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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

1. 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).

2. The following changes are proposed:

(a) Revision of grouping characteristics, including the behavior against pathogens

(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 5: Grouping of Varieties and Organization of the Growing Trial
- Chapter 7: Table of Characteristics
- Chapter 8: Explanations on the Table of Characteristics
  - 8.2 Explanations for individual characteristics
- Chapter 10: Technical Questionnaire
  - Paragraph 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).

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

[Annex follows]

ANNEX

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 - 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)
(k) Resistance to *Tomato spotted wilt virus* (TSWV) – Pathotype P0 (characteristic 52)

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. **(+)**	**Resistance to Tobamovirus**	**Résistance au Tobamovirus**	**Resistenz gegen Tobamovirus**	**Resistencia al Tobamovirus**		
48.1 **(*)**	**Pathotype P0**	**Pathotype P0**	**Pathotyp P0**	**Patotipo P0**		
QL	absent	absente	fehlend	ausente	Doux italien, Piperade	1
	present	présente	vorhanden	presente	Lamuyo, Sonar, Yolo Wonder	9
48.2 **(*)**	**Pathotype P1-2**	**Pathotype P1-2**	**Pathotyp P1-2**	**Patotipo P1-2**		
QL	absent	absente	fehlend	ausente	Piperade, Yolo Wonder	1
	present	présente	vorhanden	presente	Delgado, Festos, Novi, Orion	9
48.3 **(*)**	**Pathotype P1-2-3**	**Pathotype P1-2-3**	**Pathotyp P1-2-3**	**Patotipo P1-2-3**		
QL	absent	absente	fehlend	ausente	Piperade, Yolo Wonder	1
	present	présente	vorhanden	presente	Cuby, Tasty	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. **(+)**	**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 Morelos 334	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. **(+)**	**Resistance to *Phytophthora capsici*** 	**Résistance à *Phytophthora capsici*** 	**Resistenz gegen *Phytophthora capsici*** 	**Resistencia al *Phytophthora capsici*** 		
QL	absent	absente	fehlend	ausente	Yolo Wonder, Jupiter	1
	present	présente	vorhanden	presente	Chistera, Favolor, Phyo 636, Picador, 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. **(+)**	**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

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. **(*)** **(+)**	**Resistance to *Tomato spotted wilt virus* (TSWV)** Pathotype P0	**Résistance au *Tomato spotted wilt virus* (TSWV)** Pathotype P0	**Resistenz gegen *Tomato spotted wilt virus* (TSWV)** Pathotype P0	**Resistencia al *Tomato spotted wilt virus* (TSWV)** Pathotype P0		
QL	absent	absente	fehlend	ausente	Yolo Wonder	1
	present	présente	vorhanden	presente	Galileo, Jackal, Jackpot	9

Current wording:

53. **(+)**	**Resistance to *Xanthomonas campestris* pv. *vesicatoria*** **QL**	**Résistance au *Xanthomonas campestris* pv. *vesicatoria*** **QL**	**Resistenz gegen *Xanthomonas campestris* pv. *vesicatoria*** **QL**	**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. **(+)**	**Resistance to *Xanthomonas campestris* pv. *vesicatoria*** **QL**	**Résistance au *Xanthomonas campestris* pv. *vesicatoria*** **QL**	**Resistenz gegen *Xanthomonas campestris* pv. *vesicatoria*** **QL**	**Resistencia al *Xanthomonas campestris* pv. *vesicatoria*** **QL**		
53.1 **(+)**	**Resistance to *Xanthomonas campestris* pv. *vesicatoria* race 1** **QL**	**Résistance au *Xanthomonas campestris* pv. *vesicatoria*** **QL**	**Resistenz gegen *Xanthomonas campestris* pv. *vesicatoria*** **QL**	**Resistencia al *Xanthomonas campestris* pv. *vesicatoria*** **QL**		
	absent	absente	fehlend	ausente	Early Cal Wonder, Fehérözön, Yolo Wonder	1
	present	présente	vorhanden	presente	Early Cal Wonder-20R, Early Cal Wonder-30R, Early Cal Wonder-40R	9
53.2 **(+)**	**Resistance to *Xanthomonas campestris* pv. *vesicatoria* race 2** **QL**	**Résistance au *Xanthomonas campestris* pv. *vesicatoria*** **QL**	**Resistenz gegen *Xanthomonas campestris* pv. *vesicatoria*** **QL**	**Resistencia al *Xanthomonas campestris* pv. *vesicatoria*** **QL**		
	absent	absente	fehlend	ausente	Early Cal Wonder, Fehérözön, Yolo Wonder	1
	present	présente	vorhanden	presente	Early Cal Wonder-10R, Early Cal Wonder-20R	9
53.3 **(+)**	**Resistance to *Xanthomonas campestris* pv. *vesicatoria* race 3** **QL**	**Résistance au *Xanthomonas campestris* pv. *vesicatoria*** **QL**	**Resistenz gegen *Xanthomonas campestris* pv. *vesicatoria*** **QL**	**Resistencia al *Xanthomonas campestris* pv. *vesicatoria*** **QL**		
	absent	absente	fehlend	ausente	Early Cal Wonder, Fehérözön, Yolo Wonder	1
	present	présente	vorhanden	presente	Aladin X3R, Camelot X3R, Early Cal Wonder-20R, Early Cal Wonder-30R	9

