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VARIABILITY IN VIRULENCE OF WHEAT LEAF RUST (*PUCCINIA TRITICINA*) IN THE SINDH PROVINCE, PAKISTAN

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ABSTRACT

Leaf rust of wheat caused by an obligate biotrophic fungus (*Puccinia triticina*), is one of the widespread diseases of wheat. The emergence of new virulent races of fungal pathogens threatens wheat cultivars' resistance, leading to outbreaks that can cause substantial damage to crops and result in economic losses to farmers. Developing wheat cultivars that have resistance to virulent races is an effective means of reducing the frequency and impact of these outbreaks. An experiment was conducted at 5 locations (a trap nursery consisting of 37 lines of wheat leaf rust differentials and 115 commercial wheat cultivars was established against leaf rust pathogen), for evaluating the virulence pattern of leaf rust under natural field environments across wheat-producing regions of Sindh, Pakistan. Results revealed that *Lr9, Lr19* and *Lr28* genes were found effective under field environments. The moderate resistance was recorded for *Lr18, Lr23* and *Lr34* genes at two locations while *Lr36* & *Lr37* genes had moderate resistance at most of the locations. Results also revealed that Rawal-87, Marvi-2000, Bhittai, Pirsabak-08, Faisalabad-08, Benazir-13 and Shalakot-13 were the commercial wheat varieties having resistance at all locations of Sindh during (year 1 and year 2) years while most of the cultivars showed susceptibility. The scenario clue to a dire necessity to widen the genetic base of Pakistani cultivars by incorporating genes for the resistance against disease. Furthermore, strong monitoring and regular surveys should be conducted for determining current virulence status and resistance genes.

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INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is the main source of protein and energy and the majority of our population depends on it as it is a major source of nourishment (FAOSTAT, 2016). Wheat yield is seriously influenced by biotic and abiotic constraints (Singh *et al.*, 2008) and many biotic stresses are responsible for destabilizing the wheat production throughout the world. Among them, three rust species viz., yellow, brown and black rust are of major importance (Singh *et al.*, 2004; Chen, 2005).

Stem rust, leaf rust, and stripe rust have had a significant impact on cultivated cereals, causing economic losses due to the reduction in the quantity and quality of grains in various regions across the world, especially in Asia. These deadly pathogens are obligate parasites belong to the genus *Puccinia* having narrow host range and are highly specialized (Mendgen and Hahn, 2002; Kolmer *et al.*, 2009). Of these three notorious wheat diseases, brown rust is the most serious striking wheat disease (Bolton *et al.*, 2008). Characteristics of *P. triticina* are wide adaptation of the pathogen to the distinct climatic situations and increased levels of virulence distinction where wheat is grown and contribute to regular reductions in the worldwide production of wheat (Roelfs, 1992; Kolmer *et al.*, 2011).

Mains and Jackson (1926) were the first to explain the physiologic specialization of leaf rust of wheat as a pathogen has a long history of studies of population. Mains and Jackson (1926) initially described the primary differentials set, including the eight lines of wheat having distinct genes for resistance i.e. ([*Lr1* gene] Malakoff, [*Lr2b* = *Lr22*] Carina, [*Lr2c* = *Lr23*] Brevit, [*Lr2a*] Webster, [*Lr24*] Loros, [*Lr3a*] Mediterranean, [*Lr11*] Hussar, and [*Lr3a*] Democrat) and broadly utilized for investigation and monitoring structure of pathogen races globally. Dyck (1968) had initially developed lines of Thatcher differential and designated them as fundamental set for examining virulence diversity of pathogens globally. A particular differential set, comprising nine lines of wheat, was reported in the early 1980s to investigate pathogens. A constant record of knowledge regarding the evolution of diverse populations and epidemiology of the pathogen has been provided by virulence surveys of pathogen races. Until the late 1960s, single-uredinial isolates of pathogen were characterized for virulence on eight differential varieties (Kolmer *et al.*, 1995). A sequence of near-isogenic wheat lines of Thatcher were utilized by (Dyck *et al.*, 1974) that varied by single resistance genes of pathogen. The Thatcher lines have been exercised to differentiate virulence in populations of pathogen in Egypt (McVey *et al.*, 2004b) and throughout the world (Singh, 1991). Use of near-isogenic differential lines has made possible the distinction of *P. triticina* isolates that vary by an individual virulence (Samborski and Dyck, 1976).

