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Comparative Virulence and Molecular Diversity of Stripe Rust (*Puccinia striiformis* f. sp. *tritici*) Collections from Pakistan and United States

HADI BUX[1], AWAIS RASHEED†, SHER MUHAMMAD MANGRIO, SAEED AKHTAR ABRO, SYED JAWAD AHMED SHAH‡, MUHAMMAD ASHRAF¶ AND XIANMING CHEN¶¶
Institute of Plant Sciences, University of Sindh, Jamshoro 76080, Pakistan
†Department of Plant Sciences, Quaid-i-Azam University, Islamabad 44000, Pakistan
‡Nuclear Institute for Food and Agriculture, Peshawar, Pakistan
¶NUST Centre of Virology and Immunology, National University of Science and Technology (NUST), Islamabad, Pakistan
¶¶United States Department of Agriculture, Agricultural Research Service, Wheat Genetics, Quality, Physiology, and Disease Research Unit, and Department of Plant Pathology, Washington State University, Pullman, Washington, USA
[1]Corresponding author's e-mail: hadiqau@gmail.com

ABSTRACT

Information on virulence and molecular diversity of *Puccinia striiformis* f. sp. *tritici* (*Pst*) is a pre-requisite for mitigating the substantial yield losses caused by the stripe rust pathogen in Pakistan, the United States and other countries of the world. This study was undertaken to analyze both virulence and molecular diversity of 46 *Pst* isolates from Pakistan, in comparison with 9 US isolates. Avirulence to stripe rust resistance genes *Yr5*, *Yr15* and *YrSP* was common among all tested isolates. Isolates from Pakistan had low virulence frequency for differentials carrying *Yr2*, (*Yr10*, *YrMor*) and (*Yr2*, *Yr4a*, *YrYam*). Clustering based on virulence data grouped contemporary isolates together and revealed high genetic diversity among pathotypes of both countries. Molecular analysis using sequence tagged site (STS) and microsatellites markers revealed high diversity based on polymorphic information contents (PIC)and marker index (MI) which was higher for SSRs (0.78 & 39.51, respectively) than STS markers(0.04 & 0.29, respectively). Dendrogram based on molecular marker data grouped together contemporary pathotypes indicating genetic similarity. Pathotypes belonging to both countries clustered together indicating common ancestry. Furthermore, very low correlation (r=0.08) was observed between virulence and molecular diversity showing independence in both trends of diversity. The depicted virulence pattern of *Pst* isolates will guide in devising cultivar improvement efforts for stripe rust resistance.

Key Words: Comparative virulence; Molecular diversity; Stripe rust; Pakistan; United States

INTRODUCTION

Stripe rust of wheat has been a major production constraint in South Asia, North America and other parts of the world (Singh *et al.*, 2004; Chen, 2005, 2007). Due to the wider occurrence of the pathogen which induces substantial grain losses, it is a formidable threat to wheat production (Chen, 2005). In Pakistan, 5.8 m ha (70% of the total wheat acreage) is prone to stripe rust (Singh *et al*., 2004). The disease occurs frequently in the foot hills in the north and rarely prevails in wheat growing areas of the central region and western uplands of the country (Hassan, 1968). These areas encountered thirteen epidemics since 1948 where farmers have faced heavy grain losses. Prevalence of stripe rust was rare during the past in southern parts of Pakistan due to dry and warm climate but recently it was observed on wheat in southern Punjab and Sindh. Despite being a major foliar disease of wheat, limited work on the pathogen virulence has been reported from Pakistan (Ahmad, 2000; Hussain *et al*., 2004; Rizwan *et al*., 2010; Bahri *et al*., 2011; Bux *et al*., 2011). Stripe rust of wheat is historically prevalent in the western United States and has become significant in in the South-Central States since 1984 (Line & Qayoum, 1992) and especially more destructive in states east of the Rocky Mountains since 2000 (Chen *et al*., 2002, 2004; Chen & Penman, 2005, 2006). During 2005, the disease spread to more than 30 states (Chen & Penman, 2006). Virulence studies from 2000 to 2008, revealed 79 new races (PST-60 to PST-138). The number of races identified from 2000 onwards was more than that identified in last four decades and virulence spectrum seemed to be widening making the pathogen more destructive (Chen *et al*., 2010).

Stripe rust is preferably controlled by genetic

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resistance besides using fungicides and agronomic practices; however, its dynamic nature has imposed difficulties to combat it adequately. Changes in the pathogen population are brought about by mutation and somatic recombination and through selection pressure exerted by the major (R) genes deployed on larger areas resulting in the evolution of races. Sexual recombination may cause genetic variability in the pathogen where alternate hosts, *Berberis* spp., are available (Jin *et al*., 2010) or through migration into new areas bringing change in the local pathogen population (Singh & Huerta-Espino, 1996). These factors bring about shift in the virulence scenario of the pathogen. Surveillance studies to track virulence changes in the stripe rust pathogen populations were useful in the deployment of resistant genes and guide breeding programs accordingly. The most common method of studying virulence patterns of the rust pathogen is evaluating its reaction on a set of differential genotypes carrying different resistance genes. However, virulence studies reveal limited information about pathogen diversity because it is governed by a very small fraction of its genome.

In addition to virulence studies, molecular markers are widely applied to characterize genetic diversity, population structure, phylogenetic relationships, and discrimination of pathotypes in fungal plant pathogen populations (Majer *et al*., 1996; Arenal *et al*., 1999). Various molecular marker systems, such as random amplified polymorphic DNA (RAPD) (Chen *et al*., 1993), restriction fragment length polymorphism (RFLP) (Shan *et al.,* 1998), intergenic spaces (IGS) sequences (Roose-Amsaleg *et al.,* 2002) and amplified fragment length polymorphism (AFLP) (Hovmøller *et al*., 2002; 2008) have been used successfully. Recently, simple sequence repeat (SSR) markers were developed (Duan *et al*., 2003; Enjalbert *et al*., 2002) which were successful in determining the genetic variability and relationship among *Pst* populations. Such studies on the genetic diversity of *Pst* population of Pakistan are not much available (Bahri *et al*., 2011). This study was therefore designed to analyze the diversity of virulence and molecular variation of the Pakistan *Pst* population and to determine the genetic relationship of the Pakistan isolates with predominant races in the United States.

MATERIALS AND METHODS

Stripe rust collection and spore multiplication: A total of 74 stripe rust samples were used for obtaining *Pst* isolates. Of these, 38 leaf samples infected by *Pst* were collected from disease nurseries, breeding trials, and commercial fields across Pakistan during the 2008-2009 wheat growing season. Two glass vials (1 mL) containing bulk urediniospores were obtained from the Department of Plant Sciences, Quaid-e-Azam University, Islamabad, Pakistan. Urediospores of 34 *Pst* isolates (25 Pakistani & 9 U.S.) were obtained from Dr. Xianming Chen's collections. Spores were revived from diseased leaf samples by cutting them into pieces which were placed on wet filter paper in a petri dish that was kept at 4^{o}C overnight or maximally 24 h. Wheat cultivar Nugaines, known to be susceptible to all U.S. *Pst* races in the seedling stage was used for spore increase. Fresh spores produced on leaf pieces were transferred with a small brush for inoculating two-leaf stage seedlings of the susceptible cultivar. After heat shock in a glass vial or foil bag that was stored in liquid nitrogen in 50-55°C water for 2 min, urediniospores of an isolate were inoculated on to seedlings of Nugaines after mixing with talcum (Sigma, St. Louis, MO) in a ratio of 1:20. Inoculated seedlings were kept in a dew chamber at 10°C for 16-24 h and then transferred to a growth chamber programmed to change temperature gradually between a minimum of 4°C at 2:00 AM during the 8-h dark period and a maximum of 20°C at 2:00 PM during the 16-h light period as described by Chen *et al*. (2002). Urediniospores were collected 16 days after inoculation using a vacuum spore collector and stored in a desiccator at 4°C for 3 to 10 days for drying. Fresh urediniospores were further increased to obtain a sufficient quantity of spores for virulence and molecular analyses.

