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| --- | --- | --- |
|  |  | ETWV/48/38**ORIGINAL:**  EnglishDATE:  June 12, 2014 |
| INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS  |
| Geneva |

Technical working party for vegetables

Forty-eighth Session
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Partial Revision of the Test Guidelines for
Sweet Pepper, Hot Pepper, Paprika, Chili (Document TG/76/8)

Document prepared by experts from France and the Netherlands

 The purpose of this document is to present the proposals for the partial revision of the Test Guidelines for Sweet Pepper, Hot Pepper, Paprika, Chili (document TG/76/8).

 The following changes are proposed:

(a) Revision of the Grouping Characteristics in Chapter 5.3

(b) A revised format for disease resistance characteristics according to the explanations for disease resistance characteristics in Test Guidelines and eventual new proposal to update the protocol

* Chapter 7: Table of Characteristics
	+ Proposal to Revise Characteristics 48 to 53
* Chapter 8: Explanations on the Table of Characteristics
	+ 8.2 Inclusion of a Revised Format for Disease Resistance Characteristics
* Chapter 9: Literature
* Chapter 10: Technical Questionnaire
	+ Section 7: Addition of new characteristics under 7.3.1

 The proposed revisions are presented in the Annex to this document.

[Annex follows]

Proposal for a Revision of the Grouping Characteristics in Chapter 5.3

*Current wording:*

(a) Seedling: anthocyanin coloration of hypocotyl (characteristic 1)

(b) Plant: shortened internode (in upper part) (characteristic 4)

(c) Fruit: color (before maturity) (characteristic 21)

(d) Fruit: shape in longitudinal section (characteristic 28)

(e) Fruit: color (at maturity) (characteristic 33)

(f) Fruit: capsaicin in placenta (characteristic 45)

(g) Resistance to Tobamovirus - Pathotype 0 (Tobacco MosaicVirus (0)) (characteristic 48.1)

(h) Resistance to Tobamovirus - Pathotype 1-2 (Tomato MosaicVirus (1-2)) (characteristic 48.2)

(i) Resistance to Tobamovirus - Pathotype 1-2-3 (Pepper Mild Mottle Virus (1-2-3)) (characteristic 48.3)

(j) Resistance to Potato Virus Y (PVY) - Pathotype 0 (characteristic 49.1)

*Proposed new wording:*

(a) Seedling: anthocyanin coloration of hypocotyl (characteristic 1)

(b) Plant: shortened internode (in upper part) (characteristic 4)

(c) Fruit: color (before maturity) (characteristic 21)

(d) Fruit: shape in longitudinal section (characteristic 28)

(e) Fruit: color (at maturity) (characteristic 33)

(f) Fruit: capsaicin in placenta (characteristic 45)

(g) Resistance to Tobamovirus - *Tobacco mosaic virus* Pathotype 0 (TMV: 0) (characteristic 48.1)

(h) Resistance to Tobamovirus - *Pepper mild mottle virus* Pathotype 1.2 (PMMoV: 1.2) (characteristic 48.2)

(i) Resistance to Tobamovirus - *Pepper mild mottle virus* Pathotype 1.2.3 (PMMoV: 1.2.3) (characteristic 48.3)

(j) Resistance to *Potato Y virus* Pathotype 0 (PVY: 0) (characteristic 49.1)

(k) Resistance to *Tomato spotted wilt virus* Pathotype 0 (TSWV: 0) (characteristic 52)

