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Effects of powdery mildew severity *(Blumeria graminis* f. sp. *tritici)* on breeding lines of durum wheat *(Triticum turgidum* L. spp. *durum)* yield in western Andalusia

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Abstract

Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* is one of the most important diseases affecting to durum wheat (*Triticum durum* L. ssp. *durum)* production in mediterranean conditions over the last few years. Effects of high levels of this pathogenic agent during tillering, stem elongation and booting phases and their relation with yield variations between years 1998 and 2002 in western Andalusia, where this agent shows a high incidence and severity have been studied. Severity data and AUDPC (area under disease progress curve) during the vegetative crop cycle show a high correlation between the resistance of each variety and its productivity. The need for the inclusion of powdery mildew resistance genes in the new durum wheat varieties is emphasized.

Key words: AUDPC, differentials lines, resistance genes, phytopathogenic fungi, cereal breeding.

Resumen

Efecto de la infección de oidio *(Blumeria graminis* f. sp. *tritici)* sobre la producción del trigo duro *(Triticum turgidum* L. ssp. *durum)* en Andalucía occidental

El oidio del trigo duro (*Triticum turgidum* L. ssp. *durum)* causado por *Blumeria graminis* f. sp. *tritici* se ha convertido en uno de los principales factores limitantes en la producción de este cereal en condiciones de clima mediterráneo. Se investigó el efecto de elevadas infecciones de este patógeno durante las fases de ahijamiento y encañado y su relación con las variaciones del rendimiento obtenido durante las campañas agrícolas comprendidas entre 1998 y 2002 en la zona triguera de Andalucía occidental, donde la enfermedad tiene una elevada incidencia y gravedad. A partir de los datos periódicos de gravedad tomados a lo largo del ciclo vegetativo del cultivo y de sus correspondientes áreas por debajo de la curva de progresión de la enfermedad, se observó una elevada correlación entre el nivel de resistencia de cada variedad y su productividad. De los resultados obtenidos se puso de manifiesto la necesidad de incorporar genes de resistencia a oidio en las nuevas variedades de trigo duro.

Palabras clave: AUDPC, líneas diferenciales, genes de resistencia, hongos fitopatógenos, mejora de cereales.

Introduction

Genetic resistance is the most profitable control method for all leaf diseases of wheat both from economical and ecological perspectives. Disease control results in higher yields and helps to reduce yield oscillations from one year to the next. Reports on the effect of the pathogen *Blumeria graminis* f. spp. *tritici* on yield losses has generally referred to soft (bread) wheat (*Triticum aestivum* L.), where losses over 34% of the yield have been recorded (Pearce *et al*., 1996) and even over 45% (Brown, 2001). However, the effect of powdery mildew caused by *Blumeria graminis* f. spp. *tritici* on the yield of durum wheat (*Triticum turgidum* L. ssp. *durum*), is becoming a limiting factor in production of this cereal in the mediterranean climate (Nsarellah *et al*., 2000; Imani *et al*., 2002).

Winters characterized by dry periods after the first rains usually favour disease development in susceptible varieties, giving rise to early epidemics that can be very severe. Mean temperatures, high relative humidity and high plant density favour pathogenic development.

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In the wheat growing region of Andalusia, these environmental conditions are especially common during the tillering and shoot forming phases. Management of this disease should be based on the use of resistant varieties (Brown *et al.*, 1997), since the persistence of available fungicides is not sufficient to protect the plant during the whole cycle and, given the increasing importance of other leaf diseases in durum wheat, such as leaf rust fungus and septoria, which make it necessary for the farmer to treat the plants during development of the flag leaf, incorporation of resistance genes to powdery mildew in new commercial varieties of durum wheat is essential to avoid the need for additional treatment in young crop stages (Urbano, 2001).

