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LOCAL STEM RUST VIRULENCE IN PAKISTAN AND FUTURE BREEDING STRATEGY

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Abstract

Evolution and spread of stem rust race Ug-99 has created an alarming global situation. Majority of the CIMMYT germplasm protected by gene *Sr31* fell susceptible to this catastrophic strain. Like other parts of the world stem rust of wheat was very successfully controlled in Pakistan by the introduction of resistant germplasm during and after green revolution. In 2001 stem rust reappeared and hit many commercial varieties in the province of Sindh. Its sporadic infections were recorded in 2005 summer crop of Kaghan followed by 2005-06, 2006-07, 2007-08 and 2008-09 spring crops of Sindh. In a regional scenario, where by wheat crop of the whole CWANA region was threatened by the Ug-99, it was thought that the crop is attacked by Ug-99. To date data from the stem rust TRAP nurseries and analysis of the disease samples is presented here to clarify the situation about presence/absence of Ug-99 in Pakistan. Commercial cultivars resistant to local stem rust race and genes effective against local and Ug-99 races are identified and their implications to improve stem rust resistance of Pakistani cultivars are discussed.

Introduction

Stem rust of wheat caused by *Puccinia graminis* f. sp. *tritici (Pgt)*, responsible for heavy yield losses in wheat crop, has successfully been controlled throughout the world by introduction of semi-dwarf resistant cultivars during 1960s and 1970s and there has been no report of significant loss since then. Resistance in majority of the wheat cultivars sown in this region is based on gene *Sr31* which is introgressed into bread wheat from *Secale cereale* cv. Petkus (Rye) (McIntosh *et al*., 1995). These lines gave significant yield advantage in addition to powdery mildew, stem leaf and stripe rust resistance. Breakdown of stem rust resistance gene *Sr31* with the introduction of *pgt* race Ug-99 in Uganda during 1999 (Pretorius, 2000) created an alarming situation throughout the world as the gene was protecting 80% of the developing country's wheat lines derived from the CIMMYT germplasm. Virulence to this gene was subsequently reported along *Yr9* path following its first report from Uganda. On its way along the path it acquired virulences to the genes of importance and three variants were reported from Ethiopia (Jin *et al*., 2008; Jin *et al.*, 2009). Khanzada (2008) reported stem rust infection in Pakistan on mega cultivars during 2000-01 wheat cropping cycle. Sporadic infection of stem rust were recorded in 2005 summer crop of Kaghan followed by 2005-06, 2006-07, 2007-08 and 2008-09 spring crops of Sindh. Appearance of stem rust on mega wheat cultivars of Pakistan was alarming under the prevailing scenario of the region where by Ug-99 was spawning new strains and was moving ahead towards Pakistan along *Yr9* Path. Moreover

all Pakistani wheat cultivars screened under Kenyan field conditions were susceptible to Ug-99 (Anon., 1995). Analysis of the situation was realized as prime factor in adaptation of breeding strategy to incorporate genes resistant to Ug-99 in Pakistani cultivars. Our analysis indicated that the virulence found in Pakistan did not match Ug-99 but still had the capability to attack genes resistant to Ug-99. This paper discusses virulence patterns of the local stem rust race found in Sindh province of Pakistan and its importance to adopted breeding strategy to combat Ug-99 in this region.

Material and Methods

Stem rust TRAP Nursery, commercial cultivars and disease sampling: A TRAP nursery consisting of 39 stem rust isogenic lines was planted at Thatta and Matli. Details of the lines in this nursery are given in Table 1. The nursery was planted in 1m row 30cm apart from each other. Morocco was added as susceptible check and spreader.

Seventy one commercial wheat cultivars, most of them with known susceptibility to Ug-99 were cultivated at Matli, Sakrand and Karachi. Third stem rust screening nursery (3rd SRSN) received from CIMMYT was planted at Thatta and Karachi for field screening. Morocco was added as susceptible check and spreader.