Chapter 7: Table of Characteristics: Proposal to revise Characteristics 48 to 53

*Current wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **48.(+)** |  | **Resistance to Tobamovirus** | **Résistance au tobamovirus** | **Resistenz gegen Tobamovirus** | **Resistencia al tobamovirus** |  |  |
| **48.1 (\*)** |  | **Pathotype 0(Tobacco MosaicVirus (0))** | **Pathotype 0(virus de la mosaïque du tabac (0))** | **Pathotyp 0(Tabakmosaikvirus (0))** | **Patotipo 0(Virus del mosaico del tabaco (0))** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Doux italien, Piperade | 1 |
|  |  | present | présente | vorhanden | presente | Lamuyo, Sonar, Yolo Wonder | 9 |
| **48.2(\*)** |  | **Pathotype 1-2(Tomato MosaicVirus (1-2))** | **Pathotype 1-2(virus de la mosaïque de la tomate (1-2))** | **Pathotyp 1-2(Tomatomosaikvirus (1-2))** | **Patotipo 1–2(Virus del mosaico del tomate (1–2))** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Piperade, Yolo Wonder | 1 |
|  |  | present | présente | vorhanden | presente | Delgado, Festos, Novi, Orion | 9 |
| **48.3(\*)** |  | **Pathotype 1-2-3(Pepper Mild Mottle Virus (1-2-3))** | **Pathotype 1-2-3(virus de la marbrure nervaire du piment (1‑2-3))** | **Pathotyp 1-2-3(Pepper Mild Mottle Virus (1-2-3))** | **Patotipo 1–2–3(Virus del moteado suave del pimiento (1‑2–3))** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Piperade, Yolo Wonder | 1 |
|  |  | present | présente | vorhanden | presente | Cuby, Tasty | 9 |

*Proposed new wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **48.(+)** | **VG** | **Resistance to Tobamovirus** |  |  |  |  |  |
| **48.1 (\*)** |  | ***Tobacco mosaic virus* Pathotype 0(TMV: 0)** |  |  |  |  |  |
| **QL** |  | absent |  |  |  | Gordo, Pepita, Piperade | 1 |
|  |  | present |  |  |  | Lamuyo, Sonar, Yolo Wonder | 9 |
| **48.2(\*)** |  | ***Pepper mild mottle virus* Pathotype 1.2(PMMoV: 1.2)** |  |  |  |  |  |
| **QL** |  | absent |  |  |  | Lamuyo, Yolo Wonder | 1 |
|  |  | present |  |  |  | Ferrari, Orion, Solario | 9 |
| **48.3(\*)** |  | ***Pepper mild mottle virus* Pathotype 1.2.3(PMMoV: 1.2.3)** |  |  |  |  |  |
| **QL** |  | absent |  |  |  | Solario, Yolo Wonder | 1 |
|  |  | present |  |  |  | Cuby, Friendly | 9 |

*Current wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **49.(+)** |  | **Resistance to Potato Virus Y (PVY )**  | **Résistance au virus Y de la pomme de terre (PVY)** | **Resistenz gegen Kartoffel-Y-Virus (PVY)** | **Resistencia al virus Y de la papa (PVY)** |  |  |
| **49.1(\*)** |  | **Pathotype 0** | **Pathotype 0** | **Pathotyp 0** | **Patotipo 0** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Yolo Wonder | 1 |
|  |  | present | présente | vorhanden | presente | Yolo Y | 9 |
| **49.2** |  | **Pathotype 1** | **Pathotype 1** | **Pathotyp 1** | **Patotipo 1** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Yolo Wonder, Yolo Y | 1 |
|  |  | present | présente | vorhanden | presente | Florida VR2 | 9 |
| **49.3** |  | **Pathotype 1-2** | **Pathotype 1‑2** | **Pathotyp 1-2** | **Patotipo 1–2** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Florida VR2, Yolo Wonder, Yolo Y | 1 |
|  |  | present | présente | vorhanden | presente | Serrano Criollo de Morenos  | 9 |

*Proposed new wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **49.(+)** | **VG** | **Resistance to *Potato Y virus (*PVY)**  |  |  |  |  |  |
| **49.1(\*)** |  | **Pathotype 0 (PVY: 0)** |  |  |  |  |  |
| **QL** |  | absent |  |  |  | Yolo Wonder | 1 |
|  |  | present |  |  |  | Balico, Gerico, Solario | 9 |
| **49.2** |  | **Pathotype 1 (PVY: 1)** |  |  |  |  |  |
| **QL** |  | absent |  |  |  | Yolo Wonder | 1 |
|  |  | present |  |  |  | Sileno, Solario, Vidi | 9 |
| **49.3** |  | **Pathotype 1.2 (PVY: 1.2)** |  |  |  |  |  |
| **QL** |  | absent |  |  |  | Yolo Wonder | 1 |
|  |  | present |  |  |  | Fenice, Navarro, Solario | 9 |

