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IDENTIFICATION OF DURABLE RESISTANCE AGAINST YELLOW RUST

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ABSTRACT

Yellow (stripe) rust of wheat is responsible for a severe decrease in crop yield worldwide. Deployment of durable disease resistant cultivars is the best strategy being cost effective and safe. A comprehensive study was conducted to identify sources of durable resistance against stripe rust of wheat. Initially, surveillance of the crop was conducted to expose the status of the dilemma. The study discovered that disease is more prevalent in the region existing in Khyber Pakhtunkhwa (KPK) and the Northern Region of Punjab. Race identification of the pathogen under glasshouse conditions revealed high diversity and at least eleven races were designated. Prevalence of the race 70E0 was most dominating (39 %). Cultivation of multi-location trap nurseries yielded valuable information demonstrating disease-fighting genes where, at all locations, *Yr5*, *Yr10*, *Yr15*, and *YrSp* were effective. Seventy-two advanced lines collected from research institutions were screened in a glasshouse to categorize the test material based on their response to disease at the seedling stage. Test material comprising seventy-two advanced lines collected from different research institutions was screened in a glasshouse to categorize the test material on the ground of their response against disease at the seedling stage. Slow and fast rusters were categorized by studying susceptible and moderately susceptible seedlings in the field for two years. Genetic diversity in the host allows changes in the genetic organization to adapt to environmental changes. Coefficient parentage revealed the test material's restricted genetic base. In the pedigree of wheat advanced lines, Pastor, Kauz, Inqilab-91, Sokoll, Ae. Aquarosa (211), WBLL-1, Kukuna, and Millan were 60% out of a total of 72 parents. Findings of the present studies revealed persistent resistant genotypes with a broad genetic background are needed to feed a growing population.

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INTRODUCTION

Three rust diseases in wheat are the main concerns for sustainable production universally, causing significant yield depression in wheat (Badebo *et al.*, 1990) and colossal damage in various zones, at different times (Solh *et al.*, 2012). Epidemics of rusts in wheat induced losses during the early 19th century significantly (Hussain *et al.*, 2017). These diseases have been perpetual problematic for wheat farmers since the beginning of farming (Kislev, 1982). Substantial work has been accomplished in wheat rust pathosystems to counter the hazard in wheat-cultivating areas (Afzal *et al.*, 2015). The research work conducted explored principles of breeding for disease resistance being applied in the evolution of varieties resistant to diseases. Description is as under;

1- Trait of disease resistance is inherited following Mendelian genetics (Biffen, 1905)
2- Genetic diversity in pathogens causing stem rusts in wheat was first documented by Stalkman and Piemisel, 1917.
3- Flor (1956) presented the idea of gene-for-gene resistance, which is the basis for the contemporary race nomenclature and accounts for a great share of the breeding endeavors against cereal rusts.
4- Slow rusters are more durable source of resistance (van der plank 1963).

Among three rusts, Stripe (yellow) rust (Figure 1), caused by *Puccinia striiformis* f. sp. *tritici* Westend., has emerged as a prime biotic constraint to wheat production worldwide (Wellings, 2011).

Figure 1. Classical symptoms of stripe rust.

The disease has been described in particular as a growing dilemma with recurrent cases of global incursions (Solh *et al.*, 2012). Unfortunately, our efforts to control stripe rust are not very successful as in the case of stem rust and leaf rust. Genotypes when released as varieties are approved if resistant against three rusts in multilocation trials. But it has happened repeatedly that variety lost resistance against stripe rust although was resistant against the other two diseases.

Circumventing disease epidemics is a challenge, providing few cultivars are grown over vast zones with parallel parentage, a situation agrees free movement of infectious pathotypes. Deployment of more than a few durable resistant cultivars is the most practical strategy (Bux *et al.*, 2012; Ellis *et al.*, 2014). Fungicide application not only increases the cost of production but also pollutes the environment, especially in the regions where the wheat crop is cultivated dominantly (Abbasi *et al.*, 2014). That is why only those candidate lines are released for general cultivation which exhibit resistant responses against prevailing race patterns in multilocational screening trials. Despite the wide research work conducted since the beginning of the previous century into the management of this dilemma however, remains a major threat to wheat production worldwide.

Wind borne diseases do not respect state boundaries and cross borders in the regions with similar epidemiological circumstances. The war against rust needs, an organized struggle in collaboration with states in same region. The role of policymakers and international leadership is critical if we are to take an important stage onward in diminishing the effects of stripe rust. These facts indicate that rusts are 'social diseases' and can be controlled by collective strategies decided across areas (Singh *et al.*, 2016). For example, virulence pattern is not confined to the country but it should be explored within the geographic regions across the border. For this purpose, sharing data on virulence patterns within neighboring countries would be useful for each one associated with the wheat industry. Similarly, the deployment of cultivars with dissimilar genetic backgrounds in a geographic zone must be encouraged.

Generally, genotypes recognized as resistant under field conditions carried one or more *Yr* genes (Kokhmetova *et al.*, 2021). The foremost topics of research on wheat rust include exploration for new sources of genetic resistance and transmission thence into cultivars possessing desired agronomic characters, keeping aware of the

diversity of rust pathogens as well as wheat.

Genetic diversity between and within populations of the same species over time is lost or decreases of the genetic base of a species as a result of human interference, fluctuations in the environment, etc. making crops enormously susceptible to extensive disease. This phenomenon is termed genetic erosion in the discipline of plant breeding. Since plant breeding research and variety improvements are indispensable apparatuses for improving food production, hence, accessibility to diverse genetic sources will ensure that the worldwide food production network becomes more sustainable (Gessese, 2019).

Keeping in view the prevailing scenario of host and pathogen, we believe that breeding wheat for durable resistance delivers the best option. It is a continuous process that demands keeping aware of changing race patterns of disease as well as the effectiveness of wheat genes. A multidimensional study was planned with an objective to identify durable resistant genotypes against stripe rust.

