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PARTIAL REVISION OF THE TEST GUIDELINES FOR TOMATO ROOTSTOCKS

Document prepared by an expert from the Netherlands

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1. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Tomato Rootstocks (document TG/294/1 Corr. Rev. 2).

2. The Technical Working Party for Vegetables (TWV), at its fifty-first session, held in Roelofarendsveen, Netherlands, from July 3 to 7, 2017, considered a proposal for a partial revision of the Test Guidelines for Tomato Rootstocks (document TG/294/1 Corr. Rev.) on the basis of documents TG/294/1 Corr. Rev. and TWV/51/11 "Partial Revision of the Test Guidelines for Tomato Rootstocks" and proposed the following revisions to the Test Guidelines for Tomato Rootstocks (see document TWV/51/16 "Report", paragraph 115):

(a) To change the method of observation of Characteristics 24.1 and 24.2:
    (i) Characteristic 24.1 "Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) - Race 0 (ex 1)"
    (ii) Characteristic 24.2 "Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) - Race 1 (ex 2)"
(b) To change the explanation Ad. 24 by adding an alternative method to observe the resistance and by minor changes in the current method
(c) To change the method of observation of Characteristics 27.1, 27.2 and 27.3:
    (i) Characteristic 27.1 "Resistance to Tomato mosaic virus (ToMV) - Strain 0"
    (ii) Characteristic 27.2 "Resistance to Tomato mosaic virus (ToMV) - Strain 1"
    (iii) Characteristic 27.3 "Resistance to Tomato mosaic virus (ToMV) - Strain 2"
(d) To change the explanation Ad. 27 by adding an alternative method to observe the resistance and by minor typographic changes in the current method
(e) To change the explanation Ad. 30 "Resistance to Tomato yellow leaf curl virus (TYLCV)" by revision of the current methodology and by adding an alternative method to observe the resistance.
(f) To change the method of observation of Characteristic 31 "Resistance to Tomato spotted wilt virus (TSWV)"
(g) To change the explanation Ad. 31 by adding an alternative method to observe the resistance
(h) To add a reference to literature related to changes (a) – (h) to Chapter 9 "Literature".

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

Proposal to change the method of observation of Characteristics 24.1 and 24.2

Current wording

24. **(+)**		**Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**	**Résistance à *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**	**Resistenz gegen *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**	**Resistencia a *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**		
24.1 **(*)**	**VG**	**– Race 0 (ex 1)**	**– Pathotype 0 (ex 1)**	**– Pathotyp 0 (ex 1)**	**– Raza 0 (ex 1)**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente	Emperador	9
24.2 **(*)**	**VG**	**– Race 1 (ex 2)**	**– Pathotype 1 (ex 2)**	**– Pathotyp 1 (ex 2)**	**– Raza 1 (ex 2)**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente	Emperador	9
24.3 **(*)**	**VG**	**– Race 2 (ex 3)**	**– Pathotype 2 (ex 3)**	**– Pathotyp 2 (ex 3)**	**– Raza 2 (ex 3)**		
QL		absent	absente	fehlend	ausente	Emperador	1
		present	présente	vorhanden	presente	Colosus	9

Proposed new wording

24. **(+)**		**Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**	**Résistance à *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**	**Resistenz gegen *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**	**Resistencia a *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**		
24.1 **(*)**	**VG/ VS**	**– Race 0 (ex 1)**	**– Pathotype 0 (ex 1)**	**– Pathotyp 0 (ex 1)**	**– Raza 0 (ex 1)**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente	Emperador	9
24.2 **(*)**	**VG/ VS**	**– Race 1 (ex 2)**	**– Pathotype 1 (ex 2)**	**– Pathotyp 1 (ex 2)**	**– Raza 1 (ex 2)**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente	Emperador	9
24.3 **(*)**	**VG**	**– Race 2 (ex 3)**	**– Pathotype 2 (ex 3)**	**– Pathotyp 2 (ex 3)**	**– Raza 2 (ex 3)**		
QL		absent	absente	fehlend	ausente	Emperador	1
		present	présente	vorhanden	presente	Colosus	9

