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PARTIAL REVISION OF THE TEST GUIDELINES FOR PEPPER

Document prepared by an expert from the European Union

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1. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Pepper (*Capsicum annuum* L.) (document TG/76/8 Rev.).

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

(a) To change the example varieties for the following characteristics of Characteristic 48 "Resistance to Tobamovirus"
  (i) 48.1 "*Tobacco mosaic virus* Pathotype 0 (TMV: 0)"
  (ii) 48.2 "*Pepper mild mottle virus* Pathotype 1.2 (PMMoV: 1.2)"
  (iii) 48.3 "*Pepper mild mottle virus* Pathotype 1.2.3 (PMMoV: 1.2.3)"

(b) To change the methodology for Characteristic 48 "Resistance to Tobamovirus" under Ad. 48

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

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

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

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

Current wording

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

Proposed new wording

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

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

Current wording

Ad. 48: Resistance to Tobamovirus

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 untreated plant
9.5	Test facility	glasshouse or climatic chamber
9.6	Temperature	20-25°C
9.7	Light	at least 12h

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

Proposed new wording

Ad. 48: Resistance to Tobamovirus

1.	Pathogen	*Tobacco mosaic virus* and *Pepper mild mottle virus*
2.	Quarantine status	no
3.	Host species	Sweet pepper, hot pepper, paprika and chili – *Capsicum annuum* L.
4.	Source of inoculum	GEVES[1] (FR), Naktuinbouw[2] (NL) or INIA[3] (SP)
5.	Isolate	*Tobacco mosaic virus* pathotype 0 (TMV: 0) strain Vi-6 *Pepper mild mottle virus* pathotype 1.2 (PMMoV: 1.2) strain nt203 *Pepper mild mottle virus* pathotype 1.2.3 (PMMoV: 1.2.3) strain Eve The test protocols have been validated in a CPVO co-funded project[4] with these 3 isolates/pathotypes.
6.	Establishment isolate identity	genetically defined pepper differentials (reference to ISFwebsite: http://www.worldseed.org/isf/differential_hosts.html)
7.	Establishment pathogenicity	Test on susceptible plants
8.	Multiplication inoculum	
8.1	Multiplication medium	Regeneration of the virus of plant material before inoculum preparation.
8.2	Multiplication variety	On susceptible pepper variety, Tobamovirus races may be multiplied on varieties which are selective for each particular race. For TMV, because tomato and tobacco *Nicotiana tabacum* cv. Samsun have large leaves and can produce a lot of inoculum, they are recommended for the multiplication of TMV: 0.
8.3	Plant stage at inoculation	see 10.3
8.4	Inoculation medium	see 10.1
8.5	Inoculation method	see 10.4
8.6	Harvest of inoculum	Symptomatic fresh leaves
8.7	Check of harvested inoculum	option: on young leaves of *Nicotiana tabacum* “Xanthi”, check for local lesions after 5-7 days at 20-25°C.
8.8	Shelflife/viability inoculum	fresh > 1 day in fridge, desiccated > 1 year in fridge or juice > 1 year in freezer at -20°C
9.	Format of the test	
9.1	Number of plants per genotype	At least 20 plants.
9.2	Number of replicates	-
9.3	Control varieties	TMV: 0: Susceptible controls: Lamu, Pepita, Piquillo Resistant controls: Fehérözön, Yolo Wonder PMMoV: 1.2: Susceptible controls: Fehérözön, Lamu, Yolo Wonder Resistant controls: Ferrari, Novi 3 PMMoV: 1.2.3: Susceptible controls: Ferrari, Yolo Wonder Resistant controls: Friendly, Tom 4
9.4	Test design	add non inoculated plants
9.5	Test facility	Climate room or greenhouse
9.6	Temperature	20-25°C
9.7	Light	12 hours or longer

[1] matref@geves.fr

[2] resistentie@naktuinbouw.nl

[3] cardaba@inia.es

[4] Harmores 2 CPVO project (http://www.cpvo.europa.eu/main/en/home/documents-and-publications/technical-projects-reports)

9.8	Season	-
9.9	Special measures	-
10.	Inoculation	
10.1	Preparation inoculum	1 g leaf with symptoms with 10 mL PBS or similar buffer or dilution of juice in water. Homogenize, add carborundum to buffer
10.2	Quantification inoculum	-
10.3	Plant stage at inoculation	TMV: 0, cotyledons to first leaf stage PMMoV: 1.2, cotyledon stage PMMoV: 1.2.3, cotyledon stage
10.4	Inoculation method	rubbing with the virus suspension.
10.5	First observation	TMV:0: 4-7 days post-inoculation for observation of local necrosis. PMMoV: 1.2 and PMMoV: 1.2.3: 4-7 days post-inoculation for observation of local necrotic lesions which can lead to cotyledon drop. After this date these necrosis can hardly be seen on fallen cotyledons.
10.6	Second observation	TMV: 0: two weeks post-inoculation for observation of symptoms of susceptibility. PMMoV: 1.2 and PMMoV: 1.2.3: two weeks post-inoculation for observation of symptoms of susceptibility.
10.7	Final observations	TMV:0 : three weeks post-inoculation. PMMoV: 1.2 and PMMoV: 1.2.3: three weeks post-inoculation. For TMV:0, PMMoV: 1.2 and PMMoV: 1.2.3, two of these three observations may be sufficient; the third notation is optional for observation of evolution of symptoms (depending on symptoms on controls or heterogeneous behaviour).
11.	Observations	
11.1	Method	Visual
11.2	Observation scale	TMV: 0: Susceptibility: mosaic (aucuba in case of aucuba strain as Vi-6), growth reduction, death of plants. Resistance: local necrotic lesions which can lead to leave drop, systemic necrosis, vein necrosis, stem necrosis. PMMoV: 1.2 and PMMoV: 1.2.3: Susceptibility: mosaic (green), growth reduction. Resistance: local necrotic lesions which can lead to cotyledon drop, systemic necrosis.
11.3	Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls.
12.	Interpretation of data in terms of UPOV characteristic states	
	absent……	[1] susceptible
	present……	[9] resistant
13.	Critical control points	For TMV: 0, plants with no symptoms at all have to be interpreted as escapes of inoculation.
	Recommended dates of notation should be adapted depending of expression of symptoms on controls. Environmental conditions can have an effect on the expression of symptoms over time. In this case a third notation could be necessary.	

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

Current wording

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

Proposed new wording

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

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