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ASYMPTOMATIC INFECTION OF MAIZE LATE WILT CAUSED BY *Cephalosporium maydis*

El-Naggar, A. A. A. and A. M. Sabry
Maize and Sugar crops Dis. Res. Sec., Plant Pathol. Res. Inst., ARC, Giza, Egypt.

ABSTRACT

Greenhouse experiments were conducted in 2008, 2009 and 2010 growing seasons to determine the incidence of symptomatic and asymptomatic infection by *Cephalosporium maydis* the causal agent of late wilt disease of maize. The percentage of healthy appearing plants that incubating the fungus (asymptomatic) ranged from 5 to 68% whereas visually infected plants (symptomatic) was in between 0 to 100%. There was no positive correlation between the degree of resistance and asymptomatic infection. Based on the occurrence of asymptomatic infection by *C. maydis*, under inoculum of single isolate, mixture isolates and different levels of its density, on resistant or susceptible genotypes it is concluded that asymptomatic of late wilt disease is evident and maize plants used tolerance as a defense against the causal agent of late wilt disease.

INTRODUCTION

In Egypt, maize (Zea mays L.) is considered as one of the most important food and feed cereal crops. Late wilt disease caused in maize by the soil-borne as well as the seed-transmitting (Michail *et. al.,* 1999) fungus, *Cephalosporium maydis* Samra, Sabet & Hingorani (Samra *et. al*., 1962 & Samra *et. al*., 1963) is a principal limiting factor in production. This disease also has been reported from India (Payak *et. al*., 1970) and Hungary (Pecsi and Nemeth, 1998). *C. maydis* reproduces asexually and no perfect state has been identified. The pathogen population, in Egypt, contains four lineages, three of which are widely distributed throughout the country (Saleh *et. al*., 2003). At the same time, Saleh and Leslie (2004) reclassified *C. maydis* as *Harpophora maydis* based on amplified fragment length polymorphism (AFLP) profile which was distinct from the other tested species of *Cephalosporium*, *Phialophora* sensu lato and members of *Gaeumannomyces-Harpophora* species complex.

Sabet *et. al.* (1970) reported that the fungus grows superficially on maize roots, producing hyphae with short, brown, thick-walled, and swollen cells. It progresses inter- and intra-cellularly reaching to the root endodermis and the xylem after15 and 21days after sowing respectively. After the fungus penetrates the xylem, it grows slowly at first but after 5 weeks grows faster upward (El-Fangary, 1970 & Mansour, 1969). Invading plants by the pathogen result in a reduction of the vascular bundles size of maize stalks (Abd El-Ghani, 1987), in addition to blocking in many xylem vessels (Abd El-Rahim *et. al.*, 1998 and Abd El-Ghani, 1987) and finally lack of water so wilting symptoms appear. Wilting may be appearing after tasseling stage until shortly before maturity (Samra *et. al.*, 1963).

In spite of more than 40 years of research on the pathology, anatomy and genetics of this fungus, there is no report about asymptomatic or latent infection of *C. maydis* until now. The aim of this investigation is an attempt to answer the following question; is *C. maydis* having asymptomatic infection as those of Fusarium kernel rot (King and Scott, 1981) or not ?

MATERIALS AND METHODS

Three experiments were conducted during 2008, 2009 and 2010 growing seasons under greenhouse in Maize and Sugar crops Disease Research Section (MSDRS), Plant Pathology Research Institute (PPRI), Agricultural Research Center (ARC), Giza. Experiment-one; sixteen maize hybrids (Table 1) were screened for symptomatic and asymptomatic infection by *Cephalosporium maydis* the causal agent of corn late wilt disease in 2008. The inoculum was a mixture of 5 isolates (75g/25cm-pot) collected from corn the previous year from different governorates. Experiment-two, was to test the effect of inoculum density on symptomatic and asymptomatic infection % by the fungus. The pre-tested susceptible hybrid SC 3062 was used under 11 inoculum densities of one fungal isolate in 2009. Ten, 25, 50, 75, 100, 125, 150, 175, 200, 225 and 250 gm of grain sorghum colonized by *C. maydis* (one isolate) were used on 25cm diameter pot as inoculum. Experiment-three was in 2010, which ten hybrids and their parents (inbred lines) were evaluated against *C. maydis* to determine symptomatic and asymptomatic infection % by the fungus on these parents as well as their crosses. One hundred and fifty gram of grain sorghum colonized by *C. maydis* (the same isolate in experiment two) was added to 35 cm diameter pots.