*Current wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **50.(+)** |  | **Resistance to *Phytophthora capsici*** | **Résistance à *Phytophthora capsici*** | **Resistenz gegen *Phytophthora capsici*** | **Resistencia al *Phytophthora capsici*** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Yolo Wonder | 1 |
|  |  | present | présente | vorhanden | presente | Chistera, Favolor, Phyo 636, Solario  | 9 |

*Proposed new wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **50.(+)** | **VG** | **Resistance to *Phytophthora capsici* (Pc)** |  |  |  |  |  |
| **QL** |  | absent |  |  |  | Jupiter, Yolo Wonder | 1 |
|  |  | present |  |  |  | Favolor, Solario | 9 |

*Current wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **51.(+)** |  | **Resistance to Cucumber Mosaic Virus (CMV)** | **Résistance au virus de la mosaïque du concombre (CMV)** | **Resistenz gegen Gurkenmosaikvirus (CMV)** | **Resistencia al virus del mosaico del pepino (CMV)** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Yolo Wonder | 1 |
|  |  | present | présente | vorhanden | presente | Alby, Favolor | 9 |

*Proposed new wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **51.(+)** | **VG** | **Resistance to C*ucumber mosaic virus* (CMV)** |  |  |  |  |  |
| **QL** |  | absent |  |  |  | Yolo Wonder | 1 |
|  |  | present |  |  |  | Alby, Ducato, Favolor | 9 |

*Current wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **52.(+)** |  | **Resistance to Tomato Spotted Wilt Virus (TSWV)** | **Résistance au Tomato Spotted Wilt Virus (TSWV)** | **Resistenz gegen Tomato Spotted Wilt Virus (TSWV)** | **Resistencia al Tomato Spotted Wilt Virus (TSWV)** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Yolo Wonder | 1 |
|  |  | present | présente | vorhanden | presente | Galileo, Jackal, Jackpot | 9 |

*Proposed new wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **52.(+)** | **VG** | **Resistance to** ***Tomato spotted wilt virus* Pathotype 0 (TSWV: 0)** |  |  |  |  |  |
| **QL** |  | absent |  |  |  | Lamuyo, Yolo Wonder | 1 |
|  |  | present |  |  |  | Galileo, Jackal, Jackpot, Prior | 9 |

*Current wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **53.(+)** |  | **Resistance to *Xanthomonas campestris* pv. *vesicatoria*** | **Résistance au *Xanthomonas campestris* pv. *vesicatoria*** | **Resistenz gegen *Xanthomonas campestris* pv. *vesicatoria*** | **Resistencia al *Xanthomonas campestris* pv. *vesicatoria*** |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Fehérözön, Yolo Wonder | 1 |
|  |  | present | présente | vorhanden | presente | Aladin, Camelot, ECR-20R, Kaldóm, Kalorez, Lancelot, Pasa | 9 |

*Proposed new wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **53.(+)** | **VG** | **Resistance to *Xanthomonas campestris* pv. *vesicatoria* (Xcv)** |  |  |  |  |  |
| **53.1** |  | **Pathotype 1** |  |  |  |  |  |
| **QL** |  | absent |  |  |  | Fehérözön, Yolo Wonder | 1 |
|  |  | present |  |  |  | Emiro, Filidor, Gotico, San Marco, Solanor | 9 |
| **53.2** |  | **Pathotype 2** |  |  |  |  |  |
| **QL** |  | absent |  |  |  | Fehérözön, Yolo Wonder | 1 |
|  |  | present |  |  |  | Emiro, Filidor, Gotico,San Marco, Solanor | 9 |
| **53.3** |  | **Pathotype 3** |  |  |  |  |  |
| **QL** |  | absent |  |  |  | Fehérözön, Yolo Wonder | 1 |
|  |  | present |  |  |  | Emiro, Filidor, Gotico,San Marco, Solanor | 9 |

Chapter 8: Explanations on the Table of Characteristics

Chapter 8.2: Proposal to Include a Revised Format for Disease Resistance Characteristics

*Current wording:*

Ad. 48: Resistance to Tobamovirus

Maintenance of pathotypes

Type of medium: On plants or dehydrated leaves (in deep-freezer or method BOS)

Special conditions: Regeneration of the virus on plant material before inoculum preparation

