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Virulence Analysis of the Selected *Puccinia striiformis* f. sp. *tritici* Isolates

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Abstract: The study was conducted for the virulence analysis of the stripe rust disease samples collected from different wheat growing areas of Pakistan. Samples were selected out of CDRP sample collection 2004-05. The inoculum was increased and maintained on universally susceptible cultivar Morocco, inoculated at two leaf stage with urediospores under control conditions. Inoculum was collected separately for each disease sample in No. 0 capsule using a separate cyclone collector for each sample attached to a vacuum pump. Uredioospores were collected and inoculated on seedlings of differential genotypes sets. Samples analyzed for virulence showed the presence of virulence even against the breeding material considered to have resistant genes. The study also revealed the high disease response of commercial varieties against stripe rust. Virulence analysis can provide an early information to the breeders about susceptibility of their material and they can incorporate resistance gene to avoid future epidemic.

Keywords: Wheat, stripe or yellow rust, virulence analysis, Pakistan

INTRODUCTION

About 93% of the food to feed the people of the world comes from plants, two-third of which is contributed by the cereals (wheat, maize, barley, sorghum, and millet). About 80% of the global cereal production comes from wheat, maize, and rice. Among the cereals, wheat is the largest. Of the two principal types of wheat, 90% of the world's wheat is bread wheat, which accounts for 94% of the total production. Wheat is grown in 27 countries in the developing world (Stubbs *et al*., 1986). Wheat is a staple food crop of the inhabitants of Pakistan and occupies central position in the agricultural policies. Wheat cultivation encompasses a major production area of 8.176 million hectares thereby engages about 33% of the cultivated area of the country each year and exhibits production around 19.7 million tonnes (Anonymous, 2004). It contributes 12.5% to the value added in agriculture and 2.9% to the GDP. Out of the total wheat cultivated area, 20% comes under rain fed while the rest is irrigated.

Many biotic and abiotic factors limit wheat production throughout the world (Ahmad, 1997). One of the major production constraints in wheat are rust diseases. Plant breeding programs were initiated, in world, as an attempt to control them. There are three main types of rusts i.e., stem rust caused by *Puccinia graminis*, leaf rust caused *P. triticiana* and yellow rust caused by *Puccinia striiformis*. Economically rusts are very important. They extremely prolific and can spread long distances under the favorable condition. Among the three rusts stripe rust caused by the obligate parasite *Puccinia striiformis* Westend. f. sp. *tritici* Eriks. is the most important disease of wheat (*Triticum aestivum* L.) through out the world.

Cultivars often appear to lose their resistance due to cultivation of the varieties in area of its non-adaptability or change in virulence, either due to appearance of a new race (s) or change in the composition of the existing races (Kilpatrik, 1975).

The capacity to develop durable and efficient control methods against crop diseases is largely based on the knowledge of the pathogen population structure and its potential for the adaptation to new cultivars (Grant and Archer, 1983). It was reported (Javed *et al*., 2004) that field data from the yellow rust Trap nurseries showed the presence of virulence for the genes Yr1, Yr7, Yr6, Yr2 and Yr9 in Pakistan during 1998-99.

The current production target is set at slightly over 20 million tonnes. To achieve the envisaged target, sustainable productivity of this cropping system is of paramount importance in the context of food security.

The major objectives of virulence studies were to conduct virulence analysis of the selected samples of *Puccinia striiformis* from 2004-2005 collections and systematic presentation of uniform disease data.

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MATERIALS AND METHODS

Disease samples: This study was conducted at CDRP Murree Substation for the virulence analysis of the rust disease samples collected from different wheat growing areas of Pakistan. The samples used for the study were randomly selected from 2004-05 collection of CDRP, Murree substation. Total 30 samples were selected out of which 13 were from 5 location of Punjab, 13 from 3 locations of AJK and 4 from 4 location of NWFP (Table 1). The samples were given accession numbers to maintain record.

