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Sensitivity of Seven Tomato Cultivars to Fusarium Wilt under Glasshouse Conditions in Kazakhstan

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Sensitivity of seven tomato cultivars grown under glasshouse conditions in Almaty, Kazakhstan to wilt disease caused by *Fusarium oxysporum* f.sp. *lycopersici*, was evaluated. Evaluation results revealed that the tested cultivars could be classified into three groups: 1) Susceptible cultivars (Carolina Gold and Dona), 2) Highly resistant (EXP 1 and EXP 2) and moderately resistant (EXP3, EXP5 and EXP4). All investigated growth characters and fruit yield/plant were significantly lower in inoculated than un-inoculated plants of most tested tomato cultivars particularly Carolina Gold and Dona. Also, the results pronounced that the high resistant cultivars could be used as sources of resistance in the tomato breeding programs. In term of early fruit yield production, the moderately resistant EXP4 was significantly better than the highly resistant cultivars EXP1 or EXP2, so it could be subjected to further tests to introduce it as commercial cultivar in Kazakhstan.

Keywords: Fusarium wilt, growth characters and tomato cultivars.

Fusarium oxysporum f.sp. *lycopersici* (Sacc.) Snyder & H.N. Hansen is economically important wilting pathogen of tomato (Eraky *et al.*, 2007). Fusarium wilt attacks only certain tomato cultivars. Plants infected by this soil-dwelling fungus show leaf yellowing and wilting that progress upward from the base of the stem. Initially, only one side of a leaf midrib, one branch, or one side of a plant will be affected. The symptoms soon spread to the remaining parts of the plant. Wilted leaves usually drop prematurely. Management of this pathogen is difficult due to their endophytic growth and persistence in soil (Alström, 2001). Several disease management strategies are available, *e.g.* resistant cultivars, biological control, crop rotation and chemical fungicides. Furthermore, new races of the pathogen that overcome plant resistance have continued to appear (Rodríguez *et al.*, 2003). To minimize losses due to Fusarium wilt, it is advisable to plant varieties resistant to one or more races of the Fusarium fungus. Resistant varieties may become infected, but disease will not be as severe as with susceptible varieties and a reasonable yield should still be obtained (Gleason and Edmunds, 2006).

This study aimed to screen some tomato cultivars against infection with the tomato wilt fungus, *Fusarium oxysporum* f.sp. *lycopersici*. The impact of artificial inoculation with this isolate on some growth characters and fruit yield production of tested tomato cultivars was also investigated.

Materials and Methods

Tomato cultivars and pathogen inoculation:

Tomato transplants (*Lycopersicon esculentum* Mill) of different cultivars (Table 1) were used in this study. Transplants (4 weeks old) were planted in pots 30 cm diameter (3 seedlings per pot) and placed under glasshouse conditions at 25-30°C with 70% RH and watered as required.

Table 1. The tested tomato cultivars [provided by the seed company (Rijk Zwaan Ltd. - Uzbekistan)]

Used name	Experimental code	Lot number
Exp 1	EXP 8340 Tomato Seeds	088340
Exp 2	EXP 8355 Tomato Seeds	088355
Exp 3	EXP 8416 Tomato Seeds	088416
Exp 4	EXP 8420 Tomato Seeds	088420
Exp 5	EXP 8576 Tomato Seeds	088576
Carolina Gold	-	-
Dona	-	-

Preparation of fungal inoculum:

Pathogenic isolate of *Fusarium oxysporum* f.sp. *lycopersici* (designed as *FOL*), previously isolated from naturally infected tomato plants showing wilt symptoms under conditions of glasshouses in Almaty province of Kazakhstan, was used in this study. The *FOL* isolate was plated onto Potato Dextrose Agar (PDA) medium amended with streptomycin sulfate (300 mg/l) according to Amini (2009). Cultures of *FOL* (14-day-old) grown on PDA in Petri plates at 28°C were used for preparing fungal spore suspension as recommended by Youssef (2007). The resultant spore suspension was adjusted to be about "$1.0x10^6$ spores/ml" (Amini, 2009) by microscopic enumeration with the aid of a cell-counting haemocytometer. Two weeks after transplanting, spore suspension was added at the stem base of the plants (20 ml for each). Three pots (replicates) were used for each cultivar. The inoculated pots were arranged in a completely randomized block design in the glasshouse.

