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TWV/51/11

Fifty-First Session

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PARTIAL REVISION OF THE TEST GUIDELINES FOR TOMATO ROOTSTOCKS

*Document prepared by The Netherlands**Disclaimer: this document does not represent UPOV policies or guidance*

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 fiftieh session, held in Brno, Czech Republic, from June 27 to July 1, 2016, agreed that the Test Guidelines for Tomato Rootstocks (document TG/294/1 Corr. Rev.) be partially revised for disease resistance characteristics (see document TWV/50/25 "Report", Annex IV).
3. The following changes are proposed:
 - (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 method of observation of Characteristic 58 "Resistance to Tomato spotted wilt virus (TSWV) - Race 0"
 - (f) 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.
 - (g) To change the method of observation of Characteristic 31 "Resistance to Tomato spotted wilt virus (TSWV) - Race 0"
 - (h) To change the explanation Ad. 31 by adding an alternative method to observe the resistance
 - (i) To add a reference to literature related to changes (a) – (h) to Chapter 9 "Literature".
4. 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 <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol)	Résistance à <i>Fusarium</i> <i>oxysporum</i> f. sp. <i>lycopersici</i> (Fol)	Resistenz gegen <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol)	Resistencia a <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (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 <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol)	Résistance à <i>Fusarium</i> <i>oxysporum</i> f. sp. <i>lycopersici</i> (Fol)	Resistenz gegen <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol)	Resistencia a <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (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..... Naktuinbouw¹ (NL) and GEVES² (FR)
5. Isolate..... Race 0 (ex 1) (e.g. strains Orange 71 or PRI 20698 or Fol 071 1 (ex 2) (e.g. strains 4152 or PRI40698 or RAF 70 and 2 (ex 3)
Individual strains may vary in pathogenicity
6. Establishment isolate identity use differential varieties (see 9.3)
7. Establishment pathogenicity..... on susceptible tomato varieties
8. Multiplication inoculum
- 8.1 Multiplication medium..... Potato Dextrose Agar, Medium "S" of Messiaen
- 8.4 Inoculation medium..... water for scraping agar plates or Czapek-Dox culture medium
(7 d-old aerated culture)
- 8.6 Harvest of inoculum filter through double muslin cloth
- 8.7 Check of harvested inoculum spore count; adjust to 10⁶ per ml
- 8.8 Shelf-life/viability inoculum 4-8 h, keep cool to prevent spore germination
9. Format of the test
- 9.1 Number of plants per genotype..... at least 20 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 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 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.

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² GEVES; Valerie.GRIMAUULT@geves.fr

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 Naktuinbouw³ (NL), GEVES⁴ (FR) or INIA⁵ (SP)
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 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 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~~ Emperador, Colosus and (*Solanum lycopersicum*) Marporum, Larissa,
"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
- 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

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⁵ INIA: cardaba@inia.sp

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,
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 μ l $T_{10}E_{0.1}$. Dilute total DNA to 1/10 (H_2O) to obtain a DNA concentration between 1-10 ng/ μ l.

5.1 Preparation PCR use 3 μ l of each diluted DNA sample into individual PCR reactions.

Prepare the PCR master mix, 20 μ l reaction volume:

- 3 μ l of 10x diluted DNA
- 2,5 μ l of 10x reaction buffer
- 2 mM $MgCl_2$
- 0.1 μ M of resistance primers each
- 0.2 μ M of susceptible primers each
- 200 μ M 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

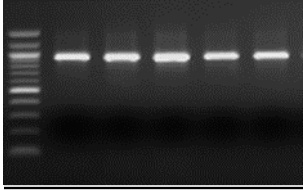
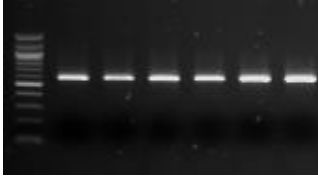
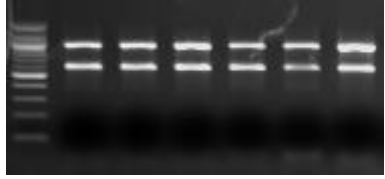
2. 35 cycles at 94°C for 1 minute, 56°C for 1 minute, and 72°C for 2 minutes

3. final extension step of 72°C for 10 minutes

7. Observations

7.1 Method visual

7.2 Observation scale

		
<u>amplicon of 940bp only</u>	<u>amplicon of 600bp only</u>	<u>amplicons of 940bp and 600bp</u>
<u>homozygous susceptible allele present</u>	<u>homozygous resistant allele present</u>	<u>susceptible and resistant allele present: heterozygous resistant</u>

