UPOV

International Union for the Protection of New Varieties of Plants

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**Technical Working Party for Vegetables**

**TWV/55/12**

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# PARTIAL REVISION OF THE TEST GUIDELINES FOR VEGETABLE MARROW, SQUASH

*Document prepared by an expert from France*

*Disclaimer: this document does not represent UPOV policies or guidance*

1. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Vegetable Marrow, Squash (document TG/119/4 Corr. 2).

2. The Technical Working Party for Vegetables (TWV), at its fifty-fourth session hosted by Brazil and organized by electronic means, from May 11 to 15, 2020, agreed that the Test Guidelines for Vegetable Marrow, Squash (document TG/119/4 Corr. 2) be partially revised for the addition of new Characteristics "Resistance to *Zucchini yellow mosaic virus* (ZYMV)" and "Resistance to *Watermelon mosaic virus* (WMV)" (see document TWV/54/9 "Report", Annex III).

3. The following changes are proposed:

(a) Addition of new Characteristic 82 "Resistance to *Zucchini yellow mosaic virus* (ZYMV)" at the end of the Table of Characteristics
(b) Addition of an explanation Ad. 82 "Resistance to *Zucchini yellow mosaic virus* (ZYMV)" in Chapter 8.2 "Explanations for individual characteristics"
(c) Addition of new Characteristic 83 "Resistance to *Watermelon mosaic virus* (WMV)" at the end of the Table of Characteristics
(d) Addition of an explanation Ad. 83 "Resistance to *Watermelon mosaic virus* (WMV)" in Chapter 8.2 "Explanations for individual characteristics"

4. The proposed changes are presented below in highlight and underline (insertion) and ~~strikethrough~~ (deletion).

Proposal to add new Characteristic 82 “Resistance to Zucchini yellow mosaic virus (ZYMV)” at the end of the Table of Characteristics

| | | English | français | Deutsch | español | Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo | Note/ Nota |
|---|---|---|---|---|---|---|---|
| **82**. **(+)** | **VS** | **Resistance to *Zucchini yellow mosaic virus* (ZYMV)** | **Resistance au *Zucchini yellow mosaic virus* (ZYMV)** | **Resistenz gegen *Zucchini yellow mosaic virus* (ZYMV)** | **Resistencia a *Zucchini yellow mosaic virus* (ZYMV)** | | |
| **QN** | | susceptible | sensible | anfällig | sensible | Cora | 1 |
| | | sudceptible to intermediate resistant | sensible à modérément résistante | anfällig bis mäßig resistent | sensible a moderadamente resistente | Not used | 2 |
| | | intermediate resistant | modérément résistante | mäßig resistent | moderadamente resistente | Mirza | 3 |
| | | intermediate resistant to resistant | modérément résistante à résistante | mäßig resistent bis resistent | moderadamente resistente a resistente | Not used | 4 |
| | | resistant | résistante | resistent | resistente | Mikonos | 5 |

Proposed addition of an explanation Ad. 82 “Resistance to *Zucchini yellow mosaic virus* (ZYMV)” in Chapter 8.2 “Explanations for individual characteristics”

Ad. 82: Resistance to *Zucchini yellow mosaic virus* (ZYMV)

