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

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| Technical Working Party for Vegetables  Fifty-Sixth Session Virtual meeting, April 18 to 22, 2022 | TWV/56/20  Original: English  Date: March 17, 2022 |

Partial revision of the Test Guidelines for VEGETABLE MARROW, SQUASH

Document prepared by the Office of the Union

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-fifth session, hosted by Turkey and organized by electronic means, from May 3 to 7, 2021, 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/55/16 “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 |
|  |  | susceptible to intermediate resistant | sensible à résistant à un niveau intermédiaire | anfällig bis mittel resistent | sensible a resistencia intermedia | - | 2\* |
|  |  | intermediate resistant | résistant à un niveau intermédiaire | mittel resistent | resistencia intermedia | Mirza | 3 |
|  |  | intermediate resistant to resistant | résistant à un niveau intermédiaire à résistant | mittel resistent bis resistent | resistencia intermedia a resistente | - | 4\* |
|  |  | highly resistant | hautement résistant | hochresistent | altamente resistente | Mikonos | 5 |
|  |  |  |  |  |  | (\*= restricted use suggested  of this level. See Ad. 82, 12. ) | |

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  = MAT/REF/06-08-02-02 |
| 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: Mirza (low threshold of intermediate resistance level): * highly resistant: Mikonos (low threshold of high resistance level): |
| 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 | keep glasshouse free of aphids |
| 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 cotyledons 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 |
| Courtesy of GEVES-SNES | | |
| 11.3 | Validation of test | The highly resistant control (Mikonos), the intermediate resistant control (Mirza) and the susceptible control (Cora) are necessary to validate the aggressiveness of test.  Results should be compared with results of controls, based on disease index AND distribution of 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 are predominantly observed on intermediate resistant plants.  - Note 5: Classes 0, 1 are predominantly observed on highly 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 concentrating 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 can be used to finalize the pathologist’s raw observation to the assessment of uniformity, and relative position regarding the controls results. |
| highly resistant  CORA  MIKONOS  susceptible  MIRZA  intermediate resistant | | |
| 13. | Critical control points | Recommended dates of notation should be adapted depending on expression of symptoms on controls.  Environmental conditions can influence the expression of symptoms over time. In this case a second notation could be necessary.  Aphids may transmit ZYMV as well as other viruses that may contaminate the ZYMV strain. Test should be in aphid-free compartment. |

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)** |  |  |
| QN |  | susceptible | sensible | anfällig | sensible | Cora | 1 |
|  |  | susceptible to intermediate resistant | sensible à résistant à un niveau intermédiaire | anfällig bis mittel resistent | sensible a resistencia intermedia | - | 2\* |
|  |  | intermediate resistant | résistant à un niveau intermédiaire | mittel resistent | resistencia intermedia | Sofia | 3 |
|  |  | intermediate resistant to resistant | résistant à un niveau intermédiaire à résistant | mittel resistent bis resistent | resistencia intermedia a resistente | Mikonos, Syros | 4 |
|  |  | highly resistant | hautement résistant | hochresistent | altamente resistente | - | 5\* |
|  |  |  |  |  |  | (\*= restricted use suggested  of this level. See Ad. 83, 12. ) | |

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  = MAT/REF/06-09-01 2 |
| 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 * Sofia (low threshold level) * Mikonos, Syros = intermediate resistant to highly resistant (intermediate resistant controls of higher level |
| 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 | Keep glasshouse free of aphids |
| 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 cotyledons 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 |
| Courtesy of GEVES-SNES | | |
| 11.3 | Validation of test | The controls for two levels of intermediate resistance and the susceptible control are necessary to validate the aggressiveness of the test.  Results should be compared with the results of controls, based on disease index AND distribution of 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 exists, 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 concentrating 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 orSyros (note 4), will be described as note 3.  An additional statistical analysis can be used to finalize the pathologist’s raw observation to the assessment of uniformity, and relative position regarding the controls results. |
| intermediate to highly resistant  CORA  MIKONOS  SYROS  susceptible  SOFIA  intermediate resistant  = **Note 3**  highly resistant | | |
| 13. | Critical control points | Recommended dates of notation should be adapted depending on expression of symptoms on controls.  Environmental conditions can influence the expression of symptoms over time. In this case a second notation could be necessary.  Aphids may transmit WMV as well as other viruses that may contaminate the WMV strain. Test should be in aphid-free compartment. |

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