EFFECT OF INFECTION WITH *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* ON SOME CHEMICAL PROPERTIES OF TOMATO PLANTS

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Abstract

Infection with tomato wilt pathogen is characterized by changes in synthesis of certain chemical components such as phenols, free amino acids and sugars. The phenolic compounds were found in high concentrations in the whole tomato seedling after 45 days from inoculation.

High levels of total free amino acids were detected in the susceptible and tolerant tomato cultivars particularly after 30 and 45 days from inoculation.

The reduced, non-reduced and total sugars decreased in the inoculated seedlings of the resistant cultivars. In addition, there was no correlation between the content of reduced or total soluble sugars and the percent of wilt infection in all tested cultivars. The concentration of such sugars gradually increased as the plant age increased. The diseased seedlings of most susceptible and tolerant cultivars contained higher amount of sugars than did the healthy seedlings, while the reverse was observed in most resistant tomato cultivars.

INTRODUCTION

It has been pointed out that, in certain vascular wilt diseases, plants defend themselves against the spread of the pathogens by a series of sequential defense re-

sponses. Thus, many phenolic substances and their oxidation products exhibit antimicrobial properties which play an important role in plant resistance (Bell 1980, Campbell *et al.* 1980, Heath 1981 and Sarhan and Kiraly 1981).

Also, induced amino acids and proteins seems to play an important role in disease resistance (Stahmann 1967 and Sidhu and Webster 1977).

Disease-induced alterations in carbohydrate metabolism may be due to the disturbance of a number of regulatory systems (Kosuge and Kimpel 1981 and Ferraris *et al.* 1987).

The present investigation has been carried out to study the relationship between pathological alterations caused by *Fusarium oxysporum* Schl. f. sp. *lycopersici* (Sacc) Snyder & Hans and in phenolic compounds, free amino acids and sugar contents of tomato plants in relation to resistance and/or susceptibility to wilt disease.

MATERIALS AND METHODS

Chemical analysis of some tomato cultivars infected with *F. oxysporum* f. sp. *lycopersici*:

Fifteen tomato cultivars, namely, Hybrid luxor, E-6203, Early pak-2, Strain-3, UC 82-B, V.F.N. Bush, UC 97-3, Marmande, Peto-86, Pearson V.F.N., Supermarmande, Cal-Ace, Pritchard, Ace and Money maker, were used in this experiment. The evaluation of these cultivars as to resistance or susceptibility was determined according to Tohamy *et al.* (1991).

Un-treated tomato seeds of different cultivars were planted in four pots (20 cm in diameter), for each cultivar, containing 2 kg sandy-clay soil (1:1). The seedlings of each tomato cultivar, were transplanted in 24 pots 25 days later (each pot received 6 seedlings). After 5 days from transplanting, 12 pots of each cultivar were infested with the most virulent isolate of *F. oxysporum* f. sp. *lycopersici* race 1 (previously isolated and identified by Tohamy *et al.* 1991). The inoculum was added at the rate of 50 cc of $4x10^8$ spores/pot containing 2 kg soil. The remaining pots were kept without infestation to serve as a control. All pots were left under greenhouse conditions at 25 $^{o}C \pm 2$.

Chemical determination of phenols, sugars and total free amino acids were recorded fifteen days following soil infestation and was replicated twice at 15 days intervals.

Extraction:

Samples of 2.5, 5 and 5 grams root, stem and leaf tissues, respectively, of both healthy and inoculated tomato cultivars were cut into small portions and immediately stored in 50 ml of 95% ethanol in brown bottles. The ethanolic extracts were subjected to air current at room temperature to approximate dryness, then the extracts were quantitatively transferred into 5 ml of 50% isoproponal. The extracts were stored in vials at 1 ^{o}C for chemical determination.

1. Determination of phenolic compounds:

Free, conjugated and total phenols were determined after 15, 30 and 45 days of inoculation according to Snell and Snell (1953). Determinations were carried out on fresh tissue of healthy and inoculated seedlings. Assessments of *Fusarium* wilt infection was carried out just before every particular determination.

Free and total phenols were determined as mg catechol /300 g fresh matter.

2. Determination of total free amino acids:

One dimensional descending paper chromatography using Whatman No. 1 filter paper and a solvent composed of n-butanol : glacial acetic acid : water (4 : 1 : 2), were used for the separation of the individual free amino acids as described by Ambe and Toppel (1961). The spots of free amino acids were visualized on chromatograms by means of 0.2% ninhydrine in acetone (W/V) according to Smith (1958).

