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VIRULENCE OF STRAINS OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *SEPEDONICUS*

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Abstract

The development of potato cultivars immune to bacterial ring rot disease (*Clavibacter michiganensis* subsp. *sepedonicus*) is a significant step towards disease eradication. The effective evaluation of selected potato hybrids for resistance/immunity to ring rot requires regular control of virulence of strains used. Application of potato tubers for differentiation of virulence of strains of the pathogen is shown. A strong correlation was observed between virulence of *Clavibacter michiganensis* subsp. *sepedonicus* strains on potato tubers and on eggplants (Pearson $r = 0.78$, $P < 0.01$).

Key words: *Clavibacter michiganensis* subsp. *sepedonicus*, ring rot, virulence.

Introduction

Potato ring rot caused by *Clavibacter michiganensis* subsp. *sepedonicus* (Cms) is one of the most harmful potato diseases /Rich, 1983/. It was first found and described in Germany by Spieckermann in 1913. Ring rot is spread in 31 countries distributed over 5 different continents /Elphinstone, 2004/.

Ring rot can cause large losses in tonnage as a result of tuber breakdown, but it may cause even greater economic losses due to rejection of entire lots for seed purposes /Rich, 1983/. Elimination of bacterial ring rot is particularly difficult because of latent infections /De Boer, McNaughton, 1986; Franc, 1999/. In addition, the effects of growth conditions on *C. michiganensis* subsp. *sepedonicus* infection and variable expression of disease symptoms complicate eradication of the disease /Laurila et al., 2005/.

The development of potato (*Solanum tuberosum*) cultivars immune to bacterial ring rot disease is a significant step towards eradication of the disease /Laurila et al., 2005/. The effective evaluation of selected potato hybrids for immunity to ring rot requires regular control of virulence of strains used.

Heterogeneity of virulence of *C. michiganensis* subsp. *sepedonicus* strains is known. Strains of the pathogen show marked differences in virulence on host plants (*S. tuberosum* L. and *S. melongena* L.) and the virulent strains of the pathogen elicit a typical hypersensitive response (HR) in tobacco /Nissinen, 1997; 2001/. Tests on eggplant (*S. melongena* L.) can be used to make quantitative distinctions among pathogenic strains of *C. michiganensis* subsp. *sepedonicus* /Bishop, Slack, 1987/. The

method of tuber injection was used by В. И. Янович (1971) for ring rot strains' virulence determination.

The objective of this study was to compare methods of differentiation of virulence of pathogen strains on potato tubers and on eggplants.

Materials and Methods

Potato tubers of 'Delphin', 'Krinitsa', 'Skarb', 'Atlant' cultivars were used in this study. Cultures of Cms were grown for 6 days at 24° C on slants of potato agar. Pure cultures of ring rot were harvested by washing with sterile distilled water and were adjusted to necessary concentration taking into account that $OD_{600} = 0.1$ correspond to 1×10^8 CFU/ml.

Potato tubers were washed up in running tap water and dried. The spot of inoculum injection was disinfected by dipping into alcohol followed by burning in burner flame. From the direction of stolon end two apertures were made by preparation needle on the tuber diagonals crossing in the same plane. Tubers were infected with pathogen suspension by syringe with 21 g needle through one of the apertures. The inoculum was carefully injected into tuber until the suspension overflowed from the second aperture. The infected material was put into plastic boxes and incubated at 21° C. After incubation period, the tubers were cut up through the spot of inoculums injection and one half was pressed by hand till bacterial exudation excretion from the spot of infection. By thin wooden stick the residual exudation was accurately extracted and affection zone width of tuber tissues was measured. For each variant of the experiment 10 tubers were used.

Eggplants (cultivar 'Patsekha') were grown under room temperature and natural lighting in the plastic cassettes, containing 24 pots (85x60) mm filled with compost. Eggplants were inoculated with bacterial suspension into longitudinal cut at leaf stage 3 (Quarantine procedure № 25, 1990).

Pure cultures of strains E1, E3, E3, E4, E6, EK11, EK15, EK18, EP1, ES4 were cultured as it was described above. Optical density of the suspension was adjusted to give an optical density of 0.2 at a wavelength 600 nm, which corresponded to a concentration 2×10^8 CFU/ml. Disease intensity on eggplants was estimated based on the peculiarities of disease symptoms expression and pathogen affection intensity of every plant leaf using 0–6 scale in which: 0 – no symptoms; 1 – leaf deformation; 2 – chlorosis of separate parts of lamina, 3 – oilness of separate parts of leaf; 4 – wilting or necrosis of separate parts of limb; 5 – entire lamina chlorosis or wilting; 6 – entire leaf complete necrosis.

