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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 rooTstocks

Document prepared by an expert from the Netherlands

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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).

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

1. To change the method of observation of Characteristics 24.1 and 24.2:
   1. Characteristic 24.1 “Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) - Race 0 (ex 1)”
   2. Characteristic 24.2 “Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) - Race 1 (ex 2)”
2. To change the explanation Ad. 24 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 27.1, 27.2 and 27.3:
   1. Characteristic 27.1 “Resistance to Tomato mosaic virus (ToMV) - Strain 0”
   2. Characteristic 27.2 “Resistance to Tomato mosaic virus (ToMV) - Strain 1”
   3. Characteristic 27.3 “Resistance to Tomato mosaic virus (ToMV) - Strain 2”
4. To change the explanation Ad. 27 by adding an alternative method to observe the resistance and by minor typographic changes in the current method
5. 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.
6. To change the method of observation of Characteristic 31 “Resistance to Tomato spotted wilt virus (TSWV)”
7. To change the explanation Ad. 31 by adding an alternative method to observe the resistance
8. To add a reference to literature related to changes (a) – (h) 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 24.1 and 24.2

*Current wording*

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

*Proposed new wording*

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

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

*Current wording*

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

1. Pathogen *Fusarium oxysporum* f. sp. *lycopersici*

3. Host species *Solanum lycopersicum*

4. Source of inoculum Naktuinbouw[[1]](#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……………………………… (*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.

*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.

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 | (*Solanum lycopersicum*) Marmande, Marmande verte, Resal |
|  | Resistant ~~for race 0 only~~ | “Marporum x Marmande verte”, ~~Marsol, Anabel~~ Motelle, Gourmet, Mohawk, Ranco, Tradiro |
|  | ~~Resistant for race 0 and 1~~ | ~~(~~*~~Solanum lycopersicum~~*~~) Motelle, Gourmet, Mohawk~~ |
|  | Remark: | Ranco is slightly less resistant than Tradiro |
| 9.3.2 | Control varieties for the test with race 1 (ex 2) |  |
|  | Susceptible | (*Solanum lycopersicum*) Marmande verte, Cherry Belle, Roma, Marporum, Ranco |
|  | ~~Resistant for race 0 only~~ | ~~(~~*~~Solanum lycopersicum~~*~~) Marporum, Ranco~~ |
|  | Resistant ~~for race 0 and 1~~ | Emperador, Colosus and (*Solanum lycopersicum*) Tradiro, Odisea, “Motelle x Marmande verte” |
|  | ~~Remark:~~ | ~~Ranco is slightly less resistant than Tradiro~~ |
| 9.3.3 | Control varieties for the test with  race 2 (ex 3) |  |
|  | Susceptible ~~for race 2~~ | Emperador and (*Solanum lycopersicum*) Marmande verte, Motelle, Marporum |
|  | Resistant ~~for race 0, 1 and 2~~ | Colosus and (*Solanum lycopersicum*) Tributes, Murdoch, “Marmande verte x Florida” |
| 9.4 | Test design | >20 plants; e.g. 35 seeds for 24 plants, including 2 blanks |
| 9.5 | Test facility | glasshouse or climate room |
| 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. |
| 12. | Interpretation of test results in comparison with control varieties |  |
|  | absent [1] | severe symptoms |
|  | present [9] | mild or no symptoms |
| 13. | Critical control points  Test results may vary slightly in inoculum pressure due to differences in isolate, spore concentration, soil humidity and temperature. Standards near borderline R/S will help to compare between labs. | |

(ii) DNA marker test

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

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | *Fusarium oxysporum* f. sp. *lycopersici* |
| 2. | Functional gene | I2 |
| 3. | Primers |  |
| 3.1 | Susceptible allele | Z1063-i2-F 5’-GTT TGA CAG CTT GGT TTT GT-3’  Z1063-i2-R 5’-CTC AAA CTC ACC ATC ATT GA-3’ |
| 3.2 | Resistant allele | TFusF1 5’-CTG AAA CTC TCC GTA TTT C-3’  TFusRR1 5’-CGA AGA GTG ATT GGA GAT-3’ |
| 4. | Format of the test |  |
| 4.1 | Number of plants per genotype | at least 20 plants |
| 4.2 | Control varieties | homozygous susceptible allele present:  (*Solanum lycopersicum*) Moneymaker  homozygous resistant allele present: (*Solanum lycopersicum*) Tradiro |
| 5. | Preparation |  |
| 5.1 | Preparation DNA | harvest per individual plant a part of a young leaf. Isolate total DNA with a standard DNA isolation protocol (CTAB/SDS based). Re- suspend in 100 µl 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 minutes  2. 35 cycles at 94°C for 1 minute, 56°C for 1 minute, and 72°C for 2 minutes  3. final extension step of 72°C for 10 minutes |
| 7. | Observations |  |
| 7.1 | Method | visual |
| 7.2 | Observation scale |  |