MATERIALS AND METHODS

Disease Surveillance

Disease sampling was carried out from different regions in Punjab and KPK following Ali and Hodson (2017). Data of stripe rust incidence were recorded representing an assessment of the percentage of diseased plants in a surveyed field (Ali *et al.*, 2014) whereas 0-9 scale (McNeal *et al.*, 1971) was used to evaluate disease severity.

Growing multilocation trap nursery to recognize effective *Yr* genes

Isogenic lines along with wheat cultivars (Banned as well which are recommended for cultivation) (Detail of entries included in trap nursery are described in Table 1 & 2) were studied during 2013–2014 and 2014–2015, under uninoculated conditions at Bahawalpur (Southern Punjab) Faisalabad, Sialkot (Central Punjab), Rawalpindi, (Northern Punjab), Nowshera, and Peshawar (KPK) to investigate the virulence patterns. Disease data was recorded near the heading stage. Response of stripe rust was assessed following (Loegering, 1959) and severity using the Modified Cobb's scale (Peterson *et al.*, 1948).

Identification of races of stripe rust

Virulence analysis of the disease samples was conducted under glasshouse conditions using differentials (Johnson *et al.*, 1972). In this method, an international and European set of differentials is used to label races according to decanal notation. The differential sets comprise a "world set" of seven genotypes formerly recognized to distinguish distinction in response over a wide terrestrial zone. The second set of "European" differentials was considered appropriate for the European regions. The sum of decennary values of the susceptible response of the World set and European set is separated by the alphabet E.

Table 1. Description of isogenic lines included in trap nursery.

Sr. No.	Loci	Location	Molecular Marker/ Gene related	Origin
1	*Yr1* (Lupton and Macer, 1962)	2AL (Bariana and McIntosh, 1993)		Common wheat. (Bariana and McIntosh, 1993)
2	*Yr2* (Lupton and Macer, 1962)	7B (Labrum, 1980)		Common wheat. (McIntosh *et al.*, 1995)
3	*Yr3* (Lupton and Macer, 1962)	5BL (Lupton and Macer, 1962)		Common wheat. (McIntosh *et al.*, 1995)
4	*Yr4* (Lupton and Macer, 1962)	3B (Lupton and Macer, 1962)		Common wheat. (McIntosh *et al.*, 1995)
5	*Yr5* (Macer, 1966)	2BL (Macer, 1963)	Xwgp-17-2B, Xwgp19-2B, Yr5STS7/8 Chen et al. 2003	Spelt wheats (Kema, 1992)

6	*Yr6* (Macer, 1966)	7B (Macer, 1975)		Common wheat (Wellings, 1986; Chilosi and Johnson, 1990)
7	*Yr7* (Macer,196)	2B (Macer, 1975)		durum cv. Iumillo (McIntosh, 1981)
8	*Yr8* (Riley *et al.,* 1968)	2A (Riley *et al.*, 1968)	Sr34 (McIntosh et al. 1998)	*T. comosum* (McIntosh *et al.*, 1995)
9	*Yr9* (Macer,197)	1BL.1RS (Macer, 1975)	Lr26, Sr31, Xwgp4, Xwgp7, Xwgp8, Xwgp9 (Shi et al. 2001)	*S. cereale* cv. Petkus. (Mettin *et al.*, 1973)
10	*Yr10*	1BS (Macer, 1975)	RgaYr10a, S26-M47, S13-M (Smith *et al.*, 2002)	Bread wheat (Kema, 1992)
11	*Yr15*	1B (Gerechter-Amitai *et al.*, 1989)	Nor1, UBC212a, Xgwm413 (Peng *et al.*, 2000)	*T. turgidum* var. dicoccoides (wild emmer) (Grama and Gerechter-Amitai, 1974)
12	*Yr17*	2AS (Bariana and McIntosh, 1993)	Lr37, Sr38, Vrga1 (Seah *et al.*, 2001)	*T. ventricosum.* (Doussinault *et al.*, 1988)
13	*Yr18*	7D (Singh, 1992)	Lr34, Xgwm295.1, Xwgm44 (Suenaga *et al.*, 2003)	Common wheat (Dyck, 1991)
14	*Yr24*	1B (Zakari *et al.*, 2003)	*Xgwm11-1B* Zakari et al. 2003	
15	*Yr*26	1B	Xgwm11, Xgwm18 Ma et al. 2001	T. turgidum Ma *et al.*, 2001
16	*Yr*27	2B	Lr13, Lr23 (McDonald *et al.*, 2004)	*Yr27* was traced to a Canadian farmer's selection from where the gene was transferred to Selkirk, and subsequently to a range of CIMMYT cultivars (McDonald *et al.*, 2004) described and named *YrSk* by Wellings (Wellings and Burdon, 1992)
17	*Yr*Sp	2BS (McDonald *et al.*, 2004)		*YrSp* is one of the 15 wheat cultivars in the set of differential cultivars proposed by Johnson *et al.* (1972) and is currently used widely for identifying *P. striiformis* f. sp. *tritici* races in the world.
18	*YrCV*	2AL		
19	*Yr28*	4DS	Xmwg634-4DS Singh et al. 2000	*Ae. Tauschii* (Ma *et al.*, 2001)

20	*Yr29*	1B	Lr46 McIntosh et al. 1998	first described in 1998 by Singh *et al.* in cultivar Pavon 76 (Singh *et al.*, 1998)
21	*Yr31*	2BS	Yr27, Yr23, Lr23 (McIntosh, 1981)	Common wheat cultivar Pastor (Singh *et al.*, 2003)

Table 2. Description of varieties included in Trap Nursery.

