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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

TECHNICAL WORKING PARTY FOR VEGETABLES

Forty-Sixth Session

near the city of Venlo, Netherlands, June 11 to 15, 2012

PARTIAL REVISION OF THE TEST GUIDELINES FOR TOMATO

Document prepared by an expert from the Netherlands

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 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; and

(b) a gene-specific marker method for examination of resistance to Tomato Spotted Wilt topovirus (TSWV) - Race 0.

2. The Technical Committee (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 was 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 TC considered document TGP/12/2 Draft 2 "Guidance on Certain Physiological Characteristics" as follows (see document TC/48/22 "Report on Conclusions", paragraphs 67 to 69):

"68. The TC agreed to amend document TGP/12/2 Draft 2 to read as follows:

"2.3.2 Quantitative characteristics

"Disease resistances for which there is a continuous range of levels of susceptibility / resistance across varieties, are quantitative characteristics. Guidance for the development of appropriate states of expressions for quantitative characteristics is provided in document TGP/9, Guidance Note GN 20, section 3.

"Example with 1 – 3 scale: Resistance to Sphaerotheca fuliginea (Podosphaera xanthii) (Powdery mildew) in Melon (UPOV Test Guidelines: TG/104/5)

"[Table]

"Example with 1 – 9 scale: Resistance to Colletotrichum trifolii in Lucerne (UPOV Test Guidelines: TG/6/5)

"[Table]"

"69. The TC agreed, subject to agreement by the CAJ at its sixty-fifth session, to be held in Geneva on March 29, 2012, to submit document TGP/12/2 Draft 2 "Guidance on Certain Physiological Characteristics" as the basis for adoption of TGP/12 by the Council, at its forty-sixth session, to be held on November 1,

2012. The TC noted that the editing of the original English text and the French, German and Spanish translations would be checked by the relevant members of the Editorial Committee prior to submission of the draft of document TGP/12/2 to the Council."

4. A new proposal for a revised format of explanations of disease resistance characteristics in the Test Guidelines for Tomato is provided in Annex I to this document.

5. Annex II to this document indicates the changes made on the basis of document TGP/12/2 Draft 2 to the proposal agreed by the TWV at its forty-fifth session. Deletions are shown in strikethrough and highlighted. Additions are underlined and highlighted.

[Annexes follow}

ANNEX I

A New Proposal for a Revised Format of Explanations of Disease Resistance Characteristics in the Test Guidelines for Tomato

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] (F)	
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		
8.2 Multiplication variety	preferably resistant to powdery mildew	
8.3 Plant stage at inoculation	see 10.3	
8.1 Multiplication medium	living plant	
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 :	Madyta, “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	
11.4 Off-types	resistant varieties may have a few plants with a few galls	
12. Interpretation of data in terms of UPOV characteristic states		
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

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

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

1. Pathogen	*Verticillium dahliae* or *Verticillium albo-atrum* (see note below)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum.....	Naktuinbouw[3] (NL) and GEVES[4] (*F*)
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.

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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] (*F*)
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
12. Interpretation of data in terms of UPOV characteristic states

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

13. Critical control points

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Test results may vary slightly in inoculum pressure due to differences in isolate, spore concentration, soil humidity and temperature. Standards near borderline R/S will help to compare between labs.

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

1. Pathogen	*Fusarium oxysporum* f. sp. *radicis-lycopersici*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw[7] (NL) and GEVES[8] (*F*)
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

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Ad 50: Resistance to *Fulvia fulva* (Ff)

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
11.4 Off-types	excessively high humidity may cause rugged
.....	brown spots on all leaves

12. Interpretation of data in terms of UPOV characteristic states

absent	[1]	symptoms
present	[9]	no symptoms

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.

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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] (F)
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

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

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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 of resistant and susceptible controls	evaluation of variety resistance should be calibrated with results
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

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

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..... (10%)	mixture of soil, e.g. (70%), sand (20%) and inoculum (10.1)
.....	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.

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

1. Pathogen	*Stemphylium* spp. e.g. *Stemphylium solani* (see note below)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	GEVES (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	spraying
10.7 Final observations	4 -10days 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*.

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

1. Pathogen	*Pseudomonas syringae* pv. *tomato* (see note below)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	GEVES[13] (FR) or Naktuinbouw[14] (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

[13] GEVES; Valerie.GRIMAULT@geves.fr
[14] Naktuinbouw; resistentie@naktuinbouw.nl

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

1. Pathogen	*Ralstonia solanacearum* (ex *Pseudomonas solanacearum)*
2. Quarantine status	yes (see note below)
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

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

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

1. Pathogen	Tomato yellow leaf curl virus (see note below)
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).

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

1. Pathogen	Tomato spotted wilt virus (see note below)
2. Quarantine status	yes (see note below)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw [15] (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.