Intensity of disease expression on plants were calculated as

$$R = \frac{\sum (n \times b)}{N},$$

where R – disease intensity, index;

$\sum(n \times b)$ – the sum of products of infected leaves quantity (n) and corresponding score of their infection (b);

N – the whole number of leaves on plants, pieces;

Disease intensity was determined on nine eggplants per every strain.

Results and Discussion

While carrying out the preliminary studies with the purpose of finding out the possibility of potato tubers using for determination of the virulence of *C. michiganensis* subsp. *sepedonicus* strains, we faced the problem of secondary infection development (dry rot) in artificially infected by pathogen tubers when incubating under conditions of hyper humidity. Moreover, measuring of tuber tissue infected zone width, based on changes of tissues colour to yellow on each side of injection on cultivars with the same colour of the flesh created difficulties. For all these reasons the method of tuber injection offered by В. И. Янович (1971) was modified. Modifications concerned inoculum concentration, conditions and duration of incubation, method of registration keeping.

Effect of inoculum dose. Determination of optimal pathogen concentration necessary to potato tuber infection was carried out on three cultivars: 'Deldhin', 'Krinitsa', 'Atlant'.

The biggest zone of infection by ring rot in 'Deldhin' and 'Krinitsa' cultivars was observed when using infection density 1×10^9 CFU/ml for inoculation. Significant difference between remaining variants was not discovered (Table 1). The maximum tuber affection of cv. 'Atlant' was observed after infection with a dose of 1×10^9 CFU/ml. However, the difference between the variants 5×10^8 CFU/ml, 1×10^9 CFU/ml, 1.5×10^9 CFU/ml is statistically not significant. The minimal tuber infection intensity of cv. 'Atlant' was determined at 1×10^8 CFU/ml.

Table 1. Effect of inoculum dose on potato tuber affection by ring rot

Inoculum dose CFU/ml	Width of tuber zone affection mm		
	'Delphin'	'Krinitsa'	'Atlant'
1×10^8	4.40^{ab*}	5.22^{ab}	3.38^{ab}
5×10^8	4.44^{ab}	4.86^{ab}	4.40^{a}
1×10^9	5.75^{a}	6.33^{a}	4.67^{a}
1.5×10^9	5.00^{ab}	5.17^{ab}	4.50^{a}

*Treatment means followed by different letters differ significantly ($P<0.05$)

Results of the test showed that for potato tubers infection by *C. michiganensis* subsp. *sepedonicus* under laboratory conditions the pathogen concentration 1×10^9 CFU/ml should be used, as in this case maximum affection of tubers tissue was recorded.

Effect of incubation period. The study of incubation period effect on potato tubers affection by ring rot was carried out on cv. 'Scarb'. It was discovered that their maximum affection was observed in two months after the infection (Figure 1). However as it may be seen (Figure 1), the value of variant 2 months significantly exceeds the value of variant 1 month and does not significantly differ from 1.5 and 3 months. At the same time there is no statistically significant difference between variants 1 and 1.5 month. So, based on presented data the incubation period of *C. michiganensis* subsp. *sepedonicus* infected tubers should not be less than 2 months.