Proposal to change the explanation Ad. 24 by adding an alternative method to observe the resistance and by minor changes in the current method

Current wording

Ad. 24: 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[1] (NL) and GEVES[2] (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 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 plants
9.2 Number of replicates…… 1 replicate
9.3 Control varieties for the test with race 0 (ex 1)
Susceptible…… (*Solanum lycopersicum*) Marmande, Marmande verte, Resal
Resistant for race 0 only…….. (*Solanum lycopersicum*) Marporum, Larissa, "Marporum x Marmande verte", Marsol, Anabel
Resistant for race 0 and 1 …… (*Solanum lycopersicum*) Motelle, Gourmet, Mohawk
Control varieties for the test with race 1 (ex 2)
Susceptible …… (*Solanum lycopersicum*) Marmande verte, Cherry Belle, Roma
Resistant for race 0 only …… (*Solanum lycopersicum*) Marporum, Ranco
Resistant for race 0 and 1 …… (*Solanum lycopersicum*) Tradiro, Odisea
Remark: ..... Ranco is slightly less resistant than Tradiro
Control varieties for the test with race 2 (ex 3)
Susceptible for race 2……. Emperador
Resistant for race 0, 1 and 2……. Colosus
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 10^6 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,

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

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 test results in comparison with control varieties

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 will help to compare between labs.

Proposed new wording

Ad. 24: Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)

Resistance to race 0 (ex 1) and race 1 (ex 2) to be tested in a bio-assay (method i) and/or in a DNA marker test (method ii). Resistance to race 2 (ex 3) to be tested in a bio-assay (method i). In case of a bio-assay, type of observation is VG. In case of a DNA marker test, type of observation is VS.

(i) Bio-assay

1.	Pathogen	*Fusarium oxysporum* f. sp. *lycopersici*
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Naktuinbouw[3] (NL), GEVES[4] (FR) or INIA[5] (ES)
5.	Isolate	Race 0 (ex 1) (e.g. strains Orange 71 or PRI 20698 or Fol 071), race 1 (ex 2) (e.g. strains 4152 or PRI40698 or RAF 70) and race 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 10^6 per ml
8.8	Shelflife/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 plants
9.2	Number of replicates	1 replicate
9.3.1	Control varieties for the test with race 0 (ex 1)	
	Susceptible	(*Solanum lycopersicum*) Marmande, Marmande verte, Resal
	Resistant ~~for race 0 only~~	“Marporum x Marmande verte”, ~~Marsol, Anabel~~ Motelle, Gourmet, Mohawk, Ranco, Tradiro
	~~Resistant for race 0 and 1~~	~~(*Solanum lycopersicum*) Motelle, Gourmet, Mohawk~~
	Remark:	Ranco is slightly less resistant than Tradiro
9.3.2	Control varieties for the test with race 1 (ex 2)	
	Susceptible	(*Solanum lycopersicum*) Marmande verte, Cherry Belle, Roma, Marporum, Ranco
	~~Resistant for race 0 only~~	~~(*Solanum lycopersicum*) Marporum, Ranco~~
	Resistant ~~for race 0 and 1~~	Emperador, Colosus and (*Solanum lycopersicum*) Tradiro, Odisea, “Motelle x Marmande verte”
	~~Remark:~~	~~Ranco is slightly less resistant than Tradiro~~
9.3.3	Control varieties for the test with race 2 (ex 3)	
	Susceptible ~~for race 2~~	Emperador and (*Solanum lycopersicum*) Marmande verte, Motelle, Marporum
	Resistant ~~for race 0, 1 and 2~~	Colosus and (*Solanum lycopersicum*) 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

