

## SUSCEPTIBILITY OF SOME TOMATO CULTIVARS TO BACTERIAL CANKER AND SPOT DISEASES AND THE ROLE OF SEEDS IN PATHOGEN TRANSMISSION\*

# [61]

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### ABSTRACT

Bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganensis* (Smith) and bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye have become important diseases of tomato in Egypt. The present work was planned to evaluate the reaction of different tomato cultivars and the role of seeds to transmit both pathogens. Cultivars of Dora, Flora-Dade, KTM141, Money Maker, Niagra, Super strain B hybrid and GS12 were moderately resistant to both diseases, but Castle rock, Peto 86 and Super strain B cultivars were susceptible. However, Castle rock, Peto 86, Super strain B and Niagra cultivars were highly susceptible for bacterial canker than spot disease. Using the Liquid assay method, *C. michiganensis* subsp. *michiganensis* bacterium could be detected in seeds of cultivars Dora, KTM141, Niagra and Super strain B hybrid, but *X. campestris* pv. *vesicatoria* was detected on seeds of cultivars GS12, Peto 86 and Super Marmand. However, both bacterial pathogens were not detected in seeds of all cultivars, except GS12, when direct planting method on selective media was used. In an *in vivo* assay, bacterial canker has developed more than bacterial spot disease on tomato seedlings produced from non-treated seeds. Bacterial canker has developed on all tested cultivars at different frequencies. Transmission of both pathogens occurred at high frequency by seeds of cultivars Niagra and Dora, however bacterial spot was not borne on samples of other tomato cultivars

**Key words:** Bacterial canker, Bacterial spot, Tomato, *Lycopersicon esculentum* Mill, *Xanthomonas campestris* pv. *vesicatoria*, *Clavibacter michiganensis* subsp. *michiganensis*, Seed-borne bacteria, Transmission

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## INTRODUCTION

Bacterial canker caused by *C. michiganensis* subsp. *michiganensis* (Smith) and Bacterial spot caused by *X. campestris* pv. *vesicatoria* (Doidge) Dye have caused major economic losses in different commercial tomato (*Lycopersicon esculentum*, Mill) production worldwide, in open-fields especially in the rainy weather conditions or under moist conditions (Jones *et al* 1991; Chang *et al* 1992; Bouzar *et al* 1994 and Quezado-Duval *et al* 2004). In Egypt, bacterial canker disease was detected on tomato plants grown in certain reclaimed areas (El-Abyad *et al* 1993 and Abd El-Sayed, 2002), while bacterial spot disease was observed in several tomato production areas (Abd El-Ghafar and Abd El-Wahab, 2001).

It was postulated that both diseases were distributed in several locations in Egypt due to: 1) cultivation of imported seeds of different hybrids and cultivars, 2) both pathogens are reported to be seed-borne and 3) favorable conditions as high relative humidity, especially when tomato plants are grown under protected cultivation system. (Abd El-Ghafar and Abd El-Wahab, 2001 and Abd El-Sayed, 2002) Several studies to detect the presence of *C. michiganensis* subsp. *michiganensis* and *X. campestris* pv. *vesicatoria* in tomato seeds were

mentioned with using two techniques *i.e.* planting seed extracts on semi-selective media and inoculation of host plant (Chang *et al* 1992; Kritzman, 1992; Valarini, 1998 and Abd Alla, 2000). Singh and Shrivastava (1990) found that *C. michiganensis* subsp. *michiganensis* was seed-borne in cultivars of Punjab and Pusa Ruby, when these seeds were extracted from infected fruits and developed marked symptoms on the developed seedlings. Chang *et al* (1992) mentioned that *C. michiganensis* subsp. *michiganensis* bacterium was transmitted at low rate from seed to transplants by sowing of infested seeds in the greenhouse and transplanting the seedlings to a production field. Both susceptible and moderately resistant tomato cultivars, Heinz 1810 and Heinz 7417, respectively, supported leaf surface populations of about  $10^7$  to  $10^9$  cfu/g fresh weight, respectively.

The present work was planned to evaluate susceptibility of various tomato cultivars to infection by bacterial canker and spot pathogens and the role of seed in transmission of the causal pathogens.