Genetic resistance against wheat leaf rust is widely classified into adult plant resistance (APR) and seedling resistance. According to various reports (Leonova *et al.*, 2020; McIntosh *et al.*, 2017) more than 100 genes conferring resistance to pathogen to date, have been effectively categorized, of them the most of confer seedling resistance. According to (Flor, 1971) usually seedling resistance is regulated by a single gene with a main influence that communicates with the pathogen in a 'gene-for-gene' association. Whereas adult plant resistance (APR) is typically efficacious at the post-seedling development phases, is either regulated by an individual gene with the main influence or several genes each with minor influence. Various researchers (Lagudah, 2011; McCallum *et al.*, 2012; Burdon *et al.*, 2014) reported that partial resistance is provided by some adult plant (APR) genes which is useful (i.e. race-nonspecific) against all races of a particular species of pathogen. In contrast, most of genes possess adult plant resistance interact additively and increase resistance to certain immunity levels (Singh *et al.*, 2014). However, pleiotropic resistance is shown by some genes of APR against numerous diseases viz., *Lr67, Lr46* and *Lr34* offer partial resistance to powdery mildew, yellow, stem and wheat leaf rust diseases (Lagudah, 2011; Risk *et al.*, 2012; Ellis *et al.*, 2014).

The virulence of the pathogen has been reported in coastal zones where wheat develops and matures in warm humid environments viz., Central Asia (Morgounov *et al.*, 2007), Uruguay (German and Kolmer, 1994; Germán *et al.*, 2007), Turkey (Kolmer *et al.*, 2013) and also in continental zones (the Great Plains of North America) which have warm to hot summer temperatures (Roelfs, 1989). In Egypt, high diversity of virulence and broad variations in populations of pathogens are recorded (McVey *et al.*, 2004a). Since the 1930s, extensive studies of physiologic specialization in pathogens have been conducted in the Canada (Johnson, 1956) and the U.S. (Johnston, 1968). On an annual basis numerous virulence phenotypes of the fungus are detected due to highly variable pathogen populations. Similarly, pathogen is found annually all over wheat-producing areas in Pakistan resulting in frequent yield reductions (Saari and Prescott, 1985; Lawlor, 2013). According to reports of researchers (Nagarajan and Joshi, 1985) pathogens can survive on the crop during the summer in the mountainous western areas and then proliferates to wheat-producing areas of the provinces of Punjab and Sindh. The objective of the study was to investigate the virulence pattern of leaf rust in various wheat-growing areas in Sindh, Pakistan.

MATERIAL and METHODS

Germplasm collection

A set of 37 near-isogenic (differential) lines and 105 cultivated wheat varieties were provided by International Wheat and Maize Improvement Centre (CIMMYT) Mexico and National Agricultural Research Center (NARC) Islamabad, Pakistan respectively, while additional 10 varieties were obtained from the Nuclear Institute of Agriculture (NIA), Tandojam for the current study.

Field experiments

A trap nursery consisting of 152 wheat genotypes, including 115 Pakistani wheat cultivars and 37 wheat near-isogenic lines containing single *Lr* gene was planted at five locations (Tandojam, Sakrand, Sanghar, Larkana and Badin) of Sindh Province. The nurseries were planted over 2 years during the 2014-15 and 2015-16 wheat cropping seasons. All locations represented distinct agroecological conditions conducive to infection and development of brown rust. Every genotype was sown in an unrepeated individual line of one-meter length where row to row distance was 30cm. Susceptible check (Morocco) two lines were sown for uniform infection.