[3] Naktuinbouw: resistentie@naktuinbouw.nl

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

[5] INIA: cardaba@inia.sp

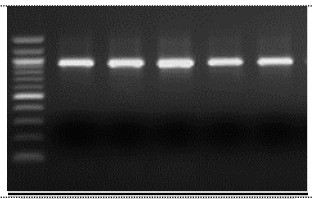
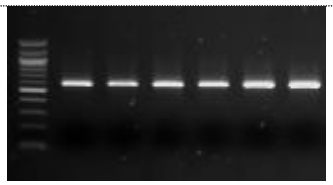
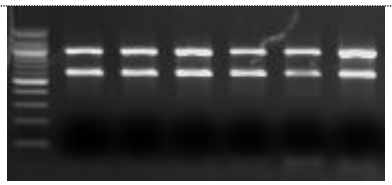
9.6	Temperature	24-28°C (severe test, with mild isolate) 20-24°C (mild test, with severe isolate)
9.7	Light	12 hours per day or longer
9.8	Season	all seasons
9.9	Special measures	slightly acidic peat soil is optimal; keep soil humid but avoid water stress
10.	Inoculation	
10.1	Preparation inoculum	aerated Messiaen or PDA or Agar Medium S of Messiaen or Czapek Dox culture or scraping of plates
10.2	Quantification inoculum	spore count, adjust to 10^6 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 test results in comparison with control varieties	
	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 will help to compare between labs.	

(ii) DNA marker test

Resistance to both race 0 (ex 1) and race 1 (ex 2) is often based on resistance gene I2. The presence of the resistant and/or susceptible allele of gene I2 can be detected by the co-dominant marker as described in this method.

1.	Pathogen	*Fusarium oxysporum* f. sp. *lycopersici*
2.	Functional gene	I2
3.	Primers	
3.1	Susceptible allele	Z1063-i2-F 5'-GTT TGA CAG CTT GGT TTT GT-3' Z1063-i2-R 5'-CTC AAA CTC ACC ATC ATT GA-3'
3.2	Resistant allele	TFusF1 5'-CTG AAA CTC TCC GTA TTT C-3' TFusRR1 5'-CGA AGA GTG ATT GGA GAT-3'
4.	Format of the test	
4.1	Number of plants per genotype	at least 20 plants
4.2	Control varieties	homozygous susceptible allele present: (*Solanum lycopersicum*) Moneymaker homozygous resistant allele present: (*Solanum lycopersicum*) Tradiro
5.	Preparation	
5.1	Preparation DNA	harvest per individual plant a part of a young leaf. Isolate total DNA with a standard DNA isolation protocol (CTAB/SDS based). Re- suspend in 100 µl $T_{10}E_{0,1}$. Dilute total DNA to 1/10 (H_2O) to obtain a DNA concentration between 1-10 ng/µl.

5.2	Preparation PCR	use 3 µl of each diluted DNA sample into individuals PCR reactions. Prepare the PCR master mix, 20µl reaction volume: • 3 µl of 10x diluted DNA • 2,5 µl of 10x reaction buffer • 2 mM MgCl2 • 0.1 µM of resistance primers each • 0.2 µM of susceptible primers each • 200 µM of each of the four dNTPs • 1 unit of Taq DNA polymerase
6.	PCR conditions	1. initial denaturation step at 94°C for 3 minutes 2. 35 cycles at 94°C for 1 minute, 56°C for 1 minute, and 72°C for 2 minutes 3. final extension step of 72°C for 10 minutes
7.	Observations	
7.1	Method	visual
7.2	Observation scale	

		
amplicon of 940bp only homozygous susceptible allele present	amplicon of 600bp only homozygous resistant allele present	amplicons of 940bp and 600bp susceptible and resistant allele present: heterozygous resistant

7.3	Validation of test	control varieties should give the expected band(s).
8.	Interpretation of test results	
	24.1 Race 0 (ex 1)	
	present ..... [9]	Homozygous or heterozygous resistant in DNA marker test. In case homozygous susceptible allele present a bio-assay on race 0 (ex 1) should be performed. In case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the resistance is absent or present for the variety (on another mechanism, e.g. gene I2 without I).
	24.2 Race 1 (ex 2)	
	absent ..... [1]	homozygous susceptible in DNA marker test
	present ..... [9]	homozygous or heterozygous resistant in DNA marker test. In case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the resistance is absent or present for the variety (on another mechanism, e.g. gene I3).

