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Virulence Structure of the Wheat Stem Rust Population in Georgia

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***Abstract* – Isolates of *Puccinia graminis* were collected from wheat field survey conducted during 2012-2014 growing season throughout five geographic zones of Georgia. In total two hundred seventy six single-pustule isolates were analyzed for virulence on the set of international differential hosts including twenty single stem rust (Sr) resistance genes. The resistance genes Sr5, Sr9g, Sr17 and SrMcN were susceptible to all tested isolates. The majority of isolates (60.9-99.1%) was virulent to genes Sr6, Sr7b, Sr9a, Sr9e, Sr9d, Sr10, Sr11, Sr38, and SrTmp. Virulence on lines with genes Sr9d, Sr24, Sr8a, and Sr30 varied from 20.7% to 40.9%. 13.0% and 14.5% of the isolates were virulent on Sr21 and Sr36, respectively. Virulence to line with Sr31 has not been found. Forty eight races were found in stem rust population during three years. The genetic diversity of population was low.**

***Keywords* – Differentials, Gene, Race, Rust, Resistance, Virulence.**

I. INTRODUCTION

The most distributed and harmful among wheat diseases are wheat rusts. Stem rust caused by *Puccinia graminis f.sp. tritici is* one of the most destructive diseases of wheat world-wide. Stem rust occurs worldwide including Georgia. It is most important where dews are frequent during and after heading and temperatures is warm - 18-30 C. Stem rust is favored by hot days 25-30 C, mild nights 15-20 C with adequate moisture for night time dews. Wind can effectively disperse spores over great distances. Rain is necessary for effective deposition of spore involved in regional spore transport. Stem rust can cause serious losses because the stem can be completely girdled by the pustules and prevent grain fill.

Protection of wheat from rust diseases has very special significance for the Georgia, which is one of the origins of wheat and its pathogens having evolved together. Presence of alternate host-plants, wild cereals–infection reserves, and optimal climatic conditions provide for stable development of rusts and consequently, severe crop losses. Wheat stem rust is a most feared disease due to its ability to inflict substantial losses. In the past few years the impact of stem rust has increased because new virulent strain of disease known as Ug99 has emerged and spread quickly across borders. FAO estimates that 30 countries are either affected by Ug99 or at potential risk. There is a chance that Ug99 will reach Georgia where barberry is widespread [1].

Resistant cultivars are the safely and economically justified way in control of the disease. The pathogen exists in a range of pathotypes pathogenic variants, which are capable of overcoming current resistance in cultivars. Successful in breeding for resistance is highly depending to understanding of genetics of resistance and pathogenicity in host-pathogen relationship. Virulence survey is necessary for monitoring the occurrence of current pathotypes and virulence factors of obligate fungus such as rusts. The objective of this study was to characterize the virulence of *Puccinia graminis f.sp. tritici* population in Georgia.

II. MATERIALS AND METHODS

The observations of wheat fields were conducted in different geographic zones of Georgia according to international methodology [5, 12]. Visual inspections for the presence of rust were made in commercial fields (2 to 40 hectares in size) every 50-100 km. Uredinial collections of stem rust was made from commercial fields and breeding nurseries from varieties: Bezostaya 1, Krasnodarskaya 99, Akhaltsikhis tsiteli Doli, Tanya, Sila etc.

Each collection was used to inoculate 7 day-old wheat seedlings of universally susceptible cultivar Morocco. After 6-10 days, the leaves were trimmed so that only one uredinium remained on each plant. Spores from single uredinia of *Puccinia graminis f. sp. Tritici (Pgt)* were collected separately; the isolates were increased through one uredinial generation and were used for characteriization the virulence of *Puccinia graminis* population. 5-20 single-pustule isolates were derived from each collection and then inoculated 1-week old seedlings of differential set consisting of 20 near-isogenic lines, each with stem rust (Sr) resistance genes. These lines used as the differentials of Pgt races were grouped into fiver subsets in the following order: I. Sr5, 21, 9e, 7b; II. Sr11, Sr6, Sr8a, Sr9g; III. Sr36, Sr9b, Sr30, Sr17; IV. Sr9a, Sr9d, Sr10, SrTmp and V. Sr24, Sr31, Sr38, SrMcN. Plants were inoculated with water-spore suspensions of each single pustule isolate and placed in a dew chamber overnight. The next morning, seedlings were maintained in a greenhouse where air temperatures varied from 20^0C to 28^0C under daily light. Twelve to fourteen days after inoculation, plant reaction types were scored using the standard scale [8]. The virulence formula was recorded according to Green [2]. A North American system of nomenclature for designating races or virulence combinations of *Puccinia graminis* was also used [4, 7]. The Gleason index (HG) was calculated to detect the number of distinct pathotypes (races) present indicating the genetic diversity of p.graminis population over five areas during 2012-2014 [3]. The differential sets were provided by the International Center of Agriculture Research for Dry Area (ICARDA).