Sr. No.	Variety	Gene	Parentage
1	MEXI.PAK. 65	2	Pj62/GB55
2	BLUE SILVER	A+6	1154388/NA/3/YT54/N10B/LR64
3	SANDAL 73	A, 6	YECORA
4	WL 711	2	S308/CHRIS//KAL
5	PAK 81	9+7	KVZ//BUHO//KAL/BB
6	KOHINOOR 83	7, 9	ORE F1 158/FDL//MFN/2*TIBA63/3/COC
7	FSD.83	7+2	FURY/KAL/BB
8	FSD.85	9+4	MAYA/MON//KVZ/TRM
9	SARSABZ	7	PI/FRND//MXP/3/PI/M20/70
10	RAWAL 87	9+	MAYA/MON//KVZ/TRM
11	KHYBER 87	9+	KVZ/TRM//PTM/ANA-CM 43930
12	PASABAN 90	9	INIA66/A. DISTT//INIA66/3/GEN
13	INQILAB 91	27	WL-711/CROW"S"
14	ROHTAS 90	6+7	INIA F 66/ A. DISTCHUM//INIA66/3/GEN
15	SOGHAT 90	6+7	PAVON MUTANT-3
16	ANMOL 91		
17	WATTAN 94	6+	LU26/HD 2179
18	ZARDANA	7	Ciano"S" 67-8156 x Tobari-66-CNO 67/NOV-66/11-12300/LR6408156-PAN-76
19	KAGHAN 93	9	TTR/JUN
20	PARWAZ 94	6+7	V.5648/PARULA or V.5648/PRL
21	SHAHKAR 95	6+	WL711//F371/TRM
22	MH 97	27	ATTILAND/VG9144/KAL/BB/3/YACO/4/VEE#5
23	SARIAB92	6+	PH-HARI Junco"S

Screening of wheat advanced material at seedling stage

The set of test material comprising seventy-two entries consisting of wheat candidate lines evolved in different agricultural research institutes as well as universities were evaluated under glasshouse conditions at Crop Disease Research Institute (CDRI), Murree, Punjab, Pakistan at the seedling stage following Shewaye and Mohammed (2021). The disease was assessed two weeks after inoculation following 0–4 scale (0 indicates immune and 4 shows a highly susceptible response) (Markel and Milus, 2008). Categorization of the test material was carried out following Shewaye and Mohammed (2021). Data generated was used to recognize genotypes possessing all stage resistance. Test entries carrying hypersensitive response in the seedling stage (all stage resistance) were not studied further. In this material, rust resistance is controlled by a single gene and is not durable.

Screening selected wheat material under field situation

Forty-eight wheat genotypes were selected (Carrying susceptible or moderately susceptible response at the seedling stage) to identify sources of slow rusting resistance to stripe rust under field conditions. Trials were conducted under field conditions to screen wheat advanced material in two cropping seasons during 2014-15 and 2015-16 (November to April). The trials were

sown in three replications laid out in RCBD (Clewer and Scarisbrick, 2013). To ensure even dissemination of disease inoculum and for sufficient disease development, Morocco (A highly susceptible wheat genotype used as a spreader in rust screening trials) was sown adjacent to the experimental areas. Inoculum of Race 70E0 (The most dominant race in irrigated and rainfed situations) suspended at the concentration of 1.5 $mg.l^{-1}$ mineral oil (0.005 kg ha^{-1}) was sprayed. The research plots were flooded with a tap with an idea to enrich moisture at micro level before inoculation. Disease severity data were recorded repeatedly with a regular interval at feekes growth stage 10.1, 10.5, and 11 (Large, 1954) using Modified Cobb's Scale (Peterson *et al.*, 1948) and rehabilitated to a coefficient of infection as designated by (Pathan and Park, 2006). Data of the following parameters were recorded.

1- Final rust severity (FRS) (Milus and Line, 1986),
2- Number of grains was counted and balanced, which was renovated to thousand kernel weights (TKW).
3- Area under the disease progress curve (AUDPC) was calculated following Milus and Line (1986). Cultivars having lower AUDPC values is acceptable parameter for practical purposes:

AURPC = [N1(X1 + X2)/2] + [N2(X2 + X3)/2] + + [N2(X3 + X4)/2], +……..

Where: X1, X2, X3, X4 – the rust intensities recorded on the first, second, third, and fourth dates; N1 – the interval day between X1 and X2, and N2 – the interval day between X2 and X3.

Evaluation of status of genetic diversity in host (wheat)

Germplasm consisting of Seventy-Two wheat candidate lines (Table 3) were evaluated for their relatedness by pedigree using the technique of Coefficient of Parentage (Cox *et al.*, 1985). Subsequently, the lineage of parents and descendants was stretched by probing their lineage over the website were scored as (1) if present or (0) if absent within each line. Line pedigree associations and Euclidian distance matrix was assessed from these scores using principal component analysis, software Minitab 15.

RESULTS AND DISCUSSION

Disease Surveillance

At the preliminary stage, surveillance of the disease was carried in the wheat growing season in major growing districts of Punjab and KPK in March and April during 2013-14 and 2014-15 to determine the extent of the disease (Table 4).

In the present study, the disease was more prevalent in Pothowar and KPK whereas it was not common in central Punjab (Table, 4). Arms of disease triangle determine the regions more prone to disease (Agrios, 1988). Surveillance data is used to evaluate the extent of the definite issues, determine the dispersal of disease, represent the natural history of ailment, form models, stimulate investigation, estimate management strategies, monitor fluctuations, and simplify forecasting.

Growing multilocation trap nursery to recognize effective *Yr* genes

Data of trap nurseries in the field condition (un-inoculated circumstances) recorded revealed the distribution of race pattern of *Puccinia striiformis* f. sp. *tritici* (*Pst*) in consecutive years. *Yr*5, *Yr*10, *Yr*15, and *YrSp* were effective at all the locations. Genes *Yr*6, *Yr*7, *Yr*8, *Yr*9, and *Yr*31 were found ineffective.