## MATERIAL AND METHODS

### 1. Source of seeds and production of transplants

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Seeds of tomato cultivars were obtained from different sources, where Castle rock, Gs12, Peto 86 and Super strain B cultivars were obtained from Horticultural Department, Faculty of Agriculture, Ain shams university, Cairo, Egypt. Flora-Dade and Money maker cultivars were obtained from Vegetables Department, Horticultural Research Institute, Agriculture Research Center Giza. Cultivars Dora, KTM 141, Niagra, Super strain B hybrid and Super Marmand were obtained from Local market, Cairo, Egypt.

Fungicide-free seeds were surface sterilized in solution of sodium hypochlorite (1%) for 2 min. The seeds were sown in seedling trays containing pasteurized peat-moss and vermiculite (1:1v/v). Trays were kept under greenhouse conditions and irrigated regularly. Tomato seedlings (4-week-old) were transplanted to clay pots (20 cm diameter) containing sandy clay soil and each pot contained three seedlings.

## 2. Preparation of inoculum

Two strains of *X. campestris* pv. *vesicatoria* (Xcv1 and Xcv5) and *C. michiganensis* subsp. *michiganensis* (Cmm4 and Cmm5), isolated from infected tomato plants in Egypt by the authors, were used throughout the study. These isolates were grown on yeast extract peptone agar (YPA) medium at 28°C for 48hr. Bacterial growth was suspended in sterile saline buffer solution (0.85 NaCl) and centrifuged at 3000g / min for 30 min. The pellet was re-suspended in sterile distilled water (SDW) to obtain concentration of  $5 \times 10^8$  and  $3 \times 10^8$  (cfu) /ml for isolates Xcv and

Cmm, respectively as determined from a standard curve based on absorbance at  $A_{600 \text{ nm}}$ , using Spectrophotometer (**Wang et al 1994** and **Hausbeck et al 2000**).

## 3. Evaluation of tomato cultivars

Ten tomato cultivars were examined for their susceptibility to bacterial canker and spot diseases, under artificial inoculation conditions. After three weeks from transplanting, tomato plants were inoculated using bacterial suspension of each isolate, individually. Stem injection method was used for *C. michiganensis* subsp. *michiganensis* where the stem was injected with a drop (0.01 ml using a hypodermic syringe) of the bacterial suspension (**Ioannou et al 2000** and **Francis et al 2001**). Leaf injection (infiltration the intercellular spaces by syringe without a needle) method was used with *X. campestris* pv. *vesicatoria* isolates (**Somodi et al 1991**). Inoculated plants were maintained in a humid chamber for 48 h before and after inoculation. Disease severity was recorded, after 10 days from inoculation on fifteen plants per cultivar, using individual disease rating scale for each pathogen. A scale from 0 to 5, where 0= no disease, 1=1-20%, 2= 21- 40%, 3= 41-60%, 4= 61-80%, 5= 81-100% of infected tissue was used for bacterial spot (**Wang et al 1994**). For bacterial canker, a scale of 0 to 5 was used, where 0= no symptoms and one point was assigned for each of the following symptoms: marginal necrosis, wilt, canker; 5= dead plants (**Francis et al 2001**).

## 4. Detection of bacteria in seeds

Detection and isolation of *C. michiganensis* subsp. *michiganensis* and *X. campestris* pv. *vesicatoria* from different tomato cultivars were carried out using two methods (direct plating on selective media (DP) and liquid assay (LA)). Hundred seeds of each cultivar were applied for detection of each pathogen and were planted on semi-selective medium (SCM) for *C. michiganensis* sub sp. *michiganensis* and tween B (TB) selective medium for *X. campestris* pv. *vesicatoria*, where seeds of each cultivar were distributed in ten plates. The plates were incubated at 26°C for 8 – 10 days and 28°C for 4 – 5 days, for Cmm and Xcv, respectively (**Fatmi and Schaad, 1988 and Gitaitis et al 1991**). In liquid assay, 0.1 gram of seeds of each cultivar was soaked in sterile saline buffer solution (10 ml) for 24 h at room temperature and aliquots (0.1 ml) of the suspension was spread onto selective medium as previously mentioned (**Fatmi and Schaad, 1988**).

