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THE VIRULENCE SPECTRUM OF *PUCCINIA GRAMINIS TRITICI* FROM DIFFERENT *BERBERIS* SPECIES

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ABSTRACT. The paper presents the virulence spectrum of the *Puccinia graminis tritici* populations from *Berberis* spp. Pathotype BBB was the most widespread (50%) whereas the rest of the pathotypes were present at considerably lower level (4,55 – 18,19%). Twelve virulence formulas were identified and the highest frequency was recorded with the virulence genes V5, V7b and V6 (45,46%). Resistance genes Sr36, Sr31 and Sr33 were 100% effective.

INTRODUCTION

Wheat, barley and a certain number of the *Poaceae* family species serve as primary and additional hosts of the causal agent of wheat stem rust (*P. graminis tritici*). The completion of the parasite's development cycle requires the presence of an intermediate host *Berberis* spp. (barberry). As early as 1920 Craigie (1931) experimentally proved the parasite hybridization on *Berberis* spp., later to be confirmed by Stakman (1944) and Newton *et al*. (1930). Convincing data on the abundance of the physiological races formed on *Barbaris* spp. were given by Johnson and Green (1957). They identified seventeen physiologic races from ten samples collected from *Berberis* spp.

The subject matter of this investigation as well was the role of *Berberis* spp. in the life cycle of the fungus and the virulence spectrum of the pathotypes produced.

Berberis sp. is important mostly from the point of view of the parasite hybridization taking place on it and resulting in the evolution of new fungus pathotypes that may disturb the existing balance in the parasite-plant system. The goal of these investigations was therefore to study the pathotypes of *P. graminis tritici* derived from *Berberis* spp. and determine their virulence.

MATERIALS AND METHODS

The investigations were carried out at the Small Grains Research Centre in Kragujevac over 1991 and 1992. The aecidiospore samples were collected from 24 species of *Berberis* spp. from the Kragujevac collection (Tab. 3). The aecidia formation on the shrubs of *Berberis*

spp. was observed and samples were collected at the time of their full development. Every seven days during the months of May and June, the sampling of *Berberis* spp. leaves with aecidia was performed, the collected samples being the average ones, considering the highly variable number of aecidia on the leaves. For that reason, the samples were taken from different sides of and heights on the *Berberis* spp. shrubs. One hundred leaf samples were collected from each species of *Berberis* spp. The leaves were dried at room temperature and then kept in refrigerator at +4°C till the beginning of the examination.

During the aecidia sampling, observation was also made on the following morphological traits in different *Berberis* species (*B. ilicifolia, B. gilgiana, B. francisci ferdinandi, B. Fischeri, B. chinensis, B. candidula, B. bidentata, B. atropurpurea, B. aridocalida, B. amurensis, B. angulosa, B. aquifolium, B. purpurea, B. vulgaris* var. *atropurpurea, B. vulgaris, B. virescens, B. vernae, B. thunbergii, B. provincialis* var. *serrata, B. oblonga, B. mutabilis, B. lycium, B. latiflora* var. *oblanceolata, B. cunawurensis*): the full flowering time, the number of inflorescence on the 30-cm-long shoot, the number of flowers per inflorescence as well as the number of leaves per nodus. The thorn length (mm) was measured and thorn number on the 30-cm-long shoot was identified. The average of ten leaves of *Berberis* spp. was taken and the average leaf length and breadth expressed in cm were measured. The shrub development was estimated visually using the following attributes: stunted, moderately vigorous, vigorous and very vigorous.

The artificial wheat inoculation was carried out during winter in the greenhouse using the isogenic wheat line seedlings. The isogenic line multiplication and inoculation method was described in detail by Kostić (1962) and is therefore not presented here. One day prior to the inoculation the leaves with aecidia were placed into Petri dishes containing wet filter paper for the purpose of fructification or releasing as many aecidiospores as possible. The susceptible wheat cultivar Little Club plants were inoculated by the aecidiospores. When the parasite on the susceptible cultivar had been fully developed, individual pustules were taken and multiplied. Pure parasite cultures used for the later inoculation of the isogenic wheat lines were obtained in this way. Twenty grains of each isogenic line were planted in flowerpots. During the first leaf full development stage, their inoculation was performed. 10-12 days later, the plant reaction was estimated by determining the infection types 0-4 (Stakman *et al.,* 1962).

