Pakistan (Table 5). The pathotype JDBQGJ from (Sanghar) had narrow spectrum while pathotype RTSTNS from (Sanghar) & pathotype RTPTPS from (Badin) were detected with broad spectrum as compare to all other pathotypes tested.

Table 5. Seedling infection types, virulence displayed by 10 pathotypes of *Puccinia triticina* on *Lr* near isogenic lines.

Differential sets	SANGHAR					BADIN			LARKANA	
	1	2	3	4	5	6	7	8	9	10
	MSCTNS	RTSTNS	RKTRGS	PNDQDS	JDBQGJ	MDPSDS	RTPTNS	MNPSDS	MJLTGS	MSPTDS
TcLr1	3	3	3+	3	2+	3	3+	3	3+	3
TcLr2a	2+	3	3	2-	3+	;2	3	0;	;1	0;
TcLr2c	0	2+	0	3	3	0;	2	0;1	;1	2
TcLr3a	3+	3	3	3	2	3	3	3	3+	3-
TcLr9	3	3	;1	3	;2	0	3+	3	;2+	3
TcLr16	3	3	3+	2	;2	2	3+	2	3+	3-
TcLr24	3	3	3+	3	3	3	3	3	3+	3

TcLr26	2+	3	3+	;1	2	2	3	12	;1	2
TcLr3ka	2	3	3+	2-	2-	3	3	3-	3	3
TcLr11	2	3	3+	;1	2-	2	2	2	2	12
TcLr17	0	3	3+	3	;1	3	3+	3	;1	3
TcLr30	3	2	3+	2+	2,2+	3	3	3	2	3
TcLrB	3+	3	3+	3+	3	3	3	3	3	3-
TcLr10	3	3	3+	3	3	3	3	3-	3	3
TcLr14a	3	3	2+	2	2+	3	3	3	3	3
TcLr18	3	3	3	2	2+	2	3	2+	3	3
TcLr21	3	3	2	2,1	2+	2+	3	0;1	;1	12
TcLr28	;2	;2	3	1	3	0;	0	0;	3	0;2
TcLr41	3	3	0	3	2	3	3	3	;2	3
TcLr42	;1	1	0	;1	;1	;1	;1	1	;1	0;1
TcLr3bg	3+	3	3	3	2+	3	3	3-	3+	3
TcLr14b	3	3	3	3	3	3	3	3	3	3
TcLr20	3	3	3	3	3	3	3	3-	3+	3
TcLr23	2+	0	2+	;1	2+,	;1	0	0;1	;2	1

Infection types: 0 = no flecks or uredinia, 0; = faint hypersensitive flecks; = hypersensitive flecks, 1 = small uredinia with necrosis, 2 = small to medium uredinia with necrosis, 3 = moderate to large size uredinia with/without chlorosis, 4 = very large uredinia without chlorosis, X = mesothetic, a mixture of resistant pustule types, ''?'' = indicates slightly larger uredinia, ''-'' = indicates slightly smaller uredinia, infection types (ITs) with two symbols denote a range: e.g., 22? = indicates a mixture of 2 sizes of uredinia with chlorosis and slightly larger uredinia with chlorosis

Results revealed that MSCTNS, RTSTNS, RKTRGS, PNDQDS and JDBQGJ pathotypes had high virulence on leaf rust resistance genes *Lr24, LrB, Lr10, Lr14b, Lr20* collected from Sanghar Sindh province, Pakistan (Table 5). High virulence was recorded for resistance genes *Lr1, Lr3a* and *Lr3bg* with MSCTNS, RTSTNS, RKTRGS and PNDQDS pathotypes, while high virulence was observed for genes *Lr2a, Lr9, Lr16, Lr17, Lr18* and *Lr41* with3 pathotypes and *Lr2c, Lr26, Lr3ka, Lr11, Lr30, Lr14a, Lr21* and *Lr28* showed high infection types with 2 pathotypes. Whereas *Lr42* and *Lr23* has showed low infection types with all MSCTNS, RTSTNS, RKTRGS, PNDQDS and JDBQGJ pathotypes (Table 5).

Result revealed that leaf rust resistance genes *Lr1, Lr3a, Lr24, Lr3ka, Lr17, Lr30, LrB, Lr10, Lr14a, Lr41, Lr3bg, Lr14b* and *Lr20* showed ineffectiveness with all (MDPSDS, RTPTPS and MNPSDS) tested pathotypes from Badin Sindh province, Pakistan and displayed high infection types (Table 5). *Lr9* had high infection types with RTPTPS and MNPSDS pathotypes. No virulence was recorded for leaf rust resistance genes viz*., Lr2c, Lr11, Lr28, Lr42* and *Lr23*as they displayed (LITs) and effectiveness to MDPSDS, RTPTPS and MNPSDS pathotypes. While *Lr2a, Lr16, Lr26, Lr18* and *Lr21* had low reactions to MDPSDS and MNPSDS pathotypes.

Result revealed that leaf rust resistance genes *Lr1, Lr3a, Lr16, Lr24, Lr3ka, LrB, Lr10, Lr14a, Lr18, Lr3bg, Lr14b* and *Lr20* demonstrated their ineffectiveness against pathogen population of *P. triticina.* Resistance genes *Lr2a, Lr2C, Lr26, Lr11, Lr21, Lr42* and *Lr23* had low reactions with MJLTGS and MSPTDS pathotypes at Larkana Sindh, Pakistan. *Lr9, Lr17, Lr30, Lr28* and *Lr41* showed intermediate response (Table 5).

