Available online at http://www.journalijdr.com

ISSN: 2230-9926

IJDR

International Journal of
DEVELOPMENT RESEARCH

International Journal of Development Research
Vol. 4, Issue, 12, pp. 2615-2619, December, 2014

Full Length Research Article

PATHOGENICITY VARIATIONS OF *PUCCINIA STRIIFORMIS* F. SP. *TRITICI* WESTEND IN WHEAT GROWING AREAS OF NEPAL

[1,2*]Syed Jawad Ahmad Shah, [3]Shaukat Hussain, [3]Musharaf Ahmad, [4]Farhatullah and [1]Muhammad Ibrahim

[1]Wheat Rust Program, Nuclear Institute for Food and Agriculture (NIFA) Tarnab, Peshawar, Pakistan
[2]Department of Plant Pathology, Washington State University, Pullman, USA
[3]Department of Plant Pathology, The University of Agriculture, Peshawar, Pakistan
[4]Department of Plant Breeding and Genetics, KPK Agricultural University, Peshawar

ARTICLE INFO

Article History:
Received 06th September, 2014
Received in revised form
18th October, 2014
Accepted 28th November, 2014
Published online 27th December, 2014

Key words:
Wheat,
Yellow Rust,
Pathotypes,
Virulences,
Nepal.

ABSTRACT

Yellow rust, caused by *Puccinia striiformis* f. sp. *tritici* (PST), is one of the most important diseases threatening wheat productions around the world. Yellow rust population from Nepal was characterized for its frequency and combination of virulences using world, European and supplemental differential sets under standard glasshouse conditions at Institute National de la Recherché Agronomique (INRA), France. A total of 62 revived isolates which were collected during 2008 were analyzed. Isolates were differentiated into six pathotypes (N1 to N6). Two pathotypes N1 and N2 represented 88% of the Nepalese population and shared virulences *v1* (virulent to resistance gene *Yr1*), *v2*, *v6*, *v7*, *v27* and *vSU*. None of the isolates was virulent to resistance genes *Yr5, Yr10, Yr15, Yr24, Yr26* and *YrEp*. The virulence frequencies of *v1, v6*, *v8*, *v9* and *vA* ranged between 26-95% in all samples analyzed while *v2, v7, v27*and *vSu* were fixed in all pathotypes. Results revealed that out of six pathotypes, five were never reported from Nepal and suggested a possibility of spore migration in the region.

INTRODUCTION

After rice and maize, wheat is the most valuable cereal crop in Nepal which is predominantly utilized for bread and biscuit making and is becoming more important in the economy of the country (Joshi *et al*., 2006). Several diseases can attack wheat crop in Nepal, but yellow rust, caused by *Puccinia striiformis* f. sp. *tritici* (PST), has posed a serious threat to its production in river basins, low to midhill areas of the country (Anonymous, 2004). Wheat growers in Nepal have faced many yellow rust epidemics in the past and can still bear yield losses upto 30% (Anonymous, 2004). Yellow rust races 4E0, 4E16, 70E16 and 68E16 were detected in late 1970s (Sharma *et al*., 1995). During the middle 1980s, the pathogen began to change and an epidemic of the rust was experienced when wheat cultivar `RR 21' (Sonalika) occupied large acerage in the country (Sharma *et al*., 1995). The change in virulence of PST was the major cause of the epidemic and a novel race, 7E150, which was according to Institute for Plant Protection, Wageningen, Netherland race data (unpublished), appeared in Afghanistan in 1981 and then moved eastwards and infected the commercially grown cv. Sonalika which appeared to be more susceptible at high elevations than at low elevations, as observed during 1986 in Nepal (Stubbs, 1988). Furthermore, additional races including 0E16, 2E0, 6E0, 6E16, 7E158, 15E158, 70E0, and 66E18 were also reported from Nepal (Sharma *et al*., 1995). Virulence *v9* evolved in Africa, migrated to Asia reaching up to Nepal during 1999 (Anonymous, 2004) where it eroded the resistance of *Yr9* based cultivars. Countries with current vulnerability to yellow rust in Asia include China, India, Pakistan and Nepal. Recent information about PST pathotypes and their associated virulences and frequencies are well documented from China, India and Pakistan (Prashar *et al*., 2007; Bahri, 2008 and Mboup, 2008), but it was lacking for Nepal. This study was

Corresponding author:** [1,2*]**Syed Jawad Ahmad Shah
[1]Wheat Rust Program, Nuclear Institute for Food and Agriculture (NIFA) Tarnab, Peshawar, Pakistan
[2]Department of Plant Pathology, Washington State University, Pullman, USA

therefore undertaken to analyze the current wheat yellow rust population from Nepal and to relate it with other regions.

