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

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| Technical Working Party for VegetablesFifty-First SessionRoelofarendsveen, Netherlands, July 3 to 7, 2017 | TWV/51/7Original: EnglishDate: June 14, 2017 |

Partial revision of the Test Guidelines for PEPPER (TG/76/8 REV.2)

Document prepared by the European Union

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 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 fiftieh session, held in Brno, Czech Republic, from June 27 to July 1, 2016, agreed that the Test Guidelines for Pepper (document TG/76/8/Rev) be partially revised for disease resistance characteristics (see document TWV/50/25 “Report”, Annex IV).

 The following changes are proposed:

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
3. To change the example varieties for Characteristic 49.1 “Resistance to *Potato Virus Y* (PVY) Pathotype 0”
4. To change the methodology for Characteristic 49 under Ad. 49.

 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, PI15225 | 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

|  |  |  |
| --- | --- | --- |
| 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

|  |  |  |
| --- | --- | --- |
| 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 |

|  |  |  |
| --- | --- | --- |
| 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* race 0 (TMV: 0) strain Vi-6*Pepper mild mottle virus* race 1.2 (PMMoV: 1.2) strain nt203*Pepper mild mottle virus* race 1.2.3 (PMMoV: 1.2.3) strain EveThe test protocols have been validated in a CPVO co-funded project[[4]](#footnote-5) with these 3 isolates/races. |
| 6. | Establishment isolate identity | genetically defined pepper differentials (reference ISF site: <http://www.worldseed.org/isf/differential_hosts.html>) |

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| --- | --- | --- | --- | --- | --- | --- |
|  | Pathotype | P0 | P1 | P1-2 | P1-2-3 | ~~P1-2-3-4~~ |
|  | Code | TMV: 0ToMV: 0TMGMV: 0BPMoV: 0 | TMV: 1TMGMV: 1PaMMV: 1 | PMMoV: 1.2 | PMMoV: 1.2.3 | ~~PMMoV: 1.2.3.4~~ |
| Variety | Gene |  |  |  |  |  |
| Lamu, Early Calwonder | - | S | S | S | S | ~~S~~ |
| Tisana, Yolo Wonder | *L1* | R | S | S | S | ~~S~~ |
| Tabasco | *L2* | R | R | S | S | ~~S~~ |
| Solario F1, Novi 3, PI159236 | *L3* | R | R | R | S | ~~S~~ |
| Tom4, PI260429 | *L4* | R | R | R | R | ~~S~~ |

S= susceptible; R= resistant; TMV= *Tobacco mosaic virus*; ToMV= *Tomato mosaic virus*; PMMoV= *Pepper mild mottle virus;* TMGMV= *Tobacco mild green mosaic virus*; BPMoV= *Bell pepper mottle virus*; PaMMV= *Paprika mild mottle virus*

|  |  |  |
| --- | --- | --- |
| 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 WonderPMMoV: 1.2:Susceptible controls: Fehérözön, Lamu, Yolo WonderResistant controls: Ferrari, Novi 3PMMoV: 1.2.3:Susceptible controls: Ferrari, Yolo WonderResistant controls: Friendly, Tom 4For PMMoV: 1.2.3, it is advised to chose Ferrari as susceptible controls because it is resistant to PMMoV: 1.2 or to add the differentials in tests to confirm the race. |
| 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 stagePMMoV: 1.2, cotyledon stagePMMoV: 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 change the example varieties for Characteristic 49.1 “Resistance to Potato Virus Y (PVY) Pathotype 0”

*Current wording*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **49.(+)** | **VG** | **Resistance to *Potato Y virus (*PVY)**  | **Résistance au *Potato Y virus (*PVY)** | **Resistenz gegen *Potato Y virus (*PVY)** | **Resistencia al *Potato Y virus (*PVY)** |  |  |
| **49.1(\*)** |  | **Pathotype 0 (PVY: 0)** | **Pathotype 0 (PVY: 0)** | **Pathotyp 0 (PVY: 0)** | **Patotipo 0 (PVY: 0)** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Yolo Wonder | 1 |
|  |  | present | présente | vorhanden | presente | Balico, Gerico, Solario | 9 |