Inoculum increase and spore collection: Inoculum of 30 diseased samples was increased and maintained on the universally susceptible cultivar Morocco. For inoculum increase seeds of Morocco were plant in disposable pots containing a mixture of peat and soil. About 10 days after planting, seedlings at the two-leaf stage were inoculated with urediniospores collected from diseased samples using smear technique. Inoculated plants were kept in a dew chamber at 10°C for 48 h and then transferred to glass house benches with temperatures between 15 and 18°C. Sporulating pustules appeared on the inoculated leaves after 15 days. Inoculum was collected separately for each disease sample in #0 capsule using a separate cyclone collector for each sample attached to a vacuum pump (Line and Qayoum, 1991).

Virulence analysis: Set of differential consisting of 9 lines of world set, 8 lines of European set and 22 supplementary lines (Table 2) were sown in 15x18 cm trays. Morocco was included as susceptible check. Ten days old seedlings were inoculated by spraying fresh urediospore suspension of *Puccinia striiformis* in mineral oil with fine Atomizer. The set was placed in a dew chamber at 10°C for 48 h and then transferred to glass house benches with temperatures between 15 and 18°C. Data was recorded after 15 days when susceptible check developed maximum infection on 0-9 scale (Johnson *et al.*, 1972) (Table 3).

Table 1: Sampling location for selected yellow rust disease samples during 2004-2005

Area	Locality	No. of samples selected
Punjab	FatahJang	1
	Chakwal	8
	Sheikhopura	1
	Talagang	1
	Khushab	2
NWFP	Nowshehra	1
	Peshwar	1
	TakthBhai	1
	Abbotabad	1
Azad kashmir	Kotli	8
	Muzaffarabad	4
	Bagh	1

Table 2: Host differential genotypes for *Puccinia striiformis*

Host cultivars	Resistance genes
World differentials	
Chinese 166	Yr1
Lee	Yr7
Heines Kolben	Yr6, Yr2
Vilmorin	Yr3V
Moro	Yr10
Strubes Dickopf	YrSD
Suwon92 × Omar YrSU	
Clement	Yr9, Yr2+
Triticum spelta	Yr5
European differential set	
Hybrid 46	Yr4+
Reichersberg42	Yr7+
Heines Peko	Yr6, Yr2+
Nord Deprez	Yr3N
Compair	Yr8, YrAPR
Carstens V	YrCV
Spaldings prolific	YrSP
Heines VII	Yr2+

Table 3: Responses of plants against stripe rust

Response	Reactions	Symbol	Observations
0	Very resistant	VR	No visible infection
1	Resistant	R	Necrotic/chlorotic flecks
2	Moderately resistant	MR	Without sporulation
3	Lightly moderate	LM	Trace sporulation
4	Moderately susceptible	MS	Light sporulation
5	Highly moderate	HM	Intermediate sporulation
6	Moderately susceptible	MS	Moderate sporulation
7	Susceptible	S	Abundant sporulation
8	Very susceptible	VS	Abundant sporulation with chlorosis
9	Very susceptible	VS	Abundant sporulation without chlorosis

RESULTS AND DISCUSSION

The results of study identified the virulence of the rust population through a set of 17 wheat genotypes that have been used to differentiate Pakistani races of the pathogen collected from diseased samples from different areas of country (Table 4). Many of the host differential genotypes for *Puccinia Striiformis* showed promising results against rust inoculum and it was needed to locate and map stripe rust resistance gene and transferred from differential to local varieties (Eriksen *et al.*, 2004). However, few of the isogenic lines showed susceptibility against some races makes might not exploited for breeding program because the number of virulence factors per race and range in virulence factors both increase considerably (Daniel *et al.*, 1994) with time.

Some of the lines in the study showed slight virulence (Table 4) against inoculum. This might be due to some sort of residual resistance , ranging from moderately to fairly high, was observed in some cultivars in which the major gene of resistance were neutralized by corresponding virulence genes (Daniel *et al.*, 1994).