III. RESULTS AND DISCUSSION

The annual disease survey was conducted across five geographic zones of Georgia: Shida Kartli, Kvemo Qartli, Kakheti, Meskheti and Djavakheti. Mainly, the bread wheat cultivars were grown there. Wheat stem rust was observed in the fall-planted wheat plots during June-July. Stem rust was occurred with different intensity in the nearly all investigated areas. During 2012-2014 survey the overall mean incidence and severity of wheat stem rust varied from 1% to 8% depending field location, environmental conditions and producing cultivars [9]. High level of incidence and severity (100% and 70%) were indicated only in Akhatsikhe and Dusheti regions on single fields. In 2012, 2013 and 2014 twenty five, twenty four and sixteen disease samples were collected from 5 zones (Meskheti, Shida Kartli, Kvemo Kartli, Kakheti, Javakheti).

The virulence of 96, 98 and 82 single uredinial isolates were analyzed on the differential set in 2012, 2013 and 2014, respectively. In total of 48 races were identified in the Georgian population during three years. Races PKFTC, PRCTF, PRCQP and PCHTP constituted 16.3%, 15.0% , 13.4% and 6.9% of all Georgian isolates, respectively and were the most prevalent races in the population in Georgia.The level of diversity of *P.graminis* races found in Georgia during 2012-2014 was low (Gleason index – 0.17) (Table 1).

Table 1. Frequency of races (pathotypes) of *Puccinia graminis f.sp tritici* identified from collections from field in Georgia during 2012-2014

