

Technical Working Party for Vegetables

TWV/51/10

Fifty-First Session

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PARTIAL REVISION OF THE TEST GUIDELINES FOR TOMATO

*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 (document TG/44/11 Rev).
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 (document TG/44/11 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 48.1 and 48.2:
 - (i) Characteristic 48.1 "Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) - Race 0 (ex 1)"
 - (ii) Characteristic 48.2 "Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) - Race 1 (ex 2)"
 - (b) To change the explanation Ad. 48 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 51.1, 51.2 and 51.3:
 - (i) Characteristic 51.1 "Resistance to Tomato mosaic virus (ToMV) - Strain 0"
 - (ii) Characteristic 51.2 "Resistance to Tomato mosaic virus (ToMV) - Strain 1"
 - (iii) Characteristic 51.3 "Resistance to Tomato mosaic virus (ToMV) - Strain 2"
 - (d) To change the explanation Ad. 51 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. 58 by adding an alternative method to observe the resistance
 - (g) To add a reference to literature related to changes (a) – (f) 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 48.1 and 48.2

Current wording

48. (+)	VG	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)		
48.1 (*)	VG	– Race 0 (ex 1)	– Pathotype 0 (ex 1)	– Pathotyp 0 (ex 1)	– Raza 0 (ex 1)		
QL		absent	absente	fehlend	ausente	Marmande verte	1
		present	présente	vorhanden	presente	Anabel, Marporum, Marsol	9
48.2 (*)	VG	– Race 1 (ex 2)	– Pathotype 1 (ex 2)	– Pathotyp 1 (ex 2)	– Raza 1 (ex 2)		
QL		absent	absente	fehlend	ausente	Marmande verte	1
		present	présente	vorhanden	presente	Motelle, Walter	9
48.3	VG	– Race 2 (ex 3)	– Pathotype 2 (ex 3)	– Pathotyp 2 (ex 3)	– Raza 2 (ex 3)		
QL		absent	absente	fehlend	ausente	Marmande verte, Motelle	1
		present	présente	vorhanden	presente	Alliance, Florida, Ivanhoé, Tributes	9

Proposed new wording

48. (+)	VG	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)		
48.1 (*)	VG/ VS	– Race 0 (ex 1)	– Pathotype 0 (ex 1)	– Pathotyp 0 (ex 1)	– Raza 0 (ex 1)		
QL		absent	absente	fehlend	ausente	Marmande verte	1
		present	présente	vorhanden	presente	Anabel, Marporum, Marsol	9
48.2 (*)	VG/ VS	– Race 1 (ex 2)	– Pathotype 1 (ex 2)	– Pathotyp 1 (ex 2)	– Raza 1 (ex 2)		
QL		absent	absente	fehlend	ausente	Marmande verte	1
		present	présente	vorhanden	presente	Motelle, Walter	9
48.3	VG	– Race 2 (ex 3)	– Pathotype 2 (ex 3)	– Pathotyp 2 (ex 3)	– Raza 2 (ex 3)		
QL		absent	absente	fehlend	ausente	Marmande verte, Motelle	1
		present	présente	vorhanden	presente	Alliance, Florida, Ivanhoé, Tributes	9

Proposal to change the explanation Ad. 48 by adding an alternative method to observe the resistance and by minor changes in the current method

Current wording

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

1. Pathogen *Fusarium oxysporum* f. sp. *lycopersici*
3. Host species..... *Solanum lycopersicum*
4. Source of inoculum..... 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..... 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..... pore count, adjust to 10⁶ spores per ml,
lower concentration for a very aggressive isolate
- 10.3 Plant stage at inoculation..... 10-18 d, cotyledon to first leaf
- 10.4 Inoculation method roots and hypocotyls are immersed in spore suspension
for 5-15 min; trimming of roots is an option
- 10.7 Final observations 14-21 days after inoculation
11. Observations
- 11.1 Method visual
- 11.2 Observation scale symptoms:
growth retardation, wilting, yellowing,
vessel browning extending above cotyledon
- 11.3 Validation of test..... evaluation of variety resistance should be calibrated with results of resistant and
susceptible controls. Standards near borderline R/S will help to compare between labs.
12. Interpretation of 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.

