

RESISTANCE OF DETERMINANT TOMATO VARIETIES TO THE CAUSAL AGENTS OF BACTERIAL WILT DISEASE

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Abstract

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Resistance of determinant tomato varieties to pathogens causing bacterial wilt disease – *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) and *Ralstonia solanacearum* (*Rs*) – was tested under greenhouse conditions. In tests to *Cmm* resistance, two inoculation methods were compared (inoculation “to the cut off top” of a plant and inoculation by three punctures into a stalk). The inoculation method “into a stalk” appeared to be most suitable. In both cases of inoculation, the highest level of resistance was found in Minigold variety. The rather high level of resistance was also found in varieties Aneta and Orange. In tests to *Rs* resistance, the most resistant varieties were Minigold, Aneta and Orange, which are recommended for direct consumption

bacterial wilt disease of tomatoes, *Clavibacter michiganensis* subsp. *michiganensis*, *Ralstonia solanacearum*, resistance

Bacteria *Clavibacter michiganensis* subsp. *michiganensis* (Smith 1910) Davis *et al.* (1984) cause wilting and drying up of leaves and fruit spot of tomatoes. They occur both under field and particularly greenhouse conditions. The first symptom consists in the sudden wilting of individual leaflets on the one side of compound leaves, so-called unilateral wilting. SÜLE (1988) found the unilateral wilting of leaflets 14–16 day after inoculation and dying of the plants during 28–30 days. The leaflets gradually get dry and turn brown, whole leaves dry out and plants finally die. The *Cmm* pathogen is wide-spread throughout the world causing serious losses in yields of field and greenhouse tomatoes. Typical symptoms of the bacterial wilt disease of tomatoes were found by OROUMCHI & LORRA (1992) in Iran in 1988 and ZHAO *et al.* (1993) in NE China in 1992. In Cyprus, the first symptoms of the bacterial wilt disease were observed in 1998 (IOANNOU *et al.*, 2000).

Another pathogen *Ralstonia solanacearum* (Smith 1896) Yabuuchi *et al.* 1995 commonly occurring in

tropical and subtropical regions could be also a potential causal agent of the bacterial wilt disease of tomatoes under greenhouse conditions. The pathogen infects the wide group of hosts, the most important of them can be potatoes and tomatoes in the temperate zone region. Symptoms of the disease differ from other manifestations of wilting, with the sudden wilting of top leaves and stalks. The plants rapidly wilt and die without any manifestations of yellowing or leaf spots. With respect to considerable requirements for temperature *Rs* bacteria were not considered to be a significant pathogen under field conditions of the Czech Republic. However, in the first half of the 90s of the last century, race 3, i.e. the causal agent of brown rot was found in a number of European countries. Some strains of this race are adapted to a colder climate, an optimum for their growth being lower ranging about 27 °C (KUDELA, 2002). In 1994–1995, DIGAT & CAFFIER (1996) noted the first occurrence of *Rs* in France on tomatoes.

In European Union, bacteria *Clavibacter michiganensis* subsp. *michiganensis* and *Ralstonia solanacearum* are included into EPPO A2 list of pests recommended for regulation as quarantine pests (ANONYMOUS, 2008). The only possible method of control of these pathogens is breeding for resistance. For this process, the suitable sources of resistance are necessary.

The aim of the paper was to determine the level of resistance of selected varieties of determinant tomatoes to both causal agents of bacterial wilt disease.

MATERIALS AND METHODS

For greenhouse experiments, varieties recommended for canning use (Denar, Odeon, Orbit, Pavlina, Salus, Titan) and varieties recommended for direct consumption (Aneta, Diana, Dulcia, Hana, Karla, Klara, Minigold, Orange) were included. Plants of the tested varieties were pre-cultivated in planting pots in steamed soil at a temperature of $20\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$.