| | | |
|---|---|---|
| 1. | Pathogen | *Zucchini yellow mosaic virus* (ZYMV) |
| 2. | Quarantine status | No |
| 3. | Host species | *Cucurbita pepo* L. |
| 4. | Source of inoculum | GEVES (FR)[1] |
| 5. | Isolate | e.g. strain E9 |
| 6. | Establishment isolate identity | - |
| 7. | Establishment pathogenicity | Symptoms on susceptible squash variety |
| 8. | Multiplication inoculum | |
| 8.1 | Multiplication medium | Living plant |
| 8.2 | Multiplication variety | e.g. Cora |
| 8.3 | Plant stage at inoculation | - |
| 8.4 | Inoculation medium | - |
| 8.5 | Inoculation method | - |
| 8.6 | Harvest of inoculum | - |
| 8.7 | Check of harvested inoculum | - |
| 8.8 | Shelf life/viability inoculum | - |
| 9. | Format of the test | |
| 9.1 | Number of plants per genotype | At least 20 |
| 9.2 | Number of replicates | At least 2 |
| 9.3 | Control varieties | • Susceptible: Cora<br>• Intermediate resistant (low threshold of intermediate resistance level): Mirza<br>• Resistant (low threshold of resistance level): Mikonos |
| 9.4 | Test design | add non inoculated plants |
| 9.5 | Test facility | Climatic room or greenhouse |
| 9.6 | Temperature | e.g. 22°C or 24°C/18°C |
| 9.7 | Light | 12h-16h |
| 9.8 | Season | |
| 9.9 | Special measures | - |
| 10. | Inoculation | |
| 10.1 | Preparation inoculum | 1 g leaf with symptoms with 4 mL of PBS with carborundum (400 mg) and activated carbon (400 mg) or similar buffer, homogenize |
| 10.2 | Quantification inoculum | - |
| 10.3 | Plant stage at inoculation | First expanded leaf |
| 10.4 | Inoculation method | Rubbing with virus suspension |
| 10.5 | First observation | 14 days post-inoculation |
| 10.6 | Second observation | - |
| 10.7 | Final observations | 21 days post-inoculation |
| 11. | Observations | |
| 11.1 | Method | Visual observation |
| 11.2 | Observation scale | Class 0: no symptoms<br>Class 1: few chlorotic patches<br>Class 2: many chlorotic patches<br>Class 3: large chlorotic areas (some patches on young leaves)<br>Class 4: mosaic and weak vein banding<br>Class 5: deformation and vein banding |