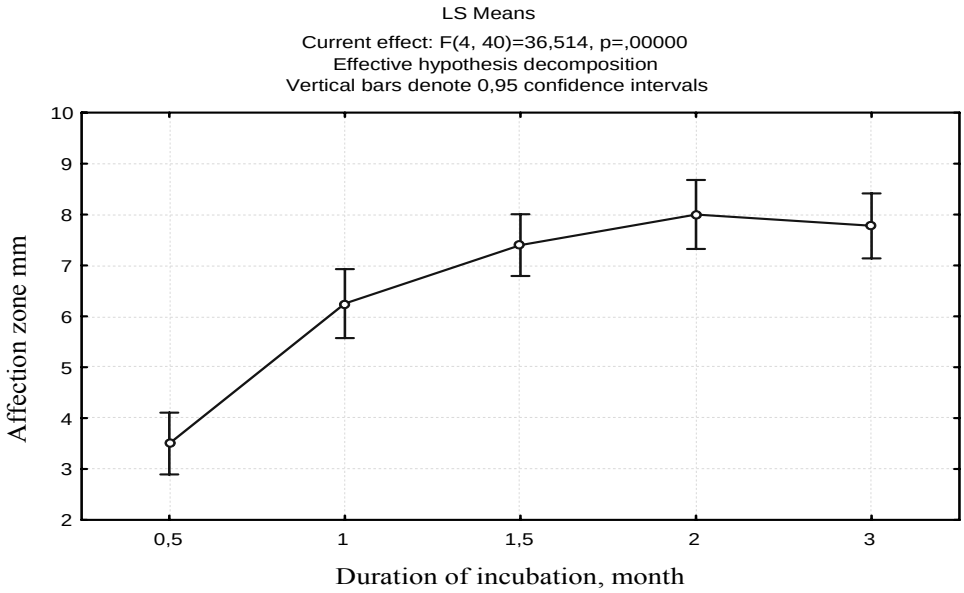

Figure 1. Effect of incubation period on potato tubers affection by *C. michiganensis* subsp. *sepedonicus*

Virulence test on potato tubers. With the help of modified method of tuber injection the width of affection zone of its tissue, typical for every pathogen strain was determined. It varied in the range from 2.2 mm – EK11 to 4.78 mm – ES4, EG1 (Figure 2).

For convenience of results interpretation we placed values of tuber tissue affection intensity by each strain in increasing order. The figure shows 3 groups of strains significantly differing by the size of tuber tissue affection. These are strains with sequence numbers 1–2, 5–7, 25–29. Based on presented data the scale was proposed and differentiation of *C. michiganensis* subsp. *sepedonicus* strains by the virulence was done.

Pathogen strains with tuber tissue affection value for more than 4 mm belonged to high-virulent group (E5, EK10, EP2, EK14, ES1, ES2, EK13, EG4, EG3, EK2, EP4, EG5, EK15, K18, ES4, EG1), 3–4 mm – middle-virulent (E7, EG6, E6, E1, EP3, E4, EK16, EK1), 2–3 mm – low-virulent (EK11, E8). Strains E6.2, E8.2 were non-virulent. Tuber tissue infected by suspension of these strains became glassy-yellow on each side of injection, and when pressing there was not typical exudation excretion.

Virulence test on eggplants. 10 strains, characterized by different virulence on potato tubers were selected with the purpose of their virulence definition on eggplants. It was determined that the least significant disease intensity on eggplants was registered after their infection with EK11 strain with respect to plants infected with EG4, K18, ES4, E3, EP1 strains. After eggplants inoculation with K18, ES4, E3, EP1 strains plants affection degree was significantly higher than after using E6 strain for infection (Figure 3).

It was found that EK11 strain both on potato tubers and on eggplants showed the lowest virulence. ES4 and EP1 on both tested crops fell within the group of strains characterized by maximum virulence. Analysis of the experimental data revealed a strong correlation between tuber affection degree and disease intensity on eggplants by

these crops infection with ten mentioned pathogen strains (Pearson r = 0.78, P < 0.01). This gives a possibility to speak about strong correlation between virulence of *C. michiganensis* subsp. *sepedonicus* strains on potato tubers and on eggplants.

