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. (HTTs 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.
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 donal 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 uredinal 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, Pirskb-04, Pirskb-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 uredinospores 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 (Mclntosh 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°C overnight. Fresh spores produced on the leaf pieces were used in inoculation for spore increase.

a) Isolation and multiplication of single-pustule
Uredinospores 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; IT1 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 |
| MJLTS      | 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 pathatypes (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* | +       | +     | +       |
| *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 |       |   |
| *TcLr2a*         | 2+| 3 | 3 | 3 | 2- | 3+| 2 | 3 | 0 |       |
| *TcLr2c*         | 0 | 2+| 0 | 3 | 3 | 3 | 0 | 2 | 0;1 |       |
| *TcLr3a*         | 3+| 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3+ | 3- |
| *TcLr9*          | 3 | 3 | 3 | 3 | 3 | 0 | 3+ | 3 | 2+ | 3 |
| *TcLr16*         | 3 | 3 | 3+ | 3 | 2 | 3 | 3+ | 3 | 2+ | 3 |
| *TcLr24*         | 3 | 3 | 3+ | 3 | 3 | 3 | 3 | 3 | 3+ | 3 |
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 with 3 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 (MDPDS, RTTPS and MNPSDS) tested pathotypes from Badin Sindh province, Pakistan and displayed high infection types (Table 5). Lr9 had high infection types with RTTPS and MNPSDS pathotypes. No virulence was recorded for leaf rust resistance genes viz., Lr2c, Lr11, Lr28, Lr42 and Lr23as they displayed (LITs) and effectiveness to MDPDS, RTTPS and MNPSDS pathotypes. While Lr2a, Lr16, Lr26, Lr18 and Lr21 had low reactions to MDPDS 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 MSPTPS 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 *Lr*1, 2a, 3a, 9, 16, 24, 26, 3ka, 11, 17, B, 10, 14a, 18, 21, 41, 3bg, 14b and 20) and phenotypes RTPTNS virulent to (*Lr*1, 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 (*Lr*1, 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 (*Lr*1, 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, MDPDS, MJLTGS and PNDQDS) had the similar virulent spectrum, each produced virulence on 15, 14, 13, 13 and 12 *Lr* genes tested respectively. However, [DBQG] proved to be the least virulent pathotype making just 8 *Lr* genes susceptible and produced compatible reaction only on *Lr*2a, 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 *Lr*3, *Lr*10, *Lr*16, *Lr*17 and *Lr*26 while PBMQQ recorded as third frequent phenotype, was virulent to gene *Lr*1, *Lr*3 and *Lr*10. (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 *Lr*9, *Lr*19 and *Lr*28. 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 \((Lr24, B, 10, 14b \text{ and } 20)\). 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 \((LITs\ 0, 1, \text{ and } 2+)\) 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.

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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
- `-` Smaller uredia
- `+` Large uredinia
- `++` Larger uredinia
- `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)

| Pt code | Host set | Infection type (ITs) produced on differential *Lr* lines |
|---------|----------|--------------------------------------------------------|
|         | 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      |

