TC-EDC/Jan13/25
ORIGINAL: English
DATE: November 29, 2012

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
Geneva

ENLARGED EDITORIAL COMMITTEE

Geneva, January 9 and 10, 2013

PARTIAL REVISION OF THE TEST GUIDELINES FOR TOMATO (DOCUMENT TG/44/11)

Document prepared by the Office of the Union

1. The Technical Working Party for Vegetables (TWV), at its forty-fifth session, held in Monterey, United States of America, from July 25 to 29, 2011, agreed to propose to the Technical Committee (TC) to adopt a partial revision of the Test Guidelines for Tomato (document TG/44/11) in order to include:

(a) a revised format for disease resistance characteristics according to the explanations for disease resistance characteristics in Test Guidelines; as set out in document TGP/12/2 Draft 2 "Guidance on Certain Physiological Characteristics", Section 2.4; and
(b) a gene-specific marker method for examination of resistance to Tomato Spotted Wilt topovirus (TSWV) - Race 0.

2. The TC, at its forty-eighth session held in Geneva from March 26 to 28, 2012, noted that, in response to a number of technical questions concerning disease resistance, raised by interested experts after the TWV session, it had been agreed by the TWV Chairperson, former TWV Chairperson, and the Leading Expert to consider a new draft of the partial revision of the Test Guidelines for Tomato at the forty-sixth session of the TWV (see document TC/48/22 "Report on Conclusions", paragraph 147).

3. The TWV at its forty-sixth session, held near the city of Venlo, Netherlands, June 11 to 15, 2012, considered document TWV/46/19 and agreed to propose the partial revision of the Test Guidelines for Tomato (document TG/44/11) as set out in the Annexes to this document:

ANNEX I Proposal to correct diseases names in Chapters: 5.3, 7, 8 and 10

ANNEX II Inclusion of a revised format for disease resistance characteristics according to the explanations for disease resistance characteristics in Test Guidelines; as set out in document TGP/12/2 Draft 2 "Guidance on Certain Physiological Characteristics", Section 2.4 (current and proposed new wording are presented on opposite pages)

ANNEX III Addition of Literature References to Chapter 9: Literature;

4. The partial revision to document TG/44/11 would be adopted as document TG/44/11 Rev..

[Annex follows]

ANNEX I

Proposal to Correct Disease Names in Chapters: 5.3, 7 ,8 and 10. TQ

Current wording:

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
49. **(+)**	**VG**	**Resistance to *Fusarium oxysporum* f. sp. *radicis lycopersici* (Forl)**	**Résistance à *Fusarium oxysporum* f. sp. *radicis lycopersici* (Forl)**	**Resistenz gegen *Fusarium oxysporum* f. sp. *radicis lycopersici* (Forl)**	**Resistencia a *Fusarium oxysporum* f. sp. *radicis lycopersici* (Forl)**		

Proposed new wording:

49. **(+)**	**VG**	**Resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl)**	**Résistance à *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl)**	**Resistenz gegen *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl)**	**Resistencia a *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl)**		

Current wording:

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
51. **(+)**	**VG**	**Resistance to Tomato Mosaic Tobamovirus (ToMV)**	**Résistance au virus de la mosaïque de la tomate (ToMV)**	**Resistenz gegen das Tomatenmosaikvirus, Tobamovirus (ToMV)**	**Resistencia al virus del mosaico del tomate (ToMV)**		

Proposed new wording:

51. **(+)**	**VG**	**Resistance to Tomato mosaic virus (ToMV)**	**Résistance au virus de la mosaïque de la tomate (ToMV)**	**Resistenz gegen das Tomatenmosaikvirus (ToMV)**	**Resistencia al virus del mosaico del tomate (ToMV)**		

Current wording:

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
54. **(+)**	**VG**	**Resistance to Stemphylium**	**Résistance à Stemphylium**	**Resistenz gegen Stemphylium**	**Resistencia a Stemphylium**		

Proposed new wording:

54. **(+)**	**VG**	**Resistance to Stemphylium spp. (Ss)**	**Résistance à Stemphylium spp. (Ss)**	**Resistenz gegen Stemphylium spp. (Ss)**	**Resistencia a Stemphylium spp. (Ss)**		

Current wording:

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
57. **(+)**	**VG**	**Resistance to Tomato Yellow Leaf Curl Begomovirus (TYLCV)**	**Résistance au bégomovirus des feuilles jaunes en cuillère de la tomate (TYLCV)**	**Resistenz gegen gelbes Tomatenblatt-rollvirus, Begomovirus (TYLCV)**	**Resistencia a Begomovirus del rizado amarillo de la hoja del tomate (TYLCV)**		

Proposed new wording:

57. **(+)**	**VG**	**Resistance to Tomato yellow leaf curl virus (TYLCV)**	**Résistance au virus des feuilles jaunes en cuillère de la tomate (TYLCV)**	**Resistenz gegen gelbes Tomatenblatt-rollvirus (TYLCV)**	**Resistencia a virus del rizado amarillo de la hoja del tomate (TYLCV)**		

Current wording:

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
58. **(+)**	**VG**	**Resistance to Tomato Spotted Wilt Tospovirus (TSWV)** **- Race 0**	**Résistance au virus de la tache bronzée de la tomate (TSWV)** **– Pathotype 0**	**Resistenz gegen das Tomatenbronzen-fleckenvirus, Tospovirus (TSWV)** **- Pathotyp 0**	**Resistencia a Tospovirus del bronceado de tomate (TSWV)** **– Raza 1**		

Proposed new wording:

58. **(+)**	**VG**	**Resistance to Tomato spotted wilt virus (TSWV)** **- Race 0**	**Résistance au virus de la tache bronzée de la tomate (TSWV)** **– Pathotype 0**	**Resistenz gegen das Tomatenbronzen-fleckenvirus (TSWV)** **- Pathotyp 0**	**Resistencia a virus del bronceado de tomate (TSWV)** **– Raza 1**		

Current wording:

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
61. **(+)**	**VG**	**Resistance to Tomato Torrado Virus (ToTV)**	**Résistance au virus Tomato Torrado (ToTV)**	**Resistenz gegen Tomato Torrado Virus (ToTV)**	**Resistencia al virus del torrado del tomate (ToTV)**		

Proposed new wording:

61. **(+)**	**VG**	**Resistance to Tomato torrado virus (ToTV)**	**Résistance au virus tomato torrado (ToTV)**	**Resistenz gegen Tomato Torrado Virus (ToTV)**	**Resistencia al virus del torrado del tomate (ToTV)**		

[Annex II follows]

ANNEX II

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

Current wording:

Ad. 46: Resistance to *Meloidogyne incognita* (Mi)

Method

Maintenance of strain

Type of medium:	on roots of susceptible varieties
Special conditions	avoid rotting of roots

Execution of test

Temperature:	not over 28° C
Growing method:	preferably in the greenhouse
Method of inoculation:	plants are sown in infested soil

Duration of test

- from sowing to inoculation:	inoculation before sowing,
- from inoculation to reading:	30 to 45 days

Number of plants tested:	10 to 20
Remarks:	avoid rotting of roots avoid high temperature
Notation:	number of root knots contaminated with eggs and root deformation
Standard varieties:	susceptible: Clairvil, Casaque Rouge moderately resistant: Madyta, Vinchy highly resistant: Anabel, Anahu, F1 Anahu x Monalbo

Proposed new wording:

Ad 46: Resistance to *Meloidogyne incognita* (Mi)