|  |  |  |
| --- | --- | --- |
|  |  |  |
| amplicon of 940bp only | 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 |  |
|  | 24.1 Race 0 (ex 1) |  |
|  | present [9] | Homozygous or heterozygous resistant in DNA marker test.  In case homozygous susceptible allele present a bio-assay on race 0 (ex 1) should be performed.  In case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the resistance is absent or present for the variety (on another mechanism, e.g. gene I2 without I). |
|  | 24.2 Race 1 (ex 2) |  |
|  | absent [1] | homozygous susceptible in DNA marker test |
|  | present [9] | homozygous or heterozygous resistant in DNA marker test. In case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the resistance is absent or present for the variety (on another mechanism, e.g. gene I3). |

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

*Current wording*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 27.  (+) |  | Resistance to Tomato mosaic virus (ToMV) | Résistance au virus de la mosaïque de la tomate (ToMV) | Resistenz gegen das Tomatenmosaikvirus (ToMV) | Resistencia al virus del mosaico del tomate (ToMV) |  |  |
| 27.1 | VG | – Strain 0 | – Souche 0 | – Pathotyp 0 | – Cepa 0 |  |  |
| **QL** |  | absent | absente | fehlend | ausente |  | 1 |
|  |  | present | présente | vorhanden | presente | Emperador | 9 |
| **27.2** |  | – Strain 1 | – Souche 1 | – Pathotyp 1 | – Cepa 1 |  |  |
| **QL** |  | absent | absente | fehlend | ausente |  | 1 |
|  |  | present | présente | vorhanden | presente |  | 9 |
| **27.3** |  | – Strain 2 | – Souche 2 | – Pathotyp 2 | – Cepa 2 |  |  |
| **QL** |  | absent | absente | fehlend | ausente |  | 1 |
|  |  | present | présente | vorhanden | presente |  | 9 |

*Proposed new wording*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 27.  (+) |  | Resistance to Tomato mosaic virus (ToMV) | Résistance au virus de la mosaïque de la tomate (ToMV) | Resistenz gegen das Tomatenmosaikvirus (ToMV) | Resistencia al virus del mosaico del tomate (ToMV) |  |  |
| 27.1 | VG/ VS | – Strain 0 | – Souche 0 | – Pathotyp 0 | – Cepa 0 |  |  |
| **QL** |  | absent | absente | fehlend | ausente |  | 1 |
|  |  | present | présente | vorhanden | presente | Emperador | 9 |
| **27.2** | VG/ VS | – Strain 1 | – Souche 1 | – Pathotyp 1 | – Cepa 1 |  |  |
| **QL** |  | absent | absente | fehlend | ausente |  | 1 |
|  |  | present | présente | vorhanden | presente |  | 9 |
| **27.3** | VG/ VS | – Strain 2 | – Souche 2 | – Pathotyp 2 | – Cepa 2 |  |  |
| **QL** |  | absent | absente | fehlend | ausente |  | 1 |
|  |  | present | présente | vorhanden | presente |  | 9 |

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

*Current wording*

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

1. Pathogen Tomato mosaic virus

3. Host species *Solanum lycopersicum*

4. Source of inoculum Naktuinbouw[[6]](#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 (*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.

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

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

*Proposed new wording*

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

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

1. Bio-assay

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | Tomato mosaic virus |
| 3. | Host species | *Solanum lycopersicum* |
| 4. | Source of inoculum | Naktuinbouw[[8]](#footnote-9) (NL) or GEVES[[9]](#footnote-10) (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 | Shelflife/viability inoculum | fresh>1 day, desiccated>1year |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | at least 20 plants |
| 9.2 | Number of replicates | 1 replicate |
| 9.3 | Control varieties |  |
|  | Susceptible | (*Solanum lycopersicum*) Marmande, Monalbo |
|  | Resistant for ToMV: 0 and 2 | (*Solanum lycopersicum*) Mobaci |
|  | Resistant for ToMV: 0 and 1 | (*Solanum lycopersicum*) Moperou |
|  | Resistant with necrosis | (*Solanum lycopersicum*) “Monalbo x Momor” |
|  | Resistant | (*Solanum lycopersicum*) Gourmet |
| 9.4 | Test design | blank treatment with PBS and carborundum or similar buffer |
| 9.5 | Test facility | glasshouse or climate room |
| 9.6 | Temperature | 24 to 26°C |
| 9.7 | Light | 12 hours or longer |
| 9.8 | Season | symptoms are more pronounced in summer |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | 1 g leaf with symptoms with 10 ml PBS or similar buffer  homogenize, add carborundum to buffer (1 g/30ml) |
| 10.3 | Plant stage at inoculation | cotyledons or 2 leaves |
| 10.4 | Inoculation method | gentle rubbing |
| 10.7 | Final observations | 11-21 days after inoculation |
| 11. | Observations |  |
| 11.1 | Method | visual |
| 11.2 | Observation scale | symptoms of susceptibility:  mosaic in top, leaf malformation  symptoms of resistance (based on hypersensitivity):  local necrosis, top necrosis, systemic necrosis |
| 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. | |