Execution of test

Growth stage of plants: When cotyledons are fully developed or at “first leaf” stage

Temperature: 20-25°C

Growing method: Sowing and raising of seedlings in boxes or soil blocks in glasshouse

Method of inoculation: Rubbing of cotyledons with a virus suspension

Duration of test

- Sowing to inoculation: 10 to 15 days

- Inoculation to reading: 10 days

Number of plants tested: 15 to 30 plants

Genetics of virus pathotypes and resistant genotypes:

 The genetic resistance to Tobamoviruses is controlled by 5 alleles located on the same locus. The table below shows the relationship between virus pathotypes and resistance genotypes:

Pepper Genotype reactions to Tobamovirus Pathotypes

|  |  |
| --- | --- |
|  | Pepper Tobamovirus Pathotypes |
| Virus: | TMV | ToMV | PMMoV |
| Strain: | U1Feldman | P11Obuda Pepper Mosaic Virus | P14Samsun latens |
| Genotype / mark | P0 | P1-2 | P1-2-3 |
| L-L- | S | S | S |
| L1L1 | R | S | S |
| L3L3 | R | R | S |
| L4L4 | R | R | R |

Legend: S = susceptible

 R = resistant

 TMV = Tobacco Mosaic Virus

 ToMV = Tomato Mosaic Virus

 PMMoV = Pepper Mild Mottle Virus

*Proposed new 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) |
| 5. | Isolate | Pathotype 0, Pathotype 1.2, and Pathotype 1.2.3 |
| 6. | Establishment isolate identity | on differentials (S = susceptible, R = resistant) |

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | 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 blank treatment |
| 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 | leaf: PBS(1:9) - grinding with mortar or juice (grinded infected leaves dilute) |
| 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 CPOV 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 |

*Current wording:*

Ad. 49: Resistance to Potato Virus Y (PVY)

Maintenance of pathotypes

Type of medium: On susceptible plants

Special conditions: For the strain PVY(0): use the line TO72(A)

 For the strain PVY(1): use the line Sicile 15

 For the strain PVY(1-2): use the line SON41

Execution of test

Growth stage of plants: Young plants at the stage of developed cotyledons -first pointing leaf

Temperature: 18-25°C

Growing method: Raising of plants in glasshouse

Method of inoculation: Rubbing of cotyledons with a virus solution

 Composition of the solution:

inoculum: 4 ml extraction solution for 1 g infected leaves + 80 g activated carbon + 80 mg carborundum;

 extraction solution: buffer solution diluted 1/20 with
 0.2% diethyl dithiocaremate of sodium (DIECA);

 buffer solution: (for 100 ml sterile water) 10.8 g NA2HPO4 +
1.18 g K2HPO4 at pH 7.1-7.2

Duration of test

Sowing to inoculation: 10 to 15 days

Inoculation to reading: 3 weeks (2 weeks minimum, 4 weeks maximum)

Number of plants tested: 60 plants

Remarks: The test should not be conducted at high temperatures.

|  |  |  |  |
| --- | --- | --- | --- |
| Standard varieties: | Pathotype 0 | Pathotype 1 | Pathotype 1-2 |
| Sensitive varieties: | Yolo Wonder | Yolo Wonder, Yolo Y | Florida VR2,\* Yolo Wonder, Yolo Y |
| Resistant varieties: | Yolo Y | Florida VR2 | Serrano Criollo de Morenos  |

\* Florida VR2 can exhibit diffused and very late symptoms.

*Proposed new 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

|  |  |  |
| --- | --- | --- |
| 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 blank treatment |
| 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) |

*Current wording:*

Ad. 50: Resistance to *Phytophthora capsici*

Scoring must be carried out under conditions of controlled infection:

Maintenance of inoculum

Inoculum and type of medium: *Phytophthora capsici* strain 101, to be cultivated on V8 juice-agar (1%) in Petri’s dishes.

Conduct of test

Growth stage of plants: around eight-week old plants, grown in greenhouse
(stage: first flower bud)

Temperature: 22°C

Light: 12 hours/day

Method of inoculation: Plants are cut just below the point of first branching. A disc of mycelium of 4 mm-diameter should be used as inoculum. The disc is placed on the freshly cut stem. The top of the stem is wrapped with a piece of aluminium foil, to keep it wet. Infected plants are transferred to a growth chamber kept at 22°C.