[15] Naktuinbouw: resistentie@naktuinbouw.nl

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
Remark:	.....	Check cleistothecia under microscope to confirm presence of
	.....	*Leveillula* and not another powdery mildew.
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

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		24°C during the day; 18°C during the night
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.

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.

Add to chapter 9; Literature:

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Bai, Y. 2004. The genetics and mechanisms of resistance to tomato powdery mildew (*Oidium neolycopersici*) in *Lycopersicon* species. Thesis Wageningen University, The Netherlands.

Barbieri, M., et al., 2010. Introgressions of resistance to two Mediterranean virus species causing tomato yellow leaf curl into a valuable traditional tomato variety. Journal of Plant Pathology 92(2):485-493

Garcia, S., et al., 2009. Resistance driven selection of begomoviruses associated with the TYLCV. Virus research 146: 66-72

Garland, S., Sharman, M., Persley, D. and McGrath, D. (2005) The development of an improved PCR-based marker system for Sw-5, an important TSWV resistance gene of tomato. Australian Journal of Agricultural Research, 56 (3): 285-289.

Gordillo, L.F. and M. R. Stevens (2008) Screening two Lycopersicon peruvianum collections for resistance to Tomato spotted wilt virus. Plant Disease 92(5): 694-704

Hubbeling, N., 1978. Breakdown of resistance to the Cf-5 gene in tomato by another new race of *Fulvia fulva*. Mededelingen van de Faculteit Landbouwwetenschappen Universiteit Gent 42/2

Martin, G. B., A. Frary, T. Wu, S. Brommonschenkel, J. Chunwongse, E. D. Earle, S. D. Tanksley (1994) A member of the tomato Pto family confers sensitivity to fenthion resulting in rapid cell death. The Plant Cell 6: 1543-1552

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

Technical Questionnaire:
In 7.3.1 correct the following disease names:
d) Tomato mosaic virus
h) *Pseudomonas syringae* pv. *tomato*
j) Tomato yellow leaf curl virus
k) Tomato spotted wilt virus
n) Tomato torrado virus

[Annex II follows]

ANNEX II

Changes Made on the Basis of Document TGP/12/2 Draft 2 to the Proposal Agreed by the TWV at its Forty Fifth Session

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

1. Pathogen	*Meloidogyne incognita*
3. Host species	~~*Lycopersicon esculentum*~~*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw (NL[1]) or GEVES[2] (F)
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	
8.2 Multiplication variety.....	preferably resistant to powdery mildew
8.3 Plant stage at inoculation	see 10.3
8.1 Multiplication medium	living plant
~~8.2 Multiplication variety~~.....	~~Delito (resistant to powdery mildew)~~
~~8.3 Plant stage at inoculation~~	~~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 :.....	Madyta, “Anahu x Monalbo”, Campeon, Madyta, Vinchy
~~Resistant:~~	~~Anabel, Anahu~~
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 ~~End of test~~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
.....	~~1-10 galls per root system may be counted~~
11.3 Validation of test.....	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls on standards
11.4 Off-types.....	resistant varieties may have a few plants with a few galls

12. Interpretation of data in terms of UPOV characteristic states

	~~[1]~~	~~severe symptoms~~
	~~[2]~~	~~mild or no symptoms~~
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

~~Literature references~~

~~Laterrot, H., 1973: Sélection de variétés de Tomate résistantes aux Meloidogyne, OEPP/EPPO Bulletin 3(1): 89-92.~~