The disease was found more prevalent in Northern Punjab and KPK as compared to central Punjab. Understanding the genetic variability and composition of *Pst* is a prerequisite for cultivar release with appropriate resistance gene combinations for sustainable disease management. The information generated through growing multilocational trap nurseries is very valuable in wheat improvement for rust resistance. However, this data is valid before the change in the virulence pattern of *Pst*. That is why monitoring for virulence patterns is an important component of the incorporation of rust resistance in wheat through breeding.

Identification of races of stripe rust

The race pattern of stripe rust was determined based on the response of seedlings of differential varieties assessed in the greenhouse. The data reveals high diversity in *Puccinia striiformis* f. sp. *tritici* in the region.

A total of 11 races were branded thereby exposing high diversity in the population of *Puccinia striiformis* f. sp. *tritici* (*Pst*) in Pakistan (Table 5). 70E0 was the most dominant followed by 4 other races (70E16; 67E0; 66E16 & 61E0). The distribution of these races has been presented in Figure 3. It is noted that 70E0 is prevalent wherever wheat crop is grown in Punjab and KPK however other races are confined to specific regions.

Table 3. Description of Wheat Genotypes used to address the dilemma of genetic erosion.

Sr. No.	Entry	Parentage
1	V-09082	S308/CHRIS//KAL/CROW`S'/FRET.2
2	V-09087	V-87094/2*S308/CHRIS//KAL/CROW 'S'/3/SH88/KVZ//BUHO// KAL/BB/ND/VG9144/KAL/BB/3/YACO/4/VEE#5
3	V-10104	S308/CHRIS//KAL/CROW`S'/CROW'S'/NAC//BOW'S'
4	V-10110	KAUZ/CMH77A-308////BAU/3/S308/CHRIS//KAL/CROW 'S'
5	V-11005	MILLION/S87230//BABAX
6	V-11160	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES/5/ T. DICOCCON P194624/AE. SQUARROSA (409) //BCN/6/2*KAUZ//ALT AR84/AOS/3/MILAN/KAUZ/4/HUITE
7	SRN 09111	PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07
8	CT 09137	SERI.1B*2/3/KAUZ-2/BOW//KAUZ/4/
9	DN-93	ESDA/ / ALTAR 84 / AE.AQUARROSA (211) /3/ ESDA/4/..
10	109384	UP2338/V4012
11	99172	KAUZ/PASTOR//V.3009
12	99346	ND/VG9144/KAL/BB/3/YACO/4/VEE#5/FAREED-06 (5)
13	99114	THELIN/2* WBLL-1//V.3006
14	SD-998	TJ-83 X 4085/3
15	NIA-MN-08	SARC-11 X Khirman
16	CIM-04-10	PBW343*2/KONK
17	AZRC-2	TRACHA 'S' //CMH76-252/PVN'S'
18	ESW-9525	KAUZ/Gen
19	AUR-0810	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP/KAUZ
20	NR-402	KBIRD// S308/CHRIS//KAL/CROW 'S'*2/TUKURU
21	NR-411	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN
22	11C007	KS82W418/SPN/3/CHEN/AE.SQ//2*OPATA/4/FRET2
23	11C023	SOKOLL/EXCALIBUR
24	11C008	MILAN//PRL/2*PASTOR
25	DH-31	DWL5023/SNB//SNB / ATTILA /3/ HUI/CARC// CHEN/CHTO/4/ ND/VG9144/KAL/BB/3/YACO/4/VEE#5. (Double haploid)
26	6C002	SOKOLL
27	11C022	SOKOLL//SUNCO/2*PASTOR
28	06FJS3013	PASTOR//MILAN/KAUZ
29	08FJ26	BB/GGL/GTO/7C/BB/CNO/3/P20102//PIMA/SKA/3/TTR 'S' /BOW
30	v-12001	WAXWING/4/SNI/TRAP#1/3/LAUZ*2/TRAP//KAUZ
31	10FJ01	03FJ22/04FJS35
32	v-11183	SOKOLL//PBW343*2KUKUNA/3/ATTIL/PASTOR
33	10FJ16	8970/94-R30
34	NRL-0913	PRL/SARA//TSI/VEE#5/3/WBLL1
35	BARS 09	PFAU/SERI//BOW
36	09FJ34	ERA F 2000/4/ FONCHAN #3 / TRT"S"//VEE#9/3/COOK/VEES//DOVE"S/SERI
37	11FJ01	02FJ13 / KUKUNA
38	DN-84	BYJ/COC/PRL/BOW/3/URES/JUN/KAUZ
39	11FJ02	S308/CHRIS//KAL/CROW 'S'/ 03FJ13//ND/VG9144/KAL/BB/3/YACO/4/VEE#5