Andalusia has almost 56% of the cultivated surface area of durum wheat in Spain with almost 70% of the total national production (AETC, 2002). Although there are few reports on the incidence of the pathogen in Andalusia, Marin and Almacellas (1999) considered powdery mildew as one of the pathogens responsible for yield losses in this crop in Spain. For this reason, the aim of this work was to study the incidence and severity of *Blumeria graminis* f. spp. *tritici* on the yield of durum wheat (*Triticum turgidum* L. spp. *durum*) in Andalusia, on widely grown cultivated varieties and varieties preselected as resistant to the pathogen.

Material and methods

During the agricultural season 1998/1999, agronomical trials were carried out on plots of the cooperatives «San Dionisio» in Jerez de la Frontera (Cádiz), and «Campo de Tejada» in Escacena del Campo (Huelva). A total of 25 lines of durum wheat: 5 widely grown control varieties (Simeto, Gallareta, Don Pedro, Yavaros C-79 and Senadur) and 20 advanced lines from the International Maize and Wheat Breeding Centre (CIMMYT, Mexico). The statistical design was an equilibrated square lattice with 25 entries, 3 repeats and an elemental plot of 6 m^2. The assay was surrounded by plots of a commercial variety of durum wheat to avoid different conditions for experimental border plots and to minimize damage by birds and rodents. Plots were sown with machines adapted to carry out experiments, while the land was prepared and fertilized with the farmer's machinery using typical methods for that area.

To determine the part of Andalusia in which the mildew infections had reached the greatest severity, visits were made to commercial farms belonging to five cereal cooperatives in the provinces of Cádiz, Huelva and Seville. Of the areas surveyed in the 1998/1999 season, highest levels of the disease in the main commercial varieties were recorded in Conil de la Frontera (Cádiz), which was chosen as a suitable location to carry out production trials in the seasons 1999/2000, 2000/2001 and 2001/2002, to evaluate the efficacy of powdery mildew control in new varieties of durum wheat (Don Manuel, Don Rafael and Don Sebastian), previously selected as resistant in trials in Jerez de la Frontera and Escacena del Campo, together with commercial varieties studied as susceptible reference controls (Don Pedro, Gallareta and Yavaros C-79) and 19 advanced lines from the CIMMYT breeding program. The statistical design and the methodology of the trials were the same as those cited for the 1998/1999 season.

The severity of the disease was determined using a modified version of Cobb's scale (Peterson *et al.*, 1948), as the percentage of leaf surface area covered by mycelia.

From the leaf surface area data recorded over the vegetative crop cycle at fortnightly intervals from February to May, progression of the leaf epidemic was established using the parameter AUDPC [area under disease progress curve (Urbano, 2001)]. When the AUDPC was determined as a measure of efficacy, the standardized area under leaf progress curve (AUDPCs) was estimated by dividing the AUDPC by the number of days of the epidemic, calculated as follows:

$$AUDPCs = [\ \%\ (disease_i + \%\ disease_{i+1}) * 0.5 * \\ * \text{no. days interval})]\ /\text{total no. days}$$

During the trials carried out in the 2001/2002 season, a fungicide treatment was applied at the time of development of the flag leaf consisting of 12.5% epoxiconazol (commercial name: LOVIT, Basf España), at a dose of 1 L ha^{-1}, to guarantee the protection of the crop against other cryptogamic diseases that could start their activity as leaf rust or septoria.

When the plants had reached commercial maturity, the spikes of all the plots in the experiment were hand harvested and threshed. After determining the weight of the grain harvested in each 6 m^2 plot, the yield was estimated in kg ha^{-1} for each line studied. The results were analyzed by the STATISTIX 1.0 program (Analytical software, 1996) and SPSS (Lizasoain and Joaristi, 1999).

