

RECOVERY OF *Cephalosporium maydis* THE CAUSAL AGENT OF MAIZE LATE WILT DISEASE FROM VARIOUS PLANT PARTS WITH SYMPTOMATIC OR ASYMPTOMATIC INFECTION AND ANATOMICAL CHARACTERS OF TOLERANT AND SUSCEPTIBLE HYBRIDS.

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ABSTRACT

Recovery percentage of *C. maydis*, the causal agent of maize late wilt disease, was determined under greenhouse conditions in 2010 in three plant parts; i.e. second internode of stalk above the ground level (SIS), ear shank internode (EI) and tassel internode (TI), of ten maize hybrids with and without symptoms. The two maize hybrids, SC155 (susceptible) and SC124 (tolerant) were selected from the previous experiment to assess recovery percentage and seven anatomical parameters in 2011. These parameters were; vascular bundles number (VBN) / 0.49 mm² of area of stalk section, metaxylum cells number (MCN), protoxylum cells number (PCN), metaxylum cells diameter (MCD), protoxylum cells diameter (PCD), maximum of scleronchyma cell layers number (MASCLN) and minimum of scleronchyma cell layers number (MISCLN). These parameters were measured at the same time in cross sections of symptomatic (SI), asymptomatic (AI) and non infected (NI) plants by the agent. Recovery percentage in plants with symptomatic infection differed among hybrids depending on the degree of tolerance. The recovery percentage ranged from 10 to 85 % in SIS, from 10 to 85 % in EI, and from 10 to 69 % in TI. In apparently healthy plants, recovery percentage ranged from 10 to 70 % in SIS, from 0 to 50 % in EI and from 0 to 20 % in TI. However, in 2011 experiment, the recovery percentage of the susceptible hybrid was significantly higher than those of the tolerant ones in all three plant parts with or without symptoms. Concerning the measurements of anatomical parameters, the susceptible maize hybrid had number of vascular bundles / 0.49 mm² of section area significantly higher than those of the tolerant ones only in plant sections with symptomatic and asymptomatic infection. Also tolerant plants with SI, AI, and NI showed significantly higher levels in maximum number of scleronchyma cell layers than the susceptible ones indicating that disease tolerance was associated with specific structure. In summary, *C. maydis* not only had the ability to invade both the tolerant and susceptible maize plants but also moves from above ground internode to ear shank internode and tassel internode and may reach to the kernels through the cob and completing its disease cycle.

Keywords: *Cephalosporium maydis*, maize, Symptomatic, asymptomatic, anatomical parameters.

INTRODUCTION

Late wilt disease of maize incited by the soil-borne as well as the seed-transmitting (Michail *et. al.*, 1999) agent *Cephalosporium maydis* Samra, Sabet & Hingorani (Samra *et. al.*, 1962 & Samra *et. al.*, 1963). In Egypt, The disease is a principal limiting factor in production. This disease also has been reported in India (Payak *et. al.*, 1970) and Hungary (Pecsi and Nemeth, 1998). The agent 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).

Sabet *et al.* (1970) reported that the agent grows superficially on maize roots, producing hyphae with short, brown, thick-wall, and swollen cells. It progresses inter- and intra-cellularly reaching to the root endodermis and the xylem after 15 and 21 days after sowing respectively. After the agent penetrates the xylem, it grows slowly at first but after 5 weeks grows faster upward (El-Fangary, 1970 & Mansour, 1969). Previous investigators revealed that invading plants by the pathogen result in a reduction of the number (Abd El-Rahim *et al.*, 1998) and the size of the vascular bundles (Abd El-Ghani, 1987) in maize stalks, 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 teazeling stage until shortly before maturity (Samra *et al.*, 1963). Anatomical structure of corn roots in relation to their resistance to late wilt disease was investigated by Saeed *et al.* (1990). They reported that the susceptible corn inbred lines had less collenchyma cell layers in their exoderms than those of the resistant inbred lines. Also they found that the resistant lines had higher content of sclerenchyma cells in vascular bundles and higher number of xylem vessels than those of the susceptible inbred lines. El-Naggar and Sabry (2011) demonstrated that *C. maydis* invaded both the susceptible and tolerant plants and symptomless infection of maize plants by the agent was evident.