To test the resistance of particular varieties to *Cmm* two methods of inoculation were used: (1) “into a stalk” – in the stage of the 3rd to the 4th leaf by three punctures by a diabetic needle at a various height along the stalk circumference and (2) “to a top” – in the stage of the first leaf, the plant top was removed and an inoculum was inserted into vascular bundles by one puncture from above. Plants were inoculated by the bacterial suspension of 10^6 cfu.ml⁻¹ concentration of the mixture of *Cmm* isolates CCM 1636, 1/1/5/01 and 1/2/5/01 from the bacterium collection in the Research Institute of Plant Production, Praha - Ruzyně. After the inoculation, plants were covered by a PE sheet for a period of 48 hours to increase relative humidity and placed in a cultivation room with 12-hour light regime and a temperature of $23\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$. The health status of plants was evaluated the

10th day after inoculation “into a stalk” and in two dates (the 10th and the 20th day) after inoculation “to a cut off top” of the stalk. To assess the vascular wilt disease of tomato plants a four-point disease rating scale was created: degree 1 – healthy plant without symptoms, degree 2 – marginal wilting of leaves (30% of plant), degree 3 – wilting to a half of the plant leaf blade (50% of plant), degree 4 – wilting and drying up in more than half of the leaf (> 75% of plant). Disease intensity was determined from all tested tomato plants including degree 1. The experiment was established in three repetitions 20 plants of each variety and inoculation method. The disease incidence (percentage of infected plants) and disease intensity of infected plants were evaluated.

To evaluate the level of resistance of determinant varieties of tomatoes to *Rs* the same assortment was used. Inoculation was carried out in the stage of fully developed leaves to the cut off top of the plant stalk. Plants were inoculated by the mixture of two isolates (*Rs* NCPPB 2505 and *Rs* NCPPB 2796) of a concentration of 10^6 cfu.ml⁻¹. The climate chamber temperature was set to $28\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$. The health status of plants was assessed the 10th and the 20th day after inoculation on the basis of a four-point disease rating scale of the evaluation of vascular wilting of tomatoes (percentage of the wilted or dried part of a plant – see *Cmm*). The percentage of infected plants was also determined.

Both experiments were established in three repetitions 20 plants of each of the variety. Results were statically processed by the single-factor analysis of variance and subsequently tested by the Tukey test using the UPAV program. In assessing the disease incidence transformation $y' = \arcsin \sqrt{y} / 100$ was used. Correlation coefficients were calculated in MS EXCEL.

I: Reaction of tomato varieties to inoculation with *Clavibacter michiganensis* subsp. *michiganensis* “into a stalk” – the 10th day after inoculation

varieties	use	disease incidence		disease intensity	
		%	P ≤ 0.01	index	P ≤ 0.01
KLÁRA	Z	91.67	A	2.95	A
DULCIA	Z	88.33	AB	3.00	A
DIANA	Z	86.67	AB	2.70	ABC
SALUS	K	81.67	ABC	2.47	BCDEF
TITAN	K	81.67	ABC	2.60	ABCDE
KARLA	Z	81.67	ABC	2.77	AB
ODEON	K	78.33	ABC	2.67	ABCD
DENÁR	K	75.00	ABCD	2.27	DEFG
PAVLÍNA	K	73.33	ABCD	2.30	CDEFG
ORBIT	K	43.33	ABCD	2.35	CDEFG
HANA	Z	73.33	ABCD	2.23	EFG
ORANGE	Z	66.67	BCD	2.15	FGH
ANETA	Z	61.67	CD	2.02	GH
MINIGOLD	Z	50.00	D	1.78	H

Z – direct consumption, K – canning use

RESULTS AND DISCUSSION

Evaluation of the resistance of varieties of determinant tomatoes to *Clavibacter michiganensis* subsp. *michiganensis*

In assessing the response of plants to *Cmm* inoculation “into a stalk”, Minigold variety was least infected. Statistically highly significant differences were demonstrated between this variety and varieties Klara, Dulcia, Diana, Salus, Titan, Karla and Odeon in disease incidence of infected plants. Statistically highly significant differences were also found between the second least attacked variety

Aneta and varieties Diana, Dulcia and Klara (Tab. I). As for the disease intensity of infected plants, statistically highly significant differences were proved between Minigold and other varieties (with the exception of varieties Aneta and Orange) and a statistically highly significant difference between variety Aneta and varieties Odeon, Karla, Titan, Salus, Diana, Dulcia and Klara.