*Proposed new wording*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **49.(+)** | **VG** | **Resistance to *Potato Y virus (*PVY)**  | **Résistance au *Potato Y virus (*PVY)** | **Resistenz gegen *Potato Y virus (*PVY)** | **Resistencia al *Potato Y virus (*PVY)** |  |  |
| **49.1(\*)** |  | **Pathotype 0 (PVY: 0)** | **Pathotype 0 (PVY: 0)** | **Pathotyp 0 (PVY: 0)** | **Patotipo 0 (PVY: 0)** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Ferrari, Piquillo, Yolo Wonder | 1 |
|  |  | present | présente | vorhanden | presente | Andalus, Vidi, Yolo Y ~~Balico, Gerico, Solario~~ | 9 |

## Proposal to change the example varieties for Characteristic 49.1 “Resistance to *Potato Virus Y* (PVY) Pathotype 0”

*Current wording*

Ad. 49: Resistance to *Potato Y virus* (PVY)

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | *Potato Y virus* (PVY) |
| 2. | Quarantine status | no |
| 3. | Host species | *Capsicum annuum* |
| 4. | Source of inoculum | GEVES (FR),Naktuinbouw (NL) |
| 5. | Isolate | Pathotypes 0, 1, and 1.2 |
| 6. | Establishment isolate identity | on differential table (S = susceptible; R = resistant) |

|  |  |
| --- | --- |
|  | PVY pathotypes |
| Pepper variety | 0 | 1 | 1.2 |
| Yolo WonderYolo YFlorida VR2Serrano Criollo de Morelos 334, Solario, W4 | SRRR | SSRR | SSS \*R |

 \* Florida VR2 may show vague and very late symptoms with pathotype 1.2

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| 7. | Establishment pathogenicity | on susceptible plant (e.g. on *Nicotiana tabacum* 'Xanthi' and *N. glutinosa)* |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | living plant |
| 8.2 | Multiplication variety | on susceptible variety (e.g. *N. tabacum* 'Xanthi') |
| 8.3 | Plant stage at inoculation | 3 leaf stage |
| 8.4 | Inoculation medium | ice-cold buffer solution0.03 M PBS + Carborundum + 0.2% DIECA |
| 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 | - |

|  |  |  |  |
| --- | --- | --- | --- |
| Resistance | PVY: 0 | PVY: 1 | PVY: 1.2 |
| absent | Yolo Wonder | Yolo Wonder | Yolo Wonder |
| present | Balico, Gerico, Solario | Sileno, Solario, Vidi  | Fenice, Navarro, Solario |

|  |  |  |
| --- | --- | --- |
| 9.4 | Test design | to add untreated plant |
| 9.5 | Test facility | glasshouse or climatic chamber |
| 9.6 | Temperature | 22°C constant |
| 9.7 | Light | at least 12h |
| 9.8 | Season | - |
| 9.9 | Special measures | - |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | leaf in PBS - grinding with mortar |
| 10.2 | Quantification inoculum | - |
| 10.3 | Plant stage at inoculation | cotyledons fully developed or at “first leaf” stage or 3 leaf stage |
| 10.4 | Inoculation method | rubbing with a virus solution |
| 10.5 | First observation | 6 - 14 days post inoculation |
| 10.6 | Second observation | 14 - 21 days post inoculation |
| 10.7 | Final observations | 21 days post inoculation |
| 11. | Observations |  |
| 11.1 | Method | visual, comparative |
| 11.2 | Observation scale |  |
|  | [1] absent | growth retardation, leaf malformation, light mosaic in youngest leaves, or red veins; stem necrosis, plant death |
|  | [9] present | no symptoms. |
| 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 | remark: avoid high temperatures (>30°C) |

*Proposed new wording*

Ad. 49: Resistance to *Potato Y virus* (PVY)

