

## Partial resistance to yellow rust in introduced winter wheat germplasm at the north of Pakistan

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### Abstract

Thirty-seven winter wheat lines introduced from Oklahoma State University, along with local check were studied to assess their partial resistance level, at Hazara Agricultural Research Station (HARS), Abbottabad. Pathotypes of *Puccinia striiformis* West. *tritici* (*Pst*) prevalent at the site were found virulent on all of the introduced winter wheat lines. Partial resistance level was assessed through final rust severity (FRS), area under rust progress curve (AURPC) and coefficient of infection (CI). A considerably high disease pressure was observed with a maximum FRS up to 95% recorded for line F<sub>5</sub>-34. Six lines were having FRS value more than 70%, while remaining 24 lines along with local check were having FRS value less than 70%. Seventeen lines were having higher AURPC values while remaining 21 lines were having AURPC values ranging from 300 to 1100, marked to be partially resistant. Based on CI value, four lines (F<sub>5</sub>-64, F<sub>5</sub>-71, F<sub>5</sub>-15 and OK00611W) were marked as highly partially resistant, having CI value up to 20; F<sub>5</sub>-4, F<sub>5</sub>-21, F<sub>5</sub>-69, F<sub>5</sub>-78, F<sub>5</sub>-103, F<sub>5</sub>-122, F<sub>5</sub>-141, OK99212, OK00608W, Intrada and Ghaznavi, with CI value 21-40, were marked as moderately resistant; and F<sub>5</sub>-5, F<sub>5</sub>-9, F<sub>55</sub>-38, F<sub>5</sub>-70, F<sub>5</sub>-83, F<sub>5</sub>-99, F<sub>5</sub>-110, F<sub>5</sub>-139, OK95616-56, OK98G508W, OK00421, OK00514 and OK00618W, with CI value 41-60, were designated to have low level of partial resistance. Line OK00611W performed better, producing maximum grain yield with an increase of 2736 kg ha<sup>-1</sup> over the local check as well as having better level of partial resistance. Cluster analysis of the OSU introduced winter wheat lines revealed four major groups/cluster, based on partial resistance parameters and grain yield. Considerable diversity was observed for level of partial resistance of these introduced winter wheat breeding lines, which may be advanced further for manipulation in breeding or inheritance studies.

**Key words:** Winter wheat, yellow rust, introduced lines, partial resistance.

### Introduction

Wheat being a staple food in Pakistan has a prime importance in our economy. It is cultivated in Pakistan as a winter crop. Actually the spring wheat is sown in winter season in plane areas of Pakistan, where there is no winter snowfall, mainly for grain purpose. However, forage availability for livestock is reduced during the winter period of reduced forage production (Arif et al.,

2005). Winter wheat could be cultivated in Northern areas of Pakistan, which could serve as a dual purpose crop to provide both fodder during winter season and grains during the late spring. This system could have even more income stability due to overall net gain from both fodder and grain (Redmon et al., 1995). Attempts have been made to develop winter wheat varieties from

introduced winter wheat germplasm. However, the increased growth period along with more green leaf content in such genotypes could increase the chances of wheat diseases, especially yellow rust. Similarly, the climatic conditions of our northern areas are even more favorable for the disease (Ali et al., in preparation). Yellow rust is the most important one. Its regular outbreaks have caused severe losses to Pakistan in past and present (Saari et al., 1995 and Kissana et al., 2003) and remain a potential risk (Singh et al., 2004). The only economic and practical control of rust diseases can be achieved through genetic resistance (Pathan and Park, 2006). Thus searching out the most appropriate genotypes for dual purpose wheat production with special consideration of their disease resistance is crucial for sustainability of this new dual purpose winter wheat system.

Several researchers have evaluated wheat germplasm of Pakistan for rust resistance (Shah et al., 2003; Mirza et al., 2000). However, they focused mainly on vertical resistance, based on major genes, which could rapidly be overcome due to the evolution of virulence by pathogen to these genes. The alternative is to search for partial resistance based on minor genes, which is considered to be more durable (Singh et al., 2004). The present study was thus designed to assess the levels of partial resistance in winter wheat breeding lines introduced from Oklahoma State University, USA. The understanding and exploitation of partial yellow rust resistance in Pakistan will further assist in controlling rust disease globally.