Table 1. Different lines of *Blumeria graminis* f. spp. *tritici*

Resistance genes	Origin/pedigree
Pm1	Axminster / 8xChancellor (Cc)
Pm2	Ulka / 8xCc
Pm3a	Asosan / 8xCc
Pm3b	Chul / 8xCc
Pm3c	Sonora / 8xCc
Pm4a	Khapli / 8xCc
Pm4b	Ronos
Pm5	Rector
Pm6	Nk 747
Pm8	Disponent
Pm17	Amigo
Pm2,6	Maris Hunts
Pm2,4b,8	Apollo
Pm3d	Ralle
Pm7	Transfed
Pm1,2,9	Normandie

Similarly, to obtain information about the pathogenic races present in the wheat growing region where the trails were carried out, 16 lines of bread wheat were sown as differenciator varieties during the seasons 1999/2000, 2000/2001 and 2001/2002. The first 6 lines are almost isogenic, obtained by cross-breeding with the variety Chancellor (Cc). The rest were varieties with one or more resistance genes to powdery mildew (Pm). The pedigree of these lines and of the resistance genes assigned to their genetic load is shown in Table 1. The material was distributed by the scientific and technical cooperation program of the European Union COST 817.

To determine the efficacy of the genes present in the different varieties to control the mildew, the percentage leaf area covered by mycelia was recorded every fortnight in each of these as described previously.

Table 2. Incidence of the severity (% leaf area covered by mycelia) of *Blumeria graminis* f. spp. *tritici* on durum wheat yield (kg ha^{-1}) in Jerez de la Frontera (Cádiz) and Escacena del Campo (Huelva) farms

Variety	Jerez de la Frontera		Escacena del Campo	
	Yield	% leaf area	Yield	% leaf area
Simeto	2749	13.3	1337	6.7
Gallareta	2133	43.3	1036	36.7
Don Pedro	1903	46.7	1131	46.7
Yavaros C-79	2165	26.7	971	26.7
Senadur	2734	46.7	1120	43.3
TDA 1	2244	43.3	920	43.3
TDA 2	2555	16.7	997	20.0
TDA 3	2520	16.7	870	40.0
TDA 4	2071	3.3	795	0.0
TDA 5	2751	10.0	1116	16.7
TDA 6	2898	13.3	1079	6.7
TDA 7	2049	56.7	891	63.3
TDA 8	2277	36.7	953	43.3
TDA 9	1890	53.3	841	43.3
TDA 10	1915	20.0	693	36.7
TDA 11	2125	33.3	695	33.3
TDA 12	2109	60.0	879	36.7
TDA 13	2075	40.0	741	30.0
TDA 14	1897	53.3	851	50.0
TDA 15	2189	56.7	856	43.3
TDA 16	2475	20.0	1114	36.7
TDA 17	2689	26.7	1043	26.7
TDA 18	1803	56.7	970	46.7
TDA 19	1695	43.3	722	40.0
TDA 20	2225	36.7	1053	43.3
Mean	2245	34.9	947	34.4
Standard deviation	337	17.14	159	14.88
Error	67.3	3.43	31.86	2.97

Table 3. Incidence of *Blumeria graminis* f. spp. *tritici* on differential wheat lines during the seasons 1998/1999, 1999/2000 and 2001/2002 in Jerez de la Frontera (Cádiz)

Resistance genes	Origin/pedigree	% leaf area covered with mycelia		
		1999/2000	2000/2001	2001/2002
Pm1	Axminster / 8xCc	0	0	40
Pm2	Ulka / 8xCc	0	0	0
Pm3a	Asosan / 8xCc	0	0	0
Pm3b	Chul / 8xCc	0	0	0
Pm3c	Sonora / 8xCc	0	0	0
Pm4a	Khapli / 8xCc	0	0	10
Pm4b	Ronos	0	0	10
Pm5	Rector	0	0	10
Pm6	Nk 747	0	0	10
Pm8	Disponent	0	0	0
Pm17	Amigo	0	0	0
Pm2,6	Maris Hunts	0	0	0
Pm2,4b,8	Apollo	0	0	0
Pm3d	Ralle	0	0	0
Pm7	Transfed	0	0	0
Pm1,2,9	Normandie	0	0	0

