

Nonrelatedness Between Symptoms and Cucumber Virus 4 Content in Different Cucumber Cultivars

M. ČECH and HANA BRANIŠOVÁ

Institute of Experimental Botany, Department of Plant Pathology,
Czechoslovak Academy of Sciences, Praha*

Abstract. The virus replication capacity (determined by density gradient centrifugation and spectrophotometric virus assay) and severity of symptoms were compared in ten cucumber (*Cucumis sativus* L.) cultivars infected by cucumber virus 4. Except the expected resistant susceptible and symptomless virus carrier plants a new paradoxical group of cultivars was found. This group is characterized by low virus reproduction rate but simultaneously associated with severe symptoms and is responsible for difficulties in the virus transmission and purification. It is concluded that the critical evaluation of the resistance against viruses of TMV-group must include symptomatic and quantitative virus assays, as there need not be a direct relationship between virus symptoms and the virus increase.

Quantitative virus assay methods available for the viruses of the tobacco mosaic virus (TMV) group allow more detailed and accurate investigating the host-virus interactions as well as effects of various physiological and environmental conditions. Thus TMV analysis of 58 artificially inoculated plant species (ČHEO and GERARD 1971) showed, besides the disclosing of new hosts, that the virus replicating capacity as a single point feature can classify the relationship to the systemically infected plant sufficiently and precisely, when it is determined under the identical set of environmental conditions. It is not influenced by the virus content in the inoculum but it may vary with the virus strain used (BAWDEN 1950, LEBEURIER and HIRTH 1966). Scattered information exists about quantitative responses of different cultivars of the same species on the systemic infection with viruses or strains of the TMV-group. In the breeding usually the disease symptoms are followed, being sometimes supplemented with serological or more laborious dilution end point assays on the susceptible hosts or by local lesion counts on the hypersensitive plants. WALTER (1956) developed a TMV-resistant tomato line (CStMV-18) which became infected without visible symptoms. McRITCHIE and ALEXANDER (1963) used this tomato and other selected wild tomato species for the isolation and characterization of four tomato strains of TMV. These

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* Address: Na Karlovce 1, Praha 6, Czechoslovakia.

strains revealed 3 different reactions when inoculated on CStMW-18-15 tomato: susceptible with symptoms, susceptible without symptoms and highly resistant. In his long-termed virus assay performed by quantitative electronmicroscopic particle counting, DAWSON (1965) has shown a delayed increase of a new tomato strain of TMV which was selected in the resistant tomato and which was able to break the resistance. Quantitative analysis of TMV infected tulip cultivars (MOKRÁ *et al.* 1973) suggested variable virus content which in some cases did not agree with symptom expression. Similar observations reported KOOLSTRA (1968) in his paper on the resistance in cucumber cultivars to *Cucumis* virus 2 (cucumber green mottle mosaic virus belonging to the group of TMV viruses; HARRISON *et al.* 1971). By dilution end point assays he did not establish any substantial virus content differences in both the susceptible and symptomless cultivars.

A detailed study of the interaction of the TMV viruses with different cultivars of the same plant species would be beneficial. Replication capacity of the cucumber virus 4, orig. Knight (a yellow strain of cucumber green mottle mosaic virus, BAWDEN and KASSANIS 1968, KADO and KNIGHT 1970) and severity of symptoms were therefore followed in ten different cucumber cultivars under identical growing conditions using quantitative density gradient centrifugation and spectrophotometric assay of the virus.

Materials and Methods

Ten pregerminated cucumber cultivars (*Cucumis sativus* L.) listed in Table 1 were grown simultaneously in the glasshouse during the sunny summer period at 18–28 °C. All plants were inoculated at the same time with glass spatula on both cotyledons, using a stock suspension of purified cucumber virus 4 (CV 4) (0.2 mg ml⁻¹ in 0.05 M phosphate buffer pH 7.0) as the inoculum. They were harvested 15–16 days later (*i.e.* at the time of the highest virus content as determined in preliminary experiments) and immediately worked out. Leaves from 10 plants of each cultivar without cotyledons and midribs were being ground for 5 min with pestle and mortar in 0.05 Tris-HCl, pH 7.5 buffer, containing 0.001 M disodium EDTA and 0.01 M 2-mercaptoethanol (1 ml : 1 gram leaf). The expressed sap was clarified by double emulgation with equal amount of Freon 113 (1,1,2-trifluoro-1,2,2-trichloroethane) in a syringe and centrifuged 10 min at 10 000 g. Aliquots (1.8 ml) of the clear extract (corresponding to 1 g leaf) were layered on 10–40 % (w/v) linear sucrose gradients in 0.05 M Tris-HCl, pH 7.5 buffer and centrifuged 2 h at 24 000 rpm in a Spinco SW 25.1 rotor. The tube content was scanned with an ultraviolet flow-through photometer and collected into 28 fractions. The virus peak fractions (4–6 tubes) were quantitatively pooled and after proper dilution assayed spectrophotometrically, taking $A_{1\text{ cm}/260\text{ nm}} = 3.2$ for 1 mg CV 4/ml. Two fractions immediately adjoining the virus peak were omitted and the next four fractions (two from both sides of the virus peak respectively) were pooled and proportionally diluted. They served as an internal blank for spectrophotometric assay. Control blanks, obtained independently by gradient centrifugation of equivalent healthy cucumber saps, agreed within 5 per cent error limit with above mentioned internal blanks. If freshly prepared leaf extracts were analysed, the absorbancy values did not have to be corrected for light scattering. The calculated virus content was expressed in mg virus per gram fresh leaf area. The results represent the mean value of two independent experiments.

