ESTABLISHMENT OF ECONOMICALLY IMPORTANT VIRUSES ON WATERMELONS BY ELISA METHOD IN BULGARIA

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ABSTRACT
The reaction of widespread watermelon varieties to economically important viral pathogens was studied by ELISA method in Bulgaria. It was established that Crimson Suit, Sugar Baby and Mramorna 17 were susceptible to Zucchini Yellow Mosaic Virus (ZYMV), Papaya Ringspot Virus watermelon strain W - (PRSV-W), Watermelon Mosaic Virus 2 (WMV2), Squash Mosaic Virus (SqMV) and Cucumber Mosaic Virus (CMV). ZYMV reached the highest viral concentration in watermelon plants infected by artificial inoculation. PRSV-W reached the highest viral concentration in natural infection of commercial crops from Crimson Suit. This virus was followed by WMV2, SqMV, ZYMV, CMV, or followed by SqMV, ZYMV, CMV, WMV2. For the first time in Bulgaria, it was established, that SqMV was a pathogen for watermelon, because it appeared in high viral concentration in plants. The grown watermelon varieties were not immune or tolerant to CMV, because CMV reached high viral concentration in some plants from these varieties.

Introduction
The most widespread watermelon variety in Bulgaria nowadays is Crimson Suit. This variety and also the Bulgarian varieties Borjana and Bojura are with high resistance to antracnosa and fusarium wilt. (1, 2).

In Bulgaria, there is not much data of how the spread watermelon varieties react to economically important for cucurbit crops virus diseases. Before several decades, Kovatchevski (1965), established that watermelon species (Citrullus vulgaris) was very sensitive to Watermelon Mosaic Virus 2 (WMV 2). But to Cucumber Mosaic Virus (CMV) it manifested high resistance (4). During the next years Dikova, 1995 proved identical contents of both WMV2 and CMV viruses in 43 % of watermelon plants from different crops. It was established that ZYMV was the most spread virus – in 45 % of the watermelon plants, and PRSV-W - in 36 % (6). There is no data for Squash Mosaic Virus (SqMV) on watermelon crops, which is very harmful for the other cucurbit species.

The studies of the reaction of different watermelon species and varieties are orientated to economically important viruses. Gillaspie & Wright, 1993 detected 10 resistant and 5 tolerant to WMV 2 watermelon specimens after the studying of genofond, including 670 watermelon species (7). It was established resistance of the varieties Ouricuri and Charleston Gray to WMV 2 and PRSV-W (9). It was found in other examinations that watermelon BT 85 was tolerant to WMV 2 and ZYMV (10).

The aim of the present development is to establish contents of each of the five viruses in most widespread variety or varieties in Bulgaria. The results would be of an interest for selectionists and watermelon growers.

Materials and Methods
Watermelon mosaic plants from two production areas – village Polykraiste (the...
Central part of North Bulgaria) and village Koslovez (near Danube river) were gathered. Both crops were sowed with Crimson Suit variety. Samples with conspicuous mosaic symptoms on the leaves were only tested. Except plants of the production crops, artificially infected plants of Crimson suit variety and from other widespread in the commercial net varieties were also tested. The watermelon plants were inoculated in phase –first /second leaf by artificial mechanic inoculation with isolates of five economically important viruses. The isolates originated from different cucurbit cultures, but all were multiplied in cucumber variety Levina. The analyses carried out by ELISA, adapted by Koening, 1998 (8) . The existence of each of the viruses was established by comparing the measured extinction values after graphic presentation on Figs. 1 and 2. The virus’ content in both, natural and in artificial infected plants, was proved after processing the results by statistical analysis through Student’s Criterion, mentioned from Lidanski, 1988 (5) . It was accepted for negative control measured extinction values for each of the viruses in uninoculated plants of Crimson suit variety. Mosaic Zucchini Marrow was used – a plant taken from neighbouring of watermelons crop for positive control in the test with production crops. But for positive control of artificially infected watermelon varieties from commercial net, a cucumber plant Levina variety was taken. Levina plant was infected with the same inoculum as the watermelon varieties. The antisera was granted to us by different researchers: for ZYMV, WMV 2 and PRSV-W - from Dr. Lisa (Italy), for SqMV - from Dr. Maat (The Netherlands), and for CMV - from Dr. Hristova (Bulgaria).

We are extremely grateful for the granted antisera.