Virulence analysis: Out of 74 samples used for spore increase, 55 (46 from Pakistan, 9 from the U.S.) could be revived and analyzed for virulence profile (Table I). For virulence analysis, 5-7 seeds of each US wheat stripe rust differential or Yr single-gene line (Table II) were planted in 7×7 cm plastic pot filled with a potting mixture of 24 L peat moss, 8 L perlite, 12 L sand, 12 L commercial potting soil, 16 L vermiculite and 250 g 14-14-14 Osmacote. Seedlings were grown in a rust-free greenhouse and were inoculated at the two-leaf stage with urediniospores of each isolate after mixing with talcum (Sigma, St. Louis, MO) at a ratio of about 1:20. Seedlings were incubated following inoculation in a dew chamber at 10°C under dark for 18 to 24 h and then kept in a growth chamber (Chen & Line, 1992). Infection types (IT) score based on a 0-9 scale was recorded after 16 to 18 days of inoculation following Line and Qayoum (1992). Infection type 0-5 was considered avirulent and 6-9 were considered virulent.

Molecular analysis: Fifty five *Pst* isolates (46 from Pakistan, 9 from the US) (Table I), which were used in the virulence tests, were also studied for molecular analysis. Urediniospores were harvested from infected leaves 15 days after inoculation. DNA was extracted from dried urediniospores (10 mg) according to the CTAB method described by Chen *et al*. (1993). Quantification of DNA was carried out on a 0.8% agarose gel and a spectrophotometer (NanoDrop ND-1000, Thermo scientific, Wilmington, DE, USA). DNA concentration was adjusted to a 10 ng/µL by adding sterilized deionized and distilled water to use as working dilution for PCR amplification.

Genotyping of the isolates was carried out using 10 SSR and 1 STS primer (Table III). PCR was performed in a Gene Amp(R) PCR System 9700 Thermo-cycler. PCR reaction was carried out in 15 µL reaction mixture consisted

of final concentration of 0.4 uM each primer, 0.2 mM each of dCTP, dGTP, dTTP and dATP (Sigma Chemical Co., St. Louis, MO, USA), 2.5 mM $MgCl_2$, 1X PCR Buffer,1 unit/µL of Taq DNA polymerase (Promega Madison WI, USA), and 2 ng/µL genomic DNA. After 5 min of denaturation at 94°C, amplifications were programmed for 35(for SSR) and 38(for STS) consecutive cycles each consisting of 30 sec at 94°C, 30 sec at 50- 60°C (depending on the individual primers), 30 sec at 72°C and followed by a 10 min extension step at 72°C. After electrophoresis of PCR products, polyacrylamide gel was silver-stained according to the manufacturer's recommendation (Promega, Madison, WI, USA) and allowed to dry overnight at room temperature. A gel photograph was produced using a computer attached scanner.

Data analysis

Virulence frequency: Frequency of virulence was determined as percentage of the isolates virulent for specific gene from total of isolates virulent for that gene in the study.

Cluster analysis: Clusters were constructed on virulence and molecular data using NTSYSpc software (version 2.02a, Applied Biostatistics Inc., New York). Virulence or avirulence on each wheat differential genotype was coded as 1 and 0, respectively. Similarly binary (0 or 1) data generated from molecular analysis was used to construct a dendrogram. A similarity matrix based on simple matching was generated by the SIMQUAL (similarity for qualitative data) program, and cluster analysis was done using the unweighted pair group arithmetic mean (UPGMA) method in the SAHN program of the software (Rohlf, 1992) based on both virulence and molecular data. The dendrogram with the best fit to a similarity matrix based on the cophenetic (COPH) values using a matrix comparison (MXCOP) program of NTSYS-pc was chosen. Groups and subgroups were determined using arbitrary points of similarity coefficients. Common virulence and avirulence factors were identified for each group and subgroup.

Polymorphism information content (PIC): The PIC value for each SSR marker locus (*i*) was calculated following the formula described by Keim*et al.* (1992). PIC(*i*)=1 $-\sum P_{ij}2$, where P_{ij} is the frequency of the *j*th allele of the *i*th SSR locus and summation extends over *n* alleles.

Marker index (MI): MI was calculated as: MI = EHp; MI = nHep; MI = nb Hp; where n is a number of bands (n = np + nnp).

RESULTS

Virulence analysis: Forty six Pakistani isolates had low virulence frequencies for Yamhill (*Yr2, Yr4a, YrYam*) (22%) and high (61-96%) for Lemhi (*Yr21*), Chinese 166(*Yr1*), Heines VII (*Yr2, YrHVII*), Paha (*YrPa1, YrPa2, YrPa3*), Produra (*YrPr1, YrPr2*), Stephens (*Yr3a, YrS,YrSte*), Lee (*Yr7, Yr22, Yr23*), Fielder (*Yr6, Yr20*), Express (*YrExp1, YrExp2*) and Compair (*Yr8, Yr19*) (Fig. 1). Similarly, these 46 isolates had low virulence frequencies (18-19%) for single-gene lines containing *Yr17,Yr24* and *Yr25* and high frequencies (61-100%) for *YrA, Yr1, Yr2, Yr6, Yr7, Yr8, Yr9, YrUkn, Yr27, Yr28, Yr31* and *YrExp2*. None of the isolates from Pakistan was found virulent to genes*Yr5, Yr10, Yr15, Yr32* and *YrSP*. Virulence to gene *Yr31*was fixed in all isolates (Fig. 2).

Nine representative races of *Pst* from the United States displayed low virulence frequencies (11-22%) for differentials Tyee (*YrTye*), Hyak (*Yr17, YrTye*), Chinese 166(*Yr1*) and Duchamp (*Yr3a, YrD, YrDru*) and high (67-100%) for Yamhill (*Yr2, Yr4a, YrYam*), Lee (*Yr7, Yr22* & *Yr23*), Fielder (*Yr6, Yr20*), Lemhi (*Yr21*) and Heines VII (*Yr2, YrHVII)* (Fig. 1). On the other hand, these races demonstrated low virulence frequencies (22%) for single-gene lines containing *Yr1* and *Yr25*, whereas, frequencies were was high (67-100%) for virulences to*Yr2, Yr7, Yr17, Yr31* and *Exp2*. None of the races was virulent to *Yr5, Yr15* and *YrSP*. However, virulence for *Yr31* and two differentials including (Lemhi (*Yr21*) and Heines VII (*Yr2, YrHVII*) was fixed in all of the races (Fig. 2).