DISCUSSION

Screening of wheat germplasm for resistance and identification of new pathotypes of *Puccinia triticina* with the wide climatic adaptation and experimentation on virulence variation have been ongoing from past years (Jain *et al.*, 2004). Because of pathogen has high degree of virulence variation associated with adaptation to broader climatic conditions (Morgounov *et al.*, 2012), stable leaf rust resistance become difficult to achieve (Kolmer and Hughes, 2013). And due to the most of resistant genes have lost their resistance after the quick emergence of virulent pathotypes as were originally derived from common bread wheat and could have

provided durable resistance (Kolmer *et al.*, 2008). The population of *P. triticina* is greatly diverse with a huge of number of virulence phenotypes in Pakistan, but very few earlier reports (Rehman, 2006; Fayyaz *et al.,* 2008; Rattu *et al.,* 2009) are present for virulence in population of *P. triticina*. One of the earlier reports have been presented by (Nagarajan and Joshi, 1985) in which they identified and listed leaf rust races in Pakistan utilizing international standard differentials.

In current study, 24 near isogenic (differential) lines were used to differentiate races by their phenotypic reactions to various pathogenic strains and relatively distinct population of *P. triticina* was recorded for virulence phenotype in Pakistan. Collections of leaf rust from distinct wheat cultivating regions of Sindh, Pakistan were evaluated and result of each location was compared to data of other. Result of locations revealed that ten isolates of leaf rust were collected, tested for virulence and further identified as ten virulence phenotypes. Determination of virulence spectrum was carried out by the number of differential lines that the pathotype displayed virulence. A pathotype having virulence on fewer resistance genes of leaf rust was considered to have narrow spectrum as compared to those pathotypes with virulence to relatively higher number of differential lines (Mebrate *et al.*, 2008). Approximately 90% of the pathotypes (9 of 10) were recorded having wider spectrum of virulence ranging from 12 to 19 (out of 24) leaf rust resistance genes while just one pathotype had narrow spectrum virulence against 8 leaf rust resistance genes.

Result revealed that Sanghar and Badin locations collectively with eight pathotypes tested, had the highest (19) number of virulence phenotypes on differential lines and caused most of leaf rust resistance genes ineffective (Table 4). Data analysis showed the most broad spectrum virulences were recorded for phenotype RTSTNS (virulent to *Lr1, 2a, 3a, 9, 16, 24, 26, 3ka, 11, 17, B, 10, 14a, 18, 21, 41, 3bg, 14b* and *20*) and phenotypes RTPTNS virulent to (*Lr1, 2a, 3a, 9, 16, 24, 26, 3ka, 17, 30, B, 10, 14a, 18, 21, 41, 14b* and *20*) genes. The second most phenotype with broad spectrum virulence was RKTRGS from Badin (2nd highest 17 number of virulence phenotypes) with broad spectrum was virulent to genes (*Lr1, 2a, 3a, 16, 24, 26, 3ka, 11, 17, 30, B, 10, 18, 28, 3bg, 14b* and *20*). While Larkana location with only two isolates tested, had high (16) virulence phenotypes that recorded at third most phenotype MSPTDS with broad spectrum virulence to genes (*Lr1, 3a, 9, 16, 24, 3ka, 17, 30, B, 10, 14a, 18, 41, 3bg, 14b* and *20*). The broader range of virulence among the population of *P. triticina* pathotypes existed in current study. This may be related with large population size of pathogen leads to diversification of virulence/ avirulence pattern and greater possibility of mutants existed in the crop (Schafer and Roelfs, 1985). Existence of prevalent mutants with dissimilarities to others, depends on the type of wheat varieties cultivated in region (Singh, 1991) and especially on temperature (Roelfs, 1992). Significant distinction in virulence between these pathotype may resulted from the constant evolution of pathogen by different variation processes (sexual recombination, migration, selection pressure on race specific resistance and mutation). Likewise, other five pathotypes (MSCTNS, MNPSDS, MDPSDS, MJLTGS and PNDQDS) had the similar virulent spectrum, each produced virulence on 15, 14, 13, 13 and 12 *Lr* genes tested respectively. However, JDBQGJ proved to be the least virulent pathotype making just 8 *Lr* genes susceptible and produced compatible reaction only on *Lr2a, 2c, 24, B, 10, 28, 14b* and *20* genes respectively (Table 3). Determination of virulence spectrum was carried out by the number of lines that the pathotype displayed virulence. (Kolmer *et al.*, 2017) reported that FHPSQ and KHPQQ phenotypes were characterized as common phenotypes in Pakistan and found virulent to genes *Lr3, Lr10, Lr16, Lr17* and *Lr26* while PBMQQ recorded as third frequent phenotype, was virulent to gene *Lr1, Lr3* and *Lr10.* (Rattu *et al.*, 2009) recorded high (>80%) virulence frequency to most of isogenic lines in Pakistan in the population of *P. triticina* while just <10% of isolates were virulent on lines with genes *Lr9, Lr19* and *Lr28*. Diversity and distribution of leaf rust pathotypes indicated that similar virulent spectrum among pathotypes existed. This might be due to fact that extended period of cultivation of single cultivar in a particular region, their geographic proximity which have played important role for pathotype similarity. According to (Ahmad *et al.*, 2010) leaf rust pathogen is possibly more destructive when huge region are cultivated with single, genetically homogeneous cultivars which play important role for its susceptibility. (McVey *et al.*, 2004), also recorded genetic similarity between leaf rust populations from (southern and central plains) United States and Egypt. Results revealed that all five (5) pathotypes evaluated were virulent to