Disease assessment

All experimental fields had a natural infection of pathogen since the pressure of inoculum in the investigational region was high due to the continuous cultivation of wheat and suitable environments that occur in these localities. Observations were recorded at the initial manifestation of pathogen infection on the susceptible check Morocco. Disease severity data was documented based on the modified Cobb's scale as explained by (Peterson *et al.*, 1948) and was noted twice according to leaf area (%) affected on plants at the flowering and tillering phase. Reaction types (Table. 1) were noted during the flowering to soft dough stages (Jin *et al.*, 2007).

Table 1. Modified cobb's scale used for data recording during present study.

Reaction	Infection type	Field response
No disease	0	No visible infection
Resistant	R	Necrotic areas with or without minute uredia
Moderately resistant	MR	Small uredia present surrounded by necrotic area
Moderately resistant, moderately Susceptible	MRMS	Small uredia present surrounded by necrotic areas as well as medium uredia with no necrosis but possible some distinct chlorosis
Moderately susceptible	MS	Medium uredia with no necrosis but possible some distinct chlorosis
Moderately susceptible-susceptible	MSS	Medium uredia with no necrosis but possible some distinct chlorosis as well as large uredia with little or chlorosis present
Susceptible	S	Large uredia and little or no chlorosis present

RESULTS

Leaf rust virulence data obtained in both growing seasons revealed that leaf rust resistance genes *Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr3bg, Lr10, Lr12, Lr14a, Lr14b, Lr15, Lr17, Lr18, Lr20, Lr22b, Lr24, Lr25, Lr29, Lr30* and *Lr33* showed susceptible reactions demonstrating that pathotype virulent on these *Lr* genes were prevalent under natural field conditions at all five tested locations. The lines containing resistance genes *Lr3ka, Lr11, Lr13, Lr16, Lr21, Lr26, Lr32* and *Lr35* showed susceptible reaction at four locations. Similarly, virulence for *Lr18, Lr22a, Lr23* and *Lr10, 27+* was detected at three locations while avirulence for *Lr34* was recorded at Larkana and Badin (Table 2).

Virulence results revealed that most leaf rust resistance genes had susceptible reactions against the prevailing rust population in Sindh Province. Leaf rust resistance genes *Lr9, Lr19* and *Lr28* were effective and no virulence was observed while *Lr36* and *Lr37* were found moderately resistant across all tested locations in both years. Leaf rust resistance genes *Lr10, Lr11, Lr16, Lr22a, Lr27+Lr31, Lr32* and *Lr34* showed resistant reactions at one location while *Lr3ka, Lr13, Lr21, Lr22a, Lr26, Lr27* and *Lr35* showed moderate resistance at one location and *Lr18, Lr23* and *Lr34* had moderate resistance at two locations and *Lr36, Lr37* had moderate reactions at all tested locations. The infection on susceptible Morocco was recorded up to 90S at all locations (Table 2).

Table 2. Field response of near-isogenic lines against leaf rust (2014-15 & 2015-16).

Reaction types	**Leaf rust resistance genes**
Resistant (R)	*Lr9, Lr19, Lr28,*
Moderately resistant (MR)	*Lr16, Lr22a, Lr10,27+, Lr32, Lr34, Lr3ka, Lr13, Lr21, Lr26, Lr35 Lr18, Lr23, Lr36, Lr37*
Moderately susceptible (MS)	*Lr23, Lr24, Lr32, Lr33*
Susceptible (S)	*Lr22b, Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr3bg, Lr10, Lr12, Lr14a, Lr14b, Lr15, Lr17, Lr18, Lr20, Lr24, Lr25, Lr29, Lr30, Lr33, Lr3ka, Lr11, Lr13, Lr16, Lr21, Lr26, Lr32, Lr35, Lr22a, Lr23, Lr10,27+*
Moderately Susceptible to Susceptible (MS-S)	*Lr3bg, Lr20, Lr25, Lr26, Lr29, Lr35*

R =Resistant, MR= moderately resistant, MS= moderately susceptible, MS-S= moderately susceptible to susceptible, S= susceptible