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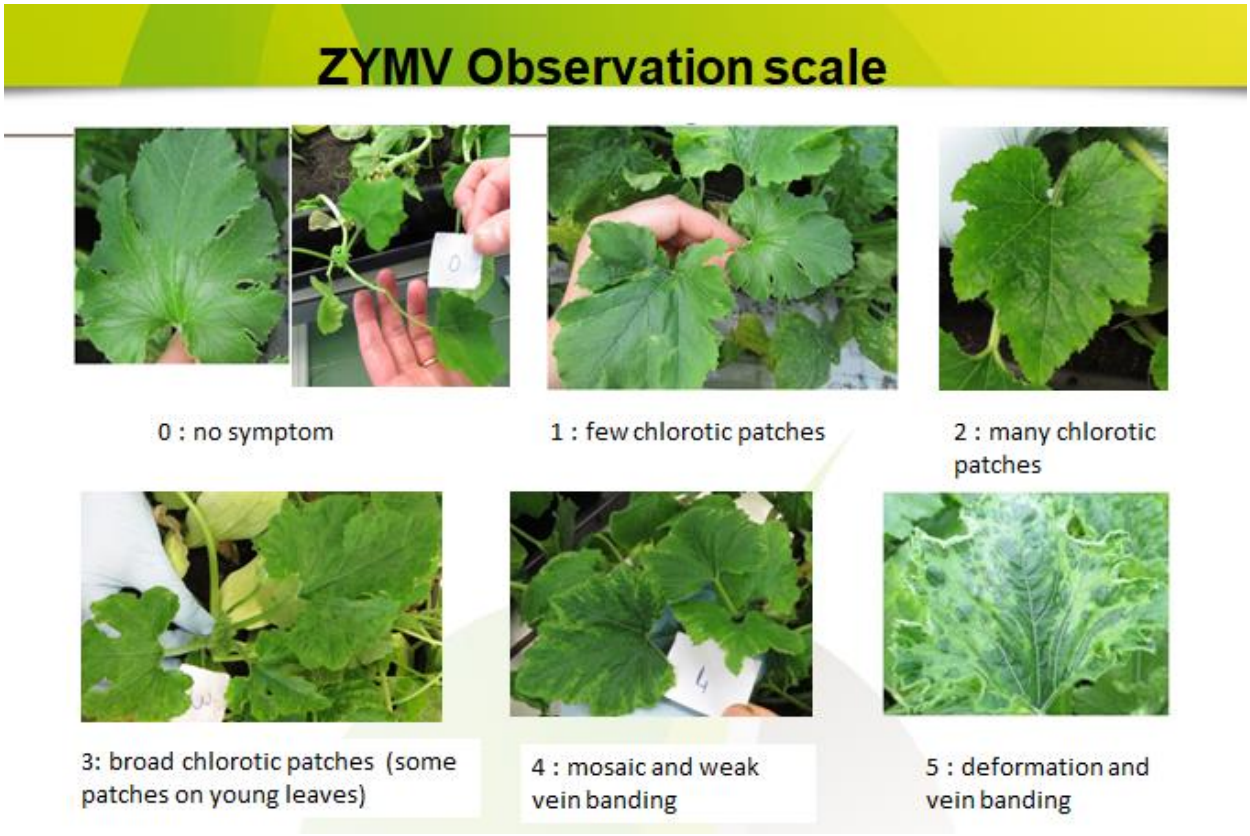
| | | |
|---|---|---|
| 11.3 | Validation of test | Results should be compared with results of controls and are depending of the aggressiveness of the test and the distribution of the plants over the classes.<br><br>The two intermediate and resistant controls are necessary to validate the aggressiveness of the test. |
| 11.4 | Off-types | - |
| 12. | Interpretation of data in terms of UPOV characteristic states | - Note 1: Classes 4 and 5 are predominantly observed on susceptible plants.<br>- Note 3: Classes 2, 3 are predominantly observed on intermediate resistant plants.<br>- Note 5: Classes 0, 1 are predominantly observed on resistant plants.<br><br>Notes 2 and 4 exist, but no control for these levels are commonly validated yet.<br><br>In the framework of harmonisation of the produced descriptions for this new quantitative characteristic, we suggest to concentrate the UPOV used notes to the notes 1, 3, and 5 only.<br><br>A variety with a lower resistance than Mirza (note 3,) will be described note 1. A variety with a lower resistance than Mikonos (note 5), will be described note 3.<br><br>An additional statistical analysis could be helpful to finalize the pathologist's raw observation to the assessment of uniformity, and relative position regarding the example varieties results. |
| 13. | Critical control points | Recommended dates of notation should be adapted depending on expression of symptoms on controls.<br>Environmental conditions can have an effect on the expression of symptoms over time. In this case a second notation could be necessary. |



Proposed addition of new Characteristic 83 “Resistance to *Watermelon mosaic virus* (WMV)” at the end of the Table of Characteristics

| | English | français | Deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|
| **83**. **VS**<br>**(+)** | **Resistance to *Watermelon mosaic virus* (WMV)** | **Resistance au *Watermelon mosaic virus* (WMV)** | **Resistenz gegen *Watermelon mosaic virus* (WMV)** | **Resistencia a *Watermelon mosaic virus* (WMV)** | | |
| | susceptible | sensible | anfällig | sensible | Cora | 1 |
| | suceptible to intermediate resistant | sensible à modérément résistante | anfällig bis mäßig resistent | sensible a moderadamente resistente | Not used | 2 |
| | intermediate resistant | modérément résistante | mäßig resistent | moderadamente resistente | Sofia | 3 |
| | intermediate resistant to resistant | modérément résistante à résistante | mäßig resistent bis resistent | moderadamente resistente a resistente | Mikonos, Syros | 4 |
| | resistant | résistante | resistent | resistente | Not identified | 5 |

Proposed addition of an explanation Ad. 83 “Resistance to *Watermelon mosaic virus* (WMV)” in Chapter 8.2 “Explanations for individual characteristics”