the members of the differential hosts including (*Lr24, B, 10, 14b* and *20*). Except JDBQGJ, all other (4) pathotypes were found virulent to *Lr* genes *Lr1, Lr3a* and *Lr3bg* (Table 2). Seven pathotypes were virulent to *Lr11, 17, 14a* and *41* while six pathotypes were virulent to genes *Lr9, 16, 30* and *18* whereas variation was recorded for virulence to all other resistance genes. Data analysis also showed that intermediate infection types (from no uredinia to moderate uredinia surround by chlorosis) were recorded for differential lines with leaf rust resistance genes *Lr2a, 2c* and *28* while (moderate to large uredinia surrounded by chlorosis- yellowish leaves with prominently green veins) with leaf rust genes *Lr30* and *Lr18* which could be considered (HITs) under certain greenhouse conditions. Of the 24 differential wheat lines utilized to distinguish the prevailing isolates just two of isogenic lines containing resistance genes (*Lr42* and *Lr23*) continuously displayed (LITs 0, 1, and 2+) resistance response with all pathotypes investigated. (Dyck and Johnson, 1983) reported that for expression of leaf rust resistance gene, *Lr23* is temperature-sensitive at seedling stage. Thatcher line with *Lr23* will produce a very low infection type if grown in cabinet with many US leaf rust isolates. The existence of dissimilar virulence phenotypes of *P. triticina* at the different localities may describe for varying levels of resistance among the tested varieties at locations. Results obtained from the virulence surveys can also be utilized to recognize those genes which provide effective resistance against leaf rust and to select germplasm having resistance against pathogen in wheat improvement strategies. Current virulence studies revealed moderate variation in virulence of leaf rust pathogen originating in southern parts of the country. However extensive virulence studies and molecular investigations of pathogen with adequate number of isolates from across wheat producing regions of Pakistan will substantially help to recognize the potential sources of diversity in pathogen population, genetical structure and epidemiology. Generated knowledge will improve in pathogen's control.

CONCLUSION

Virulence investigation of Pakistani leaf rust isolates under standard greenhouse conditions showed no virulence for resistance genes *Lr42* and *Lr23* from all tested pathotypes (Sanghar, Badin and Larkana) locations, while *Lr2c* and *Lr11* genes were found effective against all the isolates tested from two (Badin and Larkana) locations of Sindh Province, Pakistan. However, *Lr24, LrB, Lr10, Lr14b* and *Lr20* exhibited susceptibility response i.e. (HITs 3 & 4) against all pathotypes. Based on virulence data, 10 virulence phenotypes were characterized viz., MSCTNS, RTSTNS, RKTRGS, PNDQDS, JDBQGJ, MDPSDS, RTPTPS, MNPSDS, MJLTGS and MSPTDS. Among the ten pathotypes, JDBQGJ had narrow spectrum while RTSTNS & RTPTPS pathotypes had broad spectrum as compared to all other tested. These genes (*Lr42* and *Lr23*) can be exploited in breeding programmes of wheat for leaf rust resistance preferably combining minor and major genes to achieve durable resistance. Generated information about single leaf rust resistance genes status will be useful in devising effective management strategy against the prevailing population of leaf rust pathogen.

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Conflict of Interest

There is no conflict of interest among authors.

Author's Contribution

Dr. Hadi Bux, contributed to the study conception, designed research experiments and supervised, Abdul W. Channa, conducted research experiments, prepared material, collected data and analyzed and wrote the manuscript. Dr. Mahboob Ali Sial, contributed in designing of study and editing of manuscript; Dr. Ghulam H. Jatoi & Raj Kumar contributed in write up and finalized the paper. All authors have revised and approved the final manuscript.

Supplementary Table 1. Wheat near isogenic lines for leaf rust virulence test in glass house.

S. No	Isogenic Line	*Lr* Genes
1	*TcLr1*	*Lr1*
2	*TcLr2a*	*Lr2a*
3	*TcLr2c*	*Lr2c*
4	*TcLr3a*	*Lr3a*
5	*TcLr9*	*Lr9*
6	*TcLr16*	*Lr16*
7	*TcLr24*	*Lr24*
8	*TcLr26*	*Lr26*
9	*TcLr3ka*	*Lr3ka*
10	*TcLr11*	*Lr11*
11	*TcLr17*	*Lr17*
12	*TcLr30*	*Lr30*
13	*TcLrB*	*LrB*
14	*TcLr10*	*Lr10*
15	*TcLr14a*	*Lr14a*
16	*TcLr18*	*Lr18*
17	*TcLr21*	*Lr21*
18	*TcLr28*	*Lr28*
19	*TcLr41*	*Lr41*
20	*TcLr42*	*Lr42*
21	*TcLr3bg*	*Lr3bg*
22	*TcLr14b*	*Lr14b*
23	*TcLr20*	*Lr20*
24	*TcLr23*	*Lr23*