Based upon the results obtained by determining the types of the infection, the pathotypes and virulence spectrum of *P. graminis tritici* were identified.

The pathotype identification was done using Sr lines classified into three groups (Roelfs and Mc Vey, 1974). The lines containing the genes Sr5, Sr9d, Sr9e and Sr7b belong to the first group, those with the genes Sr11, Sr6, Sr8 and Sr9a and Sr36, Sr9b, Sr13 and Sr10 belong to the second and third groups, respectively. Based upon the reaction of the plants of the isogenic lines determined by the infection types 0-4, marks were established in the given group (B, C, D, F, G, H, J, K, L, M, N, P, Q, R, S and T). The pathotypes were marked collectively for all three isogenic line groups (RHT, BBB, RMN, etc.).

The isogenic wheat lines with known Sr resistance genes were used for determining the virulence formulas *P. graminis tritici* (Tab. 1).

The isogenic line plants can react in two ways: by resistant (R) types of infection (0-2) and susceptible (S) types of infection (3-4) reactions. Parasite can be virulent (V) or avirulent (A) to the isogenic lines of wheat. Based upon these interactions, the virulence formulas (V/A) as well as virulence groups, being formulas for each isolate studied, were obtained (Green, 1981).

Table. 1. Isogenic wheat lines for *P. graminis tritici* pathotypes determination

Sr genes	Genome location	Origin		Author
		cultivar	source	
5	6D/6DS	Reliance C. I. 7370	*T. aestivum*	Ausemus *et al.*, 1946
6	2D/2DS	Kenya 58	*T. aestivum*	Knott and Anderson, 1956
7b	4A/4AL	Marquis C. I. 3641 and Kota C. I. 5878	*T. aestivum*	Loegering and Sears, 1966
8a	6A/6AS	Red Egyptian	*T. aestivum*	Knott and Anderson, 1956
9b	2B/2BL	Kenya	*T. turgidum*	Green *et al.*, 1960
9e	2B/2BL	Vernal	*T. turgidum* var. *durum*	McIntosh and Luig, 1973
9g	2B/2BL	Chinese Spring	*T. turgidum* var. *durum*	McIntoshand Luig,1973
11	6B/6BL	Lee	*T. turgidum* var. *dicoccum*	Knott and Anderson, 1956
13	6A/6AL	Khapli C. I. 4013	*T. turgidum* var. *dicoccum*	Knott, 1962b
17	7B/7BL	Yaroslav	*T. monococcum*	McIntosh, 1988
21	2A/2AL	Eincorn C. I. 2433	*T. monococcum*	The, 1973
22	7A/7AL	R. L. 5244	*Th. ponticum*	The, 1973
24	3D/3DL	Amigo	*Th. ponticum*	McIntosh *et al.*, 1976
25	7D/7DL	Oasis F 86	*Th. ponticum*	McIntosh *et al.*, 1976
26	6A/6AL	Avocet	*Th. ponticum*	McIntosh *et al.*, 1976
27	3A	Imperial	*S. cereale*	McIntosh, 1988a
29	6DL/6DS	Hela	*T. aestivum*	Dyck and Kerber, 1977
30	5DL	Webster	*T. aestivum*	Knott and McIntosh, 1978
31	1BS (1BL. 1RS)	Petkus	*S. cereale*	McIntosh, 1988
32	2A/2B	W3531	*T. speltoides*	McIntosh, 1988
33	1Dl (1DS)	R. L. 5288	*T. tauschii*	McIntosh, 1988
36	2B/2BS	C. I. 12633	*T. timopheevii*	McIntosh, 1988
37	4B	W 3563	*T. timopheevii*	McIntosh, 1988