1. 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 or resistance allele | Outer primer TM2-748F:  5’CGGTCTGGGGAAAACAACTCT3’ Outer primer TM2-1256R:5’CTAGCGGTATACCTCCACATCTCC3’ TM2-SNP901misR:  5’GCAGGTTGTCCTCCAAATTTTCCATC3’ TM2-SNP901misF: 5’CAAATTGGACTGACGGAACAGAAAGTT3’ |
| 4. | Format of the test |  |
| 4.1 | Number of plants per genotype | at least 20 plants |
| 4.2 | Control varieties | homozygous susceptible allele tm2 present:  (Solanum lycopersicum) Moneymaker  resistant allele Tm2 present: (Solanum lycopersicum) Moperou  resistant allele Tm22 present: (Solanum lycopersicum) Momor, Persica, Campeon |
| 6. | PCR conditions | 1. Initial denaturation step at 94°C for 3 minutes  2. 35 cycles at 94°C for 1 minute, 55°C for 1 minute, 72°C for 2 minutes  3. Final extension step of 72°C for 10 minutes |
| 8. | Interpretation of test results | the presence of the alleles tm2, Tm2, 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). |

|  |  |  |  |
| --- | --- | --- | --- |
| Test result DNA marker test | tm2/tm2 | Tm2/tm2 or Tm2/Tm2 | Tm22/tm2 or Tm22/Tm22 orTm22/Tm2 |
|  |  | (occurs incidentally) |  |
| 27.1 Strain 0 | [1] absent | [9] resistant | [9] resistant |
| 27.2 Strain 1 | [1] absent | [9] resistant | [9] resistant |
| 27.3 Strain 2 | [1] absent | [1] absent | [9] resistant |

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

*Current wording*

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

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

2. Quarantine status yes

3. Host species *Solanum lycopersicum*

4. Source of inoculum -

5. Isolate -

8. Multiplication inoculum

8.6 Harvest of inoculum symptomatic leaves may be stored at -70°C

9. Format of the test

9.1 Number of plants per genotype 20 plants

9.2 Number of replicates……………… 1 replicate

9.3 Control varieties

Susceptible: (*Solanum lycopersicum*) Montfavet H 63.5

Resistant: (*Solanum lycopersicum*) TY 20, Anastasia, Mohawk

9.5 Test facility field with natural disease pressure

9.9 Special measures prevent spread of white-flies

10. Inoculation

10.3 Plant stage at inoculation 6-12 weeks (adult plants)

10.4 Inoculation method vector (Bemisia white-flies carrying TYLCV)

10.7 Final observations 1-2 months after inoculation

11. Observations

11.1 Method visual

11.2 Observation scale Symptoms: leaf yellowing and curling

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

12. Interpretation of test results in comparison with control varieties

absent………………………… [1] severe symptoms

present ………………………… [9] no or mild symptoms

13. Critical control points:

TYLCV is endemic in many tropical and subtropical areas and has a quarantine status in many countries with a temperate climate. TYLCV is on the EPPO alert list. Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV).