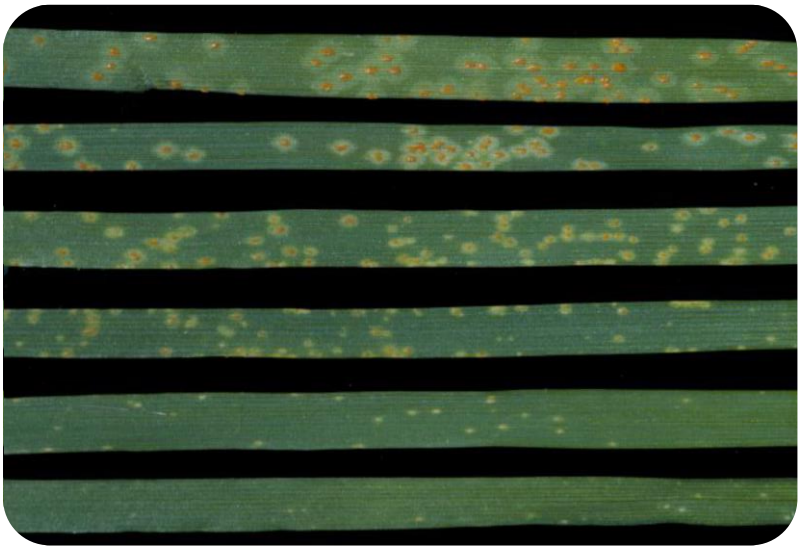
Supplementary Table 2. Description of Infection type (0-4) scale for wheat leaf rust and symptoms (Long and Kolmer., 1989)

Infection type	Host response	Symptoms
0	Low	No uredinia or other macroscopic sign of infection
0;	Low	Few faint flecks
;	Low	No uredinia, but hypersensitive necrotic or chlorotic flecks present
1	Low	Small uredinia often surrounded by a necrosis
2	Low	Small to medium uredinia often surrounded by chlorosis
Y	Low	Ordered distribution of variable-sized uredinia with largest at leaf tip
X	Low	Random distribution of variable-sized uredinia
3	High	Medium-sized uredinia without chlorosis or necrosis
4	High	Large uredinia without chlorosis or necrosis

Infection types of wheat leaf rust used in disease assessment at seedling stage adopted by (Long and Kolmer., 1989)

Modified Characters

=	low uredia	Uredinia at the lower size limit for the infection type
-	Smaller uredia	Uredinia somewhat smaller than normal for the infection type
+	Large Uredinia	Uredinia somewhat larger than normal for the infection type
++	Larger Uredinia	Uredinia at the upper size limit for the infection type
C,		More chlorosis than normal for the infection type
N,		More necrosis than normal for the infection type



Source: Long and Kolmer, 1989.

0; ; ;1 ;2 2, 2+ 3+4

Figure-1 Infection type (IT) 0; - 2+ avirulent, 3-4 virulent
(Wheat Leaf Rust Rating Scale USDA-ARS St. Paul, MN) (Long and Kolmer., 1989)

Supplementary Table 3. List of leaf rust pathotypes identified from Pakistan.

S. No	Pathotypes	Country	Year of collection	Location	Host
1	MSCTNS	Pakistan	2016	Tando Adam, Sanghar	Wheat
2	RTSTNS	Pakistan	2016	Tando Adam, Sanghar	Wheat
3	RKTRGS	Pakistan	2016	Tando Adam, Sanghar	Wheat
4	PNDQDS	Pakistan	2016	Tando Adam, Sanghar	Wheat
5	JDBQGJ	Pakistan	2016	Tando Adam, Sanghar	Wheat
6	MDPSDS	Pakistan	2016	Matli, Badin	Wheat
7	RTPTNS	Pakistan	2016	Matli, Badin	Wheat
8	MNPSDS	Pakistan	2016	Matli, Badin	Wheat
9	MJLTGS	Pakistan	2016	Naudero, Larkana	Wheat
10	MSPTDS	Pakistan	2016	Naudero, Larkana	Wheat

NIA= Nuclear Institute of Agriculture, Tandojam; WRI= Wheat research Institute, Sakrand

Supplementary Table 4. Nomenclature of *P. triticina* races on 16 North American differential hosts in ordered sets of six for race identification (based on Long and Kolmer., 1989)

	Host set	Infection type (ITs) produced on differential *Lr* lines			
Pt code	Host set 1	1	2a	2c	3
	Host set 2	9	16	24	26
	Host set 3	3ka	11	17	30
	Host set 4	B	10	14a	18
	Host set 5	21	28	41	42
	Host set 6	3bg	14b	20	23
B		L	L	L	L
C		L	L	L	H
D		L	L	H	L
F		L	L	H	H
G		L	H	L	L
H		L	H	L	H
J		L	H	H	L
K		L	H	H	H
L		H	L	L	L
M		H	L	L	H
N		H	L	H	L
P		H	L	H	H
Q		H	H	L	L
R		H	H	L	H
S		H	H	H	L
T		H	H	H	H

L =Low infection type, H=High infection type

Supplementary Table 5: Virulence pattern of leaf rust isolates collected from Tando Adam, Sanghar Sindh Province, Pakistan & its race designation based on (Long and Kolmer., 1989).

Set	Entry	Line	*Lr* gene	IT 1	Reaction	Code
1	1	*TcLr1*	1	3	H	M
	2	*TcLr2a*	2a	2+	L	
	3	*TcLr2c*	2c	0	L	
	4	*TcLr3a*	3a	3+	H	
2	5	*TcLr9*	9	3	H	S
	6	*TcLr16*	16	3	H	
	7	*TcLr24*	24	3	H	
	8	*TcLr26*	26	2+	L	
3	9	*TcLr3ka*	3ka	2	L	C
	10	*TcLr11*	11	2	L	
	11	*TcLr17*	17	0	L	
	12	*TcLr30*	30	3	H	
4	13	*TcLrB*	B	3+	H	T
	14	*TcLr10*	10	3	H	
	15	*TcLr14a*	14a	3	H	
	16	*TcLr18*	18	3	H	
5	17	*TcLr21*	21	3	H	N
	18	*TcLr28*	28	;2	L	
	19	*TcLr41*	41	3	H	
	20	*TcLr42*	42	;1	L	
6	21	*TcLr3bg*	3bg	3+	H	S
	22	*TcLr14b*	14b	3	H	
	23	*TcLr20*	20	3	H	
	24	*TcLr23*	23	2+	L	