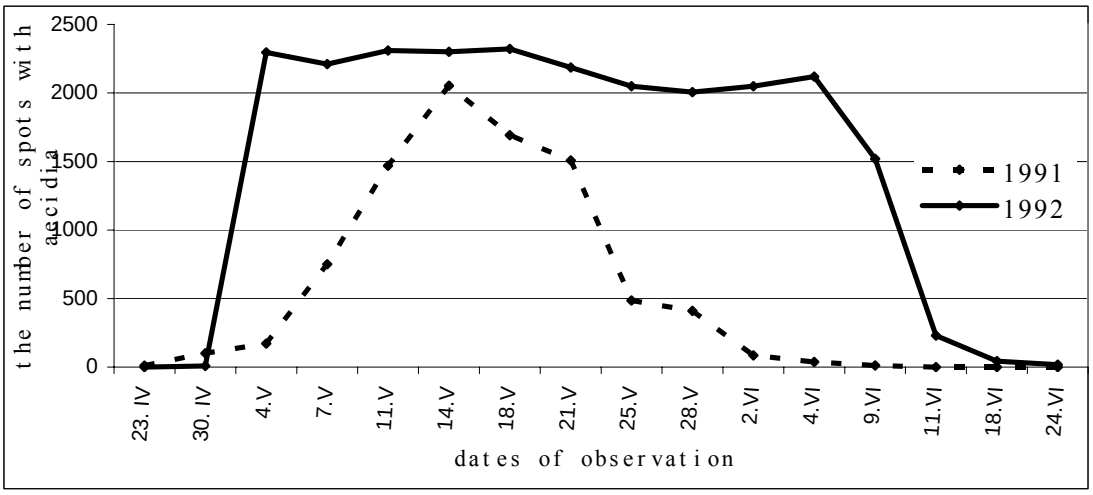
RESULTS AND DISCUSSION

During ten observations, from April 23 to June 24, 1991, a total of 94 aecidiospore samples was collected. In 1992, during seven observations, from April 23 to June 9, 64 samples were collected. Although *Berberis* spp. is not a necessary precondition for the occurrence and spreading of wheat rust, the intensity of the disease incidence is known to be higher in the presence of this intermediate host. The role of *Berberis* spp. in the *P. graminis tritici* epidemiology is primarily connected with the development and occurrence of new parasite pathotypes, due to which 158 samples were examined during 1991 and 1992.

The onset of the *Berberis* spp. leaving in the conditions of Sumadija is in the first days of April. With this in mind, the observations in both years mentioned started in the second

half of April, which is in accordance with the results of Kostić *et al*. (1970). In this period, the leaf size is such that permits infection by basidiospores. In the course of the first observation (April 23) in 1991, only traces of spermatia were identified, the number of which increased rapidly by the time of the second observation. It was only in the course of the third observation (May 7) that the first aecidiae were identified. It is noteworthy that the first spermatia in the second study year were also identified on April 23. Seven days later, the first aecidia occurred on the leaves. These investigations confirmed the previous findings on the volume and time of aecidia formation in our climatic conditions (Kostić, 1960).

Graph 1. The rhythm of aecidia formation on *Berberis* during 1991 and 1992



The Graph 1. presents the course of aecidia formation and their number by study years. The maximum total number of spots with aecidia on the leaf was recorded on May 14, 1991 and on May 18, 1992, being 2053 and 2322, respectively. From the mentioned data it could be concluded that the time difference in the aecidia occurrence between the years was not high, being only three days.

The termination of aecidia formation takes place at different periods of time. In 1991 it was on June 18 and in 1992 on June 2. Aecidia formation in 1991 and 1992 lasted 61 and 46 days, respectively.

The aecidia formation rhythm differed between the years. More intense aecidia formation was registered in 1991 characterized by fluctuating dynamics but in the following year the aecidia formation was somewhat less intense having no sudden rises.

Aecidia formation was greatly affected by climatic conditions, which is presented in Table 2.