Proposal to change the method of observation of Characteristics 27.1, 27.2 and 27.3

Current wording

27. **(+)**		**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)**		
27.1	**VG**	**– Strain 0**	**– Souche 0**	**– Pathotyp 0**	**– Cepa 0**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente	Emperador	9
27.2		**– Strain 1**	**– Souche 1**	**– Pathotyp 1**	**– Cepa 1**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente		9
27.3		**– Strain 2**	**– Souche 2**	**– Pathotyp 2**	**– Cepa 2**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente		9

Proposed new wording

27. **(+)**		**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)**		
27.1	**VG/ VS**	**– Strain 0**	**– Souche 0**	**– Pathotyp 0**	**– Cepa 0**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente	Emperador	9
27.2	**VG/ VS**	**– Strain 1**	**– Souche 1**	**– Pathotyp 1**	**– Cepa 1**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente		9
27.3	**VG/ VS**	**– Strain 2**	**– Souche 2**	**– Pathotyp 2**	**– Cepa 2**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente		9

Proposal to change the explanation Ad. 27 by adding an alternative method to observe the resistance and by minor typographic changes in the current method

Current wording

Ad. 27: Resistance to Tomato mosaic virus (ToMV)

1. Pathogen .....Tomato mosaic virus
3. Host species .....*Solanum lycopersicum*
4. Source of inoculum .....Naktuinbouw[6] (NL) or GEVES[7] (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 plants
9.2 Number of replicates…… 1 replicate
9.3 Control varieties
Susceptible .....(*Solanum lycopersicum*) Marmande, Monalbo
Resistant for ToMV: 0 and 2.....(*Solanum lycopersicum*) Mobaci
Resistant for ToMV: 0 and 1 .....(*Solanum lycopersicum*) Moperou
Resistant with necrosis .....(*Solanum lycopersicum*) "Monalbo x Momor"
Resistant.....(*Solanum lycopersicum*) 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 test results in comparison with control varieties

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.

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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

Proposed new wording

Ad. 27: Resistance to Tomato mosaic virus (ToMV)

Resistance to strain 0, 1 and 2 to be tested in a bio-assay (method i) and/or in a DNA marker test (method ii). In case of a bio-assay, type of observation is VG. In case of a DNA marker test, type of observation is VS.

(i) Bio-assay

1.	Pathogen	Tomato mosaic virus
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Naktuinbouw[8] (NL) or GEVES[9] (FR)
5.	Isolate	Strain 0 (e.g. isolate INRA Avignon 6-5-1-1), strain 1 and strain 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	Shelflife/viability inoculum	fresh>1 day, desiccated>1year
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.2	Number of replicates	1 replicate
9.3	Control varieties	
	Susceptible	(*Solanum lycopersicum*) Marmande, Monalbo
	Resistant for ToMV: 0 and 2	(*Solanum lycopersicum*) Mobaci
	Resistant for ToMV: 0 and 1	(*Solanum lycopersicum*) Moperou
	Resistant with necrosis	(*Solanum lycopersicum*) "Monalbo x Momor"
	Resistant	(*Solanum lycopersicum*) 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

[8] Naktuinbouw: resistentie@naktuinbouw.nl

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

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 test results in comparison with control varieties	
	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.	

(ii) DNA marker test

Resistance to ToMV is often based on resistance gene Tm2 (allele Tm2 or $Tm2^2$). The presence of the resistant alleles Tm2 and $Tm2^2$ and/or susceptible allele tm2 can be detected by the co-dominant markers as described in Arens, P. *et al* (2010). Specific aspects:

1.	Pathogen	Tomato mosaic virus
2.	Functional gene	$Tm2/2^2$
3.	Primers	
3.1	Assay 1 to check resistance allele Tm2 or $Tm2^2$	Outer primer TMV-2286F: 5'GGGTATACTGGGAGTGTCCAATTC3' Outer primer TMV-2658R: 5'CCGTGCACGTTACTTCAGACAA3' $Tm2^2$ SNP2494F: 5'CTCATCAAGCTTACTCTAGCCTACTTTAGT3' Tm2 SNP2493R: 5'CTGCCAGTATATAACGGTCTACCG3'
3.2	Assay 2 to check susceptible or resistance allele	Outer primer TM2-748F: 5'CGGTCTGGGGAAAACAACTCT3' Outer primer TM2-1256R:5'CTAGCGGTATACCTCCACATCTCC3' TM2-SNP901misR: 5'GCAGGTTGTCCTCCAAATTTTCCATC3' TM2-SNP901misF: 5'CAAATTGGACTGACGGAACAGAAAGTT3'
4.	Format of the test	
4.1	Number of plants per genotype	at least 20 plants
4.2	Control varieties	homozygous susceptible allele tm2 present: (Solanum lycopersicum) Moneymaker resistant allele Tm2 present: (Solanum lycopersicum) Moperou resistant allele $Tm2^2$ present: (Solanum lycopersicum) Momor, Persica, Campeon
6.	PCR conditions	1. Initial denaturation step at 94°C for 3 minutes 2. 35 cycles at 94°C for 1 minute, 55°C for 1 minute, 72°C for 2 minutes 3. Final extension step of 72°C for 10 minutes
8.	Interpretation of test results	the presence of the alleles tm2, Tm2, $Tm2^2$ lead to different interpretation for characteristics 27.1, 27.2 and 27.3, see table. In case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the resistance is absent or present for the variety (on another mechanism, e.g. gene Tm1).

Test result DNA marker test	tm2/tm2	Tm2/tm2 or Tm2/Tm2	$Tm2^2$/tm2 or $Tm2^2$/$Tm2^2$ or $Tm2^2$/Tm2
		(occurs incidentally)	
27.1 Strain 0	[1] absent	[9] resistant	[9] resistant
27.2 Strain 1	[1] absent	[9] resistant	[9] resistant
27.3 Strain 2	[1] absent	[1] absent	[9] resistant

Proposal to change the explanation Ad. 30 “Resistance to Tomato yellow leaf curl virus (TYLCV)” by revision of the current methodology and by adding an alternative method to observe the resistance

Current wording

Ad. 30: 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 plants
9.2 Number of replicates…… 1 replicate
9.3 Control varieties
Susceptible: .....(*Solanum lycopersicum*) Montfavet H 63.5
Resistant: .....(*Solanum lycopersicum*) 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 test results in comparison with control varieties

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).

Proposed new wording

Ad. 30: Resistance to Tomato yellow leaf curl virus (TYLCV)

(i) agroinoculation method

1.	Pathogen	Tomato yellow leaf curl virus (TYLCV) IL strain. (See note below)
2.	Quarantine status	yes (see 13.)
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Dr. Eduardo R. Bejarano, Plant Genetics Laboratory, IHSM UMA- CSIC)[10]
5.	Isolate	Alm:Pep:99, strain IL
6.	Establishment isolate identity	
7.	Establishment pathogenicity	
8.	Multiplication inoculum	
8.1	Multiplication medium	YEP/Kanamycin.
8.2	Multiplication variety	
8.3	Plant stage at inoculation	3-4 leaf
8.4	Inoculation medium	YEP
8.5	Inoculation method	Stem puncture agroinfiltration. Plant agroinoculation is carried out using Agrobacterium tumefaciens transformed with plasmids containing the infectious clones (Morilla, et al. 2005. Phytopathology 95: 1089-1097) The transformed Agrobacterium tumefaciens is a Geneticaly Modified Organism and requires to comply with legislation concerning the protection of the environment, human and animal health.
8.6	Harvest of inoculums	
8.7	Check of harvested inoculum	
8.8	Shelflife/viability inoculum	*A. tumefaciens* stocks are maintained frozen at -80ºC in 15-20% glycerol for long term storage. Cultures to be stored are typically started from a single colony and grown in 5 ml YEP +2.5 µl kanamycin (100mg/ml) during 48 h at 28ªC.
9.	Format of the test	
9.1	Number of plants per genotype	20
9.2	Number of replicates	2
9.3	Control varieties	Susceptible: Big Power, (*Solanum lycopersicum*) Moneymaker, Marmande Resistant: (*Solanum lycopersicum*) Delyca, Montenegro, Anastasia, TY20, Mohawk
9.4	Test design	
9.5	Test facility	Glasshouse or climatic chamber with permission to confined use of Genetically Modified Organism, confinement level 1 (N-1).
9.6	Temperature	23-25°C
9.7	Light	16 h
9.8	Season	
9.9	Special measures	Permission to confined use of Genetically Modified Organism, at least level 1 (N-1)