MATERIALS AND METHODS

Sampling and spore multiplication

Sampling of PST isolates was performed in Nepal during 2008 from areas where wheat yellow rust occurs regularly. All areas were well represented with 6 isolates from Bhairahawa (27°30′ N 83°27′ E), 1 from Dodhikot (27°64′ N, 85°40′ E), 19 from Dolakha Farm (27°40′ N 86°2′ E), 12 from Khumattar (?), 3 from Lomatar (27°62′ N 85°40′ E), 5 from Lumli (28°35′ N, 83°83′ E), 2 from Nakhipot (?), 1 from Nalduvr (?), 2 from Pythan Dhawa (?) and 11 from Ramkot (27°717′ N 85°25′ E). Infected leaves were sampled and each leaf was stored in a paper envelope to prevent contamination and shipped by express mail service. To avoid mixing of isolates, each sample was inoculated to 7-day-old seedlings of a mix of susceptible cultivars (Michigan Amber and Victo) and spores were collected from plants bearing single uredinium and were increased. Details for increasing of isolates, pre/post maintenance of inoculated plants, day/night temperature and light conditions of dew chamber/climate chambers were the same as described by (Bahri *et al*., 2011). Each pot was covered within a cellophane bag to avoid cross-contamination after 7 days of inoculation and urediniospores were collected after 18 days, dried in a desiccator at 4 ^{o}C for 3 days, and stored in microtubes at -80 ^{o}C till use.

Table 1. World, European and supplementary sets of wheat cultivars used to differentiate yellow rust pathotypes from Nepal

S.No	Host cultivars	Resistance genes
	World differentials set	
1	Chinese 166	*Yr1*
2	Lee	*Yr7*
3	Heines Kolben	*Yr6, Yr2*
4	Vilmorin 23	*Yr3V*
5	Moro	*Yr10*
6	Strubes Dickopf	*YrSD*
7	Suwon92 × Omar	*YrSU*
8	Clement	*Yr9, Yr2+*
	European differential set	
9	Hybrid 46	*Yr4+*
10	Reichersberg 42	*Yr7+*
11	Heines Peko	*Yr6, Yr2+*
12	Nord Deprez	*Yr3N*
13	Compair	*Yr8*, Yr APR
14	Carstens V	*YrCV*
15	Spaldings Prolific	*YrSP*
16	Heines VII	*Yr2+*
	Supplemental cultivars	
17	Anza	*YrA*
18	Kalyansona	*Yr2*
19	Federation X4 Fav.	*Yr9*
20	VPM1	*Yr17*
21	TP 981	*Yr25*
25	Victo	-
26	Jubilejina 2	-
27	Early Premium	-
	Avocet Isogenic Lines	-
28	AvSYr1NIL	*Yr1*
29	AvSYrYr5NIL	*Yr5*
30	AvSYrYr6NIL	*Yr6*
31	AvSYrYr7NIL	*Yr7*
32	AvSYrYr8NIL	*Yr8*
33	AvSYrYr15NIL	*Yr15*
34	AvSYrYr24NIL	*Yr24*
35	AvSYrYr26NIL	*Yr26*
36	AvSYrYr27NIL	*Yr27*

Virulence analyses

Virulence combinations of 62 revived samples were determined by using a group of 16 wheat yellow rust differentials including the world and European sets (Johnson *et al*., 1972) along with 8 supplemental wheat differential lines and 9 selected *Yr* isogenic lines in the Australian Avocet Susceptible backgroud (Table 1). Each isolate, maintained separately, was sprayed after suspending 5 mg of urediospores in 300 µl of mineral oil (Soltrol) onto five seedlings (two-leaf stage) of each variety and incubated under the conditions as previously described (Bahri *et al*., 2011). Plants were scored individually two weeks after inoculation using a standard 0-9 scale which is based on the visual assessment of chlorosis and/or necrosis and the severity of sporulation (McNeal *et al*., 1971). Classification of infection types was carried out as resistant (IT = 0-3), intermediate (IT = 4-6) or susceptible (IT = 7-9). An isolate was classified as virulent when its infection type value fell in the susceptible range.