Rust response data of different wheat cultivars revealed varying degrees of susceptibility and resistance. Among the commercial varieties Rawal-87, Marvi-2000, Bhittai, Pirsabak-08, Faisalabad-08, Benazir-13 and Shalakot-13 showed resistance at all experimental locations during both cropping seasons 2014-15 and 2015-16. While commercial varieties Rawal-87, Marvi-2000, Bhittai, Pirsabak-08, Faisalabad-08, Benazir-13, Shalakot-13, Margalla-99, GA-2002, BARS-2009, NIFA-Barsat-09, NIA-Sunder, NIA-Saarang and Johar-78 were resistant at all experimental locations during only 2014-15 cropping season. Moreover, Barani-83 and Aas-11 showed resistance at two locations whereas D-97, Chakwal-50, UP-262, C-217, C-306 and Mairaj-08 were found resistant at one location (Table 3 and 4).

Table 3. Field response of Pakistani wheat cultivars against leaf rust during cropping season 2014-15.

Reaction types	Pakistani wheat cultivars
Resistant (R)	Margalla-99, Rawal-87, Marvi-2000, GA-2002, Pirsabak-08, FSD-08, BARS-2009, NIFA-Barsat-09, NIA-Sunder, Bhittai, Johar-78, Benazir-13, NIA-Saarang, Shalakot-13
Moderately resistant to Moderately susceptible (MRMS)	Punjab-76
Moderately susceptible and Susceptible (MS & S)	Bakhtawar-94, Pasban-90, Daman-98, Kohistan-97, Sulaiman-96, Nowshera-96, Shalimar-88, Khyber-87, Sutlaj-86, Punjab-85, Kohinoor-83, Sarhad-82, Punjab-81, Zarghoon-79, BWP-79, Dirk, Tarnab-73, SA-72, Chenab-70, Yecora-70, Nuri-70, Local-White, Pothowar, SA-75, SA-42, Khushal-69, WL-711, Mexipak, Sonalika, Chakwal-86, Pirsabak-91, Inqilab-91, Barani-70, Chakwal-97, C-271, C-273, TD-1, C-518, C-591, SKD-1, Sarsabz, Sassui, Wafaq, AS-2002, Saleem-2002, Ufaq, Moomal-2003, SH-2003, Pirsabak-05, NARC-09, NARC-11, Bakhar-2002, Punjab-11, ZA-77, Mehran, Kiran, TJ-83, Abadgar, NIA-Sunehri
Moderately Susceptible to Susceptible (MSS)	Kohsar-95, Shahkar-95, Kaghan-93, Parwaz-94, MH-97, Rohtas-90, Soghat-90, Bahawalpur-97, Darawar-97, FSD-83, FSD-85, Pak-81, Zardana, Blue-Silver, Raskoh, Seher-2006, Shafaq-2006, Lasani-2008, Aari-11

R =Resistant, MR= moderately resistant, MS= moderately susceptible, MS-S= moderately susceptible to susceptible, S= susceptible

Virulence results revealed that most commercial varieties had susceptible reactions against the prevailing rust population in Sindh Province. Data revealed that the commercial varieties Bakhtawar-94, Daman-98, Kohistan-97, Sutlaj-86, Punjab-85, Zarghoon-79, BWP-79, Tarnab-73, Yecora-70, Nuri-70, Local-White, Pothowar, SA-75, SA-42, Khushal-69, WL-711, Mexipak, Sonalika, Chakwal-86, Pirsabak-91, Inqilab-91, Chakwal-97, C-271, C-273, TD-1, C-591, Wafaq, AS-2002, Saleem-2002, Ufaq, Moomal-2003, SH-2003, Pirsabak-05, NARC-09, NARC-11, Bakhar-2002, Punjab-11, ZA-77, Mehran, Kiran, TJ-83, Abadgar and NIA-Sunehri showed moderately susceptible and susceptible reactions at all 5 locations (Table 3 & 4).

Table 4. Field response of Pakistani wheat cultivars against leaf rust during cropping season 2015-16.