[10] Source of inoculum; HMS UMA (CSIC) edu_rodri@uma.es; INIA Cardaba@inia.es

10.	Inoculation	
10.1	Preparation inoculum	Streak the surface of the frozen *A. tumefaciens* stock tube and submerge in 5 ml YEP+2.5 µl kanamycin (100mg/ml) during 48 h at 28ªC. Shaking is needed. Take 100 µl and place them into 100 ml YEP and 50 µl kanamycin (100mg/ml). Shake 48 h at 28ºC. Centrifuge the saturated culture for 20 min at 3500 rpm and discard supernatant.
10.2	Quantification inoculums	Dissolve in sterile deionize water to a final OD_{600} of 1.
10.3	Plant stage at inoculation	3-4th leaf
10.4	Inoculation method	Take up into a 1 ml syringe with a 27-gauge needle and few drops (about 20 µl of the culture) were deposited on 10-15 puncture wounds made with the needle into the stem of test tomato plants. Maintain on ice while inoculating plants.
10.5	First observation	20 days post inoculation
10.6	Second observation	30 dpi
*10.7	Final observations	45 dpi
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 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-IL is the strain most widely spread worldwide. With this strain, symptoms do not appear in varieties with Ty-1 and Ty-2. 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).	

(ii) White fly inoculation method

1.	Pathogen	Tomato yellow leaf curl virus (TYLCV) IL strain
2.	Quarantine status	yes (see 13.)
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	-Spain[11]
5.	Isolate	-TYLCV-IL La Mayora
8.	Multiplication inoculum	White flies
8.6	Harvest of inoculums	
9.	Format of the test	
9.1	Number of plants per genotype	20
9.2	Number of replicates	Two replicates
9.3	Control varieties	
	Resistant	TY 20, Anastasia, Mohawk
	Susceptible	Big Power, (*Solanum lycopersicum*) ~~Montfavet H 63.5~~ Moneymaker, Marmande
	Resistant	(*Solanum lycopersicum*) Delyca, Montenegro, Anastasia, TY20, Mohawk
9.5	Test facility	~~field with natural disease pressure~~ greenhouse/plastic tunnel
9.9	Special measures	prevent spread of white-flies

[11] IHSM, CSIC guillamon@eelm.csic.es or INIA cardaba@inia.es

10.	Inoculation	
10.3	Plant stage at inoculation	~~6-12 weeks (adult plants)~~ 2-4 weeks
10.4	Inoculation method	vector (Bemisia white-flies carrying TYLCV-IL)
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.~~ TYLCV-IL is the strain most widely spread worldwide. With this strain, symptoms do not appear in varieties with Ty-1 and Ty-2. Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV).	

Proposal to change the method of observation of Characteristic 31 “Resistance to Tomato spotted wilt virus (TSWV)”

Current wording

31. **(+)**	**VG**	**Resistance to Tomato spotted wilt virus (TSWV)**	**Résistance au virus de la tache bronzée de la tomate (TSWV)**	**Resistenz gegen das gefleckte Tomaten-bronzenfleckenvirus (TSWV)**	**Resistencia al virus del bronceado de tomate (TSWV)**		
QL		absent	absente	fehlend	ausente	Big Power	1
		present	présente	vorhanden	presente	Enpower	9

Proposed new wording

31. **(+)**	**VG/ VS**	**Resistance to Tomato spotted wilt virus (TSWV)**	**Résistance au virus de la tache bronzée de la tomate (TSWV)**	**Resistenz gegen das gefleckte Tomaten-bronzenfleckenvirus (TSWV)**	**Resistencia al virus del bronceado de tomate (TSWV)**		
QL		absent	absente	fehlend	ausente	Big Power	1
		present	présente	vorhanden	presente	Enpower	9