40	99172	KAUZ/PASTOR//V.3009
41	11FJ07	S308/CHRIS//KAL/CROW 'S'/ 03FJ13//ND/VG9144/ KAL/BB/3/YACO/4/VEE#5
42	11FJ08	99FJ03 / 03FJ26
43	11FJ12	99FJ03 / KVZ//BUHO//KAL/BB
44	11FJ27	REEHAB-2
45	11FJ28	BABAGA-3
46	11FJ39	HAAMA-2/QAFZAH-16
47	11FJ45	GOUBARA-1/2*SOKOLL
48	11FJS308	D67.2/PARANA
49	11FJS309	D67.2/PARANA
50	11FJS310	H45/4/KRICHAUFF/FINSI/3/URES/PRL//BAV92
51	11FJS311	VORB/SOKOL
52	11FJS313	VORB/SOKOL
53	11FJS317	EGA BONNIE ROCK/4/MILAN/KAUZ//PRINIA/3/BAV92
54	11FJS348	KRICHAUFF/2*PASTOR//2*SOKOLL
55	11FJ09	Fin/ACS//ANA, SWM/ND/VG9144/KAL/BB/3/ YACO/4/VEE#5/3/HUI/CARC//CHEN/CHTO/4/ND/VG9144/KAL/ BB/3/YACO/4/VEE#5
56	TW96010	P20102//PIMA/SKA/3/TTR 'S' /BOW
57	NR-413	PASTOR//HXL7573/2*BAU/3/SOKOLL/WBLL1
58	TW96018	P20102//PIMA/SKA/3/TTR 'S' /BOW
59	NR-421	CROC_1/AE.SQUARROSA (210)// WL 711/CROW 'S'/ *2/KUKUNA/3/PBW343*2/KUKUNA
60	NR-409	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7
61	NR-419	OASIS/SKAUZ//4*BCN/3/2*PASTOR/4/T.SPELTA
62	UAF-9452	1154-388/NA/3/YT54/N10B/LR64/KHUSAL 69 X S308/CHRIS //KAL/CROW 'S'
63	PR-103	WBLL1*2/4/YACO/PBW65/65/3/KAUZ*2/TRAP//KAUZ
64	PR-106	MTRWA92.161/PRINIA/5/SERI*3//RL06010/4*YR/3/PASTOR/4/
65	PR-107	MTRWA92.161/PRINIA/5/SERI*3//RL06010/4*YR/3/PASTOR/4/
66	NR-439	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/4/T.DICOCCON PI94625/ AE. SQUARROSA(372)//SHA4/CHIL/5/WHEAR/KUKUNA /3/C80.1/3*BATAVIA//2*WBLL1
67	NR-407	UP2338*2/VIVITSI/3/FRET2/TUKURU//FRET2/4/OASIS/ SKAUZ//4*BCN/3/2*PASTOR
68	NR-403	KBIRD// S308/CHRIS//KAL/CROW 'S'*2/TUKURU
69	NIA-MB-02	Mutant_Sarsabz (250 Gy)
70	PR109	PBW343*2/KUKUNA/5/CNO79//PF70354/MUS/3/PASTOR/4/BA V92
71	PR-108	WHEAR/KRONSTAD F2004
72	PR-105	MILAN/S87230//BABX

Figure 2. Distribution Pattern of *Puccinia striiformis* f. sp. *tritici* in Punjab and KPK during 2014 and 2015.

Table 4. Surveillance of rust incidence in Punjab and KPK.

Location		2013		2014	
Punjab Province		Severity	Incidence	Severity	Incidence
Punjab	Rawalpindi	30S-50S	40-60	30S-60S	40-60
Punjab	Attock	40S-60S	40-70	40S-60S	30-50
Punjab	Chakwal	10S-60S	40-60	10S-70S	40-60
Punjab	Jhelum	20S-60S	30-60	20S-60S	30-60
Punjab	Gujranwala	20S-30S	30-50	20S-40S	30-50
Punjab	Faisalabad	20S-30S	20-50	20S-30S	20-50
Khyber Pakhtun Khawah (KPK) Province					
KPK	Nowshehra	40S-70S	40-60	40S-70S	40-60
KPK	Peshawar	10S-70S	20-60	10S-70S	20-50
KPK	Mardan	10S-30S	40-50	10S-30S	40-50

Table 5. Prevalence data of various races identified in sampling in Punjab and KPK during 2014-15 (Percentage).

Sr. No.	Genotype	Prevalence
1	71E0	4.35
2	70E16	8.70
3	70E0	39.1
4	68E0	4.35
5	68E16	4.35
6	67E0	8.70
7	66E16	8.70
8	64E0	4.35
9	61E0	8.70
10	6E0	4.35
11	2E0	4.35

Distribution Pattern of *Puccinia striiformis* f. sp. *tritici* in Punjab and KPK during 2014 and 2015

Long distance migration potential (Bux *et al.*, 2012; Hodson *et al.*, 2011), high rates of mutation (Hovmøller and Justesen, 2007), adaptation to diverse ecological situations (McIntosh *et al.*, 1995), the existence of recombinant and highly diverse populations and the creation of novel races through the erotic cycle (Ali *et al.*, 2014) are factors responsible for this situation. It is exciting to observe high diversity in pathogens in this region an existing situation dissimilar from Europe. However, it is justified the probable the novel races of pathogen originated in the Himalayan region, with Pakistan being the most inherited to all other global populations (Ali *et al.*, 2014).

Screening of wheat advanced material at seedling stage

A trial conducted under a glasshouse situation to categorize resistant and susceptible genotypes revealed 48 entries (66.66%) reacted with susceptible response whereas 24 (33.33%) entries were found resistant. Entries that showed resistant response was dropped in preliminary screening with the assumption that these entries hold all stage resistance and are not durable (Ellis *et al.*, 2014; Afzal *et al.*, 2015).

Screening selected wheat material under field situation

Material that exhibited resistance response at the seedling stage were dropped whereas lines that showed intermediate and susceptible reactions were studied under field conditions for their slow rusting characteristics. The test material was categorized into five groups based on the area under the disease progress curve (Figure 2) however seven entries that performed the best were selected as a durable source of resistance against stripe rust. The findings discovered that wheat lines, 11FJS310, 11FJS308, 11C023, BARS 09, NR-403, NRL-0913, and PR109 were regarded as slow rusting lines. Maximum yield was recorded in 11FJS310 and 11FJS308 during 2014 and 2015 (Table 6). Findings of the second year trial confirmed results recorded in the first year. The minor deviation is attributed to the environmental situation as the trial was conducted under field conditions.