The Pakistan and US isolates had moderate commonality of virulences. Avirulence for *Yr5*, *Yr10* and *YrSP* was common in both countries. Virulences with frequencies between 50% and 100% in both countries included those on Lemhi(*Yr21*), Heines VII (*Yr2,YrHVII*), Produra (*YrPr1, YrPr2*), Stephens(*Yr3a, YrS, YrSte*), Lee(*Yr7, Yr22, Yr23*), Fielder (*Yr6, Yr20*), Express (*YrExp1, YrExp2*), Clement (*Yr9,YrCle*), Compair (*Yr8, Yr19*), *YrA, Yr2, Yr6, Yr7, Yr8, Yr9* and *YrExp2*, while it was fixed for *Yr31*.

Cluster analysis based on virulence data: Cluster analysis using virulence/avirulence profile separated the *Pst* isolates into two main groups based on genetic similarity using simple matching coefficient (Fig. 3). First group of pathotypes (cluster-A) had 71% mean similarity and formed three sub clusters. First subcluster (cluster-A1) departed at 80% genetic similarity and grouped 18 isolates, which had virulence for Lemhi (*Yr21*), Chinese 166(*Yr1*), Heines VII (*Yr2,YrHVII*), Fielder (*Yr6, Yr20*), *Yr9, YrA, Yr1, Yr6, Yr7, Yr17, Yr27, Yr28, Yr31* and Express (*YrExp1, YrExp2*) and avirulence for Moro (*Yr10, YrMor*), *Yr5, Yr15* and *YrS.* In the second sub cluster (cluster-A2), 11 isolates grouped together at 81% genetic similarity. Isolates in this sub-cluster shared virulences to Paha (*YrPa1, YrPa2, YrPa3*), Produra (*YrPr1, YrPr2*), Stephens (*Yr3a, YrS, YrSte*), Fielder (*Yr6, Yr20*), Express (*YrExp1, YrExp2*), *Yr2, Yr7,Yr17, Yr27, Yr28, Yr31* and *YrExp2* and avirulences to Moro (*Yr10, YrMor*), *Yr5*,Clement (*Yr9, YrCle*), *Yr10, Yr15, Yr32* and *YrSP*. While the third sub cluster (cluster-A3) formed at 85% genetic similarity and 16 pathotypes grouped into it. Isolates in this group carried common virulences to Lemhi (*Yr21*), Lee (*Yr7, Yr22, Yr23*), Fielder (*Yr6, Yr20*), *Yr9, YrA, Yr2, Yr6, Yr7, Yr9, Yr31* and *YrExp2* and avirulences for Chinese 166(*Yr1*), Druchamp (*Yr3a, YrD, YrDru*), Tyee (*YrTye*), *Yr1*, and *Yr25*. In the first main group, isolate PK07-13SW was a sole member.

Fig. 1: Virulence frequency (%) of 46 Pakistan and 9 U.S. *Pst* isolates detected on US differentials

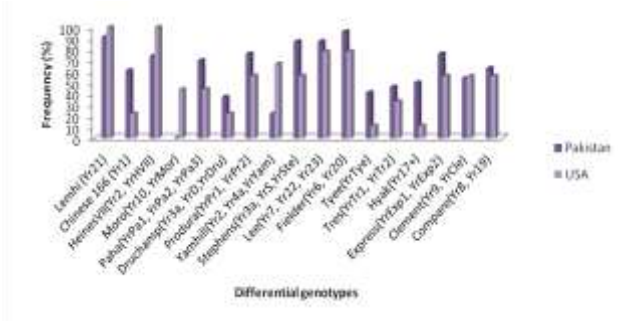


Fig. 2: Virulence frequency (%) of 46 Pakistan and 9 U.S. PST isolates detected on nearisogenic lines (NILs)

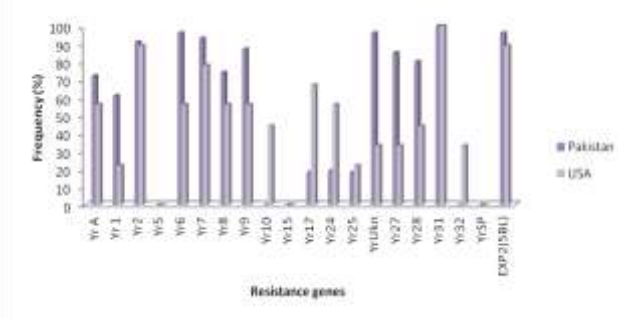
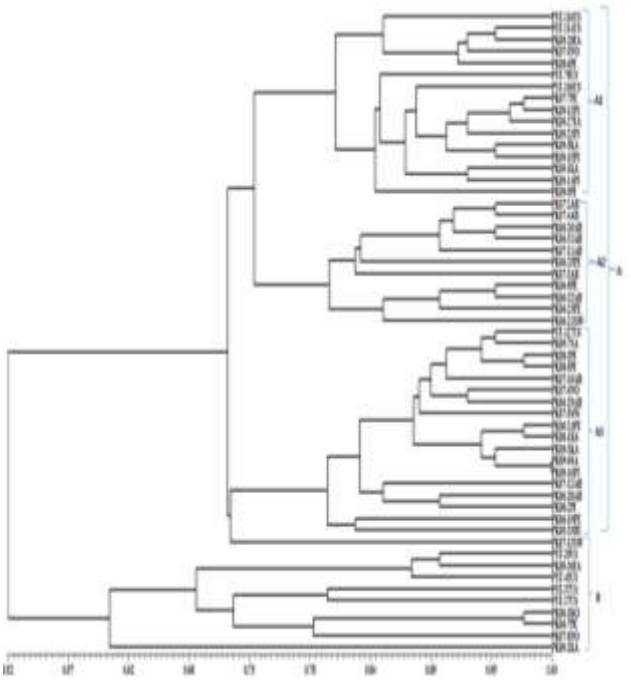
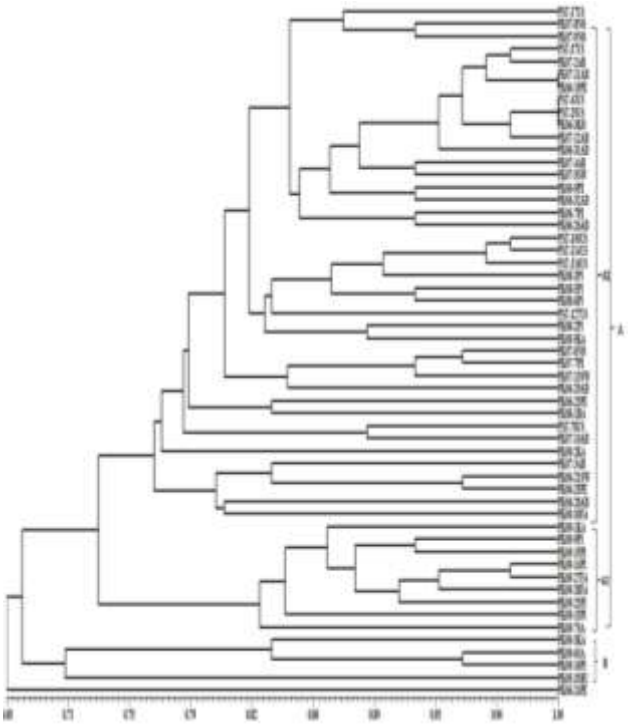


Fig. 3: Dendrogram of 55 stripe pathotypes from Pakistan and U.S.A. based on virulence data



In the second group (cluster-B), 9 isolates grouped together at 60% mean similarity. These isolates shared virulences for Lemhi (*Yr21*) and *Yr31* and avirulence for *Yr8, Yr27,* and Compair (*Yr8, Yr19*).

