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

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| Enlarged Editorial Committee  Geneva, March 26 and 27, 2018 | TC-EDC/Mar18/6  Original: English  Date: February 1, 2018 |

Partial revision of the Test Guidelines for PEPPER

Document prepared by an expert from the European 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 Pepper (*Capsicum annuum* L.) (document TG/76/8 Rev.).

The Technical Working Party for Vegetables (TWV), at its fifty-first session, held in Roelofarendsveen, Netherlands, from July 3 to 7, 2017, considered a proposal for a partial revision of the Test Guidelines for Pepper (document TG/76/8 Rev.) on the basis of documents TG/76/8 Rev. and TWV/51/7 “Partial Revision of the Test Guidelines for Pepper” and proposed the following revisions to the Test Guidelines Pepper (see document TWV/51/16 “Report”, paragraphs 107 and 108):

1. To change the example varieties for the following characteristics of Characteristic 48 “Resistance to Tobamovirus”
   1. 48.1 “*Tobacco mosaic virus* Pathotype 0 (TMV: 0)”
   2. 48.2 “*Pepper mild mottle virus* Pathotype 1.2 (PMMoV: 1.2)”
   3. 48.3 “*Pepper mild mottle virus* Pathotype 1.2.3 (PMMoV: 1.2.3)”
2. To change the methodology for Characteristic 48 “Resistance to Tobamovirus” under Ad. 48

The TWV noted that, at the same time as the partial revision of Char. 48 “Resistance to Tobamovirus”, the following correction would be made Pepper (see document TWV/51/16 “Report”, paragraph 109):

(c) To add the missing method of observation VG to Characteristic 2 “Plant: habit” (see documents TG/76/8(proj.6) and TC/42/11, Annex II).

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

Proposal to change the example varieties for the following characteristics of Characteristic 48 “Resistance to Tobamovirus”

*Current wording*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **48.  (+)** | **VG** | **Resistance to Tobamovirus** | **Résistance au tobamovirus** | **Resistenz gegen Tobamovirus** | **Resistencia al tobamovirus** |  |  |
| **48.1 (\*)** |  | ***Tobacco mosaic virus*  Pathotype 0(TMV: 0)** | ***Tobacco mosaic virus*  Pathotype 0(TMV: 0)** | ***Tobacco mosaic virus*  Pathotyp 0(TMV: 0)** | ***Tobacco mosaic virus*  Patotipo 0 (TMV: 0)** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Gordo, Pepita, Piperade | 1 |
|  |  | present | présente | vorhanden | presente | Lamuyo, Sonar,  Yolo Wonder | 9 |
| **48.2 (\*)** |  | ***Pepper mild mottle virus* Pathotype 1.2 (PMMoV: 1.2)** | ***Pepper mild mottle virus* Pathotype 1.2 (PMMoV: 1.2)** | ***Pepper mild mottle virus* Pathotyp 1.2 (PMMoV: 1.2)** | ***Pepper mild mottle virus* Patotipo 1.2 (PMMoV: 1.2)** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Lamuyo, Yolo Wonder | 1 |
|  |  | present | présente | vorhanden | presente | Ferrari, Orion, Solario | 9 |
| **48.3 (\*)** |  | ***Pepper mild mottle virus* Pathotype 1.2.3 (PMMoV: 1.2.3)** | ***Pepper mild mottle virus* Pathotype 1.2.3 (PMMoV: 1.2.3)** | ***Pepper mild mottle virus* Pathotyp 1.2.3 (PMMoV: 1.2.3)** | ***Pepper mild mottle virus* Patotipo 1.2.3 (PMMoV: 1.2.3)** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Solario, Yolo Wonder | 1 |
|  |  | present | présente | vorhanden | presente | Cuby, Friendly | 9 |