Survey of the wheat crop in districts Karachi, Thatta, Tando Muhammad Khan, Badin, Hyderabad, Mirpurkhas, Umerkot, Larkana, Sukkur, Nausheroferoze and Dadu was conducted from March 11-20, 2009 to see distribution of stem rust and collect disease samples. Disease samples were collected from farmer's fields and research stations. Some disease samples were also received from Wheat Research Institute Bahawalpur and Islamabad. Disease intensity on commercial cultivars was recorded at farmer field.

Virulence analysis and seedling screening: Virulence analysis of the disease samples was conducted under glasshouse conditions. Urediospores washed from disease samples in petroleum spirit were re-suspended in mixture of mineral oil and petroleum spirit (ratio 1: 3 v/v) before spraying them with fine atomizer on 10 day old seedlings of the susceptible cultivar Morocco grown in disposable pots. Inoculated plants were left in shade for two hours, to evaporate oil, before transferring them to growth room. The growth room was set at 18±1^{o}C temperature, 99% relative humidity and 20 hours dark followed by 4 hour florescent light. The plants were then moved to glasshouse set at 18-20^{o}C temperatures. On the appearance of pustules on 10th day all the leaves except the ones with isolated pustule were clipped, plants were washed under running water and placed in isolation chambers to sporulate the pustules. On sporulation the single pustule inoculums were multiplied on the newly grown leaves of same plant using standard procedure (Knott, 1989). Single pustule inoculums were collected for each pustule separately and inoculated on ten day old seedlings of the tester host set planted in 38 x 46 x 10 cm tray. The tester set was composed of 20 North American stem rust differentials (Roelfs & Martens, 2007) and morocco as susceptible check. Differentials in trays were inoculated with spore suspension in mixture of mineral oil and petroleum sprit (1:3 v/v) by method mentioned above. Growth room and glasshouse incubation conditions were also same as mentioned above. Stem rust data for seedling infection types described by Stakman *et al*., (1962) was recorded on 10th day of inoculation when pustules were sporulating on susceptible cultivar morocco. Seedling screening of the selected SRSN lines was conducted using conditions and methods mentioned above.

Results

Stem rust TRAP Nursery and response of commercial cultivars: Results of the TRAP nursery are given in Table 1. Susceptible filed response was recorded for genes *Sr13, 17, 25, 36, 37* and *44* in Matli and Thatta while genes *Sr22, 32, 33,* and *39* were moderately susceptible at these locations. Gene *Sr35* was highly susceptible at Matli and moderately susceptible at Thatta while gene *Sr28* and *Tmp* were moderately susceptible at Matli and susceptible at Thatta. Virulence for *Sr6, 9b, 30, 38* and *Wld-1* was absent in Kaghan.

Field screening results of the commercial cultivars are given in Table 4. Commercial cultivars Faisalabad 85, Kohinoor83, LU-26, Pasban-90, Shaheen 94, Shahkar 95, Bahawalpur 97, Abadgar 93 and Tatara which are highly susceptible to Ug-99 were immune at Karachi, Tandojam, Sakrand and Matli indicating absence of Ug-99 at these locations. Many other cultivars including Bakhtawar, Inqilab 91, Blue silver, Chakwal 86, Sindh 81, Zargoon and Faisalabad83, which are highly susceptible to Ug-99 were either immune or moderately susceptible at these locations.

Survey and sample collection: Maximum stem rust disease intensity of 40S-80S was recorded on wheat varieties Kiran, Sarsabz, unidentified cultivars named as Hira (named by seed companies), and other mixed material in Kunri, Jhuddo and neighboring fields. Disease intensity at different locations is given in Table 2. Barley infested with stem rust was observed in Districts Badin and Tando Muhammad Khan. Some pustules were observed on local variety Thori in Rattu Dero and neighboring fields of District Larkana. Disease sample were collected from Thatta, Matli and Mirpur Bathoro for further analysis.

Virulence analysis of the disease samples: Two virulence/avirulence combinations were identified in 24 stem rust isolates. These combinations differed from each other in response to *Sr9e*, *Sr24* and *SrTmp* genes and their virulence/avirulence formulae are given in Table 3.

Table 2. Intensity of stem rust disease in different districts of Sindh.