Free amino acids were quantitatively analyzed in terms of mg/100 g of fresh roots, stems and leaves (300 g/seedling).

3. Quantitative analysis of sugars:

Reduced, non-reduced and total sugars were spectrophotometrically determined by picric acid method at 540 nm as described by Thomas and Dutcher (1924). The sugar contents were determined as mg glucose /300 g fresh weight using a standard curve prepared for glucose.

RESULTS AND DISCUSSION

The development of tomato wilt disease affects the chemical constituents of the plant including phenolic compounds, total free amino acids and sugar contents. Determinations were carried out after 15, 30 and 45 days from inoculation on tomato plants grown under greenhouse conditions.

The effect of wilt infection on phenolic compounds in the whole seedlings of tested cultivars as it could be shown from Table (1) showed that the concentration of free phenols in diseased seedlings of susceptible tomato cultivars as well as the moderately resistant ones were increased, particularly after 45 days from inoculation, compared with healthy seedlings (control). The reverse was observed for the conjugated phenols. Total phenols were also increased in wilted transplants of the susceptible and moderately resistant cultivars, rather than healthy seedlings, whereas they fluctuated in the seedlings of the resistant cultivars.

The results showed continual increase of free, conjugated and total phenols in both inoculated and non-inoculated seedlings of all tested cultivars during the experimental periods. These results confirm that phenolic compounds are accumulated in tomato plants in response to *Fusarium* wilt infection. Also, these compounds that were found in conjugated form and free phenols are released after infection. Such findings are in agreement with many investigators (Kirino *et al.* 1980, Ferraris *et al.* 1987 and Shalaby 1988). On the other hand, some investigators concluded that phenolic compounds may not be responsible for wilting (Goodmann *et al.* 1967 and Sarhan and Kiraly 1981).

As for the total amino acids of the whole seedlings, data presented in Table (2) showed that total amino acids decreased after 15 days in the roots, stems and leaves of most of the inoculated resistant cultivars more than those of the healthy ones. In all inoculated moderately susceptible cultivars, the total amino acids increased in stems, while they fluctuated in the roots and leaves followed by an increase in total amino acids in the whole seedlings of such cultivars.

In the highly susceptible cultivars Ace and Money maker, it is clear that the total amino acids of the infected plants increased in its root tissues and decreased in the stems and leaves of both cultivars after 15 days from inoculation, leading to a decrease in total amino acids in the whole seedlings.

Table 1. Free, conjugated and total phenolic compounds of fifteen tomato cultivars inoculated with F. oxysporum f. sp. lycopersici measured as mg catecol/ 300 g fresh weight of tomato seedlings.