Disease severity assessments:

Percentages of diseased and dead plants as well as wilt disease severity (DS) for each particular cultivar were determined 2 months after inoculation. The plant height, root length, root fresh weight and total fruit yield/plant were also determined for all tested tomato cultivars. DS was estimated using a visual scale of 0-4 as following: 0= No wilting symptoms (healthy plant); 1= Plant slightly wilted, vascular discoloration found in main root region; 2= Plant moderately wilted, yellowing of old leaves, spreading vascular browning; 3= Plant severely wilted, all leaves are dead except end leaves; and 4= Dead plant, seedling entirely wilted (Vakalounakis and Fragkiadakis, 1999). Disease incidence was determined according to the formula suggested by Song *et al.* (2004). Plants were uprooted and

the lower stem and tap root were longitudinally dissected in order to examine the discoloration of the internal tissues. DS%= (1A+2B+3C+4D)/4T×100 where, A, B, C and D are the number of plants corresponding to the numerical grade, 1, 2,3 and 4, respectively and (T) is the total number of plants multiplied by the maximum discoloration grade (4), where T=A+B+C+D. For each treatment, 9 plants were used (3 plants per pot) to determine DS.

Reduction (%) of a given growth variable was calculated using the following formula: Reduction % = [value variable of non-inoculated (control) – value variable of the inoculated plants] / value variable of non-inoculated (control) × 100%.

Statistical analysis:

Recorded data were analyzed statistically according to Gomez and Gomez (1984).

Results

Percentage of wilted plants and wilt severity:

The tested tomato cultivars responded differently against artificial inoculation with the tomato wilt pathogen, *Fusarium oxysporum* f.sp. *lycopersici* (*FOL*) (Fig. 1). The tomato cultivar Carolina Gold and Dona showed the same percentage of diseased plants (77.8%) but they significantly varied in the wilt severity as they recorded 26.4 and 22.2%, respectively. However, the new experimental cultivars EXP 1 and EXP 2 were the most resistant and remained symptom-less or disease-free followed by EXP 3 (2.8%), EXP 5 (8.3%) and EXP 4 (12.5%), respectively.

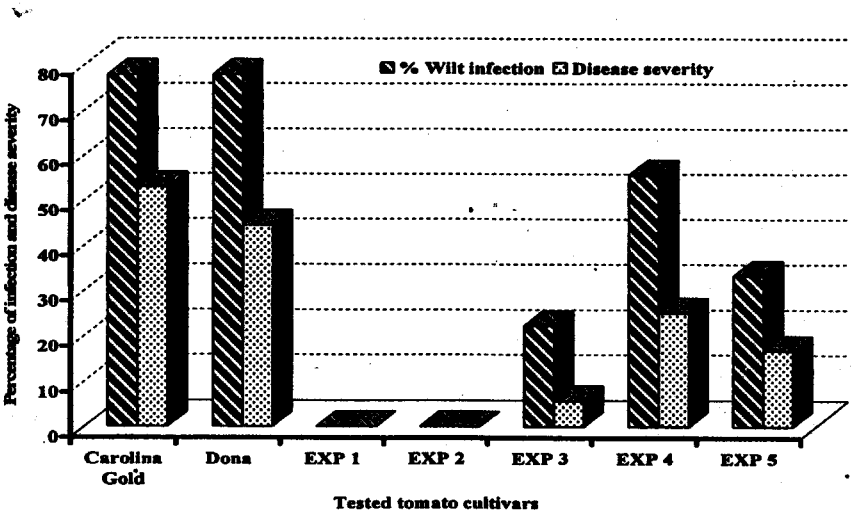

Fig. 1. Effect of inoculation with *FOL* on the percentage of disease incidence and disease severity of different tested tomato cultivars.