Location	Intensity	Variety
District Umerkot: Taluka Kunri	40S-80S	Kiran, Sarsabz and companies material Hira, and other mixture
Jhuddo and neighboring fields	40S-80S	Kiran, Sarsabz and other mixture
Distt. Thatta: Gharo, Sijawal, Mirpur Bathoro and Jhoke Sharif.	5S-60S	Companies seed Hira, Rustam and barley
Distt. Badin: Matli, Talhar, Tando Bago and neighbours field	5S-60S	Companies Rustam seed, other local varieties and barley
Distt. Tando Muhammad Khan: Bulri Shah Karim and neighbours field	5S-60S	Local wheat varieties and barley 90S Baley
District Hyderabad i.e. Kisano Mori	10S	Kiran
Distt. Larkana i.e. Rattu Dero and neighbouring fields	Trace	local variety Thori
Distt. Nawabshah and Nausheroferoze	Trace	-

Table 3. Virulence/avirulence combinations identified among isolates from Pakistan.

S. No.	Location	Virulence/avirulence combinations
1.	Thatta	*Sr5,6,7b,9a,9b,9d,9g,10,11,17,21,30,36,Tmp,McN/Sr8a,9e, 24, 31*
2.	Matli	*Sr5,6,7b,9a,9b,9d,9g,10,11,17,21,30,36,Tmp,McN/Sr8a, 9e, 24, 31*
		Sr5,6,7b,9a,9b,9d,9e,9g,10,11,17,21,24,30,36,Tmp,McN/Sr8a, 31
3.	Mirpur mathilio	*Sr5,6,7b,9a,9b,9d,9g,10,11,17,21,30,36,Tmp,McN/Sr8a, 9e, 24, 31*
4.	Bahawalpur	*Sr5,6,7b,9a,9b,9d,9g,10,11,17,21,30,36,Tmp,McN/Sr8a, 9e, 24, 31*
5.	Islamabad	*Sr5,6,7b,9a,9b,9d,9g,10,11,17,21,30,36,Tmp,McN/Sr8a, 9e, 24, 31*

Discussion

Stem rust infection found earlier in limited coastal area of Sindh spread to Distt. Umarkot, Taluka, Jhuddo, Kunri, Thattta, Sajawal, Gharo, Mirpur Bathoro, Jhoke Sharif, Matli, Talhar, Tando Bhago, Tando Muhammad Khan, Larkana, Nawabshah in Sinsh, Bahawalpur in Punjab and in Islamabad. Most popular cultivar of Sindh are susceptible to the virulence found in these areas. Field resistances in numerous Ug-99 susceptible commercial cultivars at Karachi, Tandojam, Sakarand and Matli indicate absence of Ug-99 in these locations. Moreover susceptible filed response of *Sr13, 25, 36, 37* and *44* in Matli and Thatta indicate a virulence pattern different from Ug-99. Glasshouse virulence analysis revealed that stem rust samples from all locations lacked *Sr31* virulence. Presence of Ug-99 is also nullified by data from Kaghan where virulence for *Sr31* was lacking. Among other stem rust resistant genes *Sr24,* for which virulence is detected in new strains of Ug-99, showed MS type of reaction under field conditions in Thatta and Matli. Virulence analysis confirmed *Sr24* virulent isolates in Matli which may be attacking this gene in Thatta as well. This gene in combination with other genes is still effective against local and Ug-99 races as lines of third stem rust screening nursery (SRSN) carrying this gene in combination with *SrTmp* and *Sr36* are showing very good field resistance in Thatta, seedling resistance against *Sr24* virulent isolate from Matli and isolates from Bahawalpur (Table 5). Gene *Sr25* which is closely linked with *Lr19* was thought to become more susceptible under high temperatures (Gough & Markle 1971). Genetic stocks with 7D-Ag translocations carrying this genes has never been cultivated in these areas in past but virulence to this gene was detected in Nilgiri Hills of India in 2007 (Singh *et al.*, 2008) and coastal areas of Sindh in 2008 (Mirza *et al.,* 2009). *Sr25* gave susceptible field response at Thatta and Matli. Promising level of field resistance at Thatta and their seedling resistance with isolates from Matli show that SRSN lines postulated for *Sr25* have additional genes in them (Table 5).

