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TC/51/30
ORIGINAL: English
DATE: March 5, 2015

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

TECHNICAL COMMITTEE

Fifty-First Session
Geneva, March 23 to 25, 2015

PARTIAL REVISION OF THE TEST GUIDELINES FOR
SWEET PEPPER, HOT PEPPER, PAPRIKA, CHILI (DOCUMENT TG/76/8)

Document prepared by the Office of the Union

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1. At its forty-eighth session held in Paestum, Italy, from June 23 to 27, 2014, the Technical Working Party for Vegetables (TWV) considered a partial revision of the Test Guidelines for Sweet Pepper on the basis of documents TG/76/8 and TWV/48/38 "Partial Revision of the Test Guidelines for Sweet Pepper, Hot Pepper, Paprika, Chili (Document TG/76/8)" and proposed to revise the Test Guidelines for Sweet Pepper as follows (see document TWV/48/43 "Report", paragraph 101):

(a) Revision of the Grouping Characteristics in Chapter 5.3

(b) Revision of disease resistance characteristics and explanations
- (i) Chapter 7: Proposal to Revise Characteristics 48 to 53
- (ii) Chapter 8.2: Inclusion of a Revised Format for Disease Resistance Characteristics
- (iii) Chapter 9: Literature
- (iv) Chapter 10: Technical Questionnaire

2. 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 - "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**	**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

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)**	**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
49.2		**Pathotype 1 (PVY: 1)**	**Pathotype 1 (PVY: 1)**	**Pathotyp 1 (PVY: 1)**	**Patotipo 1 (PVY: 1)**		
QL		absent	absente	fehlend	ausente	Yolo Wonder	1
		present	présente	vorhanden	presente	Sileno, Solario, Vidi	9
49.3		**Pathotype 1.2 (PVY: 1.2)**	**Pathotype 1.2 (PVY: 1.2)**	**Pathotyp 1.2 (PVY: 1.2)**	**Patotipo 1.2 (PVY: 1.2)**		
QL		absent	absente	fehlend	ausente	Yolo Wonder	1
		present	présente	vorhanden	presente	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)**	**Résistance à "Phytophthora capsici" (Pc)**	**Resistenz gegen "Phytophthora capsici" (Pc)**	**Resistencia al "Phytophthora capsici" (Pc)**	
QL		absent	absente	fehlend	ausente	Jupiter, Yolo Wonder	1
		present	présente	vorhanden	presente	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 "Cucumber mosaic virus" (CMV)**	**Résistance au "Cucumber mosaic virus" (CMV)**	**Resistenz gegen "Cucumber mosaic virus" (CMV)**	**Resistencia al "Cucumber mosaic virus" (CMV)**	
QL		absent	absente	fehlend	ausente	Yolo Wonder	1
		present	présente	vorhanden	presente	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)**	**Résistance au "Tomato spotted wilt virus" Pathotype 0 (TSWV: 0)**	**Resistenz gegen "Tomato spotted wilt virus" Pathotyp 0 (TSWV: 0)**	**Resistencia al "Tomato spotted wilt virus" Patotipo 0 (TSWV: 0)**	
QL		absent	absente	fehlend	ausente	Lamuyo, Yolo Wonder	1
		present	présente	vorhanden	presente	Galileo, Jackal, Jackpot, Prior	9

Current wording:

53. **(+)**	**Resistance to *Xanthomonas campestris* pv. *vesicatoria*** **(Xcv)**	**Résistance au *Xanthomonas campestris* pv. *vesicatoria*** **(Xcv)**	**Resistenz gegen *Xanthomonas campestris* pv. *vesicatoria*** **(Xcv)**	**Resistencia al *Xanthomonas campestris* pv. *vesicatoria*** **(Xcv)**		
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)**	**Résistance au "Xanthomonas campestris pv. vesicatoria" (Xcv)**	**Resistenz gegen "Xanthomonas campestris pv. vesicatoria" (Xcv)**	**Resistencia al "Xanthomonas campestris pv. vesicatoria" (Xcv)**		
53.1	**Pathotype 1**	**Pathotype 1**	**Pathotyp 1**	**Patotipo 1**		
QL	absent	absente	fehlend	ausente	Fehérözön, Yolo Wonder	1
	present	présente	vorhanden	presente	Emiro, Filidor, Gotico, San Marco, Solanor	9
53.2	**Pathotype 2**	**Pathotype 2**	**Pathotyp 2**	**Patotipo 2**		
QL	absent	absente	fehlend	ausente	Fehérözön, Yolo Wonder	1
	present	présente	vorhanden	presente	Emiro, Filidor, Gotico, San Marco, Solanor	9
53.3	**Pathotype 3**	**Pathotype 3**	**Pathotyp 3**	**Patotipo 3**		
QL	absent	absente	fehlend	ausente	Fehérözön, Yolo Wonder	1
	present	présente	vorhanden	presente	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:	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

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)

		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	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

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 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 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

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 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	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 standards Jupiter, 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	QL Based on the stem necrosis 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:	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	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] highly 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 °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 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), INIA (ES)
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 24h 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 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 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 13-15mm 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	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 13-20mm 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]