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| International Union for the Protection of New Varieties of Plants |  |

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| Technical Working Party for Vegetables  Fifty-Fifth Session Antalya, Turkey, May 3 to 7, 2021 | TWV/55/12  Original: English  Date: April 8, 2021 |

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

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).

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).

The following changes are proposed:

1. Addition of new Characteristic 82 “Resistance to *Zucchini yellow mosaic virus* (ZYMV)” at the end of the Table of Characteristics
2. Addition of an explanation Ad. 82 “Resistance to *Zucchini yellow mosaic virus* (ZYMV)” in Chapter 8.2 “Explanations for individual characteristics”
3. Addition of new Characteristic 83 “Resistance to *Watermelon mosaic virus* (WMV)” at the end of the Table of Characteristics
4. Addition of an explanation Ad. 83 “Resistance to *Watermelon mosaic virus* (WMV)” in Chapter 8.2 “Explanations for individual characteristics”

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

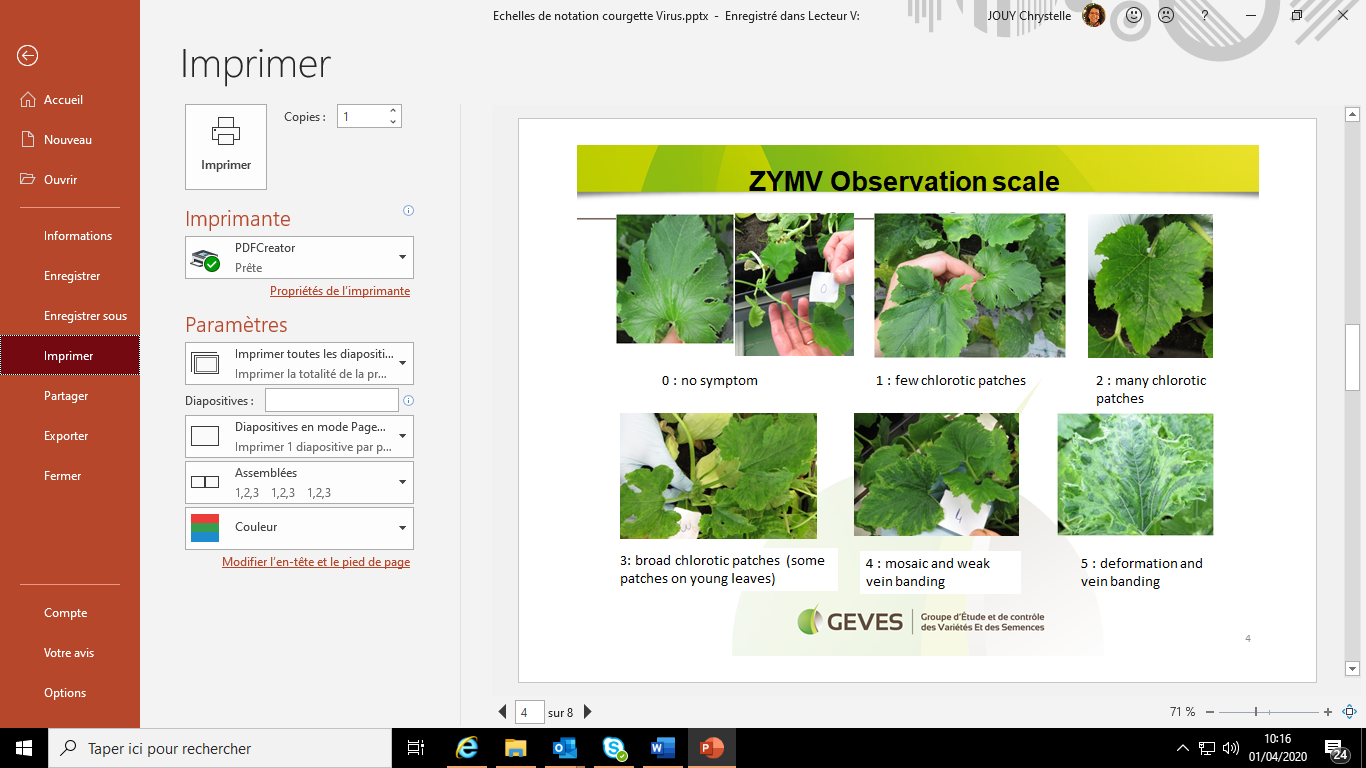
Table of Characteristics

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | 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]](#footnote-2) |
| 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 * Intermediate resistant (low threshold of intermediate resistance level): Mirza * 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  Class 1: few chlorotic patches  Class 2: many chlorotic patches  Class 3: large chlorotic areas (some patches on young leaves)  Class 4: mosaic and weak vein banding  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.  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.  - Note 3: Classes 2, 3 are predominantly observed on intermediate resistant plants.  - Note 5: Classes 0, 1 are predominantly observed on resistant plants.  Notes 2 and 4 exist, but no control for these levels are commonly validated yet.  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.  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.  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.  Environmental conditions can have an effect on the expression of symptoms over time. In this case a second notation could be necessary. |