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | *Potato virus Y* |
| 2. | Quarantine status | no |
| 3. | Host species | Sweet pepper, hot pepper, paprika and chili – *Capsicum annuum* L. |
| 4. | Source of inoculum | GEVES[[5]](#footnote-6) (FR), Naktuinbouw[[6]](#footnote-7) (NL) or INIA[[7]](#footnote-8) (SP) |
| 5. | Isolate | *For PVY: 0 strain zb6* (the test protocol has been validated in a CPVO co-funded project[[8]](#footnote-9) with this isolate/race).*PVY race 1* *PVY race 2*  |
| 6. | Establishment isolate identity | genetically defined pepper controls (extract from ISF table ISF web site: <http://www.worldseed.org/cms/medias/file/TradeIssues/DiseasesResistance/Differentials/Pepper-potyviruses_Aug2013.pdf> ) |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variety | pvr gene present | PVY: 0 | PVY: 1 | PVY: 1.2 |
| Early Cal Wonder, Yolo Wonder | pvr 0 | S | S | S |
| PI152225 | *pvr 1* | R | R | R |
| Yolo Y | *pvr11 (pvr 21)* | R | S | S |
| Florida VR2 | *pvr12 (pvr 22)* | R | R | S |
| Florida VR4, Del Rey Bell, Agronomico 10 | *pvr3* | R | R | R |
| Serrano Criollo de Morelos 334 | *pvr4* | R | R | R |

S= susceptible; R= resistant;

|  |  |  |
| --- | --- | --- |
| 7. | Establishment pathogenicity | Test on susceptible plants |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | Regeneration of the virus on plant material before inoculum preparation. |
| 8.2 | Multiplication variety | On susceptible pepper variety, PVY races may be multiplied on varieties which are selective for each particular race. For PVY: 0, because tobacco *Nicotiana tabacum* cv. *Xanthi-nc* have large leaves and can produce a lot of inoculum and have a faster multiplication, they are recommended for the multiplication. |
| 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 *Nicotiana tabacum* cv. *Xanthi-nc,* check mosaic presence and local lesion absence (contamination by Tobamovirus) after 5-7 days. |
| 8.8 | Shelflife/viability inoculum | fresh > 1 day, desiccated > 1 year. Because problem of stability of PVY: 0, shipments are recommended to be done with fresh infected leaves |
| 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 | PVY: 0:Susceptible controls: Ferrari, Piquillo,Yolo WonderResistant controls: Andalus, Vidi, Yolo YPVV: 1:Susceptible controls: Yolo Wonder, Yolo YResistant controls: Florida VR2PVY: 1.2:Susceptible controls: Florida VR2, Yolo Wonder, Yolo Y Resistant controls: Serrano Criollo de Morenos |
| 9.4 | Test design | add non inoculated plants |
| 9.5 | Test facility | Climate room or greenhouse. In case of test in greenhouse during period of low daylight, shadow should not be used. |
| 9.6 | Temperature | 18-25°C |
| 9.7 | Light | 12 hours or longer |
| 9.8 | Season | - |
| 9.9 | Special measures | For PVY: 0, it is advised to choose Yolo Y as resistant control or to add the differentials in tests to be able to observe a possible contamination by PVY: 1 or 1.2 |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | 1 g leaf with symptoms with 4 mL PBS with carborundum (80mg) and activated carbon (80mg) or similar buffer, homogenize. |
| 10.2 | Quantification inoculum | - |
| 10.3 | Plant stage at inoculation | PVY: 0 cotyledons stagePVY: 1 and 1.2: cotyledon stage or first pointing leaf stage |
| 10.4 | Inoculation method | rubbing with the virus suspension. |
| 10.5 | First observation | Three weeks post-inoculation. |
| 11. | Observations |  |
| 11.1 | Method | Visual  |
| 11.2 | Observation scale | Susceptibility: mosaic (can be very light/faint), growth reduction, Vein banding and vein necrosis.Resistance: no symptoms. |
| 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 | Recommended dates of notation should be adapted depending of expression of symptoms on controls. |

Source: ISF isf@worldseed.org

 [End of document]

1. matref@geves.fr [↑](#footnote-ref-2)
2. resistentie@naktuinbouw.nl [↑](#footnote-ref-3)
3. 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)
5. matref@geves.fr [↑](#footnote-ref-6)
6. resistentie@naktuinbouw.nl [↑](#footnote-ref-7)
7. cardaba@inia.es [↑](#footnote-ref-8)
8. Harmores 2 CPVO project (http://www.cpvo.europa.eu/main/en/home/documents-and-publications/technical-projects-reports) [↑](#footnote-ref-9)