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VIRULENCE OF THE *ERYSIPHNE GRAMINIS DC. EX MERAT F.SP. TRITICI Em. MARCHAL* GENOTYPES PROLIFERATED BY SEXUAL REPRODUCTION

VIRULENTNOST GENOTIPOVA *ERYSIPHE GRAMINIS DC. EX MERAT F. SP. TRITICI EM. MARCHAL* NASTALIH POLNIM RAZMNOŽAVANJEM

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VIRULENCE OF THE ERYSIPHE GRAMINIS DC. EX MERAT F. SP. TRITICI EM. MARCHAL GENOTYPES PROLIFERATED BY SEXUAL REPRODUCTION*)

The paper deals with the virulence of genotypes which are causal agents of powdery mildew in wheat proliferated by sexual reproduction, in 1988 and 1989. Analysis of 735 isolates showed that dominant genes had virulent formulae 1, 2, 3a, 3b, 5, 6, 8/4a and 1, 2, 3a, 3c, 5, 6, 8/3b, 4a. 45 genotypes were found in 1988 and 83 in 1989. Frequencies of V—3b and V—4a were the lowest. Most genotypes possessed 5 to 8 virulent genes.

Key words: *Erysiphe graminis*, wheat, virulence, genotypes, breeding, Serbia

Introduction

Powdery mildew induced by the fungus *Erysiphe graminis* f. sp. *tritici* is regular and economically significant disease of wheat in our country. It has been spread in all wheat growing regions reducing significantly the grain yield. According to Nikolić (1965), the yield reduction in the commercial wheat fields was as great as 45%. According to our results (Stojanović and Stojanović, 1990), the maximum yield decrease, achieved in average for three varieties which were grown in the conditions of artificial infection and different levels of plants nutrition in pots, was 56.1%. Due to its efficiency, varietal resistance is most effective, safe, and economically feasible control (Powers and Sando, 1960; Kostić *et al.*, 1987).

The successful wheat breeding for resistance to causal agent of powdery mildew is based on the identification of virulence and changes in the pathogne population. In our country, the continual population surveys started in 1961, when physiological races were identified (Smiljaković, 966) and have been continuing up to day (Stojanović, *et al.*, 1990). In early 70s, the conventional investigations of physiological races were improved by introducing the survey of the pathogen population virulence (Kostić

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and Pribaković, 1981, 1985; Stojanović and Ponoš, 1988, 1990) based on the interaction between resistant alleles and virulence according to the 'gene–for–gene' theory (Flor, 1955). Those wheat varieties and genotypes which posses identified genes for resistance were used as differentials in these investigations (Briggle, 1969; McIntosh, 1979).

The breeding for resistance is faced with the problem of the occurrence of new genotypes of parasite to which the resistant varieties may become susceptible. Different systems for resistance employment exist (Bennett, 1984) to preserve it as long as possible and to repress the occurrence of epiphytotic. New parasite genotypes in nature generate by mutations and/or recombinations during the sexual reproduction. The objective of this investigation was to study the virulence of genotypes proliferated by sexual reproduction.

Material and Methods

Cleistothecium samples, collected from different varieties of fully matured wheat at different locations (over 150) in southeast part of the country in 1988 and 1989 were used in the examination. The dry wheat leaves with visible cleistothecia were preserved in a refrigerator at 4°C. The tests were conducted according to the unique methods in the Institute of Small Grains in Kragujevac and in the Instutute of Field and Vegetable Crops in Novi Sad. From all 372 samples which we collected, 735 isolates were tested in order to provide reliable results. Wheat varieties or lines known to have Pm1, Pm2, Pm3a, Pm3b, Pm3c, Pm4a, Pm5, Pm6, and Pm8 genes for resistance were used in the investigation (Table 1).

Tab. 1. – Wheat varieties and lines used to identify specific virulence genes of *Erysiphe graminis tritici*

Sorte i linije pšenice korišćene za identifikaciju specifičnih gena virulentnosti *Erysiphe graminis tritici*

Variety or line Sorta ili linija	CI	Resistance gene Gen otpornosti	Location Lokacija	Origin Poreklo	Corresponding virulence Odgovarajuća virulentnost
Axinster/^{8}Cc	14114	Pm 1	7 AL	*T. aestivum*	V–1
Ulka/^{8}Cc	14118	Pm 2	5 DS	–	V–2
Asosan/^{8}/Cc	14120	Pm 3a	1 AS	*T. aestivum*	V–3a
Chul/^{8}Cc	14121	Pm 3b	1 AS	*T. aestivum*	V–3b
Sonora/^{8}Cc	14122	Pm 3c	1 AS	*T. aestivum*	V–3c
Khapli/^{8}Cc	14123	Pm 4a	2 AL	*T. dicoccum*	V–4a
Hope/^{8}Cc	14124	pm 5	7 BL	*T. dicoccum*	V–5
Mich. Amber/^{8}Cc	14033	Pm 6	2 B	*T. timopheevi*	V–6
Kavkaz	–	Pm 8	1 B	*S. cereale*	V–8