### 5. Seed-seedling transmission

This experiment was carried out to evaluate transmission of bacteria from seeds of ten tomato cultivars, using an *in vivo* assay. Hundred seeds of each cultivar were plated, 10 seeds/plate, on moistened-sterile filter paper in Petri plates (15cm). The plates were incubated at 25-28°C for three weeks. Development of typical symptoms of bacterial canker and spot diseases were recorded on cotyledons and stems of young tomato seedlings. Isolation from infected tissues was made to confirm the identity of the causal pathogens. Isolates obtained from this experiment were compared with standard isolates of *C. michiganensis*

subsp. *michiganensis* and *X. campestris* pv. *vesicatoria*. Disease incidence was recorded as percentage of infected seedlings with each pathogen (**Abd El-Ghafar, 2004**).

## RESULTS

### 2. Evaluation of tomato cultivars

Data in Figure (1) show that tomato cultivars Dora, Flora-Dade, GS12, KTM141, Money Maker, Niagra and Super strain B hybrid were moderately resistant to both bacterial canker and spot diseases, under artificial inoculation conditions. Cultivars of Castle rock, Peto 86 and Super strain B were susceptible to both diseases. Meanwhile, Castle rock, Peto 86 and Super strain B cultivars were more susceptible to bacterial canker than bacterial spot.

### 2. Detection of bacteria in seeds

Data in Table (1) show that seeds of all tested cultivars gave negative reaction with direct planting method on the selective media for *C. michiganensis* subsp. *michiganensis* (SCM medium) and *X. campestris* pv. *vesicatoria* (TB medium), except cultivar GS12 which gave positive reaction with *X. campestris* pv. *vesicatoria*. In case of liquid assay, cultivars Dora, KTM141, Niagra and Super strain B hybrid gave positive reaction with *C. michiganensis* subsp. *michiganensis* and cultivars of GS12, Peto86 and Super Marmand gave positive reaction with *X. campestris* pv. *vesicatoria*. Both pathogens were not detected in tomato seeds of other cultivars.

### 3. Transmission of pathogenic bacteria by seeds

Using an *in vivo* assay, results in Fig. (2) showed that bacterial canker was developed more than bacterial spot disease on tomato seedlings produced from non-treated seeds. Bacterial canker was developed on all tested cultivars at different frequencies. Transmission of both pathogens occurred at high frequency through seeds of cultivars Niagra and Dora, and at low frequency on

cultivar Castle rock. However bacterial spot was not observed on other tomato cultivars.

### DISCUSSION

Bacterial canker and spot diseases are the most important bacterial diseases of tomato in Egypt. Cultivars of Dora, Flora-Dade, KTM141, Money Maker, Niagra, Super strain B hybrid and GS12 gave resistance reaction against both

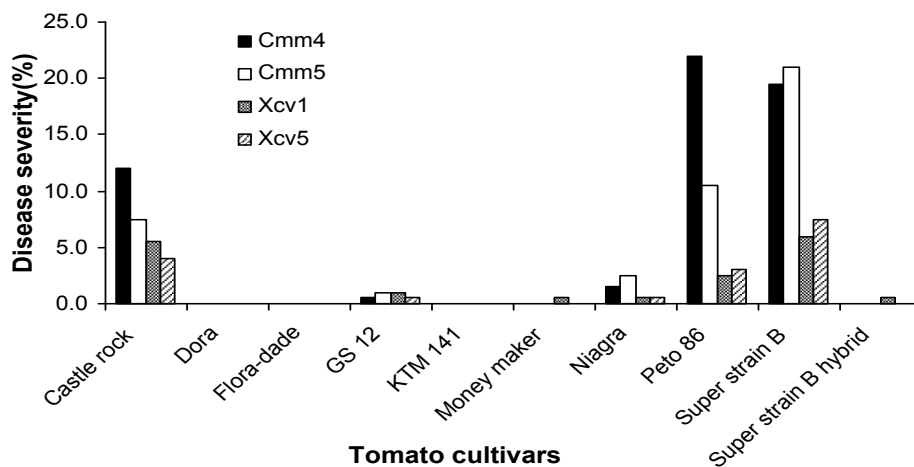


Fig. 1. Susceptibility of tomato cultivars to infection by four isolates of *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) and *Xanthomonas campestris* pv. *vesicatoria* (Xcv), under artificial inoculation conditions