Although researchers investigated pathology and anatomy of the disease, there is no report about the recovery of the agent through up-ground plant parts of tolerant hybrids with the exception of Michail *et al.* (1999). They attempted to isolate *C. maydis* from maize ear and seed parts obtained from infected plants or taken from lots of maize research section. Also there are more investigations about anatomical studies on plants with symptomatic infection but there are no reports on this in maize plants with asymptomatic infection by *C. maydis*.

The experiments described herein were conducted to determine, which plant parts are reached by the agent in tolerant and susceptible maize hybrids. The second objective was to determine whether infection of these hybrids (tolerant and susceptible) influences anatomical characters.

MATERIALS AND METHODS

Ten maize hybrids (Table 1) were screened for symptomatic and asymptomatic infection by *C. maydis* the causal agent of maize late wilt disease at greenhouse in 2010. At the same time the recovery percentage of the agent was assayed in three plant parts (second internode of stalk above the ground level (SIS), ear shank internode (EI) and tassel internode (TI)). In 2011 maize hybrids SC 124 (tolerant) and SC155 (highly susceptible) were chosen according their reaction in previous experiment (2010) to carry out the anatomical study. All experiments were conducted under greenhouse at Maize and Sugar Crops Disease Research Section (MSDRS), Plant Pathology Research Institute (PPRI), Agricultural Research Center (ARC) in Giza. Seed hybrids were sowed in 35cm-diameter clay pots infested with the agent.

Grain sorghum colonized by *C. maydis* (isolate No.29, obtained from Sids Agricultural Research Station, ARC) was added to the pots (15gm/kg soil). Inoculum of *C. maydis* was prepared by incubating inoculated autoclaved sorghum seed for about two weeks at 27 °C. Each hybrid was replicated in five randomized pots. Each pot was sowed by 7 seed. After 21 days the stand plants were thinned to 5/pot. Pots were approximately irrigated twice a week. Fifteen gram 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. Disease assessment was after 105 days after sowing.

Determination of symptomatic and asymptomatic infection percentage:

For determination of symptomatic infection percentage, the number of plants showing symptoms of *C. maydis* were counted then it was converted to percentage. The incidence of asymptomatic infection percentage was based on the number of healthy appearing plants (those with no symptoms of infection) from which the agent was isolated.

Isolation of *C. maydis* from plant parts:

All symptomatic and healthy appearing plants were cut above the ground level directly and dissected into above ground internodes (first and second internode), ear shank internode and tassel internode. Plant parts were surface-sterilized by 70% ethanol and flamed. Four pieces of plant pith taken from each plant part were plated on PDA + 5gm yeast (*Saccharomyces cerevesia*) extract amended with streptomycin and incubated at 27°C. Recovery of *C. maydis* was recorded 3-4 days after plating, and the recovery percentage in plant parts of symptomatic and asymptomatic infection was converted to a percentage.

Anatomical studies:

The experiment conducted in 2011 was to select plants with symptomatic, asymptomatic infection and non infected plants (control) of the two maize hybrids extremely differed in their reaction against *C. maydis*. The stalks, second and third internode above the ground level, of these plants were divided into two portions; the first one was used to recovery the agent as described above and the second was to the anatomical studies. Plant specimens, 3-5 cm long, was immediately immersed in the FAA solution (formalin, acetic acid, ethyl alcohol 70% and water in ratio 10: 5: 50 and 35 ml respectively) until use. The fixed specimens were dehydrated by passing through degraded series of ethyl alcohol as described by Sass (1958). Dehydration was performed in increasing concentrations of ethanol and N-butanol series, and then embedded in a paraffin wax, 58°C melting point, according to Johanson (1940). Ten microns thick sections were made by a rotary microtome. Sections were mounted on cleaned slides with Haupt,s adhesive (1gm gelatin + 100ml water + 2gm phenol + 15ml glysol) as mentioned by Johanson (1940). Slides were left to complete dryness for 24 hr in dry oven at 40°C. Sections were stained with 1% safranin and 1% light green, cleared in xylene, mounted in canda balsam and examined microscopically. Sections were photographed in OLYMPUS (BH4) microscope equipped with built in digital camera (DCM310, USB2.0) and transformed to a computer using Minise Software. Sections were tested for; fungal distribution, number of; vascular bundles / 0.49 mm² of stalk section,

protoxylum and metaxylum cells as well as their diameter and finally number of scleronchyma cell layer.