The cultures of fungi were produced by standard method (Nover, 1957) and the benzimidazole method (Wolfe, 1965). We shall briefly present the standard method detailly described by Smiljaković (1966). 50 cleistothecia were separated per each leaf sample and placed on wet filter paper fitted inside the lid of Petri dish. The lid was put on the glass cylinder which was used to

isolate the plants of the susceptible variety Little Clug grown in pot. The filter paper was regularly moistened to facilitate opening of cleistothecia and release of asci and ascospores. The primary colonies of the fungus could be seen on the leaf segments after 8–10 days. The pure monopustular cultures were separated and put on wet filter paper for fructification. Inoculum was reproduced after 24 hours and was used for inoculation of differential-plants which were planted in the boxes according to definite order. The plants were inoculated at the two–leaf stage by shaking the conidia. 8 to 10 days after the inoculation, the seedlings reaction was scored determining the infection type on the scale from 0 to 4 (M a i n s and D i e t z, 1930), where o = immune, 1 = resistant, 2 = moderately resistant, 3 = moderately susceptible, and 4 = susceptible. Infection type of 0–2 indicates a resistant reaction, whereas infection of 3–4 indicates susceptibility.

The method of benzimidazole requires the use of leaf segments, unlike the standard method. Cleistothecia were placed on wet filter paper fitted inside the lid of Petri dish. The leaf segments of the susceptible variety Little Club were placed on the bottom of Petri dish on 4.5 g/l agar containing 0.10–0.15 g/l benzimidazole and kept at 11–15°C, illuminated at low intensity. After the fungus primary colonies developed on the leaf, the individual colonies were transferred into the tubes containing 1–2 cm^3 benzimidazole solution with the concentration of 150 ppm. After inoculum generation, the leaf segments of differential varieties were inoculated in a clear plastic box on water agar containing benzimidazole, surface upwards. The leaf segments were inoculated with the pure cultures of each isolate with the inoculum concentration such as to allow the development of between 20 and 50 colonies per cm^2, such as it was done by B r o w n and W o l f e (1990). The reaction was scored the same way as with the previous method. However, the standard method gave more typical infection types, so it was applied more frequently.

Results and Discussion

According to Tables 2 and 3, it is obvious that numerous *Erysiphe graminis tritici* genotypes are produced by sexual reproduction. The isolates with the common phenotypic virulence were prevailing in both years (1, 2, 3a, 3b, 3c, 5, 6, 8/4a and 1, 2, 3a, 3c, 5, 6, 8/3b, 4a). The similar results were achived by N a m u c o *et al.*, (1987) and

T a b. 2. – Virulence formulae of *Erysiphe granimis tritici* in 1988
Fromule virulentnosti *Erysiphe graminis tritici* u 1988. godini

No culture Br. kulture	Virulence formulae V/A*) Formule virulentnosti V/A	Number of isolates Broj izolata	Number of localities Broj lokaliteta	Frequency (%) Učestalost (%)
1	1,2,3a,3b,3c,4a,5,6	2	2	0,69
2	1,2,3a,3b,3c,5,6/4a	31	19	10,65
3	1,2,3a,3c,4a,5,6/3b	4	3	1,37
4	1,2,3a,3b,3c,4a,6/5	1	1	0,34
5	1,3a,3b,3c,4a,5,6/2	7	4	2,40
6	2,3a,3b,3c,4a,5,6/1	2	2	0,69
7	1,2,3a,3b,3c,6/4a,5	2	1	0,69
8	1,2,3a,3c,5,6/3b,4a	62	28	21,30
9	1,2,3b,3c,5,6/3a,4a	2	2	0,69
10	1,2,3c,4a,5,6/3a,3b	3	2	1,03
11	1,3a,3b,3c,5,6/2,4a	5	3	1,72
12	1,3,a,3c,4a,5,6/2,3b	2	2	0,69