The disease monitoring data suggest that stripe rust is a threat to wheat production in Pakistan as most

Table 4: Virulence analysis of differential genotypes

		Accessions No.									
Isogenic lines	Genes	06	10	31	36	52	61	120	152	166	168
Chines 166	Yr1	+	+	+	+	-	-	-	-	-	-
Lee	Yr7	+	+	+	+	-	+	-	-	+	+
Heines. kolben	Yr6, Yr2	+	-	-	+		-	+	-	-	-
Vilmorin	Yr3V	-	-	-	-	-	-	-	-	-	-
Moro	Yr10	-	-	-	-	-	-	-	-	-	-
Strubs. dickhoff	YrSD	-	-	-	+	-	-	-	-	-	-
Suwan 92	YrSU	+	+	+	-	+	+	+	-	-	x
Clement	Yr9, Yr2+	-	-	-	-	-	-	-	-	-	-
T spelta album	Yr5	-	-	-	-	-	-	-	-	-	-
Hybrid-46	Yr4+	-	-	-	-	-	-	-	-	-	-
Riecher sbera	Yr7+	-	-	-	-	-	-	-	-	-	-
Heines perko	Yr6, Yr2+	-	-	-	-	-	-	-	-	-	-
Nord desperado	Yr3N	-	-	-	-	-	-	-	-	-	-
Compare	Yr8, YrAPR	-	-	-	-	-	-	-	-	-	-
Carsten	YrCV	-	-	-	-	-	-	-	-	-	-
Spalding prolific	YrSP	-	-	-	-	-	-	-	-	-	-
Heines VIII	Yr2+	-	-	-	-	-	-	-	-	-	-

		Accessions No.									
Isogenic lines	Genes	170	172	173	175	178	182	185	191	199	205
Chines 166	Yr1	-	-	-	-	-	-	-	-	-	-
Lee	Yr7	-	+	-	-	+	-	-	-	-	-
Heines. kolben	Yr6, Yr2		+	-	-	-	-	+	-	-	-
Vilmorin	Yr3V	-	-	-	-	-	-	-	-	-	-
Moro	Yr10	-	-	-	-	-	-	-	-	-	-
Strubs. dickhoff	YrSD	-	-	-	-	-	-	-	-	-	-
Suwan 92	YrSU	-	+	-	+	+	+	+	-	+	+
Clement	Yr9, Yr2+	-	-	-	-	-	-	-	-	-	-
T spelta album	Yr5	-	-	-	-	-	-	-	-	-	-
Hybrid-46	Yr4+	-	-	-	-	-	-	-	-	-	-
Riecher sbera	Yr7+	-	-	-	-	-	-	-	-	-	-
Heines perko	Yr6, Yr2+	-	-	-	-	-	-	-	-	-	-
Nord desperado	Yr3N	-	-	-	-	-	x	-	-	-	-
Compare	Yr8, YrAPR	-	-	-	-	-	x	-	-	-	-
Carsten	YrCV	-	-	-	-	-	-	-	-	-	-
Spalding prolific	YrSP	-	-	-	-	-	x	x	x	x	x
Heines VIII	Yr2+	-	-	-	-	-	x	x	x	x	x

		Accessions No.									
Isogenic Lines	Genes	213	217	233	235	236	238	239	240	253	261
Chines 166	Yr1	-	-	-	-	-	-	+	-	-	+
Lee	Yr7	+	-	-	+	+	-	+	-	+	+
Heines. kolben	Yr6, Yr2	-	+	-	-	-	-	+	+	+	+
Vilmorin	Yr3V	-	-	-	-	-	-	-	-	-	-
Moro	Yr10	-	+	-	-	-	-	-	-	-	-
Strubs. dickhoff	YrSD	-	-	-	-	-	-	-	-	-	+
Suwan 92	YrSU	+	+	-	+	-	+	+	+	+	+
Clement	Yr9, Yr2+	-	-	-	-	-	-	-	-	-	-
T spelta album	Yr5	-	-	-	-	-	-	-	-	-	-
Hybrid-46	Yr4+	-	-	-	-	-	-	-	-	-	-
Riecher sbera	Yr7+	-	-	-	-	-	-	+	-	-	-
Heines perko	Yr6, Yr2+	-	-	-	-	-	-	+	-	-	-
Nord desperado	Yr3N	-	-	-	-	-	-	+	-	-	-
Compare	Yr8, YrAPR	-	-	-	-	-	-	-	-	-	-
Carsten	YrCV	-	-	-	-	-	-	-	-	-	-
Spalding prolific	YrSP	-	-	-	-	-	-	-	-	-	-
Heines VIII	Yr2+	-	-	-	-	-	-	-	-	-	-