Gene *Sr27* showed field resistance in Thatta. This is another gene of potential importance for which Ug-99 lacks virulence. Incorporation of this gene in Pakistani cultivars resistant to local race can enhance their resistance to Ug-99. *Sr6, 9b, 30, 38* and *Wld* are the genes for which Ug-99 carries virulence. All isolates analyzed under glasshouse conditions from Sindh and Punjab were virulent to *Sr6, Sr9b, Sr30* and *Wld* .The use of these genes should not be encouraged in Pakistani cultivars. Stem rust resistant genes *Sr13, Sr14* from *Triticum turgidum*, *Sr28, Sr29* from *T. aestivum*, *Sr33* from *T. tauschii*, *Sr36, Sr37* from *T. tempoheevi* and *Sr35* from *T. monococum* were of special interest to breeders after the evolution of Ug-99 (Singh *et al*., 2006). *Sr14* has field resistance at Matli, Thatta and Kaghan while *Sr28* was susceptible at these locations. Virulence for *Sr13* & *Sr37* was found in past among isolate collections from

Table 4. Adult plant response of commercial cultivars in Pakistan and Kenya.

Ent #.	Line	Stem rust response in			
		Karachi	Sakrand	Matli	Kenya (2005)
1.	Bakhtawar 92	0	0	5MSS	20S
2.	Blue silver	0	0	5MSS	20S
3.	Chakwal 86	0	0	5MSS	40S
4.	Sind-81	0	0	5MSS	40S
5.	Zarghoon	5MSS	0	5MSS	60S
6.	Faisalabad 83	0	0	20S	30S
7.	Faisalabad 85	0	0	0	40S
8.	Inqilab 91	0	5RMR	10MSS	50S
9.	Kaghan 93	0	5RMR	10MSS	30S
10.	Morocco	0	20MSS	30S	
11.	Kirin 95	0	0	10MSS	30S
12.	Kohinoor 83	0	0	0	40S
13.	LU-26	0	0	0	40S
14.	Nowshera 96	0	0	TMS	30S
15.	Parwaz 94	0	0	5MSS	10S
16.	Pasban 90	0	0	0	20S
17.	Mexipak 65	0	5MS	5MSS	
18.	Punjab 96	0	0	5MSS	30S
19.	Sariab-92	0	0	TMS	20S
20.	Morocco	0	0	30S	
21.	Sarsabz	0	0	5MSS	40S
22.	Shaheen 94	0	0	0	30S
23.	Shahkar 95	0	0	0	10S
24.	Soughat 90	0	0	5MSS	20S
25.	Tandojam 83	0	5MS	5MSS	20S
26.	SH-2002	0	0	20MSS	20S
27.	Pak 81	0	0	5MSS	20S
28.	Bahaw-97	0	0	0	40S
29.	MH-97	0	0	70S	30S
30.	Morocco	0	0	10MSS	_
31.	Kohistan 97	0	0	20MS	40S
32.	Kohsar 95	0	0	5MSS	_
33.	Rohtas 90	0	0	0	_
34.	Suleman 96	0	0	10MSS	_
35.	WL 711	0	0	5MSS	20S
36.	Zardana	0	0	5MSS	30S
37.	Abadgar 93	0	0	0	10S
38.	Anmol-91	0	0	5MSS	20S
39.	Bahawal-2000	0	0	5RMR	40S

Table 4 (Cont'd.).