Results and Discussion

The results of the analysis of watermelon plants from production crops by ELISA are presented on Fig. 1 and in Tables 1 and 3. The highest extinction values for the largest number of samples were measured for PRSV-W and that is a proof for the highest viral concentration of the same virus in natural infected watermelon plants. From the total number of samples (32), 10 had extinction values above 1.0 Optical Density-OD. (Fig. 1a). Second place, according to virus concentration, took WMV 2. On third and fourth places are SqMV and ZYMV with a slight priority to SqMV. The latter existed in a lot of plants with high extinction values. Each of WMV 2,SqMV, and ZYMV were remarkable with 4 number of watermelon samples with extinction above 1.0 OD. The remaining plants had extinction values and according to them, WMV 2 was second, SqMV - third, and ZYMV - fourth (Fig. 1b, 1c, 1d). Fifth, according to virus concentration, was CMV. Cucumber Mosaic Virus had a majority of samples with extinction values over 0.3 OD (Fig. 1e) . Distinction, according to virus content among both production crops was formed. The samples from Koslovez had lower extinction values, compared to the samples from Polykraiste. This distinction according to viral concentration among both crops agreed with visually established difference in the observed plant’s symptoms. Mosaic from light and dark green spots on the leaves, without symptoms on the fruits was noted on the Koslovez’s crop (Fig. 3a). Conspicuous yellow spots on the leaves were visible except the mosaic in the Polykraiste, crop. Such yellowing, we established also on growing watermelon fruits. The viruses put in order, according to the extinction values in the following downgrade row in leaf sample, number 32: PRSV-W – 1.138 OD; ZYMV – 1.006 OD; SqMV – 0.957 OD; WMV2 – 0.789 OD, and CMV – 0.626 OD. The same viruses, originated from growing yellow fruit, 32 ordered according to the extinction values in downgrade row:
Establishment of viruses by ACP - ELISA in naturally infected watermelon plants, variety Crimson suit in crops of Koslovez (samples from 1 to 20) and Polykraiste (samples from 21 to 32).

- **Fig. 1a.** PRSV-W
- **Fig. 1b.** WMV2
- **Fig. 1c.** SqMV
- **Fig. 1d.** ZYMV
- **Fig. 1e.** CMV

**Fig. 1.** Establishment of viruses by ACP - ELISA in naturally infected watermelon plants, variety Crimson suit in crops of Koslovez (samples from 1 to 20) and Polykraiste (samples from 21 to 32).

On abscissa - number of samples, on ordinate - extension values.
TABLE 1

Establishment of virus concentration in watermelon plants from production crops by ELISA

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Number samples with extinction values for:</th>
<th>v. Koslovez near Danube river</th>
<th>v. Polykraiste, central part of N. Bulgaria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>PRSV-W</td>
<td>6</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>WMV2</td>
<td>2</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>SqMV</td>
<td>0</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>ZYMV</td>
<td>0</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>CMV</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Legend:
I. Number plants with extinction above 1.0 optical units
II. Number plants with extinction from 0.8 to 1.0 optical units
III. Number plants with extinction from 0.5 to 0.8 optical units
IV. Number plants with extinction from 0.15 to 0.5 optical units

TABLE 2

Establishment of virus concentration in artificially infected watermelon plants from three varieties, distributed in commercial net by ELISA

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Number samples with extinction values for:</th>
<th>Sugar baby</th>
<th>Crimson suit</th>
<th>Mramorna 17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
</tr>
<tr>
<td>ZYMV</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>WMV2</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>PRSV-W</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>SqMV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CMV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Legend:
I. - number plants with extinction from 0.9 to 1.1 optical units
II. - number plants with extinction from 0.7 to 0.9 optical units
III. - number plants with extinction from 0.5 to 0.7 optical units
IV. - number plants with extinction from 0.3 to 0.5 optical units
V. - number plants with extinction below 0.1; 0.1; 0.15 to 0.3 optical units

TABLE 3

Results from statistical analysis

<table>
<thead>
<tr>
<th>Samples from varieties or origin</th>
<th>Optical Density - Extinction values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WMV2</td>
</tr>
<tr>
<td>Sugar baby</td>
<td>*</td>
</tr>
<tr>
<td>Crimson suit</td>
<td>0.611 ± 0.104</td>
</tr>
<tr>
<td>Mramorna</td>
<td>0.679 ± 0.158</td>
</tr>
<tr>
<td>v. Koslovez</td>
<td>0.712 ± 0.084</td>
</tr>
<tr>
<td>v. Polykraiste</td>
<td>0.790 ± 0.219</td>
</tr>
</tbody>
</table>

* - medium absorption in 492 nm; ** - standard deviation; - without possibility to find confidential interval.