Results

During the 1998/1999 season, severe infections of this pathogen were recorded in the experimental areas, with percentages of leaf covered with mycelia often above 50% (Table 2). The results of grain yield and severity of infection in the trial revealed a high degree of uniformity in Jerez de la Frontera in the three repeats and the linear regression analysis indicated a negative correlation between the yield obtained and the percentage of leaf area covered by the pathogen for each variety studied ($r = –0.580$, $p < 0.05$). Results of yield and severity of infection by powdery mildew measured in the locality of Escacena del Campo were not uniform in the three repeats but a negative correlation was found between them for each repeat analyzed ($r = 0.989$, $p < 0.1$).

The results obtained for trials of different varieties sown in Jerez de la Frontera during the seasons 1999/2000, 2000/2001 and 2001/2002 are shown in Table 3.

During the seasons 1999/2000, 2000/2001 and 2001/2002 experiments were carried out to study the changes in yield due to effects of the pathogen. The seasons 1998/1999 and 1999/2000 were characterized by the high severity of the mildew attacks and the absence of other leaf diseases, while during season 2000/2001 the pathogen had a minimum incidence. The last season studied, 2001/2002, was characterized by a mildew attack in an early stage between tillering and shoot formation, followed by the appearance of the first pustules of leaf rust during development of the flag leaf when the fungal protection was applied. In this way, the absence of other leaf pathogens in the final developmental stages of the crop is guaranteed when conditions are unsuitable for powdery mildew infections. Table 4 shows the results of the AUDPCs parameter for new varieties selected as resistant and for commercial controls sensitive to mildew. Owing to the scarcity of the pathogen in studies carried out in the 2000/2001

Table 4. Results of the parameter AUDPCs of *Blumeria graminis* f. spp. *tritici* on durum wheat during the seasons 1999/2000 and 2001/2002 in Conil de la Frontera (Cádiz)

Variety	1999/2000	2001/2002
Yavaros C-79	18.75	23.80
Gallareta	33.18	40.85
Don Pedro	53.75	45.17
Don Manuel	16.82	3.60
Don Rafael	20.21	7.21
Don Sebastián	7.14	3.54
Mean of new varieties	14.72	4.78
Mean of controls	35.23	36.61
Total mean	24.97	20.69
Standard deviation	16.38	18.88
Error	6.68	7.7

Table 5. Yield (kg ha^{-1}) of durum wheat grain during the seasons 1999/2000 and 2001/2002 in Conil de la Frontera (Cádiz)

Variety	1999/2000		2001/2002	
	Yield	% of $X_{controls}$	Yield	% of $X_{controls}$
Yavaros C-79	1758	107.4	2805	124.5
Gallareta	1913	116.9	1872	83.1
Don Pedro	1238	75.7	2079	92.3
Don Manuel	2418	147.8	3761	167.0
Don Rafael	2235	136.6	3046	135.3
Don Sebastián	2151	131.5	3316	147.2
Mean of new varieties	2268	138.6	3374	149.8
Mean of controls ($X_{controls}$)	1636	100	2252	100
Total mean (X_{total}l)	1952	119.3	2813	124.9
Standard deviation	420.9		725.3	
Error	171.8		296.1	

season, together with the severity of the following attack of leaf rust, it was not possible to study the correlation between mildew and yield in this season.

The analysis of the results obtained shows a high correlation between both parameters in the seasons studied, as shown graphically in Figures 1 and 2.

Discussion

The high negative correlation between the yield results and the leaf area percentage covered by mildew in trials carried out in Jerez de la Frontera and Escacena del Campo during the season 1998/1999 (Table 2) show us that new varieties incorporating resistance genes for this pathogen are efficient in the disease control. None of the durum wheat varieties studied showed an immune reaction against the pathogen in both regions, which is common in commercial varieties of bread wheat grown in the area (data not shown).