No.	Race	Virulence formula	No.	%
1	PKFTC	21, 9b, 11, 24, 31,36, 38,/5,9e,7b, 6,8a,9g, 30, 17, 9a,9d, 10, Tmp, McN	45	16.3
2	PRCTF	21,8a,36,9b,30,24,31/5,9e,7b,11,6, 9g, 17,10,9a,9d,Tmp,38,McN	43	15.0
3	PRCQP	21,8a,36,30, 9b,10,11,Tmp, 31/ 5,9e,7b, 6, 9g, 17,9a,9d, 24,38,McN	37	13.4
4	PCHTP	21,8a, 11,6, 36,30, 31/ 5,9e,7b, 9g, 9b, 17,9a,9d, 10, Tmp, 24,38,McN	19	6.9
5	TRCQP	21,8a, 11,6, 36,30, 31/ 5,9e,7b, 9g, 9b, 17,9a,9d, 10, Tmp, 24,38,McN	11	3.9
6	PRCQF	21,8a,36,9b,10,Tmp, 30,31/ 5,9e, 7b,11,6, 9g, 17,9a,9d, 38,McN	10	3.6
7	TKFTC	11, 9b,24, 31, 36, 38/21,5,9e,7b,6, 8a, 9g, 30,17, 10, 9a,9d, Tmp, McN	8	2.8
8	PHCTF	21,11,8a,36,9b,30,24,31/5,9e,7b, 6, 9g, 17,9a,9d,10,Tmp,38,McN	7	2.5
9	TKTTF	11,24,31/5,21,9e,7b, 6, 8a,9g, 9b, 36,30,10,17,9a,9d, Tmp,38,McN	6	2.2
10	PKTSF	21, 24, 31, Tmp,/5,9e,7b, 11, 6, 8a, 9g, 9b,10, 17, 9a,9d, 30,36, 38,McN	6	2.2
11	PRCTC	21,8a,36,9b,30,24,31,38/5,9e,7b,11,6, 9g, 17,9a,9d,10,Tmp,McN	4	1.4
12	MRCQP	21, 9e,9b, 8a, 30,36, 10,31 Tmp,/5, 7b, 6, 11,9g, 17, 9a,9d, 24, 38,McN	4	1.4
13	PHHTF	21,11,8a,36,30,24,31,38/5,9e,7b, 6, 9g,9b,17,9a,9d,10,Tmp,38,McN	4	1.4
14	PRTSF	21,8a, Tmp,24,31/5,9e,7b,11, 6, 9g, 36,9b,30,17,10,9a,9d, 38,McN	4	1.4
15	PRMQF	21,8a, 9b,10,Tmp24,30,31 /5,9e, 7b,11,6, 9g, 36,17,9a,9d, 38,McN	4	1.4
16	TRCQF	8a,36,9b,10,Tmp, 30,31/ 21,5,9e, 7b,21, 11, 6, 9g, 17,9a,9d, 24,38,McN	4	1.4
17	PTCSF	21, 8a, 9e,11, 9b,10, 24, 30, , 36, Tmp, 31/5, 7b, 6,9g, 17, 9a,9d38,McN	4	1.4
18	TRTQF	21,8a, 10,Tmp, 24, 31/ 5,9e,7b,21, 11, 6, 9g, 36,9b,30,17,9a,9d, 38,McN	4	1.4
19	PKFTP	21, 11, 9b, 31 ,36, /5,9e,7b,6, 8a, 9g, 10, 17, 9a,9d, 30, Tmp, 24, 38 ,McN	3	1.1
20	PCTTC	21, 6,8a,9b,11, 24, 31,36, Tmp, / 5,9e 7b, 9g, 30, 10, 17, 9a,9d, 38,McN	3	1.1
21	PRCQM	21,8a,36,30, 9b,10,11, Tmp, 31/ 5,9e,7b, 6, 9g, 17,9a,9d, 24,38,McN	3	1.1
22	LCHTC	21, 9e,7b, 8a, 6, 11, 30, 36, 24,31,38 /5, 9g, 9b, 17, 9a,9d, 10,Tmp, McN	3	1.1
23	PKTTF	21,11,24,31/5, 9e,7b, 6, 8a,9g, 9b,36, 30,10,17,9a,9d, Tmp,38,McN	3	1.1
24	PTCTF	21,36,9b,30,24,31/5,9e,7b,11,6, 8A,9g, 17,9a,9d,10,Tmp,38,McN	2	0.7
25	PCKTF	21, 8a, 11,36, 24,31/5, 9e,7b, 6, 9g, 9b,30,17, 9a,9d, 10, Tmp, 38,McN	2	0.7
26	PCKRP	21, 6, 11,8a,36, 10, 31/5, 9e, 7b, 9g, 17, 9b, , 30,9a,9d, Tmp,24, 38,McN	2	0.7
27	MRKTF	21, 9e,8a,24, 36, 31/ 5, 7b, 11,6, 9g, 9b, 30,17,9a,9d, 10, Tmp,38,McN	2	0.7
28	PKPTC	21, 11, 9b,24, 31 , 38 /5,9e,7b,6, 8a, 9g, 10, 17, 9a,9d, 36, 30, Tmp,McN	2	0.7
29	PTCSC	21,36,9b,30,24, Tmp,,31,38/5,9e,7b,11,6, 8A,9g, 17,9a,9d,10, McN	2	0.7
30	PHTQF	21,11,8a, 10,Tmp,24,31/5,9e,7b, 6, 9g, 36, 30, 9b,17,9a,9d, 38,McN	2	0.7
31	PCPSF	21, 11,6, 9b, 8a, , 24, Tmp, 31 /5, 9e, 7b, 9g, 36,30,17,9a,9d, 10, 38, McN	2	0.7
32	PTFTF	21, 36,9b, 24,31 /5,9e,7b,11,6, 8a,9g, 30,17,9a,9d,10,Tmp, 38,McN	2	0.7
33	PRCTP	21, 8a, 36,9b, 31/5,9e,7b, 11,6, 9g, 30,17,9a,9d,10,Tmp, 24,38,McN	2	0.7
34	LRCQF	21,9e,7b,8a,36,9b,30,10, Tmp,24,31/ 5, 11, 6,9g, 17, 9a,9d, 36, 38,McN	2	0.7
35	PRFTC	21, 8a, 36,9b, 24,31,38,/5,9e,7b, 11,6, 9g, 30,17,9a,9d,10,Tmp, McN	2	0.7
36	SRCRC	7b, 9b, 10, 36,30,24,31,38,/ 5, 21, 9e, 8a, 11,6, 9g, 17,9a,9d, Tmp, McN	1	0.6
37	PHCQF	21,11,8a, 36, 30, 9b,10,Tmp, 24,31/ 5,9e,7b, 6, 9g, 17,9a,9d, 38,McN	1	0.6
38	PRCTM	21,36,9b,30, 38,31/5,9e,7b,11,6, 9g, 17,9a,9d,10,Tmp, 24, McN	1	0.3
39	PCCJC	21, 8a, 11,6, 9a, 10,36,9b,30,24,31 /5,9e,7b, 9g, 17, 9d, Tmp,38,McN	1	0.3
40	PCKRF	21, 11, 6, 8a, 10, 36, 24, 31/5, 9e,7b, 9g, 9b, 30,17, 9a,9d, Tmp, 38,McN	1	0.3
41	NTCSF	21, 7b,36, 30,9b, Tmp,24,31 /5,9e, 11,6, 8a,9g, 17,9a,9d,10, 38,McN	1	0.3
42	MHKSF	21, 9e,8a, Tmp,24, 36, 31/ 5, 7b, 11,6, 9g, 9b, 30,17,9a,9d, 10, 38,McN	1	0.3
43	PCPTF	21, 11,6, 9b, 8a, , 24, 31/5,9e,7b, 9g, 36,30, , 17,10, Tmp,9a,9d, 38,McN	1	0.3