1. Pathogen	*Meloidogyne incognita*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw (NL[1]) or GEVES[2] (FR)
5. Isolate	non-resistance breaking
6. Establishment isolate identity	use rootstock or tomato standards
7. Establishment pathogenicity	use susceptible rootstock or tomato standard
8. Multiplication inoculum	
8.1 Multiplication medium	living plant
8.2 Multiplication variety	preferably resistant to powdery mildew
8.3 Plant stage at inoculation	see 10.3
8.5 Inoculation method	see 10.4
8.6 Harvest of inoculum.....	root systems are cut with scissors into pieces of about 1 cm length
8.7 Check of harvested inoculum	visual check for presence of root knots
8.8 Shelf life/viability inoculum.....	1 day
9. Format of the test.....	
9.1 Number of plants per genotype	20 plants
9.2 Number of replicates……	Not applicable
9.3 Control varieties.....	
Susceptible:	Clairvil, Casaque Rouge
Moderately resistant :	“Anahu x Monalbo”, Campeon, Madyta, Vinchy
Highly resistant:	Anahu, Anabel
9.4 Test design	include standard varieties
9.5 Test facility	greenhouse or climate room
9.6 Temperature	not over 28° C
9.7 Light.....	at least 12 h per day
10. Inoculation	
10.1 Preparation inoculum.....	small pieces of diseased root mixed with soil mix soil and infested root pieces
10.2 Quantification inoculum	soil: root ratio = 8:1, or depending on experience
10.3 Plant stage at inoculation	seed, or cotyledons
10.4 Inoculation method	plants are sown in infested soil or contamination of soil after sowing when plantlets are at cotyledon stage
10.7 Final observations	28 to 45 days after inoculation
11. Observations.....	
11.1 Method.....	root inspection
11.2 Observation scale.....	Symptoms: galling, root malformation, growth reduction, plant death
11.3 Validation of test.....	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls on standards

12. Interpretation of data in terms of UPOV characteristic states, to consider that resistant varieties may have a few plants with a few galls. These are not considered as off-types.

absent (susceptible).....	[1]	growth strongly reduced, high gall count
intermediate (moderately resistant).....	[2]	medium growth reduction, medium gall count
present (highly resistant)	[3]	present; no growth reduction, no galls

13. Critical control points:
Avoid rotting of roots; high temperature causes breakdown of resistance.

[1] Naktuinbou; resistentie@naktuinbouw.nl

[2] GEVES; Valerie.GRIMAULT@geves.fr

Current wording:

Ad. 47: Resistance to *Verticillium* sp.(Va and Vd)

Method

Maintenance of strains

Race 0 represented by strain Toreilles 4-1-4-1 is used. Race 0 is the common race defined by its ability to infect plants with the Ve gene.

Long term storage of strains: conidia suspended in glycerol solution at -80°C.
Strain can be subcultured on Potato Dextrose Agar (PDA) medium.

Execution of test

Growth stage of plants

Plants are grown in greenhouse or growth chamber. Inoculation can be done from the cotyledon stage (first leaves emerging) to 2 expanded leaves stage.

The following varieties can be used as controls. As a minimum, there should be one resistant and one susceptible control in the test. The heterozygous variety will help interpretation of results in case of aggressive test. Clarion could be interesting to add to susceptible controls as it is less susceptible and could also help to check the inoculation pressure of the test. These 2 varieties are optional.

Standard variety	Vd:0
Marmande verte, Flix	S
Clarion	s
Monalbo x Marmande verte	RH
Monalbo, Elias	R

R	resistance present; no symptoms
RH	resistance present; sometimes very weak symptoms
s	resistance absent; weak symptoms
S	resistance absent; clear symptoms

Temperature:
Test performed under controlled conditions at 20 to 22°C.

Inoculum:
Verticillium sp. is grown on liquid Czapek Dox Broth or S of Messiaen media for 3 to 7 days in the dark, at 20 to 25°C with aeration. Spores are harvested and adjusted to 10^6sp/ml.

Method of inoculation
Plantlets are harvested, roots are cut and soaked for 5 to 15 min in the inoculum suspension. Plantlets are then transplanted in soil.

Duration of test
At least 33 days from sowing to notation.

Number of plants tested:
At least 20 plants.

Notation:
25-30 days after inoculation.

Notation scale and interpretation of results:
R: no symptoms
S: chlorosis in the lower leaves, growth reduced and brown vessels or growth not reduced and brown vessels.
Analysis of results should be calibrated with results on R and S controls.

Proposed new wording:

Ad 47: Resistance to *Verticillium* sp. (Va and Vd)

1. Pathogen	*Verticillium dahliae* or *Verticillium albo-atrum*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum.....	Naktuinbouw[3] (NL) and GEVES[4] (FR)
5. Isolate	Race 0 (e.g. strain Toreilles 4-1-4-1)
8. Multiplication inoculum	
8.1 Multiplication medium	Potato Dextrose Agar, Agar Medium "S" of Messiaen
8.4 Inoculation medium.....	water (for scraping agar plates) or Czapek Dox broth (3-7 d-old
.....	aerated culture at 20-25°C, in darkness)
8.6 Harvest of inoculum.....	filter through double muslin cloth
8.7 Check of harvested inoculums……	spore count; adjust to 106 per ml
8.8 Shelf life/viability inoculums……	1 d at 4°C
9. Format of the test.....	
9.1 Number of plants per genotype……	35 seed for 24 plants
9.2 Number of replicates…… ...	Not applicable
9.3 Control varieties.....	
Susceptible ……	Flix, Marmande verte, Clarion, Santonio, Anabel
Resistant ……	Monalbo, Elias, Monalbo x Marmande verte, Daniela, Marmande VR
9.4 Test design……	20 plants inoculated at least, 2 blanks at least
9.5 Test facility……	greenhouse or climate room
9.6 Temperature……	optimal 20-25°C, 20-22°C after inoculation
9.7 Light……	12 h or longer
10. Inoculation	
10.1 Preparation inoculums……	aerated, liquid culture (8.4)
10.2 Quantification inoculums……	count spores, adjust to 106 per ml
10.3 Plant stage at inoculation……	cotyledon to 3rd leaf
10.4 Inoculation method……	roots are immersed for 4 to 15 min in spore suspension.
10.7 Final observations……	14-33 d after inoculation
11. Observations.....	
11.1 Method……	visual
11.2 Observation scale……	growth retardation, wilting, chlorosis, and vessel browning
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]	severe symptoms
present.....	[9]	no or mild symptoms

13. Critical control points:

All symptoms may be present in resistant varieties, but the severity will be distinctly less than in susceptible varieties. Usually resistant varieties will show significantly less growth retardation then susceptible varieties. Observation of vessel browning is important for diagnosis. Usually, vessel browning will not extend to the 1st leaf in resistant varieties. Many hybrid varieties are heterozygous and appear to have mild symptoms in the biotest.

Note: Resistance to V. dahliae based in the Ve gene is also effective to V. albo-atrum. Isolates of both fungal species may be used to evaluate the UPOV characteristic "Resistance to V. dahliae" or V. albo-atrum as long as the isolate belongs to the non-Ve breaking race 0. Resistance-breaking isolates have been described in both species.

[3] Naktuinbouw: resistentie@naktuinbouw.nl

[4] GEVES; Valerie.GRIMAULT@geves.fr

Current wording:

Ad. 48.1 + 48.2 + 48.3: Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) -Race 0 (ex 1), Race 1 (ex 2) and Race 2 (ex 3)

Method

Maintenance of strains

Long term storage of strains: at -80°C in 20% glycerol.
Race 0 (ex 1) represented by strains Orange 71 or PRI 20698 or Fol 071 and race 1 represented by strains 4152 (more aggressive) or PRI40698 or RAF 70 (less aggressive) are used.
Strains can be multiplied on PDA or S of Messiaen media.

Execution of test

Growth stage of plants

Plants are grown in greenhouse or growth chamber for 10 to 18 days (cotyledons to first leaf stages).

The following varieties are used as controls. Each line will be represented by at least one variety which can be chosen in the varieties indicated; the resistance phenotype to the two pathotypes of Fol is indicated. The heterozygous variety has a resistance phenotype usually weaker than in homozygous lines. This weak resistance can be used to calibrate the borderline between resistance and susceptibility. The heterozygous control for Fol:1 is optional.