## Materials and methods

The study was conducted at Hazara Agricultural Research Station (HARS), Abbottabad to assess the levels of partial resistance in introduced winter wheat breeding lines. Seeds of winter wheat breeding lines were received by Department of Plant Breeding and Genetics, NWFP Agricultural University Peshawar under a coordinated research project of ALP from Oklahoma State University. Two kinds of breeding material were received; one from Dr. Klute and other from Dr. Craver. Twenty-eight varieties were selected from the first material for the study, designated as OSU-K-line-no., and were sown in replicated trial laid out in randomized complete block design with two replications. The second trial was consisted of 10 lines, designated as OSU-C-line-no., including Ghaznavi as a local checks variety; and was also laid

out in completely randomized block design with three replications. In both the trials, each entry was separately planted in strips of small adjacent plots with two rows plot<sup>-1</sup> of 2 m length separated by 0.3 m within entry row-distance and 0.6 m between the entries.

Natural Infection was relied upon because the site was hot spot for yellow rust. Data on disease scoring was collected twice during the season, with one month gape. Two parameters were included during the scoring i.e., severity according to the Modified Cobb Scale (Paterson et al., 1948) and host reaction following Singh (1993). The area under rust progress curve (AURPC) was estimated by using the formula adopted by Pandey et al. (1989). The data on disease severity and host reaction was combined to calculate coefficient of infection (CI) following Pathan and Park (2006), by multiplying the severity value by a value of 0.10, 0.25, 0.50, 0.75 or 1.00 for host response ratings of R, MR, MR-MS or S, respectively. Data on grain yield was calculated for each entry by harvesting entire two rows and weighing their grains through electronic balance interpreted here as kg ha<sup>-1</sup>. Data were statistically analyzed using analysis of variance appropriate for randomized complete block design (Steel and Torrie, 1984). Diversity among these was estimated and grouping was made on the basis of cluster analysis based on grain yield and partial resistance traits i.e., AURPC, FRS and coefficient of infection. Computer packages MS Excel, SAS and NTSYS PC were used for ANOVA and cluster analysis.

## Results and discussion

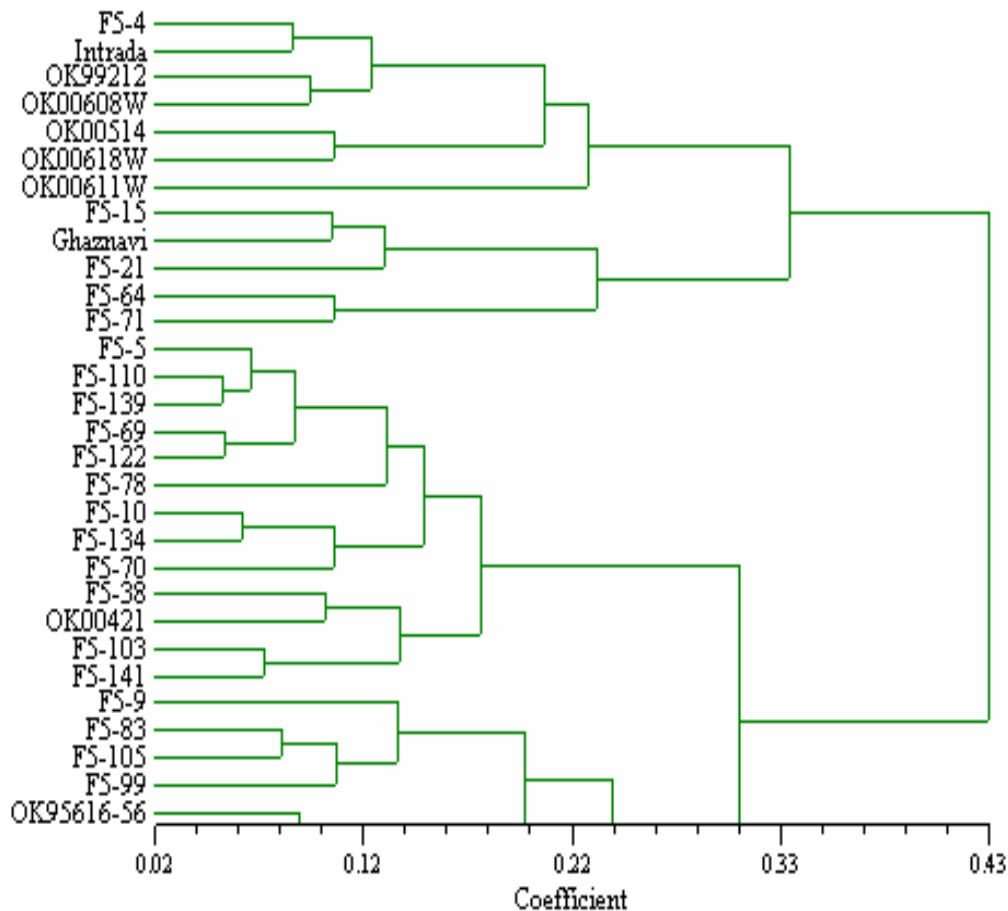
To appraise the level of partial resistance in the introduced germplasm of winter wheat, parameters used as criteria for partial resistance were final rust severity, AURPC, and coefficient of infection. Highly significant differences (<0.01%) were observed for all the parameter studied. Results regarding these parameters are described here:

### *Final Rust Severity (FRS)*

Data on final rust severity of 37 introduced winter wheat lines along with local check (Ghaznavi) are shown in table I. A considerably high disease pressure was recorded at the testing site as maximum FRS up to 95% was recorded for line F<sub>5</sub>-34, followed by F<sub>5</sub>-146 (92%), F<sub>5</sub>-117 (90%), F<sub>5</sub>-87(90%) and F<sub>5</sub>-

**Table 1.** Partial resistance traits along grain yield of OSU-introduced winter wheat breeding lines at HARS, Abbottabad, during 2005-06

Code	Entry	AUDPC value	FRS value	CI value	Yield (kg ha <sup>-1</sup> )	Yield increase over check (kg ha <sup>-1</sup> )
OSU-K-1	F <sub>5</sub> -4	637.5	30.0	22.6	3750.0	1208
OSU-K-2	F <sub>5</sub> -5	900.0	60.0	41.4	2437.5	-104
OSU-K-3	F <sub>5</sub> -9	1650.0	70.0	50.2	2645.8	104
OSU-K-4	F <sub>5</sub> -10	1050.0	70.0	58.4	2229.2	-312
OSU-K-5	F <sub>5</sub> -15	900.0	35.0	13.8	2541.7	0
OSU-K-6	F <sub>5</sub> -21	675.0	45.0	22.5	2854.2	313
OSU-K-7	F <sub>5</sub> -34	1425.0	95.0	89.6	2854.2	313
OSU-K-8	F <sub>5</sub> -38	1050.0	70.0	50.2	3083.3	542
OSU-K-9	F <sub>5</sub> -42	1650.0	90.0	79.2	3250.0	708
OSU-K-10	F <sub>5</sub> -63	1275.0	85.0	80.2	2104.2	-437
OSU-K-11	F <sub>5</sub> -64	450.0	30.0	7.5	2354.2	-187
OSU-K-12	F <sub>5</sub> -69	1050.0	70.0	35.0	2437.5	-104
OSU-K-13	F <sub>5</sub> -70	1275.0	70.0	48.3	2520.8	-21
OSU-K-14	F <sub>5</sub> -71	300.0	20.0	7.5	2729.2	188
OSU-K-15	F <sub>5</sub> -78	1275.0	60.0	30.0	2604.2	63
OSU-K-16	F <sub>5</sub> -83	1462.5	80.0	57.1	2687.5	146
OSU-K-17	F <sub>5</sub> -87	1575.0	90.0	84.6	3291.7	750
OSU-K-18	F <sub>5</sub> -99	1350.0	75.0	52.7	3250.0	708
OSU-K-19	F <sub>5</sub> -103	825.0	55.0	38.9	3229.2	688
OSU-K-20	F <sub>5</sub> -105	1350.0	75.0	66.0	2812.5	271
OSU-K-21	F <sub>5</sub> -110	1012.5	65.0	45.8	2625.0	83
OSU-K-22	F <sub>5</sub> -117	1575.0	90.0	84.6	2610.4	69
OSU-K-23	F <sub>5</sub> -122	975.0	65.0	32.5	2562.5	21
OSU-K-24	F <sub>5</sub> -134	1162.5	75.0	60.8	2358.3	-183
OSU-K-25	F <sub>5</sub> -135	1312.5	85.0	80.2	2066.7	-475
OSU-K-26	F <sub>5</sub> -139	1050.0	60.0	45.2	2416.7	-125
OSU-K-27	F <sub>5</sub> -141	900.0	60.0	30.0	3250.0	708
OSU-C-28	F <sub>5</sub> -146	1612.5	92.5	86.8	2750.0	208