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Proposed new wording

Ad. 48: 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 Marmande, Marmande verte, Resal
Resistant ~~for race 0 only~~ Marporum, Larissa, "Marporum x Marmande verte", Marsol, Anabel,
Motelle, Gourmet, Mohawk, Ranco, Tradiro
Resistant for race 0 and 1 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 Marmande verte, Cherry Belle, Roma, Marporum, Ranco
Resistant for race 0 only Marporum, Ranco
Resistant for race 0 and 1 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 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

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⁴ GEVES: Valerie.GRIMAULT@geves.fr

⁵ INIA: cardaba@inia.sp

- 10.3 Plant stage at inoculation 10-18 d, cotyledon to first leaf
- 10.4 Inoculation method roots and hypocotyls are immersed in spore suspension for 5-15 min; trimming of roots is an option
- 10.7 Final observations 14-21 days after inoculation
11. Observations
- 11.1 Method visual
- 11.2 Observation scale symptoms:
 growth retardation, wilting, yellowing,
 vessel browning extending above cotyledon
- 11.3 Validation of test evaluation of variety resistance should be calibrated with results of resistant and susceptible controls. Standards near borderline R/S will help to compare between labs.
12. Interpretation of 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.

(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: Moneymaker
 homozygous resistant allele present: 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_{10E0,1}. Dilute total DNA to 1/10 (H₂O) to obtain a DNA concentration between 1-10 ng/µl.

5.1 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

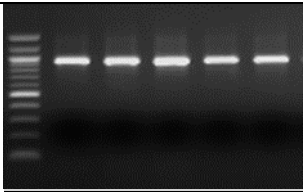
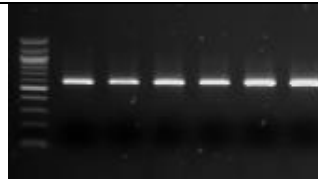
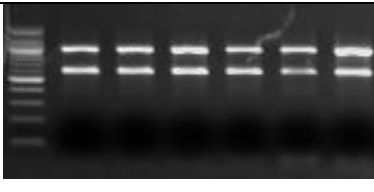
2. 35 cycles at 94°C for 1 minute, 56°C for 1 minute, and 72°C for 2 minutes

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

7. Observations

7.1 Methodvisual

7.2 Observation scale

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

48.1 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).

48.2 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 51.1, 51.2 and 51.3

Current wording

51. (+)	VG	Resistance to Tomato mosaic virus (ToMV)	Résistance au virus de la mosaïque de la tomate (ToMV)	Resistenz gegen das Tomatenmosaik-virus (ToMV)	Resistencia al virus del mosaico del tomate (ToMV)		
51.1	VG	– Strain 0	– Souche 0	– Pathotyp 0	– Cepa 0		
QL		absent	absente	fehlend	ausente	Monalbo	1
		present	présente	vorhanden	presente	Mobaci, Mocimor, Moperou	9
51.2	VG	– Strain 1	– Souche 1	– Pathotyp 1	– Cepa 1		
QL		absent	absente	fehlend	ausente	Monalbo	1
		present	présente	vorhanden	presente	Mocimor, Moperou	9
51.3	VG	– Strain 2	– Souche 2	– Pathotyp 2	– Cepa 2		
QL		absent	absente	fehlend	ausente	Monalbo	1
		present	présente	vorhanden	presente	Mobaci, Mocimor	9