In an inoculation method “to a cut off top”, varieties Minigold and Aneta did not demonstrate any symptoms of vascular wilt disease after 10 days (Tab. II). The highest disease incidence of infected leaves with the greatest disease intensity was found in variety Diana. Statistically highly significant dif-

II: Reaction of tomato varieties to inoculation with *Clavibacter michiganensis* subsp. *michiganensis* “to a cut off top” – the 10th day after inoculation

varieties	use	disease incidence		disease intensity	
		%	P ≤ 0.01	index	P ≤ 0.01
DIANA	Z	40.49	A	1.70	A
TITAN	K	14.17	B	1.18	B
KARLA	Z	13.73	BC	1.18	B
HANA	Z	11.98	BCD	1.13	BC
ODEON	K	5.57	BCDE	1.06	BC
SALUS	K	4.92	BCDE	1.06	BC
KLÁRA	Z	4.79	BCDE	1.05	BC
DULCIA	Z	4.35	BCDE	1.05	BC
DENÁR	K	3.80	CDE	1.04	BC
PAVLÍNA	K	3.31	DEF	1.03	BC
ORBIT	K	3.14	DEF	1.04	BC
ORANGE	Z	2.47	EF	1.03	BC
ANETA	Z	0.00	F	1.00	C
MINIGOLD	Z	0.00	F	1.00	C

K, Z – see Tab. I

III: Reaction of tomato varieties to inoculation with *Clavibacter michiganensis* subsp. *michiganensis* “to a cut off top” – the 20th day after inoculation

varieties	use	disease incidence		disease intensity	
		%	P ≤ 0.01	index	P ≤ 0.01
DIANA	Z	61.28	A	2.26	A
TITAN	K	58.42	AB	2.13	A
ODEON	Z	45.83	ABC	1.71	BC
KARLA	Z	41.07	BCD	1.76	B
DENÁR	K	40.40	BCD	1.51	BCD
ORBIT	K	39.14	CD	1.62	BCD
SALUS	K	37.51	CD	1.48	CD
HANA	Z	37.07	CD	1.62	BCD
KLÁRA	Z	35.39	CD	1.46	CD
PAVLÍNA	K	33.61	CD	1.52	BCD
DULCIA	Z	33.40	CD	1.45	CD
ORANGE	Z	27.14	D	1.39	DE
ANETA	Z	10.48	E	1.13	EF
MINIGOLD	Z	9.87	E	1.10	F

K, Z – see Tab. I

ferences were found between the variety and other varieties both in the disease incidence and in the disease intensity. In other varieties, marked differences in the disease incidence and disease intensity did not occur. There were significant differences between varieties in the incidence of infected leaves 20 day after inoculation (Tab. III). Variety Diana showed the highest number of infected leaves, rather high percentage of attacked leaves occurred also in varieties Titan and Odeon. The least number of attacked leaves occurred in varieties Minigold and Aneta. Significant correlations in the disease incidence ($r = 0.54724$, $P_{0.05} = 0.5139$) and disease intensity ($r = 0.534203$, $P_{0.05} = 0.5139$) were found between the evaluation of health status of plants in the inoculation method “to a top” after 10 and 20 days which means that the plant infection by *Cmm* can be assessed only within one term.

Between the inoculation method “into a stalk” and inoculation method “to a top” assessed after 10 days no significant correlation was found in the disease incidence ($r = 0.438269$, $P_{0.05} = 0.5139$) or disease intensity ($r = 0.337074$, $P_{0.05} = 0.5139$). Between the inoculation method “into a stalk” (assessed 10 days after inoculation) and inoculation method “to a top” assessed after 20 days, a highly significant correlation was already found both in the disease incidence ($r = 0.724703$, $P_{0.01} = 0.6411$) and disease intensity ($r = 0.792602$, $P_{0.01} = 0.6411$).