Fig. 4: Dendrogram of 55 isolates of *Puccinia striiformis* f. sp. *tritici* from Pakistan and the U.S.A. based on molecular marker data



Molecular analysis: The markers are presented in Table IV. The total number of amplified bands was 727 with an average of 66 bands per primer. The highest number of bands (156) was amplified with primer RJ-18, while the lowest number (13) was generated with CPS34. For individual isolates, the highest number of amplified bands was 20 in isolate PK09-23PI, while the lowest was 8 in PK07-10AB. Considering the amplified alleles, the total number was 36 with an average of 3.27 alleles per primer. The number of amplified alleles varied with different primers tested. The STS primer amplified two alleles while SSR primers amplified maximum 7 (RJ27) followed by 4 alleles by each of RB10 and RJ15. Both primers (RJ22 & CPS34) amplified the minimum number of 2 alleles each. The total polymorphic alleles were 33 with 3 loci per primer. PIC values for all primer assays were calculated and the highest (0.78) was for RJ27, which was followed by RJ18 (0.66) and RJ3 (0.66) with an average of 0.45 per primer. Marker index was determined for all primers and the highest index was for RB10 (39.51) which was followed by RJ27 (38.29). STS primer 58H22 had the least MI (0.29), while the average was 14.08 per primer.

Cluster analysis based on the molecular marker data: The molecular marker data revealed high genetic similarity of 100% among the following pair of isolates: PK06-3KO─PST-43US, PK06-3KO─PST-29US and PK06-19PE─PK07-11AB. Pathotypes, PST-43 and PST-29 were from the U.S. and collected prior 2000 while the remaining were sampled from Pakistan during 2006 and 2007.

Table I: Stripe rust isolates from Pakistan and U.S. studied for virulence and molecular diversity

Isolate*	Location	Country	Host	Year of Collection
PK07-6NW	Nowshera	Pakistan	Wheat	2007
PK07-12AB	Abbotabad	Pakistan	Wheat	2007
PK07-2AB	Abbotabad	Pakistan	Wheat	2007
PK07-4AB	Abbotabad	Pakistan	Wheat	2007
PK06-9PE	Peshawar	Pakistan	Wheat	2006
PK06-32AB	Abbotabad	Pakistan	Wheat	2006
PK06-3KO	Kohat	Pakistan	Wheat	2006
PK06-7PE	Peshawar	Pakistan	Wheat	2006
PK06-19PE	Peshawar	Pakistan	Wheat	2006
PK06-21SW	Swat	Pakistan	Wheat	2006
PK06-23PE	Peshawar	Pakistan	Wheat	2006
PK06-24PE	Peshawar	Pakistan	Wheat	2006
PK06-25PE	Peshawar	Pakistan	Wheat	2006
PK06-26AB	Abbotabad	Pakistan	Wheat	2006
PK06-28AB	Abbotabad	Pakistan	Wheat	2006
PK06-29AB	Abbotabad	Pakistan	Wheat	2006
PK06-31AB	Abbotabad	Pakistan	Wheat	2006
PK07-10AB	Abbotabad	Pakistan	Wheat	2007
PK07-11AB	Abbotabad	Pakistan	Wheat	2007
PK07-13SW	Swat	Pakistan	Wheat	2007
PK07-3AB	Abbotabad	Pakistan	Wheat	2007
PK07-5SW	Swat	Pakistan	Wheat	2007
PK07-7PE	Peshawar	Pakistan	Wheat	2007
PK07-8NO	Nowshera	Pakistan	Wheat	2007
PK07-9NO	Nowshera	Pakistan	Wheat	2007
PK08-1BA	Bahawalpur	Pakistan	Wheat	2008
PK08-2PI	Pirsabak	Pakistan	Wheat	2008
PK08-3PI	Pirsabak	Pakistan	Wheat	2008
PK08-5PI	Pirsabak	Pakistan	Wheat	2008
PK08-6PI	Pirsabak	Pakistan	Wheat	2009
PK09-1KA	Kaghan	Pakistan	Wheat	2009
PK09-2KA	Kaghan	Pakistan	Wheat	2009
PK09-3KA	Kaghan	Pakistan	Wheat	2009
PK09-5KA	Kaghan	Pakistan	Wheat	2009
PK09-6SA	Sakrand	Pakistan	Wheat	2009
PK09-7SA	Sakrand	Pakistan	Wheat	2009
PK09-9PI	Pirsabak	Pakistan	Wheat	2009
PK09-13	Pirsabak	Pakistan	Wheat	2009
PK09-14PI	Pirsabak	Pakistan	Wheat	2009
PK09-15PI	Pirsabak	Pakistan	Wheat	2009
PK09-16PI	Pirsabak	Pakistan	Wheat	2009
PK09-23PI	Pirsabak	Pakistan	Wheat	2009
PK09-27FA	Faisalabad	Pakistan	Wheat	2009
PK09-28FA	Faisalabad	Pakistan	Wheat	2009
PK09-30FA	Faisalabad	Pakistan	Wheat	2009
PK05-33BU	Bulk	Pakistan	Wheat	2005
PST 116	09-168	USA	Wheat	2009
PST 78	2k-041-Yr9	USA	Wheat	2000
PST 127	09-248	USA	Wheat	2009
PST 114	N/A	USA	Wheat	N/A
PST 100	05-464	USA	Wheat	2005
PST 29	N/A	USA	Wheat	N/A
PST 37	N/A	USA	Wheat	N/A
PST 17	N/A	USA	Wheat	N/A
PST 43	N/A	USA	Wheat	N/A

*****Isolate name consists of four parts, first letters; PK denotes country, second two values denote year of collection eg.05=2005, 06= 2006 etc., third part denotes isolate number, while last two letters denote location of collection eg. AB=Abbotabad, KO=Kohat and BU= Bulk of urediniospores etc

Among these pathotypes, the former two pairs have 5 whereas the last pair has 18 common virulences. The least genetic similarity (55.6%) was observed between following pairs of isolates: PK09-6SA─PK07-3AB, PK09-6SA─PK07-10AB, PK09-16PI─ PK07-3AB, PK09-16PI─PK07-10AB, and PK09-23AB─ PK07-10AB (data not shown). Among these pairs of isolates, the last had 21, the first and the third each had 15, while the second and fourth each had 25 common virulences. In all these pairs of pathotypes, the first was collected during 2007 while the remaining isolates were sampled during 2009. The results indicated that genetic similarity based on molecular markers does not correspond to the virulences in the pathogen.