Table 1. Detection and isolation of the pathogenic bacteria (*Clavibacter michiganensis* subsp. *michiganensis* and *Xanthomonas campestris* pv. *vesicatoria*) from seeds of different tomato cultivars, using direct plating or liquid assay methods

cultivar	<i>Clavibacter michiganensis</i> sub sp. <i>michiganensis</i>		<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i>	
	DP	LA	DP	LA
Castle rock	-	-	-	-
Diamant	-	-	-	-
Dora	-	+	-	-
Flora-Dade	-	-	-	-
GS12	-	-	+	+
KTM141	-	+	-	-
Money Maker	-	-	-	-
Niagra	-	+	-	-

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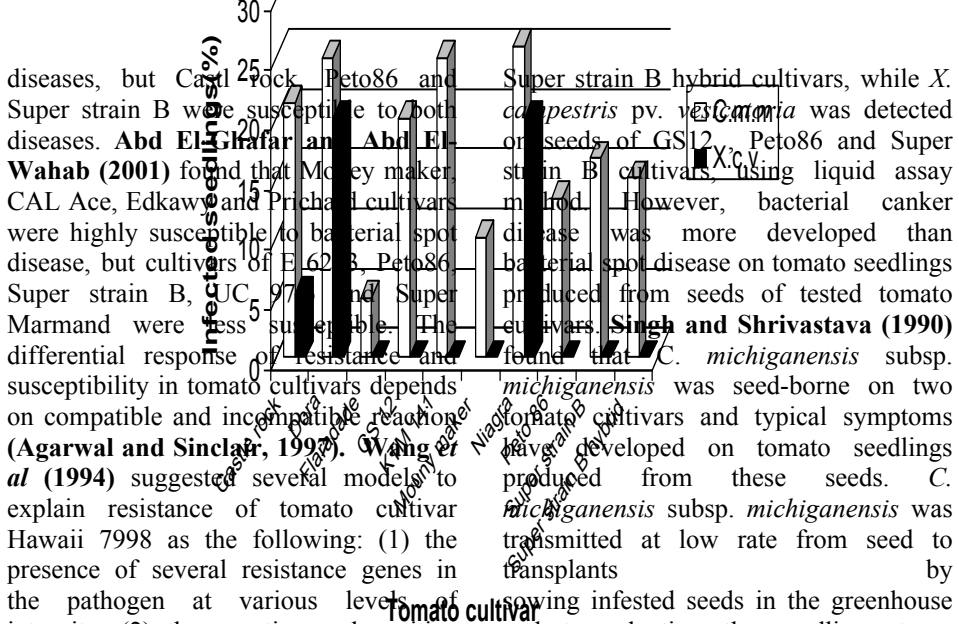
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DP= direct planting on semi selective media LA= liquid assay

(+) Positive reaction; bacteria were detected

(-) Negative reaction; bacteria were not detected

Fig. 2. Transmission of bacterial canker and spot pathogens through tomato seeds, using an *in vivo* assay



diseases, but Castle Rock, Peto86 and Super strain B were susceptible to both diseases. **Abd El-Ghafar and Abd El-Wahab (2001)** found that Money maker, CAL Ace, Edkawy and Prichard cultivars were highly susceptible to bacterial spot disease, but cultivars of E 623, Peto86, Super strain B, UC 97 and Super Marmand were less susceptible. The differential response of resistance and susceptibility in tomato cultivars depends on compatible and incompatible reactions (**Agarwal and Sinclair, 1999**). **Wang et al (1994)** suggested several models to explain resistance of tomato cultivar Hawaii 7998 as the following: (1) the presence of several resistance genes in the pathogen at various levels of intensity, (2) the negative and positive modification of a single-gene resistance by several other genes in the host and (3) the presence of two resistance systems in H. 7998, a multigenic system and a single-gene system. Synthesis and accumulation of hydrolytic enzymes were stimulated in infected tomato plants with bacterial spot pathogen, where these enzymes play important role in defense mechanisms (**Kim and Hwang, 1996**). **Pernezny and Collins (1997)** observed that epiphytic population of *X. campestris* pv. *vesicatoria* was higher on leaves and buds of susceptible cultivars (Jupiter) than on resistant cultivar (Boynton bell).