In 14 varieties of bush determinant tomatoes, resistance was studied to the causal agent of bacterial vascular wilt disease of plants – *Cmm* by various inoculation methods. Higher incidence of infected plants with the higher disease intensity was found in inoculation method carried out by three punctures “to a stalk” in the stage of the 3rd–4th leaves in comparison with the method “to a top”. Differ-

ences in the resistance between particular varieties were also pronounced as compared with the second method evaluated in the same day after inoculation. A number of authors dealt with the use of a suitable inoculation method. GITAITIS *et al.* (1991) transferred also a drop of inoculum to a cut off apical end of a stalk. Although plants were infected during two days symptoms were not obvious even after 17 days. BULK *et al.* (1991) recommend a method of the transfer of a drop of inoculum to an injured true leaf for marked differentiation of resistance. The resistance of 24 varieties of tomatoes to the systemic *Cmm* infection under greenhouse conditions was assessed by QUEZADO SOARES & LOPES (1994). In tests, they used two inoculation methods to roots and stalks and a bacterial suspension of a concentration of 10^8 cfu.ml⁻¹. KRÁTKÁ (1992) dealt with the resistance of indeterminant tomatoes to *Cmm*. She carried out inoculation in the stage of the third true leaf by means of the 18-hour dipping of roots in a bacterial suspension 10^8 cfu.ml⁻¹. The only variety resistant to *Cmm* was Slava Poryni.

Based on our results, the use of an inoculation method by means of three punctures “to a stalk” appears to be more suitable for testing the resistance to *Cmm*. The inoculation method to a cut of the top is less laborious and can serve for the preliminary differentiation of resistance to the pathogen. In both cases of artificial infection, variety Minigold appeared to be the most resistant. It is an early variety of yellow colour of a “cherry type”. High resistance was also found in varieties Aneta and Orange. It refers to varieties designed particularly for direct consumption and kitchen treatment. Variety Orange is especially valued for its high content of vitamins and increased formation of carotene being suitable as baby food.

IV: Reaction of tomato varieties to inoculation with *Ralstonia solanacearum* “to a cut off top” – the 10th day after inoculation

varieties	use	disease incidence		disease intensity	
		%	P ≤ 0.01	index	P ≤ 0.01
HANA	Z	64.71	A	2.54	A
DENÁR	K	50.59	AB	2.08	AB
PAVLÍNA	K	50.00	AB	2.04	AB
ODEON	K	48.73	AB	1.89	AB
TITAN	K	46.83	AB	1.80	AB
ANETA	Z	39.45	AB	1.80	AB
SALUS	K	38.45	AB	1.69	AB
DIANA	Z	31.16	AB	1.58	AB
DULCIA	Z	28.85	AB	1.65	AB
KLÁRA	Z	21.51	AB	1.34	AB
KARLA	Z	17.64	AB	1.42	AB
ORANGE	Z	11.45	AB	1.21	AB
ORBIT	K	7.57	B	1.16	B
MINIGOLD	Z	5.00	B	1.07	B

K, Z – see Tab. I

Evaluation of the resistance of varieties of bush determinant tomatoes to *Ralstonia solanacearum*

The tenth day after inoculation by *Rs* bacteria to the "cut off top" of determinant tomatoes, varieties Minigold and Orbit were less infected while the highest number of infected plants was found in variety Hana (Tab. IV). Statistically highly significant differences were determined between the varieties. Assessing the disease incidence corresponds with the disease intensity in this term of evaluation.

The twentieth day after inoculation, a marked increase in the number of infected plants occurred in some varieties. In variety Orbit, the number of infected plants increased from 7.5 to 64% and also in variety Minigold, the number of infected plants increased from 5 to 46%. Nevertheless, these varieties showed the lowest number of infected individuals of the low disease intensity. On the other hand, in variety Aneta the number of infected plants was higher already the tenth day after inoculation (39.5%) and until the twentieth day, it increased only by 10%. Highly significant differences in the disease incidence were proved in this term between varieties Minigold, Orange, Aneta and variety Odeon (Tab. V). Variety Minigold showed also the lowest disease intensity. Highly significant differences between this variety and varieties Odeon and Hana and highly significant differences between varieties Orange, Aneta, Klara and variety Odeon were determined. Between the evaluation of health condition of plants the 10th and the 20th day after inoculation "to a top", significant correlation was found only in the disease intensity ($r = 0.597979$, $P_{0.05} = 0.5139$) while in the disease incidence correlation was not found ($r = 0.454103$, $P_{0.05} = 0.5139$).