Plant height and root length:

Data in Table (2) reveal that both plant height and root length were significantly affected by inoculation with *FOL*. Plant height and root length were significantly decreased from 105.9 to 96.6 cm and from 37.7 to 32.4 cm on the average in inoculated and non-inoculated plants, respectively. The plant height of the three tomato cultivars, *i.e.* Carolina Gold, Dona and EXP 4 only was significantly reduced by 32.3, 13.6 and 7.8% compared to their non-inoculated plants, respectively. However, plant height in the remained tested cultivars (EXP 1, EXP 2, EXP 3 and EXP 5) was not significantly affected by inoculation with *FOL* when compared with their respective non-inoculated control. On the opposite side, the root length of all tested tomato cultivars was significantly lower in the inoculated than the non-inoculated plants. In this regard, the highest reduction in root length (20.4-25.4%) was recorded by the most susceptible cultivars (Carolina Gold and Dona), meanwhile the lowest reduction (5.2-8.2%) was recorded by the most resistant cultivars, *i.e.* EXP 1, EXP 2 and EXP 3. Inoculation with *FOL* caused intermediate reduction (15.9-16.5%) in root length of the cultivars EXP 4 and EXP 5 (moderately resistant).

Table 2. Effect of inoculation with *FOL* on plant height and root length of different tomato cultivars

Tomato cultivar	Plant height (cm)			Reduction	Root length (cm)			Reduction
	D*	H	Mean	(%)	D	H	Mean	(%)
Carolina Gold	70.0	103.3	86.7	32.3	30.3	40.7	35.5	25.4
Dona	87.0	100.7	93.8	13.6	30.0	37.7	33.8	20.4
EXP 1	107.0	109.7	108.3	2.4	41.7	44.7	43.2	6.7
EXP 2	95.7	97.3	96.5	1.7	30.3	32.0	31.2	5.2
EXP 3	114.3	115.3	114.8	0.9	37.3	40.7	39.0	8.2
EXP 4	102.3	111.0	106.7	7.8	30.0	35.7	32.8	15.9
EXP 5	100.0	103.7	101.8	3.5	27.0	32.3	29.7	16.5
Mean	96.6	105.9			32.4	37.7		
L.S.D. at 5% for:								
Inoculation	0.7				0.2			
Cultivars	2.5				0.8			
Interaction	5.0				1.7			

* D= Diseased (inoculated) plants; H= Healthy (uninoculated) control and Reduction (%)= Percentage reduction to control.

Root fresh weight and fruit yield/plant:

Inoculation of tomato plants of different cultivars by *FOL* significantly decreased the root fresh weight and fruit yield/plant of most tested tomato cultivars comparing to their respective un-inoculated control plants (Table 3).

The root fresh weight and fruit yield/plant values in the most resistant cultivars, EXP 1 and EXP 2, were the same in both inoculated and un-inoculated plants, meanwhile they were significantly decreased to different extent in the other tested tomato cultivars. The most susceptible cultivars Carolina Gold and Dona recorded

Table 3. Effect of inoculation with *FOL* on root fresh weight and fruit yield (g)/plant of different tomato cultivars

Tomato cultivar	Root fresh weight (g)			Reduction	Fruit yield (g)			Reduction
	D*	H	Mean	(%)	D*	H	Mean	(%)
Carolina Gold	13.3	22.9	18.1	41.8	24.6	71.9	48.2	65.9
Dona	12.1	20.8	16.4	42.0	30.4	69.2	49.8	56.1
EXP 1	19.3	20.3	19.8	4.9	149.0	153.8	151.4	3.1
EXP 2	14.3	14.8	14.6	3.0	75.7	78.2	76.9	3.2
EXP 3	18.3	20.2	19.3	9.3	133.3	143.3	138.3	7.0
EXP 4	18.3	19.9	19.1	7.8	205.7	236.3	221.0	13.0
EXP 5	11.2	13.6	12.4	17.1	90.3	116.7	103.5	22.6
Mean	15.3	18.9			101.3	124.2		
L.S.D. at 5% for:								
Inoculation	0.2				0.8			
Cultivars	0.8				2.9			
Interaction	1.6				5.9			