13	2,3a,3b,3c,5,6/1,4a	3	2	1,03
14	2,3a,3c,4a,5,6/1,3b	5	2	1,71
15	3a,3b,3c,4a,5,6/1,2	2	2	0,69
16	1,2,3c,5,6/3a,3b,4a	14	6	4,81
17	1,2,3a,3c,6/3b,4a,5	2	1	0,69
18	1,2,3a,3c,5/3b,4a,6	1	1	0,34
19	1,3a,3b,3c,6/2,4a,5	2	2	0,69
20	1,3a,3b,5,6/2,3c,4a	1	1	0,34
21	1,3a,3c,4a,6/2,3b,5	1	1	0,34
22	1,3a,3c,5,6/2,3b,4a	33	18	11,34
23	1,3b,3c,5,6/2,3a,4a	2	2	0,69
24	1,3c,4a,5,6/2,3a,3b	3	2	1,03
25	2,3a,3c,5,6/1,3b,4a	13	8	4,47
26	2,3b,3c,5,6/1,3a,4a	2	2	0,69
27	2,3b,4a,5,6/1,3a,3c	1	1	0,34
28	2,3c,4a,5,6/1,3a,3b	4	2	1,37
29	3a,3b,3c,5,6/1,2,4a	2	2	0,69
30	3a,3c,4a,5,6/1,2,3b	6	3	2,06
31	1,2,3c,6/3a,3b,4a,5	1	1	0,34
32	1,2,5,6/3a,3b,3c,4a	1	1	0,34
33	1,3a,3c,6/2,3b,4a,5	30	19	10,31
34	1,3a,5,6/2,3b,3c,4a	1	1	0,34
35	1,3c,5,6/2,3a,3b,4a	10	4	3,94
36	1,4a,5,6/2,3a,3b,3c	1	1	0,34
37	2,3c,5,6/1,3a,3b,4a	3	2	1,03
38	2,4a,5,6/1,3a,3b,3c	1	1	0,34
39	3a,3c,5,6/1,2,3b,4a	12	5	4,12
40	3b,3c,5,6/1,2,3a,4a	2	1	0,69
41	3c,4a,5,6/1,2,3a,3b	3	2	1,03
42	1,3c,6/2,3a,3b,4a,5	1	1	0,34
43	1,5,6/2,3a,3b,3c,4a	3	2	1,03
44	2,5,6/1,3a,3b,3c,4a	1	1	0,34
45	3a,3c,6/1,2,3b,4a,5	1	1	0,34
	Total – Ukupno	*291*		*100*

*)Virulent/Avirulent genes (Virulentni/Avirulentni geni)

Stojanović and Ponoš (1990). However, a large number of genotypes were not frequent,having only one or two isolates. Out of 45 genotypes identified in 1988, 25 were not frequent, such as was the case with 56 genotypes out of 83 identified in 1989. However, many of them were strongly virulent, because they had 6–8 virulence genes. Their further existence in the nature is unpredictable, because only 24% of the spores infecting the winter barley were derived from ascospores (Brown and Wolfe, 1990). The survival and reproduction of individual genotypes in nature depends greatly on their fitness and the host–plant. According to Vanderplank (Kostić and Pribaković, 1981), the complex races have the lower fitness which indicates that the most virulence genotypes do not necessary dominate in the population. During the sexual reproduction, tehre comes to the contacts between the cultures of different origin, and characteristics and to the recombination of different virulence alleles. Consequently, the sexual

Tab. 3. — Virulence formulae of *Erysiphe graminis tritici* in 1989
Formule virulentnosti *Erysiphe graminis tritici* u 1989. godini