RESULTS

The 62 isolates from Nepal were revived and characterized into six pathotypes which are presented along with their virulence composition in Table 2. Two predominant pathotypes, NI and N2 comprised 89% of the population (55 isolates) while N4 (*v1, v2, v3, v4, v6, v7, v9, v17, v25, v27, v32, vA, VSu, vVicto* and *vJub*) and N2 (*v1, v2, v6, v7, v9, v27* & *vSu*) had the highest and lowest number of virulence factors, respectively. All six pathotypes lacked virulences *v5, v10, v15, v24, v26* and *vEp*. Pathotypes N1, N2 and N3 had common virulences *v1, v2*, *v6*, *v7*, *v8*, *v27*, *vA* and *vSu* while *v1*, and both *v8* and *vA* were not associated with N3 and N2, respectively. The virulence and avirulence spectra of N1 and N6 were identical except virulences *v9* and *v8*, which were lacking by pathotypes N1 and N6, respectively. Pathotypes N2 and N6 shared virulences *v1*, *v2*, *v6*, *v7*, *v9*, *v27* and *vSu* with an additional virulence *vA* for N6. Pathotypes N1, N2, N3 and N6 were avirulent on cultivar TP981 (*Yr25*) as well as on an extremely susceptible cv. Victo, which is not known to have any resistance gene(s). Moreover, virulence *vSu* was not associated with virulence *v4* in these four pathotypes.

For the other two pathotypes, N4 and N5, virulences *v4* and *vSu* were associated. N4 had a composition of virulences *v1*, *v2*, *v3*, *v4*, *v6*, *v7*, *v9*, *v17*, *v25*, *v27*, *v32*, *vA*, *vSu* and *vVicto*. N5 and N4 had the same composition of virulences except that N5 lacked virulences *v6*, *v17* and *v32* but carried two additional virulences *vSD* and *vSP*. Virulence *v8* was carried by two pathotypes (N1 & N3) while *v9* was shared by four pathotypes (N2, N4, N5 and N6). However, combined virulences *v8* and *v9* were not observed in any pathotype. Out of six pathotypes, three (N1, N2 and N6) were detected from the Bhairahawa area with N2 being the major one (Table 3). Conversely, only one pathotype, N1 was identified from the Dodhikot area. Similarly, out of 19 isolates collected from the Dolakha Farm area, 4 pathotypes, N1, N3, N5 and N6, were recovered, of which, N1 was the predominant pathotype. Moreover, twelve isolates from the Khumattar area were differentiated into three pathotypes, N1, N3 and N4 with N1 being the prominent pathotype from this area. Only one pathotype, N1, was detected from three isolates collected from the Lomatar area.

Table 2. Yellow rust pathotypes and associated virulence factors in samples from Nepal during 2008

Pathotypes	No of isolates	Virulence spectrum																							No of virulence factors
		1	2	3	4	5	6	7	8	9	10	15	17	24	25	26	27	32	A	SD	SP	Su	Vic	Jub	EP	
NI	43	1	2	-	-	-	6	7	8	-	-	-	-	-	-	-	27	-	A	-	-	Su	-	-	-	8
N2	12	1	2	-	-	-	6	7	-	9	-	-	-	-	-	-	27	-	-	-	-	Su	-	-	-	7
N3	3	-	2	-	-	-	6	7	8	-	-	-	-	-	-	-	27	-	A	-	-	Su	-	Jub	-	8
N4	1	1	2	3	4	-	6	7	-	9	-	-	17	-	25	-	27	32	A	-	-	Su	Vic	Jub	-	15
N5	1	1	2	3	4	-	-	7	-	9	-	-	-	-	25	-	27	-	A	Sd	SP	Su	Vic	Jub	-	14
N6	2	1	2	-	-		6	7	-	9	-	-	-	-	-	-	27	-	A	-	-	Su	-	-	-	8

Virulence phenotype: numbers correspond to yellow rust resistance genes, A, SD, SP, Su, Vic, Jub and EP designate resistances in Anza, Strubes Dickkopf, Spaldings Prolific, Suwon 92 X Omar, Victo, Jubilejina 2 and Early Premium, respectively.
avirulence is shown by '—'

Table 3. Geographical distribution of wheat yellow rust pathotypes in Nepal during 2008

Pathotypes	No of isolates	Geographical areas									
		Bhairahawa	Dodhikot	Dolakha Farm	Khumattar	Lomatar	Lumli	Nakhipot	Nalduvr	Pythan Dhawa	Ramkot
N1	43	1	1	16	9	3	3	2	1	1	6
N2	12	4	0	0	0	0	2	0	0	1	5
N3	3	0	0	1	2	0	0	0	0	0	0
N4	1	0	0	0	1	0	0	0	0	0	0
N5	1	0	0	1	0	0	0	0	0	0	0
N6	2	1	0	1	0	0	0	0	0	0	0
Total	62	6	1	19	12	3	5	2	1	2	11