A *Pt* code comprises of the designation for set I followed by that for set 2, etc. Viz, race MSCTNS: set 1 (M) -virulent to *Lr1, 3a;* set 2 (S) - virulent to *Lr9, l6, 24;* set 3 (C) - virulent to *Lr30*; set 4 (T) - virulent to LrB, 10, 14a, 18; set 5 (N) - virulent to *Lr21, 41*; set 6 (S) - virulent to *Lr3bg, 14b, 20*

Supplementary Table 6: Virulence pattern of leaf rust isolates collected from Tando Adam, Sanghar Sindh Province, Pakistan & its race designation based on Long and Kolmer., (1989)

Set	Entry	Line	*Lr* gene	IT 1	Reaction	Code
1	1	*TcLr1*	1	3	H	R
	2	*TcLr2a*	2a	3	H	
	3	*TcLr2c*	2c	2+	L	
	4	*TcLr3a*	3a	3	H	
2	5	*TcLr9*	9	3	H	T
	6	*TcLr16*	16	3	H	
	7	*TcLr24*	24	3	H	
	8	*TcLr26*	26	3	H	
3	9	*TcLr3ka*	3ka	3	H	S
	10	*TcLr11*	11	3	L	
	11	*TcLr17*	17	3	H	
	12	*TcLr30*	30	2	H	
4	13	*TcLrB*	B	3	H	T

	14	*TcLr10*	10	3	H	
	15	*TcLr14a*	14a	3	H	
	16	*TcLr18*	18	3	H	
5	17	*TcLr21*	21	3	H	N
	18	*TcLr28*	28	;2	L	
	19	*TcLr41*	41	3	H	
	20	*TcLr42*	42	1	L	
6	21	*TcLr3bg*	3bg	3	H	S
	22	*TcLr14b*	14b	3	H	
	23	*TcLr20*	20	3	H	
	24	*TcLr23*	23	0	L	

A *Pt* code comprises of the designation for set I followed by that for set 2, etc. Viz., race RTSTNS: set 1 (R) -virulent to *Lr1, 2a, 3a;* set 2 (T) - virulent to *Lr9, l6, 24,26*; set 3 (S) - virulent to *Lr3ka, 11, 17;* set 4 (T) - virulent to *LrB, 10, 14a, 18*; set 5 (N) - virulent to *Lr21, 41*; set 6 (S) - virulent to *Lr3bg, 14b, 20, 3*

Supplementary Table 7: Virulence pattern of leaf rust isolates collected from Tando Adam, Sanghar Sindh Province, Pakistan & its race designation based on Long and Kolmer., (1989)

Set	Entry	Line	*Lr* gene	IT 1	Reaction	Code
1	1	*TcLr1*	1	3+	H	R
	2	*TcLr2a*	2a	3	H	
	3	*TcLr2c*	2c	0	L	
	4	*TcLr3a*	3a	3	H	
2	5	*TcLr9*	9	;1	L	K
	6	*TcLr16*	16	3+	H	
	7	*TcLr24*	24	3+	H	
	8	*TcLr26*	26	3+	H	
3	9	*TcLr3ka*	3ka	3+	H	T
	10	*TcLr11*	11	3+	H	
	11	*TcLr17*	17	3+	H	
	12	*TcLr30*	30	3+	H	
4	13	*TcLrB*	B	3+	H	R
	14	*TcLr10*	10	3+	H	
	15	*TcLr14a*	14a	2+	L	
	16	*TcLr18*	18	3	H	
5	17	*TcLr21*	21	2	L	G
	18	*TcLr28*	28	3	H	
	19	*TcLr41*	41	0	L	
	20	*TcLr42*	42	0	L	
6	21	*TcLr3bg*	3bg	3	H	S
	22	*TcLr14b*	14b	3	H	
	23	*TcLr20*	20	3	H	
	24	*TcLr23*	23	2+	L	

A *Pt* code comprises of the designation for set I followed by that for set 2, etc. Viz., race RKTRGS: set 1 (R) -virulent to *Lr1, 2a, 3a*; set 2 (K) - virulent to *Lrl6, 24, 26*; set 3 (T) - virulent to *Lr3ka, 11, 17, 30*; set 4 (R) - virulent to *LrB, 10, 18a*; set 5 (G) - virulent to *Lr28*; set 6 (S) - virulent to *Lr3bg, 14b, 20, 3*

Supplementary Table 8: Virulence pattern of leaf rust isolates collected from Tando Adam, Sanghar Sindh Province, Pakistan & its race designation based on Long and Kolmer., (1989)

Set	Entry	Line	*Lr* gene	IT 1	Reaction	Code
1	1	*TcLr1*	1	3	H	P
	2	*TcLr2a*	2a	2-	L	
	3	*TcLr2c*	2c	3	H	
	4	*TcLr3a*	3a	3	H	
2	5	*TcLr9*	9	3	H	N
	6	*TcLr16*	16	2	L	
	7	*TcLr24*	24	3	H	
	8	*TcLr26*	26	;1	L	
3	9	*TcLr3ka*	3ka	2-	L	D
	10	*TcLr11*	11	;1	L	
	11	*TcLr17*	17	3	H	
	12	*TcLr30*	30	2+	L	
4	13	*TcLrB*	B	3+	H	Q
	14	*TcLr10*	10	3	H	
	15	*TcLr14a*	14a	2	L	
	16	*TcLr18*	18	2	L	
5	17	*TcLr21*	21	2,1	L	D
	18	*TcLr28*	28	1	L	
	19	*TcLr41*	41	3	H	
	20	*TcLr42*	42	;1	L	
6	21	*TcLr3bg*	3bg	3	H	S
	22	*TcLr14b*	14b	3	H	
	23	*TcLr20*	20	3	H	
	24	*TcLr23*	23	;1	L	