Tomato cultivars	Days after inoculation																		AV. Percent tomato wilt infection after		
	15						30						45								
	Healthy			Inoculated			Healthy			Inoculated			Healthy			Inoculated			15 days	30 days	45 days
	F	C	T	F	C	T	F	C	T	F	C	T	F	C	T	F	C	T			
Highly resistant																					
1 - Hibrid Luxor	11.2	7.1	18.3	8.3	8.8	17.1	25.4	34.0	59.4	25.4	35.6	61.0	62.8	40.0	102.8	31.0	62.2	113.2	0.0	0.00	6.65
Resistant																					
2 - E-6203	6.4	10.8	17.2	5.8	10.7	16.5	29.0	9.0	38.0	33.8	47.6	81.4	36.0	59.6	95.6	33.6	43.4	77.0	6.65	23.10	19.60
3 - Early Pak - 7.	8.2	17.2	25.4	7.1	7.8	14.9	28.2	21.2	49.4	27.0	24.2	51.2	35.2	29.8	83.0	29.6	67.0	96.0	6.65	6.65	19.60
4 - Strain - B.	10.2	9.5	19.7	3.9	13.6	17.5	19.0	7.8	26.8	32.4	10.4	42.8	51.4	47.2	98.6	26.8	56.6	83.4	0.00	0.00	19.95
5 - UC-87-B.	7.4	9.3	16.7	8.3	7.6	15.9	23.0	20.6	43.6	27.8	6.3	34.1	61.2	66.0	127.2	23.6	44.0	67.6	0.65	19.95	19.95
6 - V.F.N. Bush.	5.7	5.0	10.7	5.4	5.4	10.8	17.2	13.2	30.4	26.8	11.6	38.4	43.2	39.8	83.0	34.8	86.0	120.8	0.00	13.30	19.95
7 - U C 97-3.	7.9	9.7	17.6	6.9	16.8	26.4	27.2	22.8	50.0	32.7	20.2	52.9	42.8	9.6	52.4	55.2	36.8	92.0	19.95	19.60	19.95
8 - Marmande.	8.1	6.4	14.5	10.3	14.8	25.1	26.5	19.0	45.5	24.7	15.3	40.0	37.1	21.3	58.4	44.0	57.0	101.0	16.45	13.30	23.10
9 - Peto - 86.	7.0	16.1	23.1	6.7	6.9	13.6	36.4	4.8	41.2	54.0	22.0	56.0	48.6	28.4	77.0	16.8	50.6	67.4	13.30	16.45	26.60
Moderately resistant	8.6	10.3	18.9	11.8	9.0	20.8	32.6	14.1	46.7	31.6	28.8	60.4	40.4	26.0	66.4	25.0	76.2	101.2	0.00	16.45	32.30
10 - Pearson V. F. N.	6.9	5.9	12.8	8.6	12.6	21.2	13.4	12.4	25.8	22.2	13.0	35.2	37.4	23.0	60.4	36.0	97.6	133.6	19.95	15.95	32.90
11 - Supermarmande.	7.0	8.3	15.3	9.7	14.2	23.9	20.6	9.6	30.2	39.4	26.0	65.4	40.4	38.8	79.2	70.8	43.6	144.4	26.25	29.75	36.05
12 - Cal - Ace.	8.3	12.6	20.9	8.4	17.3	25.7	36.4	25.4	61.8	45.2	13.6	58.8	52.0	46.4	98.4	56.4	37.2	93.6	29.75	19.95	36.05
13 - Pritchard.																					
Highly susceptible	9.0	9.3	18.3	9.2	16.9	26.1	31.8	12.0	43.8	31.9	18.7	50.6	36.8	41.6	78.1	50.6	39.4	90.0	13.30	29.75	42.10
14 - Ace.	7.1	5.2	12.3	13.0	8.9	21.9	9.8	8.8	18.6	24.9	17.6	42.8	34.8	38.8	73.6	45.8	37.8	83.6	26.25	32.90	45.00
15 - Money marker.																					
L. S. D. at 5% level for wilt infection																				18.07	

F. = Free phenols.
C. = Conjugated phenols.
T. = Total phenols.

Table 2. Total free amino acids of fifteen tomato cultivars incoculated with *F. oxysporum* f. sp. *lycopersici* and measured as mg amino acids/ 300 g fresh weight of tomato seedlings.

Tomato cultivars		Days after inoculation											
		15 days				10 days				45 days			
		R	S	L	T	R	S	L	T	R	S	L	T
Highly resistant													
1 - Hibrid Luxor	(H)	1989	638	449	2976	341	883	581	1805	558	1007	1007	2860
Hibrid Luxor	(D)	1600	861	344	2805	333	742	525	1600	372	688	1179	2239
Resistant													
2 - E-6203	(H)	562	585	544	1691	231	598	587	1416	355	598	880	1833
E-6203	(D)	350	554	390	1294	303	758	565	1706	265	609	885	1759
3 - Early Pak - 7.	(H)	322	533	522	1377	325	864	632	1621	629	1295	918	2852
Early Pak - 7.	(D)	307	530	472	1309	386	1010	709	2105	659	806	849	2314
4 - Strain - B.	(H)	562	421	540	1523	404	1142	846	2392	221	684	1117	2022
Strain - B.	(D)	350	301	632	1283	388	851	664	1903	210	475	964	1649
5 - UC-87-B.	(H)	458	297	385	1140	384	1052	466	1902	515	714	903	2132
UC-87-B.	(D)	583	277	553	1408	349	999	570	1918	342	556	953	1815
6 - V.F.N. Bush.	(H)	397	551	458	1406	966	636	465	2067	390	627	808	1825
V.F.N. Bush.	(D)	374	505	378	1257	427	647	473	1547	257	499	758	1514
7 - U C 97-3.	(H)	344	715	609	1660	578	931	738	2247	679	1006	864	2549
U C 97-3.	(D)	289	794	745	1928	592	1256	622	2470	586	909	802	2297
8 - Marmande.	(H)	1493	632	933	3058	657	772	666	2095	768	1412	777	2957
Marmande.	(D)	979	550	699	227	558	792	608	1958	1012	1167	744	2923
9 - Peto - 86.	(H)	429	861	324	1614	382	695	618	1695	287	373	917	1577
Peto - 86.	(D)	401	618	290	1309	326	383	535	1264	240	340	833	1413
Moderately resistant													
10 - Pearson V. F. N.	(H)	336	526	712	1574	289	837	527	1653	694	967	797	2458
Pearson V. F. N.	(D)	480	597	729	1806	378	1005	532	1915	573	527	813	1913
11 - Supermarmande.	(H)	592	759	874	3225	849	561	469	1879	388	556	452	1396
Supermarmande.	(D)	455	844	780	2079	1091	763	550	2404	419	878	561	1858
12 - Cal - Ace.	(H)	557	544	681	1782	506	641	517	1664	349	935	450	1734
Cal - Ace.	(D)	460	586	654	1700	489	878	534	1901	386	1057	571	2014
13 - Pritchard.	(H)	242	522	374	1138	448	867	391	1706	751	818	494	2063
Pritchard.	(D)	402	559	532	1493	790	1100	548	2438	678	625	606	1909
Highly susceptible													
14 - Ace.	(H)	372	585	704	1661	942	849	671	2462	631	746	646	2023
Ace.	(D)	374	483	631	1488	627	1119	900	2646	650	1006	791	2547
15 - Money marker.	(H)	354	707	1116	2177	498	603	621	1722	397	969	398	1764
Money marker.	(D)	426	687	612	1725	526	809	572	1907	377	1128	464	1969