44	PRFTF	21,8a, 36,30,11,6, 9b, 10,24, 31,38,/ 5,9e,7b, 9g, 17,9a,9d, McN	1	0.3
45	PRTQF	21,8a, 10,Tmp, 24, 31/ 5,9e,7b,21, 11, 6, 9g, 36,9b,30,17,9a,9d, 38,McN	1	0.3
46	PRCQC	9b, 8a, 30, 36, 10, Tmp , 24, 31, 38 /9e, 5,21, 7b, 11, 6,9g, 17, 9a,9d, McN	1	0.3
47	TRTTF	8a9d, 24, 31 / 5,21,9e, 7b, 11,6, 9g, 9b, 36,30, 17,9a, 10, Tmp, 38,McN	1	0.3
48	TKKTF	11,36, 24, 31, 38,/ 5,9e,7b,21, 6, 8a, 9g, ,9b,30,17,9a,9d, Tmp ,10, McN	1	0.3
	Total isolates no.		276	

Ten races (PCHTP, PRCTF, PRCQP, PKFTC, TKTTF, TRCQP, PRCQF, PRCTC, PRNQF and TRTQF) with different incidence (1.4-13.7%) were recorded in all three years. Races PRCTF and PRCQP were dominated in 2012-2013 and race PKFTC was the most common in 2014. Incidence of race PKFTC has increased from 6.2% in 2012 to 20.7% in 2014. Frequency of race PRCTF has decreased from 16.6% to 9.7% and of race PCHTP from 11.4% to 4.9%. The percentage of the rest races has remained about the same (Table 2). Race PKFTC was present throughout most of the area except Shida Kartli and Meskheti and its frequency varied between 11-19%. PRCTF, PCHTP and PRCQP were the most common races in Shida Kartli and Meskheti areas, respectively. However, race PCHTP was recorded only in Shida cartli area. Dominant races consist of 11-13 virulence genes. Pathotypes with low frequency (4%) consisted of 16-17 virulence genes (Table 3). The diversity index varied from 0.14 to 0.20 within geographic area. We suppose that differences in virulence frequency between the areas may have been due to differences in the sampling years, not to differences in geographical locations.

Table 2. Frequency of prevalent races and pathotype of *Puccinia graminis* in Georgia in 2012-2014

Race	Frequency of isolates of prevalent Pgt race, %			
	Years			
	2012	2013	2014	Mean
PRCTF	16.6	15.3	9.7	13.7
PRCQP	14.6	14.3	10.9	13.3
PCHTP	11.4	4.1	4.9	6.8
PKFTC	6.2	16.3	20.7	14.4
PRCTC	2.1	2.0	6.0	3.4
TRCQP	4.1	2.0	3.6	3.2
PRCQF	3.1	4.1	2.4	3.2
TKTTF	2.0	1.0	3.6	2.2
PRMQF	2.0	1.0	1.2	1.4
TRTQF	1.0	2.0	1.2	1.4

Table 3. Frequency (%) of *Puccinia graminis* prevalent races occurred in different geographic areas of Georgia in 2012-2014