*Proposed new wording*

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

1. agroinoculation method

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | Tomato yellow leaf curl virus (TYLCV) IL strain. (See note below) |
| 2. | Quarantine status | yes (see 13.) |
| 3. | Host species | *Solanum lycopersicum* |
| 4. | Source of inoculum | Dr. Eduardo R. Bejarano, Plant Genetics Laboratory, IHSM UMA- CSIC)[[10]](#footnote-11) |
| 5. | Isolate | Alm:Pep:99, strain IL |
| 6. | Establishment isolate identity |  |
| 7. | Establishment pathogenicity |  |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | YEP/Kanamycin. |
| 8.2 | Multiplication variety |  |
| 8.3 | Plant stage at inoculation | 3-4 leaf |
| 8.4 | Inoculation medium | YEP |
| 8.5 | Inoculation method | Stem puncture agroinfiltration. Plant agroinoculation is carried out using Agrobacterium tumefaciens transformed with plasmids containing the infectious clones (Morilla, et al. 2005. Phytopathology 95: 1089-1097)  The transformed Agrobacterium tumefaciens is a Geneticaly Modified Organism and requires to comply with legislation concerning the protection of the environment, human and animal health. |
| 8.6 | Harvest of inoculums |  |
| 8.7 | Check of harvested inoculum |  |
| 8.8 | Shelflife/viability inoculum | *A. tumefaciens* stocks are maintained frozen at -80ºC in 15-20% glycerol for long term storage. Cultures to be stored are typically started from a single colony and grown in 5 ml YEP +2.5 µl kanamycin (100mg/ml) during 48 h at 28ªC. |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | 20 |
| 9.2 | Number of replicates | 2 |
| 9.3 | Control varieties | Susceptible: Big Power, (*Solanum lycopersicum*) Moneymaker, Marmande  Resistant: (*Solanum lycopersicum*) Delyca, Montenegro, Anastasia, TY20, Mohawk |
| 9.4 | Test design |  |
| 9.5 | Test facility | Glasshouse or climatic chamber with permission to confined use of Genetically Modified Organism, confinement level 1 (N‑1). |
| 9.6 | Temperature | 23-25°C |
| 9.7 | Light | 16 h |
| 9.8 | Season |  |
| 9.9 | Special measures | Permission to confined use of Genetically Modified Organism, at least level 1 (N-1) |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | Streak the surface of the frozen *A. tumefaciens* stock tube and submerge in 5 ml YEP+2.5 µl kanamycin (100mg/ml) during 48 h at 28ªC. Shaking is needed. Take 100 µl and place them into 100 ml YEP and 50 µl kanamycin (100mg/ml). Shake 48 h at 28ºC. Centrifuge the saturated culture for 20 min at 3500 rpm and discard supernatant. |
| 10.2 | Quantification inoculums | Dissolve in sterile deionize water to a final OD 600 of 1. |
| 10.3 | Plant stage at inoculation | 3-4th leaf |
| 10.4 | Inoculation method | Take up into a 1 ml syringe with a 27-gauge needle and few drops (about 20 µl of the culture) were deposited on 10-15 puncture wounds made with the needle into the stem of test tomato plants. Maintain on ice while inoculating plants. |
| 10.5 | First observation | 20 days post inoculation |
| 10.6 | Second observation | 30 dpi |
| \*10.7 | Final observations | 45 dpi |
| 11. | Observations |  |
| 11.1 | Method | Visual |
| 11.2 | Observation scale | Symptoms: leaf yellowing and curling |
| 11.3 | Validation of test | evaluation of variety resistance should be calibrated with results of resistant and susceptible controls |
| 12. | Interpretation of data in terms of  UPOV characteristic states |  |
|  | absent [1] | severe symptoms |
|  | present [9] | no symptoms |
| 13. | Critical control points:  TYLCV is endemic in many tropical and subtropical areas and has a quarantine status in many countries with a temperate climate.  TYLCV-IL is the strain most widely spread worldwide. With this strain, symptoms do not appear in varieties with Ty-1 and Ty-2.  TYLCV is on the EPPO alert list. Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV). | |

1. White fly inoculation method

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | Tomato yellow leaf curl virus (TYLCV) IL strain |
| 2. | Quarantine status | yes (see 13.) |
| 3. | Host species | *Solanum lycopersicum* |
| 4. | Source of inoculum | -Spain[[11]](#footnote-12) |
| 5. | Isolate | -TYLCV-IL La Mayora |
| 8. | Multiplication inoculum | White flies |
| 8.6 | Harvest of inoculums |  |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | 20 |
| 9.2 | Number of replicates | Two replicates |
| 9.3 | Control varieties |  |
|  | Resistant | TY 20, Anastasia, Mohawk |
|  | Susceptible | Big Power, (*Solanum lycopersicum*) ~~Montfavet H 63.5~~ Moneymaker, Marmande |
|  | Resistant | (*Solanum lycopersicum*) Delyca, Montenegro, Anastasia, TY20, Mohawk |
| 9.5 | Test facility | ~~field with natural disease pressure~~ greenhouse/plastic tunnel |
| 9.9 | Special measures | prevent spread of white-flies |
| 10. | Inoculation |  |
| 10.3 | Plant stage at inoculation | ~~6-12 weeks (adult plants)~~ 2-4 weeks |
| 10.4 | Inoculation method | vector (Bemisia white-flies carrying TYLCV-IL) |
| 10.7 | Final observations | 1-2 months after inoculation |
| 11. | Observations |  |
| 11.1 | Method | visual |
| 11.2 | Observation scale | Symptoms: leaf yellowing and curling |
| 11.3 | Validation of test | evaluation of variety resistance should be calibrated with results of resistant and susceptible controls |
| 12. | Interpretation of data in terms of  UPOV characteristic states |  |
|  | absent [1] | severe symptoms |
|  | present [9] | no or mild symptoms |
| 13. | Critical control points:  TYLCV is endemic in many tropical and subtropical areas and has a quarantine status in many countries with a temperate climate. ~~TYLCV is on the EPPO alert list.~~  TYLCV-IL is the strain most widely spread worldwide. With this strain, symptoms do not appear in varieties with Ty-1 and Ty-2.  Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV). | |