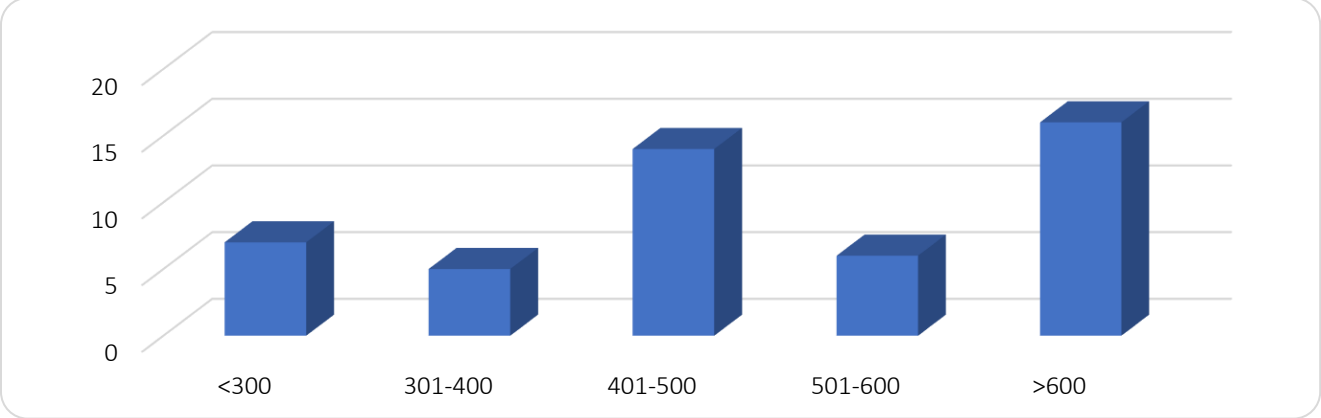

Figure 3. Categorization of susceptible entries under glass house according to audpc in field.

Table 6. Data of FDS (Final Disease Severity), Area under Disease Progress Curve (AUDPC) and Thousand Kernel Weight (TKW) recorded during two consecutive years.

	2014-15			2015-16		
genotype	FDS	AUDPC	TKW	FDS	AUDPC	TKW
V-11160	75.00bc	806.8bc	59.7vw	73.3a	754.7b	56.3qrs
V-10110	71.67bcd	774.5cd	57.7wx	66.7 ab	699.3e	55.3qrst
V-10104	71.67bcd	834.7b	60.7v	66.7ab	754.3b	55.3qrst
UAF-9452	41.7 ijk	455.0pqr	66.67st	36.7fgh	417.7p	66.33mno
TW96010	78.33 ab	742.0def	56.3xy	73.3a	698.7e	54.2rst
SD-998	56.67 fg	529.8lm	63.7u	50.0de	474.3k	63.3op
PR109	12.50 o	125.8 x	175.33d	8.33 m	111.7c	170.50 c
PR-108	70.00 cd	752.5 de	54.7 yz	66.67 ab	739 c	52.33rstu
PR-107	45.00 hij	431.7qrs	45.33 b	36.7fgh	388.7r	45.17v
PR-105	38.333 jk	401.7s	120.67 i	33. 3ghi	344.3t	110.67f
PR-103	41.67ijk	427.0 qrs	75.7pq	36.7fgh	384.7rs	70.3klm
NRL-0913	21.7 n	234.8 w	139.67g	20.0 kl	237.7 x	132.33e
NR-439	35 kl	385.2rs	90.67jk	33.33ghi	350 t	85.33h
NR-419	50.0 g	500.3mnop	83.67 m	43.3ef	438m	79.33ij
NR-413	43.3ef	432mn	64.33nop	43.3ef	432mn	64.33nop
NR-411	33.67 ghi	397.8s	157.75f	36.67fgh	380.7 rs	147.83d
NR-409	45.0 hij	462.3opq	71.67 r	36.7fgh	420.7op	70.33klm
NR-407	41.67 ijk	477.5nopq	71.67 r	36.66fgh	428mnop	68.83 lmn
NR-403	26.67 mn	284.7x	134.67g	26.67 ijk	284.7x	128.33e
NR-402	61.667 ef	652f	64.33st	56.667cd	566.7i	59.67pq
NIA-MN-08	46.67hi	460.2opq	63.7u	40.0fg	403q	59.8pq
DN-93	70.0 cd	665.3ghi	56.3xy	66.7ab	605.7g	53.5rst
DN-84	41.7 ijk	405.8rs	187.33c	36.7 fgh	350.3t	166.67c
DH-31	45 hij	509.0mno	83mn	36.6fgh	456l	79.7ij
CT 09137	66. 7 de	703.8efg	59.7vw	56.7cd	654f	56.3qrs
CIM-0410	61.67 ef	609.3jk	59.7vw	56.7cd	585h	54.3rst
BARS 09	21.67 n	289.0uv	98j	20.0kl	234.7y	94.3g
AZRC-2	30 hij	333.7u	79.67pq	30.00hij	331 u	74.83 jk
AUR-0810	26.67 ijk	316.5w	86mn	26.67 ijk	306.7w	80.50 hi
6C002	46.6 hi	517.8lmn	76p	36.6fgh	463l	73.2kl
11FJS348	41.67 ijk	468.0nopq	87.7l	36. 7fgh	376 s	82.7hi
11FJS310	12.50 o	136.5x	228.7 a	11. 7lm	123b	219.2a
11FJS309	38.33 jk	402.2s	75.3pq	33.3ghi	350.3t	72.3kl
11FJS308	26.67mn	247.5vw	197.7b	20.0kl	189a	192.8b
11FJ28	56.67 fg	564.0kl	60v	53.3cd	516j	56.5qrs
11FJ27	43.33 hij	468.7nopq	67s	43.3ef	426.7nop	63.7op
11FJ07	70.0 cd	742.7def	49a	66.7ab	705e	47.7uv
11FJ02	71. 7 bcd	749.7def	49a	66.7ab	723.7d	45.7v
11C023	28.3lmn	238.3vw	153f	23.3jk	214z	152.0d
11C022	71.7bcd	666.3ghi	54z	60.0bc	609.3g	51.0tu
11C007	35.0 kl	329.3u	97j	33.3ghi	318.7v	93.5g
10FJ16	61.67 ef	700.fgh	54z	56.7cd	652.7f	51.3stu