No culture Broj kulture	Virulence formulae V/A*) Formule virulentnosti V/A	Number of isolates Broj izolata	Number of localities Broj lokaliteta	Frequency (%) Učestalost (%)
1	2	3	4	5
1	1,2,3a,3b,3c,4a,5,6,8	33	19	7,43
2	1,2,3a,3b,3c,4a,5,6/8	2	2	0,45
3	1,2,3a,3b,3c,4a,6,8/5	1	1	0,22
4	1,2,3a,3b,3c,5,6,8/4a	53	27	11,94
5	1,2,3a,3c,4a,5,6,8/3b	13	8	2,93
6	1,2,3b,3c,4a,5,6,8/3a	32	19	7,21
7	1,3a,3b,3c,4a,5,6,8/2	1	1	0,22
8	2,3a,3b,3c,4a,5,6,8/1	1	1	0,22
9	1,2,3a,3c,5,6,8/3b,4a	50	31	11,26
10	1,2,3a,3c,4a,5,6/3b,8	1	1	0,22
11	1,2,3a,3b,3c,6,8/4a,5	6	4	1,35
12	1,2,3a,3b,3c,5,8/4a,6	1	1	0,22
13	1,2,3b,5,6,8/3a,4a	3	2	0,67
14	1,2,3c,4a,5,6,8/3a,3b	1	1	0,22
15	1,3a,3b,3c,4a,5,8/2,6	3	2	0,67
16	1,3a,3b,3c,5,6,8/2,4a	5	3	1,13
17	2,3a,3b,3c,5,6,8/1,4a	3	2	0,67
18	2,3a,3c,4a,5,6,8/1,3b	2	2	0,45
19	1,2,3a,3c,4a,6/3b,5,8	1	1	0,22
20	1,2,3a,3c,5,6/3b,4a,8	3	3	0,67
21	1,2,3a,3c,5,8/3b,4a,6	5	3	1,11
22	1,2,3a,3c,6,8/3b,4a,5	4	2	0,90
23	1,2,3b,3c,4a,5/3a,6,8	1	1	0,22
24	1,2,3b,3c,5,8/3a,4a,6	7	3	1,58
25	1,2,3c,5,6,8/3a,3b,4a	31	12	6,98
26	1,3a,3b,3c,6,8/2,4a,5	6	3	1,35
27	1,3a,3c,4a,5,6/2,3b,8	1	1	0,22
28	1,3a,3c,4a,5,8/2,3b,6	2	2	0,45
29	1,3a,3c,5,6,8/2,3b,4a	1	1	0,22
30	2,3a,3b,3c,6,8/1,4a,5	1	1	0,22
31	2,3a,3c,5,6,8/1,3b,4a	17	9	3,83
32	2,3c,4a,5,6,8/1,3a,3b	1	1	0,22
33	3a,3b,3c,4a,5,8/1,2,6	1	1	0,22
34	1,2,3a,5,6/3b,3c,4a,8	1	1	0,22
35	1,2,3a,6,8/3b,3c,4a,5	1	1	0,22
36	1,2,3c,5,6/3a,3b,4a,8	3	3	0,67
37	1,2,3c,5,8/3a,3b,4a,6	1	1	0,22
38	1,2,3c,6,8/3a,3b,4a,5	1	1	0,22
39	1,3a,3c,4a,5/2,3b,6,8	1	1	0,22
40	1,3a,3c,4a,6/2,3b,5,8	2	2	0,45
41	1,3a,3c,5,8/2,3b,4a,6	21	10	4,73
42	1,3a,3c,6,8/2,3b,4a,5	6	4	1,35

43	1,3a,5,6,8/2,3b,3c,4a	1	1	0,22
44	1,3b,3c,5,8/2,3a,4a,6	5	3	1,13
45	2,3a,3c,5,6/1,3b,4a,8	1	1	0,22
46	2,3a,3c,5,8/1,3b,4a,8	2	1	0,45
47	2,3b,3c,4a,5/1,3a,6,8	1	1	0,22
48	2,3b,3c,5,6/1,3a,4a,8	1	1	0,22
49	2,3c,5,6,8/1,3a,3b,4a	10	4	2,25
50	3a,3b,3c,6,8/1,2,4a,5	1	1	0,22
51	3a,3c,4a,5,8/1,2,3b,6	4	2	0,90
52	1,2,3c,5/3a,3b,4a,6,8	1	1	0,22
53	1,2,6,8/3a,3b,3c,4a,5	3	2	0,67
54	1,3a,3c,5/2,3b,4a,5,8	1	1	0,22
55	1,3a,3c,8/2,3b,4a,5,6	1	1	0,22
56	1,3a,5,8/2,3b,3c,4a,6	2	2	0,45
57	1,3c,5,8/2,ea,3b,4a,6	16	10	3,60
58	1,3c,6,8/2,3a,3b,4a,5	2	2	0,45
59	1,5,6,8/2,3a,3b,3c,4a	1	1	0,22
60	2,3a,3c,5/1,3b,4a,6,8	1	1	0,22
61	2,3c,5,6/1,3a,3b,4a,8	1	1	0,22
62	2,3c,5,8/1,3a,3b,4a,6	1	1	0,22
63	3a,3c,5,8/1,2,3b,4a,6	11	7	2,48
64	3a,3c,6,8/1,2,3b,4a,5	1	1	0,22
65	3b,3c,5,8/1,2,3a,4a,6	2	2	0,45
66	1,2,5/3a,3b,3c,4a,6,8	1	1	0,22
67	1,2,8/3a,3b,3c,4a,5,6	1	1	0,22
68	1,3a,8/2,3b,3c,4a,5,6	1	1	0,22
69	1,3c,5/2,3a,3b,4a,6,8	1	1	0,22
70	1,5,8/2,3a,3b,3c,4a,6	1	1	0,22
71	2,3c,5/1,3a,3b,4a,6,8	1	1	0,22
72	3a,3c,5/1,2,3b,4a,6,8	11	5	2,48
73	3a,3c,6/1,2,3b,4a,5,8	1	1	0,22
74	3a,4a,8/1,2,3b,3c,5,6	1	1	0,22
75	3a,5,8/1,2,3b,3c,4a,6	1*	1	0,22
76	1,3c/2,3a,3b,4a,5,6,8	1	1	0,22
77	1,6/2,3a,3b,3c,4a,5,8	1	1	0,22
78	1,8/2,3a,3b,3c,4a,5,6	3	2	0,67
79	3a,8/1,2,3b,3c,4a,5,6	1	1	0,22
80	3c,8/1,2,3a,3b,4a,5,6	1	1	0,22
81	5,8/1,2,3a,3b,3c,4a,6	1	1	0,22
82	6,8/1,2,3a,3b,3c,4a,5	1	1	0,22
83	5/1,2,3a,3b,3c,4a,6,8	1	1	0,22
	Total – Ukupno	*444*		*100*