Duration of test:

From sowing to inoculation: between 6 and 8 weeks

From inoculation to scoring: first scoring: 7 days

 second scoring: 14 days

 final scoring: 21 days

Number of plants tested: 20 plants

Scoring: The length of necrosis on the stem, induced by the fungus development, is recorded once a week during 3 weeks, on each plant. The aluminium foil on the top of the stem should be removed 7 days after the inoculation. The first reading should take place immediately after the removal of the aluminium foil. Subsequent scoring should be made on the 14th and 21st day counting from the day of inoculation. The distance (in mm) between the lowest point reached by the necrosis and the top of the stem should be recorded.

Standard varieties: Susceptible: Yolo Wonder

 Resistant: Chistera, Favolor, Solario, Phyo 636 (given in the order of their level of resistance)

*Proposed new wording:*

Ad. 50: Resistance to *Phytophthora capsici* (Pc)

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | *Phytophthora capsici* (Pc) |
| 2. | Quarantine status | no |
| 3. | Host species | *Capsicum annuum* |
| 4. | Source of inoculum | Naktuinbouw (NL) - INRA GAFL (FR) |
| 5. | Isolate | moderately aggressive (e.g. strain 101) |
| 6. | Establishment isolate identity | on standardsJupiter, Yolo Wonder (susceptible), Favolor (moderately resistant), Solario, Phyo 636 (resistant) |
| 7. | Establishment pathogenicity | in biotest on plants |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | V8 juice-agar (1%) or 10% V8A or PDA+ |
| 8.2 | Multiplication variety | - |
| 8.3 | Plant stage at inoculation | - |
| 8.4 | Inoculation medium | 10% V8A or PDA+ |
| 8.5 | Inoculation method | see 10.4 |
| 8.6 | Harvest of inoculum | - |
| 8.7 | Check of harvested inoculum | - |
| 8.8 | Shelflife/viability inoculum | 10% V8A 3 months, PDA+ 2 months |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | at least 20 (2 blanks) |
| 9.2 | Number of replicates | e.g. 1 |
| 9.3 | Control varieties | Jupiter, Yolo Wonder (susceptible),Favolor (moderately resistant), Solario (resistant) |
| 9.4 | Test design | - |
| 9.5 | Test facility | glasshouse |
| 9.6 | Temperature | 22°C d/n |
| 9.7 | Light | at least 12h |
| 9.8 | Season | - |
| 9.9 | Special measures | - |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | growing on Petri dishes |
| 10.2 | Quantification inoculum | - |
| 10.3 | Plant stage at inoculation | first flower bud |
| 10.4 | Inoculation method | stem is cut just below point of first branching, a 4mm- agar plug is placed carefully on the wound and covered with aluminum foil |
| 10.5 | First observation | 7 days post inoculation |
| 10.6 | Second observation | 14 days post inoculation |
| 10.7 | Final observations | 21 days post inoculation |
| 11. | Observations |  |
| 11.1 | Method | visual, comparative or measurement of stem necrosis length; for repeated measurements, the stem is marked with permanent ink |
| 11.2 | Observation scale |  |
|  | [1] absent | e.g. length increase > 0.8 cm/week |
|  | [9] present (moderately resistant) | e.g. length increase  ≥ 0.5 cm ≤ 0.8 cm/week |
|  | [9] present (highly resistant) | e.g. length increase < 0.5 cm/week |
| 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 | QLBased on the stem nectaries length increase compared to the standards.[1] susceptible: Jupiter, Yolo Wonder[9] moderately resistant: Favolor[9] resistant: Solario |
| 13. | Critical control points | absence of differential interactions between host and pathogen |

*Current wording:*

Ad. 51: Resistance to Cucumber Mosaic Virus (CMV)

Maintenance of pathotypes

Strain: Fulton

Type of medium: On susceptible plants: *Vinca rosea*

Special conditions: -

Inoculum production: Crushing of 1g of fresh leaves of *Vinca rosea* in 4 ml of Phosphate buffer 0.03M pH 7 + DIECA (diethyl dithiocaremate de sodium) (1 for 1000) + 300 mg of activated carbon + 80 mg of carborundum