OSU-C-29	OK95616-56	1550.0	80.0	59.0	3875.0	1333
OSU-C-30	OK98G508W	1375.0	81.7	52.9	4152.8	1611
OSU-C-31	OK99212	650.0	43.3	38.1	4236.1	1695
OSU-C-32	OK00421	950.0	63.3	49.4	3611.1	1069
OSU-C-33	OK00514	1000.0	53.3	43.1	4861.1	2320
OSU-C-34	OK00608W	750.0	50.0	35.1	3791.7	1250
OSU-C-35	OK00611W	750.0	40.0	20.0	5277.8	2736
OSU-C-36	OK00618W	850.0	56.7	49.9	4375.0	1833
OSU-C-37	Intrada	625.0	41.7	25.9	3888.9	1347
Local Check	Ghaznavi	1000.0	46.7	23.3	2541.6	0
Correlation with grain yield		-0.22	-0.25	-0.18		



**Fig 1.** Dendrogram showing similarity index cluster analysis of OSU introduced winter wheat breeding lines based on disease resistance and grain yield.

42 (90%), designated as susceptible; while none of the line was recorded to be immune. Similarly, based on FRS, the tested lines were grouped into three groups of partial resistance i.e., high, moderate, low levels of partial resistance having 1-30%, 31-50% and 50-70% FRS, respectively. Lines F<sub>5</sub>-71, F<sub>5</sub>-64 and F<sub>5</sub>-4 were included in first group, F<sub>5</sub>-15, F<sub>5</sub>-21, OK99212, OK00608W, OK00611W, Intrada and local check ‘Ghaznavi’ were marked as having moderate level of partial resistance, based on FRS. F<sub>5</sub>-5, F<sub>5</sub>-9, F<sub>5</sub>-10, F<sub>5</sub>-38, F<sub>5</sub>-69, F<sub>5</sub>-70, F<sub>5</sub>-78, F<sub>5</sub>-103, F<sub>5</sub>-110, F<sub>5</sub>-122, F<sub>5</sub>-139, F<sub>5</sub>-141, OK00421, OK00514 and OK00618W were marked to be having low level

of partial resistance. This final rust severity is assumed to represent the cumulative result of all resistance factors during the progress of epidemic (Parlevliet and van Omeren, 1975). Previously, Ali et al. (2007) evaluated wheat breeding material from Pakistan to assess their partial resistance in terms of slow rusting behavior. Similarly, Broers et al. (1996) also carried out field assessment of quantitative resistance to yellow rust for ranking of wheat breeding lines. According to the resistance level based on disease severity (DS) along with other slow rusting parameters, they found that resistance levels ranged from very low (in Taichung 23) to very high (in Parula) among the tested wheat breeding lines.