08FJ26	43.3 hij	403.5rs	81no	43.3ef	351.7t	78.3ij
06FJS3013	43.3 hij	475.3nopq	60v	43.3ef	429mno	56.0 qrs
99172	43.33 hij	517.2 lmn	60.7v	40.0fg	461.3 l	53.2rst
99114	70.0cd	615.0ijk	59.7vw	60.0bc	556.7i	57.0qr
Morocco	85 a	1974.5a	27 ijk	73.33ab	1688.3ab	23.50 w
LSD (0.05)	2.33	7.33	10.5	3.3	5.66	8.67

Breeding wheat for resistance against rust was initiated with the recognition of the genetic base of resistance (Biffen, 1905). The concept of breeding for disease resistance is based on the theory of gene-for-gene interaction (Flor, 1956). This method seems to be very attractive because it is easy to combine into improved germplasm. Breeding for rust resistance leads to an arms race in host and pathogen in which the pathogen is the conqueror emerging new races that are virulent against the varieties previously evolved as resistant. While the prospects have not been achieved. The phenomenon of the erosion of advantageous genes led researchers to seek alternate methods of resistance management. It is fact that the scientific community has not succeeded in generating the rust-free wheat farming circumstances that were intangible from this breeding investment continuous for more than a hundred years.

Van der Plank explained the theoretic base of ideas of resistance that is not race specific (Vander and Vlietnck, 1991) controversial to Flor's concept of gene-for-gene (Flor, 1956; Flor, 1971). However, screening on the parameter of the area under disease progress curve (Audpc) may be convenient to differentiate between slow rusters and fast rusters. Slow rusters genotypes have proved durable. It is simple to explain host and pathogen agree to live and let live. Consequently, the ultimate goal of wheat improvement through breeding is accomplished i.e., the produce of the crop is affected least in case crop is infected with disease. Moreover, this situation does not compel evolving novel races swiftly as in case of complete resistance (Hypersensitive resistance).

In the early seventies, the concept of general (race-nonspecific) resistance was restored (Caldwell, 1968). This approach was practiced for breeding leaf rust resistance (Caldwell, 1968), stem rust resistance (Borlaug, 1968), and yellow rust resistance (Johnson *et al.*, 1972). Deployment of semi dwarf varieties advanced at CIMMYT in the 1960s improved yield in the subcontinent, Afghanistan, Turkey, besides other regions of the globe. These short statured varieties did not perform for long attributed to the emergence of the novel stem rust race however, there were some exemptions also.

The varieties like Champingo 52, Champingo 53, and Yaqui 50 did not lose resistance but were evacuated from commercial cultivation by new varieties characterized by high yield (Borlaug, 1968). The genetic background of these genotypes is the factor responsible for durable resistance. Resistance to yellow rust from the Andean region evolved during the 1960's had a high level of resistance. The durable resistance of Anza is deployed widely in spring wheat and some winter wheat varieties. The presence of gene *Yr18* is source of durable resistance (Singh, 1992). The gene, *Yr7* has been postulated in several varieties e.g., Pavon76 (*Yr*6, *Yr*7, *Yr*29), Pak.81 (*Yr*7, *Yr*9), PBW12, Barani 83, Seri 82, WL2265 (*Yr*2, *Yr*7, *Yr*9), (Badebo *et al.*, 1990). Pavon and Veery possessing *Yr*7 have been released in 16 31countries, respectively with changed names which express the usage of *Yr*7 gene widely (McIntosh *et al.*, 1995). In this study, we contributed to recognizing new sources of durable resistance. Data generated is valuable in breeding wheat for rust resistance and the genotypes selected may be tested in multilocation yield trials directly to release as a variety for farmers.

Evaluation of status of genetic diversity in host (wheat)

Pedigree of wheat advanced material evolved in different research institutes and universities was studied intensely using the technique of Coefficient of Parentage to explore the extent of genetic diversity in the material. The finding of the study to discover genetic diversity in the host is not very optimistic (Figure 4).

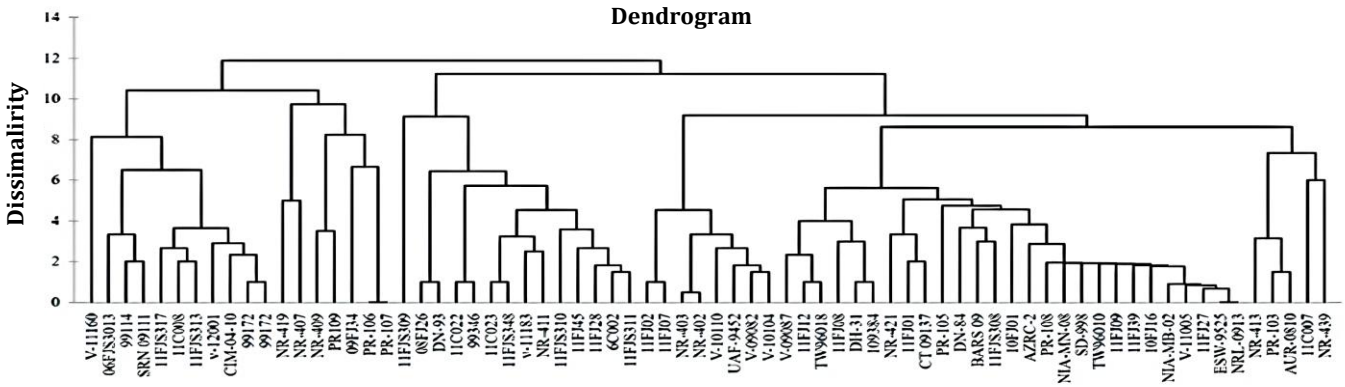

Figure 4. Dendrogram illustrating status of genetic diversity in wheat advanced material.