* As described in footnote of Table (2).

the highest reduction in root fresh weight (41.8-42.0%) and fruit yield/plant (56.1-65.9%). In the moderately resistant cultivars (EXP 3, EXP 4 and EXP 5), their root fresh weight and fruit yield/plant were decreased due to inoculation with *FOL* by 7.8-17.1 and 7.0-22.6%, respectively.

Discussion

Tomato Fusarium wilt is caused by *Fusarium oxysporum* f.sp. *lycopersici*. The first symptoms are a yellowing and dropping of lower leaves on a single stem. A progressive yellowing and wilting of the leaves occur, and the plants may die. When the stem is cut open, the vascular (water conducting) tissues under the surface are frequently discoloured brown. This vascular browning extends from the roots to the upper portions of the plant and into the leaf petioles (Arthur and Martin, 1992).

The uses of resistant cultivars or rootstocks are the most reliable way to prevent the diseases. For this reason, to select the most appropriate cultivar for the next growing season and identify the form and race of the pathogen emerging in the field is important. Even though, *Fusarium* species can be identified by their morphological characteristics on selective media, the pathogenic types or *formae specials*, and races of *FOL* cannot (Nelson *et al.*, 1983). In the present study, the tested commercial and experimental tomato cultivars were responded differently against inoculation with *Fusarium oxysporum* f.sp. *lycopersici*. In term of wilt severity, they could be classified into three groups: 1- Highly resistant (EXP 1 and EXP 2), 2- Moderately susceptible (EXP3, EXP5 and EXP4) and 3- Highly susceptible cultivars (Carolina Gold and Dona).

It is well known that the development of wilt is depending upon the final interaction among host plant, pathogen and the surrounded environmental conditions. *FOL* has three physiological races (1, 2, and 3) hereafter (r1, r2 and r3) are distinguished by their specific pathogenicity on tester plants carrying dominant race-specific resistance genes (Cai *et al.*, 2003). After reporting r1 and r2 (Alexander and Tucker, 1945), r3 of *FOL* was determined in Australia in 1978 (Giraud *et al.*, 2006). The report was followed by studies in several U.S.A. states and Mexico that showed that the lack of *I*-genes, conferring resistance to *FOL* races in commercially cultivated tomatoes (*L. esculentum*), were concerned with plant susceptibility [Davis *et al.*, 1988]. Resistant tomato plants with the *I*-gene against r1 were used to control the disease in breeding varieties (Bohn and Tucker, 1940) and (Grattidge and ÓBrien, 1982). However, r2 overcame the resistance of r1-resistant cultivars, as reported in Korea and in Ohio, USA (Valenzuela-Ureta *et al.*, 1996). Then, the *I2* gene was found in tomato (*Lycopersicon peruvianum*) resistant to both r1 and r2 (Hirano and Arie, 2006). R3 was also observed in Australia and Florida (Grattidge and ÓBrien, 1982). Resistance to r3 in *Lycopersicon pennellii* genotype harboring the *I3* gene was found (McGrath and Bhrs, 2005) and (Reis *et al.*, 2004). Races of *FOL* could be distinguished by their differential virulence on tomato cultivars containing different dominant resistance genes (McGrath and Bhrs, 2005). Therefore, the use of resistant varieties is suggested as the best strategy for disease control, compared to biological control measurements (Akköprü and Demir, 2005). The 3 *FOL* physiological races are distinguished by their specific pathogenicity to different tomato cultivars. But Horinouchi *et al.* (2007) showed that even if an isolate shows the properties of a specific race, it can genetically be different and may has the tendency to change its genetic properties. The virulence profile of *FOL* isolates affecting tomatoes has been grouped into three races according to their ability to infect varieties carrying distinct resistance loci. This information, even today, is valid, and displaying different responses of the varieties to isolates can be related to genetic differences of the pathogen screening test carried out under *in vivo* conditions. R1 and r2 are distributed throughout the world, whereas r3 has a more limited geographic distribution (Peña, 2005). In this study, in addition to formally report the presence of *FOL* races, some genetic changes on the pathogen in Turkey are also has been reported. The existence of r3 was observed in Australia in 1978 and was subsequently observed in several U.S.A. states (Cai *et al.*, 2003).