*) Virulent/Avirulent genes (Virulentni/Avirulentni geni)

reproduction is responsible for the presence of many genotypes in the fungus population of the southeast part of Yugoslavia. We identified some other genotypes (unpublished data) using the varieties with resistance genes Pm 7, Pm 9, Pm 4b and Mld. For the comprehensive analysis of the population structure, the greatest number of varieties with individual major resistance genes or varieties with different combinations, such as line CI

12633 (Pm 2 + Pm 6), Normandie (Pm 1, Pm 2 and Pm 9), etc., should be used. According to B e n n e t t (1984) and L e b e d e v a (1986) eleven resistance genes have been identified so far, which is insufficient to have a real picture of the virulence of individual genotypes. A large number of the parasite's genotypes possessing different virulence makes the breeding for resistance difficult. It calls for an efficient determination of resistance sources and their incorporation in new varieties. In Yugoslavia, the most varieties grown in commercial fields are susceptible to powdery mildew agent. Our investigations showed that the same genotypes were obtained from different varieties and localities by sexual reproduction, which indicates that genotypes formation is not limited to the local climatic conditions and variety.

It is important to know the individual frequency of virulence alleles, because their recombination is performed in sexual reproduction (Table 4). The obtained results showed that numerous genotypes had V–1, V–2, V–3a, V–5, V–6 and V–8 virulence alleles. In 1988 each one was represented in more than 56%, while in 1989 in more than 66%. Regardless the differences which occurred in different years, it is obvious that V–3b and V–4a had lower frequency than the others. This agrees with the results achieved by L i m p e r t *et al.* (1987) analyzing the population virulence in Europe. According to the results of H e u n (1987), virulence alleles for resistance genes Pm 1, Pm 4a and Pm 8 are present in northern Germany, while the gen Pm 3b is more effective in southern part. According to N a m u c o *et al.* (1987), the most of the isolates had virulence to Pm 2, Pm 3c, Pm 4, Pm 6 and Pm 8. A small number of isolates had virulence to Pm 1 and Pm 3a (below 3%), while none of the isolates had virulence to Pm 3b. A high frequency of allele V–8 is underfined, because the gen Pm 8 was obtained in transgression from rye. This gene was successfully used at producing commercial varieties Aurora and Kavkaz in the

T a b. 4. — Frequency of the virulence genes in the isolates of *Erysiphe graminis tritici* reproduced sexually

Učestalost gena virulentnosti u izolatima *Erysiphe graminis tritici* nastalih polnim razmnožavanjem

Virulence genes Geni virulent.	1988		1989	
	No of isolates in each culture Br. izolata u svakoj kulturi	Frequency Učestalost %	No of isolates in each culture Br. izolata u svakoj kulturi	Frequency Učestalost %
V–1	228	78,35	320	72,07
V–2	163	56,01	305	68,69
V–3a	233	80,07	293	65,99
V–3b	35	12,03	170	38,29
V–3c	225	77,32	406	91,44
V–4a	49	16,84	106	23,87
V–5	265	91,06	391	88,06
V–6	290	99,66	316	71,17
V–8	–	–	403	90,76

T a b. 5. – Relation between some virulence genes in genotypes of *Erysiphe graminis tritici*