Pathotype absence is shown by '0'

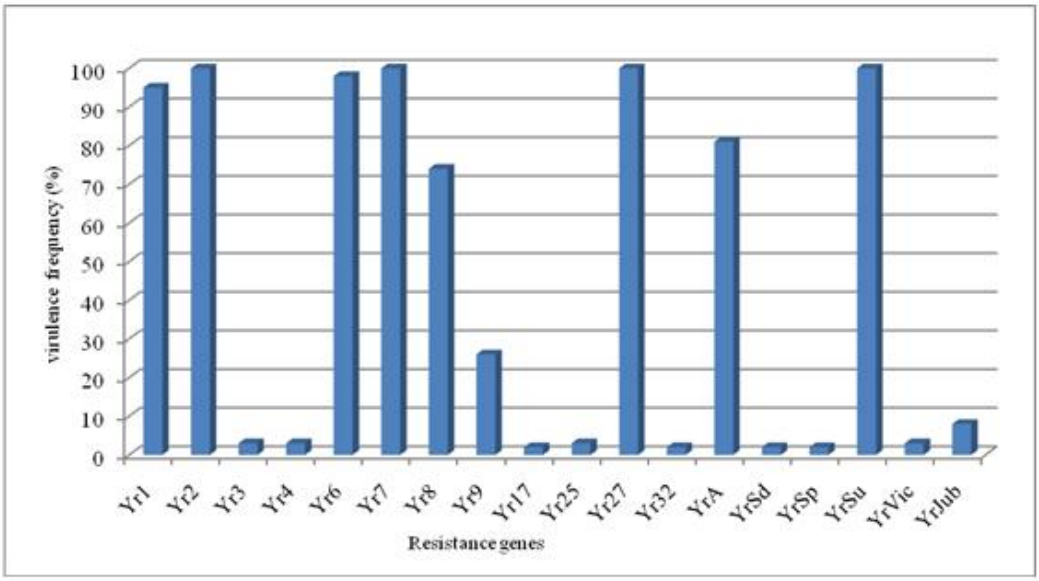

Fig. 1. Virulence frequency of wheat yellow rust pathotypes collected from Nepal during 2008

Likewise, five isolates from Lumli area were classified into two pathotypes, N1 and N2. Surprisingly only N1 was detected from the three samples collected from the Nakhipot and Nalduvr areas. Additional pathotypes, N1 and N2, were identified from the two samples of the Pythan Dhawa area. Finally, eleven isolates from the Ramkot area were differentiated into two pathotypes, N1 and N2 at similar frequencies. Virulence frequencies of *v3*, *v4*, *v17*, *v25*, *v32*, *vSd*, *vSP* and *vVic* were very low at 3 or <3% of the total pathotypes while that of *vJub* was 8%. Virulence frequencies of *v1*, *v2*, *v6*, *v7*, *v27*, *vA* and *vSu* were high (>80%) and those of *v8 and v9* were 74 and 26%, respectively (Fig. 1). Virulences *v2, v7, v27*and *vSu* were fixed in all pathotypes.

DISCUSSION

Analyses of the PST population from Nepal revealed a considerable variability in the virulence composition of six pathotypes. Pathotype N3 was reported from Nepal under the name 70E16 during late 1970s (Sharma *et al*., 1995). Pathotypes N2 (67E0), N3 (70E16) and N6 (64E0) with an identical virulence pattern have been reported previously from Pakistan during 1973-76 and 1981-93, respectively (Hussain *et al*., 2004). Moreover, these three pathotypes were also detected recently from India and Pakistan (Prashar *et al*., 2007 and Shah, 2010). Virulence composition of pathotype N4 is close to a Chinese (CYR32), French (237E141*v17*), Pakistani (P12) and Algerian (M7) pathotypes (Wan *et al*., 2002 and Bahri, 2008). As long-distance disease spread of yellow rust is an established phenomena (Chen, 2005) within and between different regions so urediniospore migration might explain the commonality of the above mentioned pathotypes in Nepal. Detection of three old pathotypes (N2, N3 and N6) in the current study and lack of previous information impose limitations on their probable survival mechanism in Nepal.