All investigated growth characters and fruit yield/plant of most tested tomato cultivars were significantly lower in inoculated than in un-inoculated plants particularly the most susceptible cultivars Carolina Gold and Dona. Similar results were reported by Khan and Khan (2001) and Borkowski *et al.* (2007). Youssef (2007) tabulated that the fresh weight was significantly lower in the wilted tomato plants than healthy (un-inoculated) ones.

The present results concluded that the new experimental cultivars EXP1 and EXP2 which showed resistant responses against *FOL* could be used as sources for wilt resistance in the tomato breeding programs. In term of weight of fruit yield/plant, the moderately resistant EXP4 was significantly better than the highly resistant cultivars EXP1 or EXP2 then it could be subjected to further tests to introduce it as commercial cultivar in Kazakhstan.

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حساسية سبعة أصناف من الطماطم للإصابة بمرض ذبول الفيوزاريوم تحت ظروف الصوب الزجاجية في كازاخستان

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استهدفت هذه الدراسة توضيح تأثيرات التلقيح بالفطر فيوزاريوم أوكسيسبورم ليكوبيرسيكى على شدة الذبول، نمو النبات والخسارة اللاحقة فى محصول الثمار تحت ظروف الصوب الزجاجية بمحافظة ألماتى - كازاخستان. استخدم في هذه الدراسة سبعة أصناف من الطماطم منها صنفين تجاريين هما ذهب كارولينا ، دونا بالأضافة إلى خمسة أصناف تجريبية جديدة هى: إي إكس بي ٨٣٤٠ (إي إكس بي ١) ، إي إكس بي ٨٣٥٥ (إي إكس بي ٢) ، إي إكس بي ٨٤١٦ (إي إكس بي ٣) ، إي إكس بي ٨٤٢٠ (إي إكس بي ٤) ، إي إكس بي ٨٥٧٦ (إي إكس بي ٥) . وطبقا لشدة الإصابة بمرض الذبول يمكن وضع الأصناف المختبرة فى ثلاثة مجموعات كالتالى: أولا- أصناف حساسة للإصابة (ذهب كارولينا و دونا) ، ثانيا- أصناف عالية المقاومة (إي إكس بي ١ ، إي إكس بي ٢). ثالثا - أصناف متوسطة المقاومة (إي إكس بي ٣، إي إكس بي ٥ وإي إكس بي ٤). أظهرت النتائج أيضا انخفاضا معنويا فى ارتفاع النبات ، طول الجذر ، الوزن الطازج للجذر/نبات ، وزن الثمار على النبات الواحد في نباتات معظم الأصناف المختبرة المحقونة بالفطر مقارنة بغير الملقحة خصوصاً فى الأصناف الحساسة ذهب كارولينا ودونا. من النتائج يمكن استخدام الأصناف التجريبية عالية المقاومة (إي إكس بي ١ ، إي إكس بي ٢) كمصادر للمقاومة فى برامج التربية وطبقا لمعيار محصول الثمار فإن الصنف التجريبى متوسط المقاومة (إي إكس بي ٤) كان محصوله عاليا وبشكل ملحوظ عن الصنفين التجريبين المقاومين للإصابة (إي إكس بي ١ ، ، إي إكس بي ٢) ومن ثم يمكن وضعه تحت الإختبار كصنف تجارى في كازاخستان.