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

*Current wording*

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

*Proposed new wording*

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

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

*Current wording*

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

1. Pathogen Tomato spotted wilt virus (see note below)

2. Quarantine status yes (see note below)

3. Host species *Solanum lycopersicum*

4. Source of inoculum Naktuinbouw [[12]](#footnote-13) (NL), GEVES[[13]](#footnote-14) (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.

*Proposed new wording*

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

1. Bio-assay

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | Tomato spotted wilt virus (see note below) |
| 2. | Quarantine status | yes (see note below) |
| 3. | Host species | *Solanum lycopersicum* |
| 4. | Source of inoculum | Naktuinbouw [[14]](#footnote-15) (NL), GEVES [[15]](#footnote-16) (FR) |
| 5. | Isolate | race 0, preferably a thrips-transmission deficient variant |
| 7. | Establishment pathogenicity | biotest |
| 8. | Multiplication inoculum |  |
| 8.6 | Harvest of inoculum | symptomatic leaves may be stored at -70°C |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | 20 plants |
| 9.2 | Number of replicates | 1 replicate |
| 9.3 | Control varieties |  |
|  | Susceptible | Big Power and (*Solanum lycopersicum*) Monalbo, Momor,  Montfavet H 63.5 |
|  | Resistant | Enpower and (*Solanum lycopersicum*) Tsunami, Bodar, Mospomor, Lisboa |
| 9.5 | Test facility | glasshouse or climatic chamber |
| 9.6 | Temperature | 20°C |
| 9.7 | Light | 12 hours or longer |
| 9.9 | Special measures | prevent or combat thrips |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | press symptomatic leaves in ice-cold buffer 0,01 M PBS, pH 7.4, with 0,01 M sodium sulfite or similar buffer  option: sieve the leaf sap through double muslin |
| 10.3 | Plant stage at inoculation | one or two expanded leaves |
| 10.4 | Inoculation method | mechanical, rubbing with carborundum on cotyledons, inoculum suspension < 10° C |
| 10.7 | Final observations | 7-21 days after inoculation |
| 11. | Observations |  |
| 11.1 | Method | visual |
| 11.2 | Observation scale | symptoms: top mosaic, bronzing, various malformations, necrosis |
| 11.3 | Validation of test | evaluation of variety resistance should be calibrated with results of resistant and susceptible controls |
| 12. | Interpretation of test results in comparison with control varieties |  |
|  | absent [1] | symptoms |
|  | present [9] | no symptoms |
| 13. | Critical control points  TSWV has a quarantine status in some countries. TSWV is transmitted by *Thrips tabac*i and Western flower thrips (*Frankliniella occidentalis*). Pathotype 0 is defined by its inability to break resistance in tomato varieties carrying the resistance gene Sw-5. | |

1. DNA marker test

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

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

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

*Proposed addition to 9. Literature*

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

[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)
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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. Source of inoculum; HMS UMA (CSIC) edu\_rodri@uma.es; INIA Cardaba@inia.es [↑](#footnote-ref-11)
11. IHSM, CSIC guillamon@eelm.csic.es or INIA cardaba@inia.es [↑](#footnote-ref-12)
12. Naktuinbouw: resistentie@naktuinbouw.nl [↑](#footnote-ref-13)
13. GEVES; Valerie.GRIMAULT@geves.fr [↑](#footnote-ref-14)
14. Naktuinbouw: [resistentie@naktuinbouw.nl](mailto:resistentie@naktuinbouw.nl) [↑](#footnote-ref-15)
15. GEVES; Valerie.GRIMAULT@geves.fr [↑](#footnote-ref-16)