Table. 2. Meteorological data for Kragujevac and its surroundings

Meteorological elements	Year	April			May			June		
		Decade								
		I	II	III	I	II	III	I	II	III
Mean temperature °C	1991	10.0	9.8	9.3	14.0	12.0	11.9	18.0	22.6	20.1
	1992	12.2	8.2	15.2	15.6	15.0	15.7	18.8	18.4	19.4
Precipitation in mm	1991	15.7	10.0	33.7	19.3	30.7	19.5	20.6	1.5	2.0
	1992	13.9	38.0	36.1	0.0	15.0	34.7	39.3	29.4	57.9
Number of days with rain	1991	3	6	5	4	4	5	6	1	3
	1992	4	5	4	1	3	5	6	8	7
Number of days with dew	1991	1	4	5	6	2	1	1	3	1
	1992	1	3	6	7	5	5	1	3	4
Relative air humidity	1991	68	72	76	68	81	77	74	62	65
	1992	59	70	60	59	66	72	74	78	76

Data on the presence of the pathotypes in 1991 are not shown because no aecidiospores infection of the sensitive wheat cultivar was registered, the primary reason for that being unfavourable climatic conditions during the sampling period. The incessant rain caused washing off and decay of the aecidiospores (Tab. 2). Optimal temperature for teleutospore and basidiospore germination ranged from 20-22°C and from 15-20°C, respectively (Vennot-Bourgin, 1949). Humidity necessary for spore germination was positively affected by evenly distributed precipitations as well as by dew.

Although the aecidia were present almost more than two months, the aecidiospores did not exert almost any effect on the occurrence of *Puccinia graminis tritici* on wheat in the observation region. The reason for this should be sought in low virulence of the pathotypes obtained from *Berberis* spp. as well as in unfavourable conditions for the development of the infection. Early infection and numerous uredopustules occurred on some *Poaceae* species and particularly on *Agropyron repens* growing under or by the shrubs of *Berberis* spp.

All *Berberis* spp. species are characterized by uniform flowering onset time. In all species it started around April 7 on average. The number of flowers per shrub on the 30-cm-long shoot highly varied ranging from the absence of inflorescence (*B. aridocalida, B. oblonga* and *B. cunawurensis*) to 23 inflorescences (*B. vernae).* Besides having the highest number of inflorescences, the species *B. vernae* had also the highest number of flowers per inflorescence, being 24 on average. The number of leaves per nodus was rather uniform ranging from 7 to 11. In the species *B. francisci ferdinandi* leaving was not recorded. The average leaf length was greatest in *B. chinensis* species (4, 29cm) and smallest in *B. aridocalida* (1, 65 cm). *B. aridocalida* species had also the smallest average leaf breadth (0, 59 cm), the highest being recorded in *B. candidula* species (2, 12 cm). The vigour of shrubs of the *Berberis* spp. species ranged from very vigorous (*B. Fischeri, B. amurensis, B. angulosa, B. vulgaris var. atropurpurea* and *B. vernae*) to stunted species (*B. francisci ferdinandi, B. atropurpurea, B. aridocalida, B. aquifolium* and *B. purpurea)* (Tab. 3).