Controls for Fol:0 resistance test	Fol:0	Fol:1*
Marmande, Marmande verte, Resal	S	S
Marporum x Marmande verte (heterozygous)	R	S
Marporum, Larissa	R	S
Motelle, Gourmet, Mohawk	R	R

** For information*

Controls for Fol:1 resistance test	Fol:0*	Fol:1
Cherry Belle, Roma, Marmande verte	S	S
Ranco**, Marporum	R	S
Motelle x Marmande verte	R	R
Tradiro, Odisea	R	R

** For information*
*** For Ranco: weak resistance to Fol:0 with many escapes*

R = resistance present
S = resistance absent

Temperature:
Test performed in climatic chambers or greenhouse at 24-28°C. In case of aggressive test, temperature can be decreased to 20-24°C.

Inoculum:
Fusarium oxysporum f. sp. *lycopersici* is grown on PDA or S of Messiaen media or in aerated Czapek-Dox liquid cultures for 7 to 10 days. Spores are harvested and adjusted to 10^6sp/ml for strains grown on media. In case of very aggressive isolate, inoculum concentration can be decreased.

Method of inoculation
Soaking of roots (cutting of roots optional) and of hypocotyls axis for 5 to 15 min in the inoculum suspension and transplantation of inoculated plantlets in soil.

Duration of test
At least 28 days from sowing to notation.

Number of plants tested:
At least 20 plants.

Notation:
At least 21 days after inoculation.

Notation scale:
4 classes:

- 0: no symptoms,
- 1: external healthy aspect of plant (without growth reduction) with brown vessels (sometimes extending above cotyledons, generally remaining below cotyledons),
- 2: growth reduction and brown vessels above cotyledons,
- 3: dead plant.

Interpretation of scale:
Generally, 0 and 1 are equivalent to resistant, 2 and 3 are susceptible but analysis of results should be calibrated with results of R and S controls.

Proposed new wording:

Ad 48: Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)

1. Pathogen	*Fusarium oxysporum* f. sp. *lycopersici*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw[5] (NL) and GEVES[6] (FR)
5. Isolate	Race 0 (ex 1) (e.g. strains Orange 71 or PRI 20698 or Fol 071 1 (ex 2) (e.g. strains 4152 or PRI40698 or RAF 70 and 2 (ex 3) Individual strains may vary in pathogenicity
6. Establishment isolate identity	use differential varieties (see 9.3)
7. Establishment pathogenicity	on susceptible tomato varieties
8. Multiplication inoculum	
8.1 Multiplication medium	Potato Dextrose Agar, Medium "S" of Messiaen
8.4 Inoculation medium	water for scraping agar plates or Czapek-Dox culture medium (7 d-old aerated culture)
8.6 Harvest of inoculum	filter through double muslin cloth
8.7 Check of harvested inoculum	spore count; adjust to 106 per ml
8.8 Shelf-life/viability inoculum	4-8 h, keep cool to prevent spore germination
9. Format of the test	
9.1 Number of plants per genotype	at least 20
9.2 Number of replicates	Not applicable
9.3 Control varieties for the test with race 0 (ex 1)	
Susceptible	Marmande, Marmande verte, Resal
Resistant for race 0 only	Marporum, Larissa, "Marporum x Marmande verte", Marsol, Anabel
Resistant for race 0 and 1	Motelle, Gourmet, Mohawk
Control varieties for the test with race 1 (ex 2)	
Susceptible	Marmande verte, Cherry Belle, Roma
Resistant for race 0 only	Marporum, Ranco
Resistant for race 0 and 1	Tradiro, Odisea
Remark:	Ranco is slightly less resistant than Tradiro
Control varieties for the test with race 2 (ex 3)	
Susceptible for race 0, 1 and 2	Marmande verte, Motelle, Marporum
Resistant for race 0, 1 and 2	Tributes, Murdoch, Marmande verte x Florida
9.4 Test design	>20 plants; e.g. 35 seeds for 24 plants, including 2 blanks
9.5 Test facility	glasshouse or climate room
9.6 Temperature	24-28°C (severe test, with mild isolate) 20-24°C (mild test, with severe isolate)
9.7 Light	12 hours per day or longer
9.8 Season	all seasons
9.9 Special measures	slightly acidic peat soil is optimal; keep soil humid but avoid water stress
10. Inoculation	
10.1 Preparation inoculums	aerated Messiaen or PDA or Agar Medium S of Messiaen or Czapek Dox culture or scraping of plates
10.2 Quantification inoculums	spore count, adjust to 106 spores per ml, lower concentration for a very aggressive isolate
10.3 Plant stage at inoculation	10-18 d, cotyledon to first leaf
10.4 Inoculation method	roots and hypocotyls are immersed in spore suspension for 5-15 min; trimming of roots is an option
10.7 Final observations	14-21 days after inoculation
11. Observations	
11.1 Method	visual
11.2 Observation scale	Symptoms: growth retardation, wilting, yellowing, vessel browning extending above cotyledon
11.3 Validation of test……	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls. Standards near borderline R/S will help to compare between labs.

12. Interpretation of data in terms of UPOV characteristic states

absent	[1]	severe symptoms
present	[9]	mild or no symptoms

13. Critical control points

Test results may vary slightly in inoculum pressure due to differences in isolate, spore concentration, soil humidity and temperature.

[5] Naktuinbouw: resistentie@naktuinbouw.nl

[6] GEVES; Valerie.GRIMAULT@geves.fr

Current wording:

Ad. 49: Resistance to *Fusarium oxysporum* f. sp. *radicis lycopersici* (Forl)

Method

Maintenance of race

Type of medium: on PDA or synthetic medium (according to Messiaen)

Special conditions: fridge 4° C

Execution of test

Growth stage of plants: appearance of third leaf

Temperature: day: 22° C, night: 16° C

Light: 14 hours

Growing method: climate room or glasshouse

Method of inoculation: soaking of roots and of hypocotyl axis for five minutes in the inoculum.

Duration of test
- from sowing to inoculation: 18 to 20 days
- from inoculation to reading: 10 days

Number of plants tested: 10 to 20 plants

Remarks: need for frequent renewal of races because of loss of pathogenicity

Standard varieties:

- susceptible: Motelle
- resistant: - Momor (homozygote)
  - F1 Momor x Motelle (heterozygote)
  - the Frl gene does not completely control the disease in the heterozygous stage.

Proposed new wording:

Ad 49: Resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl)

1. Pathogen	*Fusarium oxysporum* f. sp. *radicis-lycopersici*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum.....	Naktuinbouw[7] (NL) and GEVES[8] (FR)
5. Isolate	
7. Establishment pathogenicity.....	symptoms on susceptible tomato Multiplication inoculum
8.1 Multiplication medium	Potato Dextrose Agar or Medium agar “S” of Messiaen
8.4 Inoculation medium.....	water for scraping agar plates or Czapek-Dox (7 d-old aerated culture)
8.6 Harvest of inoculum.....	filter through double muslin cloth
8.7 Check of harvested inoculum	spore count; adjust to 10^6 per ml
8.8 Shelf life/viability inoculum.....	4-8 h, keep cool to prevent spore germination
9. Format of the test.....	
9.1 Number of plants per genotype	at least 20
9.2 Number of replicates……	Not applicable
9.3 Control varieties.....	
Susceptible:	Motelle, Moneymaker
Resistant:	Momor, “Momor x Motelle”
Remark:	“Momor x Motelle” has slightly weaker resistance than Momor
9.4 Test design	>20 plants; e.g. 35 seeds for 24 plants, including 2 blanks
9.5 Test facility.....	glasshouse or climate room
9.6 Temperature	24-28°C (severe test, with mild isolate) 17-24°C (mild test, with severe isolate)
9.7 Light	at least 12 hours per day
9.8 Season.....	all seasons
9.9 Special measures	slightly acidic peat soil is optimal; keep soil humid but avoid water stress
10. Inoculation	
10.1 Preparation inoculum.....	aerated culture or scraping of plates
10.2 Quantification inoculum	spore count, adjust to 10^6 spores per ml
10.3 Plant stage at inoculation	12-18 d, cotyledon to third leaf
10.4 Inoculation method	roots and hypocotyls are immersed in spore suspension for 5-15 min
10.7 Final observations	10-21 days after inoculation
11. Observations.....	
11.1 Method.....	visual; a few plants are lifted at the end of the test
11.2 Observation scale.....	Symptoms: plant death, growth retardation caused by root degradation root degradation, necrotic pinpoints and necrotic lesions on stems
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]	symptoms
present.....	[9]	no symptoms