| Pt code | Host set | Infection type (ITs) produced on differential *Lr* lines |
|---------|----------|--------------------------------------------------------|
| 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           | L         | 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   | 4     | TcLr3a  | 3a      | 3+   | H        |      |
| 5   | 6     | TcLr9   | 9       | 3    | H        |      |
| 6   | 7     | TcLr16  | 16      | 3    | H        |      |
| 8   | 9     | TcLr24  | 24      | 3    | H        |      |
| 10  | 11    | TcLr26  | 26      | 2+   | L        |      |
| 2   | 13    | TcLr1   | 1       | 3    | H        |      |
| 14  | 16    | TcLr2a  | 2a      | 2+   | L        |      |
| 15  | 18    | TcLr3a  | 3a      | 3+   | H        |      |
| 19  | 21    | TcLr1   | 1       | 3    | H        |      |
| 22  | 24    | TcLr2a  | 2a      | 2+   | L        |      |
| 23  | 26    | TcLr3a  | 3a      | 3    | H        |      |
| 4   | 5     | TcLr9   | 9       | 3    | H        |      |
|     | 7     | TcLr16  | 16      | 3    | H        |      |
|     | 8     | TcLr24  | 24      | 3    | H        |      |
|     | 10    | TcLr26  | 26      | 3    | H        |      |
|     | 11    | TcLr1   | 1       | 3    | H        |      |
|     | 12    | TcLr2a  | 2a      | 2+   | L        |      |
|     | 13    | TcLr3a  | 3a      | 3    | H        |      |
|     | 14    | TcLr16  | 16      | 3    | H        |      |
|     | 15    | TcLr24  | 24      | 3    | H        |      |
|     | 16    | TcLr26  | 26      | 3    | H        |      |
|     | 17    | TcLr1   | 1       | 3    | H        |      |
|     | 18    | TcLr2a  | 2a      | 2+   | L        |      |
|     | 19    | TcLr3a  | 3a      | 3    | H        |      |
|     | 20    | TcLr1   | 1       | 3    | H        |      |
|     | 21    | TcLr2a  | 2a      | 2+   | L        |      |
|     | 22    | TcLr3a  | 3a      | 3    | H        |      |

A Pt code comprises of the designation for set 1 followed by that for set 2, etc. Viz, race MSCTNS: set 1 (M) - virulent to Lr1, 3a; set 2 (S) - virulent to Lr9, 16, 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   | 4     | TcLr2c  | 2c      | 2+   | L        |      |
| 5   | 6     | TcLr9   | 9       | 3    | H        |      |
| 7   | 8     | TcLr16  | 16      | 3    | H        |      |
| 9   | 10    | TcLr24  | 24      | 3    | H        |      |
| 11  | 12    | TcLr26  | 26      | 3    | H        |      |
| 2   | 5     | TcLr1   | 1       | 3    | H        | T    |
| 6   | 7     | TcLr9   | 9       | 3    | H        |      |
|     | 8     | TcLr16  | 16      | 3    | H        |      |
|     | 9     | TcLr2a  | 2a      | 2+   | L        |      |
|     | 10    | TcLr3a  | 3a      | 3    | H        |      |
|     | 11    | TcLr16  | 16      | 3    | H        |      |
|     | 12    | TcLr24  | 24      | 3    | H        |      |
|     | 13    | TcLr26  | 26      | 3    | H        |      |
|     | 14    | TcLr2a  | 2a      | 2+   | L        |      |
|     | 15    | TcLr3a  | 3a      | 3    | H        |      |
|     | 16    | TcLr16  | 16      | 3    | H        |      |
Int. J. Phytopathol. 10 (02) 2021. 101-123  
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| Set | Entry | Line | Lr gene | IT 1 | Reaction | Code |
|-----|-------|------|---------|------|----------|------|
| 1   | 1     | TcLr1| 1       | 3+   | H        | R    |
| 2   | 2     | TcLr2a| 2a      | 3    | H        |      |
| 3   | 3     | TcLr2c| 2c      | 0    | L        |      |
| 4   | 4     | TcLr3a| 3a      | 3    | H        |      |
| 5   | 5     | TcLr9| 9       | 1    | L        |      |
| 6   | 6     | TcLr16| 16      | 3+   | H        | K    |
| 7   | 7     | TcLr24| 24      | 3+   | H        |      |
| 8   | 8     | TcLr26| 26      | 3+   | H        |      |
| 9   | 9     | TcLr3ka| 3ka     | 3    | H        | T    |
| 10  | 10    | TcLr11| 11      | 3+   | H        |      |
| 11  | 11    | TcLr17| 17      | 3+   | H        |      |
| 12  | 12    | TcLr30| 30      | 3+   | H        |      |
| 13  | 13    | TcLrB| B       | 3+   | H        | R    |
| 14  | 14    | TcLr10| 10      | 3+   | H        |      |
| 15  | 15    | TcLr14a| 14a     | 2+   | L        |      |
| 16  | 16    | TcLr18| 18      | 3    | H        |      |
| 17  | 17    | TcLr21| 21      | 2    | L        | G    |
| 18  | 18    | TcLr28| 28      | 3    | H        |      |
| 19  | 19    | TcLr41| 41      | 0    | L        |      |
| 20  | 20    | TcLr42| 42      | 0    | L        |      |
| 21  | 21    | TcLr3bg| 3bg    | 3    | H        | S    |
| 22  | 22    | TcLr14b| 14b     | 3    | H        |      |
| 23  | 23    | TcLr20| 20      | 3    | H        |      |
| 24  | 24    | TcLr23| 23      | 2+   | 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, 16, 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)