For measuring number of vascular bundles, ten microscopic fields with three sections (replicates) of each hybrid were used with the aid of ocular micrometer, after calibrated using 0.01mm stage micrometer slide (POLAND, WARSZAW Company) at 10X. Then the values converted to the number by area (0.49 mm²). Concerning of the number of metaxylum and protoxylum cells as well as the number of scleronchyma cell layer, they were selected randomly using 10 vascular bundles with three replicates. Also, metaxylum and protoxylum diameters were performed with the aid of ocular micrometer after its calibration using 0.01mm micrometer slide.

Statistical analysis:

All obtained data of greenhouse experiments were transformed to arcsine before carrying out analysis of variance (ANOVA) plus 0.01 to normalize and stabilize variance. Greenhouse experiments were designed as a complete randomized design in four replicates of 35cm-diameter clay pots in 2010. Each hybrid was analyzed individually by ANOVA and Duncan's multiple range test to compare means of recovery percentage among the three plant parts; first with symptomatic alone and the second with asymptomatic infection by the agent. The histograms were obtained using Excel program, Windows XP 2003. Concerning the experiment of 2011 having complete randomized design, was also performed with five replicates (pots). Analysis was performed in the two hybrids with three plant parts once in symptomatic infection and the second in asymptomatic infection. Finally, number of vascular bundles, metaxylum and protoxylum cells, diameter of protoxylum and metaxylum cells as well as number of scleronchyma cell layers were analyzed as in experiment of 2011 for the two hybrids with symptomatic, asymptomatic infection and healthy plant (non infected plant). ANOVA was performed with COSTAT version 9 software.

RESULTS

In 2010 experiment, figs 1 & 2, all examined hybrids showed symptoms of late wilt disease and varied in their levels of symptomatic infection percentage. The lowest degree of symptomatic infection, exhibited by SC124 and SC129, were 10 and 15 % respectively. At the same time the remaining examined hybrids showed high level of symptomatic infection with the range of 50 %, SC122, to 85 %, SC123. All plant stalks with symptomatic infection were colonized by the agent where *C. maydis* was recovered. Results, figs 1 & 2, show also that *C. maydis* reached to ear shank internode (EI) and tassel internode (TI) of most symptomatic plants in tolerant or susceptible hybrid with the percentage ranged from 10 %, SC124 to 85 %, SC123, and from 10 %, SC124, to 69 %, SC123, respectively. The recovery percentage of the agent obtained from tassel internode always lower than that either of ear shank internode or above-ground internode with the exception of the resistant hybrid SC124, the percentage of fungal recovery was equal in all three plant parts.

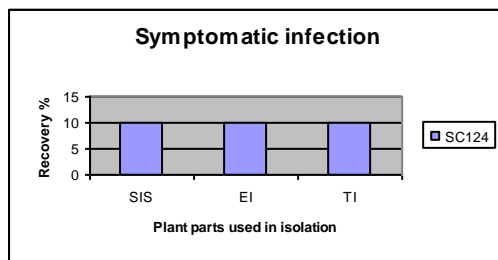


Fig.1: Recovery percentage of *C. maydis* from three plant parts of five maize hybrids with symptomatic and asymptomatic infection of plants grown in infested soil in 2010. Second internode above ground level (SIS), ear branch internode (EI) and tassel internode. a, b, ab and c indicates a significant differences among plant parts for recovery percentage.

Fig.2:Recovery percentage of *C. maydis* from three plant parts of five maize hybrids, with symptomatic and asymptomatic infection of plants grown in infested soil in 2010. Second internode above ground level (SIS), ear branch internode (EI) and tassel internode. a, b, ab and c indicates a significant differences among plant parts for recovery percentage.