*Proposed new wording*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **48.  (+)** | **VG** | **Resistance to *Tobamovirus*** | **Résistance au tobamovirus** | **Resistenz gegen Tobamovirus** | **Resistencia al tobamovirus** |  |  |
| **48.1 (\*)** |  | ***Tobacco mosaic virus*  Pathotype P0(TMV: 0)** | ***Tobacco mosaic virus*  Pathotype 0(TMV: 0)** | ***Tobacco mosaic virus*  Pathotyp 0(TMV: 0)** | ***Tobacco mosaic virus*  Patotipo 0 (TMV: 0)** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | ~~Gordo, Pepita, Piperade~~ Lamu, Pepita, Piquillo | 1 |
|  |  | present | présente | vorhanden | presente | ~~Lamuyo, Sonar,~~ Fehérözön, Turia, Yolo Wonder | 9 |
| **48.2 (\*)** |  | ***Pepper mild mottle virus* Pathotype P1.2 (PMMoV: 1.2)** | ***Pepper mild mottle virus* Pathotype 1.2 (PMMoV: 1.2)** | ***Pepper mild mottle virus* Pathotyp 1.2 (PMMoV: 1.2)** | ***Pepper mild mottle virus* Patotipo 1.2 (PMMoV: 1.2)** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | ~~Lamuyo,~~ Fehérözön, Lamu, Turia, Yolo Wonder | 1 |
|  |  | present | présente | vorhanden | presente | ~~Ferrari, Orion, Solario~~ Candela, Ferrari, Novi 3, PI152225 | 9 |
| **48.3 (\*)** |  | ***Pepper mild mottle virus* Pathotype P1.2.3 (PMMoV: 1.2.3)** | ***Pepper mild mottle virus* Pathotype 1.2.3 (PMMoV: 1.2.3)** | ***Pepper mild mottle virus* Pathotyp 1.2.3 (PMMoV: 1.2.3)** | ***Pepper mild mottle virus* Patotipo 1.2.3 (PMMoV: 1.2.3)** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | ~~Solario,~~ Candela, Ferrari, Yolo Wonder | 1 |
|  |  | present | présente | vorhanden | presente | ~~Cuby,~~ Bisonte, Friendly, Tom 4 | 9 |

## Proposal to change the methodology for Characteristic 48 “Resistance to Tobamovirus” under Ad. 48

*Current wording*

Ad. 48: Resistance to Tobamovirus

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| --- | --- | --- |
| 1. | Pathogen | Tobamovirus (the genus containing *Tobacco mosaic virus* (TMV), and *Pepper mild mottle virus* (PMMoV)) |
| 2. | Quarantine status | no |
| 3. | Host species | *Capsicum annuum* |
| 4. | Source of inoculum | GEVES (FR), Naktuinbouw (NL), INIA (ES) |
| 5. | Isolate | Pathotype 0, Pathotype 1.2, and Pathotype 1.2.3 |
| 6. | Establishment isolate identity | on differentials (S = susceptible, R = resistant) |

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| --- | --- | --- | --- | --- | --- |
|  |  | Tobamovirus Pathotypes on Pepper | | |  |
|  |  | TMV: 0 | PMMoV: 1.2 | PMMo: 1.2.3 |  |
| Resistance code | Resistance gene | 0 | 1.2 | 1*.*2*.*3 | Differentials |
|  | L0 | S | S | S | Lamu, Pepita |
| Tm0 | L1 | R | S | S | Explorer, Lamuyo, Sonar, Yolo Wonder |
| Tm1 | L2\* | R | S | S | *C. frutescens* ‘Tabasco’\* |
| Tm2 | L3 | R | R | S | Ferrari, Novi 3, Orion, Solario |
| Tm3 | L4 | R | R | R | Cuby, Friendly, Tom 4 |

\*no seed of L2 varieties available; L2 is not used in breeding

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| --- | --- | --- |
| 7. | Establishment pathogenicity | use susceptible pepper standard or lesions on *Nicotiana tabacum* 'Xanthi' 2 days after inoculation |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | on living plant or desiccated leaves |
| 8.2 | Multiplication variety | tomato or pepper (e.g. Lamu) or *Nicotiana tabacum* (cv. Samsun) |
| 8.3 | Plant stage at inoculation | cotyledons fully developed or at “first leaf” pointed stage or 3-5 leaf |
| 8.4 | Inoculation medium | ice-cold PBS + carborundum |
| 8.5 | Inoculation method | rubbing |
| 8.6 | Harvest of inoculum | - |
| 8.7 | Check of harvested inoculum | - |
| 8.8 | Shelflife/viability inoculum | freeze-dried leaves dry storage at 4°C for ten years |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | at least 20 plants |
| 9.2 | Number of replicates | e.g. 1 |
| 9.3 | Control varieties | see table of example varieties below |

|  |  |  |  |
| --- | --- | --- | --- |
| Resistance to | ToMV: 0 – TMV: 0 | PMMoV: 1.2 | PMMoV: 1.2.3 |
| absent | Gordo, Pepita, Piperade | Lamuyo, Yolo Wonder | Solario, Yolo Wonder |
| present | Lamuyo, Sonar, Yolo Wonder | Ferrari, Orion, Solario | Cuby, Friendly |