- Shows no virulence, + Shows high virulence, x Shows slight virulences

cultivated commercial varieties like Inqalab91, Bakhtawar92, SH-2002, Wattan94, Chakwal86, AS-2002, Fakhre Sarhad, Punjab96 (Table 5) showed a susceptible response against many isolates. These varieties are resistant when first released often become susceptible later due to the spread of previously undetected races but the time taken for this to occur in very variable. It often occurs so rapidly as to curtail the commercial use of

Table 5: *In vitro* evaluation of wheat varieties against stripe rust (*Puccinia striiformis*)

	Accession No.																													
Variety	06	10	31	36	52	61	120	152	166	168	170	172	173	175	178	182	185	191	199	205	213	217	233	235	236	238	239	240	253	261
Inqalab-91	8	9	8	6	7	0	8	0	7	0	6	9	6	1	8	7	9	6	0	7	7	8	0	6	1	0	7	0	8	9
Bakhta-War 92	7	2	0	0	1	6	5	0	2	0	2	7	0	6	0	1	8	1	0	1	8	3	0	1	1	4	5	0	5	2
SH-2002	9	8	7	6	8	0	5	2	0	0	2	9	4	6	7	8	0	7	2	0	2	8	0	7	1	6	9	8	7	9
Wattan94	9	7	6	7	6	0	9	2	0	2	1	8	0	0	7	5	9	6	0	6	2	0	0	1	1	8	9	8	7	9
Chakwal-86	9	6	6	4	6	3	6	1	1	2	0	6	0	6	5	5	8	1	8	0	6	7	0	6	1	0	8	0	5	7
Marvi-2000	2	5	2	2	5	3	1	0	2	2	1	0	1	1	2	2	5	1	9	0	2	4	0	2	1	0	2	0	2	5
AS-2002	9	6	4	7	6	2	9	1	1	2	1	9	4	2	7	6	8	2	0	7	4	8	1	6	1	1	8	1	8	7
Fahre-Sarhad	8	6	0	7	2	3	0	1	1	2	0	2	1	1	2	2	3	1	8	5	5	0	0	1	1	0	3	1	1	8
Punjab96	0	6	6	7	6	0	0	1	1	1	0	0	0	0	3	2	5	1	9	1	2	0	1	5	1	1	5	0	7	8

otherwise satisfactory varieties (Johnson, 1978). Monoculture of these varieties results for pathogenic variability and might result future epidemic due to uniform genetic pool (Agrios, 1997). The wheat variety Marvi-2000 showed resistance to stripe rust (Table 5) and low disease response as compare to other commercial varieties. This variety evolved through pyramiding different rust resistant genes in a high yielding genotype PK-1600. It combines both high yield and resistance against the prevailing races of leaf and steam rust. It is not only resistant to rust but has 13.5 percent protein content with high gluten (http://www.pakissan.com/english/news/2002/November/new.wheat.shtml). Disease pyramiding has the potential to be exploited in breeding for rust resistance.

Stripe rust has the lowest temperature requirements for infection of the three wheat rust pathogens. Prolonged cool and wet conditions of glass house may favors disease development on commercial varieties which means that the varietal tendency is towards disease, and their resistance is not based on the genetic makeup (Agrios, 1997).

The presence of several races of each and ever changing behavior of these pathogens further complicate breeding for rust resistance. Virulence analysis is to provide the breeders early information about behavior of their material towards major wheat diseases so that they can decide which material should be retained for further use in their breeding program. Secondly we need to refine our pathotype surveys in order to more closely monitor adult resistances; currently, only seedling resistances are being monitored.

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