Table II: Differential genotypes used for virulence analyses

US differentials		Nearisogenic lines(NILSs)	
Differential	**Gene**	**Differential**	**Gene**
Lemhi	*Yr21*	AvSYrANIL	*YrA*
Chinese 166	*Yr1*	AvSYr1NIL	*Yr1*
Heines VII	*Yr2, YrHVII*	SieteCerros T66	*Yr2*
Moro	*Yr10, YrMor*	AvSYr5NIL	*Yr5*
Paha	*YrPa1, YrPa2, YrPa3*	AvSYr6NIL	*Yr6*
Druchamp	*Yr3a, YrD,YrDru*	AvSYr7NIL	*Yr7*
AvSYr5NIL	*Yr5*	AvSYr8NIL	*Yr8*
Produra	*YrPr1, YrPr2*	AvSYr9NIL	*Yr9*
Yamhill	*Yr2, Yr4a,YrYam*	AvSYr10NIL	*Yr10*
Stephens	*Yr3a, YrS,YrSte*	AvSYr15NIL	*Yr15*
Lee	*Yr7, Yr22, Yr23*	AvSYr17NIL	*Yr17*
Fielder	*Yr6, Yr20*	AvSYr24NIL	*Yr24*
Tyee	*YrTye*	TP981	*Yr25*
Tres	*YrTr1, YrTr2*	AvSYrUknNIL	*YrUkn*
Hyak	*Yr17,YrTye*	AvsYr27NIL	*Yr27*
Express	*YrExp1, YrExp2*	AvSYr28NIL	*Yr28*
AvSYr8NIL	*Yr8*	AvSYr31NIL	*Yr31*
AvSYr9NIL	*Yr9*	AvSYr32NIL	*Yr32*
Clement	*Yr9, YrCle*	AvSYrSPNIL	*YrSP*
Compair	*Yr8, Yr19*	AvS/Exp1/1-1 Line74	*YrExp2*

The cluster constituted two main groups based on the genetic similarities using the simple matching coefficient (Fig. 4). The first group (cluster-A) of isolates were separated at 72% mean similarity and formed two subclusters. The first subcluster (cluster-A1) diverged at 75.8% genetic similarity and grouped isolates PST17US, PK07-8NO, PK07-9NO, PST37US, PK07-2AB, PK07-11AB, PK06-19PE, PST43US, PST29US, PK06-3KO, PK07-12AB, PK06-31AB, PK07-4AB, PK07-5SW, PK06-9PE, PK06-32AB, PK06-7PE, PK06-26AB, PST100US, PST114US, PST116US, PK08-3PI, PK08-5PI, PK08-6PI, PST127US, PK08-2PI, PK09-5KA, PK07-6NO, PK07-7PE, PK07-13SW, PK06-29AB, PK06-23PE, PK08-1BA, PST78US, PK07-10AB, PK09-2KA, PK07-3AB, PK06-21SW, PK06-25PE, PK06-28AB and PK09-30FA. In the second subcluster (cluster-A2), isolates including PK09-1KA, PK09-9PI, PK09-15PI, PK09-14PI, PK09-27FA, PK09-28FA, PK09-23PI, PK09-13PI and K09-7SAwere grouped at 83% genetic similarity. Subcluster (A1) grouped 41 isolates, 9 from the U.S. and 32 from Pakistan. In this cluster, most of the isolates (24) from Pakistan were sampled during 2006 and 2007. All isolates belonging to this cluster had a common virulence for *Yr31*.

Table III: Virulence/avirulence pattern of Pakistan and U.S. stripe rust isolates detected at seedlings of U.S. differentials and near isogenic lines (NILs)