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 Lr9, 16, 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   | 2     | TcLr2a| 2a      | 2    | L        |      |
| 3   | 3     | TcLr2c| 2c      | 3    | H        |      |
| 4   | 4     | TcLr3a| 3a      | 3    | H        |      |
| 5   | 5     | TcLr9 | 9       | 3    | H        | N    |
| 6   | 6     | TcLr16| 16      | 2    | L        |      |
| 7   | 7     | TcLr24| 24      | 3    | H        |      |
| 8   | 8     | TcLr26| 26      | ;1   | L        |      |
| 9   | 9     | TcLr3ka| 3ka    | 2    | L        |      |
| 10  | 10    | TcLr11| 11      | ;1   | L        | D    |
| 11  | 11    | TcLr17| 17      | 3    | H        |      |
| 12  | 12    | TcLr30| 30      | 2+   | L        |      |
| 13  | 13    | TcLrB | B       | 3+   | H        | Q    |
| 14  | 14    | TcLr10| 10      | 3    | H        |      |
| 15  | 15    | TcLr14a| 14a   | 2    | L        |      |
| 16  | 16    | TcLr18| 18      | 2    | L        |      |
| 17  | 17    | TcLr21| 21      | 2,1  | L        |      |
| 18  | 18    | TcLr28| 28      | 1    | L        | D    |
| 19  | 19    | TcLr41| 41      | 3    | H        |      |
| 20  | 20    | TcLr42| 42      | ;1   | L        |      |
| 21  | 21    | TcLr3bg| 3bg   | 3    | H        | S    |
| 22  | 22    | TcLr14b| 14b   | 3    | H        |      |
| 23  | 23    | TcLr20| 20      | 3    | H        |      |
| 24  | 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   | 2     | TcLr2a| 2a      | 3+   | H        |      |
| 3   | 3     | TcLr2c| 2c      | 3    | H        |      |
| 4   | 4     | TcLr3a| 3a      | 2    | L        |      |
| 5   | 5     | TcLr9 | 9       | ;2   | L        | D    |
| 6   | 6     | TcLr16| 16      | ;2   | L        |      |
| 7   | 7     | TcLr24| 24      | 3    | H        |      |
| 8   | 8     | TcLr26| 26      | 2    | L        |      |
| 9   | 9     | TcLr3ka| 3ka   | 2    | L        | B    |
| 10  | 10    | TcLr11| 11      | 2    | L        |      |
| 11  | 11    | TcLr17| 17      | ;1   | L        |      |
| 12  | 12    | TcLr30| 30      | 2,2+ | L        |      |
| 13  | 13    | TcLrB | B       | 3    | H        | Q    |
| 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 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)

*A Pt code comprises of the designation for set I followed by that for set 2, etc. Viz, race MDPDS: 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, 16, 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    |
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        |      |
### 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 |
| *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     | 40S     |
| *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       | 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     | 30MSS   |
| *Lr14b*             | 80S     | 70S     | 60S     | 70MSS   | 80MSS   | 40MS    | 60MS    | 60MS    | 70S     | 20S     |
| *Lr15*              | 80S     | 60S     | 60MSS   | 80MSS   | 80MS    | 80MSS   | 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       | 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    | 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    | 80MSS   | 80MSS   | 80MS    | 20MR    | 25MR    | 80S     | 60S     |
| Lr10,27+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| 80MS| 40MS| 80MSS| 40MSS| 50S  | 60S   |
| Lr34       | 10MR | 10MR | 20MR| 20MR | 40MS| 30MSS| 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| 20MR | 15MR| 40MR| 20MR | 30MR | 20MR | 30MRMS|

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