*Clavibacter michiganensis* subsp. *michiganensis* bacterium was detected in seeds of Dora, KTM141, Niagara and

Super strain B hybrid cultivars, while *X. campestris* pv. *vesicatoria* was detected on seeds of GS12, Peto86 and Super strain B cultivars, using liquid assay method. However, bacterial canker disease was more developed than bacterial spot disease on tomato seedlings produced from seeds of tested tomato cultivars. **Singh and Shrivastava (1990)** found that *C. michiganensis* subsp. *michiganensis* was seed-borne on two tomato cultivars and typical symptoms developed on tomato seedlings produced from these seeds. *C. michiganensis* subsp. *michiganensis* was transmitted at low rate from seed to transplants by sowing infested seeds in the greenhouse and transplanting the seedlings to a production field (**Chang et al 1992**). *Xanthomonas campestris* pv. *vesicatoria* was detected in 12% of imported tomato seed lots of different cultivars in Egypt (**Abd Alla, 2000**). Seed infection may occur systemically, either through the vascular system or plasmodesmatic connections or directly through floral infection or penetration of the ovary wall, seed coat or natural opening. Seed transmission of pathogens and the establishment in the host were influenced by environmental conditions, with moisture and temperature being the most important factors (**Agarwal and Sinclair, 1997**). Phytopathogenic bacteria invade seeds through the seed coat or ovule wall, where *C. michiganensis* subsp.



*michiganensis* began at the chalazal end and continued into the innermost cells of the seed coat (Patino-Mendez, 1967) and *X. campestris* pv. *vesicatoria* penetrated flowers and resulted in bacterial proliferation in warts of pepper (Bashan and Okon, 1986).

It could be concluded that tomato cultivars, commonly grown in Egypt, varied in their susceptibility to bacterial canker and spot diseases. Meanwhile, the causal pathogens proved to be seed-borne and could be transmitted to seedlings or soil in pathogen-free areas. Therefore, integrated management strategies for these diseases could consider such issues.

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رويويتك بعق اب تل اهرق تل بيض رم قبا اصل ال مظا مل فان ص من عي بل باق  
ض رم مل لق ني هو و ذبل ا

[61]

دياس ل دب ع دم حم افو و اف غل دب ني سي يي جان ولي زع لد دب ني دب اع لن ي ز ف ا ف ع  
ل ي م ح دب ع ي و ج ن دي ز ي ل و ب ا دم ح ا

قدها ق ل د ق م ي خ ل ل ا ب ش - س مشن ي ع ة ع م ا ج - ة ع ا ر ز ل ق ي ل ك ت ا ب ن ل ا ض ا ر م س ق - 1  
قدها ق ل د ق م ي خ ل ل ا ب ش - س مشن ي ع ة ع م ا ج - ة ع ا ر ز ل ق ي ل ك ي ت ا س ب ل م س ق - 2

