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GENETIC STRUCTURE OF POWDERY MILDEW DISEASE PATHOGEN *BLUMERIA GRAMINIS* F. SP. *HORDEI* IN THE BARLEY FIELDS OF CUKUROVA IN TURKEY

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ABSTRACT

This work was conducted in order to investigate the frequency of virulence genes, gene complexities, and pathotype frequencies of powdery mildew (*Blumeria graminis* f. sp. *hordei*) populations on two different barley production fields in the Çukurova Region. For this purpose, the barley leaves, prior to the pre-harvest period, which were infected with pathogen at the sexual period developmental stage as *Cleistothecium* were collected in 2007 and stored in the laboratory at room temperature. Pathogenic isolates were obtained in laboratory conditions with the aid of susceptible control variety (Bülbül-89) by stimulating ascospore output from each foliar. Every isolate was further purified through multiplying from a single spore. A total of 138 *Blumeria graminis* f. sp. *hordei* single spores were obtained. The differential isogenic lines of 25, each possessing a single resistance genes were inoculated with the single spore isolates obtained from *Cleistothecium*. The aggressiveness of gene frequencies and virulence gene complexities, and pathotype frequencies of the isolates were determined according to the scale 0-4, developed by Welz. The pathotypes were established through the formula developed by Habgood. Virulence gene frequencies varied from 0 % to 94.33 % in powdery mildew populations. Gene frequencies of Va1, (Va7+Vk) were at 0.0 % in both populations. In the populations of Adana and Hatay 47 and 56 pathotypes were obtained, respectively. The isolate with Habgood pathotype index of 04667601 showed 14.8 % gene frequency in Adana population.

KEYWORDS:
Barley, *Blumeria graminis* f. sp. *hordei*, Virulence gene frequency, Pathotype frequency, Habgood analysis

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the most important crop following wheat, corn and paddy in the world. Turkey possesses a significant position by planting area 2.783.583 hectares of barley and 136.5 million tons of yield in the world [1]. Powdery mildew (*Erysiphe graminis* DC. f. sp. *hordei* Em Marchal Synamorph, *Blumeria graminis* DC. Golovin ex Speer f.sp. *hordei*) is one of the primary diseases affecting yield and quality in barley production. Conidia spores, as a disease factor, may infect barley plants in all growth stages of the vegetation. A number of investigators reported that powdery mildew causes 25-30 % yield losses, especially in Central Europe and, more than 50 % in USA if the climatic conditions are pertaining to spreading of the disease [2, 3, 4, 5]. The infected field ratio varies from 25 % to 70 % when the coastal belt areas of the Aegean and Mediterranean Regions dominated by the Mediterranean climate. On the other hand the intensity of the infection was stated between 1-100 %.

Several strategies were improved in the battle against powdery mildew such as use of fungicides, planting resistance varieties, mosaic planting techniques, rotated planting practices and combined fighting systems in Europe possessing moist and cool climate conditions [6, 7]. A number of studies were carried out on distribution of powdery mildew and occurrence of physiologic race in order to develop early warning systems to control crop

diseases, therefore almost all barley cultivars produced in Europe was tested in view of resistance against to powdery mildew [8, 9, 10].

It is revealed that European barley cultivars possess mostly two or three resistance genes and rarely more than three [7, 11, 12, 13]. Emergence of new physiologic races in powdery mildew populations results in resistance-breaking in spite of planting newly developed varieties in relatively short time period [14]. At the same time use of fungicides is also being ineffective due to increased resistance by pathogen [6, 15, 16, 17].

The aim of modern agriculture is to produce permanently and securely within the means of minimally polluting the environment with fungicides. Therefore, the use of resistant varieties through plant breeding or the implementation of chemical control methods is considered inadequate. In developing more effective combat systems, the in-depth studies in the interactions of host-parasite systems and means of integrated cross-disciplinary should be conducted. The early warning systems in the control against diseases, a systematic and collaborative virulence spectrum of pathogens world-wide should also be determined. To this end, powdery mildew virulence surveys are carried out in barley crop fields in Europe and in the different regions of the world every year [12, 18, 19, 20, 21, 22, 23, 24]. However, to this date a periodical survey for investigating the virulence pattern of barley powdery mildew has not been implemented.

The purpose of this research is to determine virulence spectrum in spore population of powdery mildew in the sexual period, *Cleistothecia*, on the barley fields of Çukurova Region. The data gathered will help facilitate protection strategies in the region.

MATERIALS AND METHODS

Plant materials. Differential test varieties of 25 isogenic lines are used to identify virulence genes in *Blumeria graminis* f. sp. *hordei* populations, developed by Kolster et al. [25], widely used in Europe and a Turkish isogenic line carrying no resistance (Table 1). Isogenic lines were amplified at the Adnan Menderes University farms, which were obtained from ICARDA (The International Center for Agricultural Research in the Dry Areas). Barley leaves with *Cleistothecium*, from the barley fields in the Çukurova Region were collected prior to harvest and stored at 4ºC.

TABLE 1
Differential test varieties for the virulence of *Blumeria graminis* f. sp. *hordei* collected in the Çukurova Region.