Execution of test:

Growth stage of plants: Young plants at the stage of developed cotyledons. First leaf non pointing

Number of plants: 50 plants

Growing conditions: 22C, 12 hours of light

Growing method: Raising of plants in climatised room

Method of inoculation: Mechanical rubbing of cotyledons with a virus solution, the plants are kept in darkness for 48 hours

Duration of test:

From sowing to inoculation: 12 to 13 days

From inoculation to reading: 3 readings at 10, 15 and 21 days after inoculation

Standard varieties:

Susceptible variety: Yolo Wonder

Tolerant (T) or resistant (R) Milord (T)

varieties: Vania (R)

*Proposed new wording:*

Ad. 51: Resistance to *Cucumber mosaic virus* (CMV)

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | *Cucumber mosaic virus* (CMV) |
| 2. | Quarantine status | no |
| 3. | Host species | *Capsicum annuum* |
| 4. | Source of inoculum | INRA GAFL (FR) |
| 5. | Isolate | e.g. ‘Fulton’ |
| 6. | Establishment isolate identity | - |
| 7. | Establishment pathogenicity | - |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | living plant |
| 8.2 | Multiplication variety | e.g. *Vinca rosea* |
| 8.3 | Plant stage at inoculation | - |
| 8.4 | Inoculation medium | 0.03 M PBS + 0.1% DIECA |
| 8.5 | Inoculation method | rubbing with carborundum |
| 8.6 | Harvest of inoculum | 1 g on 4 ml buffer |
| 8.7 | Check of harvested inoculum | - |
| 8.8 | Shelflife/viability inoculum | - |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | 50 |
| 9.2 | Number of replicates | e.g. 1 |
| 9.3 | Control varieties | Yolo Wonder (susceptible), Ducato (moderately resistant), Alby, Favolor (resistant) |
| 9.4 | Test design | - |
| 9.5 | Test facility | - |
| 9.6 | Temperature | 20-22°C |
| 9.7 | Light | 12h |
| 9.8 | Season | - |
| 9.9 | Special measures | - |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | - |
| 10.2 | Quantification inoculum | - |
| 10.3 | Plant stage at inoculation | cotyledon, before emergence of first leaf (12-13 days after sowing) |
| 10.4 | Inoculation method | rubbing cotyledons with carborundum, followed by 48h darkness |
| 10.5 | First observation | 10 days post inoculation |
| 10.6 | Second observation | 15 days post inoculation |
| 10.7 | Final observations | 21 days post inoculation |
| 11. | Observations |  |
| 11.1 | Method | visual, comparative |
| 11.2 | Observation scale |  |
|  | [1] susceptible | many local lesion, mosaic |
|  | [9] moderately resistant | intermediate symptoms |
|  | [9] resistant | few local lesions, no or light 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 | - |

*Current wording:*

Ad. 52: Resistance to Tomato Spotted Wilt Virus (TSWV)

Maintenance of patothypes:

Type of medium: Pepper fruit in deep-freezer (-70 oC)

Special condition: Regeneration of the virus on *Nicotiana rustica* or *Nicotiana benthamiana* plants before inoculation

Execution of test:

Growth stage of the plants: Two leaves expanded

Temperature: 20 - 22 oC

Light: Extra light in winter

Growing method: Sowing in greenhouse

Method of inoculation: Mechanical, rubbing on cotyledons, inoculum suspension 10 oC

Duration of test:

from sowing to inoculation: 20 days

from inoculation to reading: 14 days

Number of tested plants: 20 plants

Standard varieties.