A *Pt* code comprises of the designation for set I followed by that for set 2, etc. Viz., race PNDQDS: set 1 (P) -virulent to *Lr1, 2c, 3a*; set 2 (N) - virulent to *Lr9, 24*; set 3 (D) - virulent to *Lr, 17*; set 4 (Q) - virulent to *LrB, 10*; set 5 (D) - virulent to *Lr41*; set 6 (S) - virulent to *Lr3bg, 14b, 20*

Supplementary Table 9: Virulence pattern of leaf rust isolates collected from Tando Adam, Sanghar Sindh Province, Pakistan & its race designation based on Long and Kolmer, (1989)

Set	Entry	Line	Lr gene	IT 1	Reaction	Code
1	1	*TcLr1*	1	2+	L	J
	2	*TcLr2a*	2a	3+	H	
	3	*TcLr2c*	2c	3	H	
	4	*TcLr3a*	3a	2	L	
2	5	*TcLr9*	9	;2	L	D
	6	*TcLr16*	16	;2	L	
	7	*TcLr24*	24	3	H	
	8	*TcLr26*	26	2	L	
3	9	*TcLr3ka*	3ka	2-	L	B
	10	*TcLr11*	11	2-	L	
	11	*TcLr17*	17	;1	L	
	12	*TcLr30*	30	2,2+	L	
4	13	*TcLrB*	B	3	H	Q

	14	*TcLr10*	10	3	H	
	15	*TcLr14a*	14a	2+	L	
	16	*TcLr18*	18	2+	L	
5	17	*TcLr21*	21	2+	L	G
	18	*TcLr28*	28	3	H	
	19	*TcLr41*	41	2	L	
	20	*TcLr42*	42	;1	L	
6	21	*TcLr3bg*	3bg	2+	L	J
	22	*TcLr14b*	14b	3	H	
	23	*TcLr20*	20	3	H	
	24	*TcLr23*	23	2+, 3	L	

A *Pt* code comprises of the designation for set I followed by that for set 2, etc. Viz., race JDBQGJ: set 1 (J) -virulent to *Lr2a, 2c*; set 2 (D) - virulent to *Lr24*; set 3 (B) - avirulent; set 4 (Q) - virulent to *LrB, 10*; set 5 (G) - virulent to *Lr28*; set 6 (J) - virulent to *Lr14b, 20*

Supplementary Table 10: Virulence pattern of leaf rust isolates collected from Matli, Badin Sindh Province, Pakistan & its race designation based on Long and Kolmer, (1989)

Set	Entry	Line	Lr gene	IT 1	Reaction	Code
1	1	*TcLr1*	1	3	H	M
	2	*TcLr2a*	20	0;	L	
	3	*TcLr2c*	2c	0;1	L	
	4	*TcLr3a*	3a	3	H	
2	5	*TcLr9*	9	2	L	D
	6	*TcLr16*	16	2	L	
	7	*TcLr24*	24	3	H	
	8	*TcLr26*	26	12	L	
3	9	*TcLr3ka*	3ka	3-	H	P
	10	*TcLr11*	11	2	L	
	11	*TcLr17*	17	3	H	
	12	*TcLr30*	30	3	H	
4	13	*TcLrB*	B	3	H	S
	14	*TcLr10*	10	3-	H	
	15	*TcLr14a*	14a	3	H	
	16	*TcLr18*	18	2+	L	
5	17	*TcLr21*	21	0;1	L	D
	18	*TcLr28*	28	0;	L	
	19	*TcLr41*	41	3	H	
	20	*TcLr42*	42	1	L	
6	21	*TcLr3bg*	3bg	3-	H	S
	22	*TcLr14b*	14b	3	H	
	23	*TcLr20*	20	3-	H	
	24	*TcLr23*	23	0;1	L	

A *Pt* code comprises of the designation for set I followed by that for set 2, etc. Viz, race MDPSDS: set 1 (M) -virulent to *Lr1, 3a;* set 2 (D) - virulent to *Lr24;* set 3 (P) - virulent to *Lr3ka, 17, 30*; set 4 (S) - virulent to *LrB, 10, 14a;* set 5 (D) - virulent to *Lr41*; set 6 (S) - virulent to *Lr3bg, 14b, 20*

Supplementary Table 11: Virulence pattern of leaf rust isolates collected from Matli, Badin Sindh Province, Pakistan & its race designation based on Long and Kolmer., (1989)

Set	Entry	Line	Lr gene	IT 1	Reaction	Code
1	1	*TcLr1*	1	3+	H	R
	2	*TcLr2a*	2a	3	H	
	3	*TcLr2c*	2c	2	L	
	4	*TcLr3a*	3a	3	H	
2	5	*TcLr9*	9	3+	H	T
	6	*TcLr16*	16	3+	H	
	7	*TcLr24*	24	3	H	
	8	*TcLr26*	26	3	H	
3	9	*TcLr3ka*	3ka	3	H	P
	10	*TcLr11*	11	2	L	
	11	*TcLr17*	17	3+	H	
	12	*TcLr30*	30	3	H	
4	13	*TcLrB*	B	3	H	T
	14	*TcLr10*	10	3	H	
	15	*TcLr14a*	14a	3	H	
	16	*TcLr18*	18	3	H	
5	17	*TcLr21*	21	3	H	N
	18	*TcLr28*	28	0	L	
	19	*TcLr41*	41	3	H	
	20	*TcLr42*	42	;1	L	
6	21	*TcLr3bg*	3bg	3	H	S
	22	*TcLr14b*	14b	3	H	
	23	*TcLr20*	20	3	H	
	24	*TcLr23*	23	0	L	