Within the tested range of determinant tomatoes, varieties recommended for direct consumption

Minigold, Aneta and Orange had the highest level of resistance to the causal agent of bacterial brown rot *Rs*. IN *et al.* (1996) dealt with the resistance of varieties of tomatoes to *Rs*. Of 31 varieties, only three were moderately resistant, other varieties were assessed as susceptible. During 1986–1991, SHARMA & KUMAR (1997) and SHARMA *et al.* (1997) tested in India new bred lines of tomatoes for resistance to *Rs*. Three of them, medium resistant with high yields, were recommended for waterlogged regions. SCOTT *et al.* (2009) obtained an array of large-fruited breeding lines from eight crosses at the F5 generation, which demonstrated high level of resistance to *Rs*. One of the possible ways is also using of wild species from the genus *Lycopersicon* spp. CARMEILLE *et al.* (2006) and TRUONG THI HONG HAI (2008) tested some introductions from this genus and found some accessions with high level of resistance to *Rs*. A suitable inoculation method is necessary for the determination of resistance to the pathogen. VUDHIVANICH (1997) tested six levels of the bacterial suspension concentration (10^3 – 10^8 cfu/ml) on 10 varieties of tomatoes (aged 30 days). Inoculation was carried out by means of a micropipette containing 0.1 ml inoculum diagonally to a stalk in the axil of the third leaf from the plant top. After three weeks, the author evaluated the degree of attack. At the low concentration of bacteria, varieties appeared to be resistant, however, with the increasing concentration of the pathogen their resistance proportionally decreased. The knowledge of isolate races is also needed for the determination of resistance to the pathogen. Race 1 attacks potato, tomato, tobacco and banana. Its temperature optimum ranges from 35 to 37 °C. Under conditions of a temperate zone, it can occur only in greenhouses. Race 3 shows lower temperature optimum (27 °C) and attacks mainly potato and tomato (KUDELA *et al.*, 2002). Temperature only max. 28 °C could be set in

V: Reaction of tomato varieties to inoculation with *Ralstonia solanacearum* "to a cut off top" – the 20th day after inoculation

varieties	use	disease incidence		disease intensity	
		%	P ≤ 0.01	index	P ≤ 0.01
ODEON	K	97.56	A	3.67	A
SALUS	K	88.99	AB	3.01	ABC
HANA	Z	86.89	AB	3.27	AB
DULCIA	Z	82.77	AB	3.18	ABC
KARLA	Z	79.53	AB	2.83	ABC
TITAN	K	67.04	AB	2.45	ABC
DENÁR	K	68.20	AB	2.64	ABC
ORBIT	K	64.13	AB	2.48	ABC
PAVLÍNA	K	61.58	AB	2.50	ABC
DIANA	Z	61.61	AB	2.36	ABC
KLÁRA	Z	53.23	AB	2.06	BC
ANETA	Z	50.73	B	2.17	BC
MINIGOLD	Z	46.08	B	1.78	C
ORANGE	Z	46.48	B	1.92	BC

K, Z – see Tab. I

a cultivation room where plants were placed after *Rs* inoculation during our experiments. It is possible that *Rs* isolates which were used in our tests belong to race 1 and a temperature in the cultivation room was too low. Therefore, a period necessary to the manifestation of symptoms was longer. If we do not know exactly the race of an isolate it is more suitable

to evaluate the health condition of tested plants in the course of several dates.

Based on the results of tests of tomato varieties resistance to pathogens causing bacterial wilt disease it can be concluded that varieties Minigold, Orange and Aneta had higher level of resistance to both *Clavibacter michiganensis* subsp. *michiganensis* and *Ralstonia solanacearum*.

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