Proposal to change the explanation Ad. 31 by adding an alternative method to observe the resistance

Current wording

Ad. 31: 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 [12] (NL), GEVES[13] (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 plants
9.2 Number of replicates…… 1 replicate
9.3 Control varieties
Susceptible: .....Big Power and (*Solanum lycopersicum*) Monalbo, Momor, Montfavet H 63.5
Resistant: .....Enpower and (*Solanum lycopersicum*) 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 test results in comparison with control varieties
absent…… [1] symptoms
present…… [9] no symptoms
13. Critical control points:
TSWV has a quarantine status in some countries. TSWV is transmitted by *Thrips tabaci* 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.

[12] Naktuinbouw: resistentie@naktuinbouw.nl

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

Proposed new wording

Ad. 31: Resistance to Tomato spotted wilt virus (TSWV)

(i) Bio-assay

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 [14] (NL), GEVES [15] (FR)
5.	Isolate	race 0, preferably a thrips-transmission deficient variant
7.	Establishment pathogenicity	biotest
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 plants
9.2	Number of replicates	1 replicate
9.3	Control varieties	
	Susceptible	Big Power and (*Solanum lycopersicum*) Monalbo, Momor, Montfavet H 63.5
	Resistant	Enpower and (*Solanum lycopersicum*) 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 test results in comparison with control varieties	
	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.	

[14] Naktuinbouw: resistentie@naktuinbouw.nl

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

(ii) DNA marker test

Resistance to TSWV strain 0 is often based on resistance gene Sw-5. The presence of the resistant allele and/or susceptible allele(s) can be detected by the co-dominant markers as described in Dianese, E.C. *et al* (2010). Specific aspects:

1.	Pathogen	Tomato spotted wilt virus
2.	Functional gene	Sw-5b
3.	Primers	
3.1	Susceptible alleles	Sw5-Vat1-F: 5'-ACAACATCAAACAATGTTAGCC-3' Sw5-Vat2-F: 5'-CATCAAACAATGCAGTTAGCC-3'
3.2	Resistant allele	Sw5-Res-F: 5'-ATCAACCAATACAGCCTAACC-3
3.3	Universal reverse	Sw5-universal-R: 5'-TTTCTCCCTGCAAGTTCACC-3'
3.4	Allele specific probes	Sw5-Sus1: 5'-VIC-TACATTATGAAGGGTTAACAAG-MGB-NFQ-3' Sw5-Sus2: 5'-6FAM-ACAACAGAGGGTTAACAAGTTTAGG-BHQ1-3' Sw5-Res: 5'-TEXAS RED-TGGGCGAAAATCCCAACAAG-BHQ2-3'
4.	Format of the test	
4.1	Number of plants per genotype	at least 20 plants
4.2	Control varieties	homozygous susceptible allele 1 present: (*Solanum lycopersicum*) Moneymaker homozygous susceptible allele 2 present: (*Solanum lycopersicum*) Mountain Magic homozygous resistant allele present: (*Solanum lycopersicum*) Montealto
6.	PCR conditions	1. Initial denaturation step 10 min 95 °C 2. 40 cycles 15 sec 95 °C and 1 min 60°C. Every cycle ends with a plate reading.
8.	Interpretation of test results	
	absent .....[1]	susceptible allele(s) present and resistant allele absent
	present .....[9]	resistant allele present (homozygous or heterozygous) In case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the resistance is absent or present for the variety (on another mechanism).

Proposal to add a reference to literature related to changes (a) – (h) to Chapter 9 “Literature”

Proposed addition to 9. Literature

Dianese, E.C. et al, 2010: Development of a locus-specific, co-dominant SCAR marker for assisted-selection of the Sw-5 (Topovirus resistance) gene cluster in a wide range of tomato accessions. Molecular Breeding, 25(1), pp. 133-142.

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