A *Pt* code comprises of the designation for set I followed by that for set 2, etc. Viz., race RTPTNS: set 1 (R) -virulent to *Lr1, 2a, 3a;* set 2 (T) - virulent to *Lr9, l6, 24, 26*; set 3 (P) - virulent to *Lr3ka, 17, 30;* set 4 (T) - virulent to *LrB, 10, 14a, 18*; set 5 (N) - virulent to *Lr21, 41*; set 6 (S) - virulent to *Lr3bg, 14b, 20, 3*

Supplementary Table 12: Virulence pattern of leaf rust isolates collected from Matli, Badin Sindh Province, Pakistan & its race designation based on Long and Kolmer., (1989)

Set	Entry	Line	Lr gene	IT 1	Reaction	Code
1	1	*TcLr1*	1	3	H	M
	2	*TcLr2a*	2a	0;	L	
	3	*TcLr2c*	2c	0;1	L	
	4	*TcLr3a*	3a	3	H	
2	5	*TcLr9*	9	3	H	N
	6	*TcLr16*	16	2	L	
	7	*TcLr24*	24	3	H	
	8	*TcLr26*	26	12	L	
3	9	*TcLr3ka*	3ka	3-	H	P
	10	*TcLr11*	11	2	L	
	11	*TcLr17*	17	3	H	
	12	*TcLr30*	30	3	H	
4	13	*TcLrB*	B	3	H	S

	14	*TcLr10*	10	3-	H	
	15	*TcLr14a*	14a	3	H	
	16	*TcLr18*	18	2+	L	
5	17	*TcLr21*	21	0;1	L	D
	18	*TcLr28*	28	0;	L	
	19	*TcLr41*	41	3	H	
	20	*TcLr42*	42	1	L	
6	21	*TcLr3bg*	3bg	3-	H	S
	22	*TcLr14b*	14b	3	H	
	23	*TcLr20*	20	3-	H	
	24	*TcLr23*	23	0;1	L	

A *Pt* code comprises of the designation for set I followed by that for set 2, etc. Viz, race MNPSDS: set 1 (M) -virulent to *Lr1, 3a;* set 2 (N) - virulent to *Lr9, 24;* set 3 (P) - virulent to *Lr3ka, 17, 30*; set 4 (S) - virulent to *LrB, 10, 14a;* set 5 (D) - virulent to *Lr41*; set 6 (S) - virulent to *Lr3bg, 14b, 20*

Supplementary Table 13: Virulence pattern of leaf rust isolates collected from Naudodero, Larkana Sindh Province, Pakistan & its race designation based on Long and Kolmer, (1989)

Set	Entry	Line	Lr gene	IT 1	Reaction	Code
1	1	*TcLr1*	1	3+	H	M
	2	*TcLr2a*	2a	;1	L	
	3	*TcLr2c*	2c	;1	L	
	4	*TcLr3a*	3a	3+	H	
2	5	*TcLr9*	9	;2+	L	J
	6	*TcLr16*	16	3+	H	
	7	*TcLr24*	24	3+	H	
	8	*TcLr26*	26	;1	L	
3	9	*TcLr3ka*	3ka	3	H	L
	10	*TcLr11*	11	2	L	
	11	*TcLr17*	17	;1	L	
	12	*TcLr30*	30	2	L	
4	13	*TcLrB*	B	3	H	T
	14	*TcLr10*	10	3	H	
	15	*TcLr14a*	14a	3	H	
	16	*TcLr18*	18	3	H	
5	17	*TcLr21*	21	;1	L	G
	18	*TcLr28*	28	3	H	
	19	*TcLr41*	41	;2	L	
	20	*TcLr42*	42	;1	L	
6	21	*TcLr3bg*	3bg	3+	H	S
	22	*TcLr14b*	14b	3	H	
	23	*TcLr20*	20	3+	H	
	24	*TcLr23*	23	;2	L	

A *Pt* code comprises of the designation for set I followed by that for set 2, etc. Viz, race MJLTGS: set 1 (M) -virulent to *Lr1, 3a;* set 2 (J) - virulent to *Lr9, 24;* set 3 (L) - virulent to *Lr3ka*; set 4 (T) - virulent to *LrB, 10, 14a,18;* set 5 (G) - virulent to *Lr28*; set 6 (S) - virulent to *Lr3bg, 14b, 20*

Supplementary Table 14: Virulence pattern of leaf rust isolates collected from Naudodero Larkana Sindh Province, Pakistan & its race designation based on Long and Kolmer., (1989)