Susceptible: Lamuyo

Resistant: Galileo, Jackal, Jackpot

*Proposed new wording:*

Ad. 52: Resistance to *Tomato spotted wilt virus* Pathotype 0 (TSWV: 0)

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | *Tomato spotted wilt virus*, Pathotype 0 (TSWV: 0) |
| 2. | Quarantine status | yes |
| 3. | Host species | *Capsicum annuum* |
| 4. | Source of inoculum | GEVES (FR),Naktuinbouw (NL) |
| 5. | Isolate | e.g. LYE 51 or Br-01 |
| 6. | Establishment isolate identity | - |
| 7. | Establishment pathogenicity | on susceptible plant or *Nicotiana benthamiana, N. rustica* |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | living plant |
| 8.2 | Multiplication variety | Yolo Wonder or *N. benthamiana*, *N. rustica* |
| 8.3 | Plant stage at inoculation | cotyledons fully developed or at “first leaf” pointed stage or 1- 3 leaves |
| 8.4 | Inoculation medium | ice-cold buffer suspension or 0.03 M PBS + optional addition of 0.1% sodium sulfite freshly added |
| 8.5 | Inoculation method | rubbing with carborundum |
| 8.6 | Harvest of inoculum | - |
| 8.7 | Check of harvested inoculum | - |
| 8.8 | Shelflife/viability inoculum | stability in ice cold suspension ca. 15-20 minutes |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | at least 20 |
| 9.2 | Number of replicates | e.g. 1 |
| 9.3 | Control varieties | Lamuyo, Yolo Wonder (susceptible), Galileo, Jackal, Jackpot, Prior (resistant) |
| 9.4 | Test design | - |
| 9.5 | Test facility | growth chamber or insect proof glasshouse |
| 9.6 | Temperature | 18-20°C or 20-22°C |
| 9.7 | Light | 12 h |
| 9.8 | Season | all seasons, but winter reduce the risk of thrips infestation |
| 9.9 | Special measures | biohazard sign on compartment for countries with a TSWV quarantine status |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | - |
| 10.2 | Quantification inoculum | - |
| 10.3 | Plant stage at inoculation | cotyledons fully developed / at “first leaf” pointed stage or 1- 3 leaves |
| 10.4 | Inoculation method | rubbing with carborundum, then apply shading or darkness for 24hoption: repeat the inoculation 2-3 days later to reduce accidental escapes |
| 10.5 | First observation | 5-6 days to10 - 15 days post inoculation |
| 10.6 | Second observation | 10-11 days post inoculation to 15 - 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  | mosaic on young leaf, some leaf malformation |
|  | [9] present | necrosis or only mechanical damage |
| 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 | Monitor and control the presence of thrips.TSWV is transmitted by thrips (*Thrips tabaci* and *Frankliniella occidentalis*.).TSWV has a broad host range.After a few multiplication the virus could be ineffective. New isolates can be obtained from practice by harvesting fruits of L4 pepper varieties infected naturally with TSWV. The fruits are kept at -70°C temperature. The presence of other viruses must be checked before using this material. |

*Current wording:*

Ad. 53: Resistance to *Xanthomonas campestris* pv. *vesicatoria*

Maintenance of pathotypes

Type of medium: PDA (Potato, Dextrose, Agar ) medium

Special conditions: 48 hours *Xanthomonas campestris* pv. *vesicatoria* culture. Adjusting inoculum concentration of bacteria-cellular 107 .

Execution of test

Growth stage of plants: 6th to 8th true leaves

Temperature: 24 °C night, 25°C day

Relative humidity: 80%

Light: 30 000 lx, day length 16 hours

Growing method: Sowing in boxes in climate chamber or in glasshouse

Method of inoculation: Infiltration into abaxial surface of a leaf in 13-15 mm diameter spots

Duration of the test: 10-14 days

Number of plants tested: 15 to 30 plants

Remarks

Genetics of bacteria pathotypes and resistant genotypes:

Resistant varieties: Aladin, Camelot, ECR-20R, Kaldóm, Kalorez, Lancelot, Pasa

*Proposed new wording:*

Ad. 53: Resistance to *Xanthomonas campestris* pv. *vesicatoria* (Xcv)Pathotype 1, Pathotype 2, Pathotype 3

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | *Xanthomonas campestris* pv. *vesicatoria* (Xcv) |
| 2. | Quarantine status | - |
| 3. | Host species | *Capsicum annuum* |
| 4. | Source of inoculum | natural; to be taken from any source of infection in the field |
| 5. | Isolate | expected reactions on resistant standard varieties |
| 6. | Establishment isolate identity | on differentials |