Proposal to Include a Revised Format for Disease Resistance Characteristics

Please see next page, current and proposed new wording are presented on opposite pages.

Proposal to Include a Revised Format for Disease Resistance Characteristics
(Current and Proposed New Wording are presented on opposite pages)

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:	U1 Feldman	P11 Obuda Pepper Mosaic Virus	P14 Samsun latens
Genotype / mark	P_0	P_{1-2}	P_{1-2-3}
L^-L^-	S	S	S
L^1L^1	R	S	S
L^3L^3	R	R	S
L^4L^4	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 (Tm)

1. Pathogen	**Tobamovirus** (the genus containing *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), 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 P0, P1-2, and P1-2-3
6. Establishment isolate identity	on differentials
7. Establishment pathogenicity	use susceptible pepper standard or lesions on *Nicotiana tabacum* 'Xanthi' 2 d 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 N. 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	
9.3 Control varieties	see table of example varieties below
9.4 Test design	Optional: to add blank treatement
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 dpi to 15 - 20 dpi
10.7 Final observations	20 dpi
11. Observations	
11.1 Method	Visual, comparative; necrosis signifies hypersensitivity and resistance;
11.2 Observation scale	
[1] Susceptible: Mosaic	(sometimes developing late, sometimes early and leading to plant death without hypersensitivity)
[9] Resistant	Systemic necrosis, stunting
[9] Resistant	Local necrosis, leaf dropping
[9] Resistant	No virus symptoms, only mechanical damage
11.3 Validation of test	On standards
11.4 Off-types	
12. Interpretation of data in terms of UPOV characteristic states	QL
13. Critical control points	Tobamovirus pathotype P0 is defined on differentials and may belong to ToMV or TMV Pathotype P1-2 and P1-2-3 belong to PMMoV

Differential table (S = susceptible; R = resistant)

		Tobamovirus Pathotypes on Pepper		
Resistance code	**Resistance gene**	**P0**	**P1-2**	**P1-2-3**
	L0	S	S	S
Tm0	L1	R	S	S
Tm1	*L2**	*R*	*S*	*S*
Tm2	L3	R	R	S
Tm3	L4	R	R	R

Example varieties:

L0: Pepita, Doux Italien, Piperade, Lamu
L1: Explorer, Lamuyo, Sonar, Yolo Wonder
*L2: C. frutescens 'Tabasco'**
L3: Ferrari, Delgado, Festos, Novi, Orion, Solario,
L4: Friendly, Cuby, Tasty, Tom 4

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

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 NA_2HPO_4 + 1.18 g K_2HPO_4 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 virus* Y (PVY)

1. Pathogen ..... *Potato virus* Y (PVY)
2. Quarantine status ..... NO
3. Host species ..... *Capsicum annuum*
4. Source of inoculum ..... GEVES (FR), Naktuinbouw (NL)
5. Isolate ..... Pathotype 0, 1, and 1-2
6. Establishment isolate identity ..... on differentials: Yolo Wonder, Yolo Y, Florida V2, W4, Solario
7. Establishment pathogenicity ..... on susceptible plant (e.g. on *Nicotina 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 solution
0,03 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
9.3 Control varieties

Differential table (S = susceptible; R = resistant)

	PVY pathotypes		
Pepper variety	0	1	1-2
Yolo Wonder	S	S	S
Yolo Y	R	S	S
Florida VR2	R	R	S *
Serrano Criollo de Morelos 334, Solario, W4	R	R	R

* Florida VR2 may show vague and very late symptoms with pathotype 1-2

9.4 Test design ..... Optional: to add blank treatement
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 dpi
10.6 Second observation ..... 14 - 21 dpi
10.7 Final observations ..... 21 dpi
11. Observations
11.1 Method ..... Visual, comparative
11.2 Observation scale
[1] Susceptible: ..... Growth retardation, leaf malformation, light mosaic in youngest leaves, or red veins; stem necrosis, plant death
[9] Resistant ..... No symptoms.
11.3 Validation of test ..... on standards
11.4 Off-types
12. Interpretation of data in terms of UPOV characteristic states ..... QL
13. Critical control points ..... Remark: Avoid high temperatures