Concerning healthy appearing plants, it ranged from 15 to 90%. Figs 1 & 2 show also that asymptomatic infection was in between 10 %, SC166 – 70 %, SC124. On the other hand, the agent reached also to tassel internode with recovery 20 %. The hybrids with the lowest degree of symptomatic infection, SC124 and SC129, exhibited the highest % of fungal recovery in plants without symptoms (asymptomatic infection), since the recovery % in above ground internode were 70 and 55 % respectively compared with the other tested hybrids. Finally, most healthy appearing plants of the tested hybrids showed asymptomatic infection and the agent was approximately recovered from most of the three plant parts with some exception.

Concerning the experiment conducted in 2011, Table 1, show that the selected two hybrids, SC124 and SC155, varied significantly in their symptomatic infection since it was 14 and 54 % respectively. Also they varied significantly in the recovery percentage from the three plant parts of plants with symptomatic infection. In contrast to results obtained in 2010, recovery % of the second internode of the tolerant hybrid SC124 with asymptomatic infection was less than that of the susceptible ones. Results of table 1, also revealed that there were significant differences within and between hybrids in *C. maydis* recovery percentage obtained from the three plant parts under symptomatic infection.

Table 1: Mean of infection and recovery percentage in three plant parts of two maize hybrids, SC124 tolerant and SC155 susceptible, with symptomatic and asymptomatic infection by *C. maydis*, planted in infested soil under greenhouse in 2011.

Cultivars	Symptomatic			Apparently healthy plants				
	Infection %	*Recovery % in			%	**Recovery % in		
		Second internode	Ear shank internode	Tassel		Second internode	Ear shank internode	Tassel
SC124	14	14 (22)***	14 (22)	14 (22)	86 (68)	18 (25)	13 (21.1)	4 (11.5)
SC155	54	54 (47.3)	54 (47.3)	50 (45)	46 (42.7)	33 (35.1)	23 (28.7)	18 (25.1)

LSD (0.05) for symptomatic infection; plant parts= 8.1, for hybrids= 6.6 and for interaction = 11.6. LSD (0.05) for asymptomatic infection; plant parts= 8.5, for hybrids= 7.0 and for interaction = 12.6. LSD assayed from transformed data to arcsine.

* Recovery % represents the number of plants with symptoms of late wilt disease exhibited *C. maydis* on PDYA divided by the total grown plants then multiply by 100.

** Recovery % represents the number of plants exhibited *C. maydis* on PDYA from the healthy appearing plants multiply by 100. *** transformed data.

Anatomical study:

The anatomy of stained, transverse sections of maize stalk fits the previous description by Esau (1965) (Fig.4). Fungal hyphae were not visible in any of the two non infected maize hybrids, SC124 and SC155, (control plants). In both sections of the tolerant hybrid, SC124, and the susceptible ones, SC155, vessel elements of plants with symptomatic infection were occluded by the agent. However, the susceptible hybrid was more occluded than the resistant ones (Fig.4-1&2).Whereas, sections of the two hybrids with asymptomatic infection showed slight hyphal growth into vessel elements (Fig.4-3&4).

Fig. 3: Mean of vascular bundles number / 0.49 mm² of area of maize stalk section (A), metaxylum and protoxylum cells number and diameter (B&C) and (D&E) respectively and maximum & minimum of scleronchyma cell layers number (F&G) in maize hybrids SC124 and SC155, with symptomatic and asymptomatic infection by *C. maydis* compared with their healthy plants. * indicates a significant differences between measured anatomical parameters in the two hybrids.