Ent #.	Line	Stem rust response in			
		Karachi	Sakrand	Matli	Kenya (2005)
40.	Morocco	0	5S	40S	_
41.	Bahkhar-2002	0	0	10MSS	30S
42.	Fakhr-e-Sarhad	70S	10MSS	10MSS	20S
43.	Marvi-2000	0	0	TMS	20S
44.	Mehran-89	0	5MS	TMS	_
45.	Soorab-96 (Barley)	0	Missing	TMS	20S
46.	Tatara	0	0	0	20S
47.	Takbeer	0	10MS	60S	20S
48.	AS-2002	0	5MS	5MSS	10S
49.	Iqbal 2000	0	0	5MSS	10S
50.	Morocco	0	5MSS	30S	
51.	Auqab-2000	0	0	30S	20S
52.	Chakwal-97	0	0	0	_
53.	Durum-97	0	0	TMS	40S
54.	Watan 94	0	0	10MSS	50S
55.	Moomal 2002	0	0	10S	30S
56.	Zarlashata	0	0	TMS	40S
57.	GA-2002	0	0	5MSS	20S
58.	Wafaq-01	0	0	5MSS	40S
59.	Margalla-99	0	0	5MS	20S
60.	Morocco	0	0	20S	_
61.	Manthar-3	0	0	0	30S
62.	Saleem 2000	0	0	5MS	_
63.	Khyber 87	0	0	0	_
64.	Pirsabak 2004	0	0	0	_
65.	Pirsabak 2005	0	0	TMS	_
66.	Punjnad-1	0	0	0	_
67.	Darawar-97	0	0	0	_
68.	V-87094 (Wattan)	0	0	TS	_
69.	Shafaq 2006	0	0	5MSS	_
70.	Morocco	0	0	20S	_
71.	Sehar 2006	0	0	30MSS	_
72.	Bhittai	0	0	5MSS	_
73.	Chakwal-50	0	0	0	_
74.	Saussi	0	0	5MSS	_
75.	Lasani-08	5MSS	0	10MSS	_
76.	Meraj-08	0	0	5MSS	_
77.	Fareed-06	0	0	0	_
78.	Faisalabad-08	20S	0	20MSS	_

India and Pakistan (Luig, 1983) and for *Sr35* was found in collection from Ethiopia, Kenya, Nepal (Huerta-Espino, 1992; Admassu & Fekadu, 2005). Virulence for *Sr36* is present in collections from Ethiopia since 1987 (Huerta Espino, 1992, Admmassu & Fekadu 2005) and among new variants of Ug-99 (Jin *et al*., 2009). Field susceptibility of genes *Sr36* and *37* at Thatta, Matli and seedling virulence for *Sr36* in all isolates analyzed discourage their use for our breeding program. Similarly field virulence for *Sr35* at Matli, *Sr28, Sr29* & *Sr13* at Thatta, renders them useless if used alone to protect wheat against local and Ug-99 races. These genes can however be incorporated in commercial cultivars, resistant to local races, to protect them against both Ug-99 and local races. Virulence for *Sr29* was detected in isolates from Western Asia (Huertta-Espino, 1992) and three races from Ethiopia (Admassu and Fekadu, 2005). Confirmation of the presence of high virulence at seedling stage for genes *Sr28, 29 & 13* and studies on their effectiveness when used in combination with other genes need to be conducted prior to their use in our lines. Virulence for *Sr33* which conferred moderate level of resistance to Ug-99 in past (Jin *et al.*, 2007) is reported in seven races from Ethiopia (Admassu & Fekadu, 2005). This gave MS-MSS reactions under field conditions in Thatta and Matli this year. *Sr39* resistant to Ug-99 have effective field resistance at Thatta and Matli. This gene can be deployed in Pakistani wheat cultivars to enhance their resistance. *Sr40, 44, dp-2, Gt* and *Tmp* appear to have virulence in Thatta. *SrTmp* virulent isolates were found in samples analyzed under glasshouse conditions. The gene is showing effective level of field resistance in combination with *Sr24* in 3rd SRSN lines (Table 5).

Wide spread of local stem rust race/s up to Bahawalpur in southern Punjab and susceptibility of mega cultivars planted in Sindh emphasize replacement of these cultivars and adaptation of a breeding strategy which fulfils our requirement to meet upcoming challenge of Ug-99 and local race/s. Out of 17 commercial cultivars, identified as immune to local stem rust, Faisalabad85, Kohinoor83, LU-26, Pasban90, Shahen94, Shahkar95, Bahawalpur97, Rohtas90 and Abadgar, are highly susceptible to Ug-99 and resistant to local race/s. These lines should be included in breeding program to improve their resistance by incorporating genes resistant to Ug-99 in them. Genes susceptible both to local and Ug-99 races should be avoided in our breeding program. Stem rust resistant genes *Sr13, 25, 36, 37* and *44* should very carefully be used in combination with genes resistant to local race/s. Lines resistant to local and Ug-99 races identified in 3rd stem rust screening nursery should also be included in our breeding program and be focused for direct adaptation and release.