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*
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 standards
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	
9.3 Control varieties	Jupiter, Yolo Wonder (Susceptible), Favolor, Chistera (IR) Solario, Picador, Phyo 636 (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 4 mm- 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 dpi
10.7 Final observations	21 dpi
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] Susceptible: Jupiter, Yolo Wonder	e.g.length increase > 0,8 cm / week
[9] Intermediate Resistant: Favolor, Chistera	e.g.length increase ≥ 0,5 en ≤ 0,8 cm / week
[9] Resistant: Solario, Picador, Phyo 636	e.g.length increase < 0,5 cm / week
11.3 Validation of test	on standards
11.4 Off-types	
12. Interpretation of data in terms of UPOV characteristic states	QL
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:	22°C, 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) varieties:	Milord (T) 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	
9.3 Control varieties.....	Yolo Wonder (Susceptible), Milord (IR), Vania, 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 d after sowing)
10.4 Inoculation method	Rubbing cotyledons with carborundum, followed by 48 h darkness
10.5 First observation	10 days post inoculation
10.6 Second observation	15 dpi
*10.7 Final observations	21 dpi
11. Observations	
11.1 Method	Visual, comparative
11.2 Observation scale	
[1] Susceptible: Yolo Wonder	mosaic
[9] Intermediate Resistant: Milord	intermediate symptoms
[9] Resistant: Vania, Alby, Favolor	No or light symptoms
11.3 Validation of test	on standards
11.4 Off-types	
12. Interpretation of data in terms of UPOV characteristic states	QL
13. Critical control points	The Fulton isolate causes local lesion on **all pepper leaves** at optimal concentration. The difference between the susceptible and resistant is only in the number of lesions on the inoculated leaves.

Current wording:

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

Maintenance of patothypes:

Type of medium:	Pepper fruit in deep-freezer (-70 °C)
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 °C
Light:	Extra light in winter
Growing method:	Sowing in greenhouse
Method of inoculation:	Mechanical, rubbing on cotyledons, inoculum suspension 10 °C

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 P0 (TSWV)

1. Pathogen	*Tomato spotted wilt virus* P0 (TSWV: P0)
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 (see remark)
6. Establishment isolate identity	
7. Establishment pathogenicity	on susceptible plant e.g. *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 / at “first leaf” pointed stage or 1- 3 leaves
8.3 Plant stage at inoculation	
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	
9.3 Control varieties	Yolo Wonder, Lamuyo (Susceptible) CHI7, Galileo, Jackal, Jackpot, Prior (Resistant)
9.4 Test design	
9.5 Test facility	growth chamber or insect proof glasshouse
9.6 Temperature	18-20° 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 24 h option : 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 to 15 - 21 dpi
*10.7 Final observations	21 dpi
11. Observations	
11.1 Method	Visual, comparative
11.2 Observation scale	
[1] Susceptible: Yolo Wonder, Lamuyo	Mosaic in young leaf, some leaf malformation
[9] Resistant: CHI7,Galileo, Jackal, Jackpot, Prior	Necrosis or only mechanical damage
11.3 Validation of test	on standards
11.4 Off-types	
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.

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

(Ed.), Progress in Research on Capsicum and Eggplant, Eucarpia conference proceedings, Warsaw, pp. 231-236

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 10^7 .

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)* race 1, race2, race 3

1. Pathogen	*Xanthomonas campestris* pv. *vesicatoria*		
2. Quarantine status			
3. Host species	*Capsicum annuum*		
4. Source of inoculum			
5. Isolate			
6. Establishment isolate identity	on differentials		
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			
9.3 Control varieties			
Differential	Race 1	Race 2	Race 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
9.4 Test design			
9.5 Test facility			
9.6 Temperature	25/24°C d/n		
9.7 Light	30.000 lx suggested, 16 h/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	10^7 -10^8 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 13-20 mm diameter spots		
10.5 First observation	2-5 d		
10.6 Second observation	6-8 d		
*10.7 Final observations	10-14 d		
11. Observations			
11.1 Method	visual , comparative		
11.2 Observation scale			
[1] Susceptible	Water soaking near infiltration site		
[9] Resistant	Necrotic reaction at infiltration site		
11.3 Validation of test	on standards		
11.4 Off-types			
12. Interpretation of data in terms of UPOV characteristic states	QL		
13. Critical control points			

Proposed changes to Chapter 10 “Technical Questionnaire”

To add characteristics 52 “Resistance to Tomato spotted wilt virus (TSWV) - Pathotype P0” to Chapter TQ 5.

[End of Annex and of document]