Results and Discussion

Experimental results, summarized in Table 1, represent comparable responses of examined cucumber cultivars to CV 4 infection, as other factors were minimized by identical set of environmental conditions. All plants used for the assay were infected as judged by symptoms or indirectly after the back transmission on susceptible 'Chinesische Schlangen' cultivars. Effects of possible virus inhibitors were eliminated by the sucrose gradient centri-

TABLE 1

VIRUS REPLICATION CAPACITY AND SYMPTOM SEVERITY WITH CUCUMBER CULTIVARS (*Cucumis sativus* L.), 15–16 DAYS AFTER INOCULATION WITH CUCUMBER VIRUS 4.

Cucumber cultivar	Virus content mg virus/gram leaf	Symptom severity ^a
'Chinesische Schlangen'	0.83	+++
'Spottresisting'	0.68	++
'Jedinečná'	0.47	+
'Marketer'	0.39	+++
'Bilská III'	0.35	0 ^b
'Židovická produkta'	0.32	+
'Salátnice'	0.16	+
'Unikát'	0.02	+++
'Mladoboleslavská polní salátovka'	< 0.01	0 ^b
'Reforma I pařeništní'	< 0.01	+++

- ^a 0 — no symptoms
 + — no more than one yellow spot per leaf
 ++ — vein clearing and yellow flecks
 +++ — yellow (white) mottle and mosaic

^b A small samples from each plant were taken and tested separately for infectivity by back transmission on susceptible cucumber

fugation and spectrophotometric assay of the pure virus fraction. All plant extracts were prepared shortly before they were analysed by sucrose gradient centrifugation. Consequently a single virus peak without aggregates occurred and no material was pelleted on the bottom of the centrifugation tubes. Therefore the gradient centrifugation could not cause any virus losses.

Responses of ten randomly selected cultivars differ both in the virus replication capacity and in the degree of symptom expression; no direct relationship could be derived. The analysed cultivars, however, can be more or less arranged into four groups representing all possible combinations of extreme responses:

1) Cultivars reacting with high virus reproduction rate and with severe symptoms (cv. 'Chinesische Schlangen', 'Spottresisting', 'Marketer'). This group represents plants which could be unequivocally classified as susceptible.

2) Cultivars with high virus reproduction rate and slight or no symptoms (cv. 'Bilská', 'Jedinečná'). This group represents symptomless virus carriers.

3) Cultivars with low virus reproduction rate and severe symptoms (cv. 'Unikát', 'Reforma I pařeništní'). This strange group comprises plants with paradoxically high symptomatic sensitivity and high resistancy to virus increase. It follows that the severity of symptoms and the virus replication need not necessarily be simply interrelated as suggested originally by BANCROFT and POUND (1954) for TMV. Such feature would deserve further study.

4) Cultivars with low virus reproduction rate and slight or no symptoms (cv. 'Mladoboleslavská polní salátovka'). Only this group can be classified as resistant.

It is remarkable that, when cucumber cultivars belonging to groups 3 and 4 were inoculated with the stock virus suspension (0.2 mg CV 4/ml) under not optimal growing conditions (*i.e.* in early spring, without supplement-

ing illumination and at 12–18 °C), many of those cucumber plants failed to become infected. Both groups behave therefore like resistant to the virus infection and spread. On the other hand all seedlings belonging to groups 1 and 2 became easily infected under such conditions, though the plants of group 2 did not show any symptoms. Therefore it seems that the resistance of a given cultivar to CV 4 infection is associated with the low replication capacity.

Searching for resistance to *Cucumis* virus 2 (a green strain of CV 4) KOOSTRA (1968) experienced that Japanese cucumber cultivar 'Natsufushinari' behaves like cultivars from the above described group 2. He warns against introducing such varieties as basic breeding materials as they might act as unrecognized virus carriers.

From the foregoing it follows that the exact evaluation of resistance or susceptibility to CV 4 and most probably to other TMV viruses must include both the symptoms evaluation and the quantitative virus assay. Lower virus assay limits especially in the TMV group can be further extended as experiments with subliminal infection of cotton suggest (CHEO 1970).