Reaction types	Pakistani wheat cultivars
Resistant (R)	Rawal-87, Marvi-2000, Pirsabak-08, FSD-08, Bhittai, Benazir-13, Shalakot-13
Moderately resistant to Moderately susceptible (MRMS)	Punjab-76
Moderately susceptible and Susceptible (MS & S)	Bakhtawar-94, Sairab-92, Anmol-91, Pasban-90, Margalla-99, Daman-98, Kohistan-97, Suleman-96, Sutlaj-86, Punjab-85, Zarghoon-79, Bahawalpur-79, Tarnab-73, Lyp-73, Yecora-70, Nuri-70, Local-White, Pothowar, SA-72, SA-42, Khushal-69, WL-711, Mexipak, Sonalika, Chakwal-86, Pirsabak-91, Inqilab-91, Chakwal-97, C-271, C-273, TD-1, C-250, C-591, Wafaq, AS-2002, Saleem-2002, Ufaq, Moomal-2003, SH-2003, Pirsabak-05, NARC-09, NARC-11, Bakhar-2002, Punjab-11, Imdad, NIA-Sunder, ZA-77, Mehran, Kiran, TJ-83, Abadgar, NIA-Sunehri, Shalimar-88
Moderately Susceptible to Susceptible (MSS)	Kohsar-95, Shahkar-95, Kaghan-93, Parwaz-94, Dera-98, MH-97, Rohtas-90, Soghat-90, Bahawalpur-97, FSD-83, FSD-85, Pak-81, Zardana, Blue-Silver, Raskoh, GA-2002, Seher-2006, Shafaq-2006, Lasani-2008, Aari-11

R =Resistant, MR= moderately resistant, MS= moderately susceptible, MS-S= moderately susceptible to susceptible, S= susceptible

While the commercial cultivars Anmol-91, Pasban-90, Suleman-96, Nowshera-96, Shalimar-88, Khyber-87, Punjab-85, Sarhad-82, Punjab-81, LYP-73, Chenab-70, LU-26, Barani-70, Chakwal-50, C-250, C-518, C-591, SKD-1, Ufaq, Pirsabak-04, Imdad and Khirman showed moderately susceptible and susceptible reactions at four locations. Furthermore, results revealed that commercial cultivars Sairab-92, Darawar-97, Kohinoor-83, Dirk, Pari-73, UP-262, SA-72, C-217, C-228, C-306, Sassui and Mairaj-08 had moderately susceptible and susceptible reactions at three locations while Dera and Pavon at two locations and Kaghan-93, Parwaz-94, Soghat-90 and Zardana at one location respectively (Table 3 & 4).

Partial resistance (moderately resistance) (10-30MR) was recorded for commercial cultivars Sulaiman-96, Tarnab-73, Pari-73, C-228, AS-2002, Ufaq, Imdad and Pavon at one location while Punjab-76 had a moderate response at three locations. Similarly, cultivars D-97, C-217 and C-306 showed (10-20MRMS) moderately resistant to moderately susceptible reactions at one location and Punjab-76 (10MR-20MRMS) gave the same moderate reactions at two locations (Tables 3 & 4).

DISCUSSION

Breeding for rust resistance is a continuous procedure as the constant evolution and variability of wheat leaf rust pathogen urges enormous pressure on plant breeders to be attentive against new emergent virulence phenotypes that overcome resistance genes (McIntosh *et al.*, 1995; Figlan *et al.*, 2020; Pretorius *et al.*, 2015; Pretorius *et al.*, 2007). This needs regular surveillance of variations of virulence patterns and timely monitoring of rust pathogens in wheat-growing areas (Park *et al.*, 2011; Figlan *et al.*, 2020).