PRSV-W – 0.648 OD; ZYMV – 0.462 OD; SqMV – 0.245 OD; WMV2 – 0.218 OD, and CMV – 0.109 OD (Fig. 3b). The data from the statistical analysis (medium extinction quantity from the extinction values of the watermelon samples and standard deviations) are presented in Table 3. They supported the results from Table 1 as they showed the distinction in virus concentration in samples of both production crops. Higher content of PRSV-W, ZYMV and CMV in Polykraiste samples is probably the reason for the yellow symptoms on the leaves and fruits (Fig. 3b). For yellow spots on the leaves and yellowing on the fruits one of the viruses might be responsible. Another possibility is a mixed infection of several viruses. It was estab-
lished by statistical analysis in degree of reliability 0.05 distinctions between the virus concentration in the Koslovez’s samples and the Polykraiste’s samples only among ZYMV and CMV. At the other viruses did not prove distinctions in concentrations. The most sufficient reason for the yellowing of the growing watermelon fruits on Polykraiste, crop is ZYMV, or ZYMV and one of the following: PRSV-W, SqMV and CMV. The comparing of the medium quantities of the extinction values proved the distinction in viral concentration of the samples from both crops. At the Koslovez’s samples according to the medium quantities of the extinction values the viruses ranged in downgrade row in this way: PRSV-W, WMV2, SqMV, ZYMV and CMV. At the Polykraiste’s samples, according to the middle quantities, the viruses ranged in the following downgrade row: PRSV - W, SqMV, ZYMV, CMV and WMV2. (Table 3). The data from Tables 1 and 3 showed, that the species Citrullus lanatus (vulgaris), variety Crimson Suit could not be considered tolerant to CMV, since 5 out of 32 samples had very high extinctions above 1.0 OD. Only 9 samples from production crops had low extinctions for CMV below 0.3 OD. Therefore on the field CMV appeared as a serious pathogen for watermelon species. In support of our results for CMV we present the result of Varveri & Boutsika (1999) in Greece (11). They isolated CMV in very high viral concentration – above 1.0 OD. from watermelon plant with mosaic symptoms. For the first time in Bulgaria, we received data for infection of watermelon production crops with SqMV. Out of 32 plants, 21 had extinction values for SqMV near 0.5 OD, but 4 of them all from Polykraiste had extinctions above 1.0 OD.

It was established in artificial infection of widespread in commercial net of Bulgaria watermelon varieties the following conclusion: about varieties Crimson Suit, Sugar Baby and Mramorna 17 was reported the largest number of samples with the highest extinction values for ZYMV (Table 2, Fig. 2a). Both, Crimson Suit and Mramorna 17, had 5 plants with high extinction values from 0.9 to 1.1 OD. for ZYMV. According to virus concentration, the following viruses distributed themselves so: on second place was WMV2, on third place was PRSV – W, on fourth place was SqMV, and on fifth place was CMV (Fig. 2b, 2c, 2d, 2e). The CMV infection was very low in plants of the three varieties. Out of 27 artificially infected plants, 26 had extinction values below 0.3 OD. and for only one watermelon plant was noted extinction value of 0.306 OD. According to Kovatchevski, 1965, in artificial inoculation watermelon endured CMV latently (4). Our results show a change in watermelon reaction to CMV. In production watermelon crops of Crimson Suit, tolerance to CMV was not manifested. The comparing of the medium quantities of extinction values, showed no statistical proved distinction (difference) among virus concentration in the three watermelon varieties. Therefore, widespread in Bulgaria varieties of Citrullus lanatus reacted almost equally to viruses.

The difference between our and Kovatchevski’s data (4) was possibly caused by the appearance of aggressive strains of CMV, or by the susceptibility of the watermelon varieties. Squash Mosaic Virus (SqMV) was established in highest concentration after PRSV-W concentration from the crop of Polykraiste. This result shows SqMV as a serious pathogen for watermelon. Because of the circumstance that SqMV transmits itself through the seeds, it was found, that SqMV is economically significant for watermelon. SqMV transmission through the watermelon seeds in extent 2 % was proved (3). PRSV-W, known also as WMV1, and WMV2 were in considerable concentration in both cases: in naturally and in artificially infected watermelons.
Fig. 2a. ZYMV

Fig. 2b. WMV2

Fig. 2c. PRSV-W

Fig. 2d. SqMV

Fig. 2e. CMV

Fig. 2. Establishment of viruses by ACP-ELISA in artificially infected three watermelon varieties: from 1 to 7 - Sugar baby, from 8 to 16 - Crimson suit and from 17 to 27 - Mramorna 17.

on abscissa - number of samples, on ordinate - extension values.
Fig. 3. Plant sample № 32. 3a - Mosaic from light and darkgreen spots on the leaves from v. Koslovetz; 3b - Mosaic from yellow spots on the leaves and yellowing on the watermelon fruit from v. Polykraste; 3c - Yellow colored fruit, from watermelon plant - sample № 32, containing viruses.

Conclusions
Propagated in Bulgaria watermelon varieties: Crimson Suit, Sugar Baby and Maramorna 17 are susceptible to economically important for cucurbit crops virus pathogens: ZYMV, PRSV-W, WMV2, SqMV, CMV. The highest viral concentration in artificially inoculated watermelon plants,
reached ZYMV. In naturally infected watermelons from production crops, the highest virus concentration reached PRSV-W. SqMV is established for the first time in Bulgaria as a pathogen for watermelons. The varieties Crimson Suit, Sugar Baby and Mramorna 17 are not immune to CMV. Crimson Suit is not tolerant to CMV.

REFERENCES