Virulence *vSu* is not associated with *v4* in these three Nepali pathotypes which was common in old Pakistani, Indian, Australian, United Kingdom and Danish pathotypes (Prashar *et al*., 2007 and Hovmoller *et al*., 2008). The association between these two virulences has not been detected so far in Northwestern European pathotypes. Dissociation between *vSu* and *v4* has been observed in the United States, Eritrea and Yemen (De Vallavieille-Pope and Line 1990 and Hovmoller *et al*., 2008). The association of virulences *v8* and *v9* was absent in the population studied from Nepal. Virulence on wheat genotypes with *Yr8* and *Yr9* resistance genes was first discovered in the United States during 2000 (Chen *et al*., 2002) and in Australia during 2002 (Wellings *et al*., 2003). Several pathotypes from Pakistan have this combination which was also reported from India (Prashar *et al*., 2007), Iran (Afshari, 2008), Middle East and West Mediterranean regions (Bahri, 2008).

Intensive deployment of *Yr2, Yr6*, and *Yr7* in East Africa, North Africa, Middle East and South Asia created genetically vulnerable situation which resulted in successive erosion of these resistance genes. Cultivar `RR 21' carrying *Yr2* was grown extensively in Nepal and pathotype 7E150 was responsible for its resistance break down during middle 1980s (Anonymous, 2004). All pathotypes detected from Nepal carried virulence for *Yr2, Yr6*, and *Yr7*. The 1B/1R translocation carrying *Yr9* resistance was intensively introgressed into CIMMYT lines in the 1980s which were introduced and became very popular in Asian countries including Nepal where cultivars 'Annapurna-1' and 'Annapurna-4' carrying *Yr9* were released during 1988 and 1991, respectively, which created a situation of monoculture. Both these cultivars with *Yr*9 became susceptible when the new virulent race 46S119 (46E151*v9*) appeared during 1999 (Anonymous, 2004). Several pathotypes possesing virulences *v2*, *v6*, *v6*+, *v7*, *v7*+, *v8* and *v9* were common in the region (Hakim *et al*., 2002).

Virulence *v9* is still present in Nepal and its frequency was 26% in the rust population analyzed in the present study. Two most important cultivars Inqilab-91(*Yr27*) and PBW-343 (*Y9* and *Yr27*) were released in South Asia following epidemics on *Yr9* and occupied more than 11 million ha in Pakistan and India (Singh *et al*., 2004). Similarly, 30% (0.2 million ha) of the wheat acerage in Nepal was under one main variety "NL-297" carrying *Yr2*+ (CIMMYT, 2001). Evidence of virulence *v27* and resistance breakdown of NL-297 was observed with a severe epidemic in Kathmandu valley of Nepal during 2004 (www.globalrust.org/db/attachments/pathogen/62/2/Nepal,Dr%20Saral-ICARDA-presentation.swf; Anonymous, 2004) suggesting the presence of *v2* and *v27* and both these virulences in addition to *v6*, *v7* and *vSu* were fixed in all pathotypes analyzed in the current study. Pathotypes carrying both *v2* and *v27* were reported from Australia (C. R. Wellings, personal communication), India (Prashar *et al*., 2009), Pakistan (Shah, 2010), Europe, Central & West Asia, Eritrea and Yemen (www.globalrust.org/db/attachments/ resources/ 843/10/ HOVMOLLERObregon%20March_09%20(I).pdf). As migration and virulences are common in the region and pathogen change continues to be a major factor, strong collaborative efforts must be taken for developing yellow rust resistant wheat varieties for commercial production in South Asia.

REFERENCES

Afshari, F., 2008. Prevalent Pathotypes of *Puccinia striiformis* f.sp. *tritici* in Iran. *Journal of Agricultural Science and Technology*, 10:67-78.

Anonymous, 2004. A Quarterly Newsletter of Nepal Agricultural Research Council (NARC). 3:1-8. www.narc-nepal.org

Bahri, B., 2008. Adaptation et Structuration spatiale des populations me´diterrane´ennes de rouille jaune du ble´ (*Puccinia striiformis* f. sp. *tritici*). Ph.D. Thesis. Universite´ d_Orsay Paris Sud, Paris, France,

Bahri, B., Shah, S.J.A., Hussain, S., Leconte, M., Enjalbert, J. and De Vallavieille-Pope, C., 2011. Genetic diversity of wheat yellow rust population in Pakistan and its relationship with host resistance. *Plant Pathology*, 60:649-660.

Chen, X.M., 2005. Epidemiology and control of stripe rust (*Puccinia striiformis* f.sp. *tritici*) on wheat. *Canadian Journal of Plant Pathology*, 27:314-337.