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| 9.4 | Test design | to add untreated plant |
| 9.5 | Test facility | glasshouse or climatic chamber |
| 9.6 | Temperature | 20-25°C |
| 9.7 | Light | at least 12h |
| 9.8 | Season | - |
| 9.9 | Special measures | - |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | juice: PBS(1:9). To obtain the juice, it is preferable to use a mortar for grinding infected leaves |
| 10.2 | Quantification inoculum | 150 plants with 100 ml virus suspension |
| 10.3 | Plant stage at inoculation | cotyledons fully developed or at “first leaf” pointed stage or 3-5th leaf |
| 10.4 | Inoculation method | rubbing with a virus suspension or using of brush for more equable inoculation and avoiding mechanical damage |
| 10.5 | First observation | 5-6 days to 10 - 15 days post inoculation |
| 10.6 | Second observation | 10-11 days post inoculation to 15 - 20 days post inoculation |
| 10.7 | Final observations | 20 days post inoculation |
| 11. | Observations |  |
| 11.1 | Method | visual, comparative; necrosis signifies hypersensitivity and resistance |
| 11.2 | Observation scale |  |
|  | [1] absent: | mosaic (sometimes developing late, sometimes early and leading to plant death without hypersensitivity) |
|  | [9] present | All these observations could be made:   * systemic necrosis, stunting * local necrosis, leaf dropping * no virus symptoms, only mechanical damage   They can be linked to several factors such as the earliness of contamination, the strain use for example (see CPVO project HARMORES 2 – 2012-2015), but not due to particular genotypes. |
| 11.3 | Validation of test | on standards |
| 11.4 | Off-types | maximum 1 on 20 plants |
| 12. | Interpretation of data in terms of UPOV characteristic states | QL |
| 13. | Critical control points | Tobamovirus pathotype is defined on differentials and may belong to TMV: 0, PMMoV: 1.2, PMMoV: 1.2.3 |