13. Critical control points:

Temperature should never exceed 27°C during the test period; frequent renewal of races may be needed because of loss of pathogenicity

[7] Naktuinbouw: resistentie@naktuinbouw.nl

[8] GEVES; Valerie.GRIMAULT@geves.fr

Current wording:

Ad. 50.1 – 50.6 Resistance to *Fulvia fulva* (Ff) (ex *Cladosporium fulvum)*

Method

Maintenance of races

Type of medium:	PDA or synthetic medium
Special conditions:	subculturing of isolates

Execution of test

Growth stage of plants:	3 leaves expanded
Temperature:	day: 24° C, night: 16° C
Light:	12 hours
Growing method:	in climate room, highest possible humidity, with reduced growth a few days before inoculation by irrigation of roots with ALAR 85 (daminazoide), or in glasshouse with high humidity, for example under a polyethylene cover.
Method of inoculation:	spraying of a solution with the fungus on leaves.

Duration of test

- from sowing to inoculation:	22 to 25 days
- from inoculation to reading:	20 to 25 days
Number of plants tested:	30 plants
Remarks:	the level of expression of symptoms may vary between plants due to complex resistance genetics

Standard varieties:

- susceptible: Monalbo
- resistant: has to be chosen with the concerned alleles
  - cf1: Stirling Castle
  - cf2: Vetomold
  - cf3: V 121
  - cf4: Purdue 135
  - cf5: IVT 1149
  - cf2 cf4: Vagabond
  - cf2 cf5: F1 “Vetomold x IVT 1149”
  - cf2 cf4 cf5: F1 “Vagabond x IVT 1149”
  - cf6: F 77-38
  - cf9: IVT 1154

Race 0:	Angela, Estrella, Sonatine, Sonato, Vemone
Group A:	Angela, Estrella, Sonatine, Sonato
Group B:	Angela, Estrella, Sonatine, Sonato, Vemone
Group C:	Angela, Estrella, Sonatine
Group D:	Estrella, Sonatine, Vemone
Group E:	Sonatine

Proposed new wording:

Ad 50: Resistance to *Fulvia fulva* (Ff) (ex *Cladosporium fulvum)*

1. Pathogen	*Fulvia fulva* (ex *Cladosporium fulvum)*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum.....	Naktuinbouw[9] (NL) or GEVES[10] (FR)
5. Isolate	Race group 0, A, B, C, D, and E
6. Establishment isolate identity	with genetically defined differentials from GEVES (FR) A breaks Cf-2, B Cf-4, C Cf-2&4, D Cf-5, E Cf-2&4&5
7. Establishment pathogenicity.....	symptoms on susceptible tomato
8. Multiplication inoculum	
8.1 Multiplication medium	Potato Dextrose Agar or Malt Agar or a synthetic medium
8.8 Shelf life/viability inoculum.....	4 hours, keep cool
9. Format of the test.....	
9.1 Number of plants per genotype	more than 20
9.2 Number of replicates……	Not applicable
9.3 Control varieties.....	
Susceptible:	Monalbo, Moneymaker
Resistant for race 0:.....	Angela, Estrella, Sonatine, Sonato, Vemone, Vagabond, IVT 1149, Vagabond × IVT 1149, IVT 1154
Resistant for race group A:	Angela, Estrella, Sonatine, Sonato
Resistant for race group B:	Angela, Estrella, Sonatine, Sonato, Vemone
Resistant for race group C:	Angela, Estrella, Sonatine
Resistant for race group D:	Estrella, Sonatine, Vemone
Resistant for race group E:	Sonatine, Jadviga, Rhianna, IVT 1154
9.5 Test facility.....	glasshouse or climate room
9.6 Temperature	day: 22° C, night: 20° or day: 25°C, night 20°C
9.7 Light	12 hours or longer
9.9 Special measures	depending on facility and weather, there may be a need to raise the humidity e.g. humidity tent closed 3-4 days after inoculation and after this, 66% until 80% closed during day, until end
10. Inoculation	
10.1 Preparation inoculum.....	prepare evenly colonized plates, e.g. 1 for 36 plants; remove spores from plate by scraping with water with Tween20; filter through double muslin cloth
10.2 Quantification inoculum	count spores; adjust to 10^5 spores per ml or more
10.3 Plant stage at inoculation	19-20 d (incl. 12 d at 24°), 2-3 leaves
10.4 Inoculation method	spray on dry leaves
10.7 Final observations	14 days after inoculation
11. Observations.....	
11.1 Method.....	visual inspection of abaxial side of inoculated leaves
11.2 Observation scale.....	Symptom: velvety, white spots
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] symptoms
present.....	[9] no symptoms
	Excessively high humidity may cause rugged brown spot on all leaves. These are not to be considered as off-types.

13. Critical control points:
Ff spores have a variable size and morphology. Small spores are also viable.
Fungal plates will gradually become sterile after 6-10 weeks. Store good culture at -80°C.
For practical purposes, it is not possible to keep plants longer than 14 days inside a tent.

[9] Naktuinbouw: resistentie@naktuinbouw.nl

[10] GEVES; Valerie.GRIMAULT@geves.fr

Current wording:

Ad. 51.1 – 51.3: Resistance to Tomato Mosaic Tobamovirus (ToMV)- Strains 0, 1 and 2

Method

Maintenance of strains

Strains are long term stored as desiccated leaves below 10°C.
Race 0 represented by isolate INRA Avignon 6-5-1-1 (aucuba mosaic strain) is used.
Virus should be multiplied on the susceptible control before being used for inoculation of the test.

Execution of test

Growth stage of plants

Plants are grown in greenhouse or growth chamber until cotyledons (first leaves emerging) to two expanded leaves have appeared.

Within each test at least one resistant and one susceptible standard variety is included.

The following varieties are used as controls. Each line will be represented by at least one resistance phenotype which can be chosen from the varieties indicated; the resistance phenotype to the 3 pathotypes of ToMV is indicated. Mobaci and Moperou will allow checking the pathotype identity of the virus. Monalbo x Momor will help the interpretation of the distinct resistance phenotype with necrosis.

Variety	Resistance phenotype ToMV:0	ToMV:1	ToMV:2
Marmande, Monalbo	S	S	S
Mobaci	R	S	R
Moperou	R	R	S
Monalbo x Momor	RN	RN	RN
Momor, Gourmet	R	R	R

R = resistance present; no symptoms
RN = resistance present; a variable proportion of plants showing some or extensive necrosis; all other plants have no symptoms.
S = resistance absent; mosaic symptoms

Temperature:

Test performed in climatic chambers or greenhouse at 24 to 26°C. At higher temperatures, resistance can break down.

Inoculum and method of inoculation

Mechanical inoculation by rubbing cotyledons (first leaves emerging) or two expanded leaves with an inoculum solution consisting of symptomatic leaves grinded in a buffer with carborundum added. Leaves can be rinsed after inoculation. Light is important for symptom expression.

Duration of test
24 to 42 days from sowing to notation.