The results presented indicate that from the virological point of view the choice of proper varieties, cultivars or lines as suitable hosts is very important. Susceptible plants represented by group 1 are convenient in general. Plants belonging to the above mentioned group 3 may, however, cause serious difficulties in characterizing as well as in indexing of virus diseases as they may be responsible for troubles or even failures of transmission experiments. For purification purposes such plants are fully unsuitable as the virus yields may be several orders lower. Preliminary quantitative assays offer more reliable information than the mere symptomatical evaluation.

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M. ČECH, HANA BRANIŠOVÁ (Praha): **Nesouvislost mezi příznaky a obsahem viru zelenoskvvrnitě mozaiky okurky (Cucumber virus 4, Knight) v různých kultivarech okurky.** — Biol. Plant. 18 : 58—62, 1976.

V deseti kultivarech okurky (*Cucumis sativus* L.) infikovaných virem zelenoskvvrnitě mozaiky okurky (CV 4, Knight) se porovnával maximální obsah viru (stanovený spektrofotometricky po centrifugaci v hustotním gradientu) s intenzitou příznaků. Vedle rezistentních, náchylných kultivarů a bezpříznakých nosičů viru byla nalezena nová paradoxní skupina, vyznačující se nízkou reprodukcí viru a velmi silnými příznaky. V této skupině lze obtížně přenášet i čistit virus. Z pokusů vyplývá, že ke kritickému posouzení rezistence vůči virům ze skupiny viru tabákové mozaiky je nutné doplnit sledování příznaků kvantitativní analýzou obsahu viru, protože mezi příznaky a reprodukcí viru v rostlině nemusí být přímý vztah.

BOOK REVIEW

ZIMMERMANN, U., DAINTY, J. (ed.): **Membrane Transport in Plants.** — Springer-Verlag, Berlin-Heidelberg-New York 1974. 473 pp.

This book represents the proceedings of the symposium "International Workshop on Membrane Transport in Plants" held at the Nuclear Research Centre, Jülich, FRG in February 1974. More than two hundred and fifty scientists from fourteen countries took part in this meeting. The members of the programme committee (U. Zimmermann, J. Dainty, F. Führ, N. Higginbotham, A. B. Hope, A. Lev, U. Lüttge, H. W. Nürnberg, E. A. C. MacRobbie, H. Stieve, M. Thellier, K. Wagener) decided on a very broad spectrum of topics including thermodynamics of transport processes, water relations, primary reactions of photosynthesis, as well as the more conventional aspects of membrane transport. Like the symposium, the book is divided into nine sessions; each session contains 4 to 13 papers and a record of the round table discussion.

The contributions to Session 1 (Thermodynamics and Electrochemistry of Membrane Transport) give basic knowledge of the transport processes and chemical reactions across membranes. Session 2 (Water Transport and Osmotic Processes) dealt with transport of water at cellular level as well as bulk water movement in the shoots. Special attention was given to some parameters such as the hydraulic conductivity or the volume elastic modulus and their volume- and pressure-dependence and to osmoregulation and turgor pressure regulation. The next, Session 3 (Electrical Properties of Membranes), is focussed on the nature of biological membrane potentials and the relationship of those potentials to metabolism, on the transport of ionic substances and the transport of uncharged substrates. Special attention was given to the light-dependent changes in membrane potentials closely linked to the photosynthetic reactions. The biggest Session 4 (Solute Transport in Algae and Cellsuspension Cultures) contains 13 papers dealing with transport of ions (Cl^- , H^+ , phosphate, sulphate, etc.) and organic substances connected with electron transport processes of photosynthesis, photophosphorylation, permeability, and thermodynamics. The objects were mostly algae. Photosynthesis is the subject of Session 5 (Transport in Isolated Chloroplasts). Proton transport in chloroplasts is explained by chemiosmotic theory as a process connected with photosynthetic electron transport and characterized by changes in chlorophyll energetics *in vivo* (variable fluorescence and delayed fluorescence). Session 6 (ATPases and Transport) contains only four papers dealing with stimulation of ATPase activity by monovalent or divalent cations and of another membrane bound ATPase by anions and correlations between those ion-stimulated ATPases and ion uptake. The round-table discussions are included in Session 7 (Kinetics of Transport). In this session special attention was given to interpretation and use of influx isotherms. Session 8 (Transport in Organs of Higher Plants) is very wide because it includes various systems of supply and demand of particular substances, their structure and ultrastructure, transport mechanisms and methodical approaches to these problems. The last, Session 9 (Regulating Factors in Membrane Transport) is devoted to the influence of plant hormones, growth regulators and metabolic inhibitors on transport processes.

The book may be recommended as a very recent source of information for research workers, particularly because it is intended to bring advanced thermodynamical concepts to the attention of biologists and to show physical chemists what the more complex biological systems are like. Thanks to the great effort of the editorial board to make the submitted manuscripts uniform, to redraw figures and so on, the book is a homogeneous unit, understandable to a wide circle of readers working in this field. It is well produced and its text is illustrated with 252 graphic figures and 49 tables. A subject index is also included.

JANA POSPÍŠILOVÁ (Praha)