[1] Naktuinbouw; resistentie@naktuinbouw.nl
[2] GEVES; Valerie.GRIMAULT@geves.fr

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

1. Pathogen	*Verticillium dahliae* ~~or *Verticillium albo-atrum*~~ (see note below)
3. Host species	~~*Lycopersicon esculentum*~~*Solanum lycopersicum*
4. Source of inoculum.....	Naktuinbouw[3] (NL) and GEVES[4] (*F*)
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~~, 20-25°C, in darkness~~
.....	
~~8.6 Harvest of inoculum~~.....	(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 ……	~~**Marmande,**~~ Flix, ~~**Planet**~~ Marmande verte, Clarion, Santonio, Anabel
~~**Weakly resistant**~~ Resistant ……..	Monalbo, Elias, Monalbo x Marmande verte, Daniela,
~~**Resistant**~~	~~**Monalbo, Elias**~~ Marmande VR
9.4 Test design ……..	~~22~~ 20 plants inoculated~~,~~ at least, 2 blanks at least
9.5 Test facility……	greenhouse or climate room
9.6 Temperature……	optimal 20-25°C ~~for germination~~, 20-22°C after inoculation
9.7 Light ……	~~16~~ 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.~~5 First observation~~	7 Final observations……~~14 d after inoculation~~
~~10.6 Second observation~~.....	~~21 d after inoculation~~
~~10.7 End of test~~……	~~21~~-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	~~compare~~ 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 ~~a relatively weak resistance~~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.

~~Literature references~~ ~~Denby, L. G., Wooliams, G. E., 1962: The Development of Verticillium Resistant Strains of Established Tomato Varieties, Canadian Journal Plant Science 42,681-685.~~

[3] Naktuinbouw; resistentie@naktuinbouw.nl
[4] GEVES; Valerie.GRIMAULT@geves.fr

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

1. Pathogen	*Fusarium oxysporum* f. sp. *lycopersici*
3. Host species	~~*Lycopersicon esculentum*~~*Solanum lycopersicum*
4. Source of inoculum.....	Naktuinbouw[5] (NL) and GEVES [6]
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
.....	~~Long term storage: 80°C in 20% glycerol~~
6. Establishment isolate identity	use differential varieties (see 9.3)
7. Establishment pathogenicity.....	on ~~suscpetible~~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)
~~8.5 Inoculation method .....~~	~~immersion of roots in spore suspension, 5-15 min~~
8.6 Harvest of inoculum……	~~7 d-old aerated culture~~ 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 ……..	~~at least 16~~ 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 ~~culture 7-10 days~~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
~~10.5 First observation.....~~	
10.7 Final observations ……	14 ~~days after inoculation~~
~~10.7 End of test~~	-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 ……..	~~on standards~~ 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]	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. Standards near borderline R/S ~~are essential~~will help to compare between labs.

~~Literature references~~

~~Laterrot, H., 1972: Sélection de tomates résistantes à Fusarium oxysporum f. sp. lycopersici, Phytopathologia Mediterranea, Volume XI, No. 3, p. 154-158.~~

[5] Naktuinbouw; resistentie@naktuinbouw.nl
[6] GEVES; Valerie.GRIMAULT@geves.fr

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

1. Pathogen	*Fusarium oxysporum* f. sp. *radicis-lycopersici*
3. Host species	~~*Lycopersicon esculentum*~~ *Solanum lycopersicum*
4. Source of inoculum.....	Naktuinbouw[7] (NL) and GEVES[8] (*F*)
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.....	~~Czapek-Dox culture~~ water for scraping agar plates or
~~8.5 Inoculation method .....~~	~~immersion of roots in spore suspension, 5-15 min~~
……	Czapek-Dox (7 d-old aerated culture)
8.6 Harvest of inoculum.....	~~7 d-old aerated culture~~
.....	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)
.....	~~20~~ 17-24°C (mild test, with severe isolate)
9.7 Light	at least ~~16~~ 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 ~~7-10 days~~ 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
~~10.5 First observation.....~~	~~14 days after inoculation~~
10.7 ~~End of test~~.....	~~14~~ 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

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Ad 50: Resistance to *Fulvia fulva* (Ff)

1. Pathogen	*Fulvia fulva* (ex *Cladosporium fulvum)*
3. Host species	~~*Lycopersicum esculentum*~~*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
~~9.4 Test design .....~~	~~2 plants per pot~~
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	~~more than~~ 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
.....	~~After~~ 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 ~~2-3 times~~ with ~~1 ml~~
.....	~~demi~~ water with ~~0,01%~~ Tween20;
.....	filter through double muslin cloth
10.2 Quantification inoculum.....	count spores; adjust to ~~5.~~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 ~~End of test~~ 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	~~on standard varieties~~evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
11.4 Off-types	excessively high humidity may cause rugged brown spots on all
.....	leaves

12. Interpretation of data in terms of UPOV characteristic states

absent	[1]	symptoms
present	[9]	no symptoms

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.