In all experiments, inoculum of *C. maydis* was prepared by incubating inoculated autoclaved sorghum seed for about two weeks at 27C. Each hybrid was replicated in five randomized pots. Each pot was sowed by 7 seed. After 21days the stand plants were 5/pot. Pots were approximately irrigated twice a week. Fifteen gm of P2O5 (15%) were added before sowing meanwhile 10 gm urea (46.5%), as a source of nitrogen, were added twice at 15 and 30 days after planting. Isolates were identified by MSDRS and seeds of maize were supplemented by Maize Research Section, Crops Research Institute, ARC, Egypt.

For determination the incidence of symptomatic infection %, the number of plants showing symptoms of *C. maydis* was counted after 95 days from sowing, then it was converted to percentage. The incidence of asymptomatic infection % was based on the number of healthy appearing plants (those with no symptoms of infection) from which the fungus was isolated. All healthy appearing plants were cut and surface-sterilized by 70% ethanol. Three pieces of plant pith, with three replicates, taken from the above ground internodes, were plated on PDA + 5gm yeast extract amended with streptomycin and incubated at 27C. Incidence of *C. maydis* was recorded 3-4 days after plating, and the incidence of asymptomatic infection was converted to a percentage. According to Maize and Sugar crops Disease Research Section, maize hybrids would be considered susceptible if percentage of

symptomatic infection is more than 10%. Data were subjected to analysis of variance by COSTAT virgin 3.

RESULTS

All the tested hybrids (Table 1), artificially inoculated with a mixture of five *C. maydis* isolates in 2008, showed symptomatic infection with the exception of SC 122 did not show any symptoms. Symptomatic infection percentage ranged from 0 to 100%. The single crosses 122, 103, 125, 124 and 3080 exhibited a resistant reaction since the symptomatic infection was 0, 4, 4, 8 and 9% respectively. All screened hybrids (Table 1), resistant or susceptible, showed asymptomatic infection by the fungus with a different levels ranged from 8 to 68%. The relationship between the degree of resistance and the incidence of asymptomatic infection percentage by *C. maydis* was significantly negatively correlated among the tested hybrids (r =-0.41).

With the using of 11 inoculum densities of *C. maydis* (Table 2), the susceptible hybrid SC3062 exhibited different levels of symptomatic and asymptomatic infection ranged from 15 to 95 % and from 5 to 45% respectively. Increasing inoculum density of the fungus resulted in increasing the symptomatic infection %, meanwhile asymptomatic infection was decreased. Beginning of 50 gm of fungus inoculum, asymptomatic infection % completed the % of symptomatic infection (Table2). There was a negative correlation among symptomatic infection % and asymptomatic infection%
(r = -0.7).

Table 1. Means of symptomatic and asymptomatic infection (%) on 16 maize hybrids caused by *Cephalosporium maydis* evaluated under greenhouse conditions in 2008 growing season.

Hybrids[a]	Symptomatic infection		Disease reaction[c]	Asymptomatic infection	
	%	Transformed values[b]		%	Transformed values[b]
SC 10	16	1.75	S	36	3.36
SC 12	20	1.91	S	40	3.23
SC 103	4	0.51	R	68	4.56
SC 122	0	0	R	56	4.21
SC 124	8	1.02	R	52	3.65
SC 125	4	0.51	R	36	3.07
SC129	60	4.21	S	32	2.50
SC 3062	100	5.74	S	-[d]	-
SC 3080	9	1.09	R	26	1.84
SC Drakhma	51	3.47	S	45.3	2.99
TWC 311	53	4.05	S	19	1.90
TWC 323	43	3.60	S	14.7	1.69
TWC 325	24	2.13	S	12	1.24
TWC 327	12	1.24	S	20	1.62
TWC 351	31	2.47	S	22.7	2.06
TWC 352	35	8.64	S	8	1.02
LSD 5%		**1.99**			**2.32**

[a] SC= Single Cross, TWC= Three Way Cross. [b] values of infection percentages were transformed into degrees of Angle.

