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| International Union for the Protection of New Varieties of Plants |  |

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Partial revision of the Test Guidelines for TOMATO

Document prepared by an expert from the Netherlands

Disclaimer: this document does not represent UPOV policies or guidance

 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.).

 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 (*Solanum lycopersicum* L*.*) on the basis of documents TG/44/11 Rev. and TWV/51/11 “Partial Revision of the Test Guidelines for Tomato” and proposed the following revisions to the Test Guidelines for Tomato (see document TWV/51/16 “Report”, paragraph 114):

 The following changes are proposed:

1. To change the method of observation of Characteristics 48.1 and 48.2:
	1. Characteristic 48.1 “Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) - Race 0 (ex 1)”
	2. Characteristic 48.2 “Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) - Race 1 (ex 2)”
2. To change the explanation Ad. 48 by adding an alternative method to observe the resistance and by minor changes in the current method
3. To change the method of observation of Characteristics 51.1, 51.2 and 51.3:
	1. Characteristic 51.1 “Resistance to Tomato mosaic virus (ToMV) - Strain 0”
	2. Characteristic 51.2 “Resistance to Tomato mosaic virus (ToMV) - Strain 1”
	3. Characteristic 51.3 “Resistance to Tomato mosaic virus (ToMV) - Strain 2”
4. To change the explanation Ad. 51 by adding an alternative method to observe the resistance and by minor typographic changes in the current method
5. To change the method of observation of Characteristic 58 “Resistance to Tomato spotted wilt virus (TSWV) - Race 0”
6. To change the explanation Ad. 58 by adding an alternative method to observe the resistance
7. To add a reference to literature related to changes (a) – (f) to Chapter 9 “Literature”.

 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 *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) |  |  |
| **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 *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) |  |  |
| **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 Naktuinbouw[[1]](#footnote-2) (NL) and GEVES[[2]](#footnote-3) (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.

*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.

1. Bio-assay

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | *Fusarium oxysporum* f. sp. *lycopersici* |
| 3. | Host species | *Solanum lycopersicum* |
| 4. | Source of inoculum | Naktuinbouw[[3]](#footnote-4) (NL), GEVES[[4]](#footnote-5) (FR) or INIA[[5]](#footnote-6) (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 106 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 | Marmande, Marmande verte, Resal |
|  | Resistant ~~for race 0 only~~ | Marporum, Larissa, “Marporum x Marmande verte”, ~~Marsol, Anabel,~~ Motelle, Gourmet, Mohawk, 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 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 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. |

(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. | Quarantine status | 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: Moneymakerhomozygous 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 T10E0,1. Dilute total DNA to 1/10 (H2O) 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 minutes2. 35 cycles at 94°C for 1 minute, 56°C for 1 minute, and 72°C for 2 minutes3. final extension step of 72°C for 10 minutes |
| 7. | Observations |  |
| 7.1 | Method | visual |
| 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 Resistance to 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 Resistance to 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 | 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 Naktuinbouw[[6]](#footnote-7) (NL) or GEVES[[7]](#footnote-8) (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 (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>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 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

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.

*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.

1. Bio-assay

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | Tomato mosaic virus |
| 3. | Host species | *Solanum lycopersicum* |
| 4. | Source of inoculum | Naktuinbouw[[8]](#footnote-9) (NL), GEVES[[9]](#footnote-10) (FR) or INIA[[10]](#footnote-11) (ES, 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 standardsMobaci (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 | 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 | 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 bufferhomogenize, 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 malformationsymptoms 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. |

 (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 |
| 3. | Primers |  |
| 3.1 | Assay 1 to check resistance allele Tm2 or Tm22 | Outer primer TMV-2286F: 5’GGGTATACTGGGAGTGTCCAATTC3’Outer primer TMV-2658R: 5’CCGTGCACGTTACTTCAGACAA3’Tm22 SNP2494F: 5’CTCATCAAGCTTACTCTAGCCTACTTTAGT3’Tm2 SNP2493R: 5’CTGCCAGTATATAACGGTCTACCG3’ |
| 3.2 | Assay 2 to check susceptible orresistance 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: Moneymakerresistant allele Tm2 present: Moperouresistant allele Tm22 present: Momor, Persica, Campeon |
| 6. | PCR conditions | 1. Initial denaturation step at 94°C for 3 minutes2. 35 cycles at 94°C for 1 minute, 55°C for 1 minute, 72°C for 2 minutes3. Final extension step of 72°C for 10 minutes |
| 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). |

|  |  |  |  |
| --- | --- | --- | --- |
| Test result DNA marker test | tm2/tm2 | Tm2/tm2 or Tm2/Tm2 | Tm22/tm2 or Tm22/Tm22 orTm22/Tm2 |
|  |  | (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)- Race 0 | Résistance au virus de la tache bronzée de la tomate (TSWV) – Pathotype 0 | Resistenz gegen das Tomatenbronzen­fleckenvirus (TSWV) - Pathotyp 0 | Resistencia al virus del bronceado del tomate (TSWV)– Raza 1 |  |  |
| QL |  | absent | absente | fehlend | ausente | Montfavet H 63.5 | 1 |
|  |  | present | présente | vorhanden | presente | Lisboa | 9 |

*Proposed new wording*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 58. (+) | VG/VS | Resistance to Tomato spotted wilt virus (TSWV)- Race 0 | Résistance au virus de la tache bronzée de la tomate (TSWV) – Pathotype 0 | Resistenz gegen das Tomatenbronzen­fleckenvirus (TSWV) - Pathotyp 0 | Resistencia al virus del bronceado del tomate (TSWV)– Raza ~~1~~ 0 |  |  |
| QL |  | absent | absente | fehlend | ausente | Montfavet 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 Naktuinbouw [[11]](#footnote-12) (NL), GEVES [[12]](#footnote-13) (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 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.

*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 | Naktuinbouw [[13]](#footnote-14) (NL), GEVES [[14]](#footnote-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 | 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 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. |

(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: Moneymakerhomozygous susceptible allele 2 present: Mountain Magichomozygous resistant allele present: Montealto |
| 6. | PCR conditions | 1. Initial denaturation step 10 min 95 °C2. 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) – (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.

[End of document]

1. Naktuinbouw: resistentie@naktuinbouw.nl [↑](#footnote-ref-2)
2. GEVES; Valerie.GRIMAULT@geves.fr [↑](#footnote-ref-3)
3. Naktuinbouw: resistentie@naktuinbouw.nl [↑](#footnote-ref-4)
4. GEVES: Valerie.GRIMAULT@geves.fr [↑](#footnote-ref-5)
5. INIA: cardaba@inia.sp [↑](#footnote-ref-6)
6. Naktuinbouw: resistentie@naktuinbouw.nl [↑](#footnote-ref-7)
7. GEVES: Valerie.GRIMAULT@geves.fr [↑](#footnote-ref-8)
8. Naktuinbouw: resistentie@naktuinbouw.nl [↑](#footnote-ref-9)
9. GEVES: Valerie.GRIMAULT@geves.fr [↑](#footnote-ref-10)
10. INIA: cardaba@inia.sp [↑](#footnote-ref-11)
11. Naktuinbouw: resistentie@naktuinbouw.nl [↑](#footnote-ref-12)
12. GEVES; Valerie.GRIMAULT@geves.fr [↑](#footnote-ref-13)
13. Naktuinbouw: resistentie@naktuinbouw.nl [↑](#footnote-ref-14)
14. GEVES; Valerie.GRIMAULT@geves.fr [↑](#footnote-ref-15)