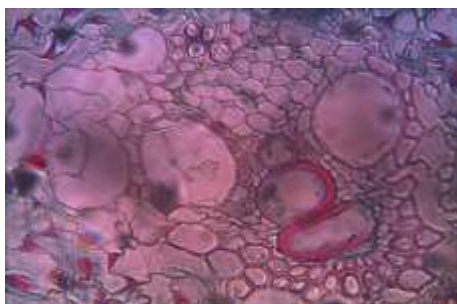


Fig. 4: Portions of a transverse sections of maize stalk of; 1, tolerant maize hybrid, SC124 with symptomatic infection by *C. maydis* showing vessel elements, one of which is semi occluded and others contain a few mycelium (X40). 2, susceptible maize hybrid, SC155 with symptomatic infection showing collapsed cortex, and vessel elements one of which is occluded and others contain mycelium (X40 with slight zoom). 3 &4, tolerant and susceptible maize hybrids, SC124 and SC155 with asymptomatic infection showing vessel elements contain a slight mycelium (X40). 5&6, tolerant and susceptible maize hybrids, SC124 and SC155 with no infection showing vessel elements free from the agent. Cortex (C), mycelium (M), metaxylem (MX), protoxylem (PX) and sclerenchyma cell layer (SCL).

The susceptible hybrid, SC155, showed more significant differences in number of their vascular bundles / 0.49 mm² than that of the tolerant ones, SC124, under symptomatic and asymptomatic infection by *C. maydis* (Fig.3A). At the same time there were no differences between the two hybrids with non infected plants. However, the number of metaxylum and protoxylum cells of sections of the two hybrids were not varied significantly with symptomatic, asymptomatic infection and noninfected plants (Fig.3B&C). With respect to metaxylum and protoxylum cells diameter (Fig. 3D&E), only with non-infected plants (control) protoxylum cell diameter of SC155 had more diameters (32.8um) than SC124 (26um). Finally, the tolerant hybrid SC124 had more significant variation in their maximum scleronchyma cells layer (5.7, 5.6 and 5.6 / vascular bundle) than the susceptible ones (4, 4 and 4 / vascular bundle) with plants of symptomatic, asymptomatic and no infection respectively (Fig. 3F&G).

DISCUSSION

Late wilt is one of the most persistent and destructive diseases of maize in Egypt. The higher recovery percentage of *C. maydis* from symptomatic and asymptomatic maize plants in the above ground parts revealed that the agent not only had the ability to invade both the most tolerant and susceptible maize plants but also takes away from above ground internode to ear shank internode and tassel internode. Our finding is some consistent with the observation of Sabet *et al.* (1966) they reported that *C. maydis* was observed close to the nodes up to the fourteenth one and also present in the cob stalk of naturally and artificially infected plants with symptoms of late wilt disease. At the same time, this result is in contrast to earlier work in which *C. maydis* attacks only the susceptible hybrids (EL-Fangary, 1970). *C. maydis* has been documented by El-Naggar and Sabry (2011) as a pathogen causing asymptomatic infection of maize plants and the plants depend on the tolerance as a tool of defense against the agent.

Anatomical study revealed that the number of vascular bundles / 0.49 mm² of area in section of susceptible maize hybrid SC155 more significant in plants with symptomatic and asymptomatic infection than non infected ones. This may be due to the shrinking occurred during development of *C. maydis* symptoms which resulted in collection of vascular bundles near to each other in plants with symptoms. Our results also are inconsistent with results of Abd El-Rahim *et al.* (1998) who found that infection by *C. maydis* resulted in a reduction of the number of vascular bundles in the cross section of maize internode.

Our anatomical study revealed that scleronchyma cell layers number serve as a specific structural factor in tolerant hybrid which may be play a role in decreasing infection or delaying symptoms development caused by the agent in symptomatic and asymptomatic infection respectively. This results agree with those of Saeed *et al.* (1990) who concluded that vascular bundles of maize roots of resistant inbred lines to late wilt disease had higher contents of sclerenchyma cells than those of susceptible lines.

Because of, the presence of *C. maydis* inside maize kernels was demonstrated in earlier work (Michail *et al.*, 1999 and Samra *et al.*, 1966), its wide spread in tested plant parts under study and cob (unpublished data) of symptomatic and seemingly healthy plants (plants with asymptomatic infection) of most tested hybrids, as well as fungal colonized in vascular bundles as obtained in our study, so the agent causes systemic infection.