Number of plants tested:
At least 20 plants.

Notation:
12-21 days after inoculation when symptoms are well developed on susceptible control.

Notation scale and interpretation results:

R: without symptoms or with necrosis (necrosis can be observed on plants heterozygous for resistance gene, these plants are noted resistant)
S: mosaic symptoms.

Proposed new wording:

Ad 51: Resistance to Tomato mosaic virus (ToMV)

1. Pathogen	Tomato mosaic virus
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw[11] (NL) or GEVES[12] (FR)
5. Isolate	Strain 0 (e.g. isolate INRA Avignon 6-5-1-1) 1 and 2
6. Establishment isolate identity	genetically defined tomato standards Mobaci (Tm1), Moperou (Tm2), Momor ($Tm2^2$)
7. Establishment pathogenicity	on susceptible plant
8. Multiplication inoculum	
8.1 Multiplication medium	living plant
8.2 Multiplication variety	e.g. Moneymaker, Marmande
8.7 Check of harvested inoculum	option: on *Nicotiana tabacum* "Xanthi", check lesions after 2 days
8.8 Shelf life/viability inoculum	fresh>1 day, desiccated>1year
9. Format of the test	
9.1 Number of plants per genotype	at least 20
9.2 Number of replicates…… ...	Not applicable
9.3 Control varieties	
Susceptible	Marmande, Monalbo
Resistant for ToMV: 0 and 2	Mobaci
Resistant for ToMV: 0 and 1	Moperou
Resistant with necrosis	"Monalbo x Momor"
Resistant	Gourmet
9.4 Test design	blank treatment with PBS and carborundum or similar buffer
9.5 Test facility	Glasshouse or climate room
9.6 Temperature	24 to 26°C
9.7 Light	12 hours or longer
9.8 Season	symptoms are more pronounced in summer
10. Inoculation	
10.1 Preparation inoculum	1 g leaf with symptoms with 10 ml PBS or similar buffer homogenize, add carborundum to buffer (1 g/30ml)
10.3 Plant stage at inoculation	cotyledons or 2 leaves
10.4 Inoculation method	gentle rubbing
10.7 Final observations	11-21 days after inoculation
11. Observations	
11.1 Method	visual
11.2 Observation scale	Symptoms of susceptibility: mosaic in top, leaf malformation Symptoms of resistance (based on hypersensitivity): local necrosis, top necrosis, systemic necrosis
11.3 Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls

Remark: in some heterozygous varieties a variable proportion of plants may have severe systemic necrosis or some necrotic spots while the other plants have no symptoms. This proportion may vary between experiments

12. Interpretation of data in terms of UPOV characteristic states

absent	[1]	symptoms of susceptibility
present	[9]	no symptoms, or symptoms of hypersensitive resistance

13. Critical control points:

Temperature and light may influence the development of necrosis. More light means more necrosis. At temperatures above 26°C the resistance may break down.

Resistant heterozygous varieties may have symptomless plants and plants with severe necrosis; in spite of apparent segregation the sample may be evaluated as uniform for resistance

Note: Strain INRA Avignon 6-5-1-1 is recommended for ToMV: 0. This strain causes a striking yellow Aucuba mosaic.

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[12] GEVES: Valerie.GRIMAULT@geves.fr

Current wording:

Ad. 52: Resistance to *Phytophthora infestans* (Pi)

Method

Maintenance of race

Type of medium:	on agar medium
Special conditions:	18° C

Execution of test

Growth stage of plants:	10 leaves developed
Temperature:	18° C
Light:	after inoculation darkness during 24 hours, thereafter 10 hours darkness per day
Growing method:	climatic room or glasshouse
Method of inoculation:	spraying of spore suspension, isolate harvested freshly from leaves

Duration of test

- from sowing to inoculation:	6 to 7 weeks
- from inoculation to reading:	7 to 8 days
Hygrometry:	very high during the first four days after inoculation (cover plants with polyethylene cover)
Remarks:	heterozygotes may show a lower level of expression of resistance

Standard varieties:

- susceptible:	Saint Pierre, Heinz 1706
- resistant:	Pieraline, Heline, Pyros, F1 “Pieraline x Pieralbo”

Proposed new wording:

Ad 52: Resistance to *Phytophthora infestans* (Pi)

1. Pathogen	*Phytophthora infestans*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum.....-	
5. Isolate	highly pathogenic on tomato
6. Establishment isolate identity	biotest
7. Establishment pathogenicity.....	biotest
8. Multiplication inoculum	
8.1 Multiplication medium	V8 Agar or PDA or Malt Agar medium
8.2 Multiplication variety	susceptible tomato variety
8.3 Plant stage at inoculation	4 weeks
8.4 Inoculation medium.....	water
8.5 Inoculation method	spraying
8.6 Harvest of inoculum.....	wash spores from wetted plates
8.7 Check of harvested inoculum	count sporangiospores
8.8 Shelf life/viability inoculum.....	4 h after chilling at 8-10°C
9. Format of the test.....	
9.1 Number of plants per genotype	20
9.2 Number of replicates……	Not applicable
9.3 Control varieties……	
Susceptible	Saint Pierre, Heinz 1706
Resistant.....	Pieraline, Heline, Pyros, “Pieraline x Pieralbo”, Fline

Remark: heterozygote varieties may have a slightly lower level of expression of resistance.

9.5 Test facility.....	glasshouse
9.6 Temperature	18°C
9.7 Light	after inoculation darkness during 24 hours, thereafter 10 hour darkness per 24 hours
9.9 Special measures	humidity tent during four days after inoculation
10. Inoculation	
10.1 Preparation inoculum.....	wash spores from sporulating leaves, chill at 8-10°C Chilling will induce zoospore release
Remark	Use fresh spores from repeated infection cycles on tomato plants during 3 weeks before inoculation
10.2 Quantification inoculum	count sporangiospores; adjust to 104 spores per ml
10.3 Plant stage at inoculation	10 leaves developed (6 to 7 weeks)
10.4 Inoculation method	spraying
10.7 Final observations	5-7 days after inoculation
11. Observations.....	
11.1 Method.....	visual
11.2 Observation scale.....	Symptoms: water-soaked lesions, yellowing, and death
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] severe symptoms
present.....	[9] no or mild symptoms

13. Critical control points:
Resistance is only well-expressed in the adult plant.

Current wording:

Ad. 53: Resistance to *Pyrenochaeta lycopersici* (Pl)

Method

Maintenance of race: method 1: on roots obtained from plants grown in the greenhouse on naturally contaminated soil (or with enforced natural contamination);

method 2: inoculum grown on sand or mould, mixed with oat-meal and sterilized in the autoclave (artificial infection)

Execution of test:

Growth stage of plants: method 1: on adult plants around fruit maturity
method 2: 4 to 6 weeks after sowing (first flowering inflorescence)

Temperature: day: 24° C; night: 14° C

Light: 12 hours minimum

Growing method and Method of inoculation:

method 1: plants are planted in contaminated soil mixed with cut contaminated roots
method 2: plants are sown in steam-disinfected sandy mould mixed with inoculum

Duration of test

- from sowing to inoculation: method 1: 6 weeks
  method 2: when sowing
- from inoculation to reading: method 1: 3 to 4 months
  method 2: 4 to 6 weeks

Number of plants tested: 10 as a minimum

Remarks: method 1: is more efficient to clearly separate susceptible from resistant plants
method 2: pathogenicity of the strains has to be tested before inoculation on roots of young plants

Standard varieties: susceptible: Montfavet H 63.5
resistant: Kyndia, Moboglan, Pyrella

Proposed new wording:

Ad 53: Resistance to *Pyrenochaeta lycopersici* (Pl)