Differential Pathotype 1 Pathotype 2 Pathotype 3

Early California Wonder S S S

Early California Wonder-10R (gene Bs1) S R S

Early California Wonder-20R (gene Bs2 R R R

Early California Wonder-30R (gene Bs3) R S S

PI 235047 (gene Bs4) R S R

|  |  |  |
| --- | --- | --- |
| 7. | Establishment pathogenicity | - |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | a bacterial growth medium, e.g. LPGA |
| 8.2 | Multiplication variety | - |
| 8.3 | Plant stage at inoculation | - |
| 8.4 | Inoculation medium | - |
| 8.5 | Inoculation method | - |
| 8.6 | Harvest of inoculum | 48h culture |
| 8.7 | Check of harvested inoculum | - |
| 8.8 | Shelflife/viability inoculum | - |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | at least 20 |
| 9.2 | Number of replicates | e.g. 1 |
| 9.3 | Control varieties | Fehérözön, Yolo Wonder (susceptible), Emiro, Filidor, Gotico, San Marco, Solanor (resistant) |
| 9.4 | Test design | - |
| 9.5 | Test facility | - |
| 9.6 | Temperature | 20-26°C day/night |
| 9.7 | Light | 30.000 lux suggested, 16h/day |
| 9.8 | Season | - |
| 9.9 | Special measures | 80% RH |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | harvest cells from LPGA plate after 48 h growing |
| 10.2 | Quantification inoculum | 107 -108 cells per ml (Stronger reaction with the higher concentration.) |
| 10.3 | Plant stage at inoculation | 6-8 true leaves |
| 10.4 | Inoculation method | infiltration into abaxial surface of the interveinal region on either side of the midrib of a fully expanded leaf in 13-20mm diameter spots |
| 10.5 | First observation | 2-5 days post inoculation |
| 10.6 | Second observation | 6-8 days post inoculation |
| 10.7 | Final observations | 10-14 days post inoculation |
| 11. | Observations |  |
| 11.1 | Method | visual, comparative |
| 11.2 | Observation scale |  |
|  | [1] absent | water soaking near infiltration site |
|  | [9] present | necrotic reaction at infiltration site |
| 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 | - |

Proposed changes to Chapter 9 “Literature”

To add the following literature references to Chapter 9, part “General Information”:

Smilde, W.D. and D. Peters (2007) Pathotyping TSWV in pepper and tomato. In: Niemorowicz-Szczytt, K.

2007: Progress in Research on Capsicum and Eggplant, Eucarpia conference proceedings, Warsaw, pp. 231-236 (<http://www.eucarpia.org/03publications/#Abstracts>)

Proposed changes to Chapter 10 “Technical Questionnaire”

To add an option “Not tested” to Characteristic 52 in Section 5:

|  |
| --- |
| 5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds). |
|  | Characteristics | Example Varieties | Note |
|  | […] |  |  |
| **5.11(52)** | Resistance to *Tomato spotted wilt virus* Pathotype 0(TSWV: 0) |  |  |
|  | absent | Lamuyo, Yolo Wonder | 1[ ] |
|  | present | Galileo, Jackal, Jackpot, Prior | 9[ ] |
|  | not tested |  | [ ] |

To add the following to Chapter 7 “Additional information which may help in the examination of the variety”:

7.3.1 Resistance to pests and diseases (please specify Pathotypes/strains if possible)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | absent | present | not tested |
| (a) | Resistance to *Potato Y virus* (PVY) | [ ] | [ ] | [ ] |
|  | (1) Pathotype 1 (char. 49.2) | [ ] | [ ] | [ ] |
|  | (2) Pathotype 1.2 (char. 49.3) | [ ] | [ ] | [ ] |
| (b) | Resistance to *Phytophthora capsici* (Pc) (char. 50) | [ ] | [ ] | [ ] |
| (c) | Resistance to *Cucumber mosaic virus* (CMV) (char. 51) | [ ] | [ ] | [ ] |
| (d) | Resistance to *Xanthomonas campestris* pv. *vesicatoria* (Xcv) | [ ] | [ ] | [ ] |
|  | (1) Pathotype 1 (char. 53.1) | [ ] | [ ] | [ ] |
|  | (2) Pathotype 2 (char. 53.2) | [ ] | [ ] | [ ] |
|  | (3) Pathotype 3 (char. 53.3) | [ ] | [ ] | [ ] |

[End of Annex and of document]