Isolate	Virulence/avirulence[a]	Virulence/avirulence[b]
PK07-6NW	1,2,3,5,6,8,9,10,12,14,15,16,17,18,19,20/4,7,11,13	1,2,3,5,6,7,8,13,14, ,15, 16, 17, 20/4,9,10,11,12,18,19
PK07-12AB	1,2,3,5,8,11,12,17,18,19,20/4,6,7,9,10,13,14,15,16	1, 2, 3, 5, 6, 7, 8, 12, 13, 14, 15, 16, 17, 20/4,9,10,11,18,19
PK07-2AB	1, 2, 3, 5, 8, 10, 11, 12, 16, 17, 18, 20/4,6,7,9,13,14,15,19	1, 2, 3, 5, 6, 7, 8, 11, 14, 15, 16, 17, 20/4,9,10,12,13,18,19
PK07-4AB	2, 3, 5, 8, 10, 11, 12, 15, 16, 17, 18, 20/1,4,6,7,9,13,14,19	1, 2, 3, 5, 6, 7, 8, 14, 15, 16, 17, 20/4,9,10,11,12,13,18,19
PK06-9PE	1, 2, 5, 8, 10, 11, 12, 16, 18/3,4,6,7,9,13,14,15,17,19,20	1, 2, 3, 5, 6, 8, 14, 15, 16, 17, 20/4,7,9,10,11,12,13,18,19
PK06-32AB	1, 2, 5, 6, 8, 10, 11, 12, 16, 18/3,4,7,9,13,14,15,17,19,20	2, 3, 5, 6, 8, 14, 15, 16, 17, 20/1,4,7,9,10,11,12,13,18,19
PK06-3KO	1, 3, 12, 13, 15/2,4,5,6,7,8,9,10,11,14,16,17,18,19,20	3, 5, 14, 17/1,2,4,6,7,8,9,10,11,12,13,15,16,18,19,20
PK06-7PE	1, 3, 12, 13, 15/2,4,5,6,7,8,9,10,11,14,16,17,18,19,20	3, 5, 14, 17, 20/1,2,4,6,7,8,9,10,11,12,13,15,16,18,19
PK06-19PE	1, 2, 3, 5, 6, 10, 11, 12, 13, 14, 15, 18, 19/4,7,8,9,16,17,20	1, 2, 5, 6, 8, 13, 14, 15, 16, 17, 20/3,4,7,9,10,11,12,18,19
PK06-21SW	1, 5, 8, 10, 11, 12, 14, 16, 18/2,3,4,6,7,9,13,15,17,19,20	3, 5, 6, 8, 14, 15, 17, 20/1,2,4,7,9,10,11,12,13,16,18,19
PK06-23PE	1, 2, 5, 8, 10, 11, 12, 14, 16/3,4,6,7,9,13,15,17,18,19,20	2, 3, 5, 6, 11, 14, 15, 16 ,17, 20/1,4,7,8,9,10,12,13,18,19
PK06-24PE	1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 15, 17, 18, 19, 20/4,7,14,16	1, 2, 5, 6, 7, 8, 13, 14, 15, 16, 20/3,4,9,10,11,12,17,18,19
PK06-25PE	2, 5, 6, 8, 10, 12, 13, 14, 16, 17, 18, 20/1,3,4,7,9,11,15,19	2, 3, 5, 6, 7, 8, 12, 14, 15, 16, 17, 20/1,4,9,10,11,13,18,19
PK06-26AB	1, 2, 5, 8, 10, 11, 12, 14, 16, 17, 18, 20/3,4,6,7,9,13,15,19	2, 3, 5, 6, 7, 8, 11, 14, 15, 17, 20/1,4,9,10,12,13,16,18,19
PK06-28AB	1, 2, 3, 5, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20/4,6,7,9,19	1, 2, 3, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16, 17, 20/4,9,10,18,19
PK06-29AB	1, 2, 3, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20/4,5,7	1, 2, 3, 5, 6, 7, 8, 13, 14, 15, 16, 17, 20/4,9,10,11,12,18,19
PK06-31AB	1, 2, 5, 8, 10, 11, 12, 15, 16, 17, 18, 20/3,4,6,7,9,13,14,19	2, 3, 5, 6, 7, 8, 14, 15, 16, 17, 20/1,2,4,9,10,11,12,13,18,19
PK07-10AB	1, 2, 3, 5, 6, 8, 10, 11, 12, 13, 16, 17, 18, 19, 20/4,7,9,14,15	1, 2, 3, 5, 6, 7, 8, 13, 14, 15, 16, 17, 20/4,9,10,11,12,18,19
PK07-11AB	2, 3, 5, 8, 10, 11, 12, 14, 16, 17, 18, 20/1,4,6,7,9,13,15,19	2, 3, 5, 6, 7, 8, 13, 14, 15, 16, 17, 20/1,4,9,10,11,12,18,19
PK07-13SW	1, 3, 5, 6, 9, 10, 11, 14, 15, 16, 18, 19/2,4,7,8,12,13,17,20	3, 5, 6, 8, 13, 14, 15, 16, 17, 20/1,2,4,7,9,10,11,12,18,19
PK07-3AB	2, 5, 8, 10, 11, 12, 14, 16, 17, 20/1,3,4,6,7,9,13,15,18,19	2, 3, 5, 6, 7, 14, 15, 17, 20/1,4,8,9,10,11,12,13,16,18,19
PK07-5SW	1, 2, 3, 5, 6, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19/4,7,8,20	1, 2, 3, 5, 6, 7, 8, 12, 13, 14, 15, 16, 17, 20/4,9,10,11,18,19
PK07-7PE	1, 3, 8, 10, 11, 12, 16, 17, 20/2,4,5,6,7,9,13,14,15,18,19	1, 3, 5, 6, 7, 8, 14, 15, 16, 17, 20/2,4,9,10,11,12,13,18,19
PK07-8NO	1, 11, 12, 16/2,3,4,5,6,7,8,9,10,13,14,15,17,18,19,20	3, 5, 6, 12, 14, 16, 17, 20/1,2,4,7,8,9,10,11,13,15,18,19
PK07-9NO	1, 5, 8, 10, 11, 12, 14, 16, 17, 18, 19, 20/2,3,4,6,7,9,13,15	1, 3, 5, 6, 7, 8, 14, 15, 17, 20/2,4,9,10,11,12,13,16,18,19
PK08-1BA	1, 2, 3, 5, 6, 8, 10, 11, 12, 13, 15, 17, 18, 19, 20/4,7,9,14,16	1, 2, 5, 6, 7, 8, 13, 14, 15, 16, 17, 20/3,4,9,10,11,12,18,19
PK08-2PI	1, 2, 3, 5, 6, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20/4,7,9	1, 2, 3, 5, 6, 7, 8, 12, 13, 14, 15, 16, 17, 20/4,9,10,11,18,19
PK08-3PI	1, 2, 3, 5, 6, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20/4,7,8,14	1, 2, 3, 5, 6, 7, 8, 12, 13, 14, 15, 16, 17, 20/4,9,10,11,18,19
PK08-5PI	1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20/4,7,14	1, 2, 3, 5, 6, 7, 8, 13, 14, 15, 16, 17, 20/4,9,10,11,12,18,19
PK08-6PI	1, 3, 8, 10, 11, 12, 16, 17, 18, 19, 20/2,4,5,6,7,9,13,14,15	1, 3, 5, 6, 7, 8, 14, 15, 16, 17, 20/2,4,9,10,11,12,13,18,19
PK09-1KA	1, 3, 8, 10, 11, 12, 14, 16, 17, 18, 20/2,4,5,6,7,9,13,15,19	1, 3, 5, 6, 7, 8, 14, 17, 20/2,4,9,10,11,12,13,15,16,18,19
PK09-2KA	1, 5, 8, 12, 14, 16, 18, 19/2,3,4,6,7,9,10,11,13,15,17,20	1, 3, 5, 6, 8, 11, 12, 17, 20/2,4,7,9,10,13,14,15,16,18,19
PK09-3KA	1, 2, 3, 5, 6, 9, 10, 11, 12, 13, 15, 17, 18, 19/4,7,8,14,16,20	1, 2, 5, 6, 7, 8, 13, 14, 15, 16, 17, 20/3,4,9,10,11,12,18,19
PK09-5KA	1, 3, 5, 8, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20/2,4,6,7,9,13	1, 3, 5, 6, 7, 8, 14, 15, 16, 17, 20/2,4,9,10,11,12,13,18,19
PK09-6SA	1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 15, 17, 18, 19/4,7,14,16,20	1, 2, 3, 5, 6, 7, 8, 13, 14, 15, 16, 17, 20/4,9,10,11,12,18,19
PK09-7SA	1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 15,1 6, 17, 18, 19 20/4,7,14	1, 2, 3, 5, 6, 7, 8, 13, 14, 15, 16, 17, 20/4,9,10,11,12,18,19
PK09-9PI	1, 3, 8, 10, 11, 12, 16, 18, 19/2,4,5,6,7,9,13,14,15,17,20	1, 3, 5, 6, 8, 14, 15, 17, 20/2,4,7,9,10,11,12,13,16,18,19
PK09-13	1, 3, 8, 10, 11, 12, 16, 17, 18, 19, 20/2,4,5,6,7,9,13,14,15	1, 3, 5, 6, 7, 8, 11, 14, 15, 16, 17, 20/2,4,9,10,12,13,18,19
PK09-14PI	1, 10, 11, 12, 16, 17, 18, 20/2,3,4,5,6,7,8,9,13,14,15,19	1, 3, 5, 6, 7, 8, 14, 17, 20/2,4,9,10,11,12,13,15,16,18,19
PK09-15PI	1, 3, 8, 10, 11, 14, 15, 16, 17, 18, 19, 20/2,4,5,6,7,9,12,13	1, 3, 5, 6, 7, 8, 14, 15, 16, 17, 20/2,4,9,10,11,12,13,18,19
PK09-16PI	1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 15, 17, 18, 19/4,7,14,16,20	1, 2, 3, 5, 6, 7, 8, 11, 13, 14, 15, 16, 17, 20/4,9,10,12,18,19
PK09-23PI	1, 3, 8, 10, 11, 12, 14, 17, 18, 19, 20/2,4,5,6,7,9,13,15,16	1, 3, 5, 6, 7, 8, 14, 15, 16, 17, 20/2,4,9,10,11,12,13,18,19
PK09-27FA	1, 3, 8, 10, 11, 12, 14, 16, 17, 18, 20/2,4,5,6,7,9,13,15,19	1, 3, 5, 6, 7, 8, 14, 15, 16, 17, 20/2,4,9,10,11,12,13,18,19
PK09-28FA	1, 3, 8, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20/2,4,5,6,7,9,13	1, 3, 5, 6, 7, 8, 12, 14, 15, 16, 17, 20/2,4,9,10,11,13,18,19
PK09-30FA	1, 2, 3, 5, 14/4,6,7,8,9,10,11,12,13,15,16,17,18,19,20	2, 3, 17, 20/1,4,5,6,7,8,9,10,11,12,13,14,15,16,18,19
PK05-33BU	1, 2, 3, 5, 10, 12, 13, 15, 17, 18/4,6,7,8,9,11,14,16,19,20	1, 2, 3, 5, 6, 7, 8, 13, 14, 15, 16, 17, 20/4,9,10,11,12,18,19
PST 116	1, 3, 4, 5, 8, 9, 10, 11, 12, 14, 16, 17, 18, 19, 20/2,6,7,13,15	1, 3, 5, 6, 7, 8, 9, 14, 17, 18, 20/2,4,10,11,12,13,15,16,19
PST 78	1, 3, 8, 11, 12, 16, 17, 18, 19, 20/2,4,5,6,7,9,10,13,14,15	1, 3, 5, 6, 7, 8, 12, 14, 15, 16, 17, 20/2,4,9,10,11,13,18,19
PST 127	1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20/4,7,14	1, 2, 3, 5, 6, 7, 8, 11, 12, 13, 15, 16, 17, 20/4,9,10,14,18,19
PST 114	1, 3, 4, 8, 9, 10, 11, 12, 14, 16, 17, 18, 19, 20/2,5,6,7,13,15	1, 3, 5, 6, 7, 8, 9, 11, 12, 14, 15, 16, 17, 18, 20/2,4,10,13,19
PST 100	1, 3, 8, 9, 10, 11, 12, 16, 17, 18, 19, 20/2,4,5,6,7,13,14,15	1, 3, 5, 6, 7, 8, 11, 16, 17, 18, 20/2,4,9,10,12,13,14,15,19
PST 29	1, 3, 4, 5/2,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20	3, 9, 11, 12, 17/1,2,4,5,6,7,8,10,13,14,15,16,18,19,20
PST 37	1, 3, 6, 8, 9, 10, 11, 12/2,4,5,7,13,14,15,16,17,18,19,20	6, 11, 17, 20/1,2,3,4,5,7,8,9,10,12,13,14,15,16,18,19
PST 17	1, 2, 3, 9, 11/4,5,6,7,8,10,12,13,14,15,16,17,18,19,20	2, 3, 6, 11, 13, 17, 20/1,4,5,7,8,9,10,12,14,15,16,18,19
PST 43	1, 3, 4, 5, 12, 14/2,6,7,8,9,10,11,13,15,16,17,18,19,20	3, 9, 12, 17, 18, 20/1,2,4,5,6,7,8,10,11,13,14,15,16,19