Isogenic Lines*	Parent	Origins-owned	Resistance Gene
Pallas-01	Iso 1R	CI 1637 Mla1, +?	Mla1, +?
Pallas-02	Ricardo	CI 6306	Mla3
Pallas-03	Iso 20R	CI 16151	Mla6, Ma14
Pallas-04a	Nordal	Calsberg	Mla7, Mlk, +?
Pallas-04b	Nordal	Calsberg	Mla7, +?
Pallas-05	Filler	Calsberg	Mla1
Pallas-06	Iso 10R	CI 16147	Mla7, Ml(LG2)
Pallas-07	Mona	Svalof	Mla9, Mlk
Pallas-08a	Senat	Svalof	Mla9, Mlak
Pallas-08b	Senat	Svalof	Mla9
Pallas-09	Iso 12R	CI 16149	Mla10, Ml(Du2)
Pallas-10	Emir	Cebeco	Mla12
Pallas-11	RuPl	Svalof	Mla13, Ml(Ru3)
Pallas-12	Hor 1657	Hor 1657	Mlc
Pallas-13	Hor 1402	Hor 1402	Ml (1402)
Pallas-14	W.41/145	WeihenstePhen	Ml(41/145)
Pallas-15	RuPee	Svalof	Ml(Ru2)
Pallas-19	Iso 5R	CI 16145	MlP
Pallas-20	Atlas	CI 4118	Mlat
Pallas-21	Deba	Abed	Mlg, Ml (CP)
Pallas-22	Riso 5678	CI 15219	mlo5
Pallas-23	Lofa	Abed	Ml (La)
Pallas-24	Iso 3R	CI 16141	Mlh
Pallas-30	Filler	Svalof	Mla1
Pallas	Pallas	Svalof	Mla8
Bülbül-89	Bülbül-89	Turkish cultivar	none known genes

*obtained from ICARDA

Method. Pathogen isolates were obtained by stimulating ascospore formations on each leaf of the susceptible variety, Bülbül-89, in laboratory conditions. Each isolate was purified from a single spore [26]. Virulence tests were carried out on the 26 differential test lines (Table 1) grew under controlled laboratory conditions at the 1-2 leaf periods according to Pons [6]. The leaves of isogenic lines were cut in a 3 cm length, placed in 9 cm in diameter Petri dishes containing BA (benzimidazol agar) (5 ppm) in the counterclockwise direction with the order in Table 1, where the bottom surface of the leaves were in contact with the feeding environment. As a control, variety Bülbül-89 leaf particles were placed at the center of the Petri dishes.

Inoculation of single spore isolates on test varieties was implemented with the aid of home-made inoculation tower in a sterile cabinet. Inoculated pieces of leaves on Petri dishes were incubated at 17°C / 14-hour dark and 10-hour light periods in a climate chamber for 8-10 days. The test plates were assessed for full inoculation and sufficient colony formation and on the Bülbül-89 susceptible variety according to the 0-4 scale developed by Welz [27]. Following the tests determining the genetic composition of pathotypes, the virulence gene frequencies and the level of complexities in virulence genes were determined using MS-Excel version 5.0 developed by Habgood [28]. The identification of powdery mildew isolates were determined using the software by Hermann et al. [29]. According to this method, the 0-4 scale results in evaluating the test sets were turned into a binary code system. As a result, two-type outcomes were obtained; the scale values of 0, 1, 2, were translated as 0 (resistant), the values of 3 and 4 were translated as 1 (sensitive). The following formula by Habgood [28] was used to determine the pathotypes.

$$PTi = \sum_{nr=1}^{25} 2^{(nr-1)} \cdot kpi \quad (1)$$

Pti: Pathotype index, *nr*: order # of differential lines (Table 1), *kpi*: binary code # of differential lines (0 or 1).

The values of the gene frequency aggression were divided into 5 different groups according to this style of grouping, if virulence gene frequency in population is bigger than 90 %, the population is considered fixed. If it is between 60 % and 90 %, it is high; if it ranges between 30 % to 60 %, it is medium; if the frequency is between 10 % and 30 %, it is very low degree and if it is smaller than 10 %, it is called recessive. Virulence complexity is a number of genes in a single isolate. The number of virulence genes was determined per isolate. Afterwards, the number isolates having the same complexity values as percentages are determined as complexity frequencies.