In the differential varieties of bread wheat, symptoms of the disease were not observed over the vegetative crop cycle during the seasons 1999/2000 and 2000/2001, probably due to the fact that resistan-

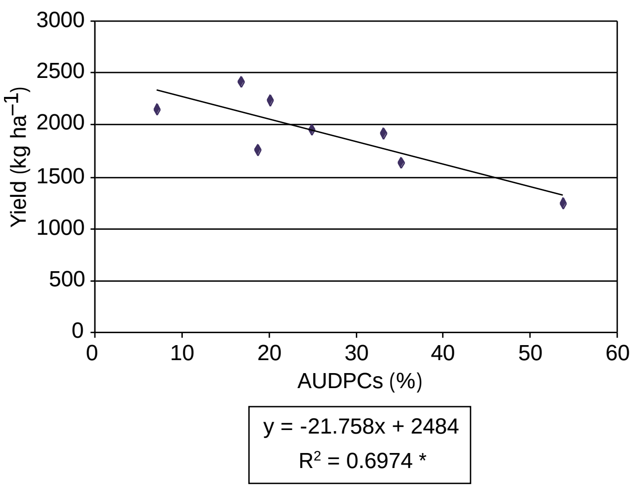

Figure 1. Regression analysis between yield factors (kg ha^{-1}) and AUDPCs of mildew on durum wheat in Conil de la Frontera (Cádiz) during the season 1999/2000. Significance level: * ($p \leq 0.05$).

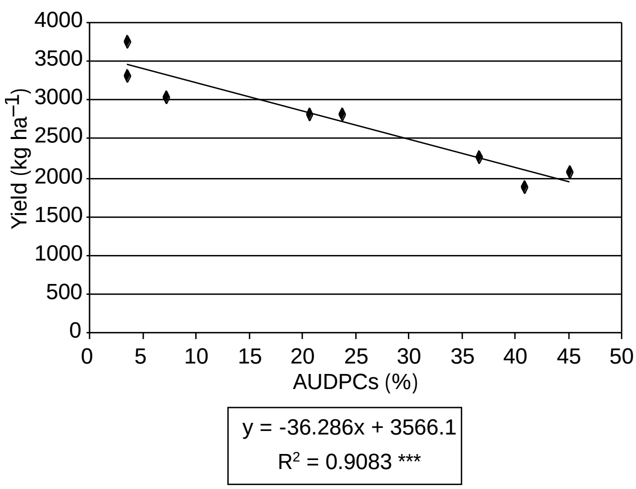

Figure 2. Regression analysis between yield factors (kg ha^{-1}) and AUDPCs of mildew on durum wheat in Conil de la Frontera (Cádiz) during the season 2001/2002. Significance level: *** ($p \leq 0.001$).

ce genes were effective against the races of mildew present in the area during these seasons. In the season 2001/2002, gene Pm1 was ineffective at controlling mildew races, as also described in Italy in the 1996 season (Zhou and Casulli, 1998), and little efficacy was demonstrated by the Pm4a, Pm4b, Pm5 and Pm6 genes. The remaining genes individually studied (Pm2, Pm3a, Pm3c, Pm3d, Pm7, Pm8 and Pm17) provided immunity to the infection in the presence of large amounts of pathogen inoculum.

To compare the severity of epidemics of different duration during the seasons 1999/2000, 2000/2001 and 2001/2002, AUDPCs has been used to eliminate the error that can occur in the case of a severe but short-lived epidemic and a long lasting epidemic of intermediate severity.

The negative correlation between yield and AUDPCs in both campaigns is statistically significant. Therefore, the changes in grain yield observed in the 1999/2000 season could be due in 70% of cases to variations in the AUDPCs of mildew as a pathogenic agent over the vegetative cycle. Similarly, during the 2001/2002 season, 91% of changes in yield can be explained by the severe infection suffered in sensitive control varieties, while the new varieties Don Manuel, Don Rafael and Don Sebastian which incorporate resistance genes result in an average increase in yield of around 50%.

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