### ***AURPC value***

Data pertaining AURPC values of introduced winter wheat breeding material introduced from Oklahoma State University is given in table I. The tested breeding lines were categorized into three distinct groups for partial resistance, based on their AURPC values. Winter wheat lines exhibiting AURPC values up to 500 were grouped as having high level of partial resistance, consisted of two lines F<sub>5</sub>-71 and F<sub>5</sub>-64; while those having AURPC values from 500 to 800 were grouped as moderately resistant lines, included F<sub>5</sub>-4, F<sub>5</sub>-21, OK99212, OK00608W, OK00611W and Intrada. Similarly, lines having AURPC ranging from 800 to 1100 were marked as having low level of partial resistance, which included F<sub>5</sub>-5, F<sub>5</sub>-10, F<sub>5</sub>-15, F<sub>5</sub>-38, F<sub>5</sub>-69, F<sub>5</sub>-103, F<sub>5</sub>-122, F<sub>5</sub>-139, F<sub>5</sub>-141, OK00421, OK00514 and local check (Ghaznavi). These lines first shown rust infection and sporulation but the final host reaction was characterized as chlorotic and necrotic strips (MR and/or MS - infection types). Subsequently, the progress of rust development remained slower and restricted. Parlevliet (1988) reported that breeding lines with such traits are expected to possess genes that confer partial resistance. Such partial resistance has been advocated to be more durable than race-specific vertical resistance (Singh et al., 2004). Such partially resistant lines restrict the evolution of new virulent races of the pathogen because multiple point mutations are extremely rare in nature (Schafer & Roelfs, 1985). Winter wheat breeding lines F<sub>5</sub>-9, F<sub>5</sub>-42, F<sub>5</sub>-87, F<sub>5</sub>-117, F<sub>5</sub>-146, F<sub>5</sub>-34, F<sub>5</sub>-63, F<sub>5</sub>-70, F<sub>5</sub>-78, F<sub>5</sub>-83, F<sub>5</sub>-99, F<sub>5</sub>-105, F<sub>5</sub>-110, F<sub>5</sub>-134, F<sub>5</sub>-135, OK95616-56 and OK98G508W were having higher AURPC values and were marked as susceptible.

### ***CI Value:***

The data on disease severity and host reaction was combined to calculate coefficient of infection (CI) following Pathan and Park (2006). Cultivars with CI values of 0-20, 21-40 and 41-60 were regarded as possessing high, moderate and low levels of APR, respectively. Breeding lines F<sub>5</sub>-64, F<sub>5</sub>-71, F<sub>5</sub>-15 and OK00611W were grouped in first category; F<sub>5</sub>-4, F<sub>5</sub>-21, F<sub>5</sub>-69, F<sub>5</sub>-78, F<sub>5</sub>-103, F<sub>5</sub>-122, F<sub>5</sub>-141, OK99212, OK00608W, Intrada and local check 'Ghaznavi' were marked to be having moderate level of Partial resistance; and F<sub>5</sub>-5, F<sub>5</sub>-9, F<sub>5</sub>-38, F<sub>5</sub>-70, F<sub>5</sub>-83, F<sub>5</sub>-99, F<sub>5</sub>-110, F<sub>5</sub>-139, OK 95616 - 56, OK 98G508 W,

OK00421, OK00514 and OK00618W were designated to be having low level of partial resistance. F<sub>5</sub>-34, F<sub>5</sub>-42, F<sub>5</sub>-63, F<sub>5</sub>-87, F<sub>5</sub>-117, F<sub>5</sub>-135 and F<sub>5</sub>-146 exhibited CI value greater than 60 and were grouped as susceptible. Previously, Pathan and Park (2006) appraised adult plant resistance, a kind of partial resistance, to wheat leaf rust in European wheat lines using average coefficient of infection (ACI) by calculating average of CI across different sites. They reported the presence of different partial resistance conferring genes in these lines. Similarly, different researchers reported the presence of varying levels of partial/general resistance in wheat breeding material (Singh, 1992 and McIntosh, 1992).