~~Literature references~~

~~Hubbeling, N., 1978. Breakdown of resistance to the Cf-5 gene in tomato by another new race of *Fulvia fulva*. Mededelingen van de Faculteit Landbouwwetenschappen Universiteit Gent 42/2~~

~~Laterrot, H., 1981. La lutte génétique contre la Cladosporiose de la Tomate en France, P.H.M. Revue Horticole, No. 214, February 1981.~~

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Ad 51: Resistance to Tomato mosaic virus (ToMV)

1. Pathogen	Tomato mosaic virus
3. Host species	~~*Lycopersicum esculentum*~~ *Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw [11](NL) or GEVES[12] (F)
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	~~25~~24 to 26°C ~~day, 23°C night~~
9.7 Light	~~16~~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 ~~PBS~~ buffer (1 g/30ml)
10.3 Plant stage at inoculation	cotyledons or 2 leaves
10.4 Inoculation method	gentle rubbing ~~with sponge wetted with inoculum~~
~~10.5 First observation~~	~~11 days after inoculation~~
10.7 ~~End of test~~	~~19~~ Final observations 11-21 days after inoculation
11. Observations	
11.1 Method	visual
11.2 Observation scale	Symptoms of susceptibility:
	Mosaic in top, ~~Leaf~~ 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

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

~~Literature references~~

~~Laterrot, H., 1973: Résistance de la Tomate au virus de la Mosaïque du Tabac. Difficultés rencontrées pour la sélection de variétés résistantes, Ann.Amelior.Plantes, 1973, 23(4), 287-313.~~

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

1. Pathogen	*Phytophthora infestans*
3. Host species	~~*Lycopersicon esculentum*~~ *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~~ Agar or PDA or Malt Agar medium
8.2 Multiplication variety	~~Moneymaker~~ 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
~~10.5 First observation	~~5 days after incoulation~~
~~10.6 Second observation.....	~~6 days after inoculation~~
10.7 ~~End of test~~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	~~on standards~~ 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

~~Literature references Laterrot, H., 1975: Sélection pour la résistance au Mildiou, Phytophthora infestans Mont. De Bary chez la Tomate, Ann.Amelior.Plantes, 1975, 25(2), 129-149.~~

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

1. Pathogen	*Pyrenochaeta lycopersici*
3. Host species	~~*Lycopersicon esculentum*~~ *Solanum lycopersicum*
4. Source of inoculum	-
5. Isolate	-
7. Establishment pathogenicity	biotest
8. Multiplication inoculum	
8.1 Multiplication medium	~~V8A~~ V8 Agar
8.2 Multiplication variety	susceptible tomato variety
8.3 Plant stage at inoculation	seed
8.4 Inoculation medium..... (10%)	mixture of soil, e.g. (70%), sand (20%) and inoculum (10.1)
.....	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 ~~loose~~ 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
..... turning	e.g. Incubate for 10-14 d at 20°C with occasional, repeated
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
~~10.5 First observation .....~~	~~6 weeks after transplanting~~
.....	or naturally infected soil
10.7 ~~End of test~~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	~~on standard varieties~~ 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 ~~looses~~ loses its pathogenicity quickly after isolation on an agar medium. It is advisable to keep the isolate alive on living plants.

~~Literature references~~

~~Laterrot, H., 1983: La lutte génétique contre la maladie des racines liégeuses de la Tomate, P.H.M. Revue Horticole, No. 238, June-July 1983.~~

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

1. Pathogen	*Stemphylium* spp. e.g. *Stemphylium solani* (see note below)
3. Host species	~~*Lycopersicon esculentum*~~ *Solanum lycopersicum*
4. Source of inoculum	GEVES (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~~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	spraying
~~10.5 First observation	~~4 days after inoculation~~
~~10.6 Second observation	~~5 days after inoculation~~
10.7 ~~End of test6~~ 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	~~on standard varieties~~ 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*.

~~Literature references~~

~~Laterrot, H. and Blancard, D., 1983: Criblage d'une série de lignées et d'hybrides F1 de Tomate pour la résistance à la Stemphyliose, Phytopath. medit. 1983, 22, 188-193.~~

~~Laterrot, H. and Blancard, D., 1986: Les Stemphylia rencontrés sur la Tomate, Phytopath. medit. 1986, 25, 140-144.~~

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

1. Pathogen	*Pseudomonas syringae* pv. *tomato* (see note below)
3. Host species	~~*Lycopersicon esculentum*~~ *Solanum lycopersicum*
4. Source of inoculum	GEVES[13] (FR) or Naktuinbouw[14] (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
~~8.3 Plant stage at inoculation~~	
8.4 Inoculation medium.....	water
~~8.5 Inoculation method~~	
~~8.6 Harvest of inoculum~~.....	
~~8.7 Check of harvested inoculum~~...	
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
~~9.4 Test design~~	
9.5 Test facility.....	greenhouse ~~in winter~~ or growth chamber ~~in summer~~
9.6 Temperature	day: 22° C, night: 16° C or 20°C
9.7 Light	12 hours
~~9.8 Season~~	
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 ~~that~~ 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 ~~End of test~~ 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	~~on standards~~ 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