1. Pathogen	*Pyrenochaeta lycopersici*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	-
5. Isolate	-
7. Establishment pathogenicity	biotest
8. Multiplication inoculum	
8.1 Multiplication medium	V8 Agar
8.2 Multiplication variety	susceptible tomato variety
8.3 Plant stage at inoculation	seed
8.4 Inoculation medium	mixture of soil, e.g. (70%), sand (20%) and inoculum (10.1) (10%) or soil mixed with diseased roots cut to small pieces
8.5 Inoculation method	sowing, or transplanting at fruit maturity
8.6 Harvest of inoculum	diseased roots are harvested after 2-4 months
8.7 Check of harvested inoculum	visual inspection of lesions on roots
8.8 Shelf-life/viability inoculum	the fungus will not die quickly, but may lose its pathogenicity within a week after isolation on an agar medium
9. Format of the test	
9.1 Number of plants per genotype	20
9.2 Number of replicates	Not applicable
9.3 Control varieties	
susceptible:	Montfavet H 63.5
resistant:	Kyndia, Moboglan, Pyrella
9.5 Test facility	greenhouse or climate cell
9.6 Temperature	day 24°C, night 14°C
9.7 Light	12 h minimum
10. Inoculation	
10.1 Preparation inoculum	e.g. double-autoclaved mixture of soil with 10% oatmeal added e.g. Incubate for 10-14 d at 20°C with occasional, repeated turning
10.3 Plant stage at inoculation	6 weeks
10.4 Inoculation method	transplanting into mixture of soil, sand and inoculum (8.4) or soil mixed with diseased roots cut to small pieces or naturally infected soil
10.7 Final observations	6-8 weeks after transplanting (flowering plant)
11. Observations	
11.1 Method	visual
11.2 Observation scale	Symptoms: brown lesions on roots
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]	symptoms
present	[9]	no symptoms

13. Critical control points:

The fungus loses its pathogenicity quickly after isolation on an agar medium. It is advisable to keep the isolate alive on living plants.

Current wording:

Ad. 54: Resistance to *Stemphylium*

Method

Maintenance of isolate

Type of medium:	on PDA or synthetic medium
Special conditions:	fridge 4° C without light

Execution of test

Growth stage of plants:	three leaves expanded
Temperature:	constant, day: 24° C, night: 24° C
Light:	12 hours
Growing method:	glasshouse or climate room
Method of inoculation:	pulverisation on leaves

Duration of test

- from sowing to inoculation:	20 to 22 days
- from inoculation to reading:	10 days
Number of plants tested:	30 plants
Remarks:	production of inoculum on medium V8 under light
Standard varieties:	susceptible: Monalbo resistant: Motelle, F1 Motelle x Monalbo

Proposed new wording:

Ad 54: Resistance to *Stemphylium* spp. (Ss)

1. Pathogen	*Stemphylium* spp. e.g. *Stemphylium solani*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	GEVES [13] (FR)
5. Isolate .....-	
7. Establishment pathogenicity	biotest
8. Multiplication inoculum	
8.1 Multiplication medium	PDA (12 hours per day under near-ultraviolet light to induce sporulation) or V8
9. Format of the test.....	
9.1 Number of plants per genotype	20 at least
9.2 Number of replicates……	Not applicable
9.3 Control varieties.....	
Susceptible:	Monalbo
Resistant:	Motelle, F1 Motelle x Monalbo
9.5 Test facility.....	greenhouse or climate cell
9.6 Temperature	24°C
9.7 Light	12 hours minimum
9.9 Special measures	incubation in tunnel with 100 % relative humidity or humidity tent closed 5 days after inoculation, after this, 80% until end
10. Inoculation	
10.1 Preparation inoculum.....	sporulating plates (8.1) are scraped and air-dried overnight The next day plates are soaked and strirred for 30 min in a beaker with demineralized water, or sporulating plates are scraped with water with Tween The spore suspension is sieved through a double layer of muslin.
10.2 Quantification inoculum	5.10^3 – 10^5 spores per ml
10.3 Plant stage at inoculation	20-22 days (three expanded leaves)
10.4 Inoculation method	praying
10.7 Final observations	4 -10 days after inoculation
11. Observations.....	
11.1 Method.....	visual
11.2 Observation scale.....	Symptoms: necrotic lesions on cotyledons and leaves; yellowing of leaves
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] symptoms (11.2)
present	[9] no symptoms, or less than resistant standard
13. Critical control points:	8.1 and 10.1

Note: Some isolates of *Stemphylium* cannot be classified easily as either *Stemphylium solani* or a related species. These *Stemphylium* isolates may still be useful for identifying resistance to *Stemphylium solani*.

[13] GEVES : Valerie.GRIMAULT@geves.fr

Current wording:

Ad. 55: Resistance to *Pseudomonas syringae* pv. tomato (Pst)

Method

Maintenance of races

Type of medium:	on King B medium
Special conditions:	20 - 22° C in the dark, transplantation every 10 days

Execution of test

Growth stage of plants:	three leaves expanded
Temperature:	day: 22° C, night: 16° C
Light:	12 hours
Growing method:	climatic room in summer, glasshouse in winter
Method of inoculation:	pulverisation on leaves

Duration of test

- from sowing to inoculation:	20 to 22 days
- from inoculation to reading:	8 days
Number of plants tested:	30 plants
Remarks:	races to be renewed each year
Standard varieties:	susceptible: Monalbo resistant: Ontario 7710, F1 Monalbo x Ontario 7710

Proposed new wording:

Ad 55: Resistance to *Pseudomonas syringae* pv. *tomato* (Pst)

1. Pathogen	*Pseudomonas syringae* pv. *tomato*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum.....	GEVES[14] (FR) or Naktuinbouw[15] (NL)
5. Isolate	
6. Establishment isolate identity	
7. Establishment pathogenicity.....	biotest
8. Multiplication inoculum	
8.1 Multiplication medium	King's B agar medium, darkness
8.2 Multiplication variety	Susceptible variety
8.4 Inoculation medium.....	water
8.8 Shelf life/viability inoculum.....	plates become old after 10 days
9. Format of the test.....	
9.1 Number of plants per genotype	20 at least
9.2 Number of replicates……	Not applicable
9.3 Control varieties.....	
Susceptible:	Monalbo
Resistant:	Ontario 7710, "Monalbo x Ontario 7710", Tradiro, Hypeel 45
9.5 Test facility.....	greenhouse or growth chamber
9.6 Temperature	day: 22° C, night: 16° C or 20°C
9.7 Light	12 hours
9.9 Special measures	humidity tent needed for 3 days or longer
10. Inoculation	
10.1 Preparation inoculum.....	wash off spores from plate. Plate should be less than 2-4 days old.
10.2 Quantification inoculum	dilution plating, density 10^6 colony forming units per ml
10.3 Plant stage at inoculation	three leaves expanded (20-22 days)
10.4 Inoculation method	spraying a bacterial suspension on leaves
10.7 Final observations	8 days after inoculation or longer
11. Observations.....	
11.1 Method.....	visual
11.2 Observation scale.....	bacterial speck, greasy in appearance with marginal chlorosis pinpoint lesions < 1.0 mm
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] bacterial speck
Present	[9] no symptoms or pinpoint lesions

13. Critical control points: ..... Strains may lose virulence in storage

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[15] Naktuinbouw; resistentie@naktuinbouw.nl

Current wording:

Ad. 56: Resistance to *Ralstonia solanacearum* (Rs) – Race 1

Method

Maintenance of race :	Two races may affect Tomato: race 1 (active between 25-30° C) and race 3 (active between 20-23° C)
Type of medium:	Freezing at -80° C; culture in PYDAC immersed in oil; suspension in sterile distilled water
Special conditions:	conservation at 15° C in sterile distilled water