[c] **R=Resistant, S= Susceptible** [d] **All plants showed symptoms.**

In 2010 experiment, all tested inbred lines and some of their hybrids (Table 3) showed symptomatic infection ranged from 5 to 100% with the exception of inbred line 629 did not show any symptoms. Inbred lines 655, 653 and the hybrid SC124 exhibited a resistant reaction since the symptomatic infection % was 5, 6 and 10% respectively. Asymptomatic infection % of all inbred lines and tested hybrids ranged from 5 to 55%. Also there was a fluctuation in symptomatic infection percentage of the hybrids SC10, SC122, SC124 and SC129 previously evaluated in 2008 (Tables, 1&3). These hybrids exhibited symptomatic infection percentage 16, 0, 8 and 60% respectively in 2008. Meanwhile in 2010 experiment they exhibited 100, 50, 10 and 20% of symptomatic infection respectively. There was no positive correlation between inbred lines or hybrids reaction (resistance or susceptible) and asymptomatic infection percentage of them (r=-0.4).

Table 2. Means of symptomatic and asymptomatic infection (%) on maize SC3062 caused by *C .maydis* using 11 inoculum densities in greenhouse, 2009 growing season.

Inoculum density (gm / 25cm pot)	Symptomatic infection		Asymptomatic infection	
	%	Transformed values [a]	%	Transformed values [a]
10	15	1.4	38.3	3.0
25	30	2.3	45	3.3
50	68.3	4.2	23.3	2.0
75	70	4.3	20	2.0
100	78.3	5.0	21.6	2.0
125	68.3	4.7	26.6	3.0
150	95	5.6	5	1.0
175	95	5.6	5	1.0
200	80	5.0	20	2.0
225	88.3	5.4	11.6	1.2
250	90	5.4	10	1.2
LSD 5%		**1.8**		**NS**

[a] **values of infection percentages were transformed into degrees of Angle.**

DISCUSSION

Fluctuations in symptomatic infection by *C. maydis* from year to year at the same location make the tested hybrids varied in their susceptibility resulting erratic reaction against the disease. These fluctuations in symptomatic infection previously exclusive by environmental factors (Singh & Siradhana, 1987) unfavorable to fungus development and affecting on symptom appearance. Also, by the changing of inoculum isolates as reported by Mansour (1969) who stated that, inbred line K6 showed a considerable degree of resistance against the late wilt disease. Meanwhile, in another experiment it showed inconsistent reaction when different strains of the fungus were used. In our research asymptomatic infection of *C. maydis* is evident and this as well as changing of inoculum isolates may be explaining the reasons of these fluctuations. In asymptomatic infection, however, the absence of symptoms is probably due to the host-induced quiescence of the

pathogen. Quiescence may be induced by some aspect of host physiology, such as low pH or a deficiency of oxygen or sugar in infected tissues (Mussell, 1980). Since the pathogen, *C. maydis*, invade resistant or susceptible inbred lines and hybrids as well as there was no positive correlation between the type of disease reaction (resistance or susceptible) and the incedince of asymptomatic infection, so the defense type of maize plants against *C. maydis* is tolerance.

Table 3. Means of symptomatic and asymptomatic infection percentage on 10 maize hybrids and their parents caused by *C. maydis* under greenhouse conditions in 2010 growing season.