Table. 3. Morphological traits of the *Berberis* species

Berberis spp.	A*	B	C	D	E	F	G	H	I
B. amurensis	7. V	2	13	9	17	20	3.70	1.79	very vigorous
B. angulosa	7. V	8	14	11	17	27	3.55	1.49	very vigorous
B. aridocalida	–	–	–	7	59	17	1.65	0.59	stunted
B. atropurpurea	7. V	19	17	8	25	8	2.63	1.14	stunted
B. aquifolium	14. V	1	13	9	53	24	2.24	1.40	stunted
B. bidentata	7. V	15	18	9	42	7	3.38	1.48	moderately vigorous
B. candidula	7. V	7	18	9	36	15	3,91	2,12	moderately vigorous
B. chinensis	7. V	13	15	9	47	23	4,29	1,98	vigorous
B. cunawurensis	–	–	–	9	19	11	3.24	1.44	moderately vigorous
B. Fischeri	7. V	3	15	9	35	23	2,85	1,44	very vigorous
B. francisci ferdinandi	-	2	15	9	38	10	-	-	stunted
B. gilgiana	14. V	7	11	7	18	20	3,92	1,64	moderately vigorous
B. ilicifolia	7.V	10	13	9	39	15	3,35	1,77	vigorous
B. latiflora var. oblanceolata	7. V	3	16	8	12	5	4.12	1.95	moderately vigorous
B. lycium	7. V	14	15	10	24	18	2.51	1.01	moderately vigorous
B. mutabilis	7. V	15	17	7	18	10	2.90	1.42	vigorous
B. oblonga	–	–	–	7	35	20	2.92	1.17	moderately vigorous
B. provincialis var. serrata	7. V	11	14	9	20	20	3.34	1.27	vigorous
B. purpurea	14. V	15	17	11	22	14	2,66	1,14	stunted
B. thunbergii	30.IV	11	9	11	18	11	2,60	1,37	moderately vigorous
B. vernae	7. V	23	24	11	14	17	4,24	1,75	very vigorous
B. virescens	7. V	9	15	8	32	19	3,28	1,96	moderately vigorous
B. vulgaris	7. V	4	14	8	28	28	3,73	2,03	vigorous
B. vulgaris var. atropurpurea	7. V	6	18	10	15	13	3,44	1,77	very vigorous

(*A-flowering time; B-the number of inflorescences per shrub on the 30-cm-long shoot; C-number of flowers per inflorescence; D-the number of leaves per nodus; E-the number of thorns on the 30 cm-long shoot; F-thorn length in mm; G-average leaf length in cm; H-average leaf breadth in cm; I- shrub development)

In 1992, in the population of the *P. graminis tritici* fungus from *Berberis* spp. nine pathotypes were identified, namely: BBB, RRK, RGD, RTH, RQB, CBB, MQB, NHC and PHB. The dominant pathotype was pathotype BBB with eleven isolates or 50%, followed by RKK with four isolates or 18.19%. The rest of the pathotypes were present with one isolate each or 4.55% (Tab. 4). There are very few literature data on the presence of the pathotypes of the fungus on *Berberis* spp. Stojanovic *et al.* (1994) investigated 98 isolates from *Berberis* spp. in a three-year period and identified 21 pathotypes. The pathotype BBB predominated (44.90%), which is in accordance with the results of this study.

Table. 4. The incidence of *P. graminis tritici* pathotypes obtained from *Berberis* spp. in 1992

Pathotype	Number of isolates	%
BBB	11	50.00
RRK	4	18.19
RGD	1	4.55
RTH	1	4.55
RQB	1	4.55
CBB	1	4.55
MQB	1	4.55
NHC	1	4.55
PHB	1	4.55
		Number of isolates: 22

In 1992 by the analysis of 22 isolates of *P. graminis tritici* from *Berberis* spp., twelve virulence formulas were detected. The most widespread was the pathotype with the virulence formula (V/A): / 5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17, 13, 22, 24, 25, 26, 27, 29, 31, 32, 33, 37 (50%) that did not contain virulence alleles for the Sr wheat resistance genes studied. The rest of the pathotypes were represented by one isolate each or 4.55%. The number of virulence genes in them ranged from 1 to 15 (Tab. 5).

Table. 5. Virulence formulas of *P. graminis tritici* from *Berberis* spp. in 1992