Trap nurseries were planted during 2014-15 and 2015-16 to detect/characterize avirulences and virulences for the leaf rust pathogen populations present in different regions of Sindh. Two years data revealed that *Lr9, Lr19* and *Lr28* were effective and displayed resistance against the leaf rust populations while *Lr34* showed moderate resistance at three locations and the same response was recorded for *Lr36* and *Lr37* at all tested locations. Based on a comparison of field response, avirulence /virulence pattern of leaf rust revealed that *Lr* genes *Lr9, Lr19* and *Lr28* were effective while *Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr3bg, Lr10, Lr12, Lr14a, Lr14b, Lr15, Lr17, Lr18, Lr20,* *Lr22b, Lr24, Lr25, Lr29, Lr30* and *Lr33* were susceptible during both years. Similarly, (Rattu *et al.*, 2009) also recorded high frequencies (75-100%) of virulence to most of near-isogenic lines under natural field conditions in Pakistan whereas just *Lr9, Lr19, Lr28* and *Lr34* were reported resistant to leaf rust pathogen population. Various studies have been documented on the effectiveness of *Lr19* and *Lr28* in the last two decades (Fayyaz *et al.*, 2008; Rattu *et al.*, 2009). Many of the single gene Thatcher lines had high frequency (>80%) of virulence in Pakistan against the *P. triticina* population (Rattu *et al.*, 2009) only *Lr9, Lr19* and *Lr28* have been reported as effective.

During both years of 2014-15 and 2015-16, most of the commercial varieties (Bakhtawar-94, Daman-98, Kohistan-97, Sutlaj-86, Punjab-85, Zarghoon-79, BWP-79, Tarnab-73, Yecora-70, Nuri-70, Local-White, Pothowar, SA-75, SA-42, Khushal-69, WL-711, Mexipak, Sonalika, Chakwal-86, Pirsabak-91, Inqilab-91, Chakwal-97, C-271, C-273, TD-1, C-591, Wafaq, AS-2002, Saleem-2002, Ufaq, Moomal-2003, SH-2003, Pirsabak-05, NARC-09, NARC-11, Bakhar-2002, Punjab-11, ZA-77, Mehran, Kiran, TJ-83, Abadgar, NIA- Sunehri) and leaf rust resistance genes had moderately susceptible and susceptible reactions against prevailing leaf rust population at all experimental locations. Out of 115, only seven commercial varieties namely (Rawal-87, Marvi-2000, Bhittai, Pirsabak-08, Faisalabad-08, Benazir-13 and Shalakot-13) have been found resistant at all experimental locations. Recently, (Iqbal *et al.*, 2010) identified 9 Pakistani commercial cultivars e.g. Abadgar, Bahawalpur-97, Shahkar-95, Shahen-94, Pasban-90, LU-26, Kohinoor-83, Faisalabad-85 and Rohtas-90 as effective and immune against local rust diseases.

Information on rust resistance recorded in the current study at each location can be advantageous for wheat breeders as it would ultimately provide a warning about the virulence variation of the pathogen (Singh *et al.*, 2011; Pretorius *et al.*, 1984). It has been difficult to achieve long-lasting and stable resistance against leaf rust because *P. triticina* has wide adaptation to environmental conditions (Roelfs, 1992; Sharma-Poudyal *et al.*, 2020). Therefore, it is essential to widen the genetic base to leaf rust resistance in wheat cultivars by pyramiding multiple leaf rust resistance genes (Kthiri *et al.*, 2018), especially those effective against local prevailing strains and their variants. Further, a consistent model of forecasting and effective monitoring should be established in the region for the identification of resistant germplasm and keeping track of variation occurring in the pathogen population.

CONCLUSION

Current work revealed that *Lr9, Lr19* and *Lr28* resistance genes demonstrated their effectiveness and *Lr36* and *Lr37* were found moderately resistant whereas most of the resistance genes were ineffective against the population of (*P. triticina*) pathogen across all tested locations during both cropping years. However, seven commercial wheat cultivars (Rawal-87, Marvi-2000, Bhittai, Pirsbak-08, Faisalabad-08, Benazir-13 and Shalakot-13) were found resistant out of 115 cultivars tested. Strong monitoring should be established and regular surveys should be conducted for determining genes for resistance and the current status of virulence. As virulence analysis provides a timely warning before the pathogen becomes a menace to crop production.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.

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