RESULTS AND DISCUSSION

Virulence genes frequencies and complexities obtained from powdery mildew population in barley field of Çukurova Region are presented in Table 2 and Table 3, respectively, according to which, both of the populations here called as Adana and Hatay locations, had 22 virulence genes. The similarities were observed in both populations in fixed and recessive genes. The Va8 gene frequency was over 90 % in both of the populations indicating the gene is fixed, the genes; Va1, Va7+Vk, Va9+Vk, and Va9 were recessive in both populations. The crucial differences were observed in Vp and Vh genes, while frequency of Vp gene was 81.64 % in Adana, it is 32.34 % in Hatay, on the other hand the frequency of Vh virulence gene was 18.30 % in Adana, it was 67.62 % in Hatay. The lowest virulence gene frequencies were found in Va1, Va7+Vk, Va9+Vak virulence genes (0.00 %) and detected in Adana population, whereas the highest one was in Va8 gene (94.33 %). Virulence gene frequencies of Va1+?, Va7+Vk, Va7, Va1, Va9+Vk, Va9+Vak, Va9, Va13+(V(Ru3), Vo5, Va1 genes were found to be recessive (<10%), virulence gene frequencies of Va3, Va7+V(LG2), Vc, V(Ru2), Vg+V(cp), Vh genes were low (10-30 %); Va6+Va14, Va12, V(41/145) genes were medium (30-60 %); Va10+V(Du2), Va(1402), Vp, Vat, V(La) genes (60-90 %) were high in the same population. In the Hatay population, virulence gene frequencies of Va1+?, Va7+Va14, Va1 genes were 0.00 %, virulence gene frequency of Va8 was 91.14 %. It is detected that Va7, Va1, Va7+V(LG2), Va9+Vak, Va9, V(41/145), V(CP) virulence genes were found to be recessive with having less than 10 % gene frequencies. The frequencies of Va3, Va6+Va14, Va9+Vk, Va13+V(Ru3), Vc, V(Ru2), Vo5 virulence genes were low (10-30 %), frequencies of Va12, Vp were medium (30-60 %), and frequencies of Va10+V(Du2), Va(1402), Vat, V(la), Vh genes were high (60-90 %).

TABLE 2
The virulence gene frequencies.

Virulence Genes	Virulence Gene Frequencies (%)	
	Adana	Hatay
Va1, +?	0.00	0.00
Va3	29.56	26.46
Va6, Va14	36.68	27.93
Va7, Vk	0.00	0.00
Va7	1.40	1.47
Va1	4.22	7.35
Va7, V(LG2)	12.67	4.41
Va9, Vk	1.40	14.70
Va9, Vak	0.00	1.47
Va9	7.04	7.35
Va10, V(Du2)	84.48	85.26
Va12	30.97	33.81
Va13, V(Ru3)	2.81	4.41
Vc	15.48	8.82
Va (1402)	83.07	82.32
V(41/145)	36.60	7.35
V(Ru2)	25.34	16.17
VP	81.64	32.34
Vat	83.07	79.38
Vg, V (CP)	19.71	8.82
Vo5	4.22	14.70
V (La)	74.62	79.38
Vh	18.30	67.62
Va1	1.40	0.00
Va8	94.33	91.14

TABLE 3
The virulence gene complexity values.

Virulence Genes	Virulence Gene Complexities (%)	
	Adana	Hatay
5	2.81	0.00
6	23.80	7.35
7	18.30	20.58
8	39.42	27.93
9	8.44	4.44
10	5.56	7.35
11	1.40	1.47
12	0.00	2.94

TABLE 4
The pathotype frequencies.

Pathotypes		Populations	
No	Habgood number	Adana	Hatay
1	04667601	14.08	0.00
2	00467611	8.44	0.00
3	04667611	8.44	0.00
4	04667631	8.44	0.00
5	0667611	8.44	0.00
6	24667611	7.04	0.00
7	0067611	4.22	0.00
8	0067615	2.81	0.00
9	04667611	2.81	0.00
10	20067603	2.81	0.00
11	404776721	0.00	7.35
12	410676320	0.00	4.41
13	004276320	0.00	2.94
14	006756721	0.00	2.94
15	204774721	0.00	2.94
16	400676321	0.00	2.94
17	400676720	0.00	2.94
18	404776721	0.00	2.94
Total frequencies of other pathotypes (%)		49.28	70.56
Number of pathotypes		47	56
Number of isolates tested		71	68

The virulence gene complexity in Adana changed from 5 to 11, and varied from 6 to 12 in Hatay (Table 3). The gene complexity values of this study are in consistent with the other studies conducted on the cultured barley fields [30, 31, 32, 33].

The pathotypes commonly seen in the powdery mildew populations in the locations of Adana and Hatay are presented in Table 4. The numbers of pathotypes are 47 and 56 in from Adana and Hatay locations, respectively. The pathotype with the Habgood number 0466760 from the Adana had the highest frequency 14.08 %. As for the Hatay location, the pathotype 404776721 has the highest frequency as 7.35 %.

A study conducted more than a decade and a half ago by Braun et al. [15] and Zeybek [34] showed 9 genes were fixed on wild type barley. The fixed genes in this study are consistent with those cited reports above. Since then, the frequencies of the virulence genes Va1, Va9, Va13, and Vo5 decreased substantially as shown in this study, and they became recessive by a negative selection.

CONCLUSION

The findings produced by the samples of powdery mildew obtained from two different barley fields in the Çukurova Region showed differences regarding the virulence gene frequencies, virulence gene complexities and pathotype frequencies. One striking similarity is that Va8 virulence gene is fixed in both of the population followed by Va10 with high degree appearance. Moreover, examining the distribution and frequency of virulence genes during the vegetation period each year will contribute towards a better understanding and dissemination of information regarding the genetic structure of powdery mildew populations.

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