~~Option for testing without using the pathogen~~

~~Resistance to *Pseudomonas syringae* pv. *tomato* is often based on the *Pto* resistance gene. The presence of the *Pto* resistance gene may be detected without a biotest by spraying 10 µl/ml of the fungicide fenthion to small plants or to detached leaves (Martin et al 1994). This test should be performed on a minimum of 20 plants.~~

~~Literature references~~

~~Martin, G. B., A. Frary, T. Wu, S. Brommonschenkel, J. Chunwongse, E. D. Earle, S. D. Tanksley (1994) A member of the tomato Pto family confers sensitivity to fenthion resulting in rapid cell death. The Plant Cell 6: 1543-1552~~

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

1. Pathogen	*Ralstonia solanacearum* (ex *Pseudomonas solanacearum)*
2. Quarantine status	yes (see note below)
3. Host species	~~*Lycopersicon esculentum*~~ *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 End of test~~	
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
~~11.1 Method~~	
~~11.2 Observation scale~~	
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

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

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

1. Pathogen	Tomato yellow leaf curl virus (see note below)
2. Quarantine status	yes
3. Host species	~~*Lycopersicon esculentum*~~ *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:	~~**local varieties**~~ 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 ~~End of test~~ 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	~~on standard varieties~~ 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. ~~Maintenance of TYLCV is only possible in living plants and *Bemisia* white flies. Transmission of TYLCV is only possible through *Bemisia* white flies. Mechanical transmission is not possible.~~ Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV).

~~Literature references~~

~~Barbieri, M., et al., 2010. Introgressions of resistance to two Mediterranean virus species causing tomato yellow leaf curl into a valuable traditional tomato variety. Journal of Plant Pathology 92(2):485-493~~

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

1. Pathogen	Tomato spotted wilt virus (see note below)
2. Quarantine status	yes (see note below)
3. Host species	~~*Lycopersicon esculentum*~~ *Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw [15](NL), GEVES (FR)[16]
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 ..... ~~16~~	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 < 10º C
10.~~5 First observation~~	~~7 days after inoculation~~
~~10.6 Second observation~~	~~14 days after inoculation~~
~~10.~~Final observations	7~~End of test -~~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	~~on standard varieties~~ 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. ~~TSWV resistance based on Sw-5 may be detected without using the pathogen.~~

~~**Note:** Option for testing without using the pathogen~~

~~Resistance to TSWV:0 is often based on the resistance gen Sw-5. The presence of the resistance gene *Sw-5* can be detected by molecular marker Sw-5b LRR (Garland et al., 2005). This molecular test is validated to be used instead of a pathotest, as foreseen in UPOV document TC/38/14 Add. – CAJ/45/5 Add. under Option 1(a). Each molecular marker should be applied to a minimum of twenty plants and validated with proper controls.~~

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

1. Pathogen		*Leveillula taurica*
3. Host species		~~*Lycopersicon esculentum*~~ *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		
~~10.1 Preparation inoculum .....~~		
~~10.2 Quantification inoculum .....~~		
10.3 Plant stage at inoculation		adult plants
10.4 Inoculation method		natural infection, mainly by wind dispersal of spores
10.7 ~~End of test~~ 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
Remark:		Check cleistothecia under microscope to confirm presence of
.....		*Leveillula* and not another powdery mildew.
11.3 Validation of test.....		~~on standards~~ 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

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

1. Pathogen		*Oidium neolycopersici* (Powdery mildew)
3. Host species	.....	~~*Lycopersicon esculentum*~~ *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	.....	24°C during the day; 18°C during the night
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
~~10.5 First observation~~	~~.....~~	~~7 days after inoculation~~
~~10.6 Second observation~~	~~.....~~	~~14 days after inoculation~~
10.7 Final observations	.....	7~~End of test~~ -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	.....	~~on standard varieties~~ 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.

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.

Add to chapter 9; Literature~~references~~:

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Technical Questionnaire:
In 7.3.1 correct the following disease names:
d) Tomato mosaic virus
h) *Pseudomonas syringae* pv. *tomato*
j) Tomato yellow leaf curl virus
k) Tomato spotted wilt virus
n) Tomato torrado virus

[End of Annex II and of document]