References

Admassu, B. and E. Fekadu. 2005. Physiological races and virulence diversity of *Puccinia graminis* f. sp. tritici on wheat in Ethopia. *Phytopathologia Mediterranea.* 44: 313-318.

Anonymous. 1995. *Expert Panel on the Stem Rust Outbreak in Eastern Africa*. 2005. Sounding the Alarm on Global Stem Rust: An assessment of race Ug-99 in Kenya and Ethiopia and the potential for impact in neighboring regions and beyond. Mexico City: International Maize and Wheat Improvement Center (CIMMYT). (Sept. 8). Available at http://www.cimmyt.org /english/ wps/ news /2005/aug /pdf/Expert_Panel_Report.pdf.

Goh, F.J. and O.G. Markle. 1971. Inheritance of Stem and Leaf rust resistance in Agent and Argus cultivars of *Triticum aestivum*. *Phytopathology*. 61: 1501-1505.

Huerta-Espino, J. 1992. *Analysis of wheat leaf and Stem Rust Virulence on worldwide basis*. Ph.D. Thesis University of Minnesota, USA.

Jin, Y., R.P. Singh, R.W. Ward, R. Wanyera, M. Kinyua, P. Njau, T. Fetch, Z.A. Pretorius and A. Yahyaoui. 2007. Characterization of seedling infection types and adult plant infection

response on monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f.sp. *tritici. Plant Disease* 91(9): 1096-1099.

Jin, Y., L. J. Szabo, Z. A. Pretorius, R. P. Singh, R. Ward and T. Jr. Fetch. 2008. Detection of virulence to gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. tritici. *Plant Disease*. 92(6): 923-926.

Jin, Y., L. J. Szabo, M. N. Rouse, T. Jr. Fetch, Z.A. Pretorius, R. Wanyera and P. Njau. 2009. Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp. tritici. *Plant Disease*. 93(4): 367-370.

Khanzada, S. 2008. Re-occurrence of new stem rust race (Kiran virulence) in southern Pakistan and its potential danger for National food security. *Abstract in the Proceedings of International Conference of Plant Scientists.* April 21-24, 2008. Page 129.

Knott, D.R. 1989. *The Wheat Rust- Breeding for resistance*. Spring Verlag. New York.

Mirza, J.I., A. Gul and A. Mujeeb-Kazi. 2009. New Stem rust virulence detected in Paksitan: A potential threat to adopted breeding strategy against Ug-99. *Annual Wheat News Letter* (55): 153-155.

McIntosh, R.A., C.R. Welling and R.F. Park. 1995. *Wheat Rusts: An atlas of resistance genes*. CSIRO. Austrailia.

Pretorius, Z.A. 2000. Detection of virulence to stem rust resistance gene *Sr 31* in *Puccinia graminis* f. sp. *tritici* in Uganda. *Plant Disease,* 84: 203.

Roelfs, A.P. and J.W. Martens. 2007. An International System of Nomenclature for *Puccinia graminis* f. sp. Tritici. Available on http://www. cdl.umn. edu/ nomenclature/ pgt_nomen.html.

Singh, R.P., P.D. Hodson, J. Huerta-Espino, Y. Jin, P. Njau, R. Wanyera, S.A Herrera-Foessel and R.W. Ward. 2008. Will Stem Rust Destroy the World's Wheat Crop. *Advances in Agronomy*, 98: 271-309.

Singh, R. P., P.D. Hodson, Y. Jin, J. Huerta-Espino, M.G. Kinyua, R. Wanyera, P. Njau and R.W. Ward. 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from Ug-99 (TTKS) of stem rust pathogen. *CABI Reviews.*1(504). pp 13. Available from http://www. cababstractsplus. org/cabreviews.

Stakman, E.C., D.M. Stewart and W. Q. Loegering. 1962. Identification of physiologic races of *Puccinia graminis* var. tritici. U.S. Dept. Agric. Res. Serv. E-617. 53pp.

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