R: Roots
S: Stems
L : Leaves.
T : Total free amino acids

H: Healthy seedlings
D : Diseased seedlings.

After 30 days from inoculation, it could be concluded that the total amino acids of the resistant cultivars showed fluctuating values in the whole seedlings. Meanwhile, the total amino acids generally increased in the infected seedlings of the susceptible and moderate resistant cultivars compared with the healthy ones.

After 45 days from inoculation, the inoculated seedlings of all the resistant cultivars contained less amino acids relative to the healthy ones. The moderate susceptible cultivars revealed fluctuated results. The development of *Fusarium* wilt infection in the highly susceptible cvs. namely Ace and Money maker, resulted in an increase of the total amino acids. In general, data suggested that the high content of the total amino acids detected in the susceptible and moderately resistant tomato cultivars may be due to that *Fusarium* infection reduced the protein content causing release of amino acids. Also, the pathogen may convert some amino acids to others which are used in its protein synthesis. This conclusion is in agreement with Raggi *et al.* (1974), Zazzerini (1976), Sidhu and Webster (1977) and Noguera Gonzalez (1980).

Data represented in Table (3) indicate that there was no correlation between the reduced, non-reduced and total sugar contents and the percentage of tomato wilt infection of all cultivars. The sugar contents in seedlings of all tested cultivars increased with age. The inoculated seedlings of most susceptible and moderately resistant cultivars contained higher sugar contents if compared with the non-inoculated seedlings. The reverse reaction was true in most resistant tomato cultivars. The high contents of sugars in both susceptible or moderately resistant cultivars found after inoculation with *F. oxysporum* f. sp. *lycopersici* race 1 may be due to the glucosidase activities of the pathogen which is capable of hydrolizing the cell walls of cortical cells and xylem elements to soluble sugars. These finding are in agreement with those obtained by Noguera Gonzalez (1980) and Ferraris *et al.* (1987).

Table 3. Reduced, non-reduced and total sugars of fifteen tomato cultivars inoculated with *F. oxysporum* f. sp. *lycopersici* expressed as gram glucose /300g fresh weight of tomato seedlings.