Odnos između pojedinih gena virulentnosti u genotipovima *Erysiphe graminis tritici*

Relation / Odnos	1988		1989	
	No of common isolates / Br. zajedničkih izolata	%	No of common isolates / Br. zajedničkih izolata	%
V–1 : V–2	129	44,33	261	58,78
V–1 : V–3a	187	64,26	230	51,80
V–1 : V–3b	55	18,90	150	35,81
V–1 : V–3c	221	75,94	330	74,32
V–1 : V–4a	24	8,25	95	21,40
V–1 : V–5	191	65,63	306	68,92
V–1 : V–6	230	79,04	270	60,81
V–1 : V–8	–	–	352	79,28
V–2 : V–3a	128	43,99	203	45,72
V–2 : V–3b	50	17,18	145	32,66
V–2 : V–3c	157	53,95	298	67,12
V–2 : V–4a	23	7,90	90	20,27
V–2 : V–5	155	53,26	286	64,41
V–2 : V–6	160	54,98	281	63,29
V–2 : V–8	–	–	285	64,19
V–3a : V–3b	62	21,30	118	26,58
V–3a : V–3c	230	79,04	282	63,51
V–3a : V–4a	33	11,34	70	15,76
V–3a : V–5	196	67,35	256	57,66
V–3a : V–6	234	80,41	222	50,00
V–3a : V–8	–	–	266	59,91
V–3b : V–3c	64	21,99	170	38,29
V–3b : V–4a	16	5,50	76	17,12
V–3b : V–5	75	25,77	155	34,91
V–3b : V–6	81	27,84	148	33,33
V–3b : V–8	–	–	165	37,16
V–3c : V–4a	45	15,46	102	22,97
V–3c : V–5	227	78,01	368	82,88
V–3c : V–6	265	91,06	302	68,02
V–3c : V–8	–	–	373	84,01
V–4a : V–5	46	15,81	101	22,75
V–4a : V–6	49	16,84	110	24,77
V–4a : V–8	–	–	136	30,63
V–5 : V–6	248	85,22	254	57,21
V–5 : V–8	–	–	310	69,82
V–6 : V–8	–	–	296	66,66

USSR, which were grown in Yugoslavia as well. These two varieties were also used in our breeding programs, so it is likely that our new varieties have the resistance gen Pm 8, which explain the high frequency of alleles V–8. The gene Pm 6 originates from *T.*

timopheevi (Jorgensen and Jensen, 1972) and is present in hexaploid line CI 12633 with the gene Pm 2. Because of its high resistance, this line has been widely used in breeding programs all over the world (Bennett, 1984), and in our country as well (Javor, 1981; Stojanović and Ponoš, 1988). However, there are no data about the presence of the gene Pm 6 in Yugoslav varieties, especially in those occupying the largest producing acreages. It can be supposed that the gene Pm 6 is widely spread, according to the results which indicate that the virulence allele V–6 was present in 290, out of 291 isolates. The high frequency of the allele V–5 was expected because of the recessive gene Pm 5 (Law and Wolfe, 1966).

The relationship between individual virulence alleles is not accidental (Table 5). The most collective V–1 and V–2 isolates were in the combination with V–3a, V–3c, V–5, V–6 and V–8, and the least with the alleles V–3b and V–4a. It indicates that the most cultivated varieties possess some of Pm 1, Pm 2, Pm 3a, Pm 3c, pm 5, Pm 6 and Pm 8 resistance genes. Virulent genes proliferated by sexual reproduction existed for all Pm genes. Consequently, the cumulation of already known resistance genes in one variety would not be sufficient protection. The sources of resistance should be searched for in the wheats relatives. The resistant hybrids of soft wheat should be bred through the transgression of their genes (Urazaliev and Kožahmedov, 1980), which could be furthermore used as resistance donors in breeding new commercial varieties.

As a results of the recombination of genes for virulence, the genotypes with different number of genes are forming during the sexual reproduction (Figure 1). The most genotypes, according to data from 1988, possessed 4 (21.99%), 5 (29.89%), and 6