Ad. 83: Resistance to *Watermelon mosaic virus* (WMV)

| | | |
|---|---|---|
| 1. | Pathogen | *Watermelon mosaic virus* (WMV) |
| 2. | Quarantine status | No |
| 3. | Host species | *Cucurbita pepo* L. |
| 4. | Source of inoculum | GEVES (FR)[2] |
| 5. | Isolate | e.g. strain LL1A |
| 6. | Establishment isolate identity | - |
| 7. | Establishment pathogenicity | Symptoms on susceptible squash variety |
| 8. | Multiplication inoculum | |
| 8.1 | Multiplication medium | Living plant |
| 8.2 | Multiplication variety | e.g. Cora |
| 8.3 | Plant stage at inoculation | - |
| 8.4 | Inoculation medium | - |
| 8.5 | Inoculation method | - |
| 8.6 | Harvest of inoculum | - |
| 8.7 | Check of harvested inoculum | - |
| 8.8 | Shelf life/viability inoculum | - |
| 9. | Format of the test | |
| 9.1 | Number of plants per genotype | At least 20 |
| 9.2 | Number of replicates | At least 2 |
| 9.3 | Control varieties | • Susceptible: Cora<br>• Intermediate resistant (low threshold level): Sofia<br>• Intermediate resistant to resistant (intermediate resistant controls of *higher level)*: Mikonos or Syros<br><br>The two levels of intermediate resistant controls are necessary to validate the aggressiveness of the test. |
| 9.4 | Test design | add non inoculated plants |
| 9.5 | Test facility | Climatic room or greenhouse |
| 9.6 | Temperature | e.g. 22°C or 24°C/18°C |
| 9.7 | Light | 12h-16h |
| 9.8 | Season | |
| 9.9 | Special measures | - |
| 10. | Inoculation | |
| 10.1 | Preparation inoculum | 1 g leaf with symptoms with 4mL of PBS with carborundum (400mg) and activated carbon (400mg) or similar buffer, homogenize |
| 10.2 | Quantification inoculum | - |
| 10.3 | Plant stage at inoculation | First expanded leave |
| 10.4 | Inoculation method | Rubbing with virus suspension |
| 10.5 | First observation | 14 days post-inoculation |
| 10.6 | Second observation | - |
| 10.7 | Final observations | 21 days post-inoculation |
| 11. | Observations | |
| 11.1 | Method | Visual observation |

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| | | |
|---|---|---|
| 11.2 | Observation scale | Class 0: no symptoms<br>Class 1: few chlorotic patches<br>Class 2: many chlorotic patches<br>Class 3: large chlorotic areas (some patches on young leaves)<br>Class 4: mosaic, weak vein banding<br>Class 5: deformation and vein banding |
| 11.3 | Validation of test | Results should be compared with results of controls and are depending of the aggressiveness of the test and the distribution of the plants over the classes. |
| 11.4 | Off-types | - |
| 12. | Interpretation of data in terms of UPOV characteristic states | - Note 1: Classes 4 and 5 are predominantly observed on susceptible plants.<br>- Note 3: Classes 2, 3, 4 are predominantly observed on intermediate resistant plants.<br>- Note 4: Classes 0, 1, 2, 3 are predominantly observed on plants with a higher level of intermediate resistance (intermediate to resistant level).<br><br>Up to now, no complete resistance is identified against this virus. It is the reason why NO example variety is provided to illustrate the Note 5.<br><br>Note 2 could exist, but no control for this level is commonly validated yet.<br><br>In the framework of harmonisation of the produced descriptions for this new quantitative characteristic, we suggest to concentrate the UPOV used notes to the notes 1, 3, and 4 only.<br><br>A variety with a lower of resistance than Sofia (note 3), will be described note 1. A variety with a lower resistance than Mikonos (note 4), will be described note 3.<br><br>*An additional statistical analysis could be helpful to finalize the pathologist's raw observation to the assessment of uniformity, and relative position regarding the example varieties results.* |
| 13. | Critical control points | Recommended dates of notation should be adapted depending on expression of symptoms on controls.<br>Environmental conditions can have an effect on the expression of symptoms over time. In this case a second notation could be necessary. |

## WMV Observation scale

0 : no symptom

1 : few chlorotic patches

2 : many chlorotic patches

3: broad chlorotic patches (some patches on young leaves)

4 : mosaic and weak vein banding

5 : deformation and vein banding

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