Tomato cultivars	Days after inoculation																		AV. Percent tomato wilt infection after		
	15						30						45								
	Healthy			Inoculated			Healthy			Inoculated			Healthy			Inoculated			15 days	30 days	45 days
	R	NR	T	R	NR	T	R	NR	T	R	NR	T	R	NR	T	R	NR	T			
Highly resistant																					
1 - Hibrid Luxor	1.92	2.40	4.32	1.54	2.06	3.60	2.90	3.46	6.36	2.00	3.10	5.10	3.98	7.62	10.60	2.80	5.12	7.92	0.0	0.00	6.65
Resistant																					
2 - E-6203	1.04	0.40	1.44	0.76	0.84	1.60	1.62	1.18	2.80	1.20	2.00	3.20	2.86	3.24	6.10	2.48	2.10	4.58	6.65	23.10	19.60
3 - Early Pak - 7.	1.46	0.52	1.98	0.70	0.57	1.27	1.60	3.40	5.00	1.92	2.60	4.52	3.94	4.64	8.58	4.30	6.18	10.48	6.65	6.65	19.95
4 - Strain - B.	1.42	0.82	2.24	0.50	0.94	1.44	0.79	0.27	1.06	1.22	1.98	3.20	3.44	3.28	6.72	2.74	3.32	6.06	0.00	0.00	19.95
5 - UC-87-B.	0.56	0.37	0.93	0.64	0.56	1.20	1.54	1.69	3.23	1.50	1.67	3.17	2.86	4.54	7.40	2.86	3.63	6.49	6.65	19.95	19.95
6 - V.F.N. Bush.	0.92	0.12	1.04	0.44	0.56	1.00	1.68	0.92	2.60	1.80	1.82	3.62	4.78	5.18	9.96	4.30	4.66	8.96	0.00	13.30	19.95
7 - U C 97-3.	1.14	0.38	1.52	1.04	0.40	1.44	1.54	1.50	3.04	1.48	1.16	2.34	1.90	2.66	4.56	3.84	4.48	8.32	19.95	19.60	23.10
8 - Marmande.	1.81	0.45	2.26	1.38	1.51	2.89	1.17	1.73	2.90	1.42	1.58	3.00	3.54	3.92	7.47	3.44	3.56	7.00	19.45	13.30	23.10
9 - Peto - 86.	1.52	0.46	1.98	0.54	0.45	0.99	2.00	1.68	3.68	1.84	1.76	3.60	2.90	2.90	5.80	1.52	2.16	3.68	13.30	16.45	26.60
Moderately resistant																					
10 - Pearson V. F. N.	1.68	0.74	2.42	1.81	1.17	2.98	1.74	2.26	4.00	1.86	2.22	4.08	3.70	5.66	9.36	6.04	3.52	9.56	0.00	16.45	32.30
11 - Supermarmande.	0.97	0.36	1.33	1.36	0.74	2.10	1.38	0.51	1.89	1.64	0.58	2.52	3.78	3.62	7.40	4.20	4.60	8.80	19.95	16.95	32.90
12 - Cal - Ace.	0.92	0.21	1.13	1.26	1.14	2.40	1.58	0.50	2.08	3.08	1.12	4.20	3.48	4.68	8.16	4.90	3.78	8.68	26.25	29.75	36.05
13 - Pritchard.	1.32	0.40	1.72	2.26	1.76	4.02	1.42	2.07	3.49	2.72	1.86	4.58	3.58	5.28	8.86	5.20	4.49	9.69	29.75	19.95	36.05
Highly susceptible																					
14 - Ace.	1.64	0.62	2.26	1.44	0.86	2.30	1.74	0.68	2.42	3.00	1.16	4.16	3.44	7.58	11.02	3.96	4.96	4.16	13.30	29.75	42.10
15 - Money marker.	1.56	0.38	1.94	1.22	0.28	1.50	0.58	0.38	0.96	2.60	2.08	4.68	3.36	3.14	6.50	4.84	4.48	9.32	26.25	32.90	45.00
L. S. D. at 5% level for wilt infection																				18.07	

R : = Reduced sugars.
NR: Non - reduced sugars.
T. = Total sugars.

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تأثير اصابة نباتات الطماطم بالفيوزايوم أوكسيسبوم ليكوبيرسى على بعض صفاتها الكيميائية

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٢ – معهد بحوث أمراض النبات – مركز البحوث الزراعية – الجيزة

تميزت الاصابة بمرض ذبول الطماطم بتغيرتمثيل بعض المركبات الكيميائية مثل الفينولات والاحماض الامينية الحرة والسكريات. ولقد قدرت المركبات الفينولية فوجدت بتركيزات عالية فى كل البادرات وذلك فى جميع الاصناف التى اختيرت بعد ٤٥ يوما من العدوى.

وجد ايضا ان هناك محتوى عالى من الاحماض الامينية الحرة وذلك فى اصناف الطماطم القابلة للاصابة وكذلك الاصناف التى تتحملها وخاصة بعد ٣٠ و ٤٥ يوما من العدوى.

انخفض محتوى البادرات المعدية من السكريات المختزلة والغير مختزلة والسكريات الكلية وذلك للأصناف المقاومة للمرض بالاضافة لذلك لم توجد أى علاقة بين المحتوى من السكريات المختزلة أو المحتوى الكلى للسكريات الذائبة وبين نسبة الاصابة فى جميع الاصناف المختبرة ويزداد تركيز هذه السكريات تدريجيا بتقدم عمر النبات. وجد أن بادرات الطماطم المصابة لاغلب الاصناف القابلة للاصابة والتى تتحمل المرض تحتوى على كمية اكبر من السكر عن البادرات السليمة بينما لوحظ أن التفاعلات كانت عكسية فى معظم أصناف الطماطم المقاومة للمرض.