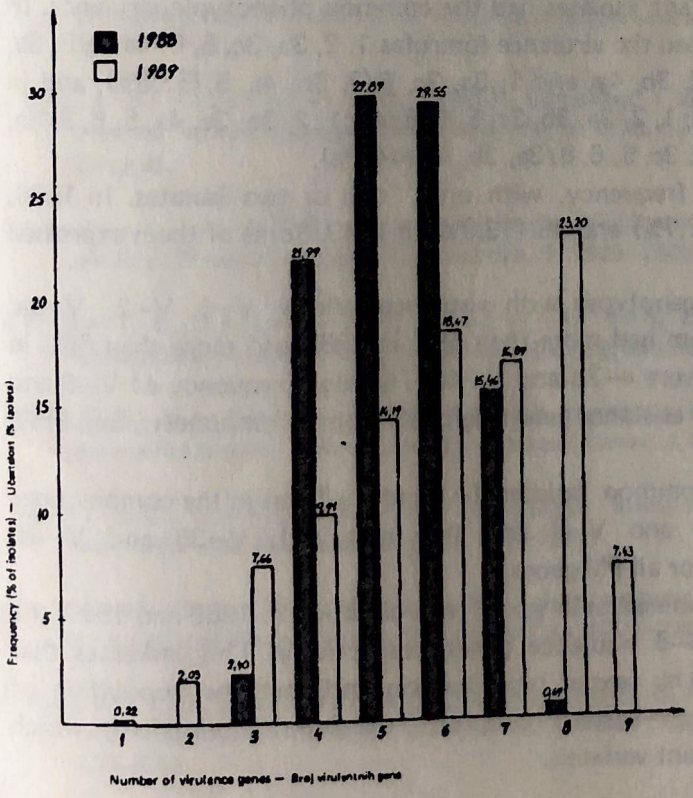

Fig. 1. – Frequency of isolates of *Erysiphe graminis tritici* with different number of the virulence genes

Učestalost izolata *Erysiphe graminis tritici* sa različitim brojem gena virulentnosti

(29.55%) genes for virulence and in 1989, 6 (18.47%), 7 (16.89%) and 8 (23.20%). Therefore, a part of the population originated from ascospores formed by sexual reproduction, possesses a large number of genes for virulence. According to S m i l j a k o v i ć (1966), the number of conidial generations reaches 18 in autumn and spring, in our agroecological conditions. Mutagenic processes are possible in every generation, which indicate the possibility to form genotypes with numerous genes for virulence during the asexual reproduction of the fungus. Our previous investigations (S t o j a n o v i ć and P o n o š, 1990) proved that the largest part of the population consists of genotypes with more genes for virulence. This is in contradiction with N a m u c o *et al.* (1987), because they found out that in 1984, the isolates without our with one gene for virulence were the most frequent in New York. Such great differences indicate to investigate the domestic population originated from sexual and asexual reproduction, which would enable the successful breeding for resistant varieties to powdery mildew.

C o n c l u s i o n s

According to the investigations and the obtained results, the following conclusions may be drawn:

It was found that numerous genotypes of different virulence are formed by sexual reproduction. In 1988 and 1989, we identified 45 and 83 genotypes, respectively. One additional Pm gene waa used in 1989.

In both years, the dominant isolates had the common phenotypic virulence. In 1988, the most frequent isolates had the virulence fomrulae 1, 2, 3a, 3c, 5, 6/4a; 1, 2, 3a, 3c, 5, 6/3c, 4a; 1, 3,a 3c, 5, 6/2, 3b, 4a, and 1, 3a, 3c, 6/2, 3b, 4a, 5 (53.6%), and in 1989, 1, 2, 3a, 3b, 3c, 4a, 5, 6, 8; 1, 2, 3a, 3b, 3c, 5, 6, 8/4a; 1, 2, 3b, 3c, 4a, 5, 6, 8/3a; 1, 2, 3a, 3c, 5, 6, 8/3b, 3a and , 2, 3c, 5, 6, 8/3a, 3b, 4a (44.8%).

Numerous genes had low frequency, with only one or two isolates. In 1988, there were 25 such genotypes (12.7%) and 45 (13.9%) in 1989. Some of them expressed high virulence.

The most frequent were genotypes with virulence alleles V–1, V–2, V–3a, V–5, V–6 and V–8. Each of them had more than 56% in 1988 and more than 66% in 1989. The least frequent alleles were v–3b and V–4a. The high frequency of V–6 and V–8 is undefined, because Pm 6 resistance gene originates from *T. timopheevi,* and Pm 8 from rye.

The highest number of common isolates V–1 and V–2 was in the combinations with V–3a, V–3c, V–5, V–6 and V–8 and the least with V–36 and V–4a. Corresponding virulences existed for all PM genes.

. As a result of gene recombination in sexual reproduction in 1988 and 1989, the most genotypes had 4–7 and 5–8 virulence genes, respectively. This indicates that the complex genotypes are formed by sexual reproduction and that the population of powdery mildew agents in the south–eastern part of Yugoslavia is viruelent, which complicates the breeding for resistant varieteis.