Execution of test

Growth stage of plants:	three to four well-developed leaves
Temp. (in climatic chamber):	day: 26-30° C; night: 25° C
Light:	10 - 12 hours
Growing method: 2 possibilities:	- in climatic chamber: rapid test - in the field: long test (applicable in tropical climate only)
Method of inoculation:	deposit of at least 2 ml of inoculum, adjusted to 10^7 colonies per ml, at the foot of each plantlet prior to planting

Duration of test

- from sowing to inoculation:	3 to 4 weeks
- from inoculation to reading:	- 3 weeks for the fast test - 2 months for the long test
Number of plants tested:	minimum of 30
Remarks:	maintain high humidity
Standard varieties:	- susceptible: Floradel - resistant: Caraïbo

Proposed new wording:

Ad 56: Resistance to *Ralstonia solanacearum*, race 1 (Rs)

1. Pathogen	*Ralstonia solanacearum* (ex *Pseudomonas solanacearum)*
2. Quarantine status	yes
3. Host species	*Solanum lycopersicum*
4. Source of inoculum.....	
5. Isolate	Race 1 has a wide host range, including tomato. Race 3 has a narrow host range, also including tomato
8. Multiplication inoculum	
8.1 Multiplication medium	Yeast Peptone Glucose (YPG) Agar or PYDAC
Special conditions:.....	25-30°C (Race 3 usually needs 20-23°C)
8.5 Inoculation method	2 ml of inoculum placed at the foot of each plantlet prior to transplanting
8.8 Shelf life/viability inoculum.....	suspension in sterile distilled water at 15°C (<1 year)
9. Format of the test.....	
9.1 Number of plants per genotype	20
9.2 Number of replicates……	Not applicable
9.3 Control varieties.....	
Susceptible:	Floradel
Resistant:	Caraibo
9.5 Test facility.....	climate room
9.6 Temperature	day: 26-30° C; night: 25° C
9.7 Light.....	10 - 12 hours
9.9 Special measures	high humidity
10. Inoculation	
10.2 Quantification inoculum	density 10^7 colony forming units per ml
10.3 Plant stage at inoculation	three to four well-developed leaves (3 weeks)
10.4 Inoculation method	
10.7 Final observations	3 weeks after inoculation
11. Observations.....	In intermediate resistance varieties, bacteria could be present in the lower part of the plant
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]	symptoms
present.....	[9]	no symptoms, or less than resistant standard

13. Critical control points:

Ralstonia solanacearum has a quarantine status in some countries and is on the EPPO alert list.

Current wording:

Ad. 57: Resistance to Tomato Yellow Leaf Curl Begomovirus (TYLCV)

Method

Execution of test:	Plants are tested under field crop conditions respecting a period of planting and a place where the disease has been proven to exist. 100% contaminated plants are grown of susceptible local varieties to ensure natural transmission by *Bemisia* insect and repeatability of the results
Growth stage of plants:	on adult plants of field crop outside
Method of inoculation:	natural inoculation by *Bemisia*

Duration of test

- from sowing to inoculation:	6 weeks minimum
- from inoculation to reading:	2.5 months maximum
Number of plants tested:	20 plants minimum
Remarks:	
Standard varieties:	- susceptible: local varieties - resistant: TY 20 or accessions from *L. pimpinellifolium* and from *L. peruvianum*

Proposed new wording:

Ad 57: Resistance to Tomato yellow leaf curl virus (TYLCV)

1. Pathogen	Tomato yellow leaf curl virus
2. Quarantine status	yes
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	-
5. Isolate	-
8. Multiplication inoculum	
8.6 Harvest of inoculum	symptomatic leaves may be stored at -70°C
9. Format of the test.....	
9.1 Number of plants per genotype	20
9.2 Number of replicates…….	Not applicable
9.3 Control varieties.....	
Susceptible:	Montfavet H 63.5
Resistant:	TY 20, Anastasia, Mohawk
9.5 Test facility.....	field with natural disease pressure
9.9 Special measures	prevent spread of white-flies
10. Inoculation	
10.3 Plant stage at inoculation	6-12 weeks (adult plants)
10.4 Inoculation method	vector (Bemisia white-flies carrying TYLCV)
10.7 Final observations	1-2 months after inoculation
11. Observations.....	
11.1 Method.....	visual
11.2 Observation scale.....	Symptoms: leaf yellowing and curling
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]	severe symptoms
present.....	[9]	no or mild symptoms

13. Critical control points:

TYLCV is endemic in many tropical and subtropical areas and has a quarantine status in many countries with a temperate climate. TYLCV is on the EPPO alert list. Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV).

Current wording:

Ad. 58: Resistance to Tomato Spotted Wilt Tospovirus (TSWV) - Race 0

Method

Maintenance of races

Type of medium:	on tomato plants or frozen at –70° C
Special conditions:	

Execution of test

Growth stage of plants:	one or two leaves expanded
Temperature:	day: 20° C, night: 20° C
Light:	extra light in winter
Growing method:	glasshouse
Method of inoculation:	mechanical, rubbing with carborundum on cotyledons, inoculum suspension < 10° C

Duration of test

- from sowing to inoculation:	20 days
- from inoculation to reading:	14 to 20 days
Number of plants tested:	15 to 30 plants
Remarks:	be aware of thrips
Standard varieties:	- susceptible: Monalbo - resistant: Tsunami, Bodar, Lisboa

Proposed new wording:

Ad 58: Resistance to Tomato spotted wilt virus (TSWV)

1. Pathogen	Tomato spotted wilt virus
2. Quarantine status	yes (see note below)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum.....	Naktuinbouw [16] (NL), GEVES (FR)
5. Isolate	race 0, preferably a thrips-transmission deficient variant
7. Establishment pathogenicity.....	biotest
8. Multiplication inoculum	
6 Harvest of inoculum.....	symptomatic leaves may be stored at -70°C
9. Format of the test.....	
9.1 Number of plants per genotype	20
9.2 Number of replicates……	Not applicable
9.3 Control varieties	
Susceptible:	Monalbo, Momor, Montfavet H 63.5
Resistant:	Tsunami, Bodar, Mospomor, Lisboa
9.5 Test facility.....	glasshouse or climatic chamber
9.6 Temperature	20°C
9.7 Light.....	12 hours or longer
9.9 Special measures	prevent or combat thrips
10. Inoculation	
10.1 Preparation inoculum.....	press symptomatic leaves in ice-cold buffer 0,01 M PBS, pH 7.4, with 0,01 M sodium sulfite or similar buffer Option: sieve the leaf sap through double muslin
10.3 Plant stage at inoculation	one or two expanded leaves
10.4 Inoculation method	mechanical, rubbing with carborundum on cotyledons, inoculum suspension < 10° C
10.7 Final observations	7-21 days after inoculation
11. Observations.....	
11.1 Method.....	visual
11.2 Observation scale.....	Symptoms: top mosaic, bronzing, various malformations, 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]	symptoms
present.....[9]	no symptoms

13. Critical control points:

TSWV has a quarantine status in some countries. TSWV is transmitted by *Thrips tabac*i and Western flower thrips (*Frankliniella occidentalis*). Pathotype 0 is defined by its inability to break resistance in tomato varieties carrying the resistance gene Sw-5.