Our findings and those reported by other workers showed that the disease cycle of this agent could be divided into the following stages; first, the agent 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 after 15 and 21 days after sowing respectively. After the agent penetrates the xylem, it grows slowly at first but after 5 weeks grows faster upward (El-Fangary, 1970 & Mansour, 1969). After 90 days of sowing the agent reaches to ear shank, cob and tassel (our findings). After the causal agent penetrates the cob through the ear branch, extends through the funiculus to the kernels (Michail *et al.*, 1999). Emergent plants from colonized kernels is infected (Samra *et al.*, 1966). Also the agent returned again from debris of infected plants to the soil and the cycle returned at the following season.

Finally maize plants with asymptomatic infection by *C. maydis* create a dilemma for evaluating materials and seed production even though symptomatic plants are avoided. So we need an effective method(s) for fungal elimination from maize kernels.

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تتبع الفطر سيفالوسبوريم مايدس المسبب لمرض الذبول المتأخر في الذرة الشامية في أجزاء من النباتات المصابة ذات الأعراض الظاهرية أو المستترة وكذلك الصفات التشريحية لهجين متحمل وآخر حساس للإصابة.

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أجرى الكشف عن الفطر سيفالوسبوريم مايدس المسبب لمرض الذبول المتأخر في الذرة الشامية في ثلاثة أجزاء نباتية وهي العقلة الثانية من الساق فوق مستوى سطح التربة ، عقلة حامل الكوز وعقلة النورة المذكورة لنباتات عشرة هجن من الذرة الشامية مظهرة وغير مظهرة للأعراض المرضية تحت ظروف الصوبة في موسم 2010. أيضا تم تقدير سبعة صفات تشريحية وكذلك الكشف عن الفطر المسبب للمرض لكل من الهجينان 155 الحساس و 124 المتحمل للإصابة والمختاران من التجربة السابقة بعد زراعتهما في موسم 2011. القياسات التشريحية موضع الدراسة هي عدد الحزم الوعائية/ 49م. 2م من حجم قطاع الساق، عدد خلايا الميتازيلم ، عدد خلايا البروتوزيلم ، قطر خلايا الميتازيلم ، قطر خلايا البروتوزيلم وكذلك أقل وأقصى عدد لطبقات الخلايا الإسكلرنشيمية وذلك لنباتات تحمل أعراضا ظاهرة وأخرى مستترة والثالثة بدون إصابة بالفطر. اختلفت النسبة المئوية لتواجد الفطر في الأجزاء الثلاثة لنباتات الهجن ذات الإصابة الظاهرة بناء على درجة تحملها للمرض، فقد تراوحت من 10-85% في كل من العقلة الثانية من الساق وعقلة حامل الكوز ، 10-69% في عقلة النورة المذكورة . بينما تراوحت النسبة في النباتات السليمة ظاهريا من 10-70% في العقلة الثانية من الساق، من صفر- 50% في عقلة حامل الكوز ، من صفر-20% في عقلة النورة المذكورة . أظهرت التجربة المقامة في الموسم 2011 أن نسبة تواجده الفطر في الهجين الحساس أعلى معنويا من نسبة تواجده في الهجين المتحمل في الثلاثة أجزاء للنباتات ذات الأعراض وكذلك التي بدون أعراض. أظهرت الصفات التشريحية أن الهجين الحساس يحتوى على عدد من الحزم الوعائية أعلى معنويا من الهجين المتحمل ذو الإصابة الظاهرية أو المستترة فقط، كما أظهرت أيضا أن العدد الأقصى من طبقات الخلايا الإسكلرنشيمية للنباتات ذات الإصابة الظاهرية والمستترة وغير مصابة للهجين المتحمل للإصابة يفوق الهجين الحساس معنويا مما يعكس تركيبا متخصصا له علاقة بالتحمل للمرض. نستخلص من ذلك أن الفطر سيفالوسبوريم مايدس ليست لديه القدرة فقط على غزو كل من النباتات الحساسة والمتحملة للمرض ولكن أيضا يأخذ طريقه من العقل السفلية للنبات التي فوق سطح التربة إلى العقلة الحاملة للكوز ثم إلى حامل السنبله وربما يصل إلى الحبوب عبر قولحة الكوز مكمل دورة حياته.

قام بتحكيم البحث

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