Set	Entry	Line	*Lr* gene	IT 1	Reaction	Code
1	1	*TcLr1*	1	3	H	M
	2	*TcLr2a*	2a	0;	L	
	3	*TcLr2c*	2c	2	L	
	4	*TcLr3a*	3a	3-	H	
2	5	*TcLr9*	9	3	H	S
	6	*TcLr16*	16	3-	H	
	7	*TcLr24*	24	3	H	
	8	*TcLr26*	26	2	L	
3	9	*TcLr3ka*	3ka	3	H	P
	10	*TcLr11*	11	12	L	
	11	*TcLr17*	17	3	H	
	12	*TcLr30*	30	3	H	
4	13	*TcLrB*	B	3-	H	T
	14	*TcLr10*	10	3	H	
	15	*TcLr14a*	14a	3	H	
	16	*TcLr18*	18	3	H	
5	17	*TcLr21*	21	12	L	D
	18	*TcLr28*	28	0;2	L	
	19	*TcLr41*	41	3	H	
	20	*TcLr42*	42	0;1	L	
6	21	*TcLr3bg*	3bg	3	H	S
	22	*TcLr14b*	14b	3	H	
	23	*TcLr20*	20	3	H	
	24	*TcLr23*	23	1	L	

A *Pt* code comprises of the designation for set I followed by that for set 2, etc. Viz, race MSPTDS: set 1 (M) -virulent to *Lr1, 3a;* set 2 (S) - virulent to *Lr9, 16, 24;* set 3 (P) - virulent to *Lr3ka, 17, 30*; set 4 (T) - virulent to *LrB, 10, 14a, 18;* set 5 (D) - virulent to *Lr41*; set 6 (S) - virulent to *Lr3bg, 14b, 20*

Supplementary Table. 15 Field response of near isogenic lines during 2014-15 and 2015-16 crop cycles at five different locations in Sindh Province, Pakistan.

Near isogenic lines	Reaction types and severity of wheat leaf rust disease									
	NIA-Tandojam		Sakrand		Sanghar		Larkana		Badin	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
Lr22b	80S	60S	20MS	50MS	80S	70S	30MS	40MS	50S	10S
Lr1	80S	50S	60S	30MS	80S	60S	50MS	40MS	40S	20S
Lr2a	70MSS	50MSS	60S	40MS	70S	70S	40MS	50MS	60S	40MS
Lr2b	70MSS	80MSS	70S	80MS	80S	80S	60MS	70MS	70S	50MS
Lr2c	80MSS	60MSS	70S	80S	80MSS	80MSS	60MS	60MS	70S	40MS
Lr3	80S	50S	50MS	80S	80MSS	70MSS	30MS	30MS	80S	40S
Lr3ka	80S	60S	20MR	20MR	80MSS	50MSS	60MS	60MS	80S	30S
Lr3bg	70MSS	80MSS	80S	50MS	80MSS	70MSS	70S	70S	70S	40S
Lr9	10R	0	10R	0	0	0	10R	0	0	0
Lr10	80S	50S	50MS	80S	80S	80S	60MS	60MS	60MSS	50MSS
Lr11	80S	60S	70S	80S	80S	80S	15R	15R	70S	30S
Lr12	80S	40S	70S	70MSS	80S	80S	60MS	60MS	70S	30S
Lr13	80S	50S	20MR	15MR	70S	70S	60MS	60MS	60S	30MSS
Lr14a	80S	60S	70S	70S	70MS	70MS	50MS	80MS	60S	30MS
Lr14b	80S	70S	60S	70MSS	80MSS	40MS	60MS	60MS	70S	20S
Lr15	80S	60S	60MS	60MSS	80MS	80MS	40MS	40MS	60MSS	40MSS
Lr16	80S	70S	70S	80MS	70MS	70MS	15R	15R	60S	30MSS
Lr17	80S	50S	80MS	60S	70MS	70MS	70S	70S	70S	40S
Lr18	20MR	20MR	20MS	50MSS	60MS	60MS	20MR	20MR	70S	30S
Lr19	10R	0	10R	0	0	0	10R	0	0	0
Lr20	70MSS	60MSS	80S	60S	20MS	50MS	70S	70MSS	60S	70S
Lr21	50MS	40MS	80S	50MS	40MS	50MS	20MR	30MR	60MSS	30MSS
Lr22a	10R	15R	30MR	20MR	40MS	40MS	70S	60Ms	70S	40MSS
Lr23	40MS	20MS	20MR	30MR	50MS	70MS	15MR	20MR	70S	30S
Lr24	20MS	30MS	80S	80MSS	20MS	80MS	60MS	80MS	80S	40S
Lr25	50MSS	60MSS	80S	60MSS	80MSS	40MS	50MS	60MS	60MSS	70S
Lr26	60MSS	70MSS	80MS	80MS	80MSS	80MS	20MR	25MR	80S	60S

Lr 10,27+31	20MR	20MR	70S	30MS	20R	15R	40MS	50MS	70S	50MSS
Lr28	10TR	0	0	0	0	0	10R	0	0	0
Lr29	30MSS	40MSS	70MS	10MS	80S	80MS	70MS	80MS	40MSS	40S
Lr30	60MSS	70MSS	80S	70MSS	60MS	80MS	60MS	70MS	50S	30S
Lr32	60MS	40MS	10R	15R	50MS	80MS	50MSS	80MSS	20MS	70MS
Lr33	50MS	60MS	60MS	60MSS	60MS	80MSS	40MS	80MSS	50S	60S
Lr34	10MR	10MR	20MR	20MR	40MS	30MS	40MS	80MS	15R	20R
Lr35	40MSS	40MSS	20MR	40MRMS	50MS	40MS	50MS	50MS	60S	20S
Lr36	20MR	10MR	20MR	20MR	15MR	40MR	20MR	30MR	10MR	10MRMS
Lr37	20MR	20MR	20MR	30MRMS	20MR	30MR	20MR	30MR	30MRMS	30MRMS

R= Resistant, MR= Moderately Resistant, MRMS= Moderately Resistant to Moderately susceptible, MS= Moderately susceptible, S= susceptible, MSS= Moderately susceptible to susceptible