Chen, X.M., Moore M.K., Milus E.A., Long D.L., Line R.F., Marshall D. and Jackson L., 2002. Wheat stripe rust epidemics and races of *Puccinia striiformis* f. sp. *tritici* in the United States in 2000. *Plant Dis*., 86: 39-46.

CIMMYT. 2001. Research Highlights of the CIMMYT Wheat Program, 1999-2000. Mexico, D.F.

De Vallavieille-Pope, C. and Line, R.F., 1990. Virulence of North American and European races of *Puccinia striiformis* on North American, world and European differentials. *Plant Disease,* 74:739-43.

Hakim, M.S., Yahyaoui, A., El-Naimi, M. and Maaz, I., 2002. Wheat yellow rust Pathotypes in Western Asia. *In*: Johnson R., Yahyaoui A, Wellings C, Saidi A. and Ketata H. (eds). Proceedings of the first regional conference on yellow rust in the Central and West Asia and North Africa region, 8-14 May 2001, Karaj, Iran, p. 55.

Hovmøller, M.S, Yahyaoui, A., Milus, E.A. and Justesen, A.F., 2008. Rapid global spread of two aggressive strains of a wheat rust fungus. Molecular Ecology 17:3818-26.

Hussain, M., Kirmani, M.A.S. and Haque, E.U., 2004. Pathotypes and man guided evolution of *Puccinia striiformis* West and sp *tritici* in Pakistan. In: Abstracts, Second Regional Yellow Rust Conference for Central & West Asia and North Africa, 22–26 March 2004, Islamabad, Pakistan, Syria, ICARDA. pp 2.

Johnson, R., Stubbs, R.W., Fuchs, E. and Chamberlain, N.H., 1972. Nomenclature for physiologic races of Puccinia striiformis infecting wheat. *Transactions of the British Mycological Society,* 58: 475-80.

Joshi, B.K., Mudwari, A. and Bhatta, M.R., 2006. Wheat Genetic Resources in Nepal. Nepal Agric. Res. J. 7:1-9.

McNeal, F.H., Konzak, C.S., Smith, E.P., Tate, W.S. and Russel T.S., 1971. A uniform system for recording and processing cereal research data. USDA, ARS 34-121.

Mboup, M., 2008. Influence des pressions de selection et de la suivie inter-epidemique sur la diversité genetique des populations de rouille jaune du blé *Puccinia striiformis* f.sp. *tritici*. Ph.D. Thesis. Universite´ d_Orsay Paris Sud, Paris, France.

Prashar, M., Bhardwaj, S.C., Jain, S.K. and Datta, D., 2007. Pathotypic evolution in *Puccinia striiformis* in India during 1995-2004. *Austrilian Journal of Agricultural Research,* 58:602-604.

Prashar, M., Bhardwaj, S.C., Jain, S.K., Sharma, Y.P. and Shoran, J., 2009. Wheat Rusts in India - Pathogenic Changes. Poster presented at '2009 Technical Workshop' Borlaug Global Rust Iniciative (BGRI), Cd. Obregon, Sonora, Mexico, March 17-20.

Shah, S.J.A., 2010. Characterization of *Puccinia striiformis* Westend. f. sp. *tritici* Eriks population and its control through host resistance. Ph.D. Thesis. NWFP Agricultural University, Pakistan.

Sharma, S., Louwers, J.M., Karkil, C.B. and Snijders, C.H.A., 1995. Postulation of resistance genes to yellow rust in wild emmer wheat derivatives and advanced wheat lines from Nepal. *Euphytica,* 81:271-277.

Singh, R.P., Duveiller, E. and Huerta-Espino, J., 2004. Virulence to yellow rust resistance gene *Yr27*: A new threat to stable wheat production in Asia. Page 16, *In*: Abstracts, Second Regional Yellow Rust Conference for Central & West Asia and North Africa, 22-26 March 2004, Islamabad, Pakistan.

Stubbs, R.W., 1988. Pathogenicity analysis of yellow (stripe) rust of wheat and its significance in global context. p. 23-38. In: N.W. Simmonds & S. Rajaram (Eds). Breeding Strategies for Resistance to Rusts of Wheat. CIMMYT, Mexico, 151 p

Wellings, C. R., Wright D.G., Keiper F. and Loughman R., 2003. First detection of wheat stripe rust in Western Australia: Evidence for a foreign incursion. *Australasian Plant Pathology,* 32:321-322.

****