It was exposed that the material advanced under the supervision of the same research organization was uniform genetically. Pastor was most common (13.28%) while, Kauz (8.59%), Inqilab-91(7.81%) Sokoll (7.03%), *Ae. Aquarosa* (211) (6.25%), WBLL-1(5.46%), Kukuna (4.68%), and Millan (4.68%) were among other frequent parentages out of one hundred and twenty-eight (128) sharing 57.78%. Investigation indicated that the accessions could be allocated into two groups at 12 dissimilarity indexes, wherein one group contained only eighteen genotypes while the lingering fifty-five accessions relate to the alternative group (B).

Genetic diversity makes available the basic adaptability to the ecological circumstances, and revolution in the genetic masterpiece to handle changes in the circumstances. On the other hand, growing a single cultivar over a vast region participates in the evolution of novel races virulent against the genes which performed resistance in the already virulence pattern. It has happened repeatedly during previous decades. Here are a few cases to demonstrate our opinion.

1- Wheat genotype SST44 was broadly cultivated in the 1980s and the prevalence of race 2SA100, which is virulent to *Sr24* possessed by genotype, increased dramatically (Roux, 1985).
2- Potato Famine in Ireland in the 1840s was caused by the absence of biodiversity. Since Potato is propagated vegetatively, no genetic diversity is developed, and the entire crop is basically a copy of one potato, it is particularly disposed to an epidemic They cultivated the "lumper" variety of potato, which was susceptible to late blight (James and Donnelly, 2002).
3- Development of high yielding wheat varieties with narrow genetic bases disposed of by grower/buyer affection has led to growing few cultivars over the vast zone producing genetic susceptibility to stress. This has been experienced repeatedly in Pakistan when the foremost cultivars derived from Veery Pak 81 and Pirsabak 85 were affected extremely by the appearance of virulence for the stripe rust genes *Yr*9 and *Yr*7 respectively **(Ahmad, 2000)**.
4- Stem rust had been controlled for three decades throughout the planet during the last half of the past century. In 1999 advent and spreading of the stem rust race Ug-99 in Uganda (a virulent race against Sr31) created an alarming situation universally. Extensive germplasm possessed gene Sr31 found susceptible to this terrible strain. The emergence of the Ug99 race of stem rust stimulated a focal struggle to identify sources of stem rust resistance genes against novel virulent strains and incorporate these genes into wheat lines. The scientific community addressed the dilemma and endeavors did not go waste and achieved a lot. This achievement serves as a model for scientists in the future (Afzal *et al.*, 2018).
5- Attila cross having resistance gene (*Yr 27*) against stripe rust was grown in 70% area under wheat cultivation during the era of the beginning of recent century formed a serious hazard led to the epidemic (Solh *et al.*, 2012).

These examples in history support how the lack of genetic diversity in the host is supportive to pathogen to progress towards the epidemic. Assessment of genetic diversity declared a narrow genetic base predominantly amongst the genotypes promoted by the team working under the same authority. This situation can be

explained that breeding in different institutes is conducted with a specific mandate. Consequently, it becomes tough to achieve the mandate of wheat breeding at an institute with broader genetic diversity against rusts including stripe rust. This matter must be focused with attention to attain desired objectives. The slow ruster lines recognized from diverse clusters could be exploited to improve wheat through breeding.

CONCLUSION

Substantial work has been accomplished in wheat rust pathosystems to counter the hazard of wheat rusts in wheat-cultivating areas. The foremost topics of research on wheat rust include exploration for new sources of genetic resistance and transmission thence into cultivars possessing desired agronomic characters, keeping aware of the diversity of rust pathogens as well as wheat. The plant genetic diversity (PGD) is a precise area since exploding population and diminishing arable domains are the serious aspects causal to nutrition insecurity. To decrease the worst impact of disease, suitable actions should be taken for gene deployment across the zone, it is the race pattern of pathogen that suggest the deployment of genotypes in a specific geographic zone. However, race alteration in the rust race pattern follows in response to mega genotypes being cultivated for a substantial era. This phenomenon is natural that is the struggle for survival. The concept of durable resistance is that host and pathogens agree to live and let live that creating the situation the variety remains in the field for longer durable. Consequently, wheat breeding for durable rust resistance occupies the status of the most effective disease management strategy. Awareness of the prevailing race pattern of pathogen across the region, and preservation of a suitable degree of diversity in the host are essential components to improve the efficiency of the breeding programs to alleviate resistance to the predominant cereal rust.

NOVELTY STATEMENT

The stripe rust of wheat remains a threat to wheat production worldwide. In the present study genetic erosion has been addressed side by side and the scientific community is invited to utilize the potential of genetic diversity in the host to achieve determining productivity. Genetic diversity in *Puccinia striiformis* and wheat germplasm has not been explored together already in Pakistan. Our research advocate that future stripe rust epidemics are likely to occur due to viz, the presence and emergence of aggressive strains, limited availability of resistant wheat cultivar with narrow genetic background, and recurrent favorable meteorological conditions. Landraces of wheat and wild relatives are a source of new genes effective against stresses including stripe rust.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

Amir Afzal designed research, collected samples and performed race phenotyping wrote manuscript under the supervision of Abid Riaz. Sharmin Ashraf recorded disease data, Javed Iqbal surveyed farmers field and compiled data. Muhammad Ijaz analysed data and contributed in improvement of manuscript. Farah Naz contributed in manuscript write up and revisions. Syed K. N. Shah contributed assessment of genetic diversity in wheat.