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VIRULENTNOST GENOTIPOVA *ERYSIPHE GRAMINIS DC. EX MERAT F.SP. TRITICI EM. MARCHAL* NASTALIH POLNIM RAZMNOŽAVANJEM

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Rezime

Pepelnica, koju prouzrokuje biotrofna gljiva *Erysiphe graminis tritici* je česta i ekonomski značajna bolest pšenice u nas. Za zaštitu otpornih sorata od značaja je poznavanje virulentnosti genotipova ove gljive i promena koje nastaju u njenoj populaciji.

Pri polnom razmnožavanju dolazi do rekombinacije različitih gena virulentnosti i formiranja novih genotipova. Zato su za ova proučavanja korišćene kleistotecije prikupljene u velikom broju lokaliteta u jugoistočnom delu Jugoslavije i sa različitih sorata. Tokom 1988. i 1989. godine proučena je virulentnost 752 izolata u Institutu za strna žita u Kragujevcu i Institutu za ratarstvo i povrtarstvo u Novom Sadu. Za ova proučavanja su korišćene sorte i linije pšenice sa genima otpornosti Pm 1, Pm 2, Pm 3a, Pm 3b, Pm 3c, Pm 4a, pm 5, Pm 6 i Pm 8 (Tab. 1). Njihovi sejanci su inokulisani čistim kulturama dobijenih od askospora, a reakcija je određivana pomoću tipova infekcije od 0 do 4 (M a i n s i D i e t z, 1930). Pored standardne metode rada (N o v e r, 1957) primenjena je i metoda benzimidazola (W o l f e, 1965).

Dobijeni rezultati pokazuju da se polnim putem formira veliki broj genotipova *Erysiphe graminis tritici* (Tab. 2 i 3). U 1988. godini je identifikovano 45, a u 1989. godini 83 genotipa. Dominantni u obe godine ispitivanja su bili izolati sa zajedničkom fenotipskom virulentnošću 1, 2, 3a, 3b, 3c, 5, 6, 8/4a i 1, 2, 3a, 3c, 5, 6, 8/3b, 4a. Veliki broj genotipova je imao malu učestalost, ali su mnogi od njih bili jako virulentni, jer su posedovali 6–8 odgovarajućih alela virulentnosti. Neizvesno je kakva je njihova sudbina u prirodi, jer zavisi kako će se adaptirati u uslovima sredine i domaćina. Ova istraživanja su pokazala da formiranje genotipova polnim razmnožavanjem nije usko vezano za mikroklimat i sortu, jer su dobijeni isti genotipovi sa različitih sorata i više lokaliteta.

Polnim razmnožavanjem se vrši rekombinacija postojećih alela virulentnosti, pa je značajno poznavati njihovu pojedinačnu učestalost (Tab. 4). Najveću učestalost su imali V–1, V–2, V–3a, V–5, V–6 i V–8 aleli virulentnosti. Svaki od njih je u 1988. godini bio zastupljen sa više od 56%, a u 1989. godini sa više od 66%. I pored izvesnih razlika po godinama jasno se uočava da je učestalost V–3b i V–4a znatno manja od ostalih.

Odnos između pojedinih alela virulentnosti nije slučajan (Tab. 5). Najveći broj zajedničkih izolata V–1 i V–2 je bio u kombinacijama sa V–3a, V–3c, V–5, V–6 i V–8, a najmanji sa V–3b i V–4a alelima. To ukazuje na mogućnost da većina gajenih sorata poseduje neke od Pm 1, Pm 2, Pm 3a, Pm 3c, pm 5, Pm 6 i Pm 8 gena otpornosti.

Kao rezultat rekombinacija gena virulentnosti pri polnom razmnožavanju formiraju se genotipovi sa različitim brojem gena (Graf. 1). Najveći broj genotipova je prema podacima iz 1988. godine posedovao 4 (21,99%), 5 (29,89%) i 6 (29,55%), a iz 1989. godine 6 (18,47%), 7 (16,89%) i 8 (23,20%) gena virulentnosti. To govori da deo populacije koji nastaje od askospora poseduje veliki broj gena virulentnosti.

Analizu strukture populacije ove gljive treba vršiti u toku vegetacije pšenice, jer veliki broj genotipova nastaje i bespolnim razmnožavanjem. Postojanje genotipova različite virulentnosti predstavlja otežavajući faktor pri selekciji otpornih sorata. Vezan je sa potrebom brzog otkrivanja novih izvora otpornosti i njihovog ugrađivanja u nove sorte.