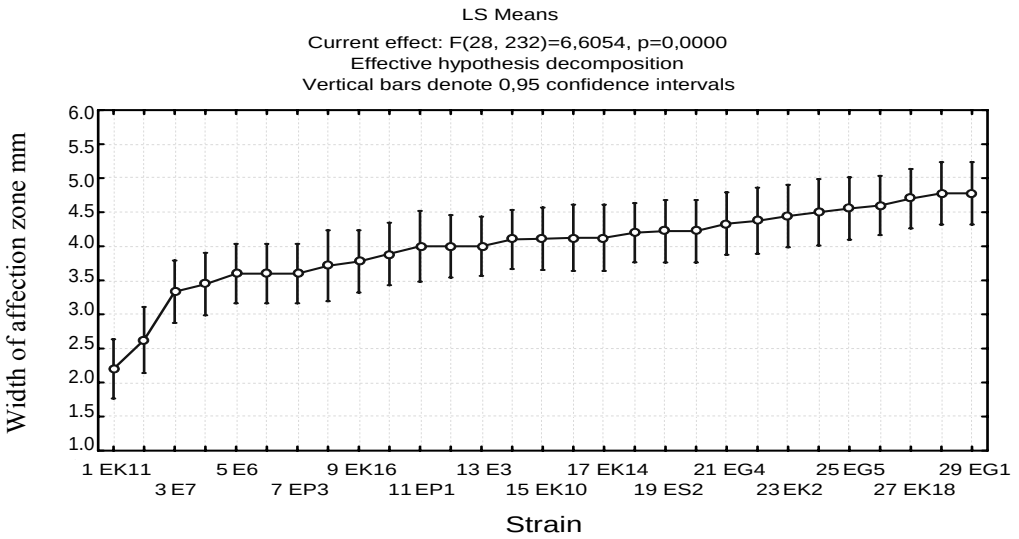

***Figure* 2.** Tubers affection intensity by *C. michiganensis* subsp. *sepedonicus* strains (**1** EK11, **2** E8, **3** E7, **4** EG6, **5** E6, **6** E1, **7** EP3, **8** E4, **9** EK16, **10** EK1, **11** EP1, **12** ES3, **13** E3, **14** E5, **15** EK10, **16** EP2, **17** EK14, **18** ES1, **19** ES2, **20** EK13, **21** EG4, **22** EG3, **23** EK2, **24** EP4, **25** EG5, **26** EK15, **27** K18, **28** ES4, **29** EG1)

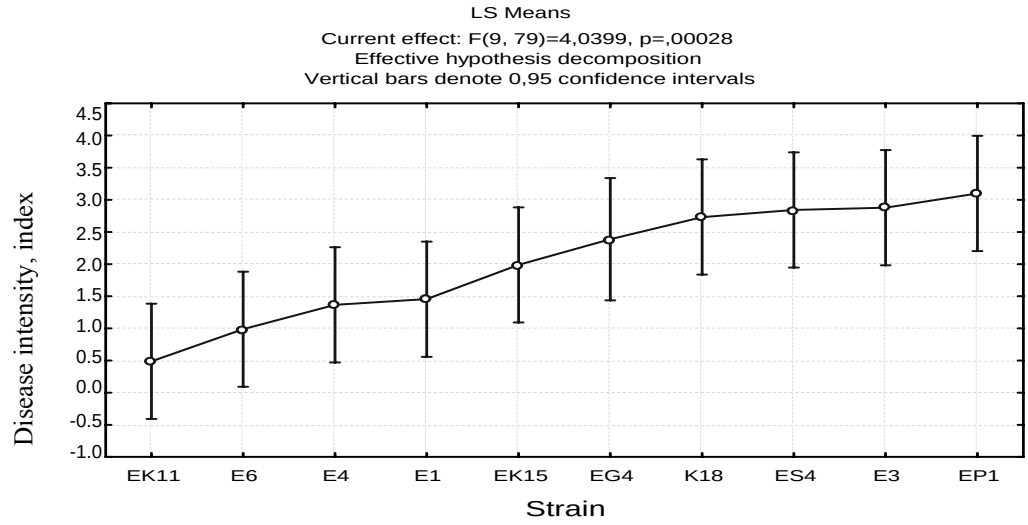

Figure 3. Disease intensity on eggplants as affected by inoculation with different strains of *C. michiganensis* subsp. *Sepedonicus*

One of the main disadvantages of tuber injection method is a long incubation period of the affected material. However, taking into account simplicity of the method, lack of special requirements for conditions under which the definition of pathogen strains virulence is carried out, sufficiently simple pathogen reisolation from infected tissues in pure culture, it may be said that this method is worthy of notice. Moreover, by evaluation the selection material under field conditions the duration of *C. michiganensis* subsp. *sepedonicus* strains virulence definition is not decisive, as it is carried out during potato storage period.

In our opinion, for determination strains virulence of potato ring rot causal agent except eggplants, potato tubers may be used according to the purpose of studies.

Conclusions

1. The breeding of potato cultivars immune to bacterial ring rot disease (*Clavibacter michiganensis* subsp. *sepedonicus*) is one of the important steps toward disease eradication. The effective work in this direction requires knowledge of the pathogen biology and ecology, understanding of mechanism of *Clavibacter michiganensis* subsp. *sepedonicus* pathogenicity, regular control of pathogen strains virulence used for evaluation for resistance/immunity to disease.

2. Along with generally accepted method of virulence of ring rot causal agent strains on eggplants, a possibility of potato tubers use for this purpose is shown. It was found that the optimal pathogen concentration necessary for potato tuber infection is 1×10^9 CFU/ml, and the incubation period is not less than 2 months. *C. michiganensis* subsp. *sepedonicus* strains showed marked difference in virulence on potato and eggplants. Analysis of the data obtained showed a strong correlation between virulence pathogen strains on both plants. Taking into account the main disadvantage of the method – a long period of incubation and its simplicity, the method of tuber injection can also be used for maintenance of pathogen strains collection in a good order and their virulence differentiation.

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