Prevalent races	Sampling area				
	Meskheti	Shida Kartli	Javakheti	Kvemo Kartli	Kakheti
PRCQP	26.6	12.0	0	0	0
PRCTF	16.0	19.0	0	0	0
PHCTF	9.6	0	0	0	0
TKTTF	8.5	0	8.3	0	0
PHHTF	7.4	0	0	0	0
PCHTP	0	20.0	0	0	0
TRCQP	0	7.0	0	0	0
PRCQF	0	5.0	0	0	0
PKFTC	0	0	42.8	41.0	52.0
PKPTC	0	0	0	35.0	0
PKTSF	0	0	0	0	15.0
PKTTF	0	0	8.3	0	0
TKFTC	0	0	24.4	0	0
Isolates (No)	94	150	49	19	38
Hg	0.20	0.16	0.14	0.21	0.16

Very high frequency (51.1-100 %) of isolates virulent to Sr5, Sr6, Sr7b, Sr9a, Sr9e, Sr9d, Sr9g, Sr10, Sr11, Sr17, Sr38, SrTmp and SrMcN was recorded. Virulence on lines with genes Sr9d, Sr24, Sr8a, and Sr30 varied between 20.7-40.9%. 13.0% and 14.5% of the isolates were virulent on Sr21 and Sr36, respectively. Virulence to line with Sr31 has not been found. The factor of virulence (Fv) that indicates the average number of virulence genes in the population was 13.0. While comparing the virulence structure of uredinial populations of stem rust distributed in Georgia during 2012-2014, few difference was found in their virulence frequencies by years. Namely, virulence has increased on Sr9b from 13.5 % in 2012 to 31.7 % in 2014, on Sr 30 from 19.8% to 60.9% and on Sr36 from10.4% to 23.2% (Table 4).

Table 4. Frequency of virulence of *Puccinia graminis* isolates on differential lines with single resistance genes in Georgia in 2012-2014

Virulence	2012		2013		2014		Total	
	Number of isolates	%	Number of isolates	%	Number of isolates	%	Number of isolates	%
5	96	100	98	100	82	100	276	100
6	69	71.9	90	91.8	69	84.1	228	82.6
7b	90	93.8	95	97	82	100	267	96.7
8a	40	41.7	28	28.6	40	42.8	108	39.1
9a	95	98.9	97	99	82	100	274	99.3
9b	13	13.5	18	18.4	26	31.7	57	20.7
9e	88	91.7	83	85	81	98.8	252	91.3
9d	96	100	97	99	82	100	275	99.6
9g	96	100	98	100	82	100	276	100
10	62	64.6	64	65.3	62	75.6	188	68.1
11	31	32.3	83	85	27	32.9	141	51.1
17	96	100	98	100	82	100	276	100
21	14	14.6	10	10.2	12	14.6	36	13.0
24	36	37.5	27	27.6	21	25.6	59	21.3
30	19	19.8	44	44.9	50	60.9	113	40.9
31	0	0	0	0	0	0	0	0
36	10	10.4	11	11.2	19	23.2	40	14.5
38	74	77.1	68	69	57	69.5	199	72.1
TMP	55	57.3	58	59	55	67.1	168	60.9
MCN	96	100	98	100	82	100	276	100

In 1999 new stem rust race Ug99 was discovered in Uganda [6]. This race destroyed most previously disease resistant wheat varieties with genes Sr24, Sr25, Sr26, Sr27 and Sr31 in other countries [10]. According to our previous [11] and present studies the virulence to lines with genes Sr25, Sr26, Sr27 and Sr31 has not yet been detected in Georgia but virulence to Sr24 was found in 2013-2014. The race structure of stem rust Georgian population differed from populations of other countries (Singh et al. 2015).

IV. CONCLUSION

Thus, population of wheat stem rust widespread in Georgia in 2012-2014 was highly virulent as it consists of virulence to nineteen resistance genes out of twenty analyzed genes. So far, considerable changes in the populations from year to year have not been detected. Gleason index of population diversity was between 0.28 - 0.32. On the basis of the virulence of pathogen, there was no significant difference in virulence genes and races distribution between populations occurred in the different geographic areas (Shida Kartli, Kvemo Qartli, Kakheti, Meskheti and Djavakheti). Population of *P. graminis* distributed in Georgia is much differed with populations from other countries by virulence structure.

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