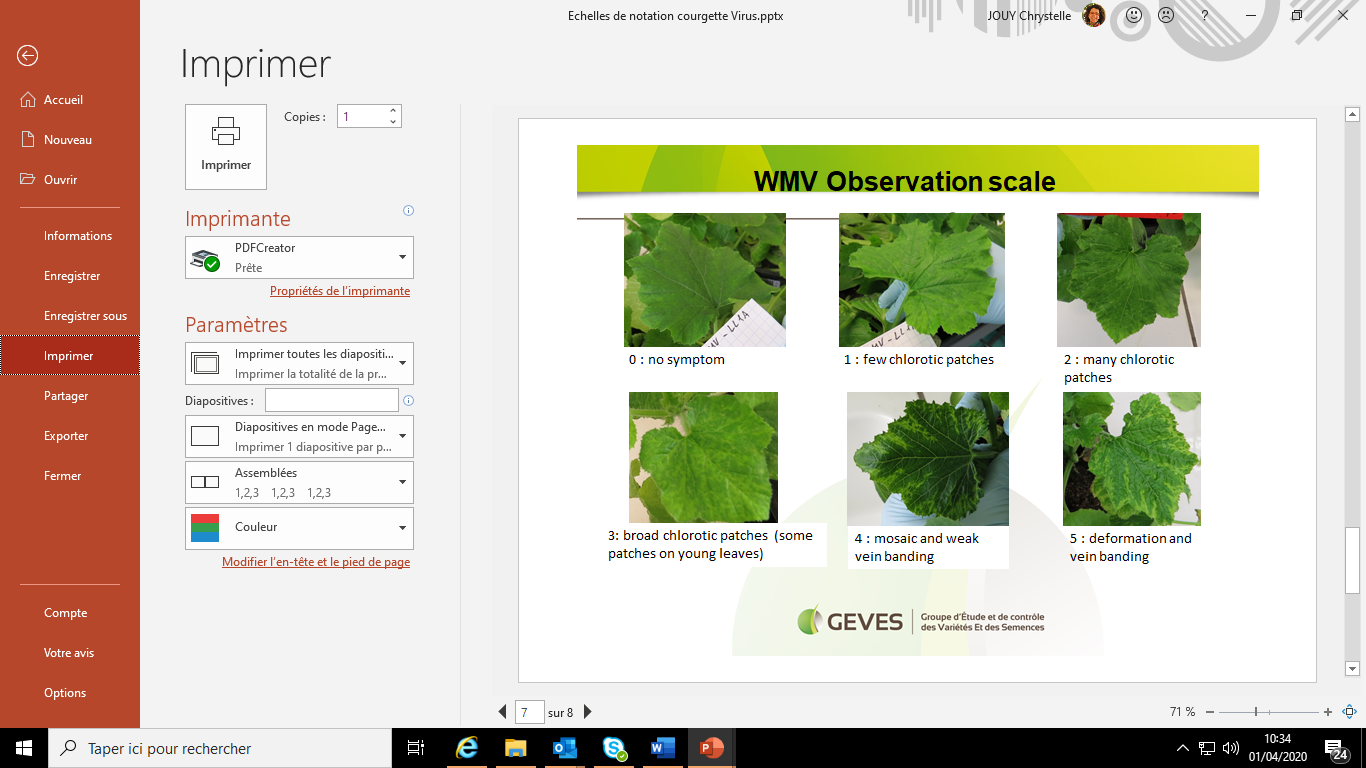
Proposed addition of new Characteristic 83 “Resistance to *Watermelon mosaic virus* (WMV)” at the end of the Table of Characteristics

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | English | français | Deutsch | español | Example Varieties Exemples Beispielssorten Variedades ejemplo | Note/ Nota |
| **83**.  **(+)** | VS | 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]](#footnote-3) |
| 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 * Intermediate resistant (low threshold level): Sofia * Intermediate resistant to resistant (intermediate resistant controls of *higher level)*: Mikonos or Syros   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 |
| 11.2 | Observation scale | Class 0: no symptoms  Class 1: few chlorotic patches  Class 2: many chlorotic patches  Class 3: large chlorotic areas (some patches on young leaves)  Class 4: mosaic, weak vein banding  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.  - Note 3: Classes 2, 3, 4 are predominantly observed on intermediate resistant plants.  - Note 4: Classes 0, 1, 2, 3 are predominantly observed on plants with a higher level of intermediate resistance (intermediate to resistant level).  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.  Note 2 could exist, but no control for this level is commonly validated yet.  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.  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.  *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.  Environmental conditions can have an effect on the expression of symptoms over time. In this case a second notation could be necessary. |



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1. matref@geves.fr [↑](#footnote-ref-2)
2. matref@geves.fr [↑](#footnote-ref-3)