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Current wording:

Ad. 59: Resistance to *Leveillula taurica* (Lt)

Method

Maintenance of races

Type of medium:	tomato plants
Special conditions:	

Execution of test

Growth stage of plants:	on adult plants of field crop outside
Method of inoculation:	natural infection

Duration of test

- from sowing to inoculation:	infection possible from planting stage to full grown plants
- from inoculation to reading:	before harvest
Number of plants tested:	20 plants
Remarks:	Yellow chlorotic spots on upper side of leaves, mycelium on lower side of leaves. Check cleistothecia under microscope if it really concerns *Leveillula* and not another powdery mildew.
Standard varieties:	- susceptible: Monalbo - resistant: Atlanta

Proposed new wording:

Ad 59: Resistance to *Leveillula taurica* (Lt)

1. Pathogen		*Leveillula taurica*
3. Host species		*Solanum lycopersicum*
4. Source of inoculum		no long term storage method is available
5. Isolate		
8.1 Multiplication medium		detached leaves of a susceptible host plant
9. Format of the test.....		
9.1 Number of plants per genotype		20
9.2 Number of replicates……		Not applicable
9.3 Control varieties.....		
Susceptible:	..	Monalbo , Montfavet H 63.5
Resistant:	..	Atlanta
10. Inoculation		
10.3 Plant stage at inoculation		adult plants
10.4 Inoculation method		natural infection, mainly by wind dispersal of spores
10.7 Final observations		before harvest
11. Observations.....		
11.1 Method.....		visual
11.2 Observation scale.....		Symptoms: Yellow chlorotic spots on upper side of leaves, mycelium on abaxial side of leaves
:		
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]	symptoms
present.....	[9]	no symptoms, or less than resistant standard

13. Critical control points:
Check cleistothecia under microscope to confirm presence of *Leveillula* and not another powdery mildew.

Current wording:

Ad. 60: Resistance to *Oidium neolycopersici* (On) (ex *Oidium lycopersicum* (Ol))

Method

Maintenance of races

Type of medium:	on tomato plants
Special conditions:	climatic room

Execution of test

Growth stage of plants:	3 weeks
Temperature:	24°C during the day; 18°C during the night
Light:	12 hours
Method of innoculation:	- by spraying (10^4 conidia/ml) on leaves - by dredging (uncontrolled inoculum) on leaves

Execution of test

Duration of test

- from sowing to inoculation:	18 - 20 days
- from inoculation to reading:	15 – 18 days
Number of plants tested:	30 plants/lot
Remarks:	

Scale of notes:	- no sporulation	}
	- sporulation without extension (necrotic points)	}Resistant }
	- moderate sporulation	}
	- abundant sporulation	}Susceptible

Standard varieties:	- susceptible: Momor (L. *esculentum*) - resistant: *L. hirsutum* PI-247087 (accession), Romiror

Proposed new wording:

Ad 60: Resistance to *Oidium neolycopersici* (On)

1. Pathogen		*Oidium neolycopersici* (Powdery mildew)
3. Host species		*Solanum lycopersicum*
4. Source of inoculum		
5. Isolate see remark under 13		
7. Establishment pathogenicity		biotest
8. Multiplication inoculum		
8.1 Multiplication medium		plant
8.3 Plant stage at inoculation		3 weeks
8.4 Inoculation medium		water
8.5 Inoculation method		see 10.4
8.6 Harvest of inoculum		by washing off
8.7 Check of harvested inoculum		check for contaminants under microscope
8.8 Shelf-life/viability inoculum		1-2 hours
9. Format of the test		
9.1 Number of plants per genotype		20
9.2 Number of replicates……		Not applicable
9.3 Control varieties		
Susceptible:		Momor, Montfavet H 63.5
Resistant tomato:		Atlanta, Romiro, PI-247087
9.5 Test facility		glasshouse
9.6 Temperature		20°C or 18/24°C
9.7 Light		12 hours
10. Inoculation		
10.1 Preparation inoculum		collect spores in water
10.2 Quantification inoculum	..	10^4 conidia/ml
10.3 Plant stage at inoculation		3 weeks
10.4 Inoculation method		by spraying on leaves or dredging of leaves
10.7 Final observations		7-18 days after inoculation
11. Observations		
11.1 Method		visual
11.2 Observation scale		0. no sporulation 1. necrotic points and sometimes locally restricted sporulation 2. moderate sporulation 3. abundant sporulation
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]	Moderate or abundant sporulation
present	[9]	No or restricted sporulation

13. Critical control points:

Resistance-breaking isolates should be avoided. Resistance to O. neolycopersici is usually race-specific. However, as long as a differential series of tomato genotypes with well defined resistances is lacking, it will remain hard to conclude that different races of O. neolycopersici exist.

Current wording:

Ad. 61: Resistance to Tomato Torrado Virus (ToTV)

Method

Maintenance of races

Type of medium:	plant material with symptoms, stored at –80° C
Multiplication:	on N. tabacum 'Xanthi' 3 weeks before start of experiment
Special conditions:	use Quarantine procedures
Remarks:	white fly may be a vector of ToTV

Execution of test

Growth stage of plants:	inoculate when cotyledons are fully grown, re-inoculate 7 days later on first true leaves one or two leaves
Temperature:	day: 23° C, night: 21° C; avoid temperature above 25°C
Light:	extra light in winter, 16 h day, 8 h night
Growing method:	Quarantine facilities; glasshouse
Method of inoculation:	with ice-cold 0,01 M PBS pH 7 and carborundum

Duration of test

- from sowing to inoculation:	14 days
- from inoculation to reading:	14-21 days
Number of plants tested:	20 to 30 plants
Remarks:	Necrotic spots on the top leaves of susceptible plants
Standard varieties:	Resistant standard variety: Matias

Note: Patents pending on part of the method: WO2006/085749 and WO2008/150158 and equivalents. Use solely for DUS purposes and for the development of variety descriptions by UPOV and authorities of UPOV members, courtesy of De Ruiter Seeds R&D B.V./Monsanto Invest N.V.

Proposed new wording:

Ad 61: Resistance to Tomato torrado virus (ToTV)

1. Pathogen	Tomato Torrado Virus
2. Quarantine status	in regions with temperate climate
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	-
5. Isolate	-
7. Establishment pathogenicity	biotest
8. Multiplication inoculum	
8.1 Multiplication medium	Nicotiana tabacum 'Xanthi'
8.3 Plant stage at inoculation	cotyledon to first leaf
8.5 Inoculation method	see 10.4
8.6 Harvest of inoculum	after 3 weeks
8.7 Check of harvested inoculum	plants yellow, systemic infection
8.8 Shelf-life/viability inoculum	instable at room temperature
9. Format of the test.....	
9.1 Number of plants per genotype	20
9.2 Number of replicates……	Not applicable
9.3 Control varieties.....	
Susceptible:	Daniela
Resistant tomato:	Matias
9.5 Test facility.....	glasshouse
9.6 Temperature	23°C during the day; 21°C during the night
9.7 Light	16 hours
10. Inoculation	
10.3 Plant stage at inoculation	14 days
10.4 Inoculation method	with ice-cold 0,01 M PBS pH 7 and carborundum
10.5 First observation	7 days after inoculation
10.6 Second observation	14 days after inoculation
10.7 Final observations	18 days after inoculation
11. Observations.....	
11.1 Method	visual
11.2 Observation scale	necrotic spots on the top leaves
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]	necrotic spots present
present	[9]	No symptoms

13. Critical control points:
ToTV is transmitted by white fly (*Bemisia tabaci*). Produce inoculum with ice-cold mortar and pestle. During inoculation the temperature should be below 25°C

Note: Patents pending on part of the method: WO2006/085749 and WO2008/150158 and equivalents. Use solely for DUS purposes and for the development of variety descriptions by UPOV and authorities of UPOV members, courtesy to De Ruiter Seeds R&D B.V./Monsanto Invest N.V.

[Annex III follows]

ANNEX III

Proposal to Add the Following Literature References to Chapter 9: Literature

Arens P., Mansilla C., Deinum D., Cavellini L., Moretti A., Rolland S., van der Schoot H., Calvache D., Ponz F., Collonnier C., Mathis R., Smilde D., Caranta C,; Vosman B., 2010. Development and evaluation of robust molecular markers linked to disease resistance in tomato for distinctness, uniformity and stability testing. Theoretical and applied genetics. 120(3): 655-64

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http://www.worldseed.org/isf/pathogen_coding_3.html (International Seed Federation (ISF), Trade Issues, Phytosanitary Matters, Pathogen coding, Strain Denomination, Differential sets)

[End of Annex III and of document]