[a]Virulence/avirulence formulae according to the U.S. differetials and [b]according to NILs given in Table II

Clustering of isolates from both countries indicate their genetic relationship.

In the second group (cluster-B), isolates PK09-3KA, PK09-6SA, PK09-16PI and PK05-33BU were grouped at 71% mean similarity. Isolate PK06-24PE did not cluster with any group and remained independent at 68% genetic similarity. In the second subcluster (A2), nine isolates from Pakistan were grouped at 83% genetic similarity. All these isolates were collected during 2009 and shared 13 virulences. In the second group, four isolates (PK09-3KA, PK09-6SA, PK09-16PI and PK05-33BU) from Pakistan were grouped at 71% mean similarity, which were collected

during 2009 except PK05-33BU collected in 2005. These isolates shared 21 virulences. Isolates PK06-24PE was separated from others at 68% genetic similarity and shared same number of virulences (21) with the isolates of the second group.

Mantel's Z test: Correlation analyses using the Mantel's *Z*-test between molecular and virulence data revealed a positive but weak association. Among the isolates collected from Pakistan, correlation was positive (r=0.24) and statistically non-significant (P =0.05). The same trend was observed for isolates from the U.S. (r=0.13) and isolates from Pakistan and USA combined (r=0.08) with non-significant probability values (P = 0.05) respectively.

DISCUSSION

Virulence analysis: Virulence analysis of the isolates from Pakistan revealed higher diversity. However, all the isolates carried avirulence to *Yr5*, *Yr10*, *Yr15*, *Yr32* and *YrSP*. Results of this study are in agreement with those reported from Pakistan by Bahri *et al*. (2011) and Bux *et al*. (2011). Virulence to *Yr5* and *Yr15* genes rarely occur in most wheat producing areas worldwide (Chen, 2005; Afshari, 2008).

Virulence for *Yr27* was observed in PK05-33BU, collected during 2005 and other isolates sampled afterwards with a high virulence frequency of 85%. This data is consistent with the previous speculation that the *Yr27* resistance became ineffective in Pakistan during 2005 (Duveiller *et al*., 2007). Loss of resistance in cultivars carrying *Yr27* impacted severely and brought over 11.0 million hectares of wheat growing areas of Indo-Pakistan subcontinent alone under threat of stripe rust epidemics due to the cultivation of three mega cultivars, Inqilab-91, MH-97 and PBW343 (Attila) possessing *Yr27*. Virulence for this gene was widespread in India, Iran, Yemen, Egypt, Ethiopia, Eritrea, Tajikistan, Uzbekistan and Kyrgyzstan (Singh *et al*., 2004; Afshari, 2008). To reduce such losses, mono-culturing or use of single resistant gene should be avoided. However, varieties with different resistant genes should be deployed in different wheat growing regions to ensure sustainable control against stripe rust.

Virulence assessment of the isolates from Pakistan displayed the highest virulence frequency (87%) for *Yr9*. Virulence for *Yr9* was first detected in Pakistan during 1994 because predominant cultivars having *Yr9* such as Pak-81, Pirsabak-85, Pasban 90, Rohtas 90, Kaghan 90, Khyber 87,

Table IV: Primers used for genetic diversity evaluation of stripe rust isolates

Marker name	Repeats	Forward primer sequence (5' - 3')	Reverse primer sequence (5' - 3')	Expected band (bp)	Anneal. Temp. (C)	Reference
58H22	beta-tubulin	GCCCAATCCACTCATCTA	GCTTCCTCTTCGTAACCC	2000bp	60°C	Wang, M unpublished
RJ-3	(TGG)8	GCA GCA CTG GCA GGT GG	GAT GAA TCA GGA TGG CTC C	208bp	52°C	Enjalbert *et al.*, 2002
RJ-18	(TGT)5	CTG CCC ATG CTC TTC GTC	GAT GAA GTG GGT GCT GCT G	358bp	52°C	Enjalbert *et al.*, 2002
RJ-20	(CAG)4	AGA AGA TCG ACG CAC CCG	CCT CCG ATT GGC TTA GGC	294bp	52°C	Enjalbert *et al.*, 2002
RJ-22	(CA)8	CCC TTC GTC TGT CAT CCG	ATC AAG AAG ATT CCT GGG TGA G	363bp	52°C	Enjalbert *et al.*, 2002
CPS34	(TC)9	GTT GGC TAC GAG TGG TCA TC	TAA CAC TAC ACA AAA GGG GTC	90bp	55°C	Chen *et al.* 2009
RJ-4	(TGG)8	GTG GGT TGG GCT GGA GTC	GCT AAT CCA TTC CAC GCA CC	198bp	52°C	Enjalbert *et al.*, 2002
RB-10	(GT)7+4+4	TAAGATTGGTGGTATGTGGTGGA	TTGTCTTTCATCTCATCCAGCC	218bp	52°C	Duan *et al*., 2003
RJ27	(TC)10	CGT CCC GAC TAA TCT GGT CC	ATG AGT TAG TTT AGA TCA GGT CGA C	229bp	52°C	Enjalbert *et al.*, 2002
RJ15	(TG)7	ATC GAG CAC GTC CAA ATC G	CAC TGG ACA GAC GAC GGT TG	246bp	52°C	Enjalbert *et al.*, 2002
RJ21	(GTT)6	TTC CTG GAT TGA ATT CGT CG	CAG TTC TCA CTC GGA CCC AG	170bp	52°C	Enjalbert *et al.*, 2002