Hybrids and inbred lines [a]	Symptomatic infection		Disease reaction [c]	Asymptomatic infection	
	%	Transformed values		%	Transformed values [b]
SC162(639x653)	70	4.8	S	20	2.2
SC163(649x654)	55	4.1	S	25	2.5
SC164(639x655)	70	4.7	S	30	2.7
SC166(639x656)	80	5.1	S	10	1.0
SC168(639x658)	75	5.0	S	25	2.5
SC10 (7x 63)	100	5.7	S	-	-
SC122 (628x603)	50	4.0	S	10	1.0
SC123 (628x602)	85	5.1	S	15	1.5
SC124 (629x603)	10	1.0	R	55	4.0
SC129 (628x612)	20	1.8	S	50	4.0
IL 639	100	5.7	S	- [d]	-
IL 649	100	5.7	S	-	-
IL 653	6.3	1.0	R	6.3	1.0
IL 654	21.3	2.3	S	5	1.0
IL 655	5	1.0	R	10	1.3
IL 656	30	2.7	S	15	1.5
IL 501	20	2.0	S	23.3	2.7
IL 63	75	4.3	S	25	1.4
IL 628	44	3.2	S	22	1.8
IL 602	55.4	4.2	S	44.6	3.4
IL 603	13.3	1.5	S	23.3	2.0
IL 629	0	0	R	23.8	1.9
IL 613	100	5.7	S	-	-
IL 7	100	5.7	S	-	-
LSD 5%		**1.8**			**2.3**

[a] SC= single cross and IL = Inbred line [b] = values of infection percentages were transformed into degrees of Angle.

[c] R= Resistant and S= Susceptible. [d] = All plants showed symptoms.

Finley, based on the occurrence of asymptomatic infection by *C. maydis* the causal agent of maize late wilt disease, by using inoculum of a single isolate, a mixture of isolates and different levels of its density, it is concluded that asymptomatic infection of late wilt disease is evident. Because of transmitting *C. maydis* by the seed (Michail *et. al.*, 1999), asymptomatic infection can exist throughout the plant, and the fungus can develop systemically to infect the kernels as those of *Fusarium moniliforme* (Kedera *et. al.*, 1992 & Munkvold *et. al.,* 1997). So, additional studies are

recommended to study its occurrence under field conditions and to determine if it plays a role to transmit the fungus by the kernel or not especially we isolated the fungus from healthy appearing field plants (unpublished data).

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الإصابة المستترة للذبول المتأخرفى الذرة الشامية المتسبب عن الفطر سيفالوسبوريم مايدز

عبدالله أحمد على النجار و أحمد محمدبشير صبرى

قسم بحوث امراض الذرة الشامية والمحاصيل السكرية - معهد بحوث أمراض النباتات - مركز البحوث الزراعية - الجيزة – مصر

أجريت خلال الأعوام ٢٠٠٨، ٢٠٠٩، ٢٠١٠ ثلاث تجارب تحت ظروف الصوبة لتحديد نسبة الإصابة المستترة (نسبة النباتات السليمة ظاهريا والتى تحتضن الفطر) والظاهرة بفطر سيفالوسبوريم مايدز المسبب لمرض الذبول المتأخر فى الذرة الشامية. تراوحت نسبة الإصابة المستترة من ٥-٦٨% بينما تراوحت نسبةالإصابة الظاهرة من صفر- ١٠٠%. لم يكن هناك إرتباطا موجبا بين درجة المقاومة وبين نسبة الإصابة المستترة. بناءً على حدوث الإصابة المستترة (بإستخدام عزلة فطرية واحدة أو مجموعة من العزلات وأيضا تحت ظروف إستخدام سلسلة تركيزات من الفطر المسبب) للأصناف الحساسة والمقاومة نخلص بأن الإصابة المستترة مثبتة وأن نباتات الذرة الشامية تستخدم التحمل كوسيلة دفاعية ضد الفطر المسبب لمرض الذبول المتأخر.

قام بتحكيم البحث

أ.د / محمود احمد المزاتى	**كلية الزراعة – جامعة المنصورة**
أ.د / مايسه عبد الحميد مرسى	**مركز البحوث الزراعية**