Proposed new wording

51. (+)	VG/ VS	Resistance to Tomato mosaic virus (ToMV)	Résistance au virus de la mosaïque de la tomate (ToMV)	Resistenz gegen das Tomatenmosaik-virus (ToMV)	Resistencia al virus del mosaico del tomate (ToMV)		
51.1	VG/ VS	– Strain 0	– Souche 0	– Pathotyp 0	– Cepa 0		
QL		absent	absente	fehlend	ausente	Monalbo	1
		present	présente	vorhanden	presente	Mobaci, Mocimor, Moperou	9
51.2	VG/ VS	– Strain 1	– Souche 1	– Pathotyp 1	– Cepa 1		
QL		absent	absente	fehlend	ausente	Monalbo	1
		present	présente	vorhanden	presente	Mocimor, Moperou	9
51.3	VG/ VS	– Strain 2	– Souche 2	– Pathotyp 2	– Cepa 2		
QL		absent	absente	fehlend	ausente	Monalbo	1
		present	présente	vorhanden	presente	Mobaci, Mocimor	9

Proposal to change the explanation Ad. 51 by adding an alternative method to observe the resistance. And by minor typographic changes in the current method

Current wording

Ad. 51: 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 replicates1 replicate
 - 9.3 Control varieties
 - Susceptible.....Marmande, Monalbo
 - Resistant for ToMV: 0 and 2.....Mobaci
 - Resistant for ToMV: 0 and 1Moperou
 - Resistant with necrosis....."Monalbo x Momor"
 - ResistantGourmet
 - 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. 51: 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), GEVES⁹ (FR) or INIA¹⁰ (SP, strain 0)
 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 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 test results in comparison with control varieties
 - absent [1] symptoms of susceptibility
 - present [9] no symptoms, or symptoms of hypersensitive resistance

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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 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. Pathogen Tomato mosaic virus

2. Functional gene Tm2/2²

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: Moneymaker
resistant allele Tm2 present: Moperou
resistant allele Tm2² present: Momor, Persica, Campeon

8. Interpretation of test results the presence of the alleles tm2, Tm2, Tm2² lead to different interpretation for characteristics 51.1, 51.2 and 51.3, 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)	
51.1 Strain 0	[1] absent	[9] resistant	[9] resistant
51.2 Strain 1	[1] absent	[9] resistant	[9] resistant
51.3 Strain 2	[1] absent	[1] absent	[9] resistant

Proposal to change the method of observation of Characteristic 58 “Resistance to Tomato spotted wilt virus (TSWV) - Race 0”

Current wording

58. (+)	VG	Resistance to Tomato spotted wilt virus (TSWV)	Résistance au virus de la tache bronzée de la tomate (TSWV)	Resistenz gegen das Tomatenbronzenfleckenvirus (TSWV)	Resistencia al virus del bronceado del tomate (TSWV)		
		- Race 0	- Pathotype 0	- Pathotyp 0	- Raza 1		
QL		absent	absente	fehlend	ausente	Montfavit H 63.5	1
		present	présente	vorhanden	presente	Lisboa	9

Proposed new wording

58. (+)	VG/ VS	Resistance to Tomato spotted wilt virus (TSWV)	Résistance au virus de la tache bronzée de la tomate (TSWV)	Resistenz gegen das Tomatenbronzenfleckenvirus (TSWV)	Resistencia al virus del bronceado del tomate (TSWV)		
		- Race 0	- Pathotype 0	- Pathotyp 0	- Raza 1		
QL		absent	absente	fehlend	ausente	Montfavit H 63.5	1
		present	présente	vorhanden	presente	Lisboa	9

Proposal to change the explanation Ad. 58 by adding an alternative method to observe the resistance

Current wording

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

1. Pathogen Tomato spotted wilt virus
2. Quarantine status yes (see note below)
3. Host species *Solanum lycopersicum*
4. Source of inoculum Naktuinbouw¹¹ (NL), GEVES¹² (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 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 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.

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¹² GEVES; Valerie.GRIMAULT@geves.fr

Proposed new wording

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

Resistance to strain 0 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 spotted wilt virus
2. Quarantine status yes (see note below)
3. Host species *Solanum lycopersicum*
4. Source of inoculum Naktuinbouw¹³ (NL), GEVES¹⁴ (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 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 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: Moneymaker
homozygous susceptible allele 2 present: Mountain Magic
homozygous resistant allele present: Montealto
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).

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Proposal to add a reference to literature related to changes (a) – (f) 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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