ة ح اس م ل ة د ا ي ز ع م ق ر ي خ ل ل ة ن و ا ل ي ف  
ن ي ا ب ت و مظا مل ا ل و ص ح م ن ع ر ز ن م ل ا  
ا م ت ع ا ر ق ر ت ك و ا م ر د ا ص ع و ن ت ف ا ن ص ا ل ا  
ض ا ر م ا ل ن ي د ع ل ا ش ت ن ا و ر و ه ظ ح و ل  
ي ر ي ت ك ب ل ح ا ر ق ت ل ا ض ر ق ص ة م ي ي ت ك ب ل ا  
*vibacter* ت ك ب ن ي ع س ت م ل ا  
*michiganensis* subsp. *michiganensis*  
ا ي ر ت ك ب ن ي ع س ن ي ل ا ي ت ك ب ع ل ا ب ت ل ا ض ر م و  
*Xanthomonas campestris* pv. *vesicatoria*  
ي ع ر ا ز م ل ل ك ا ش م ل ا ن ي د ع ل ا ب س ا م  
ث ح ب ل ا ا ذ ه ي ر ج ا د ق و . مظا مل ا ل و ص ح م  
ق م و ا ق م ي ل باق ي د م ة س ا ر ض ر غ ب  
ا ل ك ق ب ا ص ا ل ة ث و ر ت س م ل ا و ي ل ح م ل ف ا ن ص ا ل ا  
ر و ذ ب ل م ي ن ا ك م ك ل ن ا ي ق ب ا س ا ن ا ي ض ر م ل ا  
ن ي ض ر م م ل ل ك ل ا ل ق ن و ف ا ن ص ا ل ا ل ل ت  
ق ي ل ا ت ل ف ا ن ص ا ل ا ن س ا ر د ل ع ل ا ت ن ت ح ض و ا

Dora, Flora-Dade, KTM141, Money  
maker, Niagra, Super strain B hybrid,  
GS12 ق م و ا ق م ت ر ه ظ ا  
astl ف ا ن ص ا ل ا ل ي ب س ا ر د ل ت ح ت ي ض ر م ل ا ة ي ك ي ت س ا ل ب ا ق ا ف ن ا ل ا و ب و ص ل ا ف و ر ط ح ت  
ت ر ه ظ ا , Peto86 , Super strain B  
rock ,  
ف ا ن ص ا ل ا ن الك ي ن ي ض ر م ل ل ا ك ق ب ا ص ا ل ا ل باق  
Castl rock, Peto86, Super strain B,  
Niagra ح ر ق ت ل ض ر م ق ب ا ص ا ل ا ل باق ث ك ا  
ن ي ك ي م ل ي ت ك ب ع ل ا ب ت ل ا ض ر م ي ن ع ي ت ك ب ل ا  
ر و ذ ب خ و ق ل ل ا و ا ت ك ب ل ز ع و ق ظ ح ا ل م  
Dora, KTM141, Niagra, Super  
Strain B hybrid ع ق ب ل ل ا و ا ت ك ب ت ظ ح و ل م ن ي ب  
GS12, Peto86 , ف ا ن ص ا ل ا و ذ ب ن م ت ل ز ع و  
ق ي ر ط ل م ا د خ ت س ا د ن ع ك ل ذ و Super marmand  
ر ه ظ ت م ل ن ي م ي ف ب و ر ك ي م ل ل ز ع ل ا س ل ا  
S12 ف ن ص ا د ع ا ق ر ب ت خ م ل ف ا ن ص ا ل ا ل ك  
ا م ر و ذ ب ل ي ف ت ك ب ل ا ن ي ع و ن ل ا ل ك د و ج و ،

أفغش كبتوتك أن الرائي تكب لجر قتل ا ضررم  
 مطامطلتار دابي ان عيتك عى لبتل ا ضررم  
 ،تربت خم لفان صأل الكو وذب نة جت ان ل ا  
 Dora, Castl rock, فان صأ تره ظأو  
 ضار عأل احضا فلوش كت KTM141, Niagra  
 حرق تل ا ضررم ضار عأ نأ عومي ضررم ل الك  
 فان صأ ا قبعش كتل قأتين الرائي تكب ل ا  
 ت حض و أو Flora-dade, Money maker.  
 تريب ت خم لفان صألها ق اب نألض جيا ت ن ل ا  
 ةي ج ذومن ضار عأل ا ر دابي في عيش ك تي  
 ن اف ةم اف ع صر يويو ي تك عى لبتل ا ضررم ل  
 ي فم مطامطل ل وذب روهك و تفس ار دلخ ط ا ت ن  
 ةي ر ص م ل افور ظل ت ح ت ا ضررم ل لفل بلق ن  
 ن م ح ل فل ا عوفي ب اد ت فا خ ت اج و ت س ي ام  
 ضار م ل لفل و ت ل ش ت ن ا

يسوم دمحا دمحا د: أمي كحت  
 تاكرب دمحق وراف دأ

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