Pathotype	Virulence formula (V/A)	Number of isolate	%	V/A
BBB	/ 5 21 9e 7b 11 6 8a 9g 36 9b 30 17 13 22 24 25 26 27 29	11	50.00	0:23
RRK	5 21 7b 11 6 9g 9b 30 17 13 22 25 27 29 37 / 9e 8a 36 24	1	4.55	15:8
	5 21 7b 11 6 9g 9b 30 17 13 22 27 32 37 / 9e 8a 36 24 25	1	4.55	14:9
	5 21 7b 11 6 9g 9b 30 17 13 22 27 29 37 / 9e 8a 36 24 25	1	4.55	14:9
	5 21 7b 11 6 9g 9b 30 17 13 22 / 9e 8a 36 24 25 26 27 29	1	4.55	11:12
RGD	5 21 7b 6 30 13 22 27 / 9e 11 8a 9g 36 9b 17 24 25 26 29	1	4.55	8:15
RTH	5 21 7b 11 6 8a 9g 9b 17 13 22 24 27 29 / 9e 36 30 25 26	1	4.55	14:9
RQB	5 21 7b 11 6 13 22 26 27 / 9e 8a 9g 36 9b 30 17 24 25 29	1	4.55	9:14
CBB	7b / 5 21 9e 11 6 8a 9g 36 9b 30 17 13 22 24 25 26 27 29	1	4.55	1:22
MQB	5 7b 11 6 13 22 27 / 21 9e 8a 9g 36 9b 30 17 24 25 26 29	1	4.55	7:16
NHC	5 9e 6 9g 17 37 / 21 7b 11 8a 36 9b 30 13 22 24 25 26 27	1	4.55	6:17
PHB	5 9e 7b 6 9g 22 25 27 / 21 11 8a 36 9b 30 17 13 24 26 29	1	4.55	8:15
			Total: 107 : 169	

From the data in Table 6. it could be seen that the highest frequency in the population of parasite *P. graminis tritici* in 1992 was registered with virulence genes V5, V7b and V6 (45.46%) and the lowest one with the genes V36, V31 and V33 (0%). The frequency of other genes ranged from 4.55% to 40.91%.

Table. 6. The effectiveness of Sr genes against isolates of *P. graminis tritici* from *Berberis* spp. in 1992

Sr genes	R		S	
	Number of isolates	%	Number of isolates	%
5	12	54.55	10	45.46
6	12	54,55	10	45,46
7b	12	54,55	10	45,46
8a	21	95,46	1	4,55
9b	17	77,28	5	22,73
9e	21	95,46	1	4,55
9g	15	68,19	7	31,82
11	15	68,19	7	31,82
13	14	63,64	8	36,37
17	16	72,73	6	27,28
21	15	68,19	7	31,82
22	13	59,09	9	40,91
24	21	95,46	1	4,55
25	20	90,91	2	9,09
26	21	95,46	1	4,55
27	14	63,64	8	36,37
29	19	86,37	3	13.64
30	17	77,28	5	22,73
31	22	100,00	0	0,00
32	21	95.46	1	4.55
33	22	100.00	0	0.00
36	22	100,00	0	0,00
37	18	81.82	4	18.19

The Sr gene effectiveness against the isolates of *P. graminis tritici* from *Berberis* spp. in 1992 is shown in table 6. The most effective genes were the genes Sr36, Sr31 and Sr33 which did not have virulence alleles in the parasite population, followed by genes Sr9e, Sr8a, Sr24, Sr26 and Sr32, their effectiveness being 95.46%. The rest of the genes were more or less ineffective.

Although *Berberis* spp. is not a necessary precondition for the rust incidence and spreading, the intensity of the disease is known to be higher in the presence of this intermediate host. Relatively low variability of *P. graminis tritici* is caused by low presence in nature of the intermediate host *Berberis* spp. where the sexual stage of the life cycle of the fungus takes place. Barberry destruction was performed with the aim of preventing the hybridization of races and therefore the virulence gene recombination. However, the spreading of the disease and incidence of novel pathotypes have not been stopped.

CONCLUSIONS

Based upon the results obtained of the study of the *P. graminis tritici* population structure in accordance with the research goal, the following conclusions may be drawn:

- The maximum total number of spots with aecidia on the leaf was recorded on May 14, 1991 and on May 18, 1992, being 2053 and 2322, respectively. The aecidia formation rhythm differed between the years. More intense aecidia formation was registered in 1991

characterized by fluctuating dynamics but in the following year the aecidia formation was somewhat less intense having no sudden rises.

- Data on the presence of the pathotypes in 1991 are not shown because no aecidiospores infection of the sensitive wheat cultivar was registered, the primary reason for that being unfavourable climatic conditions during the sampling period.