Table V: Analysis of banding pattern generated by STS and SSR primers

Marker	Marker Type	Total Alleles	Polymorphic Alleles	Polymorphism (%)	Polymorphism Information Content (PIC)	Marker Index (MI)
58H22	STS	2	2	100	0.04	0.29
RJ-3	SSR	3	3	100	0.66	17.73
RJ-18	SSR	3	0	0	0.67	0.67
RJ-20	SSR	3	3	100	0.58	15.75
RJ-22	SSR	2	2	100	0.12	1.00
CPS34	SSR	2	2	100	0.36	2.84
RJ-4	SSR	3	3	100	0.53	14.34
RB-10	SSR	4	4	100	0.62	39.51
RJ27	SSR	7	7	100	0.78	38.29
RJ15	SSR	4	4	100	0.16	10.47
RJ21	SSR	3	3	100	0.52	14.00
Total	**11**	**36**	**33**	**1000**	**5.04**	**154.89**
average		**3.27**	**3.00**	**90.91**	**0.46**	**14.08**

Table VI: Correlation between similarity matrices based on virulence and molecular marker data

Mantel Test (Virulence-Marker)	Pakistan	US	Combined
Correlation (r)	0.24	0.13	0.08
P value (0.05)	0.93	0.87	0.86

Punjab 85 and Rawal 87 created a scenario of mono-culture. Consequently, conducive varietal pattern facilitated the pathogen to adopt. The failure of *Yr9* in cultivars grown on a considerable acreage in Khyber Pakhtunkhwa province of Pakistan caused heavy grain losses (Rizwan *et al*., 2010). Historically, virulence for *Yr9* evolved in East Africa in the late 1980s and migrated to North Africa, West Asia, and South Asia. Migration of this virulence caused heavy epidemics in Ethiopia, Turkey, Iran, Afghanistan and Pakistan (Singh *et al*., 2004).

For the virulence analysis, two differential sets (20 US differential cultivars & 20 *Yr* single-gene lines) were used. It was observed that near isogenic differentials for stripe rust resistance genes were highly useful in separating virulence/avirulence composition of the stripe rust pathogen population. Using these differentials, specific information can be provided with regard to individual genes in the prevailing pathogen population. Thus, virulence pattern guide wheat improvement programmes to design which genes to target in specific wheat growing areas. For virulence studies, use of near isogenic differentials for rust resistance genes have been published previously (Kolmer & Liu, 2000; Kosman *et al*., 2004; Goyeau *et al*., 2006; Ordoñez & Kolmer, 2007). However, this debatable issue needs further investigation to devise an appropriate and consensus based differential set for virulence studies of *Pst* populations worldwide.

Genetic diversity based on virulence data: The similarity matrix and dendrogram based on virulence data showed considerable diversity among isolates from Pakistan and the U.S. Virulence relatedness showed clustering with respect to number of virulences associated with isolates. Accordingly, isolates carrying a low number of virulences grouped together; similarly, those having a high number of virulences were placed together. It was observed that isolates tend to acquire more virulences with time, making them more aggressive. The clustering of Pakistani isolates revealed some evolutionary patterns. Isolates in cluster A1 with more number of virulences may have probably evolved from those in sub-cluster A2, A3 or cluster B containing less diverse isolates. Similarly, isolates from USA were clearly clustered in pre-and post-2000 periods, similar to the findings of Markell and Milus (2008). It is likely that these complex and aggressive isolates have evolved by single step mutations or somatic recombinations from simpler isolates (Chen *et al*., 2002; Chen, 2005, 2007).

Molecular analysis: Stripe rust isolates from Pakistan and United States were analyzed for genetic diversity using 10 SSR and 1 STS primer. All these primers showed polymorphism except for SSR primer RJ-18. Diversity of each primer locus in stripe rust isolates was determined by PIC. Efficiency of each primer locus was further determined by using MI values. Both these tools were efficient in assessing diversity of each locus in the studied population. Assessment of genetic diversity through two different primer sets demonstrated that SSRs are more efficient for stripe rust fungus as compared to STS primer. These primers can also be employed for genotype identification, evolutionary studies and epidemiological investigations of the stripe rust fungus. SSR assay has been successfully used to study stripe rust and other rust fungi (Enjalbert *et al.*, 2002; Duan *et al.*, 2003; Ordoñez & Kolmer 2007; Mboup *et al.*, 2009).

Clustering pattern showed that most of the isolates belonging to the same period grouped together with sporadic distribution regarding their location. Grouping of isolates of the same period showed their genetic similarity and also indicated that the group is genetically unstable and is undergoing changes through mutation, somatic hybridization or migration. Similarly, Bahri *et al*. (2011) hypothesized that migration and varietal diversity factors might contribute in maintaining the currently high genetic diversity in the Pakistani *Pst* population.

Molecular diversity is not strongly related to the virulence polymorphism among isolates. Previously, Chen *et al*. (1993) reported that DNA polymorphism is independent of pathogenicity and whole genome of the pathogen evolves at a much faster rate than genes governing pathogenicity. Koch *et al*. (1991) proposed that a direct relationship between pathogenicity and molecular data would be observed if pathogenicity was controlled by many genes distributed throughout the genome.

Most importantly, grouping of some isolates from Pakistan and the US in cluster A1 based on molecular data indicated their common ancestry. Such assumption was also proposed by Humphrey *et al*. (1924) who agreed that stripe rust migrated from its Asiatic gene pool to North America where it became established on American grasses. Much later, it was adapted to cereals in the new world. Later on, this hypothesis was supported by Hassebrauk (1965) and recently by Line (2002). However, thorough investigations based on molecular studies using a sufficient number of stripe rust isolates from North America and Pakistan and other wheat growing Asian countries will reveal reliable information about the evolutionary history of pathogen.

Comparatively, genetic diversity revealed by virulence was higher than molecular markers. Lower genetic diversity shown by molecular markers indicates that virulence and molecular diversity are independent of each other. Low molecular polymorphism in stripe rust pathotypes may be attributed to the clonal multiplication and migratory nature of the pathogen. Villareal *et al*. (2002) investigated genetic polymorphism in French *Pst* populations using AFLP and virulence tests which revealed low molecular polymorphism assigning all isolates to a single clonal lineage. Hovmøller *et al*. (2002) used AFLP markers and described that stripe rust as a clonal population in Northern Europe. In order to get clear picture of genetic diversity and population structure, the newly discovered alternative host of the stripe rust pathogen and sexual recombination needed to be explored in Pakistan.

In conclusion, the stripe rust pathogen in Pakistan is diverse in virulence and becoming more virulent. Virulences for Lemhi (*Yr21*), Heines VII (*Yr2*, *YrHVII*), Produra (*YrPr1*, *YrPr2*), Stephens (*Yr3a*, *YrS*, *YrSte*), Lee (*Yr7*, *Yr22*, *Yr23*), Fielder (*Yr6*, *Yr20*), Express (*YrExp1*, *YrExp2*), Clement (*Yr9*, *YrCle*), Compair (*Yr8*, *Yr19*), *YrA*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9* and *YrExp2* and *Yr31* were common in Pakistan and US isolates. This commonality in Pathogen virulence is useful in designing common control measures in terms of exchange of disease resistant wheat germplasm by both the countries. The virulence patterns revealed from this study can provide guidance to wheat breeding programmes to develop resistant cultivars. The molecular analysis indicated that the stripe rust pathogen from both Pakistan and the United States have likely the common origin. This possibility still needs to be validated through similar studies with a sufficient number of pathogen samples from the United States and Asian countries including Pakistan.

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