*Proposed new wording*

Ad. 48: Resistance to Tobamovirus

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | *Tobacco mosaic virus* and *Pepper mild mottle virus* |
| 2. | Quarantine status | no |
| 3. | Host species | Sweet pepper, hot pepper, paprika and chili – *Capsicum annuum* L. |
| 4. | Source of inoculum | GEVES[[1]](#footnote-2) (FR), Naktuinbouw[[2]](#footnote-3) (NL) or INIA[[3]](#footnote-4) (SP) |
| 5. | Isolate | *Tobacco mosaic virus* pathotype 0 (TMV: 0) strain Vi-6  *Pepper mild mottle virus* pathotype 1.2 (PMMoV: 1.2) strain nt203  *Pepper mild mottle virus* pathotype 1.2.3 (PMMoV: 1.2.3) strain Eve  The test protocols have been validated in a CPVO co-funded project[[4]](#footnote-5) with these 3 isolates/pathotypes. |
| 6. | Establishment isolate identity | genetically defined pepper differentials (reference to ISFwebsite: <http://www.worldseed.org/isf/differential_hosts.html>) |
| 7. | Establishment pathogenicity | Test on susceptible plants |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | Regeneration of the virus of plant material before inoculum preparation. |
| 8.2 | Multiplication variety | On susceptible pepper variety, Tobamovirus races may be multiplied on varieties which are selective for each particular race. For TMV, because tomato and tobacco *Nicotiana tabacum* cv.Samsun have large leaves and can produce a lot of inoculum, they are recommended for the multiplication of TMV: 0. |
| 8.3 | Plant stage at inoculation | see 10.3 |
| 8.4 | Inoculation medium | see 10.1 |
| 8.5 | Inoculation method | see 10.4 |
| 8.6 | Harvest of inoculum | Symptomatic fresh leaves |
| 8.7 | Check of harvested inoculum | option: on young leaves of *Nicotiana tabacum* “Xanthi”, check for local lesions after 5-7 days at 20-25°C. |
| 8.8 | Shelflife/viability inoculum | fresh > 1 day in fridge, desiccated > 1 year in fridge or juice > 1 year in freezer at -20°C |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | At least 20 plants. |
| 9.2 | Number of replicates | - |
| 9.3 | Control varieties | TMV: 0:  Susceptible controls: Lamu, Pepita, Piquillo  Resistant controls: Fehérözön, Yolo Wonder  PMMoV: 1.2:  Susceptible controls: Fehérözön, Lamu, Yolo Wonder  Resistant controls: Ferrari, Novi 3  PMMoV: 1.2.3:  Susceptible controls: Ferrari, Yolo Wonder  Resistant controls: Friendly, Tom 4 |
| 9.4 | Test design | add non inoculated plants |
| 9.5 | Test facility | Climate room or greenhouse |
| 9.6 | Temperature | 20-25°C |
| 9.7 | Light | 12 hours or longer |
| 9.8 | Season | - |
| 9.9 | Special measures | - |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | 1 g leaf with symptoms with 10 mL PBS or similar buffer or dilution of juice in water.  Homogenize, add carborundum to buffer |
| 10.2 | Quantification inoculum | - |
| 10.3 | Plant stage at inoculation | TMV: 0, cotyledons to first leaf stage  PMMoV: 1.2, cotyledon stage  PMMoV: 1.2.3, cotyledon stage |
| 10.4 | Inoculation method | rubbing with the virus suspension. |
| 10.5 | First observation | TMV:0:  4-7 days post-inoculation for observation of local necrosis.  PMMoV: 1.2 and PMMoV: 1.2.3:  4-7 days post-inoculation for observation of local necrotic lesions which can lead to cotyledon drop. After this date these necrosis can hardly be seen on fallen cotyledons. |
| 10.6 | Second observation | TMV: 0:  two weeks post-inoculation for observation of symptoms of susceptibility.  PMMoV: 1.2 and PMMoV: 1.2.3:  two weeks post-inoculation for observation of symptoms of susceptibility. |
| 10.7 | Final observations | TMV:0 :  three weeks post-inoculation.  PMMoV: 1.2 and PMMoV: 1.2.3:  three weeks post-inoculation.  For TMV:0, PMMoV: 1.2 and PMMoV: 1.2.3, two of these three observations may be sufficient; the third notation is optional for observation of evolution of symptoms (depending on symptoms on controls or heterogeneous behaviour). |
| 11. | Observations |  |
| 11.1 | Method | Visual |
| 11.2 | Observation scale | TMV: 0:  Susceptibility: mosaic (aucuba in case of aucuba strain as Vi-6), growth reduction, death of plants.  Resistance: local necrotic lesions which can lead to leave drop, systemic necrosis, vein necrosis, stem necrosis.  PMMoV: 1.2 and PMMoV: 1.2.3:  Susceptibility: mosaic (green), growth reduction.  Resistance: local necrotic lesions which can lead to cotyledon drop, systemic necrosis. |
| 11.3 | Validation of test | evaluation of variety resistance should be calibrated with results of resistant and susceptible controls. |
| 12. | Interpretation of data in terms of  UPOV characteristic states |  |
|  | absent………………………. | [1] susceptible |
|  | present……………………… | [9] resistant |
| 13. | Critical control points | For TMV: 0, plants with no symptoms at all have to be interpreted as escapes of inoculation. |
|  | Recommended dates of notation should be adapted depending of expression of symptoms on controls.  Environmental conditions can have an effect on the expression of symptoms over time. In this case a third notation could be necessary. | |

## Proposal to add the missing method of observation VG to Characteristic 2 “Plant: habit”

*Current wording*

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **2.** |  | **Plant: habit** | **Plante: port** | **Pflanze: Wuchsform** | **Planta: porte** |  |  |
| **QN** |  | upright | érigé | aufrecht | erecto | De Cayenne,  Doux très long des Landes, Piquant d’Algérie | 1 |
|  |  | semi-upright | demi‑érigé | halbaufrecht | semierecto | Clovis, Sonar | 2 |
|  |  | prostrate | étalé | liegend | postrado | Delphin, Trophy | 3 |

*Proposed new wording*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **2.** | **VG** | **Plant: habit** | **Plante: port** | **Pflanze: Wuchsform** | **Planta: porte** |  |  |
| **QN** |  | upright | érigé | aufrecht | erecto | De Cayenne,  Doux très long des Landes, Piquant d’Algérie | 1 |
|  |  | semi-upright | demi‑érigé | halbaufrecht | semierecto | Clovis, Sonar | 2 |
|  |  | prostrate | étalé | liegend | postrado | Delphin, Trophy | 3 |

[End of document]

1. [matref@geves.fr](mailto:matref@geves.fr) [↑](#footnote-ref-2)
2. [resistentie@naktuinbouw.nl](mailto:resistentie@naktuinbouw.nl) [↑](#footnote-ref-3)
3. [cardaba@inia.es](mailto:cardaba@inia.es) [↑](#footnote-ref-4)
4. Harmores 2 CPVO project (<http://www.cpvo.europa.eu/main/en/home/documents-and-publications/technical-projects-reports>) [↑](#footnote-ref-5)