### ***Yield Potential and Partial resistance:***

Data on grain yield along with partial resistance traits of the introduced winter wheat introduced lines are given table I. Maximum grain yield was produced by OK00611W with an increase of 2736 kg ha<sup>-1</sup> over the local check 'Ghaznavi'. The line was having better partial resistance level, based on disease parameters. Breeding lines OK98G508W, OK99212, OK00514 and OK00618W were also having higher grain yields as compared to local check 'Ghaznavi'. Their levels of partial resistance were also better in terms of FRS, AURPC and CI values. F<sub>5</sub>-64 was having lower yield despite of its highly better level of partial resistance, may be due to its lower genetic yield potential and not due to rust infection. F<sub>5</sub>-63, F<sub>5</sub>-135, F<sub>5</sub>-146, F<sub>5</sub>-134, F<sub>5</sub>-117, F<sub>5</sub>-34 and F<sub>5</sub>-42 were having lower grain yield and were marked as susceptible based on disease parameters. Negative correlation was observed between yield and FRS (-0.25), AURPC (-0.22) and CI (-0.18). Previously, Allan et al. (1963) and Ali et al. (2007) have also reported the existence of association between rust infection and grain yield losses.

### ***Diversity among Breeding Lines***

Cluster analysis of the OSU-introduced winter wheat lines revealed four major groups/ cluster, based on partial resistance parameters and grain yield (Fig. 1). F<sub>5</sub>-4, Intrada, OK99212, OK00608W, OK00514, OK00618W, and OK00611W were grouped in first cluster, while F<sub>5</sub>-15, Ghaznavi, F<sub>5</sub>-21, F<sub>5</sub>-64 and F<sub>5</sub>-71 were clustered in the second group. Similarly, F<sub>5</sub>-

5, F<sub>5</sub>-10, F<sub>5</sub>-34, F<sub>5</sub>-38, F<sub>5</sub>-42, F<sub>5</sub>-63, F<sub>5</sub>-69, F<sub>5</sub>-70, F<sub>5</sub>-78, F<sub>5</sub>-87, F<sub>5</sub>-103, F<sub>5</sub>-110, F<sub>5</sub>-117, F<sub>5</sub>-122, F<sub>5</sub>-134, F<sub>5</sub>-135, F<sub>5</sub>-139, F<sub>5</sub>-141, F<sub>5</sub>-146, OK98G508W and OK00421 were grouped in third cluster, while F<sub>5</sub>-9, F<sub>5</sub>-83, F<sub>5</sub>-105, F<sub>5</sub>-99 and OK95616-56 were grouped in fourth cluster. The first cluster comprised on the lines considered as having better level of partial resistance along with higher yield performance as compared to the check. Second cluster consisted on lines having better level of partial resistance but lower yield potential. Lines clustered in third group were marked to be having moderate level of partial resistance along with modest yield. The last cluster included winter wheat lines having low yield and low level of partial resistance. Considerable diversity was observed for level of partial resistance among the introduced winter wheat breeding lines. This may be exploited in further breeding manipulation. Previously, Ali et al., (2007) evaluated wheat breeding lines from Pakistan and reported the presence of varying degree of partial resistance to stripe rust for the tested lines in terms of slow rusting.

### Conclusion

Our results showed the presence of varying levels of partial resistance among the winter wheat lines introduced from OSU, along with certain lines marked as susceptible to the prevalent races of stripe rust at North of Pakistan. None of the lines was marked as immune. F<sub>5</sub>-64 was having highly better level of partial resistance but lower yield, may be used in breeding program to transfer its better partial resistance character. Breeding lines OK98G508W, OK99212, OK00514 and OK00618W were also having higher grain yields as compared to local check 'Ghaznavi' with better levels of partial resistance may be advanced further for manipulation in breeding or inheritance studies. However, these lines should be tested over years and locations for yellow rust along with other desirable characters, before approval.