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 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.....Naktuinbouw⁶ (NL) or GEVES⁷ (FR)
 5. Isolate..... Strain 0 (e.g. isolate INRA Avignon 6-5-1-1) 1 and 2
 6. Establishment isolate identitygenetically defined tomato standards
Mobaci (Tm1), Moperou (Tm2), Momor (Tm2²)
 7. Establishment pathogenicity.....on susceptible plant
 8. Multiplication inoculum
 - 8.1 Multiplication medium.....living plant
 - 8.2 Multiplication varietye.g. Moneymaker, Marmande
 - 8.7 Check of harvested inoculumoption: on *Nicotiana tabacum* "Xanthi",
check lesions after 2 days
 - 8.8 Shelf life/viability inoculumfresh>1 day, desiccated>1year
 9. Format of the test
 - 9.1 Number of plants per genotypeat 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 facilityGlasshouse or climate room
 - 9.6 Temperature.....24 to 26°C
 - 9.7 Light12 hours or longer
 - 9.8 Seasonsymptoms are more pronounced in summer
 10. Inoculation
 - 10.1 Preparation inoculum1 g leaf with symptoms with 10 ml PBS or similar buffer
Homogenize, add carborundum to buffer (1 g/30ml)
 - 10.3 Plant stage at inoculationcotyledons or 2 leaves
 - 10.4 Inoculation method.....gentle rubbing
 - 10.7 Final observations11-21 days after inoculation
 11. Observations
 - 11.1 Methodvisual
 - 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.

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

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

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*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 Naktuinbouw⁸ (NL) or GEVES⁹ (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 (Tm2²)
 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 >1 year
 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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⁹ GEVES: Valerie.GRIMAULT@geves.fr

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 Tm2²). The presence of the resistant alleles Tm2 and Tm2² and/or susceptible allele tm2 can be detected by the co-dominant markers as described in Arens, P. *et al* (2010). Specific aspects:

1. PathogenTomato mosaic virus

2. Functional geneTm2/2²

4. Format of the test

4.1 Number of plants per genotypeat least 20 plants

4.2 Control varietieshomozygous susceptible allele tm2 present:
(*Solanum lycopersicum*) Moneymaker
resistant allele Tm2 present: (*Solanum lycopersicum*) Moperou
resistant allele Tm2² present: (*Solanum lycopersicum*) Momor,
Persica, Campeon

8. Interpretation of test resultsthe presence of the alleles tm2, Tm2, Tm2² 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).

<u>Test result DNA marker test</u>	<u>tm2/tm2</u>	<u>Tm2/tm2 or Tm2/Tm2</u>	<u>Tm2²/tm2 or Tm2²/Tm2² or Tm2²/Tm2</u>
		(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	<i>Solanum lycopersicum</i>
4. Source of inoculums	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 <i>Agrobacterium tumefaciens</i> transformed with plasmids containing the infectious clones (Morilla, et al. 2005. <i>Phytopathology</i> 95: 1089-1097)
8.6 Harvest of inoculums	
8.7 Check of harvested inoculums	
8.8 Shelflife/viability inoculums	<i>A. tumefaciens</i> stocks are maintained frozen at -80°C 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 28°C.
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, (<i>Solanum lycopersicum</i>) Moneymaker, Marmande Resistant: (<i>Solanum lycopersicum</i>) Delyca, Montenegro, Anastasia, TY20, Mohawk
9.4 Test design	
9.5 Test facility	Glasshouse or climatic chamber with permission to confined use of OGM, 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 OGM, at least level 1 (N-1)
10. Inoculation	
10.1 Preparation inoculums	Streak the surface of the frozen <i>A. tumefaciens</i> stock tube and submerge in 5 ml YEP+2.5 µl kanamycin (100mg/ml) during 48 h at 28°C. Shaking is needed. Take 100 µl and place them into 100 ml YEP and 50 µl kanamycin (100mg/ml). Shake 48 h at 28°C. 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	3-4 th 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 Source of inoculum; HMS UMA (CSIC) edu_rodri@uma.es; INIA Cardaba@inia.es

*10.7 End of test – Final observation..... 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 Spain¹¹

5. Isolate TYLCV-IL La Mayora

8. Multiplication inoculum..... White flies

8.6 Harvest of inoculum

9. Format of the test

9.1 Number of plants per genotype20

9.2 Number of replicates..... Two replicates

9.3 Control varieties Susceptible

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

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).

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

Proposal to change the method of observation of Characteristic 31 "Resistance to Tomato spotted wilt virus (TSWV) - Race 0"

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 the method of observation of Characteristic 31 "Resistance to Tomato spotted wilt virus (TSWV) - Race 0"

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 Naktuinbouw¹² (NL), GEVES¹³ (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 Naktuinbouw¹⁴ (NL), GEVES¹⁵ (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 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.

(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
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
8. Interpretation of test results [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).

¹⁴ Naktuinbouw: resistentie@naktuinbouw.nl

¹⁵ GEVES; Valerie.GRIMAULT@geves.fr

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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