- In 1992, in the population of the *P. graminis tritici* from *Berberis* spp. nine pathotypes were identified, namely: BBB, RRK, RGD, RTH, RQB, CBB, MQB, NHC and PHB. The dominant pathotype was pathotype BBB with eleven isolates or 50%, followed by RKK with four isolates or 18.19%. The rest of the pathotypes were present with one isolate each or 4.55%.

- In 1992 by the analysis of 22 isolates of *P. graminis tritici* from *Berberis* spp., twelve virulence formulas were detected. The most widespread was the pathotype with the virulence formula (V/A): / 5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17, 13, 22, 24, 25, 26, 27, 29, 31, 32, 33, 37 (50%) that did not contain virulence alleles for the Sr wheat resistance genes studied. The rest of the pathotypes were represented by one isolate each or 4.55%. The number of virulence genes in them ranged from 1 to 15.

- The highest frequency in the population of the parasite in 1992 was registered with virulence genes V5, V7b and V6 (45.46%) and the lowest one with the genes V36, V31 and V33 (0%). The frequency of other genes ranged from 4.55% to 40.91%.

- In the same year, the most effective genes were the genes Sr36, Sr31 and Sr33 which did not have virulence alleles in the parasite population, followed by genes Sr9e, Sr8a, Sr24, Sr26 and Sr32, their effectiveness being 95.46%. The rest of the genes were more or less ineffective.

-Although *Berberis* spp. is not a necessary precondition for the rust incidence and spreading, the intensity of the disease is known to be higher in the presence of this intermediate host.

References

[1] CRAIGIE, J. H. (1931): An experimental investigation of sex in the rust fungi. Phytopathology 11: 1001-1040.

[2] GREEN, G. J. (1981): Identification of physiologic races of *Puccinia graminis* f. sp. *tritici* in Canada. Can. I. Plant Pathol. 3: 33-39.

[3] JOHNSON, T., GREEN, G. J. (1957): Physiologic specialization of wheat stem rust in Canada, 1919 to 1955. Can. Journ. of Plant Sci., 37.

[4] KOSTIĆ, B. (1960): Stvaranje ecidija *Puccinia graminis* (Pers.) Erikss. et Henn. na *Berberis vulgaris* L. i njihov epidemiološki značaj. Zaštita bilja 62: 117-130.

[5] KOSTIĆ, B. (1962): Fiziološke rase *Puccinia graminis tritici* Erikss. et Henn. u jugoistočnom delu FNRJ. Zaštita bilja 69-70: 1-81.

[6] KOSTIĆ, B., JOKSIMOVIĆ, S., STOJANOVIĆ, S. (1970): Uloga šimširike (*Berberis vulgaris* L.) u stvaranju primarnog inokuluma *P. graminis* Pers. Zaštita bilja 108: 113-118.

[7] NEWTON, M., JOHNSON, T., BROWN, A. M. (1930): A study of the inheritance of spore colour and pathogenicity in crosses between physiologic forms of *Puccinia graminis tritici* . Scien. Agricul.: 775-798.

[8] ROELFS, A. P., MC VEY, D. V. (1974): Races of *Puccinia* f. sp. *tritici* in the U. S. A. during 1973. Plant Dis. Rep., 58: 608-611.

[9] STAKMAN, E. C., LEVINE, M. N., LOEGERING, W. Q. (1944): Identification of physiologyc races of *Puccinia graminis tritici.* U. S. Dept. Agr., Res. Admn. Bur. Ent. Pl. Quar., E- 617.

[10] STAKMAN, E. C., STEWART, D. M., LOEGERING, W. Q. (1962): Identification of physiological races of *Puccinia graminis* var. *tritici.* U. S. Agric. Res. Serv., ARS E 617: 1-53.

[11] STOJANOVIĆ, S., STOJANOVIĆ, J., JEVTIĆ, R., JERKOVIĆ, Z. (1994): Pathotypes of the wheat stem rust in Serbia. IV Simposium on Plant Immunity to Diseases and Pest. Plant Science, vol. 31, 7-10: 255-257, Sofia.

[12] VENNOT-BOURGIN, G. (1949): Les champingnons parasites des plantes cultivees. Tome II, Paris.