### References

- Ali S, Shah SJA, Ibrahim M, (2007) Assessment of wheat breeding lines for slow yellow rusting (*Puccinia striiformis* west. *tritici*). Pak J Biol Sci 10: 3440-3444.
- Allen RE, Vogel OA, Purdy LH (1963) Influence of stripe rust on yield and test weights of closely related lines of wheat. Crop Sci. 3: 564-565.
- Arif M, Khan A, Akbar H, Khan S, Ali S (2006) Prospect of wheat as a dual purpose crop. Pakistan Journal of Weed Science Research. 12: 13-18.
- Broers LHM, Subias XC, Atilano RML (1996) Field assessment of quantitative resistance to yellow rust in ten spring bread wheat cultivars. Euphytica 90: 9-16.
- Clifford BC (1972) The histology of race non-specific resistance to *Puccinia hodei* Oth in barley. Pages 75-78 in Proceedings of the 5<sup>th</sup> European and Mediterranean Cereal Rusts Conference, Prague, Czechoslovakia. European Mediterranean Cereal Rusts Foundation.
- Diaz MJ, Roberto ZE, Viglizzo EF (1996) Diversificación productiva y estabilidad económica de sistemas con distintas relaciones agroganaderas. Revista Argentina. Production animal 6: 603-608.
- Horn F P (1984) Chemical composition of wheat pasture. P. 47-54. In GW Horn (ed.) Proc. Natl wheat pasture symp Oklahoma Agricultural Experimental Station. MP-115.
- Kisana SN, Mujahid YM, Mustafa ZS (2003) Wheat Production and Productivity 2002-2003. A Technical Report to Apprise the Issues and Future Strategies. Published by Coordinated Wheat, Barley and Triticale Program, National Agricultural Research Center, Pakistan Agricultural Research Council, Islamabad. 19pp.
- McIntosh RA (1992) Close genetic linkage of genes conferring adult-plant resistance to leaf rust and stripe rust in wheat. Plant Pathology 41: 523-527.
- Mirza JI, Singh RP, Ahmed I (2000) Resistance to leaf rust in Pakistani wheat lines. Pakistan Journal of Biological Sciences. 3: 1056-1061.
- Parlevliet JE (1988) Resistance of the Non-Race-Specific type. In 'The Cereal Rusts. Vol. II. Diseases, Distribution, Epidemiology and Control.' (Academic Press Orlando.).
- Pandey HN, Menon TCM, Rao MV (1989) A single formula for calculating Area Under Disease Progress curve. Rachis 8: 38-39.
- Pathan AK, Park RF (2006) Evaluation of seedling and adult plant resistance to leaf rust in European wheat cultivars. Euphytica 149: 327-342.
- Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale for rust intensity on leaves and stems of cereals. Canadian Journal of Research. 26: 496-500.
- Redmon LA, Horn GW, Krenzer EG, Bernardo DJ (1995) A review of livestock grazing and wheat grain yield: Boom or bust? Agronomy J. 88: 94-97.

- Saari EE, Hashmi NI, Kisana NS (1995) Wheat and Pakistan, an update (Yr95 doc.) pp3.
- Schafer, J.F., Roelfs A.P., 1985. Estimated relation between numbers of urediniospores of *Puccinia graminis tritici* and rates of occurrence of virulence. *Phytopathol.* 75: 749-750.
- Shah SJA, Khan AJ, Azam F, Mirza JI, Rehman AU (2003) Stability of rust resistance and yield potential of some ICARDA bread wheat lines In Pakistan. *Pakistan Journal of Scientific and Industrial Research.* 46: 443-446.
- Singh RP (1992) Genetic association of leaf rust resistance gene Lr34 with adult plant resistance to stripe rust in bread wheat. *Phytopathol.* 82: 835-838.
- Singh RP, William HM, Huerta-Espino J, Rosewarne G (2004) Wheat Rust in Asia: Meeting the Challenges with Old and New Technologies. "New directions for a diverse planet". Proceedings of the 4th International Crop Sci. Cong. 26 Sep – 1 Oct 2004, Brisbane, Australia.
- Steel RGD, Torrie JH (1984) Principles and procedures of statistics: a biometrical approach. McGraw Hill Co. New York, NY, USA.